

donic acid. About 25% of the total fatty acid content of the glands is made up of this acid which is found only in small quantities in other organs. We also found pentaenes and hexaenes present in greater amounts than in other organs. These fatty acids must have a biological purpose and two hypotheses can be advanced. According to one mentioned previously, (*Note 11, Chapter VI*), the corticoids would be synthesized from arachidonic acid through cyclization. According to the second hypothesis, arachidonic acid, as well as other high fatty acids present in the adrenals, would be used as active functional fatty acids. Secreted by the adrenals, they would pass into the circulation and intervene as needed by the organism, especially for immediate defense purposes.

We have seen that an intervention of polyunsaturated fatty acids occurs in the first defense response of an organism to a noxious agent. These acids are responsible for the exaggerated oxidation processes through which the organism attacks the noxious agents themselves or the heterogenized constituents resulting from their action. We consider that some of the fatty acids intervening in this defense mechanism are liberated locally, especially if they appear in response to a condition limited to a lower entity. In this case, they would come from changes induced in the constituents of the entity itself. The local intervention of lipolytic enzymes would lead to a liberation of free fatty acids. In a defense response for the organism as the highest entity, the actively intervening fatty acids appear in the general circulation in the first phase of the diphasic phenomenon. Some of these fatty acids would be of adrenal origin, liberated at these moments. In the second phase of the defense mechanism, a further liberation of steroids by the adrenals would occur, aimed at counteracting the effects of fatty acids. The diphasic systemic process which results can be considered to represent an exaggeration of the processes which occur alternately and which, through normal oscillations, insure the dynamic systemic balance.

The adrenals conceivably control abnormal fatty acid activity by their quantitatively exaggerated intervention and by release of qualitatively abnormal products that would pass into the circulation and result in off-balances. The activity of the adrenals in counteracting the influence exercised by fatty acids has been made the subject of a special investigation in our laboratories by E. F. Taskier.

By comparing the doses of an agent required to kill normal and adrenalectomized animals, it has been possible not only to identify this intervention but to judge the degree of this specific defense mechanism. The "Adrenal Defense Index" for an agent—the ratio between the minimal lethal dose in normal animals and in adrenalectomized animals represents

a numerical estimate of this response. It could be shown that for certain fatty acids, such as conjugated trienes, which are related to trauma, or alpha hydroxy fatty acids which are related to microbial invasion, a highly effective intervention of the adrenals occurs, through release of neoglucogenic corticoids. The administration of neoglucogenic corticoids manifestly increases the resistance of the organism to the noxious effects of fatty acids. This influence is reduced for the mineralocorticoids and is nil for sodium chloride, otherwise an important factor related to adrenal intervention.
(Note 17, Chapter VI)

We will discuss later an important difference, even an antagonism, between these two groups of corticoids when their influence is exerted concomitantly with that of other agents.

Synthetic Anti-Fatty Acids

Analysis of the natural anti-fatty acid agents has revealed the importance of their positive polar groups. And this has guided us in attempts to obtain synthetic agents with anti-fatty acid effects.

An important step was a study of alcohols with lipoidic properties, the lipoalcohols, starting with the primary mono-alcohol homologous series. This study also has permitted us to recognize the importance of the lipoidic properties for their biological activity. We started with butanol which is the first member of the homologous series of aliphatic alcohols with lipoid characteristics.

Butanol

Butanol has a special place among the alcohols that have been utilized as anti-fatty acid agents, not only by virtue of its physico-chemical and biological properties but also because of interesting therapeutic results obtained in animals and humans.

Extensive studies with butanol have helped considerably in defining the physico-chemical and biological differences between lipoids and nonlipoids. According to the concept advanced previously, lipoids and nonlipoids can be distinguished by solubility characteristics which are determined by the energetic relationship between their polar and nonpolar groups. The nonpolar group is predominant in a lipoid; the polar group is predominant in a hydroid. Lipoids have greater solubility in neutral solvents than in water, and this provides a simple criterion for their recognition.

Methyl, ethyl and propyl alcohols are all equally more soluble in water than in neutral solvents and therefore are recognized as nonlipoids. Butanol,

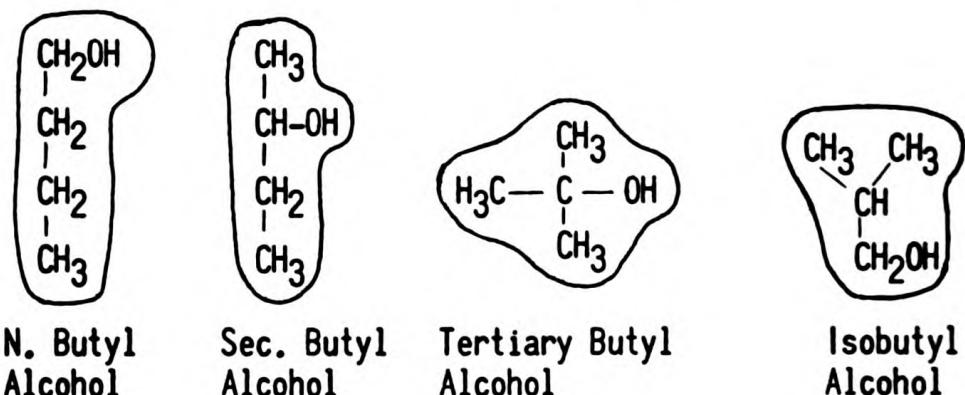


FIG. 133. Schematic representation of the molecular surfaces of the 4 isomers of butanol. The constant b of the van der Waals forces related to their surfaces are unequal. A minimum value is seen for almost spheric molecule of tertiary butanol, a fact which explains the nonpredominance of the polar group in this molecule, respectively its non-lipidic character.

however, differs from the lower members of the aliphatic alcohol series by being a lipoid, more soluble in neutral solvents than in water. This, however, is true only for three of the four isomers of butanol. n-Butanol, sec-butanol and iso-butanol are all more soluble in neutral solvents than in water, whereas tert-butanol is equally soluble in both. According to our criterion therefore, while the first three are lipoids, tert-butanol is not.

These considerations have enabled us to correlate lipoidal properties

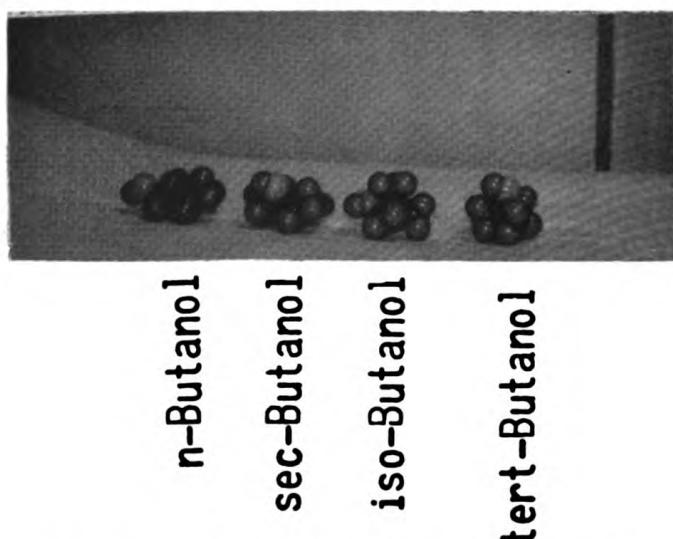


FIG. 134. The differences between the round shape of tertiary butanol and the longer form of the other isomers is evident with models of molecules.

with one more precise intermolecular factor, the predominance of one of the van der Waals cohesion forces. Comparative analysis of the structural formula of the four isomers of butanol (*Fig. 133*), reveals the importance of forces related to the surface of the molecules in determining differences in their solubility. In contrast to the three lipoid isomers, the molecule of tertiary butanol is rounder and hence has a smaller surface. The difference between tert-butanol and the other three isomers is apparently due to the cohesion forces related to the surface area of the molecule. Of the van der Waals forces, those described as related to the surface of the molecules, or

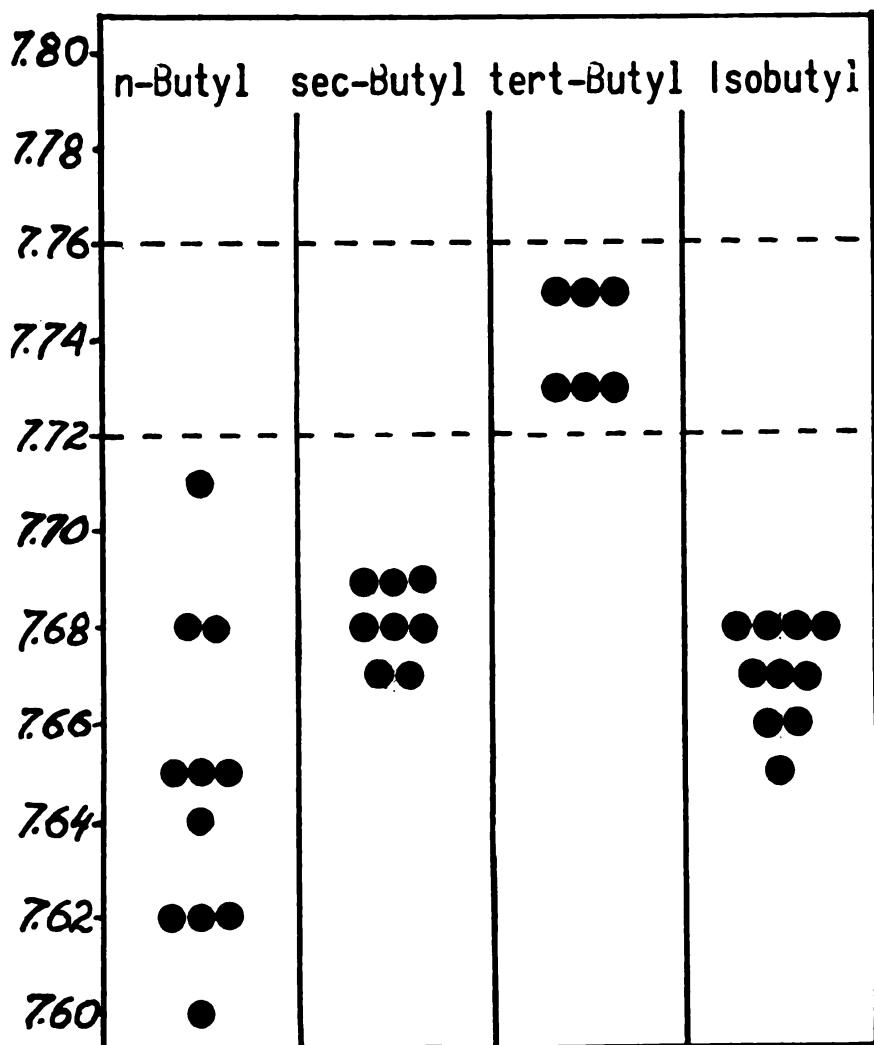


FIG. 135. While the 3 isomers of butanol which are lipoids influence the second day wound crust pH, lowering its values, tertiary butanol which is not a lipoid, does not influence it.

as the constant b of the cohesion forces, thus appeared to be most important in determining lipoidic properties. (Fig. 134)

Study of the four isomers of butanol has confirmed the importance of lipoidic properties for biological activity. Like the lower members of the homologous series of aliphatic alcohols which are not lipoids, tertiary butanol does not influence pH of the second day crust of a wound, while the three other isomers, all with lipoidic characters, lower the pH as the higher members of this series do. (Fig. 135)



FIG. 136. Effect of 0.5% solution of *n*-butanol administered instead of drinking water upon the increase in weight of young rats. The values represent the average for 20 females (.....). No differences are seen from nontreated controls (—).

The fact that a saturated water solution at 20°C still contains 7.9% *n*-butanol is of great practical importance. Because of its degree of solubility in water, *n*-butanol could be utilized in aqueous solutions in sufficiently high concentration for pharmacological studies and could be used as a therapeutic agent in this form without need for an oily solvent vehicle.

The acute toxicity dose for butanol corresponds to the narcotic dose for the respiratory centers which is related to interference with the aerobic life of these cells.

The minimal lethal dose of *n*-butanol administered subcutaneously was found to be 4.6-6.4 gm./Kgm. for mice, 3.7-5.9 gm./Kgm. for rats and 3.3-5.6 gm./Kgm. for rabbits, guinea pigs and hamsters. These values closely approximate the findings of other workers. The minimal lethal dose of *n*-butanol injected intraperitoneally is very close to that for subcutaneous

and intramuscular administration, indicating that absorption from the tissues is almost as rapid as from serous cavities.

We have administered butanol in large doses to human subjects, and these clinical studies have confirmed the laboratory findings that the toxic effect is especially manifest through the narcotic effect and is attained only with the use of very large doses. (*Note 8*)

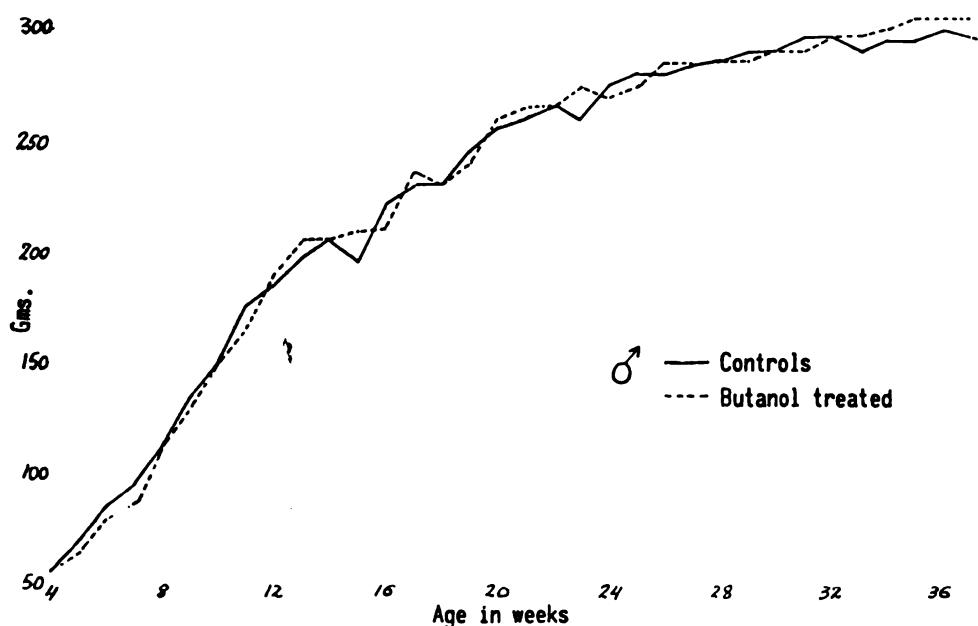


FIG. 137. The same daily change seen in male rats.

Long term use of n-butanol has virtually no influence upon normal physiology in animals. Administered continuously in the drinking water of young animals, it had no effect on growth. (*Fig. 136*) It also did not affect reproduction capabilities of mature animals or influence their off-springs.

n-Butanol shows a definite influence on white blood cells in rats. The leucocyte count is increased in adult rats receiving daily injections of a saturated solution of n-butanol. (*Note 9*)

The influence exerted by small doses of n-butanol, as for other lipoids, appears to be almost entirely confined to abnormal tissues and cells. This is evident in the influence upon the pH of experimentally induced wounds in animals. Administered before wound inductions, n-butanol showed no influence upon normal tissue, no differences were observed between pH of their normal tissues in treated and untreated animals. During the first day

following wound inductions, pH of the lesion in treated animals was no different from pH in untreated control animals. However, by the second day, pH of the wound crust was lowered by butanol, as seen in Figure 135.

n-Butanol accelerated the healing rate of wounds, although the differences between treated animals and controls was not striking. n-Butanol enhanced healing of radiation burns to some extent but the effect was not constant in different groups of animals. In several animals treated with n-butanol, radiation wounds healed within two or three weeks, while in controls healing took more than four weeks.

Butanol, when administered to patients with pain of alkaline pattern, has repeatedly provided relief within a very short time—in some cases within three to five minutes. In pain of an acid pattern, exacerbation occurs, also within a few minutes. Its quick effect has led to use of butanol as a diagnostic means for determining the pain pattern.

The anti-fatty acid action of n-butanol has led to the investigation of its effect upon shock since, as previously noted, shock appears to be related to intervention of abnormal fatty acids. Administration of butanol subcutaneously, even together with large amounts of saline, is only slightly beneficial for shock in mice with caloric burns. The addition of sodium lactate has markedly prolonged survival time in these animals. (*Note 10*) (*Fig. 138*) Still better effects upon traumatic conditions are obtained by associating butanol with glycerophosphoric acid in saline or in glucose saline solution. Especially effective and well tolerated is a solution containing 0.3-0.5 gm.% butanol with n/300 to n/200 glycerophosphoric acid and with 5% glucose in saline, used for intravenous infusions, as well as for subcutaneous clysis.

The administration of butanol in sufficient amounts to many patients having massive hemorrhages has clearly demonstrated that this substance has a hemostatic effect which will be discussed below.

After butanol studies, the effects of other aliphatic alcohols were investigated and revealed the importance of the nonpolar group in their biological activity.

Higher Alcohols

The study of aliphatic alcohols has shown that only few members of this homologous series, beginning with butanol and ending with octanol, have an effect upon the s.d.c. pH. For octanol, only half of the test animals showed changes in second day wound crust pH. (*Fig. 139*) We thought it worthwhile to study the biological effects exerted by these members of the series, including those which demonstrated no effect on the second day

wound crust pH. Comparative studies indicate definite differences between the two groups with odd or even number of carbons in their ability to act upon an existing offbalance and reduce the abnormal metabolism. Along with differences, many common properties were recognized through effects

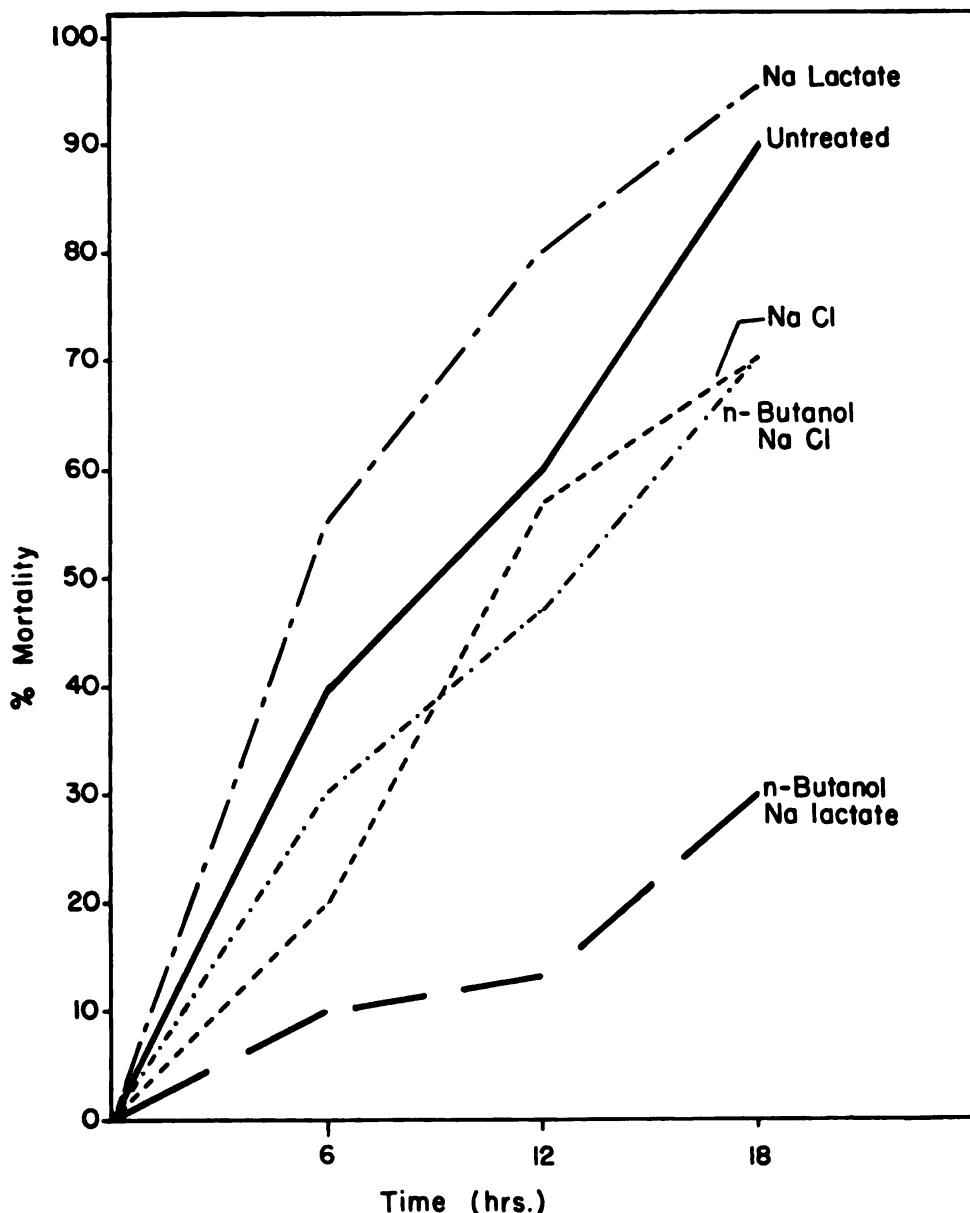


FIG. 138. Influence exerted by different agents upon the mortality of mice scalded for 3 seconds in water at 90°C. While sodium lactate seems even to increase the mortality and NaCl and butanol in saline have little influence, a marked prolongation of the survival time is induced by the mixture of n-butanol and Na lactate.

induced at various levels. In general, the effects are more profound for members with longer chains. This is true for butanol compared to hexanol in the even carbon series and for pentanol compared to heptanol in the odd carbon series. For octanol, most of the effects are diminished. In the group with odd carbon numbers, nonanol has very little or no influence.

On viruses, a protective effect against external influences such as heat or fatty acids is evident. It is more striking for the even carbon group. In microbes, little except an antibacterial effect is produced by members in the even carbon group. The odd carbon group induces Gram positivity, irregularities in form with a tendency toward roundness, and vacuolization.

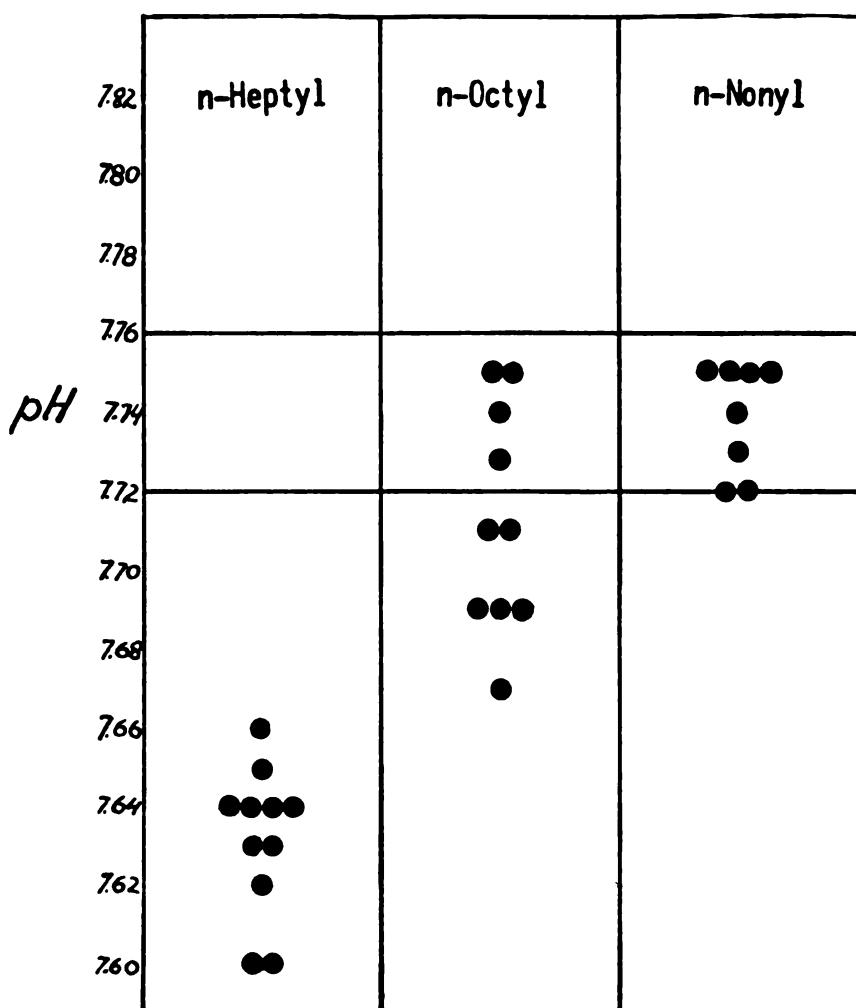


FIG. 139. The influence exerted by heptanol, octanol and nonanol upon the s.d.c. pH. While heptanol induces constantly a lowering of the alkalinity of the second day wound crust, this effect is less constant for octanol and nil for nonanol.

No important changes occur at the cellular level. We have already mentioned the different effects upon wound crust pH for these alcohols. On pain, nonanol has no effect and hexanol and octanol relatively little. But pentanol and especially heptanol show a very marked influence, both immediate and prolonged. It is interesting to note another striking effect at the tissular level, observed only for heptanol. It corresponds to an abnormal accumulation of fluid in certain abnormal tissues. This was first seen in surgical scars even several months old. Edema of the entire scar occurred followed by blistering at the surface, or even by formation of fluid-filled cavities in the scar itself. The same phenomenon appeared in other lesions such as tumors, especially when they were infected, although there had been no clinical indication of infection before the administration of heptanol. The effect sometimes was very intense, transforming an entire lesion, scar or tumor into a cavity with septic fluid exudate, but few leucocytes.

The influence of high doses of heptanol upon inflammatory processes could be judged experimentally in the gas pouch induced subcutaneously in rats or mice by injecting nitrogen and subsequently injected with a low pathogenic microbe. In controls, no unfavorable effects were noted. In animals injected with heptanol subcutaneously, fluid exudate accumulated in the pouch in a few days. (*Note 11*) Subcutaneous administration of heptanol also induced an exudate in the peritoneal cavity in mice and rats injected with the same microbial suspension. This did not occur in the controls. It must be emphasized that these effects were seen only with relatively high doses of heptanol.

At the organic level, while nonanol again showed no activity, the two higher alcohols, heptanol and octanol, had an influence upon the central nervous system. In humans, even in larger doses, such as 200 mgr. six times a day (2 cc. of a 10% solution in oil every four hours) repeated for ten days or more, the two higher alcohols produced no abnormal central nervous system manifestations. In some subjects who had previously had convulsive attacks, administration even in small doses, such as 25-50 milligrams once a day, did induce convulsive seizures. If these substances were given along with desoxycorticosterone, the latter even in doses of 1 mgr. a day, severe and even fatal convulsions were produced. Nonanol had no such effect. Somnolence followed by coma was observed with concomitant administration of cortisone and heptanol or octanol, but nonanol did not produce this effect either.

Of interest was the influence exerted by heptanol upon the different analyses. Fig. 140 shows how these values change toward the offbalance A

under the influence of heptanol. It is to be noted that of all the analyses, the urinary pH and the blood serum potassium are the first to be changed. They are followed by specific gravity, while the urinary surface tension seems to be influenced last.

M. Bier, in our laboratories, has shown that alcohols, when added *in vitro* to freshly obtained blood, reduced blood clot retractability. It is interesting to note here the relationship Bier has shown between this effect in

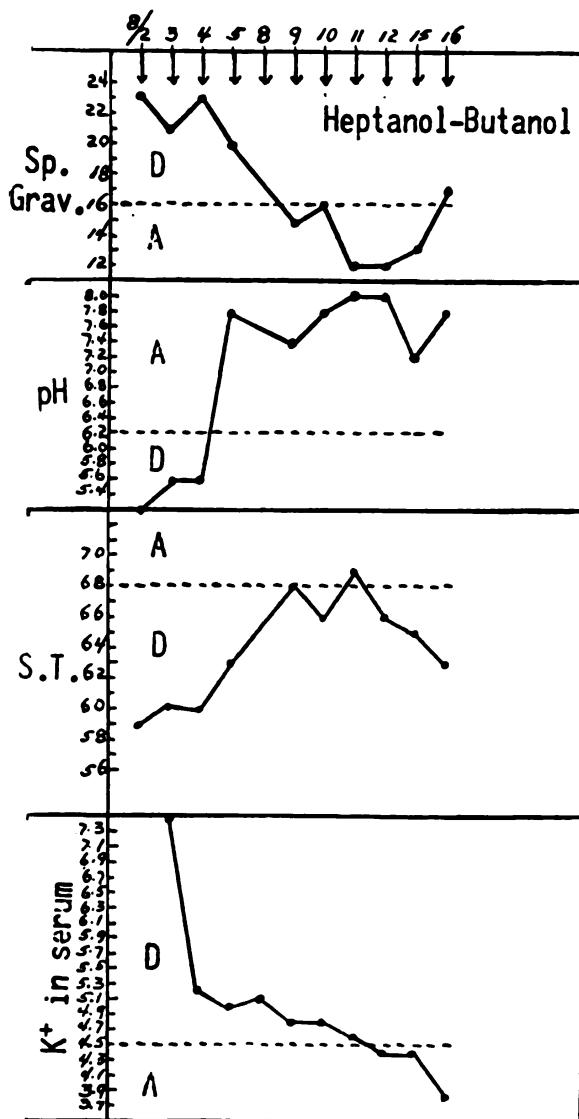


FIG. 140. In a patient with bone metastatic lesions the administration of heptanol and butanol shows a progressive decrease in the values of serum potassium and increase in the urinary pH, toward offbalance type A.

retractability and other properties of the alcohol series members. Thus, he could demonstrate that there is a critical value for the concentration of each alcohol, when mixed with fresh blood: blood clot retraction is prevented only when this value is exceeded. The critical value varies with the length of the chain, decreasing for the higher members. (Fig. 143) Bier also

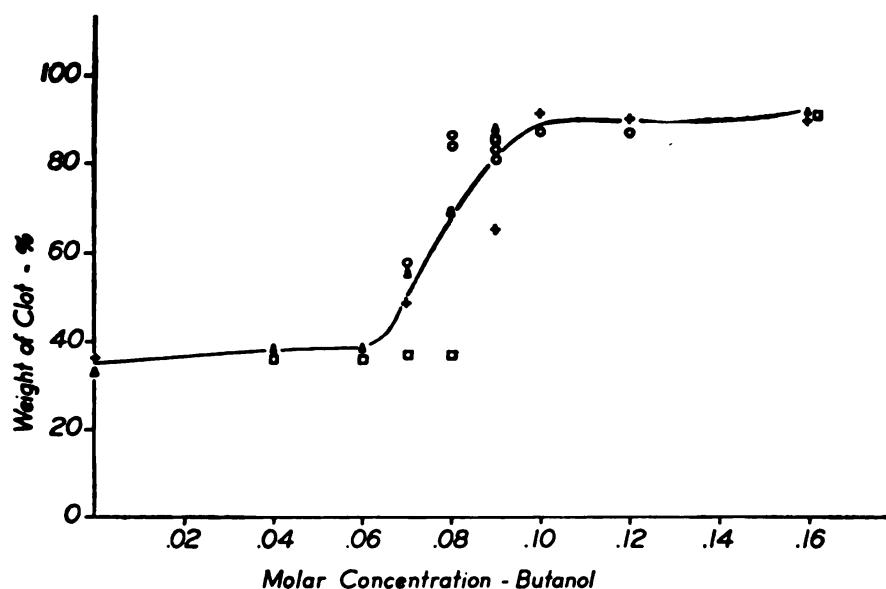


FIG. 141. Clot retraction, measured as percent weight of clot/total weight of blood, plotted against molar concentration of butanol in blood. Different symbols used for blood samples of each animal.

has shown that, since the toxicity of these alcohols seems to be related to the same factor, a correlation can be established between critical concentration values and lethal toxic doses.

This relationship, as shown in Fig. 142, applies to the members of this series of saturated alcohols, but not to alcohols of another series also studied. For the latter, the toxic dose is higher than the critical dilution at which the clot retraction is influenced, and this can be explained by the intervention of the double bond in the molecules.

Systemic effects were seen for these alcohols if administered in sufficient doses. Some special effects also were seen. Heptanol decreased the sulfhydryl index in urine analyses, especially if it had been high previously. Octanol's action was mainly to increase surface tension if it had been low. Nonanol did not show any such activity at all.

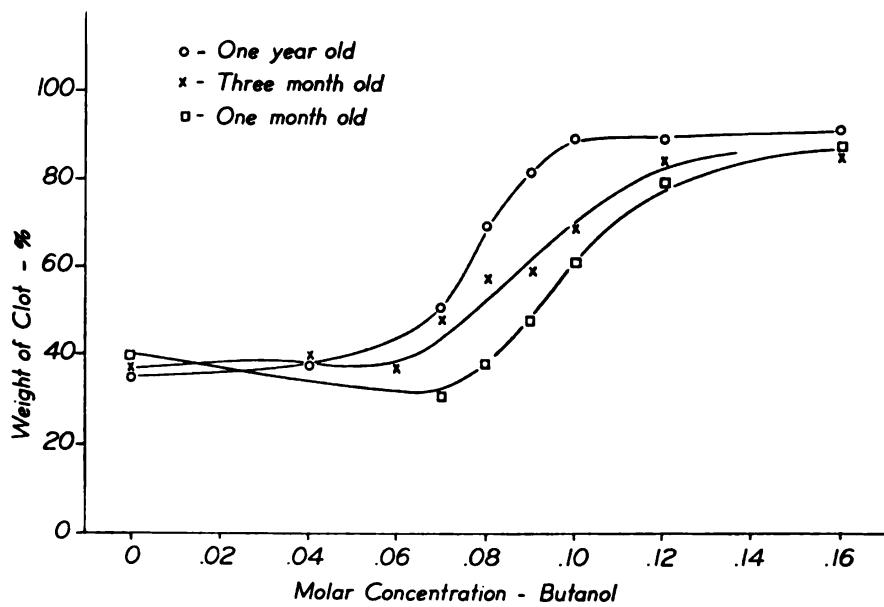


FIG. 142. Clot retraction, measured as percent weight of clot/total weight of blood, plotted against similar concentration of butanol in blood. Averages of different age group animals were studied.

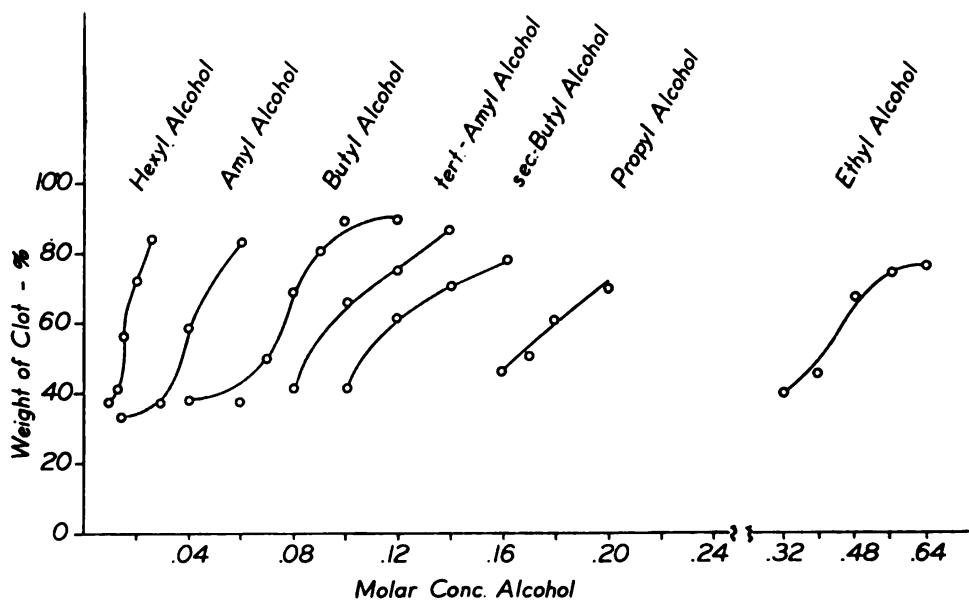


FIG. 143. Clot retraction, measured as percent weight of clot/total weight of blood, plotted against similar concentration of various alcohols in blood.

Polyols

In another study, we considered the polyalcohols, bearing in mind the important role played by glycerol in the biological activity of lipids. In animals, ethylene-glycol and diethylene-glycol proved to be too toxic for parenteral administration. However, near toxic doses produced interesting results especially in tumors. Even in relatively small subcutaneous Walker rat tumors, 2.5 cm. in diameter, for instance, necrosis was constantly induced and followed by skin ulceration. The characteristic influence of these alcohols was to induce a necrotic process not limited to the tumor alone but affecting surrounding tissues.

1.2 Diols

By following the influence exerted by more than one hydroxyl in the molecule, we tried to relate properties of glycerol to those of aliphatic lipoids. We prepared lipoids having a polar formation of 2 or 3 hydroxyls bound to the first carbons of an aliphatic chain. The lipoidic character was induced by the length of the chain. To prepare these substances, we started with corresponding alph-hydroxy fatty acids in which the carboxyl was reduced to a primary alcohol by treatment with lithium aluminum hydride. As a prototype, we studied 1.2 octanediol. The lipoidic character was recognized by its high solubility in neutral solvents and a limited solubility in water.

There were no marked differences between the effects of octanol and 1.2 octanediol in systemic analyses. Both raised surface tension values in particular. However, the new component had an effect upon the central nervous system different from most other higher alcohols. As mentioned above, these aliphatic mono-alcohols do not induce convulsions without the concomitant intervention of another factor. The second factor can be a local condition in the nervous system itself, as in subjects with cerebral tumors, or others who have had previous convulsions. It can also be another substance; desoxycorticosterol, coramine, glycerol or glucose, when administered with octanol, for example, induced convulsions in some subjects. However, 1.2 octanediol, in repeated doses of around 200 mgr. daily, induced convulsions by itself. This could be explained by the fact that 1.2 octanediol contains in its molecule a group which energetically resembles glycerol. Beyond this effect, there were no manifest differences between this substance and corresponding mono-alcohols in influence upon pain, tumor growth or systemic manifestations.

Lipoalcohols with Energetic Centers in the Nonpolar Group

We also studied other alcohols with energetic centers in their nonpolar group. From various ethenic fatty acids, we prepared the corresponding alcohols by reducing the carboxyls to primary alcohols through treatment with lithium aluminum hydride. Thus we obtained, in addition to oleic alcohol, linoleic and ricinoleic alcohols for the nonconjugated fatty acids and eleostearic alcohol for the conjugated members, as well as the corresponding alcohols of an entire series of mixtures of fatty acids from safflower oil, cotton seed oil, cod liver oil, acid lipids of organs, etc., and of conjugated fatty acids derived from them. Having two series of substances with the same common nonpolar group, but with different polar groups, COOH and OH, we could relate the effect of these alcohols to their respective fatty acids and thus ascertain once more the fundamental role played by the polar and nonpolar groups in determining the biological effects of lipoids.

Study of the effects induced upon skin viral infection has largely helped to define the differences. The polar group determines the direction of the intervention—increased receptivity or refractivity—but the extent of intervention is determined by the nonpolar group. For example, the effect is very much reduced for oleic and even for linoleic alcohols and it was similarly reduced, in the opposite direction, for the respective acids. Effects were more apparent for polyunsaturated alcohols, ricinoleic and eleostearic alcohols, polyconjugated members and corresponding acids.

The same antagonism between the corresponding acids and alcohols was very clear for systemic analyses, pain, healing of wounds, and effects upon tumors. The extent of the effects in either direction is generally determined by the nature of the nonpolar group and its energetic centers.

This comparative study of acids and alcoholic lipoids has permitted us to arrive at an important conclusion concerning the general behavior of lipoids. Thus, while the nonpolar group is extremely important for the extent of the changes induced, it appears to be secondary in importance to the polar group which determines the direction of the changes. And this explains the role attributed to the polar group in the biological activity of these agents and their separation into two fundamental groups with antagonistic biological properties, which is the basis of our approach to lipoids.

We studied the effects of alcohols and mixtures of polyunsaturated nonconjugated and polyconjugated fatty alcohols on a larger scale. These preparations were obtained from safflower and cod liver oil. The immediate effect upon alkaline pain was nearly complete relief. With prolonged treat-

ment, the relief persisted in most cases. No influence upon tumors was noted in most experiments in animals. In humans, tumor arrest in a few cases was obtained. The effect was more accentuated than with isolated members such as eleostearic, linoleic or oleic alcohols. The convulsant effect was much lower than for any other lipoalcohol of this group; even large doses did not produce convulsions except in patients who previously had had convulsions. Effects upon systemic analyses were the same as for most of the higher alcohols, manifest especially upon urinary surface tension and sulfhydryl index. The polyconjugated alcohol mixture, as an agent acting at the systemic level, produced euphoria but had very little effect upon growth and evolution of tumors.

With the intention of lowering the level at which alcohols would act, we studied a special group characterized by having a double bond between C₂ and C₃. We were particularly interested in two members of this series, allyl and crotyl alcohols.

Other Alcohols

Crotyl alcohol is a lipoid since it is miscible with neutral solvents and only slightly soluble in water. Allyl alcohol, soluble both in water and neutral solvents, appears to be an intermediary substance. Since the 1% solution of crotyl alcohol in saline was painful when given by intramuscular injection, an oily 2% solution was used. No marked differences from the effects of previously discussed lipoalcohols were seen.

We studied polyalcohols having, in addition to a primary alcohol, one or more OH attached to the molecule, such as 9,10-dihydroxy-stearic alcohol. However, this substance did not show any properties other than those noted for oleic alcohol. The alcohol obtained from ricinoleic acid, in which the carboxyl was reduced to a primary alcohol, showed a limited systemic effect. Even in larger doses, the changes were slow and not intensive, although passages from one pattern to the other could be seen more often than with other higher alcohols. A state of euphoria appeared in some subjects. The immediate effect upon pain was less than that obtained with other alcohols but, in many instances, was satisfactory. There was no favorable effect upon growth or persistence of tumors in animals or humans. In several cases, on the contrary, rapid growth of the tumor occurred despite lack of pain and even sensations of well being. In general, ricinoleic alcohol seems to act at the interstitial level and above, but not below.

Lipamines

Theoretically, it was to be expected that lipids with an amine as polar group would have a marked anti-fatty acid action. We studied several of these substances from the standpoint of their influence upon physiopathological changes considered related to lipoidic predominance. The first lipoid of the aliphatic amine series is hexylamine. A nonpolar group of at least 6 carbons is required for predominance over the potent amino radical. From commercial sources, we obtained amines corresponding to the usual saturated fatty acids with even numbers of carbons, ranging from 6 to 18; a few unsaturated with 18 carbons; and heptylamine with an odd number of carbons. All these compounds, when injected in mice and rats produced severe local reactions, often followed by skin ulcerations, even when administered in oily solutions. For this reason, we tried salts of these amines usually obtained with acetic or hydrochloric acid. Salts of the lower members of the series, no longer had lipoidic character. However, we could use hexylamine in an oily solution for intramuscular injection. It appeared to be relatively well tolerated locally even in humans. No apparent changes were seen, however, in systemic analyses, and the immediate and long range effects upon pain were minimal. No changes were obtained in experimental tumors except by local treatment, as in ascites tumor, or by injecting the product at the level of the transplant itself, in which case the growth of the tumor was slowed or even halted. A similar effect was seen when the transplant was dipped in the oily solution of the product and the procedure was repeated in successive generations.

On a larger scale, we utilized, both in animals and humans, the salt obtained from hexylamine with nicotinic acid. It showed favorable influence on pain of alkaline pattern, and exacerbated pain of acid pattern, but had no other effects. Heptylamine has been used by others as a hypertensive agent. In our studies, its hypertensive activity appeared to be weak and transitory.

The study of lipamines was the starting point for an entire series of researches into the biological role of the amino group, especially as it intervenes in a complex molecule. We have seen that, like all other polar groups, the amino group will act as a fixing group in a molecule. Its characteristics appear to be related to its capacity to bind the molecule to other molecules in a relatively stronger and more specific way than other polar groups.

The realization of more complex chemical polymers as biological entities evidently is related to this capacity of the amino group. This appears clear when the amino group is bound to various acids in the alpha position

to form the alpha amino acids that enter into the formation of complex proteins. In amino sugars, the amino group shows the same property, producing the polymer formations characteristic of connective tissues. Furthermore, it is the amino group, acting as a second polar group, which gives to alkaline amino acids their fundamental role in the biological realm, as mentioned above. The alkaline amino acids, like other amino acids, form polymers through their amino acid group. However, as these polymers, histones or protamines remain reactive through the terminal alkaline nitrogen-containing groups and it is through these groups that they realize new bonds, such as to nucleic acids.

In a molecule with two polar groups far apart the amino group will fix the molecule, while the other active radical will provide reactivity. The selective fixation upon certain constituents in various places in the organization accomplished by the amino group localizes the intervention of the other active groups of the molecule.

We have considered many of the biological substances containing an amino group and a second active group. Typical examples are the local anesthetics in which the amino group acts to fix the molecule, while the other energetic formations intervene more actively to induce the anaesthetic effect. Similarly, in epinephrine and ephedrine, the amino group serves to fix the molecule, while the hydroxyls later intervene more specifically.

In some molecules such as the alkaline amino acids, the second active group can be another amine or another nitrogen containing group. It was for this reason that we first became interested in investigating natural and synthetic compounds with an amino acid to serve for fixation, and another energetic center to intervene more actively. We studied amino-butanols, but no particular activity could be found. These agents, however, were not lipoids. We were consequently interested in substances having a lipoid character as well as two active polar groups of which one is an amine. A study of these agents is now in progress.

Procaine

In this group of agents we studied procaine. We became especially interested in procaine after we had seen some cases of ulcerations due to varicose veins healed in a few days with only two intra-arterial injections of procaine according to Leriche's method. With the idea in mind that an action through its lipoidic properties intervenes, we studied procaine base. Solutions of procaine base in sesame oil, or suspension in Tween or gum cellulose were prepared. Parallel with these procaine base preparations, we studied also the hydrochloride as well as several salts of procaine, such as

lactate, glycerophosphate, where a further effect was sought through the acids at which procaine was bound. We studied all these agents but only under the aspect of their activity in relationship to the dualistic offbalances.

No special effects were seen upon viruses and microbes from this point of view. Injected subcutaneously to rabbits, it was seen to increase in the skin corresponding to the place of injection, the manifestations of a subsequent smallpox inoculation. Added to the medium for cultures of tetrahymena pyriformis in higher doses, it led to the appearance of almost round forms. We studied the survival time through the capillary tubes method mentioned above. For progressive amounts of procaine hydrochloride added to a culture of tetrahymena, no immediate effect of the life-span of the culture could be seen. In another group of experiments, the procaine was added to the culture before its inoculation and the life-span of the tetrahymena grown on this medium was studied. Under these circumstances a prolongation of the life of the culture itself could be seen, but only in the cultures which were showing also a slow growth.

Procaine in general, and much more evident in the base preparations, was seen to reduce the pH values of the second day wound crust. In mice and rats, the lethal doses were seen to be manifested through the appearance of convulsions. Sublethal doses were seen to lead to convulsions after a few successive days of administration. In humans, an intramuscular injection with procaine base was seen to influence pain but this effect was apparently limited only to the cases with an alkaline pattern present. This and the fact that the effects were no more manifest with procaine base than with other lipoidic preparations with positive polar group, even when administered in relatively high doses such as of 2 cc. of the 5% solution of procaine in oil, indicates that its action other than at the place of the injection, has to be related largely to the intervention of procaine as lipoid. A special mention, however, has to be made for the angina pain which seems to be more evidently influenced by the injection with procaine base than other pains.

The immediate influence exerted upon systemic analyses, if any, was in general reduced even after the administration of 10 cc. of 2% procaine hydrochloride. It was manifested particularly upon the urinary pH, inducing a change toward more alkaline values. This effect was transitory and especially seen for the first injections. With repeated doses, this effect decreased even to the point of disappearing.

The study of the pharmacology of procaine has emphasized the character of its activity as a lipoid with a positive polar group rather than a direct one through its chemical constituents. This view is confirmed by the

fact that the same effects are not obtained with any one of the two constituents of procaine, para-aminobenzoic acid and diethylaminoethanol, administered separated or even together. The only difference in this case between procaine and its constituents resides in the properties of procaine as such, which we related largely to its lipoidic properties.

Furthermore, the study of the different effects induced by procaine can be seen to result from a nonspecific effect in which these agents intervene indirectly upon the metabolic processes which occur at the cells for instance, rather than to a direct influence exerted by procaine constituents upon specific metabolic processes. In this group would enter the effects seen upon different enzymatic processes indirectly influenced by procaine through the nonspecific changes induced.

We have investigated under this special aspect the use of procaine hydrochloride in solutions with a pH between 3 and 4, as indicated by A. Aslan, against the manifestations of old age. The low pH seems to intervene by preventing, as long as possible, the enzymatic hydrolysis of procaine and thus permitting its absorption and action as a nondissociated lipoid. We found that the protection exerted by the low pH would result from the chemotactic negative influence exercised by hydrosoluble acids upon the leucocytes, which thus will reduce the intervention of the leucocytic enzymes in the process of hydrolysis.

Furthermore, the fact that the bond of procaine, as such, takes place through the amino radical of the P A B A explains why a high acidity would prevent this local bond of procaine to occur at the place of the injection and thus would favor its action at other sites in the organism. This action is confirmed by the fact that the analgesic properties of procaine decrease through the low pH, while at the same time, its other metabolic effects increase.

The fact that good effects were observed with procaine in old age and arthritis, schizophrenia and many other conditions, indicates that a non-specific action would take place. The fact that the effects obtained are similar to those observed in the same conditions with other lipoidic agents with a positive polar group, permits us to see as principal factor in the pharmacological action of procaine, its nonspecific intervention as positive lipoid. Besides this nonspecific lipoidic effect, some others related to its chemical constituents and which would represent specific added factors have to be considered. The relation to the folic acid would be an example.

All these considerations led us to study various preparations in which procaine was bound to different agents, with the aim to enhance its non-specific intervention. Through procaine lactate, malate and citrate, the

chemotactic negative effect was highly increased and procaine activity enhanced. Bound to maleic or citraconic acids, the anti-fatty acid activity of procaine was further directed toward influencing more specifically, the abnormal changes occurring leading to the appearance of abnormal fatty acids.

The Elements

With the recognition that elements act biologically in two opposite directions, we became interested in those with anti-D character, capable of intervening in offbalance D. We studied the direct action of these agents upon fatty acids, as well as their indirect action upon processes and substances related to the metabolic changes characterizing the offbalance D.

We separated these inducing elements—according to their series and the compartments where they predominantly exercise their influence. The following table shows this systematization.

TABLE XIX
"A" Inducing Group or Anti-D Group

Compartments	Metals						Non Metals		
	IA	IVB	VIB	VIII	VIII'	IIB	IIIA	VA	VII A
Organism	Li						B	N	F
Metazoic	Na						Al	P	Cl
Cellular	K	Ti	Cr	Fe	Ni	Zn	Ga	As	Br
Nuclear	Rb	Zr	Mo	Ru	Pd	Cd	In	Sb	I
Submorphologic	Cs	Hf	W	Os	Pt	Hg	Tl	Bi	At
Primary	Fr								

To these elements we can add others from the lanthanum and actinium series which have anti-D characteristics. They would act in the submorphological and primary compartments.

Compartment	Anti-D Elements							
Submorphologic	La	Pr	Pm	Eu	Tb	Ho	Tm	Lu
Primary	Ac	Pa	Np	Am	Bk			

Many of these elements are known to have influences antagonistic to those of members of the D inducing group. We will consider them in more detail below.

Concerning their influence on fatty acids, some members such as those of the VII A series are known to affect fatty acids with an active nonpolar group by inactivating their double bonds, while other elements have anti-fatty acid action by binding their polar groups. Some exert this influence indirectly through the metabolism in which they take part.

We have studied these different aspects of the influence of the elements which will be presented here only in condensed form.

Monovalent Cations

One interesting aspect of monovalent cations is their correlation with organizational hierarchic compartments. Study of this correlation was first suggested by the selective distribution of monovalent cations according to levels of organization. From the previous discussion, it seems clear that the distribution into compartments can be related to positions of these elements in different periods. Sodium is the principal cation at levels above the cell, forming the metazoic compartment. Potassium, which is the next higher element in the series, is the predominant cation in cytoplasm. According to our hypothesis, ammonium, with properties resembling those of rubidium, can be considered to be the cation of the nuclear compartment.

We have only limited evidence of direct anti-fatty acid activity for members of this I A series. However, they produce characteristic changes in conditions in which an offbalance exists. They induce the type "A," as an antagonistic to the type "D" offbalance. The changes are especially evident in terms of the function of the compartment to which the cation belongs.

Sodium

Sodium, the cation of the metazoic compartment, corresponds to the environment of the sea in which the entities of this compartment developed. We have indicated previously the relationship between the time when the metazoic compartment was formed, the degree of salinity of the sea, and the concentration of the cation established as a constant in the compartment. Any excess of sodium is eliminated through the kidneys in order to conserve the metazoic constant. If an excessive supply is retained for long, it favors the appearance of manifestations, which correspond largely to an offbalance of type A in the metazoic compartment.

We could show that the appearance of aorta atheromas in animals receiving an excess of dietary cholesterol is promoted by concomitant administration of sodium. This occurs not only in rabbits but also in rats in which a cholesterol-rich diet alone does not induce such lesions. Excess of

sodium also favors the appearance of thiamine-induced convulsions in animals. In rats and mice kept on high salt intake, the convulsant dose of thiamine fell from around 150 mgr./100 grams to below 100 mgr./100 grams of body weight. Administration of sodium—in the form of sodium chloride and especially as sodium lactate—favorably influences the state of shock which, as we have seen, corresponds to a change taking place principally at the metazoic level. In superacute and acute shock, we considered the excess of sodium present at still lower levels, such as cells and tissues, to be one of the pathogenic factors. The anomaly lies not alone in the excess but in the fact that the excess is at a level to which the cation does not belong.

The study of sodium metabolism in the light of organizational systematization of elements has revealed the importance of two factors; the combination which is "proper" for an element in its normal compartment and distribution of the element among compartments. In abnormal conditions, the unusual combinations occur. At the metazoic compartment, the bond of sodium to chloride can be considered to be normal. We have seen that, when this combination does not take place, the result is an anomaly characterized by accumulation of excess amounts of sodium in the immediately higher compartment.

In state of shock, for example, the pathogenic anomaly in fatty acids leads them to bind the chloride ion. This removes the normal combining factor for sodium which then passes on to the immediately superior compartment, the gastro-intestinal tract. This explains not only the excessive passage of sodium to the duodenum but also its retention there. The fact that sodium is present in reduced amounts in blood and in excessive amounts in the duodenum in the state of shock, illustrates the rule discussed above which we believe governs the distribution between levels of elements in abnormalities.

Potassium

This same rule would explain the distribution of potassium, another element of the same series. Potassium is the principal anti-D cation for the cellular compartment, as it is one of the principal constituents of the earth's crust, the environment in which the nuclei developed. Excess of potassium in the cell results in a cellular A offbalance with consequent active proliferation. Potassium in excess appears thus to be the cellular growth-inducing factor and its role in cancer has to be considered especially at the cellular level. Through the induced growth, the excess of cellular potassium would thus represent the factor immediately responsible for the invasive phase.

Following the rule of distribution of elements between the levels and compartments, an excess of cellular potassium will result in low blood potassium. This permits us to associate excess of cellular potassium and hypokalemia in cancer with active cellular proliferation. The opposite occurs in the state of shock and in offbalance D. The amount of potassium decreases in the cells. Consequently, the element accumulates and is retained in excessive amounts in the compartment immediately above the cells, the metazoic. Teleologically speaking, while the excess of sodium in the intestines and the excess of potassium in blood could be eliminated easily by means available to the organism, they are kept in high amounts in these superior compartments as reserves, disposable when and if the abnormality disappears and they can be used properly again. We have seen how this same mechanism explains the excessive amounts of copper in the blood in cancer patients.

This redistribution between compartments explains another fact about potassium. An excess of potassium is found in cancer cells: the greater the degree of malignancy, the greater the excess of the element. Along with the cellular excess, the amount of potassium in the blood drops to low values, even below 4 mEq. The low blood value cannot be related to lack of potassium, since quantities of the elements are eliminated in the urine. It has to be considered as a kind of defense through the higher level to an excessive amount of the element at its proper level. This relationship has led to the comparative study of the potassium content of red cells and serum as a means of obtaining indications concerning the intervention of this metal at its proper cellular level. The changes in the potassium content of red blood cells are considered to parallel those occurring in the cells in general. It is not the ratio between these values which is of interest, but each value by itself. Low amounts in red cells and in serum correspond to a quantitative deficiency; high amounts in both, to an excess of the metal; low amounts in cells and high in serum indicate a metabolic anomaly corresponding to a depletion of potassium in the cells as seen in offbalance D; while a high amount in cells and with low values in the serum indicate a cellular offbalance of the type A.

Administration of sodium and potassium as therapeutic aids has to be guided by these findings. Isotonic saline appears to be adequate as a replacement product but it is useless to administer it only because hyponatremia exists, except if this hyponatremia results from a quantitative insufficiency, as in excessive perspiration. The problem to be considered is how to restore the normal bond for the cation at its proper level. Often, what is needed is chloride ions for sodium, not more sodium. Similarly,

with hyperkalemia, if it exists in cases of type D offbalance, it is not the hyperkalemia which has to be directly attacked; efforts must be made to have potassium again normally fixed in its own cellular compartment. The use of glucose, insulin and ACTH seems to accomplish this for potassium. While administration of potassium in cases of cancer with hypokalemia possibly produces some immediate subjective improvement, it is constantly followed by an exacerbation of tumor growth as long as it does not correspond to a quantitative deficiency. In cases where this potassium quantitative deficiency can be eliminated as the cause of hypokalemia, beneficial results are obtained through administration of agents such as magnesium sulfate or calcium salts.

Searching for a cation that might compete with potassium and sodium, we first chose ammonium. Theoretically, it seemed to be a likely choice since it penetrates into the cell and nucleus with ease. In the pharmacology of ammonium, the missing link is the factor or factors—the substances and conditions—which determine the role of this cation in the nuclear compartment. Ammonium proved valueless because it was taken up by the liver and transformed into urea. Therefore, we resorted to the use of another monovalent cation of the same homotropic 1A series but with a higher atomic weight, rubidium. This cation is very similar to ammonium ion. Rubidium and ammonium, in nitrates, sulfates and especially in double sulfates of aluminum or magnesium, are isomorphic. We tried rubidium salts in animals and found them to have a very low degree of toxicity.

In order to study its influence upon sodium and water retention in lesions in which fatty acids predominate, we administered 1-2 cc. of a 5% rubidium chloride solution in water, two or three times daily. In several cases, this caused diuresis and significant reduction in edema. With the idea of having rubidium act at the nuclear levels, we considered the use of rubidium compounds with anions that seem to intervene at these levels. Rubidium nucleinate was prepared and 1-2 cc. of a 10% solution in water administered to subjects two to three times daily. Although these studies are not yet sufficiently advanced to permit any conclusions to be drawn, it seems that the rubidium salts may be useful in cases of intractable edema related to a local condition.

These studies of the distribution of cations at different levels of organization appear to be extremely important if we want to reach cellular and especially nuclear levels with cations, particularly as radio-active isotopes. We tried to go still further and utilize heavier cations. Experiments with cesium salts seem to present more difficulties, at least for the present, be-

cause of the insolubility resulting from the high atomic weight of this element.

In the VI B series, we know little about chromium. *Molybdenum* appears to be an active agent. The influence exerted by molybdenum is neutralized by the action of methionine, with its active thiol group. Excess of molybdenum found in some pastures induces a deficiency in copper and calcium in animals followed by osteomalacy and bone fractures, just as it induces low fertility. (174) The antagonism between these elements is shown by the inhibitory effect of ammonium molybdate upon oxidase activity of ceruloplasmin, the form in which copper is bound to protein in blood serum. (175)

The anti-D effect of molybdenum is especially marked in microbes, in which it induces morphological and tinctorial changes. *Bac. anthracis* treated with ammonium molybdate shows a cocciform change and abnormally intensive Gram positive staining.

Iron

Iron, a member of the VIII series and an anti-D agent belonging also to the cellular level, is of special interest. Its form of activity as cytochrome oxidase or hemoglobin has facilitated the understanding of its intervention. We could thus relate the high or low amount of the red cells in hemoglobin which corresponds to the amount of iron ions to the respective offbalance. Hypochromia corresponds to a type D offbalance and hyperchromia to a type A. These patterns, which were first recognized through clinical investigations are in accord with the anti-D character of iron at the cellular level where it belongs. The existence of offbalances of A and D types in cancer explains the high and low values of Fe in the immediately higher level, the blood serum. Fever, as we have noted, corresponds to an offbalance of type A at the metazoic compartment. The fact that serum iron is low during fever would indicate increased iron activity at the cellular level, which accords with its A-inducing role. We administered iron compounds to cancer patients with hypochromic anemia to correct the anemia and also because of iron's anti-D effect which might act antagonistically on sulfhydryl groups. In several cases, with very high sulfhydryl index values, ferrous sulfate has been administered in doses as large as several grams daily. In addition to producing an increase in hemoglobin and color index, ferrous sulfate in large doses has been observed to have another effect in some cases, reducing pain of an alkaline pattern. The salutary effect upon pain was noted more often with reduced iron than with ferrous sulfate. No other influence upon systemic changes was observed.

In tumors, iron produced an increase in rate of growth as expected. This peculiar effect of iron administration could be clearly seen in a case of lymphatic leukemia.

B.V., 4 years old, came under our care with the diagnosis of subacute lymphatic leukemia. He had a count of 145,000 leucocytes, of which 96% were lymphoblasts. Butanol administered in small doses reduced the number of leucocytes considerably, even after a few days of treatment. After two weeks, the count was below 5,000 leucocytes, with the proportion of lymphoblasts decreased to 6%. A count of 3,200 leucocytes made us discontinue the administration of butanol. In three weeks the count rose progressively to previous values. Butanol was again administered and again the blood count showed the same marked decrease in leucocytes.

All through this "remission," intensive hypochromic anemia persisted and led us to administer ferrous sulfate in addition to butanol. Within a few days after iron was added, the white cell count increased to 110,000 leucocytes/cmm., and the proportion of lymphoblasts rose to 90%. When the iron was stopped and butanol continued alone, the leucocyte count fell again, to 8,000/cmm. Iron was again administered and the total white count rose a second time in three days, from 8,000 to 80,000, with 96% lymphoblasts. When the iron was discontinued, the count again fell back within a week to between 6,000 and 7,000, this time with less than 30% lymphoblasts. This continued for another two weeks, when iron therapy was instituted for the third time, the count went up again to 38,000 within a single day. When butanol was administered alone, it went back to 5,600 in another five days. The boy died a few weeks later in acute shock during a blood transfusion.

While generalizations cannot be made from these findings, it could be definitely established in this particular case that the administration of iron was followed by a marked increase in the number of leucocytes and in the proportion of lymphoblasts. We saw this repeated, although not so spectacularly, in another case of lymphatic leukemia.

In several cancer patients who received iron in large doses over a prolonged period of time, tumor growth seemed to be stimulated. In animal experiments, tumor transplants grew only slightly faster in animals fed iron than in controls. Mice and rats used in these studies received approximately 0.05 gm. daily of reduced iron per 100 grams of body weight, mixed in powdered Purina chow, or were given a corresponding amount of ferrous sulfate in drinking water. Iron was added to the diet of the animals two or three weeks before tumor transplant, and was fed after transplant. Tumor growth was slightly enhanced and survival time shortened.

Similar studies were carried out using *nickel* and *zinc* alone or mixtures of them with iron. The metals reduced by hydrogen were obtained and a powder preparation was incorporated in powdered Purina chow in amounts calculated to provide approximately 0.05 gr. metal/100 gr. of body weight daily. Significant changes occurred in the evolution of Walker tumor transplants in rats receiving 0.05 gr. daily of zinc or nickel/100 gr. of body weight. In most experiments, two different results were noted. Tumor growth was retarded in a significant proportion in one group of animals, with the tumor disappearing in some cases. In another group with the same Walker tumors, tumor growth was stimulated. It is interesting to note that retardation occurred only in tumors with a necrotic, ulcerative character, while stimulation was noted in those with white masses. The correlation of necrotic and ulcerative tumors to an offbalance of type D and of massive non-ulcerated white ones to offbalance of type A explains this paradoxical result. This would confirm the anti-D or A-inducing character of these metals.

Zinc

Zinc intervenes with a certain specificity in one group of metabolic changes, those related to carbohydrates. Both pancreas "A" cells, which manufacture glucagon, and B cells which produce insulin, contain large amounts of zinc. Zinc regulates glucose metabolism through its influence on insulin. Zinc and insulin give insoluble combinations, Zinc chloride retarding the action of insulin. (176) A diet rich in glucose depletes the Langerhans islets of zinc, (177) while protein and lipids, or even fat, increase the zinc content of the pancreas. (178) With alloxan, the islets lose their physiological capacity to store zinc. (179) Indirectly zinc appears to have anti-D activity.

A similar anti-D activity for zinc can be seen in the prostate, which is particularly rich in this element. Conceivably, zinc's role would be to favor the persistence of spermatozooids. The capacity to utilize zinc is lost in the abnormal prostate. In adenomatous hypertrophy of the prostate, zinc values decrease. (180) They decrease still more with cancer of the prostate. (181) Although cancer has been induced by excessive administration of zinc, the element's role in pancreas and prostate seems to be indirect, through the metabolic changes it influences. Calcium is antagonistic to zinc, which is to be expected considering the opposite fundamental biological groups to which they belong.

It is interesting to note the influence exerted by the oral administration of zinc powder upon radiation effects in mice of C₃H strain receiving lethal

doses of 1500 r. In different experiments in which 85 to 100% of the controls died in less than twelve days, the mortality rate for animals given zinc ranged from 25 to 50%. In one group, a mortality rate of 15% was observed. There was a much weaker effect when nickel was incorporated in food. No effect at all could be obtained with iron.

Mercury

Mercury, a member of the IIB series was studied. Theoretically, it should act upon the sulphydryl groups, and thus limit the processes in which these groups take part.

A series of compounds in which mercury is present in the anion, and which are routinely utilized as therapeutic agents because of their diuretic action and their effects on electrolyte metabolism, was investigated. Because of their well-known diuretic effect, we initially used them in patients with generalized anasarca. We extended their use to cases with localized edema which could be related to tumors with local alkalosis. It was noted that the mercurial diuretic, in addition to influencing water and sodium excretion, changed other systemic analyses toward type A, although only temporarily. However, when these substances were administered over long periods of time, other effects were observed which could not be attributed to diuretic action. In animals with slowly growing tumors, mercury was found fixed with a degree of selectivity within these lesions. After treatment for a certain time, peritumoral fats became rich in mercury, as recognized macroscopically through abnormal ash color and confirmed by histochemical analysis. It was interesting to note that mercury appeared largely in the necrotic part of tumors. A beneficial influence upon evolution of tumors was seen, if a type D offbalance was present.

Bismuth

Bismuth, from the VA series was studied. We utilized available anti-luetic compounds. Again localization in peritumoral fats was seen, with the fats this time becoming abnormally reddish. Neither in animals nor in patients could other important effects be recognized.

Arsenic

Arsenic, from the same VA series, belongs to the cellular compartment. Its manifest A inducing or anti-D effect was noted in all compartments if the amount administered was high enough. It is interesting that *Bac. anthracis*, under the influence of arsenious acid, changes to cocci

highly irregular in dimension, with intensive Gram positive staining, and a creamy character of cultures. (182)

The carcinogenic effect of arsenic has been widely investigated by many authors. In our experiments, arsenic in various preparations did not show a carcinogenic effect. It did, however, enhance the effect of various carcinogens, and thus appears to be an active co-carcinogen. Its action at the cellular level would explain this effect.

Aluminum, from the IIIA series, belongs to the metazoic compartment. In minimal doses, it produces a D systemic offbalance. *Boron*, as boric acid, shows few effects other than in gastrointestinal disorders. This effect may be due to the fact that it belongs to the organism compartment. Insoluble compounds of boron appeared to be useful in the treatment of diarrhea.

The effect of members of the VIIA series appears to be largely correlated with a direct action upon the double bonds of the nonpolar group of the fatty acids.

Chlorine

We have noted previously the capacity of chloride ions to bind fatty acids. The bond represents the first step in abnormal metabolism of sodium chloride. In a second step, sodium forms alkaline compounds by binding with the carbonate anion and, in sufficient amounts, induces local alkalosis. Accumulated in cells along with water, the sodium compound leads to the appearance of vacuoles. In interstitial fluid, the same process induces edema and pain of an alkaline pattern. At the systemic level, it results in a state of shock.

In the last analysis, the influence of the alkaline sodium compounds can be considered to result from the lack of anions other than carbonate available to bind sodium. For this reason, we were interested in studying the effects of substances able to furnish the chloride anion to the organism. Through metabolism of ammonium chloride and calcium chloride, chloride ions are liberated in the body. They have little effects at lower levels. An immediate influence on local pH is seen at the tissue level. Favorable influence on alkaline pain is part of this action. The effect of ammonium chloride in shock is related to the fact that it furnishes the needed chloride ions.

The action of chloride upon alkaline sodium compounds, however, is handicapped by another aspect of its intervention. Studies of the pathogenesis of shock has shown a noxious effect produced by compounds resulting from the bond of chloride ions to fatty acids. The gastric ulcerations

seen in the state of shock with the severe liver damage produced by several chlorine compounds—could be largely related to the bond of chloride ions to fatty acids. This led to the idea of trying such combinations *in vivo* to destroy abnormal entities such as tumoral cells. By using lipoids rich in chlorides, we hoped to achieve this without the noxious effect of free sodium ions.

We administered trichlorethylene and chlorbutanol (the last also used as an antiseptic in many pharmaceutical preparations). No effect was seen at any level in experiments on animals and humans. In a second step, we added chlorine to various lipoids, especially those with negative polar groups. We started with 9, 10-dichlorostearic and 9, 10, 12, 13-tetrachlorostearic acid. The results with these preparations in animals and in a few humans were not encouraging. Investigation of products obtained through fixation of chlorides at the double bond of conjugated fatty acids, has shown that, in large amounts, they are able to induce gastric ulcerations in rats and rabbits. This brought us back to the use of chlorides as anti-D agents—of which sodium chloride appears to be the most effective.

Fluorine and Bromine

In another series of experiments, we tried to replace the chloride ion with another halogen. We studied the influence of fluorine, bromine and iodine compounds, this time upon the processes that induce abnormal patterns related to predominance of abnormal fatty acids. The administration of sodium fluoride and of other compounds containing fluorine did not have any appreciable effect either in animals with tumors or other pathological conditions, or in humans with alkaline pattern of pain. The fact that fluorine belongs to the organism level led us to investigate it in terminal cases. Neither pain nor tumor evolution was changed. Bromine, too except for a sedative effect, did not influence systemic changes, pain or tumor growth.

Iodine

The influence of iodine was rather extensively studied because of its relationship to tumors and because it belongs to the nuclear compartment. Before the days of pathological diagnosis of cancer and serological methods of detecting syphilis, iodine salts were used to differentiate between gummatus and tongue cancers. This was the so-called "pierre de touche" treatment, since iodine was assumed to favorably influence luetic lesions but markedly enhance neoplastic growths.

In view of the dualistic concept of cancer, we were interested in ascer-

taining whether the effects of iodine were related to one of the two off-balances present, and if this was so, to try to take therapeutic advantage of it. Iodine was administered principally in the form of potassium iodide solutions and Lugol's solution. In most cases with alkaline pain, the intensity diminished and pain even disappeared soon after iodine administration. In several cases, doses as low as 3-10 drops of Lugol's solution were sufficient to induce such an effect for hours. However, larger doses or repeated doses produced effects that were distinctly undesirable. Edema within the neoplastic lesion was increased by the administration of iodine, sometimes to such an extent as to require discontinuing its use before any other changes in the tumor could be noted.

Research also was done with lipid and lipoid molecules incorporating iodine. The purpose was to determine whether these molecules would act more selectively upon abnormal cells and induce local toxic effects. 9, 10-di-iodostearic acid and 9, 10, 12, 13-tetra-iodostearic acid were prepared and tested in animals and humans. No differences were noted between these substances and inorganic iodine preparations in influence upon systemic analyses, pain or tumor growth, in both animals and humans.

Oxygen

Oxygen, an agent with a negative anti-A character, at the organism level, acts like an anti-fatty acid agent at the lower tissular level. For that reason, we will discuss it here.

At the beginning of our work, we were interested in determining the relationship between cellular membrane permeability and the pathogenesis of the two offbalances. If a change in the permeability of cell membrane was the primary mechanism involved, changing the oxygen tension in or around entities where offbalances occur might result in the correction of the abnormal manifestations present.

Clinical studies were made in which oxygen was administered to patients with acid pain pattern. The pain was not relieved as expected. Indeed, its intensity was even increased. These experiments indicate that impaired cell permeability, if it exists, is not the major factor in the pathogenic mechanism involved in the acid pain pattern. Actually, these studies indicated that another pathogenic mechanism was involved since oxygen administration increased this pain. Accordingly, it had an opposite effect on alkaline pain. The intensity of alkaline pain decreased, the pain being often entirely relieved by administration of oxygen. We found that pain produced by traumatic injuries, which was subsequently identified as invariably of the alkaline pattern, could be satisfactorily relieved by oxygen.

Such relief occurred in patients who had suffered all sorts of traumatic injuries, from superficial wounds to severe comminuted fractures.

A curious phenomenon was seen to occur which limited the practical usefulness of oxygen. After first relieving pain, continuation of oxygen administration led to appearance of a new pain. The patient was able to distinguish between original and new pain by its localization, by the different quality of the sensation, and also by the fact that instead of being relieved by oxygen, the new pain tended to increase with the continued administration of oxygen. It disappeared soon after oxygen administration was discontinued. If oxygen were administered again, the new pain returned within a short time. The new pain might become severe and even unbearable with continuation of oxygen administration. On the other hand, when administration of oxygen was stopped, the original pain again appeared within 10-20 minutes.

The appearance of a "new pain" and the resemblance between these changes and those observed with the use of lipids, suggested a change in the pain pattern itself, although the changes induced by oxygen evolved over minutes instead of days. This was confirmed by following the response to acidifying and alkalizing agents, the only adequate means to investigate the pattern in these cases. By this test the new pain was found to be of an acid pattern.

Because of the possibility of inducing a period of calm between the old alkaline and new acid pain, oxygen administration still seemed to be useful. In cases of traumatic pain, always with an alkaline pattern, successful results were obtained. A necessary condition appeared to be the physical and mental ability of the patient to guide the administration of oxygen. He had to recognize if too little or too much was being administered on the basis of the different sensations felt, and consequently, to adjust the administration to the optimum amount. Extreme pain caused by extensive traumatic injuries was controlled very successfully when the patient could be taught to utilize oxygen properly.

In addition to pain relief, an effect upon evolution of the lesion itself was manifest. In a few days with this form of oxygen treatment, guided by the subjective sensations, the healing process itself was observed to be sufficiently advanced to make the pain disappear entirely. Healing of the wounds seemed to be greatly enhanced by oxygen therapy guided by the patient. In several cases of open comminuted fractures in which amputation was considered inevitable on admission, unexpected improvement was noted. Atonic wounds were transformed, becoming rich in granulations, and healing was rapid.

However, the nature of the treatment was such that it could only be successful when properly applied. If dosages were too low, there was no sedative effect; and if doses were too large, new pain was induced. The physical and mental status of the patient thus appeared to be the determining factor for success. The use of an oxygen tent, in which the amount of oxygen can be carefully adjusted, helped in a few cases to maintain proper dosage. But even in this situation, the patient himself must furnish information not so much about intensity of pain, as about changes in the character of pain.

The relationship between the amount of oxygen administered and the clinical results, especially in pain, has suggested that this factor may be significant in other conditions in which oxygen therapy is used. The fact that, despite its general usefulness in the acute stage of myocardial infarction, oxygen does not alleviate the pain in some cases and may even increase it, suggests that the amount administered might not be adequate. In such cases, a decrease in pain intensity following temporary discontinuation of the use of oxygen would indicate that the amount utilized was too high. If the suppression of the oxygen administration is followed immediately by an increase in pain intensity, the amount previously administered has to be considered too low.

The possibility that too much oxygen can induce a proliferation of vessels and connective tissue, as seen in the fibroblastic retinopathy of premature babies kept in an atmosphere too rich in oxygen, fits in with the data mentioned above. We have noted that too much oxygen induced an anoxoxygenic process with anabolic character. This explains the abnormal type of offbalance with proliferative tendencies seen in fibroblastic retinopathy. We will return later to a discussion of this important factor in oxygen therapy.

On the basis of findings in traumatic pain, we studied oxygen in cases of painful cancer. Attempts were made to employ it as a diagnostic aid to help determine the acid or alkaline character of pain present on the assumption that oxygen would intensify the first and would relieve the second pain. In a group of subjects, we compared the diagnosis of the existing pattern through concomitant variations in pain intensity and urine pH, the response to acidifying and alkalinizing substances, and response to oxygen. In most of these cases, accuracy of the information furnished by the last method was confirmed. However, this method has shown great limitations. Whereas most of the patients were able to recognize an immediate change, they were less precise about a second change when it occurred. At the present stage of this research, it appears that judgment concerning the de-

velopment of changes could be improved by reducing the concentration of oxygen administered, thus increasing the length of time during which the changes would appear. In general, the results when applied as routine were insufficiently clear to be used as a practical means for the diagnosis of the pattern.

Oxygen also has been tried as a therapeutic agent for controlling alkaline pain pattern in cancer cases. Unfortunately, even in patients who are able to analyze the variations in pain character, the time between the decrease in intensity of the original pain and the appearance and increase in the intensity of the new pain is so variable that it is almost impossible to adjust the dosage of oxygen satisfactorily to obtain a long enough period of calm.

We sought a theoretical explanation for oxygen's effects upon pain. As mentioned above, the reduction of pain of an alkaline pattern and the appearance of a pain of an acid pattern, are in accord with our view of the pathogenesis of these pains through the intervention of the two groups of lipids, fatty acids and anti-fatty acids. A tentative explanation can be found in the active role of oxygen upon fatty acids. In the presence of increased oxygen tension, it is possible that oxygen is fixed in greater amounts on abnormal fatty acids, thus reducing their intervention in chloride metabolism. With less chlorides fixed by fatty acids, alkaline compounds would be reduced. This would explain the influence of oxygen upon pain with an alkaline pattern. The appearance of an acid pain pattern produced by increased anoxobiosis seems to be explained by the fact that inactivation of fatty acids, if it goes beyond certain limits, changes the balance toward a predominance of sterols. Besides this mechanism, another also can be considered. Any action upon fatty acids themselves, would reduce their availability as active agents. It seems possible that under higher tension, oxygen is bound to these fatty acids in a way different than the bond which leads to the appearance of activated oxygen. Through it, the role of the unsaturated fatty acid in activating oxygen for the cells would be to decrease intracellular activated oxygen and thus change cell metabolism to the anoxobiotic type. Since the bonds are labile, return of oxygen tension to normal allows the fatty acids to recover their function of activating oxygen. Teleologically, this process can be interpreted as a mechanism to prevent passage of excess oxygen into the cells when the external oxygen tension increases.

Studies of oxygen in normal and abnormal physiology have led us to consider also the possible utilization of various oxygenated compounds as therapeutic agents. A distinction must be made between hydroperoxides, which occur normally in organisms, and peroxides and epoxides. Peroxides

result from the binding of molecular oxygen, while the epoxides result from binding of atomic oxygen, both under abnormal conditions. Peroxides were administered as adjunct agents in cases highly refractory to therapy with lipoids having negative polar groups. In preliminary experiments, it could be seen that a certain condition was necessary to influence the desired processes. The peroxide used has to be a lipoid if it is to have influence upon the lipidic system. Some lipoidal peroxides were prepared, and their therapeutic value is still under investigation. The influence of lipoidal epoxides upon the process of carcinogenesis also is under study.

Peroxidases

It has been previously noted that hydroperoxides resulting from the oxidation of mono- and polyunsaturated fatty acids are found normally in the organism, but peroxides appear only under abnormal conditions and particularly when abnormal fatty acids intervene. The manifestations of the type D offbalance in its oxygen phase thus can be attributed to the presence of peroxides. Biologically, the intervention of peroxides would be counteracted by peroxidases and catalases. It was interesting to study a clinical curiosity which could be connected with a probable intervention of peroxidases.

In several patients with frequent headaches, whose analyses showed a typical acid pain pattern, pain was repeatedly intensified or even induced by eating pears. No other fruit had such effect; some had the opposite effect. This led us to consider that the pain intensifying action was not due to an acid-base change. The large amount of peroxidases in pears led us to isolate this enzyme in order to study its direct influence upon pain. Peroxidase could be obtained from pears in relatively small amounts, and showed reduced activity upon peroxides even in vitro. We were able to prepare much larger quantities of a highly active peroxidase from horse radish.

After being purified and tested for antiperoxide activity, preparations were administered orally to patients with acid or alkaline pain pattern. While the former was definitely intensified, there was no relief of the latter. It seemed that the effects obtained through the administration of isolated peroxidases were the same as those obtained when pears were eaten.

Antioxidants

The relationship between fixation of oxygen and chlorides has led to the study of antioxidants capable of acting in situations of abnormal oxidation. It was hoped that these substances also would be able to influence the fixation of chloride ions.

We have utilized several groups of known antioxidants, starting with manganese compounds, such as inorganic salts, and later binding these to lipids. It is too early yet to draw any definite conclusions from animal experiments, and we have not used the compounds clinically. However, our studies up to now do not show any influence that could be interpreted as sufficient to warrant hope that these compounds can control the fixation of chlorides on fatty acids.

In the same series of researches, other antioxidants—some of them used for the preservation of edible fats and others for the control of oxidation in other substances such as rubber—were tried. We investigated the influence of tocopherols, the natural antioxidants for vegetable oils. Alpha tocopherol in doses of 100 mg. was administered several times a day to patients having symptoms and signs corresponding to an intervention of abnormal fatty acids. A decrease in the intensity of pain of an alkaline pattern was observed.

Along the same lines, we investigated the influence exerted by maleic acid, used to prevent the rancidity of edible fats. In proportion of 1/10,000 this acid conserves these fats for months. Curiously enough, maleic and citraconic acid have shown an influence upon the abnormal manifestation of the type D.

For these reasons we utilized these two acids—maleic and citraconic—as anti-D agents. In one study, the acids were injected intravenously in proportion of from 0.1 to 1 mgr./100 cc. of saline. In others, the sodium salts of the acid were used, while in still others the butyl esters were prepared and administered intramuscularly in oily solutions. For the present it is difficult to judge the effects obtained.

All the above mentioned attempts were made on the basis of a direct action upon fatty acids and other lipoidic constituents with negative polar groups which intervene in inducing offbalances. For the present, it seems that no single agent can resolve the problems that result from the plural intervention of various abnormal fatty acids at the different levels. The use of various agents acting selectively at the different levels involved seems to be the only available path by which therapeutic intervention against the multiple manifestations at different levels can be accomplished.

Before going further, we thought it useful to have a synoptic view of this special part of the pharmacological activity as obtained through the study of the influence upon pain and the systemic level analyses as seen in humans. To this we added the effect seen upon tumors in humans. Tables XX and XXI which give this information in a very condensed form, were limited to the most important agents tested for each group studied. The effects are indicated as clinical results also for the facility of the presentation.

TABLE XX
CLINICAL RESULTS WITH AGENTS THAT ACT UPON
THE OFFBALANCE TYPE "A"

Group	Agent	Systemic Level	Pain	Influences Exerted Upon Tumor
<i>Fatty Acids</i>	Saturated	None	None	None
	Polyunsaturated	Slight	Fair	Some, not consistent, not persistent " " "
	Mixtures from organs	"	Good	Fair, not consistent, not persistent " " "
	" from cod liver oil	"	Fair	" " "
	Irradiated	"	"	" " "
	Conjugated	"	"	" " "
	α -OH Polyhydroxy Chloro-derivatives	None Slight Fair	None Slight Fair	None None Some, not consistent, not persistent None
<i>Aldehydes</i>	Oleic	Slight	None	None
	Crotonic	Slight	Slight	Slight, not consistent, not persistent
	Propionic Heptylic	Good Good	Good Slight	Fair Fair
<i>Sulfur Compounds</i>	Thiosulfates	Good	Good	Fair
	S. Colloidal	None	None	None
	Mercaptans	Slight	Slight	Good, consistent, persistent " " "
	Hydopersulfides	Fair	Fair	Fair, not consistent, not persistent " " "
	Methyl thioglycolate Tetrahydronaphthalene persulfides	Slight Fair	Fair Fair	Good, consistent and persistent
<i>Selenium Compounds</i>	Alkyldiselenide	Fair	Slight	Good, consistent, persistent " " "
	Perselenide	Fair	Good	" " "
<i>Peracids</i>	Perborate Perchlorate	Fair "	Fair "	Some, not consistent, not persistent
<i>Hormones</i>	Testosterone	Slight	None	Seldom, not consistent, not persistent
<i>Mustards</i>	Sulfur mustard	Fair	Fair	Fair, " "
<i>Hydrines</i>	Epichlorohydrin	Fair to good	Slight	Fair, consistent, persistent

TABLE XXI
CLINICAL RESULTS WITH AGENTS THAT ACT UPON
THE OFFBALANCE TYPE "D"

Group	Agent	Systemic Level	Pain	Influences Exerted Upon Tumor
<i>Sterols</i>	Cholesterol	Fair	Fair	Seldom, not consistent, not persistent
	Insapon. fraction of organs of eggs of milk	" " "	" " "	" " "
		" " "	" " "	" " "
		" " "	" " "	" " "
<i>Alcohols</i>	<i>Aliphatic saturated</i>			
	Butanol	Good	Good	" " "
	Pentanol	Fair	Fair	Fair, " "
	Heptanol	"	Slight	Good, consistent, persistent
	Octanol	Slight	"	Fair, not consistent, not persistent
	Octanediol	"	"	Slight
	Nonanol	None	None	None
	<i>Polyalcohol</i>			
	Glycerol	Slight	Good	Good, consistent, persistent
	Inositol	None	None	None
	<i>Unsaturated</i>			
	Oleic	Slight	Slight	None
	Linoleic	"	"	None
	Polyunsaturated	"	Fair	Slight, not consistent, not persistent
	Polyconjugated	"	"	Fair, " "
<i>Hormones</i>	Crotonic	"	Slight	" " "
	Ricinoleic	"	Fair	Slight, " "
	Salicylic	"	Slight	Fair, " "
	Estrogens	"	"	Slight, " "
<i>Amines</i>	Aminobutanol	"	Fair	" " "
	Hexylamine	"	"	" " "
	Heptylamine	Good	"	Fair, " "
	Glucosamine	Good	Slight	None
<i>Nicotinic acid deriv.</i>	Niketamide	"	Good	None
<i>Metals</i>	Iron	Fair	Slight	None
	Mercury	"	None	"
	Bismuth	"	"	"
<i>Halogen</i>	Iodine	Slight	Fair	"
	Oxygen	None	Good	None

CHAPTER 14

THERAPEUTIC APPROACH

BIOLOGICALLY GUIDED THERAPY

THE STUDIES PREVIOUSLY DESCRIBED have identified many factors involved in the pathogenesis of abnormal conditions and have shown that there are similar factors which also govern the pharmacological activity of various agents. Out of this peculiar relationship between abnormal conditions and agents has evolved a therapeutic method which is fundamentally different from methods in common use.

In conventional therapeutic efforts against disease—in chemotherapy of cancer for example—methods as standardized as possible are sought. The goal is to find agents capable of influencing the cancer cells, if not in all tumors, then at least in tumors of the same origin. Within the limitations imposed by the general toxicity of the agent, the conduct of treatment is standardized as far as possible. The criterion of value of a treatment is its ability to overcome individual variations.

In our approach, in clear contradistinction, the effort is to influence the complex condition as it is present at different levels of the organization. It corresponds to treatment individualized as far as possible. Therapeutic efforts are guided in all their aspects—choice of agents, doses to be used—by existing manifestations with their multiple quantitative and qualitative variations. The fact that many of these variations occur during treatment, as a response of the organism induced by the medication itself, increases the individual character of treatment. No prediction can be made about the whole course of treatment to be used for an individual patient because the individual qualitative and quantitative changes which occur during the treatment cannot be foreseen.

In this approach to therapy, the patterns of manifestations constitute the criteria which determine the choice of the group of agents, the specific members of the group, and the dosages to be used. Throughout, clinical and analytical manifestations are the major factors that guide treatment. The clear systematization of the relationship between analytical findings and therapeutic indications is vital.

The fundamental offbalances, their patterns and deviations, thus guide the therapeutic approach. The nature of the fundamental offbalance, in general, indicates which of the two basic groups of agents is to be used; the patterns show which substances from these groups are desirable; while the quantitative changes in these patterns, especially under the influence of the agents, determine the doses to be administered. Any change in biological manifestations is followed by change in therapy. We call this *biologically guided therapy*.

Through the years, although this biologically guided treatment has remained fundamentally unchanged as an approach itself, applications have evolved. Efforts have been made to find increasingly precise criteria and more efficient agents.

Each of the basic physiopathological concepts has greatly helped to develop standards for the guidance of treatment. For instance, the concept of organized conditions has led to the concomitant use of several agents from the same group instead of just one. The idea for this was derived from the fact that the concomitant manifestations at different levels show relative independence; they must be influenced separately. The concept of physiopathological dualism has indicated the need to use, according to the offbalance present, agents from one or the other of the two groups with antagonistic properties. The role of the lipids in dualism has underscored the importance of these substances as active agents able to induce fundamental changes. The dualism of other constituents has been the basis for their use. The mechanism of natural defense has shown the advantage of using two systems simulating, in part, the kind of dualistic response which occurs naturally. As more has been learned about these basic concepts, the therapeutic approach, while it has remained fundamentally the same, has evolved and become increasingly effective.

The value of biologically guided chemotherapy appears clear when we compare the effects obtained by using agents with and without the guidance furnished by this method. Several patients treated at another center of research with a well-known agent, such as a nitrogen mustard gas derivative, have been studied. Analytical data showing the patterns present in these patients were obtained but were not used to influence the conduct

of treatment. Favorable results were seen in a few of these cases—when, by happy accident, the treatment applied was suitable both to the original problem and to the changes occurring during treatment, as revealed by analyses. In the failures, there was no such fortunate coincidence. The fundamental offbalance present called for an agent other than what was used. Or changes occurring during treatment indicated that use of the agent should be discontinued, although this was not done.

We have employed the same agents in some patients but with treatment guided by individual manifestations. In some cases, the analyses indicated the need not only for more prolonged treatment, but even for the use of dosages higher than those usually accepted. In others, on the contrary, they indicated the need to reduce dosage or even to stop the treatment, although the doses were, according to the usual posology, too small. It is interesting to note that in some cases, the treatment has been stopped and restarted several times on the basis of analyses. The clinical results—immediate and long-range—were definitely better than in patients given unguided therapy.

In analyzing the development of clinical applications of this form of biologically guided chemotherapy, progress can be seen to have resulted as knowledge has increased in three areas: criteria, agents used, and method of application.

Criteria

We have discussed the different symptoms and analytical changes with dualistic character encountered in pathological conditions. At various stages, all were considered as potential criteria for guided therapy. The value attributed to them as indications for treatment has changed as research has progressed. Some of the analytical tests have been found to reflect changes limited to a special level, while others of more general value have been found to furnish indications of broader offbalances themselves. The importance of some criteria was established only after many years. Others, once considered of major usefulness, have appeared to be less reliable as time went on. Body temperature, for example, in spite of the frank dualistic aspect often seen, has been considered for a long time to be influenced by too many factors to furnish any valuable information. With the development of our research, however, temperature has become an important criterion for administration of agents but limited toward manifestations at the level of the organism.

Urinary specific gravity and pH; excretion of chlorides, sodium, calcium and sulfhydryl; total blood and serum potassium; and the count of eosinophiles in circulating blood—all have been used individually with the

hope that they would indicate the type of fundamental offbalance present, and consequently, which group of agents should be used. Recently, however, we have come to use these measurements rather as criteria of changes at different levels. Urinary surface tension has thus become important as an indication of the offbalance at the levels above the cells while potassium in blood indicates the offbalance at the level of the cells. Years of investigation have shown conclusively that proper criteria represent the principal condition for success in treatment by guided chemotherapy.

The Agents

The relative independence of the various levels involved in a condition like cancer, which originates below the level of the nuclei and progressively involves higher levels as it evolves until it terminates as a systemic condition, necessitates having several agents chosen specifically for their ability to work at these different levels.

Over the years, we have utilized many agents. The level at which they are most active, the nature of activity from the point of view of dualistic intervention, and integration of activity in the frame of the defense mechanism, represent the factors which determine the specific use of these agents. It must be emphasized that, in the study of pharmacodynamic activity, the value of information furnished by tests in laboratory animals is limited. Because changes in patterns in animals cannot be followed, we have been obliged to follow such changes in humans.

Method of Application

The critical role played by each level of organization in a complex condition—and the independence of these levels—underscores the urgency of applying therapeutic agents capable of acting at specific levels where they are needed. Such applications have been made possible through special affinities of various agents for different levels. Their structure—and especially their content of specific elements which belong to specific levels—account for their tendency to act at specific levels. However, it has often been judged necessary to insure intervention of an agent at a given level by applying it directly at the level itself. The injection of an agent into a tumor or into a region of the body, through surgical chemotherapy, represents one type of procedure insuring level activity. Another procedure has been to inject an agent prepared *in vitro* so that it is bound to specific body constituents, such as cells or nuclei. In the latter procedure, the specific defense mechanism is seen to take place at the level of the body constituent and thus desired level activity is obtained.

In the development of this research, we faced another problem which is basic to all therapeutic attempts but especially important in cancer: evaluation of the results obtained.

Evaluation

Incontestably, favorable results have been obtained in cancer with many agents. However, because of the variety of manifestations, evaluation of the changes obtained has been the subject of much controversy. Each clinical worker in cancer has his own ideas about the importance of various changes obtained and often utilizes as criterion changes less accepted by other workers. A systematization of evaluating standards appeared necessary and it seems possible to do so under the concept of the disease as an organized complex condition.

In the minds of most workers in the field of cancer today, any therapeutic procedure aimed at the control of cancer must prove itself in tumors in animals. We have already mentioned the reason why control of human and animal tumors represent two different problems. Failure to recognize that the results obtained in animal cancer do not necessarily apply at all in human cancer has led to failure of the tremendous project of screening virtually all known substances for their effects upon grafted animal tumors.

The unguided treatment of cancer in animals, as carried on today, *i.e.*, without any consideration of existing offbalances, seems to explain the limited importance placed upon many changes obtained in these experiments. But even if evaluation is confined solely to the influence exerted upon the cancerous cells in unguided treatment, the same agent can appear active or inactive, depending mainly on coincidental circumstances. Its influence may change from favorable to unfavorable during, and due to, the treatment. Because of this, it is quite probable that many useful agents are not being recognized in the simple screening method used today. Most of the agents which we have found incontestably valuable in guided therapy appeared to be entirely ineffective when used in unguided treatments on small animals. As long as not enough valuable criteria are available to permit guided therapy in experiments in animals, the results would have only a relative value: to furnish useful information about limited problems such as toxicity, special pharmacodynamic activity, etc.

Under the concept of cancer as a complex condition, all manifestations, not just those directly related to the anatomical presence of cancerous tumors, must be regarded as important.

The tendency of almost all cancer workers is to limit evaluation exclusively to changes observed in tumors. Without underestimating in any

way the importance of these changes, we must emphasize the importance of other manifestations. For a subject in the systemic terminal phase, any change obtained in the tumor will have little immediate importance, whereas a beneficial change in systemic metabolic anomalies will be of great immediate value. Similarly, for a subject in the invasive or painful phase, any influence exerted upon the organic or systemic functions will be of less immediate importance than a beneficial influence upon pain or upon the cancerous lesion. With cancer recognized as a complex condition, the decision of many scientists, especially clinicians, to limit evaluation to changes only in the tumors would appear to be unilateral and unrealistic.

We tried to systematize evalution of results obtained in cancer treatment by considering all the manifestations present in the patient and by assigning to each its relative value. In addition to any decrease or disappearance of tumor masses, various analytical changes, improvement in general well-being, gain in weight and control of pain represent accomplishments, the importance of which depends on the severity of these manifestations in the individual case.

With all these factors in mind, we have evaluated the various results obtained through biologically guided treatment over a period of many years, relating them to agents and criteria which were changed, of course, as research progressed.

The study of different complex conditions has emphasized the fact that many of the manifestations present are common to different diseases. Through the variety of pathological manifestations present in these conditions, we had the opportunity to study many problems specific for these conditions or of general interest. The therapeutic method consequently has been applied in many noncancerous conditions. Before presenting an analysis of the progress of this research in the field of cancer, it may be of interest to review results of the same therapeutic approach in other conditions, each with its specific problems.

THERAPEUTIC APPLICATIONS IN CONDITIONS OTHER THAN CANCER

From the beginning of this study, manifestations, clinical and analytical, present in a variety of abnormal conditions, have appeared to conform to the basic physiopathological concepts presented above. In these varied conditions, however, more often than in advanced cancer, some analyses have shown no abnormal patterns while others have shown them so constantly as to indicate that certain anomalies, often limited to specific levels of or-

ganization, are of great importance. In spite of individual variations, some of the conditions studied have shown patterns and interrelationships of patterns that provide characteristic analytic pictures which will be analyzed in further publications. Furthermore, some of these patterns appear to be so strongly related to a given condition that our attention was directed to the idea of a fundamental relationship between the processes related to the patterns and the pathogenesis of the condition itself. The following examples illustrate this.

An impressive relationship was found between the presence of peroxides in urine and schizophrenia. We mentioned previously the reaction which we devised for detection of these substances in urine. This reaction would indicate the existence in the body of abnormal processes in oxygen metabolism, leading to the appearance of peroxides in the urine. We saw that these processes ultimately could be related to an abnormal intervention of fatty acids, corresponding to the oxygen phase of offbalance D.

Daily urinary analytical patterns were studied in a group of 27 advanced schizophrenics over a period of three years. Over 27,000 urinary samples were examined and more than 135,000 tests performed. Oxidizing substances were found in 87% of these samples. This appears to be highly significant when compared with only 2% positive values in subjects considered clinically normal, and 4% in cancerous cases submitted to various treatments. Not a single negative analysis was seen in some of these schizophrenics during the three years of daily testing. This suggested that the metabolic abnormality, characterized by the appearance of peroxides, might play an important pathogenic role in this disease. (221)

Another example of a pattern revealing characteristic pathogenic processes was seen in geriatric cases. Old people often have a manifest abnormality in urinary S. T. Figure 261, page 648 shows the values encountered in old people compared to those in subjects of mixed ages. The high surface tension in the aged group is related to predominance of sterols at the systemic level. Such predominance is also found at the organic level of the skin, for instance. A clinical test, wheal resorption time, indicated abnormal values for almost all the aged subjects studied, as shown in Figure 68. These data, integrated into the general concept of complex conditions, led us to the pathogenic concept of old age presented above.

The urinary chloride retention index in subjects in a state of shock consistently shows such exceptionally high values that our attention was directed to the study of the role of abnormal metabolism of chlorides in the pathogenesis of the condition itself. This view has been confirmed by further research, as mentioned previously.

The study of the different conditions in terms of fundamental pathogenic concepts has had other consequences. It has established clearer relationships between these conditions and cancer than previously recognized. In fact, many of the data first obtained in the studies of these other conditions have been applied specifically to cancer.

One example is shock. Occurring in the terminal stage and usually leading to death, shock often has been considered to be one of the "complications" of advanced cancer. Under the concept of organized complex condition, however, it could be seen that, in cancer, shock is related to off-balance type D of the terminal systemic phase. The study of shock has greatly contributed to the knowledge of this offbalance in cancer.

The therapeutic approach, in which the choice of agents and the doses used are determined by the different patterns present, has been useful in many noncancerous conditions in which the anomaly apparently is limited to one level of organization or even to a special group of entities. As examples here, we will discuss conditions in which manifestations are produced by an acid-base tissular abnormality.

ABNORMAL LOCAL ACID-BASE MANIFESTATIONS

Pain, itching, vertigo and dyspnea are symptoms of many conditions often far apart etiologically. We have seen that, according to our research, these symptoms are related to an acid-base abnormality at the tissue level. The ideal treatment would be to remove the cause of the symptom, to act upon the etiological factor. This ideal treatment would be entirely different if the symptom stems from a systemic toxic condition, a cancerous lesion, a local inflammatory process or a local allergy, for example. But the great variety of causes that can induce such symptoms as vertigo, pain, itching and dyspnea, often makes this ideal therapy almost impossible, particularly in everyday medical practice.

Another approach to controlling these symptoms would be one which is not especially concerned with the etiological factors involved but with influencing the specific pathogenic changes underlying each of these symptoms. But as such specific changes have not yet been defined, symptomatic treatment has remained as the only practical recourse in these instances.

Recognition in the physiopathological changes present in pain, itching, vertigo and dyspnea of an acid-base pattern, has permitted another therapeutic approach on a different, more precise basis. These symptoms, with their dual pathogenesis, have been treated according to the two possibilities: acid or alkaline pattern. Identification of the specific pattern, as noted

previously, is simple. Furthermore, pain results from an abnormality present at one level of organization, the tissues. With two groups of antagonistic agents, both capable of acting at the tissular level, the therapeutic problem is reduced to the choice between these agents according to the pattern present. The therapeutic problem—previously so complex because of the need to consider so many possible etiological factors so often unrecognizable—is simplified by this approach to a choice between two groups of agents, determined by one easily identifiable factor.

The means used to recognize the acid-base pattern vary with each symptom. We present here a resume of the research made in this direction.

Pain

We have seen that pain can be sensorial or symptomatic, and that the latter has a dual pathogenesis with an acid or alkaline pattern. Reduced to the problem of an acid or alkaline offbalance occurring at the tissular level, the treatment of pain is greatly simplified.

The first problem was to determine the pattern present. The relationship between variations in the curve of pain intensity and concomitant changes in the urinary pH, previously discussed, has been used for such determinations. The simplified method of comparing two urine samples, one corresponding to a period of pain and the other to one of calm, has proven very useful.

Among other analyses, only changes in serum potassium content have appeared helpful in the recognition of the pain pattern. This can be explained by the fact that most of the other routine analyses do not inform us specifically about changes at the tissue level where pain occurs. A high serum potassium level, increasing during pain exacerbation, indicates an alkaline pattern; a low level, decreasing during pain exacerbation, indicates an acid pattern.

Another, sometimes simpler, technique for recognizing the pattern is the response of pain to administration of acidifying or alkalinizing agents, as seen above. Also, some substances, while being used as therapeutic agents, indicate the pattern through the responses they induce. Butanol and sodium thiosulfate in adequate dosages (1 cc. of a 6.5% solution of butanol or 1 cc. of a 4% solution of sodium thiosulfate injected intramuscularly) are examples. The intensity of an acid pattern of pain is increased by butanol, and decreased by thiosulfate. The inverse occurs in pain of an alkaline pattern.

With the pattern of the pain recognized through one of these means, agents are chosen from the two groups, anti-A for acid pattern and anti-D for alkaline. In each group however, some agents have been found to be

more effective than others because of their greater activity at the tissue level. The following agents have been found to be most effective against acid pain, in the order presented: lipoaldehydes and especially propionic aldehyde, sodium thiosulfate, sulfurized tetrahydronaphthalene, the acid lipid fraction of various organs, polyunsaturated fatty acids, epichlorohydrin, and selenium in the form of perselenide. For an alkaline pattern, agents with a positive polar group, such as butanol, nikethamide, insaponifiable fractions, glycerol and heptanol are effective in that order.

Once the agent to be used is chosen, the dose which can vary greatly from case to case, is easily determined from the clinical response. In practice, the patient is given a small dose. If the intensity of the symptom decreases but is not completely controlled, the same dose is usually given three hours later. If the symptom intensity remains the same, the dose is continuously increased more or less rapidly, in accordance with the severity of the symptoms, the medication being repeated this time at intervals varying from a half hour to every three hours.

On the other hand, if the symptom disappears after the first administration of the agent, medication is not repeated until the symptom reappears. If the symptom is controlled for less than six hours by the dose used, the same dose is given when the pain reappears. If more than six hours of relief follow a dose, the next dose is usually reduced in proportion to the period of time the effect lasted. If 24-hour relief occurred, the dose is reduced by half; if relief was of two days' duration, one-quarter of the last dose is given. However, if, with three successive doses of medication, the symptom increases in intensity each time, always during the first half hour after the medication is administered, it is discontinued and the entire group of agents to be used is reconsidered. A new test for the pattern is performed. If the pattern is the same as before, other agents from the same group are tried. However, if the pattern now is opposite to the original pattern, the group of substances has to be changed.

This technique has produced, with few exceptions, excellent pain relief. This approach to pain has been the object of several controlled studies. S. A. Barragan Martinez (183) and E. Stoopen, (184) in the journal "Pasteur," confirmed our conclusions. From Stoopen's publication we quote two characteristic observations in *Note 1*.

An intensive study of head and neck pain was made by B. Welt and published in the American Medical Assn. Journal of Laryngology (185), with the following conclusions:

"Summary and Conclusions

A series of 120 patients having the symptom of pain in the region of the head and neck has been studied.

The symptom of pain has been analyzed according to Revici's concept concerning the alkaline or acid pattern of the painful symptom.

Eleven cases were eliminated because no pattern was identified; 109 cases showed an acid or alkaline pattern. The results showed a satisfactory result in 84% of the vascular headaches, 100% in migraine headaches, and 75% in the neuralgic group. These results indicate a correlation of Revici's concepts and the results achieved. The simplicity of this method is indicated.

Additional data about Revici's views are given. The relationship to homeostasis and the biology of the cell is indicated.

The problem confronting the clinician in treating cases of this type, on account of the many factors involved, is simplified according to Revici's concept of dualism.

Two additional active products are presented, one acid and the other alcoholic. The data here presented concerned the incidence of the control of the acute symptoms while under observation. Further experience with this method will be necessary. As the method stands, it is a practical method of therapy for the control of pain. Recurrences were seen and controlled."

Our method of controlling pain was the subject of a panel discussion at the American Academy of Ophthalmology and Otolaryngology, New York, N. Y., September 21 and 22, 1954, and of an article published by B. Welt and M. Welt in *Modern Problems of Ophthalmology*. (186) (Note 2)

Trauma

A particularly interesting application of the method described above is in the treatment of pain related to traumatic lesions. The study of wounds from the standpoint of the offbalance present has revealed a definite pattern in their evolution. In all wounds, there is an initial period during which the offbalance is always type D, corresponding to a predominance of fatty acids. This can be recognized indirectly by measuring the local pH which shows alkalosis, the chloride content which shows a manifest increase, and by cytological and histological studies which indicate a rapid cellular aging process, with necrosis and sloughing. In uncomplicated wounds, in humans, the painful period usually lasts a few days. The pain in this initial period is of an alkaline pattern.

The constancy of the alkaline pattern in pain in traumatic lesions elimi-

nates need for any tests, thus simplifying therapeutic choice by reducing agents to be used to one group—those with positive character.

As already noted, some of the agents are more effective than others in influencing alkaline pain. Butanol and heptanol are particularly useful in traumatic pain, followed in order of activity by polyunsaturated alcohols, nikethamide and glycerol.

One important application of these findings is in routine treatment of postoperative pain. For surgical wounds, which represent typical traumatic lesions, butanol appears to be especially beneficial. Its use has been the subject of extensive studies by B. Welt (187) in surgery in otorhinolaryn-

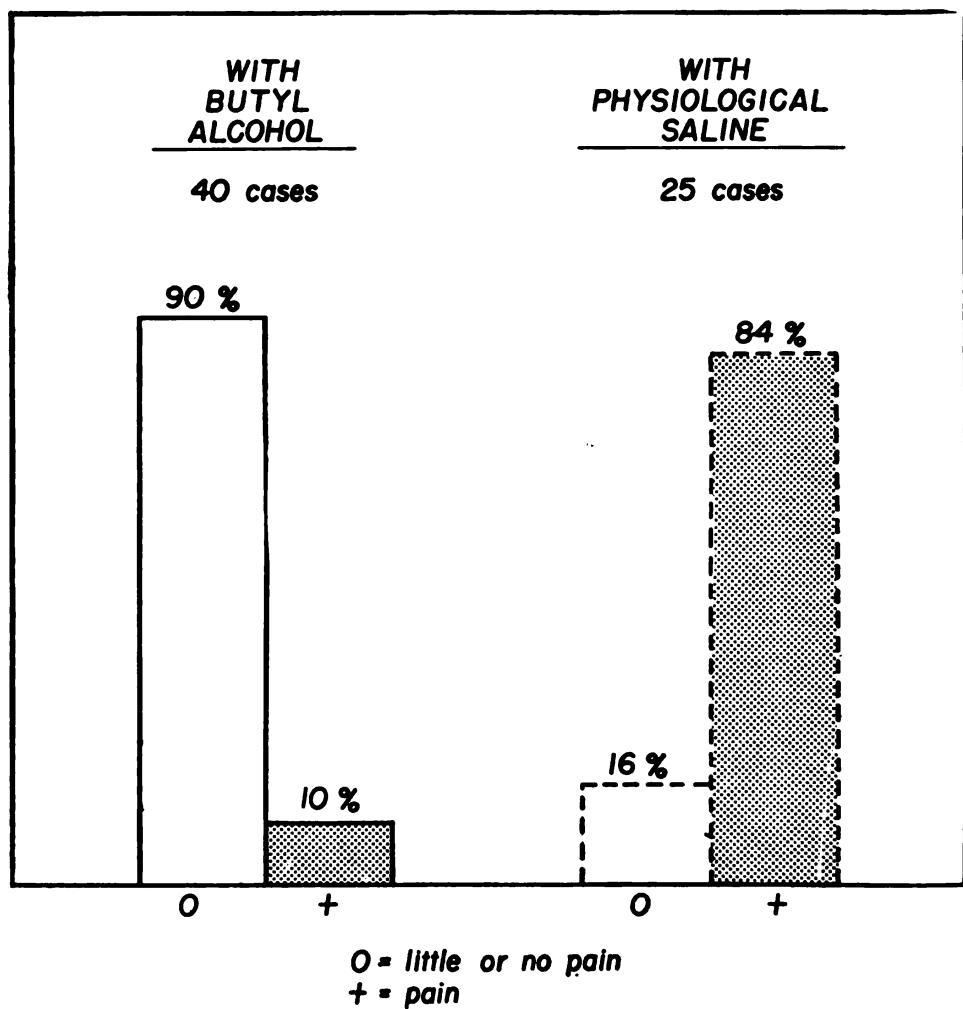
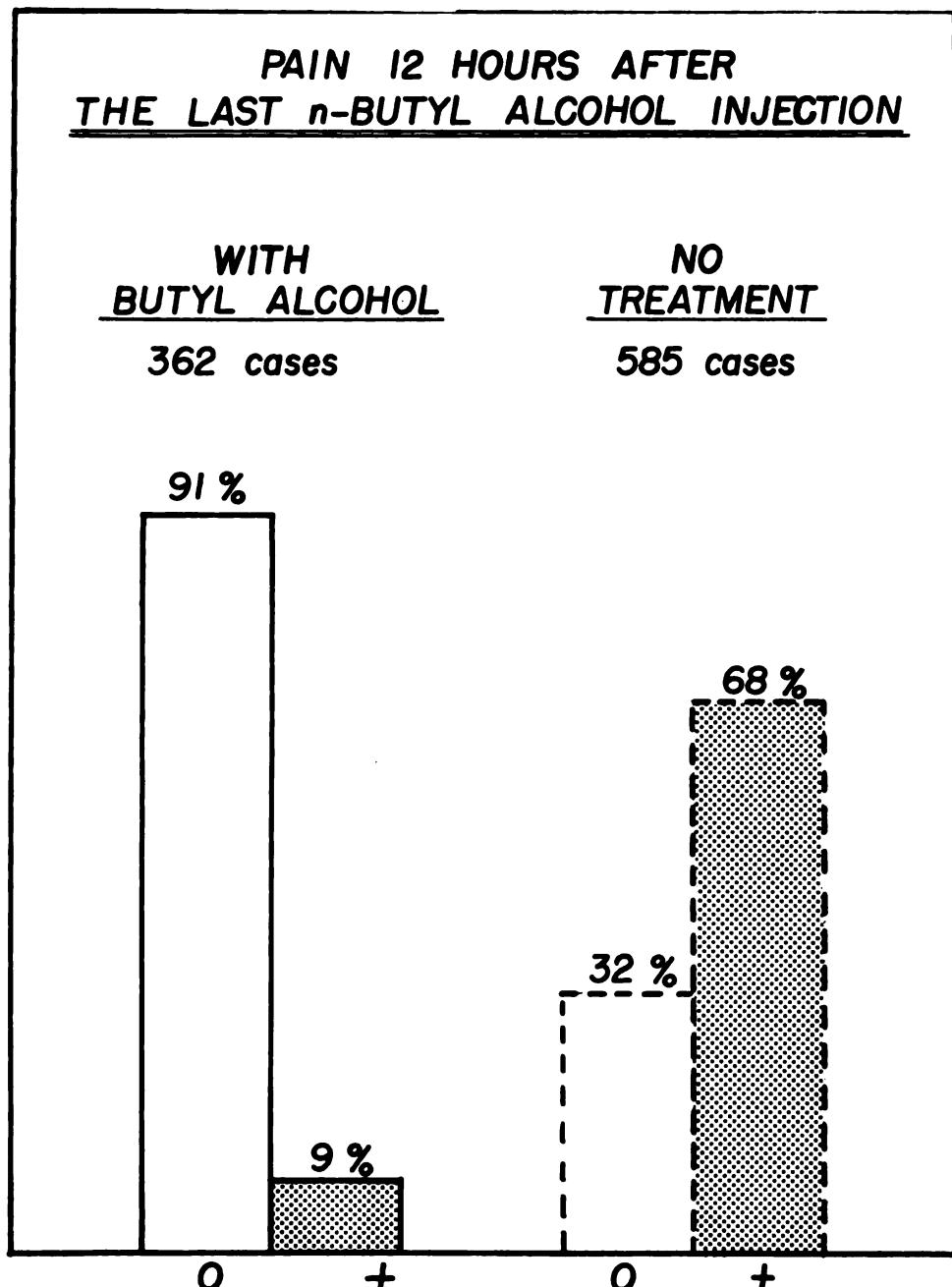


FIG. 144. Results obtained with butanol in postoperative pain—compared with subjects receiving saline as placebo—(From B. Welt—AMA Archives of Otolaryngology. 52, 540, 1950.)



0 = minimal or no pain
+= moderate or severe pain

FIG. 145. Results 12 hours after the butanol injections. (From B. Welt, AMA Archives of Otolaryngology, 50, 590, 1950.)

gology, (Note 2) (Fig. 144) by M. Welt in ophthalmology (188), by A. Ravich in urology (189) (Note 3), and by S. Sheer in plastic surgery. (190)

With more than 10,000 cases treated, butanol seems to be completely safe as well as highly effective. Not a single case of undesirable reaction due to the medication has been noted. The dose necessary to obtain pain relief varies with the amount of traumatized tissue. With an adequate dose of butanol, the patient has a minimum of pain, is conscious, and without impairment of intestinal and bladder function. Butanol also has a marked preventive action against hemorrhage, which will be discussed below.

Success with this medication depends upon adequate dosage. The addition of butanol to the saline solutions customarily used in postoperative care has done much to simplify the problem of administering it in adequate doses without resorting to supplementary injections. Doses as high as 100 cc. or even 200 cc. of 6.5% solution of butanol have been administered in the first days following surgery, with good results and no inconvenience to the patient.

Administered by injection or orally in only very small doses, heptanol is helpful. A mixture of heptanol, butanol and polyunsaturated alcohols constitutes a valuable preparation for controlling postoperative pain. *Butanol, heptanol and glycerophosphoric acid added to glucose and saline* has proved very helpful to control not only pain but also the other disagreeable *post-operative manifestations*. (Note 4)

In other forms of trauma, such as accidental wounds or fractures, the same agents have been equally successful if in sufficient doses.

With their use, the healing period seemed to be shortened, as shown in experiments in rats and rabbits. In animals with standardized wounds, the healing of connective tissue in particular is accelerated. In wounds treated with preparations of cholesterol, sterols or insaponifiable fractions of organs, the healed epithelium shows abnormally high proliferation.

Burns

The pain of burns has been successfully treated with higher alcohols such as butanol, heptanol or polyunsaturated alcohols. However, here the problem is not so simple since pain is only one, and not the most important, of the manifestations. Systemic functions are often so greatly impaired as to represent the immediate cause of death. The higher alcohols are effective in controlling pain and local troubles, while other agents are needed when the condition also involves the systemic level. Glycerol and especially sterols which are present in the insaponifiable fractions of organs such as

placenta, are very helpful in these cases. Anuria of several days' duration has been influenced in several cases, with abundant diuresis occurring only a few hours after the administration of 2-4 cc. of a 10% solution of the insaponifiable fraction of placenta, an effect not obtained with butanol alone, even in large doses. The effect was still more impressive when large doses of butanol, such as 10 cc. of the 6.5% solution every hour, were administered for a few hours before injection of the insaponifiable fraction.

The addition of sodium lactate to butanol in sufficient amount has been seen to favorably influence systemic problems related to burns. Experimentally, we have shown the value of this preparation in extending the survival time of mice.

Under ether anesthesia, adult mice were scalded in water at 90°C for three seconds up to the xiphoid appendix. The survival period for untreated animals was short. Of the various preparations used, the butanol-sodium lactate mixture seemed to be the most active in prolonging survival. (*See Note 13, Chapter 10 and Fig. 138*)

Ultraviolet ray burns, such as occur in sunburn, have been treated with butanol in several hundred cases. We started with 5 cc. of the 6.5% solution orally, and repeated the dose every 10-15 minutes until the painful sensation disappeared. When the burning sensation reappeared, treatment was resumed. It could be seen that butanol not only controlled pain rapidly and completely but also that the skin lesions healed in a shorter time than in untreated subjects.

X-ray and radium burns in animals and humans have responded to the same substances as mentioned above. Standardized ulcerated radiation lesions were inflicted in rats by inserting, between the lips of a skin incision, a needle with radium in platinum or monel metal. A needle with 25 mg. radium in monel metal was kept in place for two hours, one with 10 mg. in platinum for 96 hours. Ulcerations resulted and took four weeks or more to heal. In animals treated with sterol preparations, such as insaponifiable fractions of placenta, wounds healed in much shorter time. Similar effects could be obtained with butanol, although less consistently.

Good results were obtained with the same treatment in radiation burns in humans. In relatively new lesions, pain was relieved after a day or two and rapid healing usually followed. Even in old burns which had not healed for many years, pain was controlled in less than a week and the lesions started to heal. In some of these, scars developed in less than a month.

Vertigo

Another interesting application of the same therapeutic method has been in otological conditions. B. Welt has made a stimulating contribution to the study of ear conditions in terms of the pathogenic mechanism involved and an even more valuable contribution to therapy.

Vertigo was one of the conditions studied. In a few cases, by comparing concomitant variations in the intensity of the symptom and changes in the urinary pH, we had been able to show that acid and alkaline pathogenic patterns exist, similar to those seen in pain.

Going on from there, B. Welt, in an extensive study, has shown the presence of this dualism and has successfully applied the therapeutic method discussed above, to a significant number of cases of vertigo. The major advantage of this method over other treatments for this condition lies in its extreme simplicity.

For vertigo, which has so many different causes, an etiological approach although ideal, has seemed impossible to all workers in the field especially as a routine procedure in medical practice. An initial simplification of the approach could be made by relating vertigo to acid and alkaline patterns, thus reducing treatment choice to two groups of agents. Welt, in a further step, simplified even this procedure, making the method highly applicable in routine medical practice. He administered to patients one or the other of two agents chosen from each group, such as butanol or sodium thiosulfate, being guided in his choice by clinical aspects such as changes in the symptom with the time of day or the intake of food. A favorable change in the symptom was considered to be a confirmation of this tentative diagnosis of the pattern. The treatment was then continued with the same group of agents. An increase in vertigo led to the use of the opposite group of agents. The clinical results obtained by Welt with this simple method are impressive. (190) He arrives at the following conclusions:

"Summary and Conclusions"

A series of 44 patients having the symptom of dizziness has been studied.

The symptom of dizziness has been analyzed according to Revici's concept concerning the alkaline or acid pattern of the symptoms.

In 80% of the analyzed cases, the responses have shown the existence of an alkaline or an acid pattern; 12% showed an inconsistent relationship; 8% showed no result.

A therapeutic approach has been devised which corresponds to the presented results.

The control of the symptom of dizziness by this therapeutic approach has been obtained in 80% of the cases.

As compared with other methods of treatment, which are prolonged and involved, Revici's approach is simple in application and effective in its results.

This therapeutic approach based on the dualistic pattern of the physiopathology of the pathological tissues is especially applicable to the symptom of vertigo, which because of the multiplicity of the etiologic and pathogenic factors make a direct medical attack almost impossible.

The evidence presented in this communication relates to the control of the acute attacks of vertigo. Recurrences were observed in this series and successfully controlled.

In the cases submitted in this communication and in subsequent cases subjected to this form of therapy, further observation by this method will indicate whether or not the lesion is permanently controlled."

Welt completed his vertigo study by using many other members of the same groups of agents as they were studied by us for other conditions. The following conclusions appear in Welt's second publication on vertigo. (191)

"Summary and Conclusions

A series of 106 cases with the symptom of vertigo has been presented in this and a previous paper and analyzed according to Revici's concept of a metabolic imbalance which exists in pathological foci and manifests itself as a local alkalosis or acidosis.

Seven cases were eliminated for lack of data. This left a total of 99 case studies. This figure represents the sum of 42 cases termed the 1953 series and the present series of 57 cases. The two series were combined and analyzed according to sex, alkaline or acid character of the symptom, and clinical diagnosis.

The analysis showed an average good result of 80% for both series. This percentage indicates a consistent correlation of the use of Revici's method and substances with the results achieved. Additional substances from various sources were utilized in the present study. It is here again emphasized that I have submitted this article for the practical reason that the method described is simple and has been successful in its application. In addition, it gives a new systematization to clinical data in the field of otolaryngology. Finally, this communication points to a vast group of sub-

stances from widely differing sources and of different structures which have similar biochemical activities. The substances presented here are but a few, but they open a pathway for the development of other substances having similar biochemical activities. It is to be hoped that these too, may have a practical application in the field of otolaryngology.

Views on cell permeability and their relationship to fatty acids and sterols are added.

The role of foci of infection and psychological aspects in their relationship to the symptom of vertigo is amplified.

The simplicity of this method of therapy is emphasized, and its integration with a new method of systematization of clinical knowledge is indicated."

Hearing Impairment

Deafness remains the most important problem in otology despite good results recently obtained with surgical procedures such as fenestration and stapes mobilization. Since deafness results from long evolving processes, a logical approach to the problem would be not to wait for the advanced stage where surgery must be used but to try to control the processes before they reach this ultimate state which usually corresponds to sclerosis. Studies of the morphological changes related to progressive deafness have been too limited to present a basis for a biological or biochemical therapeutic approach that would have a chance of success. The nature of the lesions, their minuscule dimensions, and their chronic evolution, offer no opportunity for direct information. Consequently, we tried to adapt to this situation the knowledge acquired through research in the field of abnormal processes in general.

Just as for pain, it was observed that some patients with hearing impairment are aware of differences in their acoustic acuity according to the time of day, *i.e.* some hear better in the morning, some in the evening. There seemed also to be a correlation with the intake of food. Based on these observations, cases of various degrees of surdity were investigated for relationship to acid-base changes. Some subjects showed audiometric variations under the influence of acidifying or alkalinizing agents, while others did not respond. When two antagonistic agents were used in the same subject and hearing acuity was analyzed, the changes occurred in two opposite directions. We could interpret this only by analogy to changes in pain and other acid-base symptoms. Accordingly, we postulated that in some cases no activity is going on in the lesions since no changes are induced in the

audiogram by administration of acidifying or alkalinizing agents; in others, there is activity which is acid or alkaline in pattern.

Thus, the existence of a response was considered to indicate a still active lesion. Time is not the determining factor. Even in relatively recent lesions, biological activity may have ceased while in other cases, even with very advanced hearing impairment, lesions can still be evolving, with processes not yet advanced to the sclerotic scar phase. This aspect has been extensively studied because of the therapeutic implications, since only an evolving state could possibly be influenced by this method of therapy.

The influence of agents used in other conditions with acid-base pathogenesis was studied in cases of impaired hearing. The first attempt made years ago, with fatty acids and sterol preparations, was not successful. Much better results have been obtained with more recently developed synthetic agents. In the last five years, Welt has devoted much time and effort to this research. He has made thousands of complete audiograms and has used a series of active agents with placebos for control. (192) Welt has studied approximately 460 cases to date. (*Note 5*) In the simplified form, an agent from one or the other group was used for a short time and improvement in the audiogram was taken to indicate that a suspected pattern, acid or alkaline, was actually present.

Clinical results have largely confirmed theoretical views. Regardless of degree of impairment, improvement ranged from nil or almost nil in cases with no more signs of activity, to good and excellent in those with still active lesions. In young patients in whom the proportion of active lesions is high, results were particularly impressive. Hearing impairments of 60-70 decibels or more have been overcome, sometimes in only a few weeks of treatment. These results have been observed to persist in many cases, some thus far for more than three years. However, a tendency for impairments to recur, usually following rhino-pharyngeal infections, has been noted. Generally, renewed treatment has been able to restore hearing to previous values in a short time. (*Figs. 146 to 150*)

It appears superfluous to emphasize here the importance of this contribution to the medical and social problem of deafness. It represents the first approach to treat successfully the pathological processes related to impaired hearing before they lead to advanced lesions which can no longer be influenced except by surgery. Every case of incipient or even advanced hearing loss should be investigated by the simple technique devised by Welt to see if correction by this method is still possible. Surgical intervention should be reserved principally for patients with lesions which correspond to sequels and who no longer respond to biochemical treatment. But even

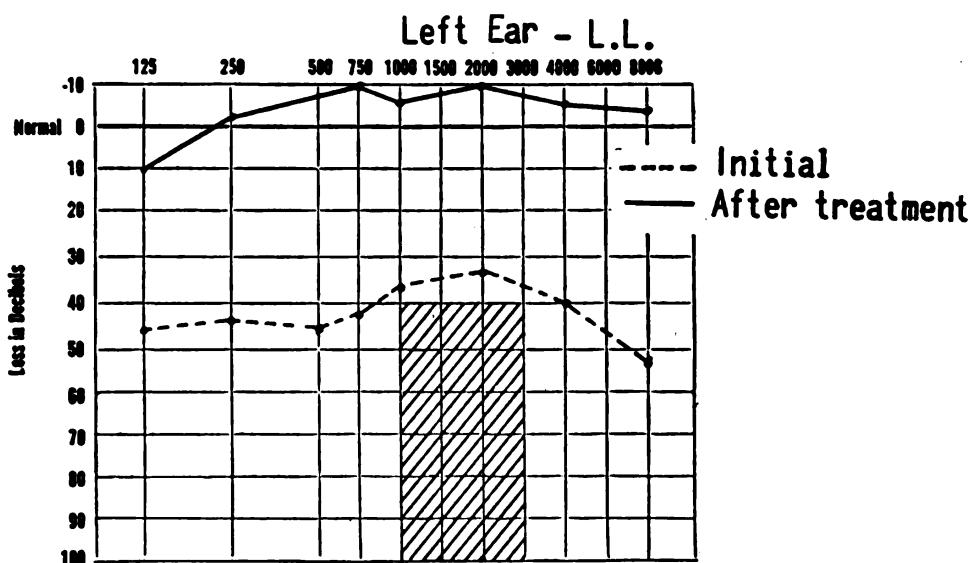


FIG. 146. Manifest improvement in the impaired hearing is obtained in a subject with acid pattern, through guided treatment with negative lipoids. (Courtesy of Dr. B. Welt.)

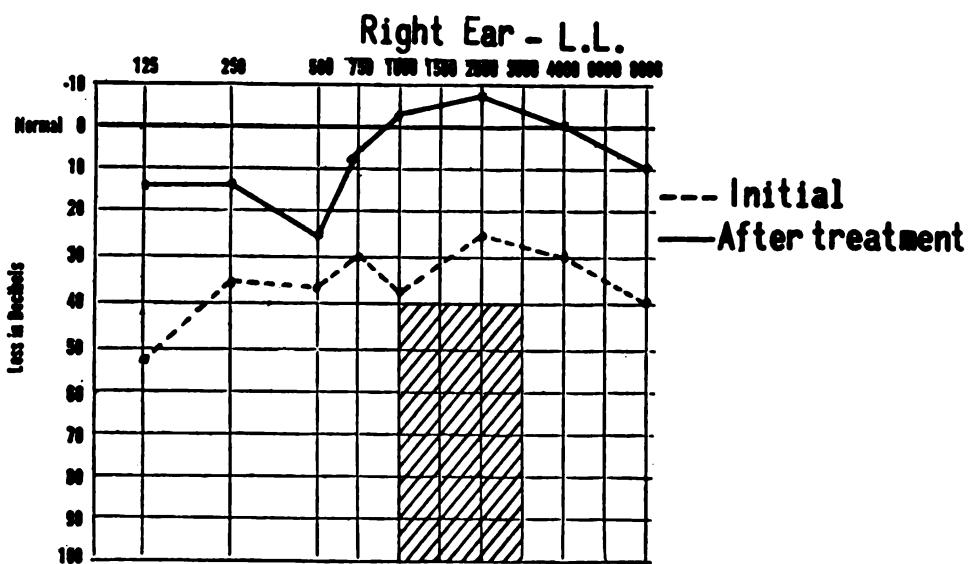


FIG. 146bis. In the same patient as in Fig. 146 the improvement is not as manifest for the right ear as for the left ear.

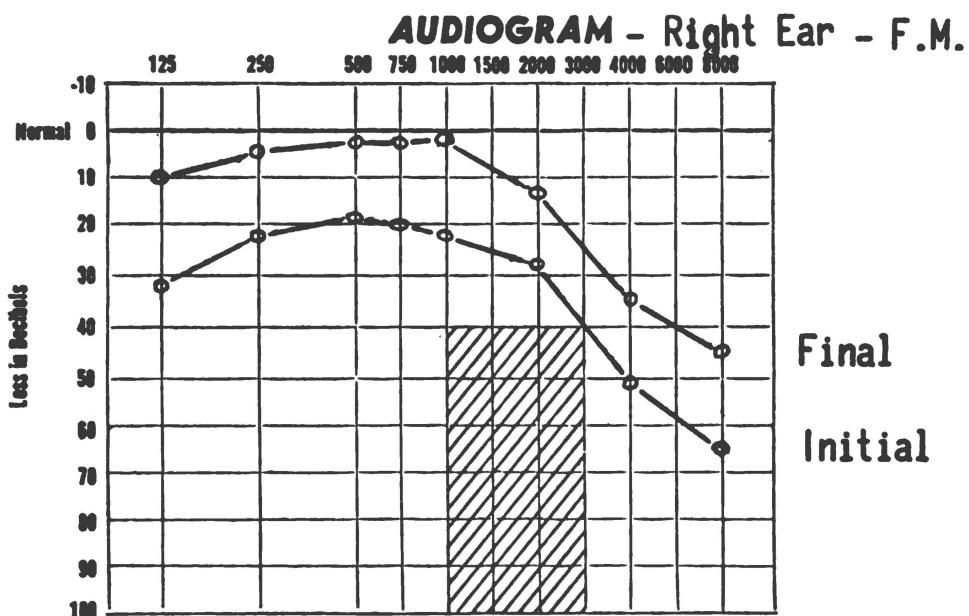


FIG. 147. Only a relative improvement is obtained in a case with alkaline pattern of impaired hearing.

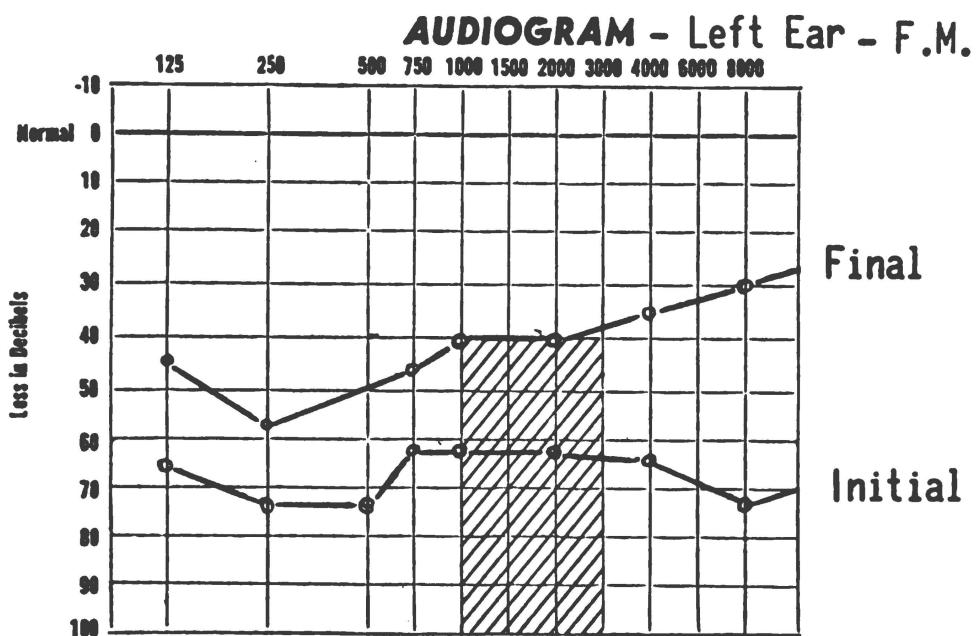


FIG. 148. A still smaller result is obtained in this case for the left ear.

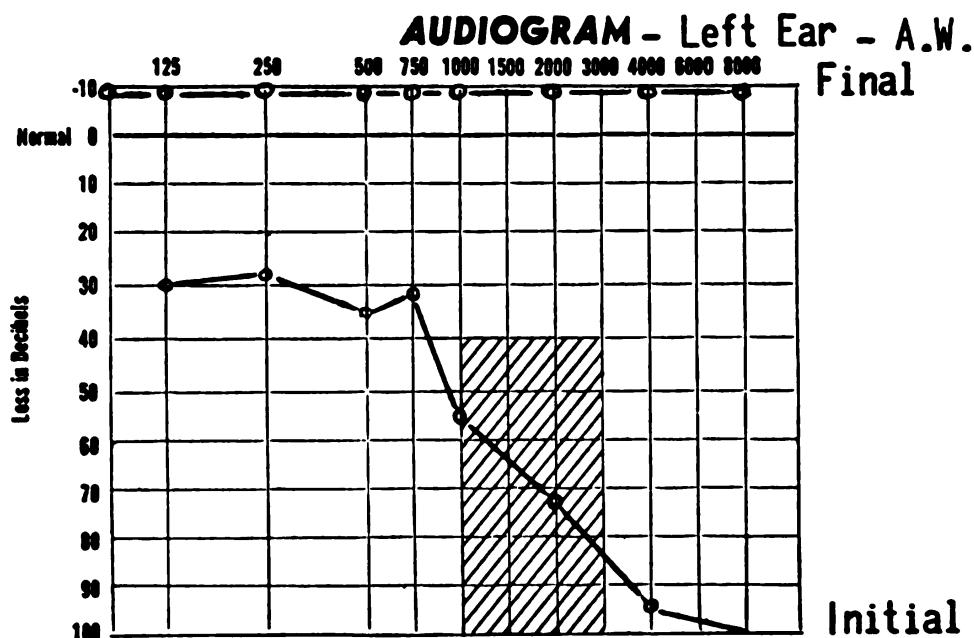


FIG. 149. An exceptionally good result is obtained for the left ear in the case A.W., in spite of an initially very impaired hearing.

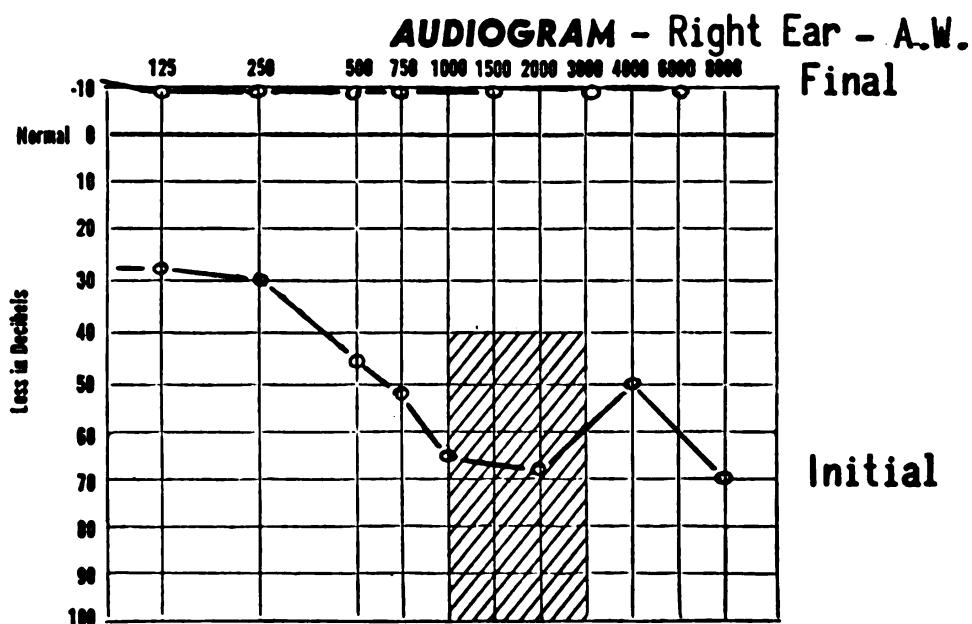


FIG. 150. Manifest improvements are obtained in this case with alkaline pattern of impaired hearing.

surgical cases can benefit from biochemical treatment which could prevent further sclerosis which often follows surgery. Application of this method in time to prevent deafness would be even more important than restoring hearing in far advanced cases.

Itching

Acid and alkaline patterns of itching have been recognized, and treatment based upon dual pathogenesis has been applied. The agent to be used is determined by the pattern found through analyses. Dosage, as for pain, is established according to the subjective response. Following these methods, many cases of intractable itching have been completely controlled. Of the group of agents with positive character, butanol and heptanol have produced the best results; in the opposite group, epichlorohydrin and sodium thiosulfate have been most effective.

Gallbladder and Renal Colic

Attempts were made to influence renal and gallbladder colic by using the same method. Butanol has shown little effect upon renal colic at least in the doses we tried. But the results in gallbladder colic were impressive. A 6.5% solution of butanol in saline injected intramuscularly, or even 15-30 cc. (one or two tablespoonsful) of 6.5% solution of butanol in water administered orally when the patient was not vomiting, controlled gallbladder colic rapidly. Only twice in more than 20 cases in which butanol was used was it necessary to give larger doses, such as 50 cc. of the same solution, to obtain the desired effect.

Arthritis

The analysis of the pattern of pain has shown that often in rheumatoid arthritis the pattern is alkaline, while in osteoarthritis, it is acid. Comparison of variations in pain intensity with changes in urinary surface tension helps to determine the pattern. However, with urinary surface tension values used as a guide both for agent and dosage, favorable clinical results have been obtained in cases resistant to other therapy. Arthritis, painful for many months, was controlled in many instances with several doses of heptanol or of sodium thiosulfate with epichlorohydrin and more recently with propionic aldehyde or sulfurized tetralin. The simplicity of treatment, total lack of undesirable side effects, and the long period of improvement

after even brief treatment in some cases, indicates that this method is worthy of further investigation. Some illustrations of the results are contained in the following observations:

Case #1

Mr. M. R., 63 years old with a long history of arthritic pains. Four years ago he was obliged to stay in bed for three months for severe pains in the right sacroiliac region. Two years ago he was again bedridden for a month and a half because of the same type of pain. A year ago, pain started again in the same sacroiliac region, to be followed by a very severe sciatica which kept him in bed for two months. Repeated X-ray examinations showed advanced arthritic lesions in the lumbar spine and around the sacroiliac articulation. About ten months ago, he suffered another similar attack, with severe pain, in the same region. Urine analysis made at this time showed an offbalance of the type D with low surface tension, low pH and high specific gravity. The blood serum potassium was high (5.3 mEq.). The patient was treated with agents of the group D—1 mg. of heptanol and 30 mg. butanol three times a day. The patient remained free of symptoms for four months, when similar severe pains were again present. This time analysis showed an offbalance of type A, with high urinary surface tension, high pH, low specific gravity and low blood serum potassium (4.0 mEq.). For this reason, different medication of the group A—5 mg. epichlorohydrin and 50 mg. sodium thiosulfate—were administered. The response was as rapid as previously and pain disappeared within a few hours. Since then the patient has been taking 5 mg. epichlorohydrin and 50 mg. sodium thiosulfate twice a day whenever the slightest pain appears. The clinical results are excellent, the patient being now able to move about entirely free of pain, as he has not been able to do for many years.

Case #2

Mrs. F. R., 60 years old, was bedridden for one and a half years about six years ago, with lumbar and sacroiliac arthritic pains. X-ray treatments, short wave, ionization with histamine and ultrasonic treatments appeared entirely ineffective. The administration of urolytic agents had helped at this time. The pains recurred repeatedly in the last three years keeping the patient in bed for from one week to three months at a time. For the past eight months, the patient has been under treatment using medication of the group A or D, according to the urine analysis. With this treatment the pain has been completely and rapidly controlled, never lasting more than a few hours.

Case #3

Mr. N. K., 55 years old, suffered for many years with generalized and very painful rheumatoid arthritis. Prior to coming under our care, he had been bedridden for six months, entirely immobilized with severe pain in arms, back and legs. The patient was entirely incapacitated, unable even to feed himself. Treatments with different cortisone preparations, ACTH, gold, etc., provided practically no relief of the severe pains. He came under our care, entirely immobilized and in severe pain. According to the analysis which showed a low urinary specific gravity, and pH, high surface tension and a low blood serum potassium, the patient received medications of the group A, sodium thiosulfate, epichlorohydrin and heptyldiselenide. Under this medication the patient made a most dramatic recovery. Within a few days, the patient was out of bed without pain, walking normally and with full functional capacity of his arms and legs. While still under treatment the patient went back to entirely normal activity. He gained 35 lbs. in the last months.

Connective Tissue Conditions

We have discussed previously the hypothesis that corticoids actively intervene in the synthesis of an entire group of substances in the organism. The energetic formation between C₁₁ and C₂₁ of these corticoids would act as a mold or template to attract and keep various radicals in special reciprocal position, thus favoring, as a second step, the synthesis of new substances. These substances would vary according to the energetic formation of the corticoid. In one group, which could be called the "gluco" group, we identified glucose, glucuronic acid, glucosamine, galactosamine, glucosaminic acid, galactosaminic acid, and some other uronic acids. Even ascorbic acid could be related to such a synthesis mechanism.

We investigated some of these substances, particularly in relation to the therapeutic properties of corticoids. In 1951, we chose glucosamine as a substance closely corresponding to the template of cortisone. Glucosamine treatment was used in patients with pemphigus, rheumatoid arthritis, psoriasis, ocular condition and allergy. The following cases illustrate the results obtained.

Mr. H. S., 46 years old, had generalized psoriasis. In the last twelve years, the lesions had progressed continuously to cover more than 75% of his skin. Local treatment had had little effect. Cortisone injections cleared the skin in two weeks but not completely. With

cessation of treatment, the condition returned. The patient remained without treatment for a few months before beginning our treatment: 5 cc. injections of glucosamine 10% solution administered daily. Changes were seen in 3 days, with the lesions becoming paler and less scabby. With two injections daily, the skin was almost entirely normal in about 10 days, only a few lesions remaining. Continuation of the treatment for another week did not make these disappear. Upon cessation of treatment, the lesions regressed to their previous dimensions. In a second course of treatment, with two daily injections of 5 cc. of a 10% solution of glucosamine in saline, the lesions cleared but reappeared when treatment was discontinued.

We also treated a series of cases of rheumatoid arthritis and found that subjects, who had previously responded well to cortisone, also responded to glucosamine.

Mrs. B. B. had rheumatoid arthritis of the left knee and both hands. Pain and swelling were severe. The patient had responded fairly well to cortisone, but due to retention of water, this treatment was discontinued. In less than 10 days, the arthritis returned. The patient was given glucosamine injections. At first, 5 cc. of the 10% solution in saline was injected once a day. Later, 3 injections daily were used. The symptoms, which had decreased with the lower doses, were fully controlled by the 3 daily injections. Pain and swelling fully disappeared. Moon face was not observed. The treatment was continued with 2 injections daily and, after one week, one injection daily. With this dose, pain and swelling returned. Resumption of 3 injections a day controlled them again. The treatment was discontinued because of the apprehension of the patient over receiving 3 injections daily of 5 cc. each. A mixed treatment with a lower dose of cortisone and one injection of glucosamine daily was continued with satisfactory results for a few weeks, after which the patient stopped her visits.

We treated cases of rheumatoid arthritis with oral glucosamine with fair to good results. Four grams of glucosamine a day appeared to be the minimum requirement. Good results could be seen in some cases after doses of 5 to 10 grams daily. These results were confirmed by B. Welt who, following his research, used the same preparation of glucosamine on a series of 15 patients bedridden with arthritis at the Greenpoint Hospital in Brooklyn, New York. Subjective and objective improvement occurred in patients with rheumatoid arthritis while no changes were seen for those with osteoarthritis.

For more than one year, Welt has treated a case of pemphigus with injections of glucosamine and has been able to prevent the appearance of new lesions.

In a few cases of iridocyclitis, treatment with glucosamine (2 injections of 5 cc. of the 10% solution daily) healed the lesions in two or three days.

Galactosamine produced results almost similar to those of glucosamine. Still better results were seen with glucosaminic acid, used in the same doses as glucosamine. Some strikingly good results in several cases of arthritis were obtained with gluconic acid administered orally in doses of a few grams a day (four tablespoons of a 25% solution).

Hemorrhage

In studying hemorrhage, it seemed necessary first to define precisely the conditions under which this important episode occurs, as we did for pain, itching, etc., since the course of hemorrhage, and particularly its response to treatment, seems to depend greatly upon certain peculiarities related to its pathogenesis. Hemorrhage results from a break in the continuity of a blood vessel, which can be induced by an external influence on a previously normal vessel, or can occur as a result of processes taking place in abnormal vessels. We have called the first type of hemorrhage "accidental" or "traumatic" and reserved the term "pathological" for the second, which appears to be a direct result of pathological changes in a vessel. The latter term is used in the same sense as it is used for fractures where "pathological" indicates only preexisting lesions in the bones.

In a traumatic hemorrhage, the therapeutic problem is limited to stopping the flow of blood. Mechanical means to close the bleeding vascular wound or agents able to increase the capacity of the blood to form clots can be used. They represent the only approach in accidental hemorrhage.

In pathological hemorrhage, other problems arise. The knowledge of the local pathological changes, which lead to the appearance of bleeding, is important both for preventing and controlling hemorrhage. The pathogenic factor involved will be considered along with the problem of hemostasis.

Hemorrhagiparous Agents

Local and general factors are involved in pathological hemorrhage. Ulcerated lesions provide a favorable condition. So does local infection. Anticoagulants may act indirectly upon blood vessels.

Hemorrhagiparous properties of some substances became evident during research on their therapeutic use in cancer patients. For example:

P. T., a patient with carcinoma of the floor of the mouth widely ulcerated in the submaxillary region, bled sporadically from the lesion.

When a dose of 20 drops of Coramine (brand of nikethamide) was given for his general condition, a relatively severe hemorrhage appeared immediately afterward. There was another hemorrhage when a similar dose was administered 12 hours later. Suspecting a correlation between hemorrhage and medication, medication was discontinued and there was no hemorrhage during the following two weeks. When only 10 drops of Coramine solution was given again, a new hemorrhage appeared. After two more weeks without Coramine and without hemorrhage, a new dose was given to check the correlation between medication and bleeding, and was followed again by hemorrhage.

Reviewing cases lost through hemorrhage, we could find several in which lethal bleeding was preceded by administration of Coramine in the usual dose. This hemorrhagiparous effect was noted so consistently as to make us discontinue use of Coramine in cases with ulcerated lesions.

Alerted by this experience, we noted hemorrhagiparous activity in other agents. Glycerol, we found, could induce severe bleeding even when administered in doses as low as 5-10 drops, and two lethal hemorrhages were traced to such doses. Thiamine chloride in therapeutic doses—such as 100 mg. injections—produced hemorrhages. A similar hemorrhagiparous effect was noted for isamine blue. Glucose, administered intravenously in large doses to patients with ulcerated and infected cancerous lesions who had previously hemorrhaged, induced new bleeding. We must emphasize that all the hemorrhagiparous agents produced the bleeding effect, especially in subjects who had previously had hemorrhages from their lesions. This would indicate the importance of local changes. In such patients, other agents also induced bleeding. In some cases of gastro-intestinal cancer, bleeding followed a normal dose of aspirin. But even in lesions which had never bled, the first group of hemorrhagiparous agents—Coramine, glycerol and thiamine—could induce bleeding.

In trying to correlate the pathogenesis of these hemorrhagiparous effects with existing offbalances, an interesting relationship was noted. A tendency to bleed was found to be promoted by sterols. Administration of cholesterol in doses as high as 5 cc. of a 2% solution in oil, two or three times a day, for several days, was followed by hemorrhage in patients with ulcerated lesions. This occurred only in a few cases. But a high sensitivity to other hemorrhagiparous agents often developed. The administration of minimal doses of glycerol produced bleeding in subjects treated with cholesterol and did so even more frequently when large amounts of insaponifiable fraction of organs were administered.

Hemorrhages which follow administration of these hemorrhagiparous agents are usually severe and of arterial character. Examination of such bleeding lesions has revealed transverse severance of small or even medium arteries. Only rarely was there oozing bleeding from capillaries or small veins. In several cases, administration of hemorrhagiparous substances induced petechiae or purpura, but this occurred only when thrombopenia was also present. The petechiae were seen at sites where local circulatory impairment already was present. We believe that this hemorrhagiparous effect must be emphasized for its clinical importance. Products such as Coramine (nikethamide) and thiamine are widely used as therapeutic agents and glycerol is a common vehicle for pharmaceutical preparations. Therefore, we must bear in mind their possible role in hemorrhages. In subjects with ulcerated lesions, their administration has to be banned or special precautions must be used. The same precautions have to be taken for the use of glucose in cases in which a previous hemorrhage has not been controlled by mechanical means.

Antihemorrhagic Agents

Butanol

In 1943, during research studies concerning the pharmacological activity of glycerol on abnormal foci, its hemorrhagiparous effect appeared as a serious handicap. Various hemostyptic substances were tested without sufficient effect. At this time, a new product, to which a hemostatic effect was attributed, appeared on the market. It was a very weak solution (around 1/10,000) of octanol in saline. We could find no therapeutic effect for it. However, we were studying butanol and other higher aliphatic alcohols with lipoidic properties, and we decided to test butanol for its antihemorrhagic activity, hoping that it might counteract the undesirable hemorrhagiparous effect of glycerol. It did and we have since added butanol to glycerol for this purpose.

We observed the remarkable hemostatic effect of butanol years later in a patient with severe hemorrhage, to whom doses of 10 cc. of a 6.5% solution were given intravenously. Hemorrhage stopped in a few minutes. Since then, we have successfully applied butanol clinically in hemorrhages of various origins.

As an antihemorrhagic agent, butanol is administered either parenterally as a 6.5% solution in saline, or orally as 6.5% solution in water. The route of administration—intravenous, intramuscular, subcutaneous or oral

—is chosen according to the severity of the hemorrhage. Doses of 5-20 cc. are given and repeated, if necessary, at intervals of a few minutes. Since butanol is not at all toxic in these large doses, we usually give them with good results in severe emergencies. The following cases illustrate the styptic effect.

R. E., a 64-year-old man with an extensive ulcerated epidermoid carcinoma of the floor of the mouth and large bilateral cervical metastases, had received intensive radiotherapy. Occasionally, there was a small amount of bleeding from the oral lesion, but a sudden hemorrhage of about 500 cc. of blood during a half hour period occurred late at night while the patient was at home. Pressure, applied to the floor of the mouth, was of value but bleeding recurred immediately upon release. Oxidized gauze, adrenalin soaked gauze, vitamin K and vitamin C in large quantities were of no value. n-Butanol solution in saline was finally obtained and 5 cc. injected intravenously. Bleeding ceased during the injection. A second equally severe hemorrhage occurred one week later and again could not be adequately controlled by pressure or oxidized gauze. 10 cc. of n-butanol solution administered intramuscularly stopped the bleeding within 2-3 minutes. Three hours later, the floor of the mouth was carefully examined preliminary to right external carotid artery ligation, and the lesion was found to be free of bleeding. Despite the ligation, bleeding later recurred but was controlled each time by n-butanol administered orally.

S. S., a 30-year-old man, had an adenocarcinoma involving the right maxillary sinus with cervical metastases. During the period of observation, this patient experienced a profuse hemorrhage from nose and mouth. Blood flowed at the rate of approximately 5-6 cc. per minute, and pressure gave little or no relief. 5 cc. of n-butanol in saline solution was injected intravenously and within two minutes, the profuse hemorrhage ceased and did not recur at that time. On several other occasions, bleeding was controlled following the administration of oral doses of 5-10 cc. of 6.5% n-butanol solution in water.

A. M., a 36-year-old man, had multiple pulmonary metastases from a primary malignant melanoma of the left foot. On several occasions, hemoptysis occurred and during three of these episodes bleeding was profuse. 5 cc. of n-butanol in saline administered intramuscularly stopped two of these episodes rapidly, but in the third, an injection of 10 cc. intravenously was needed ten minutes after an initial intramuscular dose. Although the intramuscular injection was ineffective, the bleeding was halted within two minutes after intravenous administration.

In a report in 1951 in "Angiology," (193) we presented the following statistics concerning the control of hemorrhage in cancer cases:

	Group I Untreated	Group II n-Butanol
Number of cases observed	256	344
Number of cases with profuse hemorrhage	18	25
Percent of cases with profuse hemorrhage	7	7
Number of deaths attributed to hemorrhage	12	1
Percent of deaths in cases with profuse hemorrhage *	67	4

* Death attributed directly to hemorrhage.

Since then, these results have been consistently confirmed.

Additional progress was achieved with organic acids added to butanol in adequate amounts. Completely non-toxic in the dosages used, they were observed to enhance the hemostatic effect of butanol. This hemostatic effect has been confirmed many times, particularly in Europe where, following our research, butanol has been widely used as a hemostyptic agent.

A hemostatic effect was also evident when butanol was used with the principal aim of controlling pain in postoperative cases. The hemostatic effect was especially important in cases where pathological bleeding usually represented a major complication, either because of the impossibility of obtaining hemostasis during operation or because the surgical wound could not be kept aseptic, as in tonsillectomy, prostatectomy and plastic surgery of the nose. In a study on the use of butanol for postoperative care in tonsillectomies, B. Welt has been able to show a preventive effect, and more important, a hemostatic one if hemorrhage occurs. (187) (Fig. 91) In prostatic surgery, the amount of bleeding was so reduced, that of a group of 40 cases, only one needed transfusion while in a similar number of controls, 8 had to have transfusions. (189)

Still more impressive results have been noted in pathological hemorrhages following plastic surgery, especially of the nose. In a significantly large number of cases, severe hemorrhages tend to occur around the 7th day after operation. We have discussed above the pathogenesis of these hemorrhages and the relationship to the allergic defense mechanism. Such hemorrhages have been difficult to control. The use of antibiotics has only partially reduced their frequency and gravity. S. Scher has obtained very good results with the administration of butanol in such cases, using an injection of 5 cc. of a 6.5% solution of butanol once before surgical intervention and four times daily for two days afterward, followed by oral administration of 15 cc. or one tablespoon four times a day for eight days. No hemorrhages occurred in more than two thousand cases treated. In a

few patients who neglected to take the medication, and in whom a hemorrhage appeared, bleeding was rapidly controlled by butanol. (*Note 6*)

Experimental Research on the Hemostyptic Effect: In a series of studies we sought to find the mechanism by which butanol controls hemorrhage. In an investigation carried out in our laboratory, M. Bier and P. Teitelbaum (194) showed that individual members of the homologous series of aliphatic alcohols decrease the degree of retraction of clots when added in vitro to blood. (*Figs. 141 to 143*) By varying the amounts added, this effect was observed to occur only at values above a critical concentration of the alcohol in blood. Bier could show that the critical value, which differs for members of the homologous series, is proportional to the lethal toxic dose in mice.

This non-retraction of clots in vitro also can be recognized in cases treated with butanol through the gelatinous aspect of the clots at the moment when the hemorrhage stops. However, it is interesting to note that the amount of butanol injected, considered in terms of the amount of the circulating blood, results in concentrations considerably below the critical values needed to produce this effect in vitro. However, concentration of butanol at the site of the wound may explain this. It is also of interest that the same gelatinous character typifies the clots which remain attached to bleeding lesions in animals treated with butanol. In mice, when a portion of tail was cut and butanol was used, the abnormally long clot remained adherent to the wound, differing from that seen in controls.

In spite of changes in the clot, it seems that the effect upon the blood itself, and its coagulation in a wound, represents only one of the means through which butanol controls hemorrhage. The speed with which butanol acts, often within seconds after intravenous injection, is much greater than blood coagulation time. Consequently, changes in clot formation alone do not appear sufficient to explain the mechanism through which the rapid hemostasis occurs.

M. Bier and H. Lerner in our laboratory studied the influence exerted by butanol upon hemorrhage induced by the highly active proteolytic enzyme, ficine. (195) They were able to induce standardized hemorrhages by injecting ficine solutions under the skin of the abdomen of white mice. (*Fig. 151a*) (In other animals and with other sites of injection in mice, the individual variations were too great to make the resulting bleeding useful as material for testing the effect of agents upon hemorrhage.) With adequate doses of ficine, severe hemorrhages followed by skin ulcerations were induced. The bleeding, and even the ulcerations, were almost entirely prevented when butanol was administered. Figure 151b shows the results of

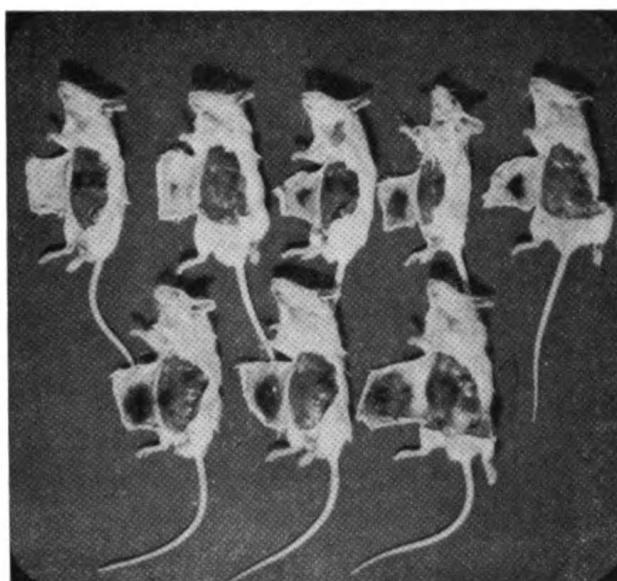


FIG. 151a. Shows graded extent of hemorrhagic infiltration obtainable with progressive amounts of ficin injected subcutaneously in the abdominal region in mice.

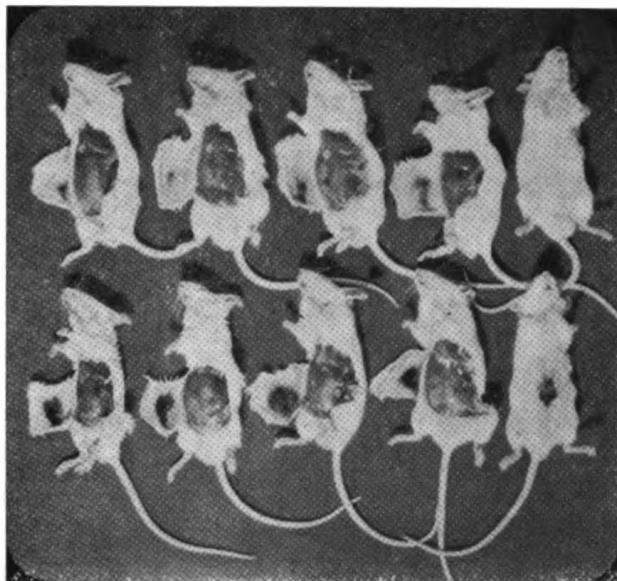


FIG. 151b. The influence exerted by butanol upon the hemorrhage induced by ficin injection.

Top Row. Comparative group treated with butanol. Last animal in each row shows the external appearance of the site of the injection while in others, the skin flap was separated.

Bottom Row: Ficin injected animals.

this experiment, with the manifest differences, following ficine injections, between animals receiving butanol intraperitoneally and controls.

The antifibrinolytic activity of butanol (196) could explain its intervention in protracted bleeding, and especially in cases where the effect of butanol appears after hours or days. However, inhibition of fibrinolytic activity cannot be conceived to intervene in an action taking place in less than a few minutes.

In trying to explain butanol's antihemorrhagic effects, we also considered its pharmacological activities. We have noted the action of butanol upon the acid-base balance of abnormal tissues, as shown by changes in the pH of the second day wound crust. In abnormal tissues, butanol reduces local pH but it does not do this to any great extent in normal tissues. This led us to investigate the difference in the influence of butanol upon pathological hemorrhages. The immediate bleeding induced by standard cutting of the tail in mice was not constant and, in general, was not markedly influenced by administration of butanol. But there was a marked effect upon hemorrhage induced by the displacement of the clots through mechanical maneuvers one to two hours after cutting of the tail. In controls, the bleeding lasted almost the same length of time as bleeding from fresh lesions, while in animals treated with butanol, it often stopped in a very short time.

These data, although interesting, did not seem to offer a completely satisfactory explanation of the mechanism by which butanol stops bleeding, especially in cases in which it acts within minutes or less. It appeared improbable that the formation of a clot alone would stop the hemorrhage under these conditions. An interesting observation led us to another hypothesis.

In several patients with wide ulcerations, we were able to examine the hemorrhaging vessel after bleeding had been stopped by butanol. Contrary to all expectations, we found that the artery, which usually was severed transversely, was not buried in a clot but remained almost isolated and somehow separated from it. It seemed that blood vessels themselves might have a role in hemostasis. The intervention of a spasm of the smooth muscles of the vessels was considered. This was indirectly confirmed when a patient with severe and prolonged bleeding from the bed of a prostate after ablation, was treated with butanol. An intravenous injection of 40 cc. of butanol was followed by such violent contraction of the bladder as to expel, with great force, the catheter together with clots and urine present in the bladder. At the same moment, bleeding, which had persisted for more than a week, stopped suddenly. We connected this sudden spasmodic contraction of the smooth muscles in the abnormal bladder with

the injection of butanol and considered that butanol might produce a spasm of the muscular walls of abnormal blood vessels—abnormal because of the hemorrhagiparous condition itself. This would explain the rapidity of the hemostatic effect and the selective action upon small and medium arteries with important muscular walls. In fact, we saw that, while very severe arterial hemorrhages were completely stopped in less than one minute after an intravenous injection of butanol, the same effect was not obtained in oozing bleeding.

We tried to investigate further this spastic effect upon blood vessels as the mechanism in butanol hemostasis. Experiments with isolated aorta preparations of rabbits and rats showed that a spastic effect cannot be induced by butanol, even if the vessels previously have been harmed by manipulation. This can be explained by the fact that the aorta does not have muscular fibers. The same lack of spastic effect is seen in normal arteries of the hind legs of rats and frogs.

The administration of butanol to an animal after a small branch of the mesenteric artery was first crushed and, after some time, cut, produced a spastic effect which transformed the jet-like hemorrhage into an oozing one, greatly reducing the blood loss. The hypothesis of vascular spastic contraction as the mechanism in butanol hemostasis has received further indirect confirmation through the study of the effect of butanol upon hemorrhage induced by single traumatic lesions in various organs. Differences were observed according to organs or tissues affected. While bleeding liver wounds are influenced only to a small degree by intravenous administration of butanol, hemorrhage from a kidney wound stopped rapidly. The fundamental difference between the liver portal circulation with minimum muscularity of the vessels, and that of the kidney, where highly developed artery muscular layers are seen, can explain the unequal response to butanol.

As we can see in these cases, the contraction of a pathological artery can insure rapid hemostasis and accords with the fact that the artery is not buried in a clot. This explains why, especially in clinical application, butanol is more active upon arterial hemorrhage and much less active upon capillary bleeding. This mechanism also would explain the same good effect upon hemorrhage from veins with important walls. Failure of butanol seen in three cases of hemorrhage from varices of the inferior esophageal veins can be explained by the almost complete lack of muscular layers in these varicose veins, which would bar a vasculo-muscular contraction.

Parallel to these studies, other possible hemostatic mechanisms have been investigated. We tried to interpret the unusual fact that butanol

stopped hemorrhage while agents such as nikethamide, thiamine, isamine blue, sterols and glycerol induced bleeding—yet all have positive polar groups in their molecules, represented by an amine or amide radical for most of them and by hydroxyls for glycerol and sterols. As we have mentioned before, all agents with such positive radicals induce a shift toward less alkaline values for the second-day-wound-crust pH. Therefore, this could not be considered to be the factor that determines the antagonistic effects on bleeding. The aliphatic or cyclic character of the nonpolar group does not seem to be a factor since glycerol and butanol both have aliphatic chains.

However, correlation with another biological effect could be noted. All the substances with hemorrhagiparous effect, also produce a convulsive effect if administered in sufficiently high doses. The convulsive effect of Coramine and thiamine has been known for a long time and we used these substances any time we wanted to induce experimental convulsions. We also have seen that convulsions can be consistently induced in animals through the use of relatively large doses of glycerol or sterols. Convulsions in several human cases have followed the use of these agents. Doses as high as 20 cc. of a 5% solution of the insaponifiable fraction of placenta or of the 2% solution of cholesterol in oil, administered for therapeutic purposes have induced convulsions in patients, with a previous history of convulsive seizures. A convulsive effect in animals has been noted for all the agents mentioned above which also have hemorrhagiparous activity. We were able to induce convulsions in rats, even by injecting 20 cc. of a solution of 10% glucose subcutaneously once a day for a few days. In humans, we also saw convulsions appearing after glucose was administered intravenously in an amount of 100 gm. in a saline preparation to patients treated concomitantly with desoxycorticosterol, although the last substance has no convulsivant effect by itself.

On the other hand, the antihemorrhagic butanol produces a hypnotic effect if administered in high doses. The dose of butanol needed to prevent hemorrhage induced by ficine in mice, for example, was enough to provoke hypnotic activity comparable to that of barbiturates, chloroform and even ether. Very interesting is the fact that these agents also are able to prevent ficine-induced hemorrhage if administered in doses sufficiently high to produce hypnotic or narcotic effects. The peculiar correlation between convulsive and hemorrhagiparous effect on the one hand, and hypnotic and hemostatic effect on the other, provides some further understanding of butanol hemostasis, but does not furnish the explanation for the mechanism through which this hemostasis takes place. The most plausi-

ble conclusion is that butanol intervenes through several mechanisms, some inducing immediate hemostasis through arterial contraction, while others have a later effect through changes in the blood clotting process, with lack of retraction of the clot and an influence upon fibrinolysis.

Contraction would explain why, with only few exceptions, it is the pathological arterial hemorrhage which responds most favorably to butanol. An immediate influence upon capillary or venous hemorrhage is less manifest and, in a very few cases, absent. The same is true for traumatic hemorrhages where, although there are some good immediate results, in general they are less rapid than for pathological arterial hemorrhage. Because of its immediate effect upon arterial bleeding, butanol became the agent of choice for those pathological hemorrhages which, through their arterial origin, could be fatal in a short time.

One of the big advantages of hemostasis induced by butanol over that obtained through other agents resides in the fact that there is no manifest increase in blood coagulability. Only in concentrations which never can be attained in vivo has butanol been seen to change the coagulability of blood. The inherent danger of thrombosis resulting from high blood coagulability limits the amount of the other agents to be administered. This danger does not exist for butanol and no such limitations are placed upon its use. The fact that normal prothrombin time is uninfluenced by butanol while high prothrombin time is reduced toward normal represents another advantage.

In further studies, we tried to enhance the hemostatic action of butanol without increasing blood coagulability. The addition of calcium salts was of no value but an enhancement was seen with potassium salts.

In a series of experiments, it was noted that, when a solution of butanol is kept for a long time in a stoppered bottle, its activity increases. For long-standing preparations, 50% smaller doses were sufficient to protect mice against the action of ficine. The narcotic and toxic effects of these preparations also increased in the same proportion. This led us to add butyric aldehyde, the product of immediate oxidation of butanol, which increased butanol's coagulating effects only very slightly but enhanced its hemostatic effect considerably. The addition of hydrogen peroxide did much the same.

Blood Mixed with Butanol, used against Hemorrhages

S. Akad, working in our laboratory, showed that the coagulation time of blood is also increased if butanol, mixed previously with blood, is added. In the clinical application of this observation, we used the patient's own blood extemporaneously mixed with butanol. In a syringe containing, for

instance, 10 cc. of the butanol solution, 2-5 cc. of the patient's blood is withdrawn. After mixing them, and without removing the needle from the vein, the contents of the syringe are injected intravenously. A similar mixture can be injected also intramuscularly or subcutaneously. The results have been very good. In some cases in which butanol alone was not able to stop a hemorrhage, the blood-butanol mixture did. With this special technique, we have been able, in recent times, to bring most hemorrhages under control within a few minutes.

The fact that agents with positive polar groups, such as sterols, glycerol, coramine, thiamine and others, have hemorrhagiparous activity led us to try to influence hemorrhage with agents considered biologically antagonistic through their negative polar group.

Many years ago, experimenting with chlorine solutions in saline, we observed a manifest effect upon coagulation time. Intravenous injection of such solutions brought coagulation time to values as low as one minute. The addition of these solutions to butanol greatly increase its effect in vitro upon coagulation time but had less effect upon hemostasis in vivo. Similar but somewhat less manifest effects were obtained by adding hydrochloric acid to butanol solution. On the other hand, organic acids such as oxalic, malonic, citric, lactic maleic or citraconic showed a favorable effect. Without changing coagulability of the blood, these acids were seen to increase the hemostatic effect.

Fatty Acids

The same antagonism to sterols and glycerol led us to use fatty acids from cod liver oil. (197) While results in severe large arterial hemorrhages were not impressive, the effect upon oozing capillary, venous and small arterial hemorrhages was very good in a large proportion of cases. For example:

N. V., 57 years old, with multiple pulmonary metastatic lesions from a hypernephroma, had frequent hemoptysis. At times, the bleeding became more accentuated, the patient expectorating clots as well as uncoagulated fresh blood. Intramuscular administration of two doses of 10 cc. of a 6.5% solution of butanol at half hour intervals had little influence upon the bleeding. Intramuscular administration of 1 cc. of a solution of 10% of the mixture of fatty acids obtained from cod liver oil stopped the bleeding in less than 20 minutes, with the effect persisting for more than two months. A new episode of oozing bleeding was again immediately controlled by injection of the fatty acid preparation.

Other cases with hemoptoic sputum, prolonged bleeding from gastric or duodenal ulcers or from rectal or uterine tumors, all corresponding to oozing rather than to acute massive hemorrhage, have responded to administration of this fatty acid preparation. Almost uniformly, these oozing hemorrhages, which had not responded to butanol, were rapidly controlled. We now use butanol mixed with small amounts of hydrogen peroxide and organic acids mentioned above, to control severe arterial hemorrhages while for the oozing type, fatty acids from cod liver oil are used.

Allergic Conditions

Allergic conditions, which as seen above, are related to the defense mechanism, also have been integrated into the general therapeutic approach. This has largely permitted us to apply to such conditions the same therapeutic measures used in general. The study of the manifestations has, however, shown that except for the anaphylactic shock, the clinical allergy corresponds to the prolonged phase with the predominance either of sterols or lipoacids. The urinary surface tension has appeared as a valuable criterion to indicate the occurring offbalance. In cases with a high urinary surface tension, sodium thiosulfate, aldehydes or epichlorohydrin have given particularly good results, while the cases with low surface tension showed favorable response to heptanol and butanol. Food allergies, asthma, urticaria which had persisted for years and were insufficiently influenced even by corticoids—have responded with the complete disappearance of their manifestations with an adequate dose of one or the other of the medications mentioned above. In most of the cases kept under continuous treatment with minimal doses, favorable results persisted even after the subjects were again under the influence of antigens. The following observations illustrate these results.

Mrs. M. S., 45 years old, with skin and eye allergic manifestations, highly sensitive for the past six years to fish, eggs, alcoholic beverages, some vegetables and fruits, and especially to dogs, showed no favorable response to corticoids. With high urinary surface tension and high eosinophiles, the patient was treated with 50 mg. sodium thiosulfate and 2 mg. epichlorohydrin, four times a day. The symptoms decreased progressively to disappear in a week. After a month's treatment, she was able to take—without any inconvenience—foods and alcohol to which she had been previously sensitive. After another two months, the acquisition of three puppies produced no disagreeable effects. The patient has continued

on the same treatment, but reduced to twice a day, for the past year without recurrence of the manifestations.

Mr. A. L., 58 years old, had frequent attacks of asthma for over four and a half years which left him unable to work for the past year. Because of low urinary surface tension, the patient was put on butanol—5 cc. of a 6.5% solution in water—to be taken every six hours. Although the first doses showed marked objective and subjective changes, the treatment was continued. The patient was free of attacks for four months at which time he stopped medication. Two weeks after stopping medication, he had an attack, the first in four and a half months and this was followed by another the following day. By resuming the medication, he has been free of symptoms for more than a year.

The possibility of preventing allergic manifestations with butanol, which appeared so clear in the traumatic lesions, opens an entirely new view for many conditions where such a pathogenesis intervenes.

The concept of a nervous tissue allergy intervening in the pathogenesis of multiple sclerosis, led us to try similar antiallergic treatments. The low surface tension led us to utilize as agents, lipoids with a positive character. Interesting—although not constant—results were obtained with insaponifiable fraction of cow brain. Good results were obtained with butanol—100 mgr. four times a day—together with an antihistamine preparation.

ARTERIOSCLEROSIS

One of the most important medical problems from all points of view—pathogenic, therapeutic and even social—since it still represents the chief direct cause of death in most civilized societies, is that of arteriosclerosis. The fact that lipids seem to intervene in its pathogenesis, has led us to consider this condition from the point of view of biological offbalances. With the development of our research, we tried to apply to this condition a systematic analysis in accordance with the basic concepts presented above. This attempt has permitted us to arrive at some new views which will be discussed briefly here.

The analysis of the specific manifestations of arteriosclerosis from the point of view of *organization*, that is, as related to the different levels, led us to recognize that it represents a condition principally limited to the level organism, and more especially to its secondary part, the circulatory system. Many of the fundamental characters of the condition could be explained by the relationship of this level to other levels of the organization, as we will see below.

From the point of view of the *dualistic concept*, it was easy to see that arteriosclerosis corresponds to an offbalance type A. All the analyses concerning the systemic level show patterns which indicate this offbalance. High urinary surface tension, low urinary specific gravity, high urinary pH, low urinary sulphydryl index, found in the routine analyses point to such an offbalance. The long persistence of the skin wheal seen in the subjects analyzed, confirms this diagnosis. The fact that some manifestations of arteriosclerosis can be induced experimentally in animals through the administration of cholesterol, has placed into limelight the pathogenic role of cholesterol. While its administration in high amounts to rabbits or cockerels induces atheromas, it does not induce the complex condition itself. Usually the animals return to normal, even with a rapid healing of their atheromas after the suppression of the administration of cholesterol. Although hypercholesterolemia represents thus only one pathogenic factor, it appeared interesting to investigate its intervention in the condition.

The relationship of cholesterolemia to arteriosclerosis is certainly not a simple one. The total amount of cholesterol in blood alone, in its free form, and the macromolecules of certain dimensions resulting from their bond to the other constituents of the blood, although related to arteriosclerosis, do not seem to represent by themselves the pathogenic factor of this condition. This latter seems to be related to a more specific intervention of this substance at the level of the blood and circulatory system. Research in this field has led us to recognize this special intervention.

It is a known fact that the appearance of crystals of cholesterol in the cells of the intima of the arteries and in the cells of the *vasa vasorum* represents an essential factor in the pathogenesis of atheromas. Study of the capacity of blood serum to dissolve or, on the contrary, to precipitate cholesterol has permitted to link it with the appearance of specific arterial lesions in this condition.

In this study, we used the procedure originally devised by Policard when he investigated the relationship of cholesterol and arthritis. Blood is obtained through venous puncture and the serum is separated aseptically. The amount of total cholesterol is determined in the blood serum. A sterile crystal of cholesterol is added to the serum. The treated serum is incubated for 6-12 hours at 37°C. The serum is then separated through filtration from the crystal added, or from those formed during the incubation. The difference between the amount of cholesterol in the serum before and after incubation with the crystal, shows that while some sera increase their content in cholesterol through this treatment, others decrease it.

We could show that the serum of those rabbits which were fed with

two grams of cholesterol daily and which have a tendency to make atheromas, precipitates cholesterol. Oppositely, the resorption of atheromas seen in rabbits after the suppression of the feeding with cholesterol, was seen to occur together with the blood serum capacity to dissolve cholesterol. The relationship between this capacity to precipitate cholesterol and the appearance of atheromas was confirmed by the fact that it could not be seen in rats fed with cholesterol, where atheromas appear very seldom. Further it was seen present in subjects prone to make vascular occlusions. The administration of high amounts of cholesterol to animals with tumors has led to the appearance of vascular occlusions followed by ischemic infarcts in the tumors. The occlusion could be related to the proliferation of the arterial endothelium and the capacity to precipitate cholesterol.

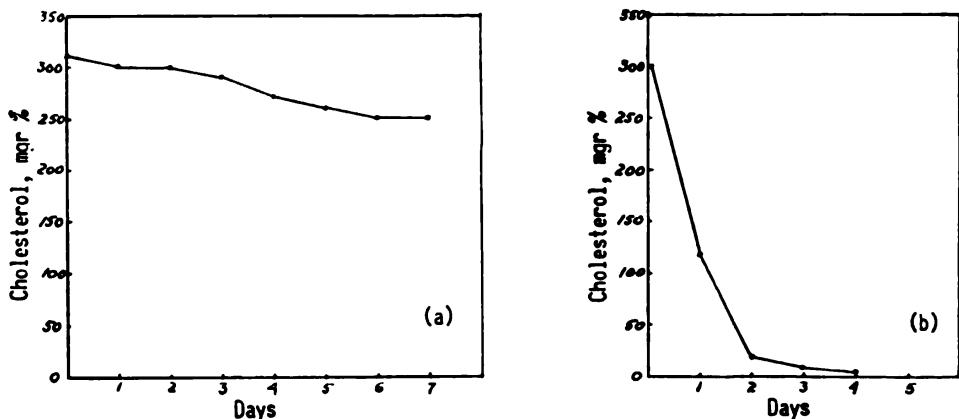


FIG. 152. The incubation of citrated blood at 37°C changes only little the amount of cholesterol in Case (a), while it determines a rapid decrease in Case (b).

In a further study of the cholesterol in blood, we investigated the capacity of the different samples of blood to cause their cholesterol to disappear after incubation at 37°C. We could show that while some samples of citrated blood kept at 37°C under sterile conditions, decrease their cholesterol content rapidly, others do not change it even after days of incubation. Figure 152 shows two such examples. We could also show that in rabbits fed with cholesterol and having atheromas, their blood lacked the capacity to make cholesterol disappear after incubation, differing from what was observed in the majority of the controls.

We then studied in another group of researches, the relationship between red cells and their cholesterol content. We could show that by washing red cells with saline, they lose their cholesterol. By using saline in amounts corresponding to the plasma, this effect could be measured. How-

ever, while in some bloods a manifest loss in cholesterol occurs with the first or second washing, in others the loss occurs very slowly. Sometimes ten such washings are necessary before the cholesterol is low enough to start hemolysis. Figure 153 illustrates two such examples. We could show that the red cells of rabbits fed with cholesterol demonstrated a higher capacity to retain cholesterol throughout saline washings, than did red cells of normal animals.

The analysis of arteriosclerotic cases from the point of view of this relationship between blood and cholesterol has shown that the tendency to precipitate cholesterol, coupled with a low capacity to make it disappear through incubation, is encountered in those cases prone to acute episodes of vascular occlusion.

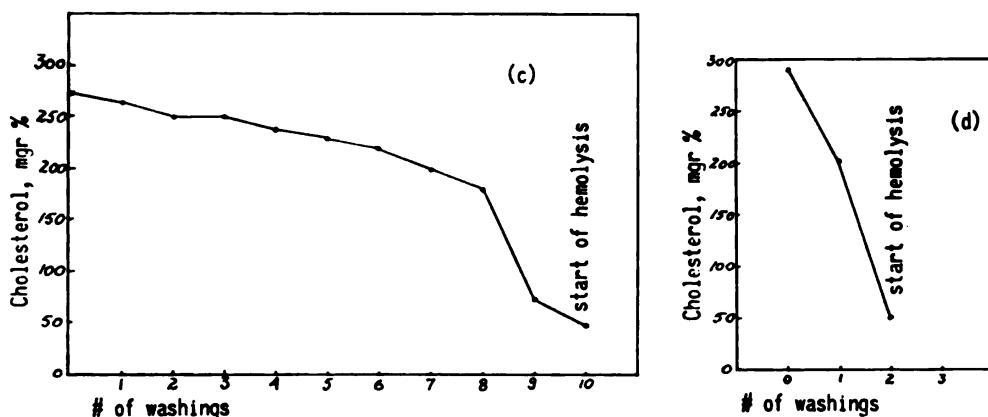


FIG. 153. The washing of human red cells with saline induces a very slow decrease in their content in cholesterol in Case (c). The hemolysis starts in this case at the 10th washing. In Case (d), such an effect appears after the second washing.

The big differences seen in the behavior of blood towards cholesterol has raised the problem of its relationship from an organizational point of view. The application of the concept of a *proper level* in the organization to cholesterol has permitted us to interpret the occurring changes.

Cholesterol represents a cellular constant and the classical studies of Shaffer have shown the importance of its amount in the cells. The quantity of water retained by the cells is largely determined by the ratio between cholesterol and fatty acids of the cells. Under this aspect, we have hypothetically considered hypercholesterolemia as corresponding to a kind of defense response of the blood level toward the changes occurring at the level below it, respectively toward an opposite lipidic offbalance occurring at cells, tissues or even organs. We investigated hypercholesterolemia under this specific

aspect. We have seen above that in the lipidic system, sterols correspond to an anti-fatty acid agent. This led to the supposition that hypercholesterolemia could represent a response at the blood level to changes taking place at a lower level in the relationship between fatty acids and cholesterol. A first fundamental finding in this direction was the fact that the amount of cholesterol in the blood represents—as shown for potassium, copper, hydrogen ion concentration, etc.—values which, while maintained constant by a regulatory system, vary very widely under different circumstances. These wide variations would correspond rather to a secondary response. The amount of blood cholesterol would change as a secondary constant. A balanced system of manufacturing and excreting insures the maintenance of this secondary blood cholesterol amount. In the case of cholesterol, the manufacturing factor seems to be represented by the R E S cells, while the excreting factor by the liver, primarily by the Kuppfer cells and secondarily by the hepatic cells, the cholesterol being ultimately excreted in the bile. We do not know the factor which directly changes the manufacturing or excretion of cholesterol in blood and which consequently maintains its balance. By analogy we consider the changes in blood cholesterol value to result from those occurring at the lower levels as seen for other substances such as potassium, or copper, for which blood does not represent the proper level. According to this view, with cholesterol having the cellular level as its proper level and the blood as superior level, the blood changes would reflect secondary responses to those occurring at lower levels. We saw such changes at the cellular level in old age.

We could find thus that organs of old animals, perfused with saline to wash them from their blood, are richer in fatty acids and poorer in sterols than those of young individuals. The cellular lipidic abnormality, corresponding to aged persons, would correspond to a quantitative predominance of fatty acids. Their analysis showed that these fatty acids correspond qualitatively to those normally encountered in the organisms and especially to the polyunsaturated members. It should be noted at this time that this predominance differs from that found in abnormal conditions which corresponds to offbalance D. In this latter case, the fatty acids are abnormal.

With the concept that cholesterol belongs to the cellular level, we studied as we did for potassium (*See Chapter 5 Note 2*), the concomitant changes in the amount of free cholesterol in plasma and in red cells. This was carried out to obtain information concerning the relationship between cholesterol and abnormal conditions. While a high or low amount in both plasmatic and cellular cholesterol would indicate an excess or lack of this substance, the discordance between these two data would correspond to

an offbalance. A high cellular, with a relatively normal or low plasmatic cholesterol, corresponds thus to an abnormal cholesterol offbalance predominant at the cellular level, while a relatively low cellular and high plasmatic cholesterol, to the opposite offbalance. By now considering cholesterol in its anti-fatty acid role, these changes permit further interpretation.

As we have seen above, cholesterol corresponds rather to the anti-fatty acid constituents controlling the normal polyunsaturated fatty acids and thus differs from the corticoids which represent agents opposing the abnormal fatty acids. The ultimate cause of hypercholesterolemia would logically be sought in a quantitatively abnormally high amount of qualitatively normal fatty acids present at the lower levels.

Although hypercholesterolemia represents by itself only a response to a fatty acid offbalance taking place at lower levels, the high amount of sterol present in blood can cause, by itself, a series of disagreeable manifestations. Hypercholesterolemia can thus induce noxious manifestations although, teleologically speaking, it is directed to correct an offbalance at a lower level. The effect of this chronic richness in blood cholesterol is manifested in the circulatory system and atherosclerosis appears as an immediate result. The precipitating tendency for any new increase in blood cholesterol explains the appearance of crystals in the cells of the intima and secondarily the appearance of atheromas.

Therapeutic Attempts

This pathogenic concept of arteriosclerosis presented above, has guided our therapeutic approach. As early as 1942, we used acid lipidic fractions of organs in a tentative therapy to control hypertension and indirectly arteriosclerosis, with interesting immediate results. We learned however, that not only were the effects temporary but also that after some time, the administration of unsaturated fatty acids induced a progressive increase in the amount of cholesterol present in blood.

The concept of hypercholesterolemia as a secondary blood response to a cellular fatty acid predominance explained this occurrence and led us also to the further development of this approach. With cholesterol as an anti-fatty acid agent which appears in high amounts in blood, as a response to an excess of fatty acids at the cellular level, we tried to influence this secondary response by acting on the existing primary cellular offbalance. A decrease of this response could be obtained by supplying substances other than cholesterol, acting as anti-fatty acid agents. If the cellular fatty acid offbalance can be controlled, the organism as an entity would no longer be obliged to respond to the occurring offbalance and manufacture blood

cholesterol in excess. Without this need, hypercholesterolemia will no longer appear. Under these conditions, the administration of anti-fatty acids has appeared as the logical way to prevent and even to combat an existing hypercholesterolemia and its consequences.

Choice of the Anti-Fatty Acid Agent

The next problem was the choice of an adequate anti-fatty acid agent. It appeared advisable to use more than one such agent. One would intervene at the lower cellular level where the primary factor—predominance of fatty acids—exists; another would act at the level of the tissue and blood itself where their presence would act as an anti-fatty acid and prevent more directly the further appearance of cholesterol.

These considerations led us to utilize as active agents, heptanol, glycerol and polyunsaturated alcohols for the tissue level. Butanol was added, being more active at the organ and systemic levels. We found that mixtures of these alcohols were advisable also in view of the plurality of fatty acids intervening in this abnormal condition. Mixtures of polyunsaturated fatty alcohols were obtained through treatment with lithium aluminum hydride, of the fatty acids present in cod liver oil, fish oil, safflower oil, sesame oil, or even in the lipoacid fraction of organs.

Clinical Results

The best clinical results were obtained with a preparation having in its constitution, glycerol, polyunsaturated fatty alcohols, heptanol and butanol. In a series of subjects with persistent high amounts of cholesterol in blood, the administration of this preparation brought the blood cholesterol to low values. The following observations are characteristic.

Mr. M. R., 60 years old, had high cholesterol in the blood for ten years, with values above 350 mgr. %, in spite of severe diet poor in fats and cholesterol. Administration of unsaturated fatty acids brought the blood cholesterol, for a short time, to values between 260 mgr. and 300, returning to values above 350 after cessation of medication. With the mixture—glycerol, polyunsaturated fatty alcohols, butanol and heptanol—the blood cholesterol went down to 150 mgr. % in less than two weeks without any side effects or restricted diet, and with a manifestly good general condition. It remained at this level for the 5 months of observation with only minimal and irregularly taken medication.

L. N., 70 years old, with blood cholesterol varying in recent years between 400 mgr. and 280 mgr. in spite of low fat and low cholesterol diet and different treatments. The mixture of glycerol, fatty alcohols, heptanol

and butanol, brought it down in three weeks to 160 mgr. It remained around this value for more than the six months of observation with no special diet and with only a very small amount of medication.

In a group of 20 subjects with cholesterol above 350 mgr. %, a descent of the cholesterol to values below 250 mgr. % was obtained in all the cases, with a treatment ranging from 10 days to 3 weeks. No inconveniences were observed.

Coronary Occlusion

The coronary occlusion with the consequent myocardial infarction represents the most important complications of arteriosclerosis. Death can occur instantaneously. In these cases it can be considered to result from a lesion of such localization or dimension as to be entirely incompatible with the function of the heart. When death occurs, not instantaneously but at any time after the occlusion has taken place, other factors have to be considered as intervening and leading to the fatal issue. Shock appears to be the most important one. Superacute shock, with death in a few minutes, an acute shock with death in 1-2 hours, or state of shock with death within hours or days, represent the other important intervening pathogenic factors added to the infarction itself. As seen above, these types of shock correspond to offbalances of the type D, this time with a predominance of abnormal lipoacids. A therapy with anti-fatty acid agents represents as shown above, the intervention which could prevent or reduce shock. While the administration of sterols appears contra-indicated, since it would increase the vascular occlusion, that of the non-sterolic anti-fatty acids is especially advisable.

The effect of these anti-fatty acid agents upon pain and the other symptoms which are of alkaline pattern, as well as upon the evolution of the condition, has fulfilled our expectations. We attributed an important role to glycerol in these cases. Its action similar to an anti-coagulant but limited to the level of the existing lesions, was able to prevent further local thrombosis without, however, the danger of a general reduction of blood coagulability. Administered in adequate amounts, guided by the pattern analyses, the mixture of the anti-fatty acid agents mentioned above has been followed by manifest clinical improvements.

L. K., 58 years old, with a history of several myocardial infarctions, was seen in a very severe state of shock. The electrocardiogram showed that besides the old lesion in the posterior wall, a new infarction of the anterior wall was present. Butanol was administered in doses of 5 cc. of the 6.5% solution together with 0.1 cc. glycerol every hour until the complete

cessation of symptoms and was continued 4 times a day afterward. The favorable effects continued in an unexpected form, the patient being without pain in less than 3 hours and without symptoms the third day. The cholesterol was found to be 135 mgr. % the fifth day.

This view of arteriosclerosis opens a new way for further research concerning many pathogenic problems, and a logical therapeutic intervention in this condition.

CHAPTER 15

THERAPEUTIC APPROACH TO CANCER

ONE OF THE ULTIMATE AIMS of our research has been to try to utilize in cancer the knowledge obtained from investigations of the general problems of pathology and therapy. Encouraged by the results of biologically guided therapy in many other conditions, we have applied it to the treatment of malignancy.

As we have mentioned before, differences between animal cancers, both experimental and spontaneous, and human cancers represent one reason why an agent, however good its results in animals, may not apply to human malignancy. Another factor, conduct of treatment, is no less important. The main characteristic of our therapeutic approach resides in the fact that treatment is continuously guided by data representing the actual condition of the subject. At least for the moment, it appears impossible to recognize, through suitable tests, the patterns present in animals so as to apply them to guided therapy. Therefore, we have been obliged to do our therapeutic research in humans, reserving animal studies for limited problems. This situation had led us to emphasize, always, the experimental nature of our therapeutic efforts in humans. Although we started with desperate terminal cases, frank immediate subjective and objective benefits, even though temporary, were obtained frequently enough to encourage us to go on. Together with the above mentioned considerations, they seemed to justify the continuation of therapeutic research in human patients. We will try to review as objectively as possible the results obtained with therapeutic methods and agents evolved over the years.

In 1927, a 33-year old woman with typical preterminal cancer of the stomach came under our care. In the highly emaciated patient, a hard,

irregular mass filling the entire epigaster was palpable. Radiological examination indicated a prepyloric gastric tumor. Laparotomy showed an inoperable tumor of the stomach, with the omentum and lymphatic glands greatly involved and evidence of multiple peritoneal and liver metastases. In view of the patient's general condition and the fact that the pylorus was only partially obstructed, no surgical procedure was performed other than biopsy of one of the metastases in the omentum. The biopsy showed an adenocarcinoma Grade III of gastric origin. Treatment was not prescribed.

I saw the patient two years later in apparently good health. Clinical and radiological examination at that time showed no tumor. The patient attested to receiving no treatment. At the time of the operation she had been two months' pregnant. We had attributed her amenorrhea at that time to the advanced cachectic condition. She had given birth at term to a normal girl. Hers was one of those cases usually catalogued as "spontaneous remission."

Since then, I have analyzed many of the published observations of cases of so-called "spontaneous remission" of cancer always to find a turning point that coincided with the intervention of some event usually considered to have no possible significance for malignancy. The fact that such events have not induced similar changes in other cancer patients has made them seem unimportant to many investigators.

While not regarding them as the only cause of favorable changes, we have not eliminated the possibility that such events may have a contributory role. We must recognize that, if such events in themselves appear to be powerless to change the course of cancer, they may intervene in conjunction with, and potentiate, another factor also powerless in itself to induce a change.

It was with this concept in mind that we reviewed the case of the woman with stomach cancer. We considered the possible effects of two factors which apparently intervened concomitantly: pregnancy and surgery. We then began a series of experiments.

Ehrlich mammary carcinoma was grafted in two groups of female mice, one pregnant and the other not. In each group, half of the mice were kept as controls while the other half was submitted to a sham surgical procedure consisting of a laparotomy in which multiple ligatures were performed. Growth of the tumors and survival times were noted. Compared with non-operated, nonpregnant mice serving as controls, both the pregnant mice and the surgically treated mice showed a slowing down in the evolution of cancer lesions. It was in the group of mice, both pregnant and surgically treated, that a temporary arrest in tumor evolution was seen. In some ani-

mals even temporary regression was noted; in 1/20 the tumor regressed entirely.

Placenta Extracts

These experiments led us to try an extract obtained from placenta autolysates which, in our opinion at the time, would reproduce, up to a point, some of the conditions present in these experiments. Human placentas were autolyzed by being maintained from several hours to a few days at 37° C, and an alcoholic extract was obtained. The alcohol was eliminated through distillation in vacuum. The residue proved non-toxic in animals and was injected intramuscularly in some terminal cancer cases.

Impressive results were observed in the first cases. Pain was markedly diminished and, in some instances, disappeared entirely. Objective changes in the tumors could be noted. While only temporary results were seen for most of these cases, for some the results appeared to last a long time. Their number would exclude pure coincidence.

Mr. H. B., 56 years old, came under our care with a cancer involving more than half of the right part of the tongue. Multiple large submaxillary and cervical gland metastases were present, two of them being approximately 8 cm. in diameter. The mouth lesion was very painful and bled occasionally; there was moderate pain in the ganglionar metastases. A biopsy performed at a much earlier stage had shown a squamous carcinoma. Considered inoperable, the subject had not received any treatment except for pain palliation.

We administered daily intramuscular injections of 5 cc. of the placenta extract. Except for a limited local reaction at the site of injection, no disagreeable effects were seen. On the contrary, after each injection, the pain in the tongue was reduced for a few hours. It disappeared entirely after one week of treatment. During the second week of treatment, the tumor of the tongue, as well as the metastases, began to decrease in size. The local reaction at the site of the injections increased, however, to such an extent that we were obliged to stop treatment after 5 weeks. In spite of this, the lesions continued to decrease so that the tongue tumor was no longer palpable after two months. At that time, the gland metastases were reduced to approximately one and a half centimeters in diameter. The patient's general condition was much improved and he gained weight. In another month, except for a scar on the tongue, no other pathology could be found. We followed this case without treatment for another year and a half during which time there was no recurrence. After that, the patient left town and we were unable to reestablish contact with him.

Mrs. B. A., 44 years old, came under our care with a massive tumor filling the entire vagina. The condition had been diagnosed 8 months previously as carcinoma of the cervix which had invaded the parametria and was propagating toward the vagina. A biopsy made at that time indicated squamous carcinoma Grade III. As the patient was considered inoperable and refused any other treatment, only sedation was prescribed. When we examined her, the tumor was protruding from the vagina as a hard mass. Rectal examination revealed invasion of the entire recto-vaginal wall. The patient received a daily injection of the placenta extract preparation for 45 days, after which she interrupted the treatment. The pain had been entirely controlled in less than a week, but no other changes had been observed.

She returned three months later, having received no treatment in the interval. Examination revealed complete disappearance of the vaginal tumor, with the cervix entirely replaced by soft scar tissue. We followed this case for two years, during which no further treatment was given and the patient showed no recurrence.

Mr. A. N., 40 years old had an extensive cancer of the cheek, with massive ulceration resulting in a large communication between oral cavity and exterior. Occasionally small hemorrhagic episodes were experienced. The patient had had several courses of radium therapy. When he came under our care, he had multiple lesions, and several biopsies performed at that time revealed active carcinoma in all lesions tested. The patient received intramuscular injections of 5 cc. of the placenta extract preparation daily for 17 days. A massive hemorrhage occurred at this point, treatment was stopped, and he went home without further medication. When the patient returned three months later, scar tissue covered all areas where the tumor had been seen previously. Clinically no trace of tumor could be found. In a few months, the patient's condition was good enough to allow his surgeon to attempt a skin graft to cover the big opening in the cheek. This was not successful. The graft from the skin of the neck unfortunately underwent necrosis.

In several other cases, similar subjective and objective changes were observed with use of the same alcoholic extract of human placenta autolysates. During this time, we attempted to substitute cow placenta, utilizing both fetal and maternal parts, which are easily separable in the cow. In a relatively small number of cases in which these products were used, we could see no differences in the influence of placenta extract according to origin. Poorer results were obtained with extracts using fresh placenta in-

stead of the autolysate. However, certain interesting clinical results indicated that fresh placenta still has a capacity to influence what can be considered to be the normal course of cancer. The following case is an example.

Mrs. C., 54 years old, had a tumor of the rectum which was considered inoperable. Only a colostomy was performed. Pain was slight and no other treatment was instituted. Several months after the colostomy, the patient came under our care. At that time, the tumor filled the entire rectal ampulla. Treatment with the fresh cow placenta alcoholic extract was started, using intramuscular injections of 5 cc. daily. After less than a month of treatment, the tumor diminished in size, leaving a small passage for the examining finger. After another month and a half, the tumor had entirely disappeared and the rectal ampulla was wide open. Proctoscopic examination showed only normal mucosa. This case was followed for two and a half years with no evidence of recurrence. Thereafter, because of the war, we lost touch with her.

In spite of such results in a few cases however, extracts of fresh placenta were judged to be much less effective in general than extracts of placenta autolysates.

At the beginning of our research, still unaware of the dualism intervening in cancer pathogenesis, we observed a series of cases in which the placenta preparations in general produced undesirable results, such as increase in pain intensity. Furthermore, when used in higher doses over a longer period of time, it induced new pains which clearly increased with each subsequent injection. For a while, this fact made us limit the use of the product to only those patients showing favorable responses in pain, until we could find an explanation for these paradoxical results.

With the progress of our research and recognition of dualism in the pathogenesis of pain, we limited the use of the placenta extracts to patients with an acid pattern of pain. This improved the subjective and objective results, and reduced the cases in which undesirable effects occurred. In over 100 terminal patients treated with this preparation between 1935 and 1938 in different hospitals in Paris, objective improvement was observed in 20%. In a few, tumors disappeared. Acid pattern pain was relieved. In many of these cases, however, after a period in which the tumor decreased in size, or even clinically disappeared, it started to grow again and could not be influenced by further treatment. Furthermore, when the dose was increased, other pathological manifestations appeared. The following cases are examples.

Mrs. B. B., 42 years old, came under our care with severe pain result-

ing from a widely ulcerated cancer of the cervix involving the parametria and the vagina. 5 cc. of the cow placenta extract was administered daily and the patient remained without pain for almost three weeks, after which time the pain returned. An increase in dosage—to two injections of 5 cc. daily and then to two injections of 10 cc. daily—resulted not only in an increase in pain but also caused the appearance of an abundant watery vaginal discharge. In a few days this reached several liters a day. Despite the fact that we stopped treatment, the exudate continued to increase. At one point, it amounted to 8 liters in 24 hours. The very concentrated urine was reduced to less than 200 cc. in 24 hours. The patient died in ten days in spite of all attempts to stop the excessive secretion.

Mrs. G. L., 48 years old, had a radical mastectomy for a left breast adenocarcinoma. A rapidly growing local recurrence was seen 6 months later. The patient came under our care with an ulcerated tumor occupying the entire left half of the chest. Administration of 5 cc. of cow placenta extract for two weeks not only increased the burning sensation present but caused the appearance of an abnormally abundant watery exudate. As is often true in such cases, an infection with *B. pyocyaneus* was seen. A clear fluid was observed surging in drops from the ulcerated lesion. By weighing the dressing, the amount excreted was measured and found to exceed 10 kilos a day. Despite use of saline infusions, calcium preparations, vitamin C in high doses, atropine, and other measures, the patient expired in less than a week. The appearance of such complications, the frequent changes toward alkaline patterns of pain, and the increase of intensity of alkaline pattern pain, made us reduce and ultimately stop use of these placenta extracts in spite of some good results obtained.

Cod Liver Oil Fatty Acids and Sterols

At the same time, progress in our research had led us to recognize, in addition to dualism in the pathogenesis of many manifestations, the special role played by lipids. In 1938, we began to use two groups of antagonistic lipids, fatty acids and sterols. We started with a mixture of fatty acids prepared from cod liver oil for one group and with cholesterol for the other. Later we utilized only the polyunsaturated members from the group of cod liver oil fatty acids.

Fatty acids were administered intramuscularly in oily solutions or in gelatinous capsules by mouth. As with administration of placenta extract, the immediate effect was favorable on pain of an acid pattern, and adverse on alkaline pain. In both cases, the effect occurred in a few minutes. Therapeutic attempts with fatty acids were consequently limited to patients with

an acid pattern pain and with this restriction, pain was efficiently controlled. We used the effect upon pain as a criterion, and we discontinued treatment in any case in which fatty acids induced or increased pain.

Subsequently, along with the effect upon pain, we used urinary pH and specific gravity as criteria for treatment with fatty acids. A persistent high urinary pH and a low specific gravity were indications for the use of these substances. In addition to the control of severe pain, interesting objective changes occurred. Unfortunately, most of them were only temporary. The following two examples taken from a group of 15 similar cases are illustrative.

L. B., 66 years old, had cancer of the right lung for which he had received only symptomatic treatment. For more than a month the patient had complained of pain in the right chest, with increasing breathing difficulty. Chest X-ray examination revealed a tumor of the right lung extending from the mediastinum into the medium lobe. A diagnosis of bronchogenic cancer was made. Subsequent X-ray examinations showed rapid growth with several tumors in the upper lobe and in the left lung. The general condition was rapidly and progressively deteriorating, the dyspnea and pain increasing. Two months after first symptoms, the patient was bedridden.

When the patient came under our care a few weeks later, he was dyspneic, slightly cyanotic, had persistent cough, was extremely fatigued and in almost continuous pain. By this time, we had started to use urinary specific gravity and pH as criteria for the recognition of the offbalance present. Because of low specific gravity and high urinary pH, the patient was given oral treatment with cod liver oil fatty acids. Gelatinous capsules containing 0.25 gm. of the fatty acid mixture were used in a starting dose of 0.5 gm. a day, and were increased progressively to 1.5 gm. a day. The patient made an impressive gain in a few days of treatment. The pain disappeared entirely, as did the dyspnea. The cough also almost disappeared in a few days, and in two weeks the patient was able to get out of bed. The improvement continued, and in less than two months, the patient was even able to go horse-back riding. Radiologically, the tumors also showed progressive regression. We continued the treatment with a relatively high dosage—2 grams of cod liver oil fatty acids daily—for a total of two months, with evidence of continued improvement. Then, suddenly, symptoms of pulmonary congestion became apparent and the general condition rapidly became worse. Urine analyses now showed a high specific gravity and a low pH. In spite of discontinuing the medication, the patient was back in bed with increasing dyspnea. He died two weeks later with symptoms of pulmonary edema.

Mrs. D. A., 68 years old, had a cancer of the left breast for which she had undergone a radical mastectomy four years previously. Pathological examination of the lesion had shown an adenocarcinoma Grade IV, with ganglionar involvement. When the patient came under our care she was bedridden with a diagnosis of multiple bone metastases. Radiological examination showed multiple osteolytic lesions in the pelvis, femur, lower spine, ribs and skull. We instituted treatment with cod liver oily fatty acids in gelatine capsules. The dose was progressively increased, by 0.25 gm. increments, until it reached 3 grams a day. Ortho-phosphoric acid was added orally in doses of $\frac{1}{4}$ cc. of a 50% solution given in water in order to control the pain which appeared after administration of the capsules and was of an alkaline pattern. Improvement began in a few days and continued so satisfactorily that in less than six weeks the patient was up and about. Five months later, with bone lesions healed, the patient went home. I saw her in 1941, almost two and a half years later, during which time no treatment had been given. When examined at that time, she appeared in excellent condition. Subsequently, because of the war, I lost contact with her.

The increase of pain, and especially the frequent appearance of pain of an alkaline pattern after extended treatment, considerably limited the use of these cod liver oil fatty acid preparations. Furthermore, an inconsistency in objective changes was seen even when administration was guided by the acid or alkaline character of the pain. In most patients, favorable objective changes were only temporary.

During this research, we observed a very favorable response in some cases of hemorrhage, especially of the long-term oozing type, treated with these preparations. Bleeding usually stopped after one injection of 1 cc. of a 10% solution of unsaturated members of cod liver oil fatty acids. We still use this preparation for this purpose, as mentioned previously.

Among the group of lipids opposed to fatty acids, we first used cholesterol with the intention of trying to influence pain having an alkaline pattern. The effect was much less impressive than that obtained with fatty acids in pain of acid pattern. In some cases, objective changes also were observed although they were less frequent and less profound than those seen with the fatty acid preparations. Cholesterol alone never produced total clinical disappearance of tumors.

Acid Lipidic Fractions and Unsaponifiable

The development of the concept of dualistic pathogenic intervention of two groups of lipids led us to a treatment employing these two types of lipid constituents of the body chosen according to the character of the

manifestations. We obtained from different organic sources the insaponifiable fraction as well as the acid lipid fraction. Human placenta was widely employed. The two preparations, acid lipid and insaponifiable fraction, were used until 1943 on about 200 patients (in France, and Mexico). Some good subjective and objective results were observed. The subjective changes were most impressive. Frequently, an injection of only 1 cc. of the human placenta acid lipid preparation (5% in oil) controlled pain in a few minutes, with relief lasting for hours or sometimes even days. With the same preparation, alkaline pain increased after only a few minutes and sometimes became unbearable. In cases treated with placenta acid preparation, using acid pain pattern as a criterion, we observed some significant objective changes.

Mrs. B. B., 54 years old, with a papillary adenocarcinoma of the ovary and multiple peritoneal metastases found during exploratory laparotomy, had rapidly reproducing ascites. The patient required repeated paracenteses at short intervals. In the month just prior to coming under our care, it had been necessary to tap her once a week or even every five days. Treatment with acid lipids of placenta was instituted, with daily injections, first of 1 cc. and then of 2 cc. of the 5% oil solution. In less than two weeks, the pain was controlled and much less fluid accumulated. The patient had two more paracenteses at two and three week intervals, after which fluid no longer was a problem. The multiple tumor masses, which were very easily felt through the skin after each paracentesis, were seen to decrease rapidly and disappeared in about two and a half months of treatment. After four months, treatment was discontinued and no recurrence was seen during the three years we followed this patient.

We saw Mrs. L. S. N., 73 years old, in 1942 in a subcomatous state, with deep jaundice and with a history of primary tumor of the stomach and multiple big metastatic lesions of the liver. Her condition, which had started a few months earlier, was getting rapidly worse. When we examined her, the liver was occupying the abdomen until the pubis, and practically each of the individual metastatic tumors present at the surface of the liver was easily palpable through the thin abdominal wall.

Guided by the urine analyses—with a high pH and a low oxireduction index—we started with a treatment with 10% solution in oil of a human placenta lipoacid preparation. The doses were increased according to the analyses until they reached 3 injections daily of 2 cc. each. The patient improved, and in less than a week she was conscious again. Her condition continued to improve for more than a month when a rapid change for the worse took place. As the analyses at this time showed the opposite offbal-

ance present, the treatment was changed. In addition to infusions with glucose and saline, glycerol in a dose of 8 drops daily with 20 drops of coramine was given. Again the response was impressively good. The general condition improved rapidly and in less than a month, the patient was out of bed and started to take care of her home. By this time, the jaundice had almost completely disappeared, although the liver remained almost of the same dimension. The patient continued to take glycerol for more than 6 months, leading an absolutely normal life. It took almost one year for the liver to come back to normal dimensions. The patient remained in perfect health for the next 9 years. Several coronary occlusions led to her death at the age of 83, from a myocardial infarction. She showed no objective or subjective signs of recurrence of either her stomach or liver condition.

Unfortunately, uncontrollable changes toward rapid tumor growth ultimately occurred in most cases treated with placenta acid lipids despite favorable objective changes at the beginning of treatment.

Clinical use of the insaponifiable fraction preparations supported observations made in animals, indicating the importance of other factors for obtaining favorable changes in tumors. With the insaponifiable fractions of placenta, marked clinical effects could be obtained only when the condition of the patient permitted the treatment to be continued for a long time. Temporary regression or even clinical disappearance was effected in several cases, only to have the tumors start growing again, this time beyond control by the medication. Even at this point, it was obvious that favorable influence with these two antagonistic groups of lipids was dependent on using the lipid which corresponded to the pattern present. It became increasingly evident that changes in the pattern occurred during treatment. Administration of acid lipidic and insaponifiable fractions induced unfavorable responses in patients with tumors in which a predominance of the same lipids was indicated by analyses. Pain increased and unfavorable changes occurred in the evolution of the disease. For example:

Mrs. A. D., a 42-year-old woman, operated on for an adenocarcinoma of the breast 18 months prior to coming under our care, presented a few skin lesions near the operation scar. Radiological study revealed a few small osteolytic lesions in two ribs, and one in the skull. The analytical data showed low urinary pH, high specific gravity and high chloride index, indicating an offbalance which we attributed to predominance of fatty acids. Treatment with 2 injections daily of 1 cc. of a 5% oily solution of the insaponifiable fraction of placenta was started. This was later increased to 2 cc. twice a day. The patient left the city for a summer vacation and took the medication with her. Disregarding our instructions that treatment must

be guided by further analysis, she continued it without interruption for four weeks, despite a rapid change in her condition and a constant increase in the pain after each injection. When we saw her again a month later, all the analyses had changed markedly, indicating the appearance of an opposite offbalance. Clinically, the condition also had changed. The skin was extensively involved in the vicinity of the operative scar and multiple, rapidly growing metastases were seen all over. Radiological study showed extensive new lesions in many bones. The condition had progressed in one month in a manner never seen before in any patient and we had to relate it to continued use of medication after a change in the offbalance. This change was similar to that seen in animals with massive administration of the insaponifiable lipidic preparation. Switching to placenta acid lipid preparations quickly changed the evolution of the condition in this patient. Pain was controlled and for three months the condition seemed arrested. In spite of treatment, however, it started to evolve rapidly again after that and the patient died five months following her return from vacation.

Similar deleterious effects were seen with acid lipid preparations obtained from organs when their administration apparently was at variance with the pattern present.

W. S., 56 years old, had a carcinoma of the cheek mucous membrane which led to wide perforation. Biopsy of the edges of the ulceration showed squamous cancerous tissue. Based upon the urine analysis, which indicated low specific gravity and high pH, treatment consisted of two daily injections of 1 cc. of the acid lipid fraction of placenta in a 5% solution in oil. As the patient was treated on an ambulatory basis, he was advised to see us in a few days. He continued the treatment without any control for two weeks. When next seen, a marked gelatinous edema of the tissues surrounding the ulceration was found and the patient complained of severe pain. Biopsy at this time revealed, in addition to interstitial edema, a high vacuolization of the cancerous cells which had not been seen in the biopsy done the day prior to beginning treatment. The pain became unbearable a few minutes after each injection. These local changes were accompanied by a marked deterioration of the general condition, the patient complaining of a sensation of weakness. The unfavorable changes which occurred in only two weeks were very impressive.

Favorable results were obtained even in terminal cases with these fraction preparations. In some cases, arrest or disappearance of tumors was noted. In most cases, however, these effects were only temporary. After being arrested for months or even years by these lipid preparations, some tumors began to grow and to become painful and could not be as readily

controlled again by the same preparation. In a small number of patients, about 3% of the group of 200 treated with these preparations, the favorable results could be maintained over a number of years.

Even with the relatively strict guidance of therapy by the analyses available at the time, results were not always favorable. We attributed this both to the agents used for therapy and the criteria employed for recognition of offbalances. For a long time, research was devoted to developing means to permit better recognition of offbalances and to ascertain the value of the various analyses used as criteria for the conduct of treatment. Each new urine or blood test was investigated as a criterion for the group of lipids to be administered. This led to better results in controlling pain, improving the general condition and even in objective changes in tumors.

However, the temporary character of the effects obtained with lipids derived from normal organs appeared more and more evident in long-term appraisal of results. We changed from human placenta to other sources for both acid and insaponifiable fractions. We prepared and used lipids from different organs of cow, pig, fish, and chicken. We also used mollusks, chicken embryos, molds and even microbes, as well as milk and eggs as source for these lipids. In one group of investigations, we even tried to use lipids of the organ from which the tumor derived. Most of these preparations satisfactorily controlled pain, and in some cases, good results were seen in the growth of tumors.

The following observation concerns a case treated with the lipoacids of human blood.

A. M., a 56-year-old man, was referred to us by his physician with a diagnosis of cancer of the rectum. Difficulty in defecation, mucosanguinolent discharges, and pain in the rectal region had been increasing in the three months prior to the diagnosis. Examination had revealed a tumor of a cauliflower type, starting at about 4 cm. from the anal orifice and almost entirely filling the rectal ampulla. A biopsy had shown it to be an adenocarcinoma, Grade III. The patient had refused surgical intervention because, years before, a minor operation on his right hand had led to local infection followed by amputation of the hand.

At the time he was referred to us, his main complaints were pain in the rectum, radiating to the left leg, and tenesmus with frequent mucosanguinolent discharges. We employed daily injections of 1 cc. of a 5% oily solution of the acid lipidic fraction obtained from human blood. The treatment was continued for six weeks, the injection being given daily during the first two weeks and twice a week thereafter. After one week, the pain and tenesmus disappeared and there was a decrease in the mucosanguinol-

ent discharge. There was also an obvious decrease in the size of the tumor. In less than a month the tumor regressed to one-fourth its original size; in six weeks, digital and proctoscopic examination showed no clinical tumor. A whitish scar could be observed in the posterior wall of the rectum. Thereafter, the condition of the patient was followed indirectly through reports from his physician. There was no tumor recurrence in spite of the fact that he received no further treatment. He died six years later from an acute paratyphoid infection.

Our clinical experience provided ample evidence that the preparations rich in polyunsaturated fatty acids would influence pain as well as the growth and evolution of human cancers. Using the same amounts of polyethenic fatty acids prepared from various sources, no differences in effects could be noted. The effect upon tumors in all instances was relatively limited.

Our next effort was to try fatty acids unlike those found in the organism. They included norbixine—the monomethyl ester of the bicarboxylic acid, bixine—which we used in a group of patients between 1938 and 1940. With urinary specific gravity and pH as criteria, daily doses of from 1 mgr. to 100 mgr. were administered to 30 preterminal and terminal patients with patterns corresponding to predominance of sterols. Even with small doses, the changes toward a predominance of fatty acids were impressive. Superficial, massive tumors were often seen to melt away within a few days, usually leaving ulceration in their place. This rapid change of a massive tumor into an ulcerated one, however, usually was followed by a manifest deterioration of the general condition. With this preparation, once the offbalance was changed from the original to the opposite type, attempts to control the new offbalance were usually unsuccessful. This led us to discontinue its use at this time in spite of the rapid and intensive changes it induced in tumors.

Groups of Agents

Parallel to these researches on fatty acids, the use of agents with positive polar groups also underwent changes. As previously mentioned, it appeared increasingly clear that no single agent could be effective in itself if an offbalance corresponding to predominance of fatty acids was present. Quite early in this research it could be observed that the simultaneous use of two agents from the same group appeared to be a better procedure than using either agent alone. In a limited number of patients treated with single agents, without impressive clinical changes, better effects were obtained when combinations were tried. Although it was difficult to ascertain in

individual cases that favorable effects were due exclusively to change in medication, the following cases are interesting.

A. Ch., a 53-year-old woman, had a radical mastectomy for an adenocarcinoma of the right breast with axillary and supra clavicular ganglionar involvement. Almost two years later—during the three months immediately prior to the time we first saw her—she showed rapidly developing multiple metastases for which only symptomatic treatment was applied. Along with multiple bone metastases, and a recent pathological fracture of the inferior third of the right femur, there were liver metastases and a right pleural effusion for which she had been tapped three times. When she came under our care, she complained especially of pain in the lower back caused by lumbar and sacrum metastases. Her condition was considered terminal so that the surgeon did not think it advisable even to apply traction for the fracture of the femur. It was under these conditions that we started to treat her with two injections a day of 1 cc. of a 5% solution in oil of the insaponifiable fraction of human placenta. Except for the unexpected survival of the patient, no apparent change was seen after two weeks of treatment. The pain, pleural effusion and general condition remained the same.

The treatment was changed to 5 drops of glycerol three times a day, and the dosage was progressively increased to 15 drops of glycerol t.i.d. After ten days without change, Coramine in doses of 15 drops was given when necessary to control the typical alkaline pain. After another week there was still no change. The pain remained almost the same, except for a decrease in intensity immediately following administration of Coramine. When all three substances were given concomitantly—the insaponifiable fraction by injection and glycerol and coramine orally—the situation changed impressively. Within a few hours, pain disappeared completely, and within one week other manifestations had totally changed. The pleural effusion, for which the patient had been tapped regularly each week or every 6 days, disappeared. The fracture which, until then, had appeared entirely inactive, showed a consolidation so rapid that in less than two weeks a solid callus was present. In three weeks the patient was out of bed on crutches. X-ray pictures taken two months after the change in medication showed most of the osteolytic lesions replaced by new bone tissue and the fracture replaced by an abnormally solid callus. There was no fluid in the pleura. All treatment, except the glycerol, was discontinued after another three months when, at the start of the war, the patient left Paris. We heard that she continued in good health without further treatment for three more years, at the end of which time she developed a recurrence, with liver metastases, and died shortly thereafter.

M. R., a 58-year old woman, the wife of a professor of gynecology, had an ulceration of the cervix two years before coming under our care. Biopsy had revealed squamous carcinoma, Grade III. After local treatment with radium, she underwent total hysterectomy. Six months before we saw her, she developed multiple abdominal metastases for which only symptomatic treatment was prescribed. When she came under our care, she had a distended abdomen in which masses of various dimensions were easily palpable. Besides several large tumors, two of them about 15-20 cm. in diameter, there were many smaller ones which gave the distended abdomen a very irregular appearance. The abdominal pain, her generally poor condition, as well as severe edema of the legs, kept the patient bedridden. She showed a high urinary specific gravity and low pH, and treatment consisting of a daily injection of 2 cc. of a 2.5% solution of cholesterol in oil was prescribed. When no subjective or objective changes were seen in three weeks, the treatment was changed to 10 drops of glycerol orally, three times a day, for another three weeks. There was still no obvious change.

After another three week period, this time without treatment, during which her general condition deteriorated, mixed treatment with cholesterol and glycerol was started. The patient now made a sudden remarkable recovery. The edema of the legs disappeared rapidly and in less than a month the tumors were no longer palpable. The abdomen, however, continued to be distended, but instead of the previous irregularity with multiple well-delineated tumors, a single huge mass was recognized. It filled practically the entire abdomen. We made the diagnosis of a large ovarian cyst which had probably been present before but had been obscured by the multiple tumors. The patient was operated on three months later and the cyst was removed. Not only were no tumors found in the abdomen, but there were no adhesions which the surgeon had feared. White patches were seen at the sites previously occupied by the tumors. There were no recurrences during several years of follow-up, after which we lost track of the patient.

Similar results were obtained during the following years, indicating the value of mixed therapy. We made it our standard clinical treatment except when the pharmacodynamic effect of a specific agent was being investigated.

As an example of the objective changes obtained through mixed treatment for type D offbalance, we give an abbreviated form of the following observation.

Miss S., 39 years of age, came under our care with bone metastases from an adenocarcinoma of the breast, for which she had a radical mastectomy 1½ years previously. At admission in very severe pain, besides other multiple bone metastases, she showed especially a marked destruction



FIG. 154. Arterioposterior view of the chest of patient S at time of admission showing an osteolytic process in the 6th and 9th left ribs.

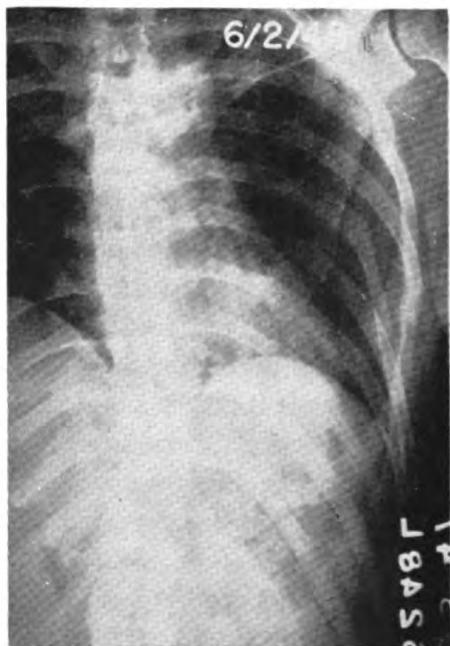


FIG. 155. Arterioposterior view of the chest of patient 5 months later, with the appearance of the new bones replacing the part of the ribs previously destroyed.

of the 6th and 9th left ribs. (*Fig. 154*) The analyses showed an offbalance type D and consequently she was treated with butanol and glycerol. The patient showed rapid changes not only in her subjective feeling, but also objectively. *Fig. 155* shows the rib osteolytic lesions healed and the missing bone replaced by new bone.

In general, however, in spite of some favorable cases, the results were not satisfactory. Even in patients in whom tumors regressed and disappeared, recurrences were seen anywhere from a few months to several years after discontinuation of treatment. By prolonging treatment over a period of years, the period of improvement was lengthened in some cases. Still, all too often the response to treatment was only temporary.

Mercaptans

Other substances with lipoidic character but with a polar group different from the carboxyl were tested. Mercaptans, as lipoids with a thiolic negative polar group, were used. In 1942, we started to study the therapeutic effect of ethyl mercaptan injected intramuscularly, in a 10% solution in oil. (326)

Use of ethyl mercaptan had to be limited to subjects with an offbalance corresponding to a predominance of sterols. Although only a few patients were treated because of the offensive odor, the results were satisfactory as indicated by the following case histories.

F. C.—This patient, at the age of 66, had a small carcinoma of the tip of the tongue resected at Columbia Presbyterian Hospital on May 6, 1941. At the same time, a second carcinoma of the left lateral border of the tongue was found infiltrating into the deeper portions. This lesion was treated with radium needles with a total dose of 2500 mg. hours. In September 1941, a prophylactic left radical neck dissection was performed. The patient was well for 8 months thereafter, and then began to experience soreness in the region of the scar on the lateral side of the tongue.

In August 1942, 16 months after the tumor had been irradiated, the patient came under our care. At the juncture of the anterior and middle third of the left lateral border of the tongue, there was an induration of 2/1 cm. in dimension with an ulceration of 1/0.5 cm. covered by necrotic tissue. The indurated base was especially developed anteriorly to the ulcerated lesion. There was no evidence of recurrence at the scar of the tip of the tongue nor at the left side of the neck. The latter lesion was very tender. A Wassermann analysis was negative. Biopsy of the edge of the ulcerated tumor revealed squamous cell epithelioma.

The patient was treated with placental acid lipid fraction for the first

week without any subjective or objective change. The treatment was changed and the patient received 1 cc. of a 10% solution of ethyl mercaptan in oil intramuscularly, three times a day. The dose was increased after three days to 2 cc., three times a day. By the end of one week under this treatment, pain had disappeared, although there was still some burning sensation. The appearance of the lesion was unchanged. The treatment was continued. By the end of the second week, the edema was reduced, and after another week of the same treatment, the induration was gone. After one month of treatment with ethyl mercaptan, epithelization of the lesion was noted. The treatment was discontinued. The lesion appeared completely healed, without induration, burning sensation or pain, less than a week later. The patient has remained without treatment since, and there has been no recurrence in the last 18 years. Three years ago, the patient had a basal carcinoma of the skin of the nose, which responded well to local treatment with radium in Mexico.

Mrs. L. F.—In June 1942, the patient then 50 years old, observed an induration on the right border of her tongue. Biopsy revealed a squamous cell carcinoma. After the biopsy, the lesion progressed so rapidly that it was judged inoperable. Without having received any other treatment, the patient came under our care in August 1942. At that time, the tumor had involved the right half of the tongue and multiple submaxillary and neck nodes, the biggest being 2 cm. in diameter. The lesion was extremely painful. Urine analyses showed low specific gravity and high pH and the patient was treated with 10% ethyl mercaptan in vegetable oil. We started with three $\frac{1}{2}$ cc. injections daily. The dose was progressively increased until it reached 6 cc. daily. The pain disappeared entirely in less than a week.

Treatment was continued for only 3½ weeks at which time there was a marked improvement in the tongue lesion and lymph nodes. Except for an inflammatory reaction at the site of injection, no side effects were seen. A month after the start of treatment, the lesions had completely disappeared. No treatment has been given since. The patient is still well today, free of recurrence, 18 years after treatment with ethyl mercaptan.

H. A.—In May 1942, at the age of 52, the patient developed hematuria, pyuria and tenesmus. In June 1942 an infiltrating tumor of the right side of the trigone was revealed by cystoscopy and cystogram. The patient came under our care at the end of August 1942. Having high urinary specific gravity, he was treated first with insaponifiable fraction of placenta, receiving 1 cc. of a 5% preparation in oil three times a day. Hematuria and the other symptoms increased under the treatment which was then changed to ethyl mercaptan, 10% solution in oil, starting with $\frac{1}{2}$ cc. three

times a day. The dose was progressively increased to 3 cc., three times a day by the tenth day. Hematuria decreased in the first 48 hours and ceased completely on the fourth day. Other symptoms disappeared. The treatment was continued with this dose for another 2½ weeks. Disappearance of the tumor was seen in follow-up cystograms. Without further treatment, the patient remained well until the beginning of 1955, 12½ years later, when a recurrence of the tumor of the bladder was noted. Being in Mexico, he did not receive treatment by this method and died from generalized carcinomatosis after 9 months. (*Figs. 156 and 157*)



FIG. 156. Cystogram of patient H.A. before treatment.

Similar favorable results were obtained for other patients with patterns corresponding to sterol predominance. We treated only three patients with patterns indicating fatty acid predominance. The use of ethyl mercaptan in these cases caused an exacerbation of symptoms and rapid deterioration of the general condition.

The odor of ethyl mercaptan was so offensive that its use posed insurmountable problems. Patients were forced to be social recluses and it was practically impossible to get nurses to administer the injections because of persistence of the odor on skin and clothes. We had many complaints that the odor of the medication polluted the atmosphere of a large area for

a long time. Obliged to discontinue its use, we sought other preparations containing thiol groups or bivalent sulfur that we hoped would have similar biological effects.

We investigated a large number of such substances in animals. Only a few were extensively studied in patients. Of the homologous series of aliphatic mercaptans, we utilized propyl, butyl and amyl mercaptans. These offered no advantage over ethyl mercaptan, being less active but hardly less offensive in odor.



FIG. 157. Cystogram of patient H.A., after treatment with ethyl mercaptan.

Hexyl mercaptan was the first of the series that had a more bearable odor but it was definitely less active. Although objective changes in cancer were observed with its use, the tumors did not disappear as they did in some cases treated with ethyl mercaptan, and many favorable responses were only temporary.

Of the higher mercaptans, dodecyl and hexadecyl were most extensively studied. While their odor was far less objectionable, they produced less favorable results than the lower homologues. Much larger doses were required to influence pain, and even then the effects were reduced and tem-

porary. The striking results obtained with ethyl mercaptan could not be duplicated with any of the higher homologues. For this reason, we reluctantly abandoned the use of mercaptans.

Sulfurized Oil

During the years that followed, we tried to achieve results similar to those obtained with ethyl mercaptan by using other agents with bivalent sulfur. The one most commonly used was the so-called "sulfurized oil" containing fatty acid hydrosulfides, whose pharmacological characteristics have been discussed previously. Sulfurized oil's effects on pain and systemic manifestations were less impressive than those of the mercaptans but better than those of unsaturated fatty acids and their derivatives. In several cases, tumor disappearance was actually observed. Generally the clinical results were neither as consistent nor as persistent as with the mercaptans. With the use of this product alone, however, long-term favorable responses were the exception. For example:

Mr. I. G.—White male, was operated on at Maimonides Hospital in January 1950 at the age of 56. A pyloric mass was found and a subtotal gastrectomy was performed. Diagnosis of adenocarcinoma of the stomach was made. Microscopic examination showed a large area of replacement of gastric mucosa by atypical glands, with a great mass of abnormal cells invading the submucosa. These cells extended into the first part of the duodenum. There were post-operative complications with abscess formations in the wound, which were incised and drained. With new complaints of pain in the upper abdomen, and rapid loss of weight, the patient was admitted to Monticello Hospital in July 1950, and to Kings County Hospital twelve days later. He was then transferred to a nursing home with a diagnosis of terminal cancer with recurrent tumor in the upper abdomen and metastases to the chest. He remained at the nursing home for seven weeks. In September, he came under our care.

At this time, he had lost 39 lbs., and complained of extreme weakness, pain in the lower chest and upper abdominal region, and cough with hemoptoic sputum. The pain was only slightly relieved by narcotics and the general condition of the patient was considered very poor. A large mass was found occupying the entire upper abdomen; X-ray examination of the chest showed masses in the right lung. No fluid was obtained from several chest punctures.

Based on the urinary analyses, the patient was treated with a preparation of hydro-persulfide. The response was excellent. Not only was his pain rapidly relieved but the mass in the abdomen progressively decreased in

size. Evidence of lung involvement slowly disappeared in X-ray studies. After November 1950, he continued the treatment at home for over 13 months after which he resumed his old job as a millinery cutter. He had gained 55 lbs. since beginning treatment. For the past 7½ years, he has worked without interruption and there has been no clinical evidence of malignancy. The extreme condition under which the patient came into our care can explain the length of time needed for general recovery and his inability, despite disappearance of the tumoral masses within a few months, to resume his job for more than a year.

Thiosulfates

We also utilized sodium thiosulfate in many patients where symptoms such as pain, vertigo, itching, etc., could be related to a local acid pattern. In these specific cases, the results were generally satisfactory. Sodium thiosulfate was administered either orally in drops of a 10% solution in water, or parenterally in a 4% solution in water. Intramuscular and subcutaneous injections were well tolerated even when doses were as high as 10 cc. Doses as small as 10mgs. were observed to influence symptoms in certain patients. However, in some cases it was necessary to give as much as 5 gr. of the substance—125 cc. of the parenteral solution—in 24 hours to obtain any effect. In these cases there were no apparent side effects even when this dosage was continued for many days. Effects upon tumors with the use of thiosulfate alone were seen in several cases but the treatment did not produce complete disappearance and results usually were only temporary.

The use of sulfurized oil in conjunction with sodium thiosulfate has been tested in a sufficient number of cases (more than 75) to enable us to recognize that the combination produces changes in pain and systemic analysis, as well as reduction in the size of tumors, especially when given in adequate amounts over a long enough period of time. In several cases, tumors disappeared for many years following this treatment. The following are illustrative cases:

G. M.—In March 1944, this patient had an ulceration of the cervix. Biopsy showed squamous cell epithelioma, Grade III. A total hysterectomy was performed. She was treated with 3,600 mg. hours of radium in and around the cervix in April 1944. Five months later, there was evidence of local recurrence and the patient was given 1800 r. of deep X-ray therapy. In July 1945, examination revealed no evidence of disease. On October 28, 1945, the patient was examined at the Scott and White clinic in Temple, Texas, and a diagnosis of extensive metastatic carcinoma of the para-

metria was made and further deep X-ray therapy was advised. The patient refused this.

She came under our care in November 1945. She was extremely weak and showed evidence of considerable weight loss, weighing only 86 lbs. Hematuria and dysuria were the principal complaints. Multiple large tumor masses were palpable in the pelvis and extended into the abdomen above the umbilicus. The largest mass palpable, about the size of a big grapefruit, was in the right lower quadrant. The tumor was found to have invaded the bladder, too.

The treatment with which we started, t.i.d. intramuscular injections of 1 cc. of placenta fatty acids, 10% in oil, was changed after one week, in view of the preterminal condition of the patient. A hydopersulfide preparation containing 1% sulfur, and sodium thiosulfate 10% was administered orally. We started with a dose of $\frac{1}{4}$ cc. three times daily of hydopersulfide and $\frac{1}{2}$ cc. thiosulfate and increased it progressively, with amelioration of the general condition and disappearance of the hematuria. After a month, the dosage reached 3 cc. of the first and 9 cc. of the latter preparation daily. Under this treatment, the patient continuously gained strength and weight. The hematuria did not reappear.

In May 1946, she was admitted to the University of Chicago clinic. A large fixed, firm, irregular tumor mass was still present in the lower abdomen rising from the pelvis to the umbilicus. Although her general condition had improved, the patient again had urinary frequency and urgency, and cystoscopy revealed a severe cystitis and several small stones in the bladder. An intravenous pyelogram showed a right hydro-nephrosis.

The treatment with hydopersulfide and sodium thiosulfate was continued for 4 months in Chicago during which time the abdominal mass became smaller, softer, less fixed and was no longer tender. By August 1946, her weight was 136 lbs., a gain of 50 lbs. since start of treatment. The treatment was continued during 1947, although abdominal examination did not reveal any palpable masses. On rectal examination, however, the pelvis appeared to be frozen but no definite mass was felt. Cystoscopy showed severe cystitis, bladder calculi and a distorted bladder. Although she passed several stones and gravel, her urinary symptoms persisted.

In December 1948, an attempt was made in Texas to remove a bladder calculus transuretherally after lithotripsy. The bladder was perforated during this procedure and a recto-vasical fistula resulted. The patient's local physician in Texas believed that the patient was terminal, but we insisted upon this. This was done, and the surgeon reported that there was no evidence of any a colostomy to divert the fecal stream as an immediate first procedure.

pelvic or abdominal masses. The patient made a slow recovery. According to reports to this date, 16 years after start of treatment, no recurrence has been noted. Recently the colostomy was closed, the patient being in good condition.

Mrs. M. L.—In November 1941, at the age of 40, the patient had a left oophorectomy for a multi-loculated ovarian tumor with ascites and peritoneal implants. The pathological finding was papillary cyst adenocarcinoma of the left ovary. Without any other treatment, she remained free

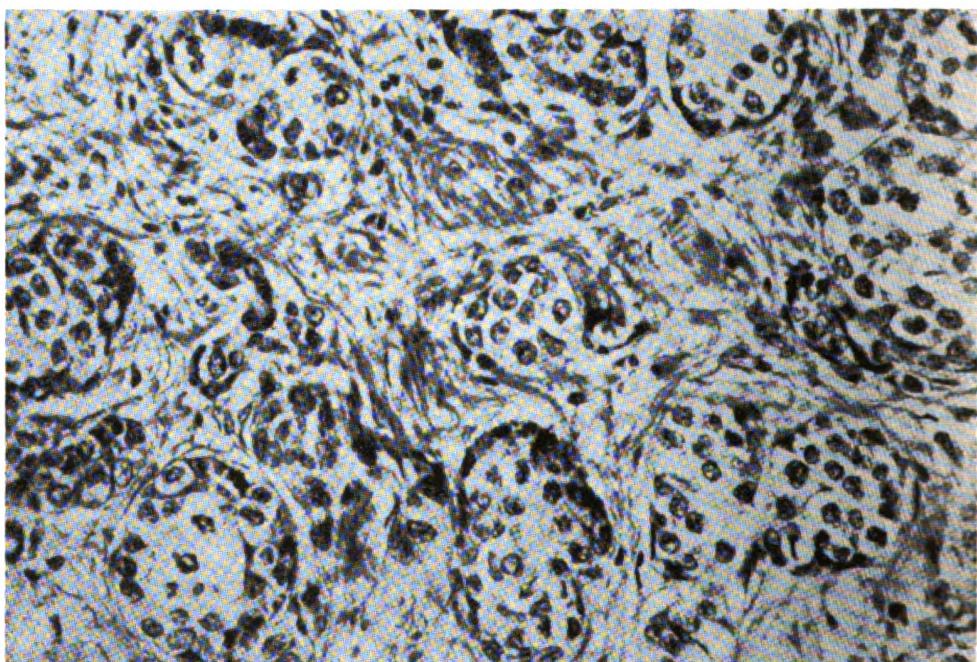


FIG. 158. Photomicrograph of lymph node from Case E.H. showing metastatic adenocarcinoma Gr III (400 x).

of symptoms until the beginning of 1945, when she started to complain of abdominal discomfort in the lower left quadrant. A mass was found at the site of operation. Growth was noted in further examinations. The patient had no treatment until December 1945, when she came under our care. On examination, a tumor of 11/6 cm. with limited mobility was found. No abdominal fluid was present at this time. She was started on amylercaptan in doses increasing to 6 cc. daily of a 10% solution in oil. The treatment was discontinued after a week because of the odor. No manifest changes could be seen. A preparation of hydopersulfide containing 1% sulfur, in a dose of 1 cc. three times a day orally, was used. The mass in the left parameter disappeared entirely in about 2 months. She

continued with the same medication for another 14 months. There has been no recurrence to date.

Mrs. E. H.—In April 1947, at the age of 46, this patient had a right radical mastectomy. The pathological diagnosis was adenocarcinoma, Grade III, with metastases to axillary lymph nodes. (Fig. 158) A course of post-operative irradiation was administered. Menses had been interrupted 8 years before by a total hysterectomy and bilateral salpingo-oophorectomy.

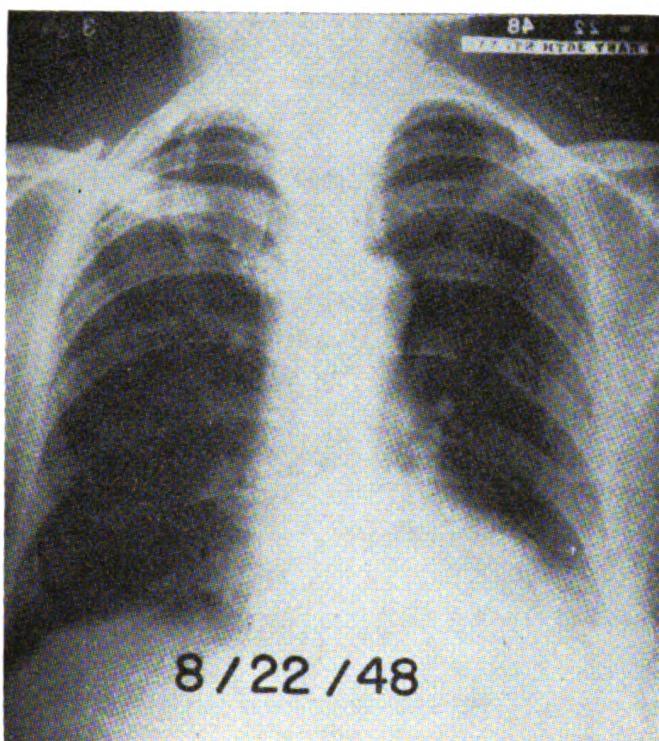


FIG. 159. Anteroposterior view of chest in Case E.H. at conclusion of testosterone and deep X-ray therapy to spine, showing destruction of medial $\frac{1}{3}$ of left clavicle and metastatic rib lesions.

The patient was free of symptoms until July 1948, when she began to complain of back pain and difficulty in walking. X-ray examination revealed osteolytic metastatic lesions in the medial third of the left clavicle, pelvis, thoracic and lumbar vertebrae, with collapse of the ninth thoracic and third lumbar vertebrae. She was hospitalized and deep X-ray therapy was applied to the thoracic and lumbar vertebral regions and the right hip, 1800 r. being delivered to each of the three fields. A total of 600 mgm. of testosterone propionate was also administered in four weeks (50 mgm. three

times a week). Clinically, there was improvement and the patient was able to walk with the aid of a back brace and cane upon discharge. X-ray examination on August 22, 1948, at the conclusion of this period of therapy, revealed continued spread of osteolytic lesions, involving the bodies of the lower cervical, lower thoracic and lumbar vertebrae and pelvis. Numerous lesions were observed in the left ribs and in the upper thirds of both femurs. There was further destruction of the left clavicle. (*Fig. 159*)

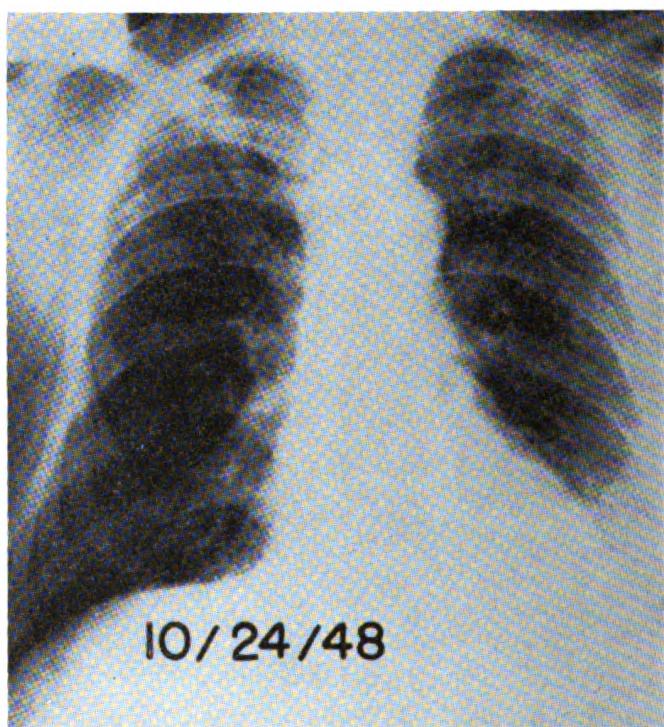


FIG. 160. Anteroposterior view of chest in Case E.H. two months after stopping testosterone and X-ray therapy, showing further involvement of medial $\frac{1}{3}$ of left clavicle.

After discharge, the patient did not receive any further X-ray therapy or testosterone. The clinical improvement lasted for only a short time, the back pain and difficulty in walking recurring within a few weeks. Radiographic examination at the end of October revealed further increase of the previously described lesions, with new areas of involvement. (*Fig. 160*)

The patient received several injections of an unknown medication at home but her condition continued to grow worse. Pain was more severe, and required increasing amounts of narcotics. The patient was confined to bed and there was evidence of increasing nerve involvement, culminating,

by the end of November, in paralysis of both lower limbs with loss of sensation below the level of the ninth thoracic vertebrae. There was no bladder or rectal dysfunction.

The patient came under our care in this condition on December 21, 1948. Pain was so severe that a body cast appeared indicated, but the orthopedic consultant considered the patient's condition too far advanced to warrant this. The urinary pH was alkaline.



FIG. 161. Lateral view of thoracic spine in Case E.H. at time of admission, showing extensive metastatic involvement of vertebrae with collapse of fifth and ninth vertebrae.

A hydropsulfide preparation with 0.5% sulfur was administered in doses ranging from several drops by mouth to 1 cc. twice a day intramuscularly. Sodium thiosulfate in a 10% aqueous solution was given at the same time by mouth, and after a few weeks a 4% solution administered intramuscularly was substituted and was given in increasing doses up to as much as 11 cc. every few hours. For a short period, colloidal sulfur was administered in doses of 100 mgm. orally every three hours.

During the first few weeks, with small oral doses of sulfur in oil and sodium thiosulfate, there was no evidence of improvement. 2 cc. doses of sodium thiosulfate provided definite pain relief for a short time after each injection and the gradual increase of the individual dose to 11 cc. over a

twelve week period gave complete pain relief. During this time, the patient's general condition improved rapidly. Motion and sensation returned to the toes, feet and finally to the whole leg. By April, the patient was able to sit out of bed. By the end of May, she was ambulatory without requiring a brace or cane. Her only complaint was a mild facial acne that developed during the course of treatment.



FIG. 162. Anteroposterior view of left shoulder region in Case E.H. four months after stopping testosterone and X-ray therapy at time of admission, showing involvement of entire left clavicle and continued spread in left seventh and eighth ribs posteriorly.

Radiographic examinations were made at the beginning of treatment and each month thereafter. At the beginning, four months after the last X-ray treatment and doses of testosterone, osteolytic metastases were observed in all the vertebrae of the cervical spine and the bodies of the lower thoracic and lumbar vertebrae, with compression fractures of the third, fifth and ninth thoracic vertebrae. The entire pelvis was involved (*Fig. 163*) and there was destruction throughout the entire length of the left clavicle, in the left acromial process, both humeri and several ribs on the left side. (*Fig. 162*) Vascular markings were moderately increased in both lung fields.

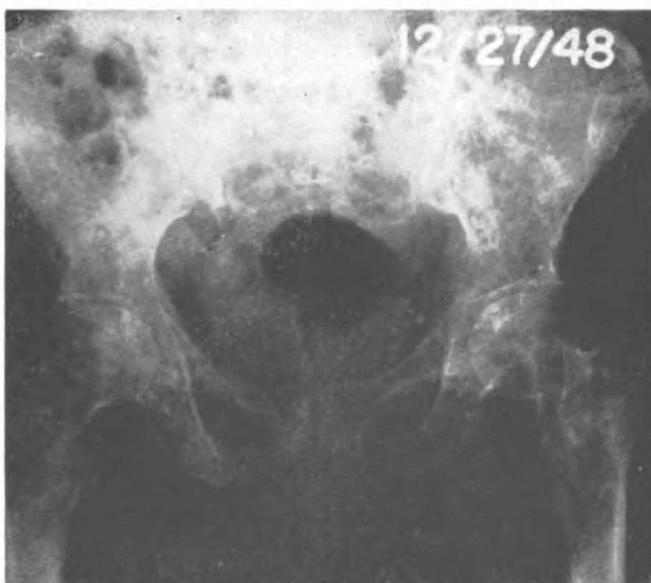


FIG. 163. Anteroposterior view of pelvis and upper $\frac{1}{3}$ of both femurs in Case E.H. at time of admission, showing widespread osteolytic process.



FIG. 164. Lateral view of thoracic spine in Case E.H. four months after beginning of treatment, showing considerable bone regeneration in all vertebrae.

Comparison with previous films showed definite evidence of continued spread of the metastatic process.

Films in February showed no change, but in March, slight regressive changes were observed, especially in the left clavicle, left humerus and pelvis. During this time the urine became acid and remained so almost continuously. In April regressive changes were observed in the ribs, pelvis,

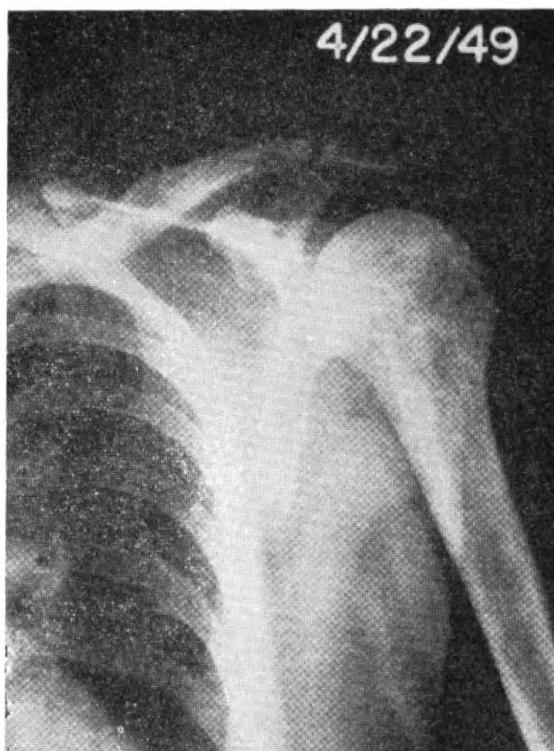


FIG. 165. Anteroposterior view of left shoulder region in Case E.H. four months after beginning of therapy, showing considerable repair of left clavicular and rib lesions.

femurs and left shoulder girdle. Elsewhere no further involvement was noted.

In July 1949, regressive changes were found to be continuing in all the involved bones. (*Figs. 164, 165, 166*)

At this time, all medication was discontinued and the patient returned home. In August, the patient again complained of pain in the back, right thigh and left shoulder, and had increasing trouble walking because of difficulty in moving the right leg. The urinary pH again was alkaline. Sodium thiosulfate and sulfur in oil again were administered with relief of

pain and considerable improvement in the ability to walk. A few months later the patient had a stroke from which she died in a few days.



FIG. 166. Anteroposterior view of pelvis and upper $\frac{1}{3}$ of both femurs in Case E.H. four months after beginning of treatment, showing considerable bone repair.

Hydronaphthalene Persulfides

The exceptionally good results obtained with mercaptans in treating cancer in humans could only partially be reproduced by the different agents with bivalent sulfur in the polar group we tested. In addition to the hydroper-sulfides of the fatty acids, we tried to prepare and study other groups of persulfides. The great ability of the products of hydrogenization of naphthalene, such as tetrahydronaphthalene (tetralin) and decahydro-naphthalene (decalin) to fix oxygen as peroxides, led us to try to fix sulfur as persulfides on their molecules. These substances were treated with sulfur in conditions similar to those which led to fixation of sulfur to fatty acids. The agents obtained through this fixation on tetralin were particularly studied.

We have mentioned the pharmacological study of these products. Based on their biological action, they appeared to be intermediary between mercaptans and fatty acids persulfides, with the big advantage of having a bearable odor. In humans, we utilized this produce in cases of which, most are still under observation. In general, the results obtained were good.

Subjective changes and objective ones upon the tumors were obtained. We found that only minimal doses are to be used, corresponding to micrograms of sulfur and milligrams of tetralin. With these doses, no side effects were observed. When given slightly higher doses, some patients felt a sensation of weakness and also, in a few cases, of dizziness.

This agent has shown a marked influence upon the existing patterns with a special facility to induce, in most cases, an offbalance of type D at the cellular level. With higher doses, the systemic offbalance which is also influenced, can be changed back to offbalance type A only with difficulty. This fact indicates the need for reduced doses, acting almost exclusively at the cellular level. The dosage must be guided by a continuous control of the analyses, and especially by those of serum potassium for the cellular level and of urinary surface tension for the systemic. With values above 5 mEq potassium in serum (and low in blood red cells) and urinary surface tension of less than 68 dynes/cm., corresponding to a systemic offbalance of the type D, the decrease or even suppression of the medication is indicated.

We utilized the agent in cancer cases where the rapid disappearance of the tumor appeared as the capital aim. More time is needed to judge the value of those results already obtained. Among the cases treated, the following observation represents an interesting example.

B. P., 32 year old male, was operated for a wart at the right ear 2½ years before coming under our care. Pathological examination had revealed malignant melanoma. A month later, a dissection of cervical glands was made. The analyses of the obtained glands showed no cancerous cells. Two months before coming under our care, a checkup revealed a new tumor in the right side of the larynx. With the growth of the tumor, edema of the right face was also increasing. Examination showed a tumor 4 cm. in diameter on the right side of the larynx. The tumor was adherent to the skin. Laryngological examination showed the tumor protruding in the pyriform sinus. Because extensive surgery would have been necessary for the tumor removal, the patient refused such surgery. He was admitted and first treated with only limited change in the tumor. Treatment was changed to tetralin persulfides in a dose of 10 drops of a 10% solution in oil, administered 3 times a day. Under this treatment, the tumor involuted very rapidly, and practically disappeared in 3 weeks. Subjective feeling, such as dizziness, made us discontinue the administration of the preparation. The treatment was changed to epichlorohydrin and sulfur in the form of fatty acid hydrosulfides, administered in small doses. The patient has continued this treatment at home for the past 2½ years with no apparent recurrences.

Butanol, Glycerol

During the period when mercaptans and other sulfur-containing agents were being studied, attention also was centered on butanol in the group of anti-fatty acid agents. While butanol's effect upon pain and other subjective manifestations appeared evident from the beginning of its use, the influence upon tumors seemed small. Together with glycerol, however, it produced several long-lasting objective changes. Characteristically, in most of these cases recurrences appeared only after many years of normal active life during which there was no clinical manifestation of cancer. In some cases, however, there were no recurrences.



FIG. 167. Myelogram of patient M.H. showing the flow of lipiodol arrested at the level of 4-T.

Mrs. M. H.—This 45-year-old patient experienced, in June 1943, sensory and motor disturbances which progressed so rapidly that in September 1943 she presented a complete paraplegia below 4-T. She came under our care in February 1944, a paraplegic for 5½ months. The myelogram taken at that time showed complete obstruction, with the flow of lipiodol arrested within the spinal canal at the level of the 4-T. (Fig. 167)

The urine analyses showed an offbalance of the type A, and she was treated with the acid lipid fraction of human placenta, and with hydro-persulfides. The symptoms continued to progress and the pain was more severe. Following a change in the urine analyses toward the offbalance D, the treatment was changed to butanol and glycerol. The pain was controlled in a few days, and a slow regression of the paraplegia occurred. After four months of this treatment, there was complete remission and the patient was again ambulatory. The myelogram repeated at the end of November 1944, showed complete disappearance of the obstruction. (*Fig. 168*) The

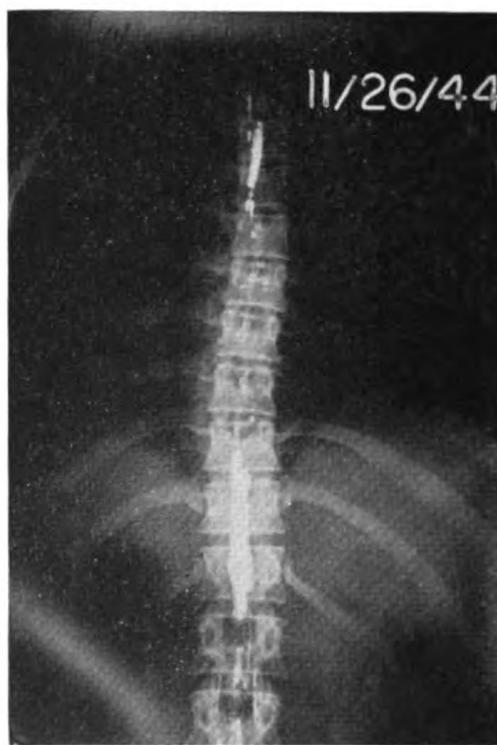


FIG. 168. Myelogram of the patient M.H., showing the complete disappearance of the obstruction.

patient remained entirely well, without treatment and free of symptoms for eleven years. We were informed that, subsequently, a recurrence appeared, followed again by paraplegia and multiple lung metastases. The patient died after four months of paraplegia.

Mr. I. H.—In 1938, at the age of 30, the patient underwent surgery for a tumor of the right parotid diagnosed as chondromyxosarcoma; in 1940, a recurrent tumor was removed and was followed this time by facial

paralysis. In 1943, another recurrence was treated surgically. In June 1945, the same procedure was repeated. Immediately after the last operation, there was still another recurrence and the tumor this time started to grow rapidly. Severe pain was only slightly relieved by narcotics. Radiotherapy was refused, in spite of the massive tumor and pain.

The patient came under our care in December 1945, with several tumors occupying the right parotid region and extending below the mandibula. He was using various narcotics with little effect. With the urinary chloride retention index, pH and specific gravity as criteria, treatment with

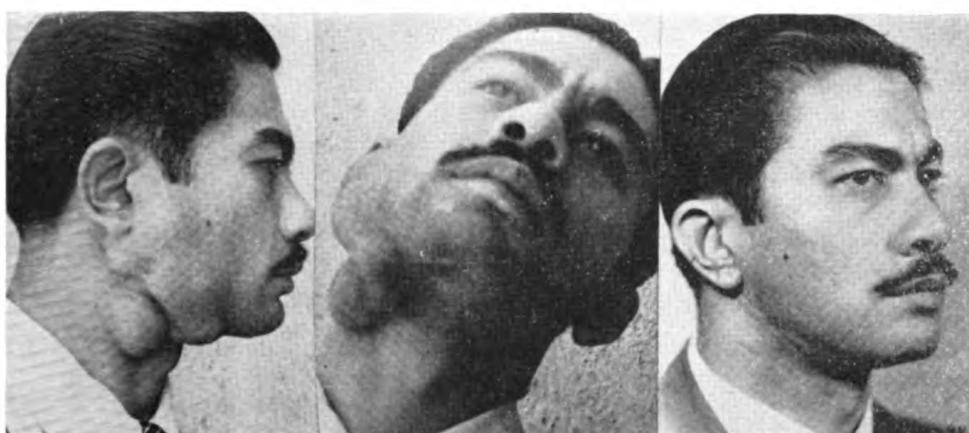


FIG. 169. Patient I.H. with a recurrent chondromyxosarcoma of the right parotid gland before and after treatment. The scar is from repeated previous surgical interventions.

sodium thiosulfate and hydrosulfides was started. Under this treatment, which lasted a week, the lesion appeared to be unfavorably influenced and pain increased. With the urine chloride index used as a criterion, the treatment was changed. 1 cc. of butanol 6.5% was administered orally three times a day along with 0.3 cc. of glycerol. After 4 days, the dose of butanol was increased to 2 cc. three times a day. Pain was relieved in a few days. Rapid disappearance of the tumor masses followed. The same treatment was continued for one year. Since then, the patient has been well and is enjoying good health without any recurrence as of this date. (Fig. 169)

E. M.—In 1935, at the age of 42, this patient had a right radical mastectomy for adenocarcinoma of the breast. In 1940, recurrent nodules appeared in the line of the scar. One of these was biopsied and showed a recurrent adenocarcinoma, Grade III. The patient was then treated with deep X-ray therapy, 3800 r. being delivered through five fields to the

right chest and axillary regions. A daily dose of 200 r. was given for nineteen days between December 1940 and January 1941, using the following factors: 200 kv., 25 ma., 50 cm., $\frac{1}{2}$ mm. Cu and 1 mm. A1 filter. In May 1941, the wound area and recurrent nodules (*Fig. 170*) were excised and a skin graft was used to repair the defect.

In July 1943, skeletal metastases, predominantly osteolytic in nature, were reported in the fourth, fifth and twelfth thoracic vertebrae and first, second, fourth and fifth lumbar vertebrae, the first sacral segment and the left ala of the sacrum. There was also involvement of the outer portion of the left ilium and the inner portion of the right ilium near the sacroiliac joint. The patient received a second course of deep X-ray therapy over the spine and posterior pelvis, the total dose being 3800 r. with the same factors. Following this, her menses ceased. The pain in her back, which had confined her to bed, was considerably relieved and she became ambulatory.

By January 1944, however, pain had recurred and the patient was again confined to bed most of the time. X-ray studies showed metastatic involvement in practically every thoracic vertebrae, especially marked in the fourth, fifth, eighth and twelfth. All the lumbar vertebrae were involved,

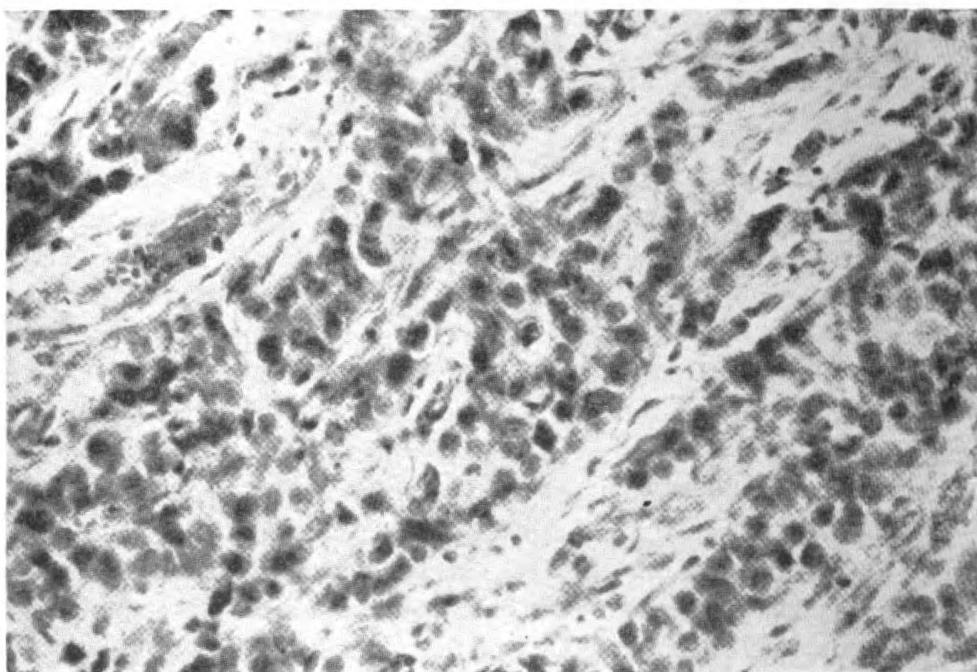


FIG. 170. Photomicrograph of recurrent skin nodule in Case E.M., showing adenocarcinoma, Gr III (400 x).

especially the first, fourth and fifth bodies and transverse processes. Deposits were present throughout the sacrum, in both iliae, the right ischium, left acetabulum and upper femurs. Another series of deep X-ray treatments was used on the same areas of the spine and posterior pelvis as before, the total dose this time being 2000 r., using the same factors. There



FIG. 171. Anteroposterior views of thoracic spine in Case E.M. at time of admission, showing metastatic involvement of vertebrae.



FIG. 172. Lateral view of the thoracic spine in Case E.M. at time of admission, showing metastatic involvement of vertebrae.

was again relief of pain, although the patient was kept in bed and a body brace was applied to reduce pain associated with motion and to avoid fracture.

We first saw the patient on March 9, 1944. She had moderately severe back pain, was confined to bed with a back brace, and complained of weakness. Blood pressure was 90 systolic, 60 diastolic. Operative scars of

the right chest region were well healed and there was no evidence of local recurrence of the tumor. There was no superficial lymphadenopathy. The liver area was tender to pressure on deep inspiration but the liver was not palpable. There was tenderness over most of the vertebrae with pressure. Patellar reflexes were hyperactive bilaterally. X-rays showed widespread skeletal metastases involving the dorsal and lumbar vertebrae, the sacrum and the pelvis. The lesions were predominantly osteoplastic. (*Figs. 171, 172, 173*)

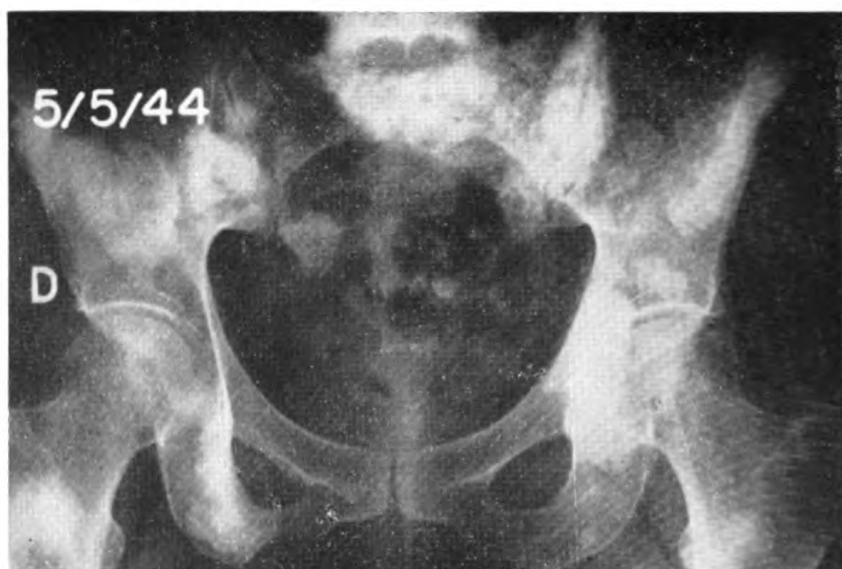


FIG. 173. Anteroposterior view of pelvis in Case E.M. showing widespread metastatic involvement in fifth lumbar vertebrae, sacrum, both iliac and femurs, two months after onset of treatment.

The patient was hospitalized and treated experimentally from March 8 to December 5, 1944, a total of nine months. The substances employed were n-butyl alcohol, cholesterol and glycerin, administered singly and concurrently at different periods. n-Butyl alcohol was administered as saturated water or saline solution (7.9%) in doses of from 3 drops every two hours to 20 cc. three times a day by mouth, or from 1 to 5 cc. three times a day. 2.5% cholesterol in neutral oil solution was administered intramuscularly in doses of 1 to 8 cc. three times a day. Glycerin was employed orally in 0.5 to 1 cc. doses three times a day. Dosages of all medication were increased progressively, the aim being to bring about and maintain alkalization of the urine.

At the time of admission, the daily urine pH was almost constantly

acid. After five months of treatment, the urine became alkaline and remained so until the patient was discharged from the hospital. (*Fig. 174*)

Clinically, pain was completely relieved after about one month. The patient's appetite improved and she began to gain weight and to feel stronger. After five months, she was mobilized with a brace and showed good progress in the ability to walk.

No changes were observed in monthly X-ray studies during the first four months of treatment. In the X-rays taken in July, the fifth month, at the time of urinary pH change, a few lesions began to show areas of decreased density. These changes progressed fairly rapidly thereafter.

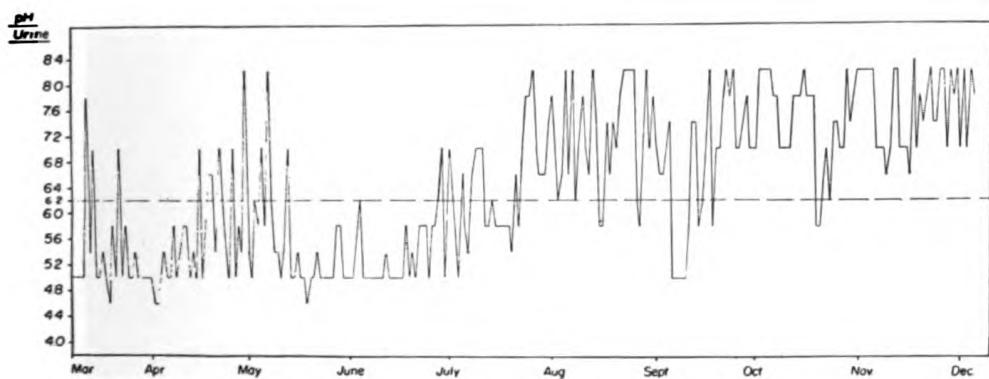


FIG. 2

FIG. 174. pH of daily morning urine specimens in Case E.M. determined colorimetrically, showing changes from acid to alkaline 5 months after the beginning of the treatment.

Following her discharge from the hospital, the patient continued to take 0.25 cc. of glycerol three times a day for another six months. All medication was discontinued after that and the patient has received no further treatment since. She returned to her old clerical position and after several years, when the X-ray changes appeared to warrant it, the brace was removed.

X-ray pictures taken fifty-seven months after beginning of the experimental treatment, (*Figs. 175, 176, 177*) no longer showed osseous pathology and indicated virtually complete restitution of normal appearing bone. The clinical condition was excellent and remained so in 1956, when we saw her. We were indirectly informed that later, in Texas, she suffered a right pleural effusion and died shortly thereafter.

M. H.—In February 1948, at the age of 18 months, this patient was admitted to the Good Samaritan Hospital in Dayton, Ohio, because of

abdominal pain. An exploratory operation revealed an obstruction caused by a tumor of the bowel that had spread to the lymph nodes throughout the abdomen. A by-passing operation was performed to relieve the obstruction. A piece of the mass and some of the involved lymph nodes were removed. The pathological diagnosis was fibro-sarcoma.



FIG. 175. Anteroposterior views of thoracic spine in Case E.M. fifty-seven months after onset of therapy, showing almost complete restitution of normal bone structure.



FIG. 176. Lateral view of thoracic spine in Case E.M., fifty-seven months after onset of therapy, showing almost complete restitution of normal bone structure.

On March 3, 1948, the patient came under our care. At that time, almost a month after surgical intervention, a mass the size of a tangerine was found in the right side of the abdomen. Based upon her analyses, the patient was treated with $\frac{1}{2}$ cc. glycerol and 2 cc. of butanol solution three times a day. She continued the same treatment without interruption for

two years, gained weight and grew, and no recurrences were noted. It is now 12 years since this little girl started treatment. She is well, attending school, and carrying on all the usual activities of a child of her age.

Paralleling these clinical investigations, a study was made of the elimination of surface-active substances. This led to the use of surface tension as a criterion for the recognition of offbalance patterns. A series of cases was treated according to this criterion. With the progress of research, new agents also were utilized.



FIG. 177. Anteroposterior view of pelvis in Case E.M. fifty-seven months after onset of treatment, showing almost complete restitution of bone structure.

Conjugated Fatty Acids

In 1947, we started therapeutic trials of conjugated fatty acids, first using eleostearic acid, the conjugated triene obtained from tung oil, administered orally or parenterally. The effects upon pain, systemic changes and particularly tumor evolution, were not up to expectation although subjective changes were immediately more manifest than for unconjugated fatty acids. In a short time, however, it was found necessary to continuously increase the dosage in order to maintain the effects. The intervention of a defense mechanism against these preparations often was evident. Cancer patients who had responded to administration of eleostearic acid with relief of pain and even with an arrest of tumor growth were found to require increasing amounts of this substance. After a while, they no longer

responded. Even very large amounts of this conjugated fatty acid, well tolerated in these cases, no longer had an effect upon the tumor and its manifestations. This fading effect limited the clinical usefulness of eleostearic acid. In this respect, it appeared to resemble many other constituents or even heterogeneous agents which have therapeutic effects that fade rapidly.

We have noted previously that intervention of the adrenals is directed especially against the conjugated trienes, substances related to traumatic noxious influences. Therefore, we tried to utilize other conjugated members, some with a higher number of double bonds, in the hope that the body would not be able to efficiently fight their intervention. We obtained conjugated fatty acids with four double bonds by treating mixtures of fatty acids rich in arachidonic acid, such as salmon oil, then fractionating the mixtures through their solubility in solvents, especially acetone, at low temperatures. We also obtained the same type of compound directly from parinarum laurinum nuces as parinaric acid, a tetraconjugated acid. Conjugated pentaenic and hexaenic acids were isolated from the mixture of conjugated fatty acids obtained from salmon, sardine and cod liver oils. When these preparations were tried in patients with cancer, no apparent improvement over the results obtained with mixtures of non-conjugated fatty acid was seen.

Having in mind the plurality of levels at which they would act, and especially considering the influence exerted by methylcholanthrene upon carcinogenic activity, mixtures of fatty acids from cod liver oil, sardine oils and from normal organs and tissues were conjugated and used. While the effects on pain and systemic changes were more intense and longer lasting, the effects upon tumors were not strikingly different from those obtained with the non-conjugated isomers. Of approximately 140 cases in which these conjugated fatty acid preparations were used, 45% showed subjective changes. In 25%, objective changes occurred, including clinical disappearance of malignant tumors in a few cases. Most of these results, however, were temporary. The tumors later grew again and no longer could be controlled by administration of these lipids. In some cases, the good results persisted and the following illustrates three of these cases.

B. T., 46 years old, had a left mastectomy in 1948, for an adenocarcinoma. 1½ years after the operation, progressively increasing generalized pain appeared, with the general condition going rapidly downhill. Pain, more than the general condition, obliged her to become totally bedridden. Successive X-ray examinations showed rapidly progressing osteolytic

lesions. X-ray treatment for three regions was started with the intention to control the pain which was most severe in skull, ribs, spine, pelvis and femurs. Because of the general condition, this was discontinued after a few treatments. When the patient came under our care, she was entirely immobilized and in severe pain. X-rays revealed (*Figs. 178, 179*) multiple osteolytic metastases in skull, femur, pelvic bones, spine. In view of the analysis

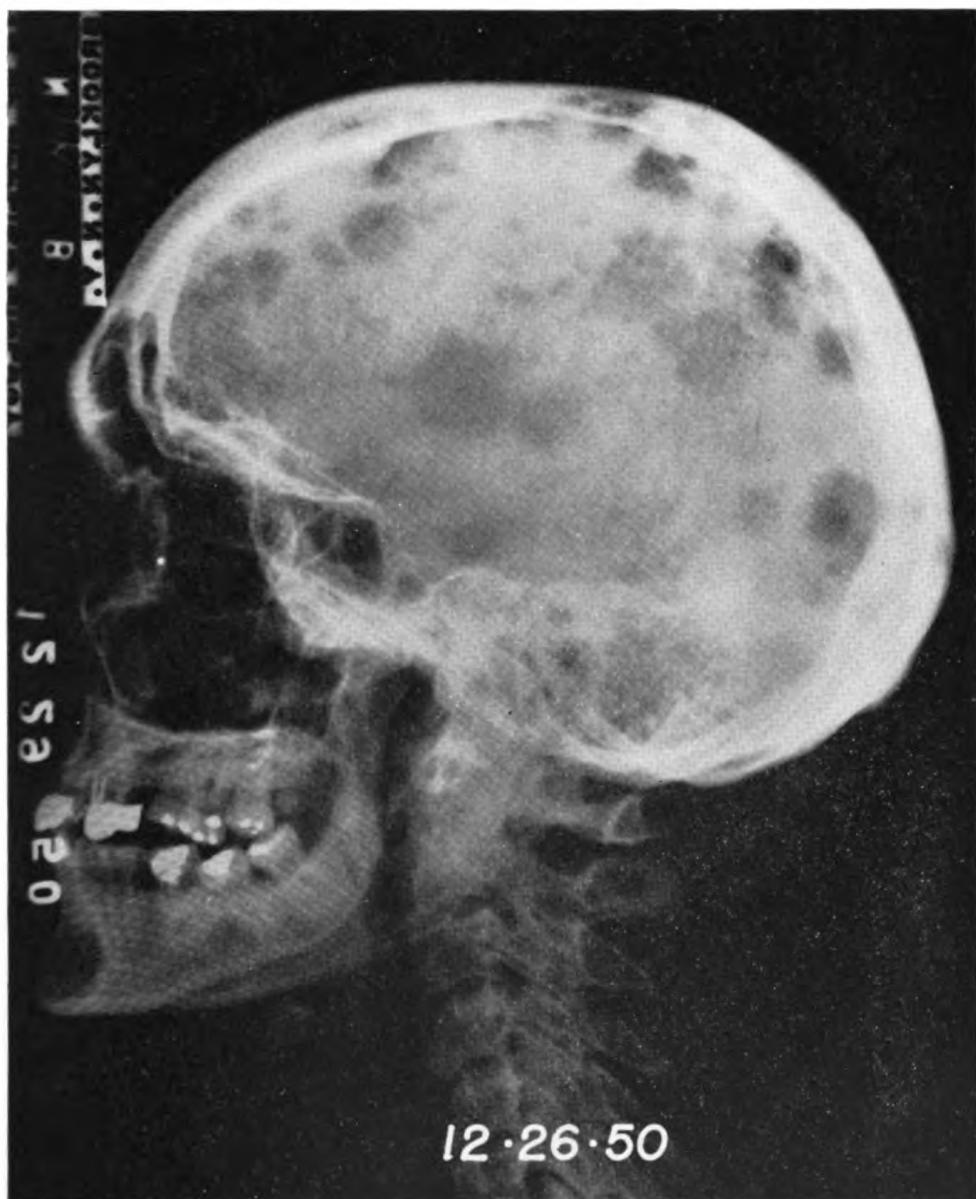


FIG. 178. Lateral view of the skull of patient (B.T.) at the time of admission, showing multiple osteolytic metastases.

as a typical A offbalance, treatment with hydrosulfide and conjugated fatty acids was instituted.

In her case we saw a peculiar form of response encountered also in several other subjects, and which we considered in general as corresponding to a favorable response. During the treatment, while pain in general was relieved, one lesion was seen to become progressively more painful. It remained severely painful for 2-3 days after which the pain disappeared. The same change was seen to occur successively in one lesion after another with the same temporary increase of pain until it became very severe, followed by disappearance after 2-3 days. Not only did the pain fail to return in the same lesion, but usually the lesion was seen to involute after such a change.

With this kind of treatment the patient made a very rapid recovery and was out of bed in less than two months. The radiological changes, although showing progressive repair of the osteolytic lesions, took more time to be completed Fig. 181 shows the healing of the bone metastases in progress, while Figs. 180 and 182 the results after almost 2 years. The patient



FIG. 179. Anteroposterior view of the pelvis and upper parts of the femurs of the patient (B.T.) showing multiple osteolytic metastases.

resumed normal life for 3 years after which recurrences appeared on skin, lung and liver. These responded less favorably to the same treatment. The patient left our care and died a few months later.

Mrs. S. T., 47 years old, came under our care in a subcomatous state, 2½ years after a left breast mastectomy for an adenocarcinoma. For three months before, the patient complained of generalized pains and especially of severe headaches, and for a month had symptoms of diabetes insipidus.



FIG. 180. Lateral view of the skull of patient (B.T.) after 22 months of treatment. Most of the lesions have disappeared.

An X-ray examination of the skull, made prior to her admission (*Fig. 183*) showed extensive skull metastases with an advanced destruction of the clinoid bones.

Because of the diabetes insipidus, the urine analyses could not furnish the needed indication for the treatment and we recurred, therefore, to the number of blood leucocytes and to the body temperature, as tests able to



FIG. 181. Anteroposterior view of the pelvis and femurs of patient (B.T.) after four months of treatment, showing the lesions decreasing.

indicate the existing offbalance. With 14,500 leucocytes and a constant temperature of above 98.6°F, we considered the offbalance to be of the type A and administered conjugated fatty acids obtained from cod liver oil, and sodium thiosulfate together with posterior pituitary hormone for her diabetes insipidus. Probably due largely also to her electrolytic balance the patient regained consciousness and made a rapid recovery. In less than



FIG. 182. Anteroposterior view of the pelvis and femurs of patient (B.T.) 2 years later, showing most of the lesions disappeared.

two weeks she was out of bed and resumed a normal life. She continued with the same treatment on an ambulatory basis. An X-ray examination four months later showed a manifest healing of the previous lesions. (*Fig. 184*)

After another four months however, without any recurrence of her malignancy the diabetes insipidus could not be adequately controlled. She refused hospitalization and left our care. She died a short time later with symptoms of electrolytic offbalance.

Mr. L. N., 64 years old, had a long history of vesical troubles, with

biopsies showing cancerous lesions. In spite of repeated fulgurations, the vesical tumors grew rapidly with constant hematuria and tenesmus. Three months before coming under our care, the patient suffered severe pains in the left groin which X-ray examination showed to be due to a bone metastatic lesion. Fig. 185 depicts the lesions upon admission. The analyses showed an offbalance type A, and a treatment with conjugated fatty acid obtained from cod liver oil and sodium thiosulfate was instituted. The pain disappeared in a few days, as did the hematuria and dysuria. The patient continued to improve. An X-ray examination, four months after treatment was started, showed the appearance of a callus at the place of the bone metastases. (Fig. 168) The patient continued the treatment for a few more months after which time we lost track of him.

Heterogeneous Agents

Because of the organism's defense against the fatty acids with which it comes in contact under normal and abnormal conditions, more hetero-

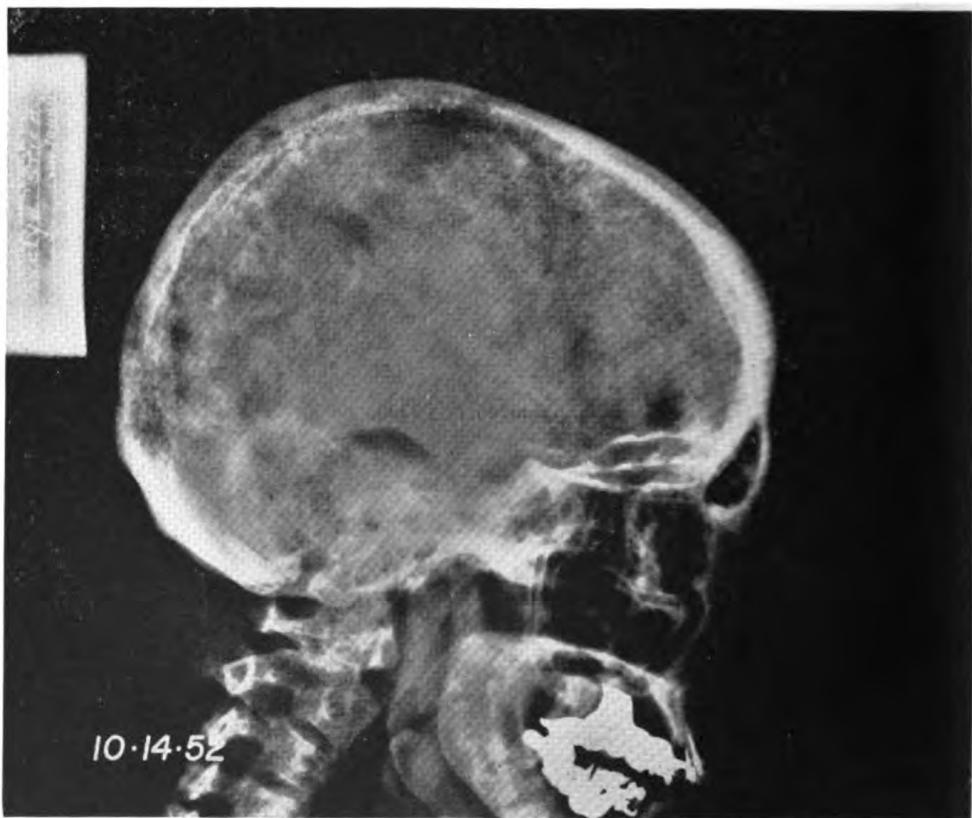


FIG. 183. Lateral view of the skull of patient (S.T.) showing multiple osteolytic processes.

geneous agents were sought. We first resorted to the alpha hydroxy fatty acids. Isolated members and mixtures of the acids, after being prepared and tested for toxicity in animals, were used in patients. We have discussed previously the striking and specific effect obtained upon lymphosarcoma in C₃H mice with alpha hydroxy caprylic acid. Although we were not able to account for this effect, we did attempt to determine whether the acid would have a similar favorable influence upon human lymphomas, especially



FIG. 184. Lateral view of the skull of patient (S.T.) showing most of the lesions disappeared.

Hodgkins' disease. Several patients with Hodgkins' disease were treated by oral administration of preparations of alpha hydroxy fatty acids from caproic to stearic acids, or with mixtures of them. Only in a few subjects were very limited effects, such as a small decrease in the lesions, observed. Changes were not considered to exceed those known to occur spontaneously in such cases. These effects were inferior to those obtained with the



FIG. 185. Anteroposterior view of the pelvis of a patient with an adenocarcinoma of the bladder, showing the destruction of the left ischion bone.

fatty acid preparations previously tested. There were no effects upon evolution of other lymphomas or in other types of cancer. Alpha hydroxy preparations produced limited subjective changes in less than 10% of cancer patients, and no marked objective changes at all.

Other heterogeneous fatty acid preparations were obtained and, after study of their pharmacological activities, were applied in humans. Polyhydroxy fatty acids, peroxides of fatty acids, and fatty acids in which chlorine was fixed at the double bonds, were tested in only a limited number of patients, but enough cases to show that effects were no different from those obtained with conjugated fatty acids, for instance.

Parallel to these efforts to find new agents for patients with the one type of offbalance, other agents for use against the opposite type of offbalance were investigated.

Sterols and nonsaponifiable fractions were treated in various ways to obtain heterogeneous substances not found in living organisms. We used heat at 300°C or ultraviolet light, according to the procedures employed by Roffo, in order to induce changes in sterols which would tend, accord-



FIG. 186. Anteroposterior view of the pelvis of patient of Fig. 185, 4 months later, showing the healing of the lesion.

ing to him (198) to make them carcinogenic. We considered these heat or ultraviolet treated sterols and nonsaponifiable fractions to represent abnormal lipoids. They were administered in oily solutions to animals and to a few patients with advanced malignancies. Injected intramuscularly daily for a few weeks, they induced no undesirable results. Their effects upon pain and systemic changes were not significantly different from those of corresponding untreated preparations. The studies of other changes in cancer patients treated with these preparations do not allow any conclusions to be drawn at this time.

We prepared and studied various sulfurized fatty acids, of which the conjugated were used on a broad scale in therapeutic trials. Clinically, these sulfurized fatty acids produced marked results in several cases, but did not constitute a significant advance over sulfurized oil in which sulfur was bound to triglycerides rather than to free fatty acids.

In the search for more active thiolipoids, we prepared several products, one of which, methylthioglycolate, was given a broad clinical trial. Although it produced some interesting objective results, they were neither sufficiently intense nor consistently reproducible nor persistent enough to make methylthioglycolate a distinct advance over the other sulfur preparations used. The compound also had the disadvantage of disagreeable odor although it is not as obnoxious as the mercaptans. As a result, we abandoned the use of this substance after a year of clinical experiments.

Of 131 patients treated with methylthioglycolate, 39—or 30% showed subjective changes and 19—or 15%, also showed objective positive changes.

Another synthetic thiolipoid, hexylthionic acid, was utilized in a few clinical cases. Only a small number of patients showed objective clinical results and these were neither consistent nor persistent enough to warrant using this substance for further research. While the results obtained with agents other than mercaptans having a thiol polar group were interesting, these compounds had too little influence upon tumors, especially in cases where the pattern indicated persistent predominance of sterols.

Selenium Preparations

We have previously noted the considerations which led us to study lipoidic compounds containing bivalent selenium. The compound used in clinical research was hexyldiselenide, a lipoid with an -Se-Se- as a polar group. Oily solutions in various concentrations were given by subcutaneous or intramuscular injection. Doses as low as 4 micrograms or as high as 400 milligrams were employed several times a day. For oral administration, capsules containing the product in solution in hydrogenated oil in amounts from 4 micrograms to 100 milligrams were employed. A short time before the experimental therapeutic use of selenium compounds, the means of recognizing the existing offbalance were implemented with the sulfhydryl index, a measure of urinary elimination of the sulfhydryl group.

Despite negative results in animal tumors, hexyldiselenide was used clinically in the hope that, with treatment guided by the data furnished by urinalyses, satisfactory results could be obtained. The sulfhydryl index served, at the beginning of this study as the principal indication for the administration of the selenium compound. Later we used urinary surface

tension as the criterion, and lately we have used the changes in serum and total blood potassium. In general, we administered the medication only if the sulfhydryl index was below 1.5, the surface tension above 68. A marked influence upon the tumor itself was seen in a relatively high proportion of cases. The results also are of theoretical interest since the compound had less influence upon symptoms at the tissue level, such as pain, or at the systemic level, and more on those at the cellular level. The effect on pain was slow to appear, often requiring days. But once relief of pain was achieved, it persisted for a long time, in contrast to the brief effect produced by other agents such as thiosulfate or hydrosulfides acting directly at the tissue level. Primarily because hexyldiselenide alters the pattern present at the cellular level, it must be emphasized that determining proper dosage at least in the beginning, appeared more difficult than for any other substance with which we have had experience.

At the beginning, we used doses in the range of 10-80 mg. but in a number of cases a persistent change to the opposite pattern occurred after one or two doses. This led to the utilization of smaller and smaller doses in order to avoid too rapid change to the opposite pattern. Also with small doses, we hoped to limit the therapeutic response to the cellular level only. We decreased the daily dose to 5 mg., then to 1 mg. and eventually even to micrograms. With these small amounts, the immediate clinical effects seemed to be almost entirely limited to the cellular level. The changes in pain and the systemic pattern were minimal. The choice of dose to be given was determined in the second part of this research primarily by urinary surface tension. It was observed that microgram doses may influence lesions without changing the values of the other analyses corresponding to the systemic level.

The clinical results obtained with the use of hexyldiselenide in humans warrants detailed consideration. Good results were obtained with greater consistency after the problem of dosage was resolved and the relationship to urinary analyses was established. Important objective changes could be achieved by using urinary analyses as a guide for dosage, as illustrated by the following cases.

A. B., 68 years old, male. In October 1949, the patient had an amputation of the left leg at the hip-joint performed at the Memorial Hospital because of a tumor of the femur. The pathological diagnosis was sarcoma. He was well until July 1955 when he began to cough and have recurrent episodes of hemoptysis. X-ray examination revealed right pleural effusion and infiltration of the right lower lobe. He was readmitted to Memorial Hospital in October 1955 and thoracentesis revealed bloody fluid. Bron-

choscopy showed partial narrowing of the right lower lobe bronchus. Examination of pleural fluid and bronchial washing by the Papanicolaou method showed cells that were suspicious for malignant disease, but not conclusive. It could not be determined whether a primary lung cancer or metastatic sarcoma was present. An exploratory thoracotomy was advised but refused by the patient, who signed himself out.

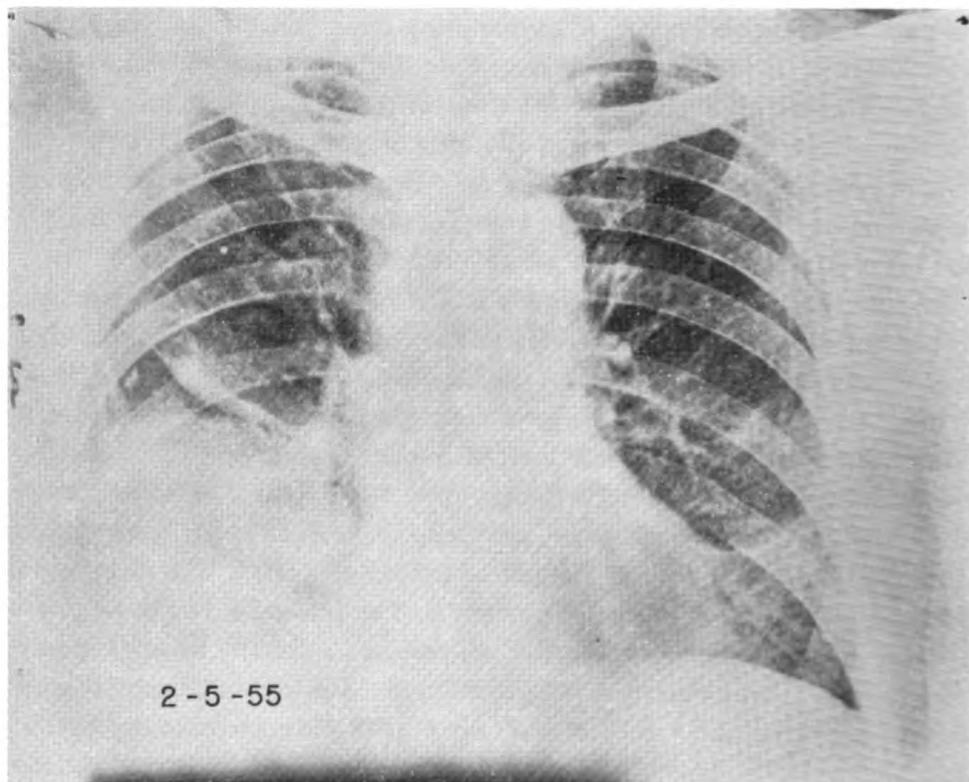


FIG. 187. Anteroposterior view of chest in Case (A.B.) at time of admission showing the presence of a mass in the lower part of the right hemithorax.

Two weeks later, he came under our care and has been an ambulatory patient since then. His chest pain had been considerably relieved with the thoracentesis and there was no bleeding. He complained of distress and tightness in the chest as well as perspiration at night. A physical examination revealed dullness in the lower right hemithorax below the seventh rib. Blood pressure was 208/165. X-ray examination revealed a large round mass occupying the right lower lobe. (*Fig. 187*)

Urinalyses showed a low surface tension, low specific gravity, high pH and low sulfhydryl excretion. Hexyldiselenide was chosen as the only chemotherapeutic agent. Treatment was started with a tenth of a milligram

twice a day, and was increased gradually to half a milligram twice a day. Chest pain and hemoptysis decreased in the following weeks. The general condition improved. He felt better and had no cough. X-rays a month and a half later showed that the right lower lobe mass was slightly smaller in size.

In January 1956, a striking reduction in size and density of the lower right lobe mass was seen. It was now about 60% as big as a month before.

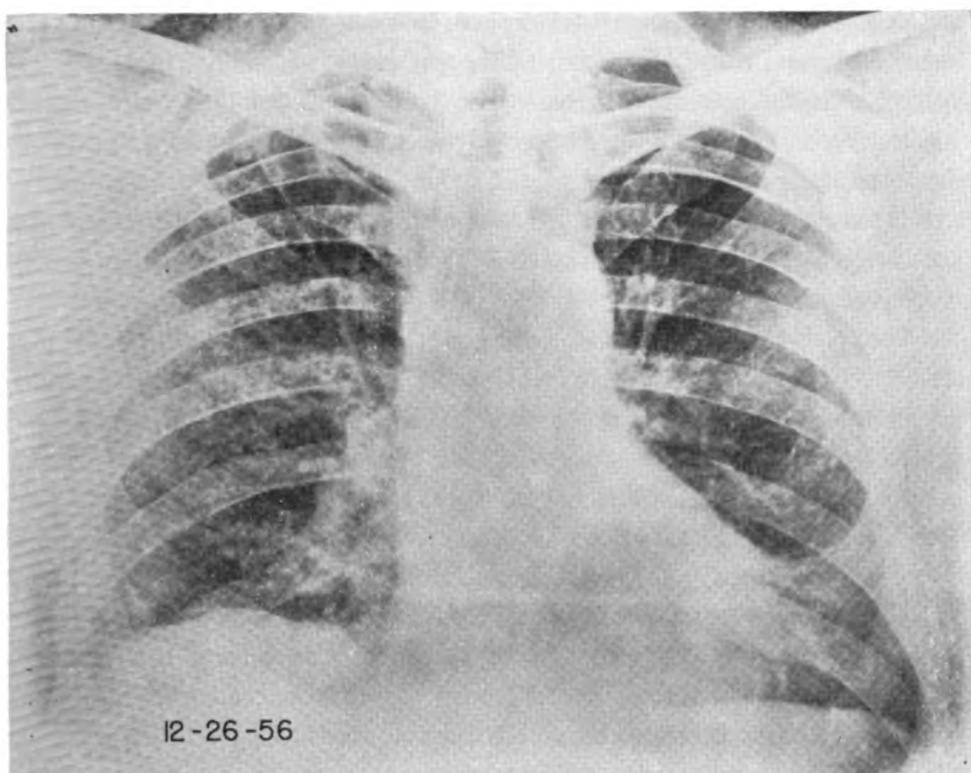


FIG. 188. Anteroposterior view of the chest of the patient A.B. after ten months of treatment with hexyldiselenide.

At the beginning of February, the mass in the right lower lobe was almost entirely gone. The interlobular thickening of the left lower lung still persisted. In April, further clearing of the right lower lobe was seen. (*Fig. 188*) The patient received treatment with hexyldiselenide for a few more months. He continued in excellent condition without further treatment for the next three years. At the end of that time, there was a recurrence of pain and the mass in the right lower lobe reappeared and grew rapidly. Treatment with hexyldiselenide was resumed. The pain disappeared again within a short time, and the tumor again regressed.

I. A.—This 58-year-old patient had diabetes beginning at the age of 30. It was controlled by insulin. At 35, he had rheumatic fever and remained in bed for 6 months. Angina developed at 53. In January 1956, he was awakened by a pain in the right side of the abdomen. The pain continued to be severe for months and the patient lost weight. Barium enema revealed nothing. He entered Jewish Hospital in Brooklyn with jaundice in April 1956. Upon operation, the gall bladder was found enlarged to twice its normal size and the common duct was dilated. With a finger inserted through the foramen of Winslow, a hard, stony mass involving the head, body and most of the tail of the pancreas, was felt. The tumor involved almost the entire pancreas. The lymph node of the common duct was enlarged. The liver showed no evidence of metastases. The general condition of the patient, in spite of the extensive involvement of almost the entire pancreas, did not justify a total pancreatectomy. Therefore, a palliative surgical procedure was done. The gall bladder was anastomosed to the jejunum.

The post-operative course was good. No other treatment was prescribed. When the patient came under our care at the end of May 1956, the jaundice had disappeared but the pain was the same as it had been before operation. A mass occupying the upper abdomen was felt. Treatment was started with a dose of 30 micrograms of hexyldiselenide a day. This dose was increased later to 300 micrograms a day. The abdominal discomfort lessened; pain in the upper abdomen disappeared. The patient remained on this treatment until 1957 when epichlorohydrin was added to the hexyldiselenide. With the mixed treatment, the patient continued to improve and the tumor, which had been palpable in the upper abdomen, disappeared after a few months. Except for diarrhea, which occurs from time to time, the patient has made a very good recovery. He has continued on hexyldiselenide and epichlorohydrin and, at present, four years later, is in good general condition. No tumor can be felt and he is at his regular job.

C. M., 52 years old, male. In 1946, the left breast of this patient was removed because of a malignant tumor. In 1947, the right breast was removed but the tumor proved to be benign. In 1954, the patient, showing blood in the urine, was admitted to the Presbyterian Medical Center where a right kidney tumor was diagnosed. In February 1955, the patient underwent surgery. A tumor of the right kidney with extensive lymph node involvement above and below the renal vessels was found. A simple nephrectomy was performed, the lymph nodes being considered inoperable. The pathological report revealed "clear cell carcinoma of the kidney with ex-

tension into the renal vessels and surrounding tissue." There was no evidence of pulmonary or other metastases.

The patient did not undergo any treatment at this time and felt well until September 1955, when he experienced increased fatigue, flatulence, slight pain in the right kidney region, and some abdominal pressure. No abdominal mass or lymph node involvement was found. He was treated with hexyldiselenide, starting with 200 micrograms a day. This dose was increased progressively until it reached 2½ milligrams a day.

Under this treatment, most of the patient's pain disappeared and no palpable mass could be found. The patient continued treatment, with the same dose, until October 1957, when epichlorohydrin was added. At first, with the new medication, the patient was more tired and there was a noticeable increase in perspiration. However, after a short time, he continued to improve. At present, he is still taking 15 milligrams of epichlorohydrin, and 100 micrograms of hexyldiselenide daily. He feels well, continues normal work, and no tumor is palpable now four and a half years since the beginning of the treatment.

W. H.—In March 1954, at the age of 11, this boy had the first of three brain operations at the Jersey City Medical Center. He had complained of persistent headaches for about a year. A brain tumor was removed and on pathological examination, was first thought to be benign, but later proved to be malignant (spongio-blastoma). By August of 1954, his symptoms recurred and a swelling appeared in the area of the scar. At the second operation, it was possible to remove only a part of the recurrent tumor. In November 1954, local swelling and headaches returned. X-ray treatments failed to give relief and a third operation was performed, but only a piece of tumor was removed. It showed the same pathology.

He came under our care May 3, 1955, and was treated, because of low sulfhydryl urinary index, with hexyldiselenide, in dosages ranging from 300 micrograms to 1 milligram per day. This treatment was continued for one year. He has done remarkably well since. He has had no headaches, is more alert and less drowsy than at any time since he first became ill. There has been no evidence of recurrence. Since treatment, there has been a definite reduction in involuntary movements of his head, extremities and body, which appear to have been related to the tumor growth. There have been no abnormal manifestations to date, five years since the beginning of the treatment. The patient goes to school and engages in all the activities of a normal boy of his age.

Mrs. A. L.—This patient first noted a lump in her neck in the summer of 1953 when she was 26 years old. In February 1954, a second lump

appeared. Surgery was performed at the Ottawa Civic Hospital. The pathological examination showed a cancer of the thyroid (papillary adenocarcinoma). It involved both sides of the gland and many of the lymph nodes. The entire thyroid gland was removed and bilateral lymph node dissection was done in two stages. Since the surgeon felt that he had not removed all the affected areas, the patient received deep neck X-ray therapy following the operation. However, by July 1954, a new mass developed on the right side of the neck and this was removed in November. The pathological examination proving it to be the same type of tumor.

The patient was first seen by us on January 10, 1955. Although it was only two months since the last operation, there were several recurrent tumor masses in the lateral right side of the back of the neck, as well as infiltrations into the area of the last operative wound. The patient was treated with hexyldiselenide, in doses ranging from 300 micrograms to 1 milligram daily. Under this treatment, the masses progressively decreased and, after 3 months, disappeared. She continued the treatment for another six months. Now, after 5½ years, she is feeling well, has had no recurrences, and is carrying on her usual activities as a housewife and mother.

Hexyldiselenide, although it produces impressive results, very often is not of itself able to provide enduring benefit. Many other patients have had recurrences, some in spite of impressive first results and continuation of treatment as shown in the following observation. J. D., 10 years old, came under our care in a preterminal state, after an exploratory laparotomy revealing an extensive carcinoma of the liver. (*Fig. 189*) The response to hexyldeselenide treatment was impressive with the patient making a perfect recovery. His liver which had filled the abdomen, returned to normal dimensions. The patient continued a normal life for 2 years when, in spite of the continuation of the treatment, generalized recurrences appeared. These could no longer be controlled.

Tetralin Perselenide

The good effects obtained with persulfides on one hand, and with selenium on the other, have led us to investigate the corresponding compound—tetraline perselenide. With its low toxicity, the compound was administered to humans in which the destruction of the tumor appeared the immediate aim. The effect upon pain was good although not immediate, the same as for the systemic level. It was at the cellular levels where these were the most manifest. Research with this agent is still in progress and for the moment the influence exerted upon the tumors seems to be very fa-

vable. Similar good results were obtained with naphthalene perselenide and other similar preparations of aromatic hydrocarbons.

Parallel to studies with synthetic negative lipoids, synthetic positive lipoids were investigated. The problem was quite different because of the fundamental differences in biological roles of the two antagonistic groups. Among negative lipoids we singled out an effect against sterol predominance and a destructive activity through the induction of rapid cellular aging.

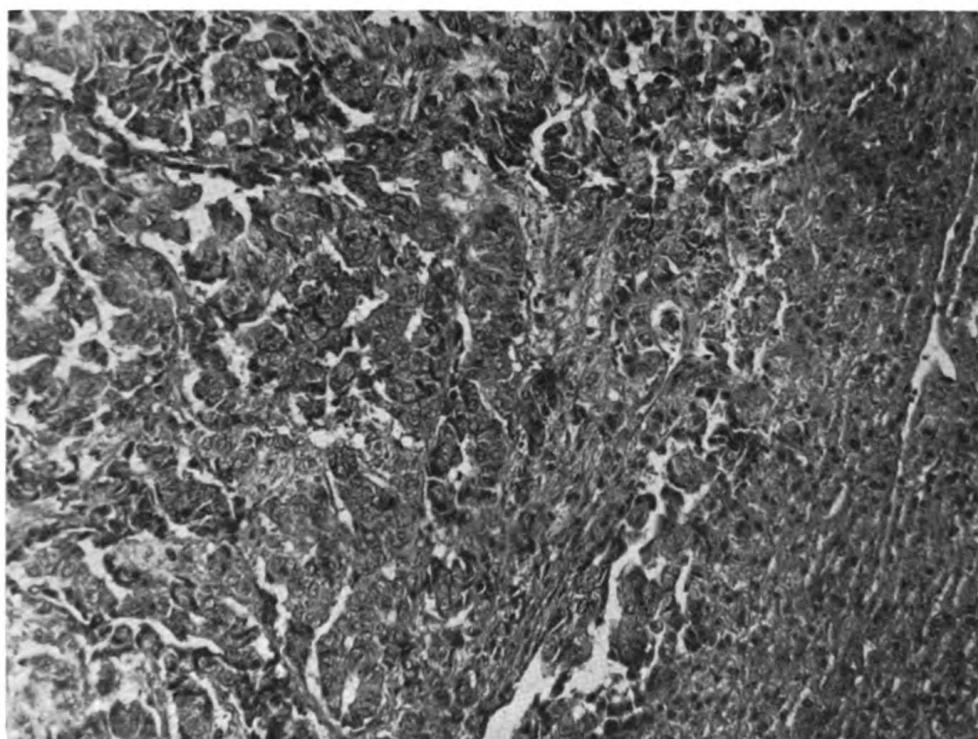


FIG. 189. Photomicrograph of a liver biopsy of patient J.D.

Among the positive lipoids, we sought agents able to correct the noxious effects induced by fatty acids. As noted previously, this led to a search in one group of experiments for substances with higher specificity for binding particular fatty acids and in other experiments for substances with broad spectrum acting against acid lipids in general. We have discussed previously the pharmacodynamic characteristics of many of these synthetic positive lipoids. Clinical results have improved with the development not only of new substances but also of new means of recognizing the pattern present and of following the changes that take place. We have mentioned the results

obtained with butanol, the first of this group of synthetic lipids with positive character to be used in humans.

Butanol was unable by itself to influence the growth of tumors, although it was effective in controlling pain of an alkaline pattern. This beneficial effect upon pain was still more manifest for other aliphatic alcohols, and especially for heptanol. In high doses, however, heptanol probably acts beyond the tissue level. Besides influencing pain, it induces severe edema and changes in the evolution of the tumor. It has little influence at the systemic level upon hemorrhage, even with high doses. For tumors with offbalance of type D, a mixed treatment appeared indicated. The use of heptanol-butanol, however, was not fully satisfactory although it has been employed in some cases with good clinical results.

During the progress of this research, it became increasingly important to have an accurate knowledge of the existing offbalance, and of the adequate use of the available therapeutic agents. The following observations show how the results obtained by the treatment are a function of the correct application of this concept of guided chemotherapy.

M. S.—This 52 year old woman started to lose weight in September 1953 and her abdomen became very distended several months later. An exploratory laparotomy was done in July 1954 at Brooklyn Hospital and revealed a large mass in the lower abdomen with metastases. A lymph node biopsy was performed and showed lymphosarcoma. (*Fig. 190*) Subsequently, she had 36 X-ray treatments, followed by another course of 12 X-rays, the last in December 1954. She felt relatively well until the first week in February when abdominal pains recurred. A mass in the middle abdomen, the size of a large grapefruit, could be felt.

Under treatment with sterols, the tumor first increased slightly in size so that by the middle of April, it extended, filling up the entire left side of the abdomen. At the end of the month, the pain became stronger while the abdominal mass remained unchanged. The patient was treated, according to her analyses, with 1 mgm. of hexyldiselenide daily. The tumor became much reduced in size. Pain recurred at intervals and, although only a small mass was still palpable after 1½ years of treatment, the patient's general condition started to deteriorate at that point. Analyses then revealed a change in the pattern present. The treatment was changed to a mixture of higher alcohols—octanol, heptanol and polyconjugated alcohols. The tumor rapidly regressed and, at the beginning of 1957, the mass had entirely disappeared. She continued in good general condition until August 1957 when she had a coronary occlusion. At present, two years later, the

patient is in good general condition and without any sign of recurrence of the tumor.

In this case, the first treatment with sterols, using urinary pH as a criterion, brought some subjective improvement but this could also be considered to be the result of the radiation. The improvement was transitory and the appearance of more symptoms, probably reflected the waning effect of radiation. The change to hexyldiselenide brought improvement but could

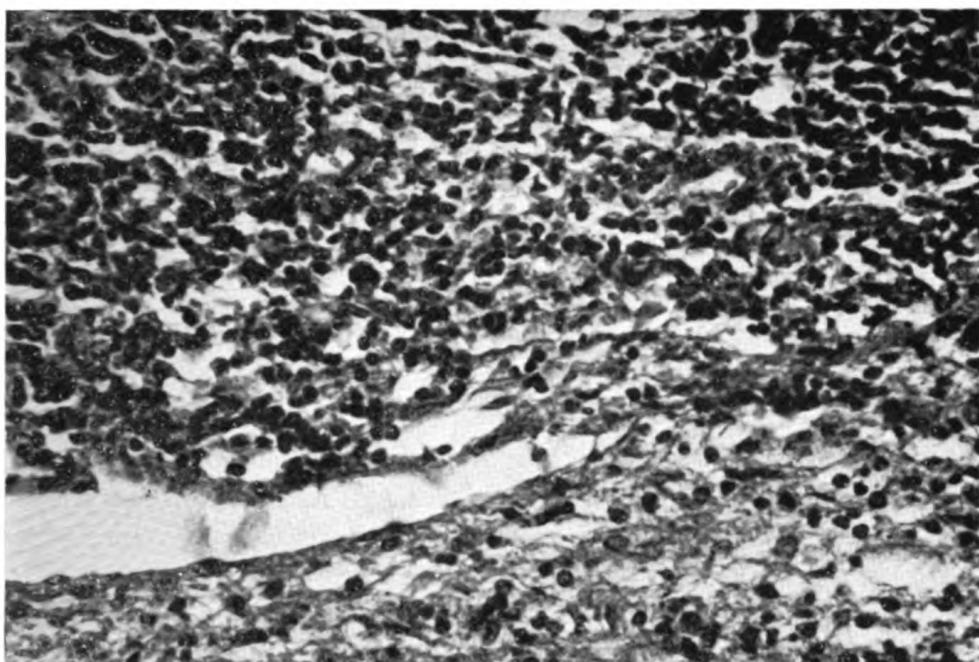


FIG. 190. Photomicrograph of a lymph node of patient M.S. showing a lymphosarcoma.

not entirely control the condition. It was with higher alcohols that important objective as well as subjective changes were achieved. The tumor decreased rapidly. Interruption of treatment, due to the cardiac condition, did not seem to influence the favorable progress of the condition.

M. B.—Toward the end of 1950, at the age of 45, the patient began to complain of right lower quadrant and intermittent left upper quadrant pain. She discovered an abdominal mass by herself. In February 1951, an exploratory laparotomy was performed at Lenox Hill Hospital with the following findings: "Situated in the left side of the pelvis, a huge cystic structure the size of a football was found. The superior wall of this mass was attached to the mesentery of the loop of the small bowel. The anterior

cyst was attached to the posterior leaf of the broad ligament and contained the left ovary. On the ovary and within the wall of the cyst were several papillomatous structures. On the right side of the pelvis was another cystic mass containing a greenish turbid fluid. This cyst contained the right tube and ovary and on its walls were several papillomatous projections. The parietal peritoneum, the liver and omentum were studded with tumor implants and several sections of bowel were matted together by the same tumoral tissue. Biopsy specimens were obtained from several areas."

The gross pathological diagnosis was papillary carcinoma of the ovary with metastases to peritoneum, liver, intestines and duodenum. The report on the frozen section was malignant. Microscopic examination confirmed the diagnosis given at the time of the operation and revealed a fibroid fatty tissue extensively infiltrated by a malignant neoplasm of epithelial origin.

The patient came under our care 11 days after her operation. The abdominal mass was palpable, rising from the pelvis to a level slightly above the umbilicus. Vaginal examination revealed large cystic masses filling both fornices and cul-de-sac. The pelvic structures were partially fixed. The urinary specimen showed a pH between 7.4 and 7.8 and the surface tension was 68 to 71 dynes/centimeter. Treatment was instituted with a mixture of conjugated fatty acids, derived from cod liver oils, and a preparation of hydopersulfides as well as sodium thiosulfate. The first preparation was administered intramuscularly in a 5% oil solution in doses of from 2 to 4 cc. daily. The sulfur preparation (corresponding to 0.5% sulfur) and 10% solution of thiosulfate were administered in doses ranging up to 6 cc. daily. The treatment was continued without change for 1 year and 8 months. The abdominal mass was found somewhat smaller a month after starting treatment. A month later, a surgeon's report showed that the tumor was about $\frac{1}{3}$ the size at the time of operation. After several months, no mass could be felt on abdominal and vaginal examination. After twenty months, the treatment was discontinued for one year. At the conclusion of this period, the patient began to relapse and a cystic mass in the cul-de-sac, palpable under finger examination, was found. Treatment with the same medications as before was instituted but did not change the dimension of the recurring tumor. Despite treatment, the tumor continued to grow and in April 1954, the abdomen was distended. X-ray also revealed a large liver and elevation of the right diaphragm. The patient was readmitted to Lenox Hill Hospital where a small abdominal incision revealed two masses, one above the liver and another one in the lower abdomen. Fluid reaccumulated rapidly and the patient's general condition became poor. On several occasions it was necessary to tap the subdiaphragmatic cyst and

lower abdomen separately as emergency procedures because of acute distress.

Because analyses now showed a low pH (around 5.2) and low surface tension (around 61 dynes/centimeters), and also because she had obviously failed to respond to conjugated fatty acids, treatment with butanol and nonsaponifiable lipids of intestine was instituted. By July the situation appeared to have been brought under control again. Fluid was no longer

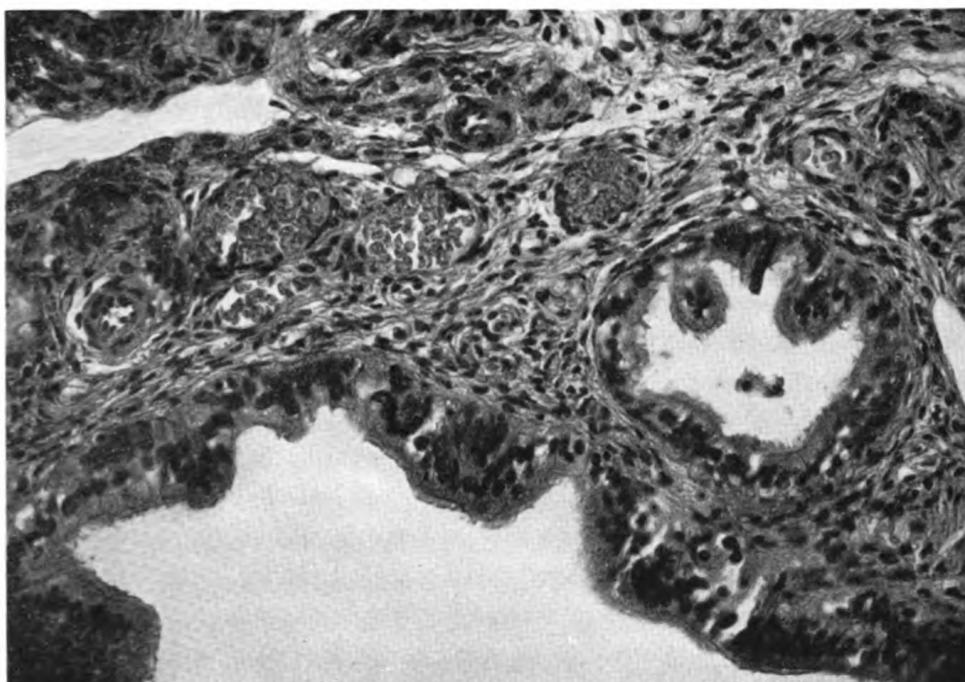


FIG. 191. Photomicrograph of a lymph node biopsied from patient M.B. showing metastatic adenocarcinoma.

accumulating and leg edema, which had been pronounced, was gone. At the end of 1954, no masses could be felt. The subjective complaints disappeared slowly. In February 1955, the nonsaponifiable medications were replaced by heptanol and octanol. The patient continued to improve with this treatment. In March, the abdomen was a little swollen. By the end of April, a mass could be found in the abdomen just above the umbilicus. At the end of May the mass had increased and the patient was hospitalized for about three weeks. A large bloody cyst was drained. A small lymph node was biopsied and microscopic examination showed the same metastatic adenocarcinoma. (Fig. 191) The patient was put on epichlorohydrin and the mass did not grow back. She has remained on epichlorohydrin

and for four years has had no subjective discomfort. No mass can be felt in the abdomen and the patient is well now.

Radiation and Chemotherapy

Parallel to the guided chemotherapy, the idea of mixed radio- and chemotherapy has led us over the years to several tentatives. In one of them, in collaboration with Dr. L. Goldman on a larger scale, chemotherapy was added to radiotherapy. (*Note 1*) In spite of the interesting immediate results, if chemotherapy was not further pursued, the ultimate fate of the patients thus treated was no better than that of the patients who had received radiation alone. Analysis of these various attempts has permitted us, however, to establish the conditions under which such a mixed treatment would appear especially indicated. Consequently, it is the type of offbalance present which is seen to determine the relationship between the two therapies, and respectively the procedure to be followed. The same relation has permitted to define the conditions under which chemotherapy would be indicated and added to radiotherapy. It should not be forgotten that through its action upon the fatty acids, radiation represents indirectly an agent of the same group to be added as the negative lipoids.

The cases with a type A offbalance which seem to respond insufficiently to medication with agents, represent an indication for the mixed treatment. Acting in conjunction with radiation, chemotherapy has appeared especially active. Chemotherapy started before, is continued actively during radiotherapy and following radiation, for a long time, even for years. The radiation acting as an adjuvant agent is usually applied in small doses, from only several hundred r to a few thousand r, in general much below those dosages known to induce by themselves alone therapeutic effect upon tumors. In cases with type D, as the radiotherapy represents an agent increasing the actual offbalance, an added chemotherapy with positive lipoids would have the role of correcting not only the existing manifestations but of preventing some of those which would be induced by radiotherapy. It would in fact have the specific aim to permit the continuation of radiotherapy while limiting its noxious influence.

The mixed therapy has to be guided by the routine test, with frequent analyses, in view of the rapid important changes which have been seen to occur. In these cases, it appears useful to follow especially the changes of the chloride index, those of peroxides in the urine which together with the surface tension, are seen to be not only particularly influenced by radiation but able to indicate the important change in offbalance.

Exceptionally good results were seen in the cases in offbalance type A treated by this mixed method when chemotherapy has been added to radiotherapy. These cases are especially interesting because of the persistent result obtained. The abnormally small amount of radiation often used, applied only on a localized spot, has served in these cases as an adjuvant to help to resolve particular local problems, while the chemotherapy, continued for a long time thereafter, to prevent the appearance of recurrences.

In the case of offbalance D, as the radiotherapy will increase the actual offbalance and the role of chemotherapy is to reduce the intensity of this offbalance and thus permit the continuation of radiation for its direct target effect the results are less interesting, differing less than in the first case from those obtained by radiation alone. The continuation of chemotherapy however after the radiation has been completed, has appeared especially helpful.

Level Chemotherapy

In recent years, the course of our research has been directed parallel toward the precise application of available agents as well as finding new substances. For it has become increasingly evident to us that it is the basic independence of the different levels of organization in the body which represents the critical factor in the pathogenesis of cancer and its manifestations and which is also critical to the most effective use of therapeutic agents. This concept has led us to focus our research efforts in certain areas. Attempts are made to find criteria capable of indicating changes at specific levels of organization. Similarly, a growing awareness that each element belongs to a specific level of biological organization and exerts its biological activity primarily at this level has led us to try to use specific elements in correcting dysfunctions at various levels. Because of the independence of the levels, we have attempted to trigger the defense mechanism at appropriate individual levels rather than indiscriminately. This is especially important when the failure of the defense to operate at some one level represents a major factor in allowing pathological states to advance.

While each of these developments has been important, it has been the use of all three which has brought marked progress in the clinical application of the method in the last years.

With the concept of independence of levels, the use of more than one agent is not simply a matter of synergistic activity, but rather of employing several agents, each capable of acting at its proper level in complex conditions where more than one level is involved. To make this possible, it

has been necessary to relate analytical data to changes occurring at specific levels. For example, changes in potassium in serum and red cells have been related to cellular level abnormalities; urinary surface tension changes, to metazoic; specific gravity to systemic; urinary pH and blood eosinophiles, to tissular. This has guided the choice of agents: selenium preparations, epichlorohydrin and heptanol for the cellular level; sulfurized hydronaphthalenes, lipoacids, unsaponifiable fractions and glycerol for the tissue level; magnesium and sodium thiosulfates, propionic aldehyde and butanol for the organ and organism levels. Through this approach, therapy has evolved toward what we can now consider to be *biologically guided level-chemotherapy*.

The following observations show the role of these level indications in the conduct of the treatment.

F. R., in March of 1957, had a left mastectomy for an adenocarcinoma of a mammary gland. In July of 1958, she began to experience pain in the lower back and legs. In the three months prior to coming under our care, several skin nodules and some progressive difficulty in breathing was evident and there was a loss of 25 lbs. in weight. On admission, multiple skin and subcutaneous lesions, some of them measuring 3-4 cm in diameter were found, as well as 3 or 4 nodules in the right breast. The ambulant patient started treatment with selenium and epichlorohydrin. Although treatment was followed with irregularity, improvement was seen in the local lesions. In December, 1959, however, the difficulty in breathing had markedly increased and oxygen administration was needed. In less than a week her condition worsened. The treatment with more selenium and epichlorohydrin appeared unable to control the situation. She was admitted to the hospital in extreme dyspnea. The suppression of oxygen for even a few minutes was followed by convulsions. Radiological examination showed a very limited exudate especially in the right hemithorax, but multiple metastases in both lungs. All the analyses showed an offbalance of type A. Considering the condition as manifested also at the organic and systemic level, to the treatment with epichlorohydrin, acting at the cellular level, we added bixine and propionic aldehyde. The first agent was considered to act at the tissular level and the latter at the systemic level. With this treatment the patient made a very good and constant recovery. In less than two weeks it became unnecessary to use oxygen and her general improvement progressed so that 3 months after treatment was started, the patient was able to resume part of her housework. With mixed treatment—epichlorohydrin acting at

the cellular level, and bixine and propionic aldehyde at the organic and systemic level—the condition seems controlled. The pulmonary metastases seen in previous X-rays have also disappeared and all the skin nodules decreased rapidly, most of them having completely disappeared. She is at present in good state of health, doing all her own housework.

Mrs. C. H., 60 years old, came under our care two years ago with a history of lymphosarcoma with two positive biopsies of the inguinal glands, complaining especially of pain in the abdomen. On examination, inguinal, axillar and cervical glands were present, some of them 4 cm in diameter. With treatment of sodium thiosulfate and fatty acid hydrosulfides in relatively small doses, the pain was sufficiently controlled. After two months of treatment, the patient experienced extreme pain and a tumor of the head of the right humerus. In view of the local lesions with analyses which all showed an intensive offbalance of type A, the treatment was changed to epichlorohydrin and heptyldiselenide. Administering these agents in relatively high doses, it was possible to not only control the pain in a few hours, but to have the lesion disappear in less than two weeks, as did also all the abnormal glands. However, in spite of these objective very good results, the general condition became unsatisfactory, especially with marked weakness. Changes in the doses of medication or the temporary discontinuation of the medication, failed to correct it. It was only when propionic aldehyde considered to act upon the organism level was administered, that a rapid change toward a feeling of general well-being was obtained. At present, with small doses of propionic aldehyde the patient is entirely free from any subjective or objective abnormal manifestations and has been so for almost a year.

Mrs. E. R., 42 years old, had a radical mastectomy for an adenocarcinoma of the breast. After 1½ years, she experienced persistent back pain which was first diagnosed as arthritis. X-ray studies and a myelogram made at Montefiore Hospital showed two metastatic lesions at the 1st and 2nd lumbar. Surgical intervention was thought to be not indicated. X-ray therapy neither relieved the pain nor made it possible for her to leave her bed. At admission under our care, the patient was in severe pain and unable to even turn in bed, although she was still able to move her legs and their sensitivity was conserved. A treatment with heptyldiselenide, propionic aldehyde and bixine made the pain disappear and up to date the patient is leading a normal life, after having been bedridden for 8 months.

Mrs. M. McB., 62 years old, came under our care for a basocellular carcinoma of the left side of the face, near the inferior eyelid. Advised to

undergo surgery, which would enucleate the left eye, she refused the operation. A biopsy was performed (*Fig. 192*) showing the presence of a basocellular carcinoma. She was treated with hexyldiselenide and with sodium thiosulfate, and the lesion disappeared within a few weeks. No recurrence was observed during the past 6 years.

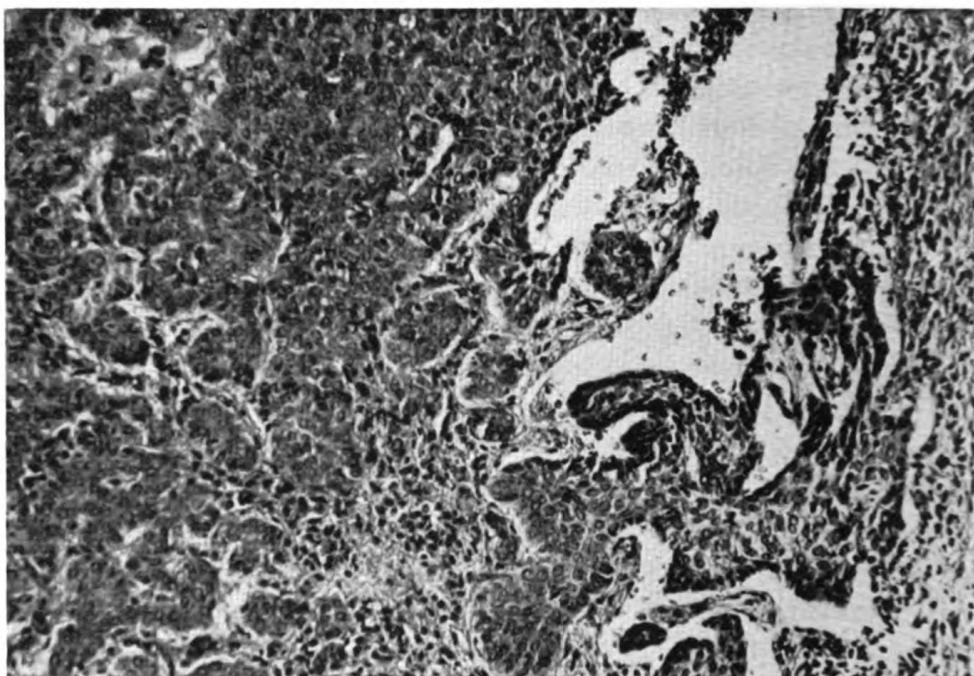


FIG. 192. Photomicrograph of the biopsy made on patient M.McB. showing a basocellular carcinoma.

Mr. G. Z., 56 years old came under our care for a lesion of the upper lip. Biopsy proved it to be a squamous cell carcinoma. (*Fig. 193*) The patient refused surgery or radiation which we advised. Following the biopsy the tumor started to grow rapidly, as seen in Fig. 194. According to the analyses, the patient was treated with fatty acid hydrosulfide. The lesion disappeared in less than 2 weeks. (*Fig. 195*) No recurrence was observed although the patient was no longer under treatment. The patient died 8 months later from a coronary occlusion.

The ability to induce changes at single specific levels has altered completely what was once a gross dualistic approach. Until recently, we had considered it inadvisable to induce therapeutic changes at a single level if imbalances were present at more than one level. With the concept of level independence, it has become part of the method to attempt to influence



FIG. 193. Patient G.Z. before treatment.



FIG. 194. The tumor grew rapidly following biopsy.



FIG. 195. Patient G.Z. after treatment.

the level in which the predominant abnormality is present rather than to induce gross changes at all levels. In cases with general offbalance A but predominant at the cellular level, therapy to induce offbalance D limited to the cellular level alone, with the metazoic remaining at offbalance A, has produced interesting objective clinical results. Similarly, good results have been obtained in cases with predominant offbalance D at the cellular level and in the metazoic compartment, with treatment directed at changing the systemic and tissular offbalance D into type A without influencing the offbalance D at the cellular level. It is interesting to note that in both types of cases the changes lead ultimately to the same overall pattern of offbalance—D for the cellular level and A for the metazoic compartment.

Level chemotherapy with selenium, heptanol, propionic aldehyde and other agents, has given highly gratifying results. It led us to the present form of treatment, with particularly good results. In a variety of tumors, some impressive because of their huge dimensions or because of their high degree of malignancy, the immediate objective response has been striking. Massive lung metastases from breast cancer, generalized abdominal metastases from colon carcinoma, bone metastases from breast or prostate, and metastatic melanoma are among the cases which have responded and we are now waiting for time to indicate whether the results have lasting value.

While we were convinced in the past of the inherent potentiality of the method in general, because of results with many different agents and criteria, we have been greatly encouraged by the most recent applications. We believe the method now provides a means of controlling a significant proportion even of preterminal and terminal cases of malignancy considered otherwise entirely beyond any hope. The particularly favorable results obtained in the cases which came under our care before the disease had progressed to advanced stages indicate that we are fully entitled to prefer this therapeutic method even for those cases where the presently used procedures might still have a chance to help.

CHAPTER 16

PRESENT FORM OF TREATMENT

THE THERAPEUTIC MEASURES against cancer which have evolved thus far out of the pathogenic and pharmacodynamic concepts described in these pages have proved, as we have shown above, to be useful. All along in their development, they have fallen short of producing uniformly, the full measure of benefit which, we have continuously hoped, one day will be attained in most and possibly in all cases of malignancy.

Yet the results achieved with biologically guided therapy, imperfect as they have been, have continuously indicated the great potential of the method.

What has been accomplished during the years has been hardly considered as a final measure of the effectiveness of this basic new approach to treatment but only of the available criteria and agents. With the criteria and agents the method itself has been evolving. In its present form, the method represents a valuable tool for helping many cancer patients who are entirely beyond help with present day methods. In spite of its accomplishments further advances along the same lines—both in techniques for recognizing fundamental offbalances at different levels of organization and even in new compounds more effective in correcting them—are to be expected.

Equal in importance with the current results with biologically guided therapy is the fact that new applications are evolving; that this approach helps us to better understanding and treatment of malignancy and of other pathological conditions as well; and that, by its very nature, the method has the capacity to furnish the guidance needed for improving it.

We are presenting here the form of treatment which we currently use.

In this form, when correctly applied, biologically guided therapy can, in many cases, bring under control even far-advanced malignancies. The importance of the correct application of agents based upon specific criteria makes it necessary to emphasize how agents are chosen and used.

Criteria Used: Recognition that no test is able to indicate by itself the existence of more than an offbalance at a specific level, and that cancer is a complex condition involving many levels, has led to the use of a group of tests able to offer the necessary information on offbalances at different levels. We use the following routinely:

- Urinary—specific gravity
- pH
- surface tension
- calcium excretion index
- Blood— potassium in serum
- potassium in total blood
- Pain pattern
- Body temperature

In exceptional instances when the routine tests do not suffice, we use:

- Urinary—chloride retention index
- Blood— chlorides in serum
- leucocytes count
- eosinophiles count

The offbalance indications provided by these tests are shown in TABLE XXII.

TABLE XXII

Test	Offbalance D	Average Values	Offbalance A
Specific gravity	high	1.016	low
pH	low	6.2	high
Surface tension	low	68	high
Serum potassium	high	4.5 mEq	low
Total blood potassium	low	38 mEq	high
Body temperature	low	37°C	high
Leucocytes	low	7000/cmm	high
Eosinophiles	low	100/cmm	high
Chloride index	high	2.1	low
Calcium index	low	2.5	high
Chlorides in serum	high	525 mg %	low
Pain pattern	alkaline	—	acid

Whereas the other analyses give direct information concerning the off-balances, the values of potassium in serum have to be correlated with the values in red cells or in total blood potassium for a recognition of the offbalance present. A serum potassium above 4.5 mEq and a low total blood potassium below 38 mEq indicate an offbalance D, while a low serum potassium below 4.2 mEq with a high total blood potassium above 40 mEq, an offbalance A. Low values for both analyses indicate a quantitative deficiency of this element in general, while high values for both an excess of this element in the organism.

As related to the different levels of the organization, specific gravity changes reflect processes taking place especially at the systemic level. The pH reveals, indirectly, changes at the tissue level. Surface tension indicates offbalances at the metazoic compartment; both serum and total blood potassium indicate cellular level offbalances. However, while potassium measurements give an indication of cytoplasm changes, calcium indicates cellular level changes, especially in the membranes. Eosinophile counts indicate acid-base changes at the tissue level, and temperature, systemic off-balances.

As we have seen, any abnormal value is most likely to accurately reflect a fundamental offbalance at its corresponding level when the same value is obtained in several repetitions of the test. Tests on two or three successive days are most helpful in ascertaining the existence of an offbalance, especially if it is limited to only a few levels.

The offbalance determines which agents are to be used.

As anti-A agents, we now use:

- propionic aldehyde
- epichlorohydrin
- hexyl or heptyl diselenide
- tetralin perselenide
- tetralin persulfide
- sodium or magnesium thiosulfate
- mixture of lipoacids

As anti-D agents, we use:

- heptanol
- polyunsaturated alcohols
- butanol
- glycerol
- insaponifiable fraction of liver
- glycerophosphoric acid

The preparations and usual doses are:

Propionic aldehyde: 10% sol.—for oral administration, used from 50-1000 mgr. daily; for parenteral administration: 2% sol. in saline.

Epichlorohydrin: 0.5% solution in isotonic saline, used from 1/10 mgr. to hundreds of milligrams daily for parenteral and oral administration.

Hexyl or heptyl diselenide: in solutions from 20 micrograms to 50 mgr./cc. for intramuscular injection. Orally, we use either capsules containing from 10 micrograms to 10 mgr. or, still better, a solution of 0.4% in oil, which corresponds to 100 micrograms per drop. The doses vary from 10 micrograms to 50 mgr. or more daily.

Tetralin perselenide: This preparation has 40 mgr. of selenium for 100 gr. of tetralin and is used orally as drops, from a solution of 10% or 1% in oil. For injections, a solution containing 10% in 100 cc. of sesame oil, is used in doses ranging from 0.1 cc. to 10 cc. daily. Instead of tetraline, naphthalene or other aromatic hydrocarbons are used. One cc. of a 10% solution of these preparations in oil, contains 0.1 mg. of selenium; the doses used range from 0.1 cc. to 10 cc. daily.

Sodium or magnesium thiosulfate is used in a 10% solution for oral administration or in a 4% solution for intramuscular injection in doses ranging from 25 mgr. to 5 grams or more daily.

Tetraline persulfide: This preparation contains 5% sulfur in oil and is used orally from $\frac{1}{2}$ cc. to more than 10 cc. daily. In intramuscular injections it is used from $\frac{1}{10}$ cc. to 2 cc. daily.

The acid lipids mixture is a solution in oil of 1% bixin, 2% cow liver lipoacids and 7% cod liver oil fatty acids. It is administered in doses of from $\frac{1}{2}$ cc. to 6 cc. daily by intramuscular injection.

Heptanol in a solution of 0.5% or 5% in oil is used for parenteral administration; a solution of 5% in oil is employed for oral administration. The doses range from 1 mg. to hundreds of mgs. a day.

Polyunsaturated alcohols are safflower oil fatty acids with the carboxyl changed to a primary alcohol. They are used in a 10% solution in oil with $\frac{1}{4}$ to 2 cc. injected intramuscularly up to four times a day.

Butanol in a 6.5% water solution is used for oral administration. A 6.5% saline solution is employed for parenteral administration. Usual doses are from $\frac{1}{4}$ cc. to more than 100 cc. daily.

Glycerol is used as a 50% solution for oral administration or as a 20% solution for parenteral administration in doses ranging from less than 0.2 gram to a few grams daily.

Insaponifiable fraction of pork liver in a 5% solution in oil is used for intramuscular injections in doses from $\frac{1}{2}$ cc. to 8 cc. daily.

A preparation of a mixture of polyunsaturated alcohol, heptanol and butanol also is used for oral or parenteral administration.

If, during treatment, any other medications appear necessary for various concomitant conditions, it is preferable to choose them with consideration given to the influence they will exert upon the patterns present.

TABLE XXIII shows the effects of some commonly used therapeutic agents upon the fundamental offbalances, which would indicate their use in cases with one or the other offbalance.

TABLE XXIII
EFFECTS UPON OFFBALANCES A AND D OF VARIOUS AGENTS
USED THERAPEUTICALLY

<i>Having an anti A effect</i>	<i>Having an anti D effect</i>
Dicoumarol	Glycerol
Digitalis	Glucose
Antipyrine	Coramine
Aminopyrine	Acetyl salicylic acid
Acetophenetidine	
Atropine	Procaine
Quinine	Codeine
Caffeine	Morphine
Liver extracts	Demerol
Vit. A, D, B ₆	Aminophylline
Testosterone	Iron
Epinephrine	Vit. B ₁ , B ₂ , K, E
Penicillin	Progesterone
Streptomycin	Stilbesterol
Aureomycin	Desoxycorticosterone
Sulfas	Glucosamine
	Cortisone
	Insulin
	Barbiturates
	Mercuhydrine
	Benzedrine
	Benadryl

In using the various agents, we have to keep in mind the effect upon the existing offbalances.

Conduct of Treatment: Although there may be some variations depending upon the circumstances of individual cases, treatment is conducted generally as follows:

Urine and blood analyses are performed. Values for two or three consecutive days are usually determined before starting treatment. If all values indicate the same pattern, the diagnosis of the offbalance is clear. If the analyses indicate the presence of different patterns, an interpretation is made on the basis of analyses showing the offbalance at specific levels. For patients in whom manifestations involve the metazoic compartment, urinary surface tension is the important criterion. Urinary specific gravity and urinary pH, are important when systemic or tissular manifestations are present. Potassium is the criterion when changes at the cellular level are most important. The body temperature and the other complementary analyses are used when discordant patterns are encountered. They help to recognize the type of offbalance at the different corresponding levels.

Once the level offbalances are determined, suitable treatment is instituted. The agent is chosen from the proper group according to the level

TABLE XXIV
AGENTS CHOSEN ACCORDING TO THE TESTS

Level	Test	AGENTS	
		Offbalance A	Offbalance D
Cellular	Potassium in blood	Selenium preparations	Heptanol
	Urinary calcium	Epichlorohydrin	
Tissue	Urinary pH	Lipoacids	Polyunsaturated alcohols
	Blood eosinophiles	Tetralin persulfides	Unsaponifiable fraction
	Surface Tension	Hydopersulfides	liver
	Pain pattern	Mg Thiosulfate	Glycerol
Organ and Organism	Urine specific gravity	Mg or Na Thiosulfate	Butanol
	Surface Tension	Propionic aldehyde	
	Body temperature		Glycerophosphoric or lactic acid

to be influenced. Since, in invasive cancer, the offbalance occurs at the cellular level, the agents used are selenium preparations and epichlorohydrin, for the offbalance A and heptanol for the offbalance D.

For the tissue level, indicated by pain as well as by urinary pH or eosinophiles, lipoacid preparations, Mg thiosulfate and Tetralinpersulfides are used for offbalance A, and polyunsaturated alcohols, unsaponifiable fractions and glycerol and butanol for offbalance D.

For the organ and systemic levels, with the offbalance recognized through urinary specific gravity, surface tension and body temperature, magnesium or sodium thiosulfate and propionic aldehyde are used for offbalance A, and glycerophosphoric acid for offbalance D. (Table XXIV)

Conduct of Treatment

If the patterns of the different tests and clinical manifestations are concordant, concerning the offbalance present, the agents chosen are from the respective group. Special attention is given however to the level which, clinically or analytically, shows the most abnormality, so that for a patient with a limited tumor, but without pain and in good general condition, the factor guiding the therapy will be the analyses related to the cellular level (as revealed by the potassium in blood). If the general condition is poor, indicating rather a predominant systemic condition, the treatment will be directed especially by the abnormalcy at the organism level revealed by the corresponding analytical tests—such as urinary specific gravity, surface tension and body temperature.

This interpretation of the most needed intervention becomes still more important when the data obtained—clinical and analytical are discordant. The treatment will follow the indication furnished by the level which appears the most important. In a case with a limited tumor, and no other clinical manifestations, the pattern of the cellular level, will determine the nature of the treatment, even if the other analyses show different patterns.

For a patient with a tumor and severe pain—it is the pattern of the pain which will indicate the agent to be used—even if this is discordant with that of other analyses. The same is true for a systemic severe condition, the respective analyses determine the agent to be used, even if these are discordant with the analyses concerning the other levels. In general, the decision of what level will represent the guiding factor in the treatment, represents seldom a problem, the condition of the patient directing the attention toward the principal anomaly.

In general, treatment is started with small doses. If the offbalances, and especially the clinical manifestations persist, increased doses are indi-

cated. Larger amounts are used and are administered more often. Once the desired analytical and clinical effect is obtained, dosage is maintained. If analyses corresponding to the offbalance pass far enough on the opposite side, the amount of the medication is first reduced. If this change persists or increases, treatment is discontinued for several days. If the new offbalance still persists, and especially if new clinical symptoms develop, use of the opposite group of agents is to be considered. A slight passage of an offbalance into the opposite is usually salutary and treatment is continued as long as clinical improvement persists.

It appears to be of great importance that treatment be continued for several months after all subjective and objective manifestations have disappeared. In a number of patients, we have been investigating the value of continuing medication for years in very small doses as a prophylactic measure to prevent recurrences. The results have been highly encouraging. No inconveniences have been noted with controlled continued use of any of the substances.

Results obtained

With this form of treatment most of the results obtained are striking. With naphthalene or tetrahydronaphthalene perselenide, epichlorohydrin and bixine as principal agents for the type A and heptanol-glycerol for the type D, the subjective and objective manifestations were seen to be well controlled. Pain, if present, disappeared in a few days, the tumors progressively diminishing until they disappear, even in cases considered as far advanced. The following few recent observations of such cases, give an idea of these results, up to date.

Mrs. M. C., 59 years old, had 3 years ago a mastectomy for adenocarcinoma. She came under our care with metastases in the right 8th rib and in the 11th and 12th dorsal vertebrae, liver metastases, ascites and pleural effusion. With dyspnea, severe pains and almost continuous vomiting, her general condition was judged very poor. Paracentesis was performed, but the fluid accumulated rapidly, needing a second paracentesis 10 days later. In offbalance A, the patient was treated with perselenide, bixine propanal and epichlorohydrin. Shortly after the beginning of the treatment, the pain decreased in intensity and later disappeared completely. The pleural effusion decreased and after the two paracentesis, the ascites no longer reproduced. The general condition changed rapidly for the best, together with the objective changes. Liver reduced in size after three months of treatment was within the normal limits. Radiological examination, two months later showed the bone metastases completely healed. The general

condition continued to improve. The patient resumed her normal life and now does not show any clinical abnormality.

Mr. J. S., 71 years old. Two years prior to admission, the patient had persistent hematuria, for which a prostatectomy was performed. Hematuria reappeared. Cystoscopic examination revealed tumors of the bladder, for which he was operated 1½ years ago. Since then, he has had almost constant hematuria, with frequent micturitions during the night and almost every hour during the day. Pain in the lower abdomen became progressively stronger. The patient was admitted and treated, in accordance with the analyses, with perselenide, bixine and epichlorohydrin. In less than a week, the hematuria disappeared, as did the dysuria, the patient being able to pass clear urine every 6 to 8 hours. With the interruption of the treatment for almost a month, the symptoms reappeared, with hematuria and dysuria. With the treatment resumed, the condition responded well again, the hematuria and dysuria being controlled and pain disappeared.

Mrs. R. A., for five years, had symptoms of gastric ulcers, more accentuated in the summer, which improved with treatments. For six months prior to coming under our care, the patient had progressively marked difficulty in swallowing anything other than fluids. Even after taking fluids, she experienced very severe retrosternal pain, almost always followed by vomiting. X-ray examination showed slight dilatation of the esophagus with a clearly visible growth in the stomach near the cardia. According to the analyses, the patient was treated with perselenide in injection, and bixin and epichlorohydrine orally. Two weeks after treatment was started, the patient was able to swallow not only fluids but also finely ground food. The improvement continued, the patient being able after 5 weeks of treatment to swallow food of almost normal consistency.

Mr. J. R., 56 years old, came under our care with severe pains in the right side of the neck and hemoptoic sputum. At examination, a submaxillary gland of 6 cm. diameter was seen. Laryngoscopic examination showed a tumor in the right pyriform fossa—Biopsy revealed a squamous cell carcinoma. The very severe and constant pain and the constant bleeding caused the patient to be hospitalized. According to the analyses, perselenide by injection, epichlorohydrin and bixine were administered. In 24 hours the pain was fully controlled. The bleeding stopped after 4 days, and the gland started to decrease—10 days after the beginning of the treatment. The laryngoscopic examination showed the tumor transformed into a graying mass, which was progressively decreasing.

Mrs. A. D., 60 years old, admitted to the hospital with dyspnea, cough and pain in the right hypochondrium, epigastrium and generalized weak-

ness. For 30 years, the patient had complained of pain in the right hypochondrium, related to the presence of gall bladder stones. In February of 1960, a laparotomy was performed and a tumor of the gall bladder with metastases to liver was found. Only a biopsy was performed which showed a carcinoma. At admission under our care, the patient was in very poor general condition with marked dyspnea, deep jaundice and severe pains in the upper abdomen, emaciated. A right pleural effusion was found and a thoracentesis performed. An irregular mass was found in the right hypochondrium arriving until the umbilicus. She had clay colored stools, typical for obstructive jaundice. In spite of thoracentesis the dyspnea continued to be severe and the patient was kept under oxygen. The patient was placed under chemotherapeutic treatment with epichlorohydrin, bixine and perselenide in accordance with her urinalyses. The patient's condition improved progressively. The stool returned to normal color; fluid in the right chest did not reproduce and the mass on the right hypochondrium decreased progressively to have the liver in normal dimensions. At present, the patient with all the subjective and objective symptoms improved considerably, is ambulatory.

Mr. S. S., 64 years old, was operated in 1953 for a hypernephroma of the right kidney. Two years later, massive metastases were seen in the left femur and pelvic bones. Pathological fracture of the neck of the left femur was treated surgically. Further X-ray examinations revealed extensive metastases of the femura, and pelvic bones, with multiple lung metastases. When the patient came under our care, he was suffering agonizing pain especially in the left hip. An X-ray examination showed an almost complete disappearance of the upper part of the left femur, with multiple metastases in the right femur and pelvic bones, and multiple metastases in both lungs. According to the analyses, the patient was treated with perselenide, bixine, and epichlorohydrin. The condition improved rapidly, the patient being able to sit up and even to walk a little. He was discharged from the hospital to follow the treatment at home which was done very irregularly. He was readmitted a month later with very severe pain and the treatment resumed. The pain subsided gradually and the general condition improved markedly. Recent X-rays revealed a manifest recalcification of the upper part of the left femur which, in previous X-ray examinations, had no longer been visible. At the same time, many of the metastatic lesions of the lung disappeared while in others, a marked decrease in their size was seen. These subjective and objective improvements are continuing constantly, up to date.

F. G., 61 year old female, in July 1960 had partial cecostomy for adenocarcinoma. The mesenteric lymph nodes were found involved. A

month later, because of vaginal bleeding and an erosion of the cervix, a biopsy was performed, showing the same malignancy. With constant bleeding and pain in the abdomen, the patient came under our care. On examination the tumor was seen to occupy all the upper part of the vagina, with infiltration of the recto-vaginal wall. According to the analyses, a treatment with perselenide in injection, epichlorohydrin and bixine was instituted. The bleeding stopped completely after one week, as did the pain. While the patient is still under treatment, the lesion has been seen to regress constantly, up to date.

W. M., 48 year old male. In 1956 he had a left nephrectomy for hypernephroma. He was well until early in 1960 when a mass was found in the left side of the abdomen which was progressively growing. At the same time, he had hemoptoic sputum. The x-ray examination of the chest showed multiple lung metastases. Five weeks before coming under our care, a very marked edema of the left leg with very severe pain in the back and leg appeared. He came under our care especially for the unbearable pain. According to the analyses, a treatment with heptanol, butanol and glycerol was instituted. The pain disappeared in 2-3 days and has not returned in the two months which have elapsed since then. The edema of the leg also disappeared. The tumor which, at the time of administration, was occupying the entire space between the ribs and the ileac crust, was seen to become first much softer, and progressively to reduce its dimension. Actually two months after the treatment was started, the patient is leading a normal life with the tumor decreasing progressively.

We want to emphasize that benefits, often impressive even in terminal cases, have been obtained only by following the above rules. Treatment guided closely by changes observed in the patterns indicated by analyses appears to be the condition *sine qua non* for the attainment of good results.

The results obtained and especially their high proportion, even in far advanced cases, permits a fair judgement of the place of the present form of application of this method in the fight against cancer. Based on these results, we are fully entitled to consider it, not only a highly beneficial treatment which can be offered now for this disease, but even a major step nearer to the solution of the problem of the therapy of cancer.

NOTES

Chapter 1, Note 1. Subnuclear Organization

The analysis of the available data concerning subnuclear particles shows that while an unsuspected number of different particles are progressively discovered, no satisfactory relationship between them—from the point of view of the organization—can be established. The recognition of a pattern concerning their organizational relationship would fill an important gap in the knowledge of this entire field. The fact that the same pattern governs the organization in general, from atomic nuclei up, has induced us to attempt through an extrapolation, to search it for the subnuclear realm.

As seen above, the study of the organization has permitted us to define the following concepts as characteristic for the organizational pattern:

- 1) All the entities in nature can be identified by their place in a hierarchic organization, in which the entities are connected through a superior-inferior relationship. An entity enters in the formation of other entities which are considered "superior" to it, and is formed by entities which are hierarchically "inferior" to it.
- 2) Each entity is formed by a principal and a secondary part, the principal part being represented by entities hierarchically inferior to it, while the secondary part, by elements taken from the immediate environment in which the entities forming the principal part have existed.
- 3) Entities with similar principal parts belong to a same level. In the hierarchic progression, there are entities of the same level which are grouped together to constitute the principal part of an entity of a level immediately superior.
- 4) From the energetic point of view, the principal part in the organization of each entity appears more positive than the secondary part to which it is bound.
- 5) The hierarchic progression of the organization from one entity to that immediately superior to it, is made through two processes with two different intervening forces. Forces of columbian nature bring and keep the electrostatically opposite principal and secondary parts together. A new entity appears however only when quantum forces intervene, organizing the relationship of the two constituents and especially their reciprocal movement. The immediate aim of this organization is to prevent a reciprocal total annihilation of the two parts, positive principal and negative secondary which would occur if the electrostatic forces alone would be present.
- 6) In the hierarchic progressive development of the organization for

each passage from one level to the immediately superior, these two kinds of forces—electrostatic and quantum—were seen to intervene alternatively. The fulfillment of one force is seen to induce the appearance of the other. From an energetic point of view, an entity will appear inactive when its electrostatic forces are fulfilled but with quantum forces present, or energetically active with quantum forces fulfilled and the electrostatic forces present. The example of atoms and ions is a typical illustration of this relationship for the atom level.

As a work hypothesis, we tried to apply the above schematically presented concept of hierarchic organization, to the subnuclear realm. It is only by using analogies that such an attempt can be made. The scarce data available seem to confirm however, this view. According to it, the electron and positron would represent the lowest entities of the subnuclear realm with the smallest mass and opposite charge. If these two corpuscles, when attracted, encounter one another, they will annihilate each other with liberation of two photons. This annihilation is prevented however, although the two corpuscles remain bound together through their electrostatic forces by the intervention of quantum forces organizing their reciprocal movements. The result is a new entity, of a level immediately superior to the positron, in which the electron is kept electrostatically bound to the positron but kept into an orbital movement. Hypothetically, it can even be conceived that through differences in the resulting movement, more than one solution would exist.

Residual Charge

Due to the intervention of this movement, the resulting electrostatic neutralization between positron and electron is incomplete. A "residual" positive charge would characterize the new entity. This charge alone would not be sufficient to keep another electron by neutralizing its charge. However several such entities grouped together can have the sum of their "residual positive charge" such as to be compensated by a new electron. The two electrostatic forces, that of the group of entities and that of the new electron will keep these two parts together, while the quantum forces will again organize the movement of this new added electron, preventing this time again the annihilation of these two parts. A new level, this time of the third order, is thus realized. It is easy to conceive that several solutions can exist for each case, since the sum of the residual positive charges does not correspond exactly to that of the negative electrons. Several solutions appear thus possible. Besides this in which a small group of entities would be compensated by one electron, a higher number of entities would be kept together with the sum of their residual charges approaching that of two or more electrons. For each level, several such solutions are conceivable. With the progressive passage toward higher levels, the number of the solutions increases.

The fact that the two electrostatic positive and negative parts of the entities do not compensate perfectly, leads to the possibility that the compensation takes place either with an excess or lack of negative charge.

The difference is induced by an additional electron. An entity with a positive or negative charge would result. With one or the other charge prevailing, two energetically active, positive or negative forms, differing by the mass of an electron exist, for each entity. The analysis confirms this existence.

According to the hierarchic organization, the different particles of the subnuclear realm can be separated in various levels. Promezons, mezzons, protons would represent such levels—each one with respective neutral, positive and negative entities. The same as for the higher levels—atoms and molecules—the different entities forming each of the subnuclear levels will differ through the number of the entities entering in the principal parts. A systematization of the subnuclear realm on this basis can be confirmed by the fact that the different entities of a level represent sums or multiples of the entities of their inferior level in the same way that the different nuclei represent multiples of entities of the protonic level protons and neutrons or the different molecule multiple of entities of the atomic level.

In the progression of the hierarchic organization, it is seen that the passage from one level to the other results in an exponential increase in the numbers of the kinds of entities (from around 100 different atoms it passes to around 100,000 different kinds of molecules, to millions of kinds of genes and trillions of individuals. This fact supposes that the number of the existing particles decrease with each inferior level, in the subnuclear realm, to arrive to two—positron and electron—at the bottom of this realm.

Chapter 2, Note 1. C-N-C-N

As far back as 1905, Kossel has had indicated the existence for the important alkaline aminoacids, arginine and histidine, of the C-N-C-N group, found also in the nitrogen containing bases of nucleic acid. The hypothesis which we advance that this C-N-C-N group would represent the starting point of the biological realm itself, can surely be subject to discussion.

Progressively more evidence is being obtained that important organic compounds can appear from the constituents of the atmosphere itself, under the influence of electrical discharges or of ionizing irradiation. While Henriet (268) was the first to show that formic acid is present in rainwater, it was Loew (269) who obtained glycine from the constituents of the atmosphere submitted to electrical discharges. By utilizing, under the same condition, mixtures similar to those considered to have been present at the time when life is supposed to have started, Miller obtained, through electrical discharges, many amino acids and other substances especially glycine and formic acid (270, 271). Miller's results were extensively confirmed (272 to 279).

The irradiation of the mixtures of gases considered present in the atmosphere millions of years ago has led to the synthesis of many other substances such as formic, acetic, propionic, succinic and even tricarboxilic acids (280, 287). From these, we consider of especial importance the

members with a second nitrogen group far in the molecule, as diaminosuccinic acid, iminodiacetic or iminoacetic-propionic acid.

The synthesis of the strongly positive C-N-C-N group which we consider as the starting point of the biological realm, seems thus to have taken place rather under the influence of radiation. This fact appears especially important since it would relate more directly the beginning of the biological realm to the intervention of the radioactive elements, which according to our systematization of the elements, form the period which corresponds to the lowest levels of the hierarchic organization. (See Chapters 2 and 5)

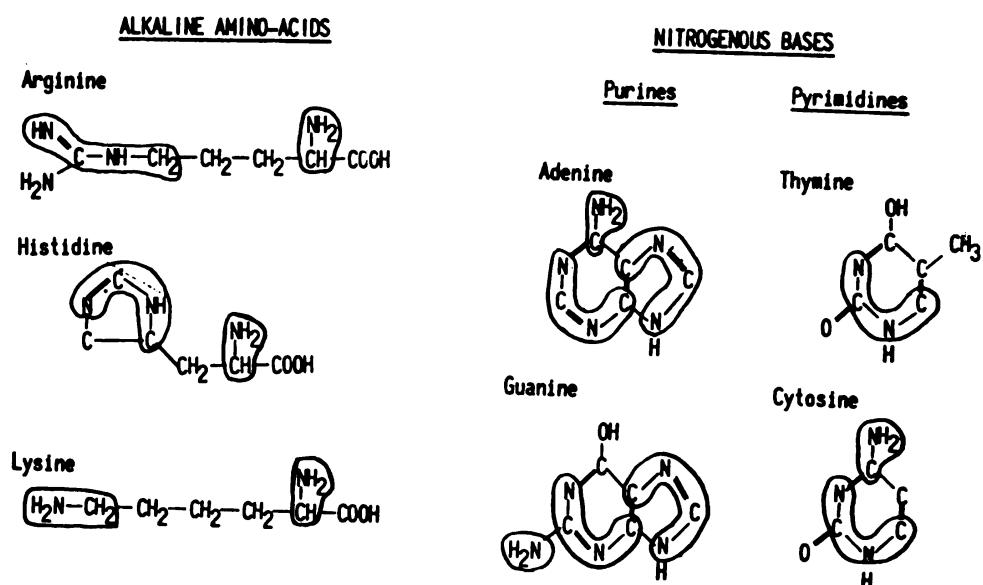


FIG. 201. The NH₂ and C-N-C-N groups appear as entities taking part in the formation of alkaline amino-acids as well as of nitrogenous bases. The bond to a chain having an amino acid group in the first case, results in a new entity—an alkaline amino acid—which polymerizes through the amino-acid group. Through the alkaline group it conserves its positive electrical character. In the nitrogenous bases, the C-N-C-N group is part of the cycle. Bound to phosphoric acid, the results are acid entities with negative charges.

The further evolution of the C-N-C-N formation seems to have taken place in two directions—one in which one or two such groups have formed a cycle and given rise to the nitrogenous bases, purines and pyrimidines, and the other in which this energetic group has bound an aliphatic amino acid chain, this last probably originated under the influence of electrical discharges. The two principal alkaline amino acids, arginine and histidine, have thus appeared. (Fig. 201) The double capacity of the alkaline amino-acids, to bond other amino-acids through their amino-acid groups and thus to form polymers, and to bond acid substances through their alkaline polar groups and make new hierarchic entities, has given these substances their peculiar organizational role. C-N-C-N, alkaline amino-acids and histones

(or in fish, the protamins) would thus represent the first hierarchic steps in the progression of the biological series.

A few words should be said about lysine, the alkaline amino-acid with an amino group as alkaline terminal group. Although together with the other alkaline amino-acids, it enters in the formation of histones, it seems to have another important biological role, that of an agent intervening in the metabolism of lipids.

Chapter 2, Note 2. Distribution of Potassium and Sodium

As mentioned above, potassium is the cation of the cytoplasm, the secondary part of cells, while sodium is the cation of the secondary part of the metazoic compartment, that is of the fluids of this compartment. According to the view presented above, the peculiar distribution of these two cations in the biological realm results from their similar distribution in the environments from which these respective secondary parts, cellular and metazoic, are considered to have been derived. As we related the cytoplasm to mud, respectively to the lithosphere, and the fluids of the metazoic compartment to the sea, we looked for a confirmation of this view in the comparison between the amount of these cations in the two biological compartments and in the two environments which we consider to correspond to them.

Although potassium and sodium are in almost equal amounts in the general constitution of the earth's crust, potassium is found almost entirely in the solid parts while sodium forms the principal constituent of the salts of the fluid part of the earth. The distribution of potassium found between cells and extracellular fluids seems very near to that which exists between lithosphere and hydrosphere. Potassium is found in a proportion of 2.46% of the lithosphere and only in 0.04% of the hydrosphere (201). The ratio of these respective concentrations corresponds to a $\frac{1}{61}$ value. This seems near enough to the ratio found in biology. While the extracellular potassium represents only 5 mEq per liter, with a total of 70 mEq (2.7 gm.) for a normal body, the intracellular part corresponds to 115 mEq per liter of cells, with a total of 4,000 mEq (160 gm.) for the body (202). The ratio of $\frac{1}{59}$ for total extracellular and intracellular is accepted today although generally considered too high when compared with the previous data given years ago by Shohl (265). This value of $\frac{1}{59}$ appears impressively near the ratio of $\frac{1}{61}$ found in the comparison of the potassium content of the lithosphere with that of the hydrosphere.

A similar resemblance is encountered when comparing the proportion between sodium and potassium in two fluids: the interstitial fluid of the body and of the sea. The two ratios of these elements appear close enough. For instance, the Atlantic Ocean has 10.464 gm. of sodium per thousand and 0.725 gr. per thousand of potassium, while the Pacific Ocean has 10.233 gr. per thousand of sodium and 0.634 gr. per thousand of sodium (266). The ratios between sodium and potassium are respectively 14 and 16. In the blood serum, the ratio is 16 when the average is considered as

320 mg.% of Na, and 20 mg.% of K. Table XXV shows these comparative values.

TABLE XXV

- (a) Comparison between the Extracellular and Intracellular Potassium and the amount present in Hydrosphere and Lithosphere.

	<i>Ratio</i>
Potassium extracellular-total body 2.7 gm.)	1/59
Potassium intracellular-total body 160 gm.)	1/61
Potassium in the hydrosphere 0.04%)	1/61
Potassium in the lithosphere 2.46%)	1/61

- (b) Potassium and Sodium in the sea and in the body fluids.

	<i>Na</i>	<i>K</i>	<i>Ratio</i>
Atlantic Ocean	10.464 gr. 0/00	0.725 gr. 0/00	14.4
Pacific Ocean	10.233 gr. 0/00	0.634 gr. 0/00	16.0
Blood Serum	320 mgr. 0/0	20 mgr. 0/0	16.0

These values seem to bind the distribution of K and Na, seen between the cellular and metazoic compartments, to that which exists between the environments from which we consider these respective secondary parts to have been derived.

Chapter 2, Note 3. Social Hierarchic Organization

The organization of nature with the characteristic hierarchic structure so evident in the biological realm, has suggested a similar structure in social organization.

All the factors which were seen to characterize hierarchic organization appear clearly in an analysis of social organization. (*Fig. 202*) The entity, immediately above that of the individual, is the family. Here, parents and children—as a grouping of entities of the same level form the principal part. The secondary part is made up of elements of the immediate environment, which are kept organized around this principal part, and as such are integrated in the new entity, the family. Housing, goods, even psychological factors, ideas and habits, characterize these added factors. A boundary formation is often much more visible than expected. Living quarters and common possessions are well delineated, and characterize the family. As expected, most of them are not considered to belong to an individual but the family as entity. "This is family property" is a common expression.

Almost always, numerous families are grouped in nearby dwellings, although this fact alone does not lead to the immediate superior entity, the community. When the group of families organizes together and limits certain possessions taken from the environment, as common to the group, the entity "community" appears. The principal part is made up of the group of families, the secondary by the material and even moral goods which are attached to the group of families in common. The community has prop-

erties which belong only to the community—streets, for example—as it has, by definition, a boundary. The limits of these social entities are well defined and these three factors—principal part, secondary added part from the environment and boundary—characterize these entities as they characterize the entities in the entire biological realm. The same pattern applies for the county where groups of communities form the principal part, and

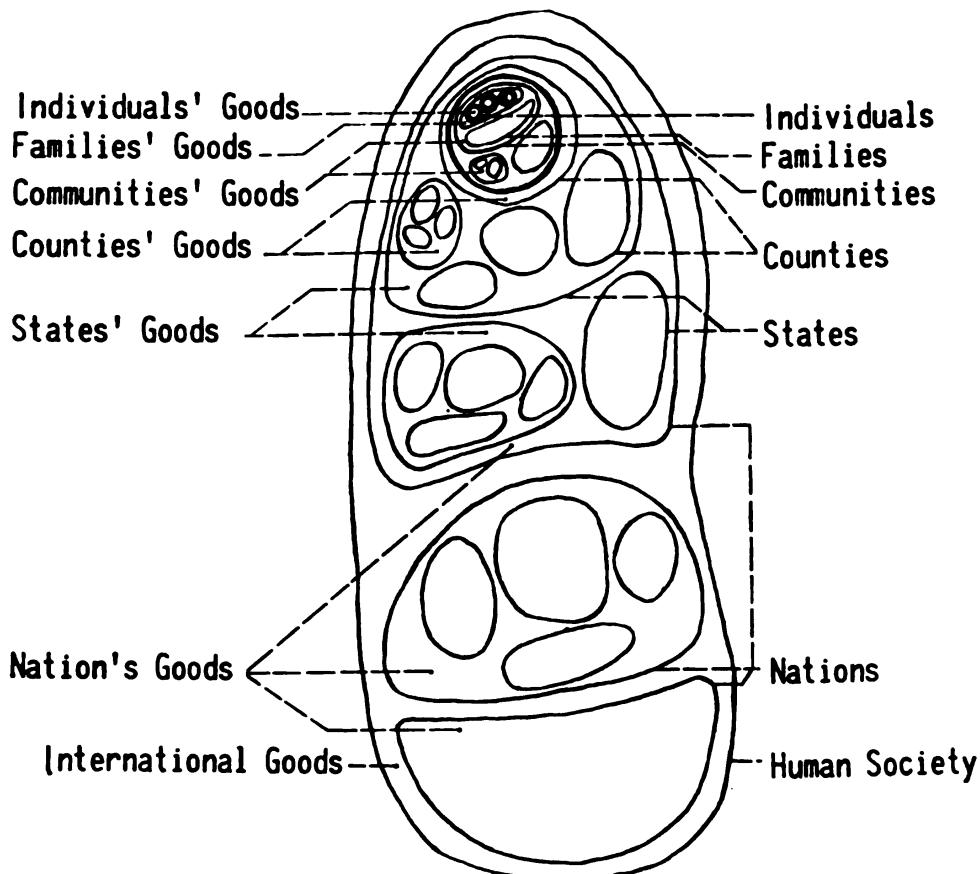


FIG. 202. The *social hierarchic organization* follows the same pattern as the organization of matter or of the biological realm. Each entity results from the bond of a group of lower entities with a secondary part taken from the environment and limited by a proper boundary.

proper parts taken from the environment and common only to this new entity form the secondary part. This entity also is defined through its boundary. It is easy to see how through the same hierarchic pattern we pass from counties to states, nations, hemispheres and world which represent successively higher hierarchic entities. It is interesting to see how, in each one of these social entities, the same manifestations which we have found to characterize the biological entities also exist. The relationship between entities and especially many of their functions shows that the

social entities are not artificial mental concepts, but are the result of the intervention of the same forces in which heterotropic organization opposes the lawless homotropy. It has appeared interesting to see how much of the knowledge of the physiological and especially the pathological manifestations of the lower entities, we can apply to understand manifestations occurring at the social hierachic levels.

Under this aspect, sociology finds a new basis not only for the analysis of many of its problems, but can have an insight to how nature, through its own organization, has tried and often succeeded in resolving problems. With the concept of unity in all organization, from subatomic to social entities, we can understand how the evolution of the environment, represented by material and intellectual goods, can produce changes in social entities. The concept of higher social entities, organized so as to conserve the characteristics of the lowest social entities, gives a new aspect to the relationship between individual, family and society. A science of social physiology can be created by systematizing hierachic social entities much as we did for entities in the biological realm. The same approach can be applied to social pathology and social therapy as well. Such an approach, will be the subject of other presentations.

Chapter 3, Note 1. Precancerous Lesions

Precancerous lesions were identified especially in cases in which cancerous lesions were induced and where a manifest polycentricity of lesions was present. (203) Polycentric lesions permit us to study the entire successive changes from normal to invasive cancer.

Induction of cancer in the stomach of rats through carcinogens and detergents (204) has furnished excellent material for such study; it has also permitted us to characterize the specific changes. Among cells which appear grossly normal, there are some in which certain morphological characteristics of the nucleus, notably size and form, appear abnormal. The existence of an anomaly is much more evident when the cell divides. It may be limited to just a few chromosomes which are abnormal in their dimension and form. This chromosomal abnormality appears still more evident when compared to cells in mitosis in controls with normal mucous membranes. (309)

Chapter 3, Note 2. Non-invasive Cancer

We have emphasized the character of the cytoplasm of the cells in non-invasive cancer. The nuclei show a number of changes which, together rather than separately characterize a cancerous entity: an irregular shape of the nucleus with a manifest increase in size; a sharp nuclear border formed by a dark pigmented nuclear membrane having fine chromatine particles; a hyperchromatism with clumps of chromatin separated in bizarre, irregular fashion; and an uneven, irregular distribution of these chromatine clumps, concentrated near the nuclear membrane. Also often

encountered is the presence of one or more irregular enlarged nucleoli, with a distinct nucleolar border and especially with a manifest acidophilic staining.

In the non-invasive cancer, all these nuclear anomalies contrast with the relatively normal cytoplasm, which has not only an acidophilic reaction—colored in orange with Papanicolaou's trichromic staining—but also a well-defined cell membrane with fairly clear cell border. The size of the cytoplasm—compared to other cells—is normal, although the nuclear-cytoplasmic ratio is increased due to the big nucleus. Due to the character of the cytoplasm, these cells were called the "third type differentiated cells" by Graham. (205) We emphasized the "normal" aspect of the cytoplasm of these non-invasive cells, in contrast to the invasive cells where the abnormality includes both the nucleus and the cytoplasm. This explains why most of the invasive cells have little cytoplasm, an indistinct cellular border and a basophil cytoplasmic staining. (206) But besides these cells with totally abnormal cytoplasm, there are some invasive cells with an apparently differentiated cytoplasm. Although their staining is orthochromatic, their cytoplasm shows marked abnormality in form. The tadpole cells found in the exfoliative cytology in epidermoid carcinoma, (207) or the fiber cells (208) with abnormally long fibrillar cytoplasm revealed in other forms of invasive cancer indicate a participation of the cytoplasm in the abnormality. The cells found in so-called "Bukhead's disease" with minimal abnormal cytoplasm thus appears to be at the boundary between non-invasive and invasive cancerous cells.

Chapter 3, Note 3. Abnormal Amino Acids

We have seen above how the concept of hierarchic organization brought us to consider the alkaline amino acids and the histones which they form as one of the first members of the biological realm. Anomalies can be conceived to result from a process of resonance which occurs constantly on a statistical basis. As work hypothesis, we consider such resonance entities as corresponding to these abnormal forms, which in hierarchic development would lead to cancerous entities.

The naturally existing levorotatory alkaline amino acids represent the constituents which, through their number and role in further organization, represent normal entities. Opposed to them, the dextrorotatory alkaline amino acids would represent abnormal entities. Their existence and their role has made the object of many discussions without, however, bringing sufficient light to this problem. The constant existence in the body of specific enzymes against these dextrorotatory amino acids in spite of the fact that they are not recognizable analytically, indicates a certain defense against them. The concept of their appearance as a resonance phenomenon would explain easily this occurrence. Dextrorotatory amino acids, although abnormal for the organism, exist in practically all individuals as a resonance form, but they are not able to develop—or develop in extremely reduced form—because of the enzymes which attack them. They are, how-

ever, able to develop the lowest levels of cancerous entities as they are recognized to exist practically in all normal individuals particularly after a certain age. There are these considerations which lead us to believe that it is the dextrorotatory resonance form of alkaline amino acids which represent the abnormal entities at the lower levels.

Chapter 4, Note 1. Physiological and Pathological Pain

Physiological Pain

Physiological pain may be defined as a specific sensorial sensation induced in normal tissues when external stimuli are applied with sufficient intensity to endanger tissue integrity. Because pain may be induced by a wide variety of stimuli, it has not always been accepted as constituting a sensorial sensation. Considerable evidence exists, however, to indicate that physiological pain is a specific sensation, similar to the other sensorial sensations. One indication that pain is a proper sensorial sensation lies in the fact that it has its special nerve system.

Blix (5) and Goldscheider (6) found that certain areas of the skin were sensitive to painful stimuli from a pinprick while others were not. Strughold (7) demonstrated that, in various areas of skin, pain points were concentrated in varying degree. Microscopic study of areas of skin showing high aggregations of spots of specific forms of sensibility has indicated that special sensory nerve and organ structures are apparently associated with different types of sensation. Thus, Krause's corpuscles are considered as receptors for cold; Ruffini's endings and Golgi-Mazzoni corpuscles for heat; and Meissner's corpuscles, Merkel's discs and the basket endings around hair roots for touch. (8) Woolard (9) described unmyelinated, finely beaded, branched free endings as the specific nerve end organs believed to be responsible for the reception of pain impulses. Certain areas such as the cornea and the mucous membrane of the nose, which are generally considered sensitive to pain alone, have been shown to have these free endings as the characteristic nerve endings at these sites. Weddell has found only this type of end structure in areas of skin sensitive solely to pain during nerve regeneration.

That pain constitutes a specific form of sensation is further indicated by the evidence that its impulses are carried along definite nerve pathways to special centers in the thalamus. By temporary asphyxia, by cocaineization, or by cooling, differential interference with conduction of the special sensations of pain, touch, heat and cold along a nerve can be produced. The existence of individuals without the sensation of pain, but with sensations of touch, cold and heat, has confirmed this view of pain as a proper sensorial sensation.

It is characteristic of the sensation of pain that it may be elicited by a wide variety of stimuli. Below the pain threshold, the incitation induces specific sensorial sensations according to the stimulus used. Above this threshold, the sensation felt is pain. When different noxious stimuli produce pain, the subject cannot distinguish the nature of the incitation. In effect,

when it is below the threshold, the incitation informs about the nature of the stimulus; above the threshold, the individual is conscious of another fact: that the stimulus is of such intensity as to endanger the integrity of the tissues. Pain thus appears to be the sensorial sensation of a specific character of stimuli—sufficient intensity to represent a danger for the tissues, and it is this which differentiates pain from the other sensorial sensations, and puts it into a special category. Pain is independent of the nature of the stimuli. By constituting a warning to the body, that its tissues are in jeopardy, physiological pain induces a general response involving brisk, rapid movements, a rise in pulse rate, and a sense of invigoration. (10)

The fact that sensorial pain results from the intensity of the external incitations has prompted investigators to study this kind of pain largely in terms of the threshold of incitation. It must be emphasized that for each stimulus there exist two thresholds, one for intensity values required to produce specific sensations, and the second for intensity needed to produce pain. There is a considerable difference, for example, between the heat intensity necessary to produce a sensation of warmth and the amount that will produce a sensation of pain.

Pathological Pain

Pathological pain differs profoundly from physiological pain. It is a psychic response to impulses originating in tissues which are abnormal either because of damage produced by external stimuli or because of inflammatory, circulatory, neoplastic, or other processes. When pain is present as a consequence of tissue damage or disease, it can no longer be considered as a warning of danger but constitutes a sign of injury.

The general response to pathological pain is totally different from the response to physiological pain. Instead of the organism being prepared for fight or flight, its efforts are directed toward placing the painful injured area, or the entire body at rest; and, to protect the painful area from further injury, the pulse rate generally slows, the blood pressure falls and often there is sweating and nausea. (10)

The local nature of the changes responsible for pathological pain has raised the problem of the several possible mechanisms of action which may intervene in inducing this pain.

1. Locally originated stimuli produced by damaged tissues themselves may act directly upon the pain end organs to induce pain impulses. Lewis has suggested that the pain associated with tissue damage is a result of the action of locally elaborated abnormal chemical substances. (10) This possibility was first considered by von Frey (11) although actually the second pain described by him was due to different rates of pain impulse transmission. Lewis (10) and his associates have studied pain in erythralgia which represents a typical form of pathological pain. (12, 13) They have shown that when skin has been injured and thus rendered hyperalgesic but not actually painful, simple arrest of circulation to this injured area may induce pain. A similar phenomenon is evident when a muscle is exercised vigorously while its circulation is arrested. If the constricting blood pressure cuff

is released, the pain that is experienced during the period of ischemia disappears, but if the cuff is reinflated, pain may recur without further exercise. In both instances, no new stimulus is required to arouse pain. It has been found that in erythralgia, neither vasodilatation nor change in skin temperature is the factor responsible for lowering the pain threshold. According to Lewis, when the circulation to the affected area is severely reduced, accumulated stable chemical substances elaborated by the damaged tissues may act directly as the pain stimulus. No definite evidence has been offered by these researchers, however, as to the chemical nature of the elaborated substances involved.

2. Local changes in damaged tissues may bring about a lowering of the nerve threshold for pain. Lewis has demonstrated the spread of the lowered threshold to nerves far beyond the site of the lesion itself. He studied the cutaneous hyperalgesia following tissue-damaging excitation of a tiny area of skin by a tapered forceps or faradic current. By producing damage in a previously anaesthetized area, he found that the local changes brought about by the damage did not produce hyperalgesia in the surrounding skin until the effects of the local anaesthetic wore off. The localized nerve changes then created a wide zone of hyperalgesia for prolonged periods. Tower (14) has presented evidence to show that the receptor end structures for pain have an arborizing rather than a plexiform arrangement, thus making unnecessary the postulation of an autonomically unidentified "nocifensor" nerve system, as proposed by Lewis, (15) to account for the type of spread of the hyperalgesia. The extent and especially the distribution of this area of hyperalgesia has clearly indicated that it is the result of a lowered threshold in the arborizing branches of the cutaneous nerve, a few branches of which were originally intensely stimulated. When a few fibers of a cutaneous nerve were directly stimulated, the same effect was observed. The findings suggested that a local tissue change lowers the threshold for pain for the nerve endings of the damaged area, and that this effect may spread through other branches of the cutaneous nerve involved as well as through larger nerve trunks so that the resultant area of hyperalgesia becomes very extensive.

3. Local changes may alter end organs ordinarily concerned with other forms of sensation in such a way that the impulses originated by them evoke the sensation of pain. Certain areas such as the appendix and the mucosa of the stomach apparently cannot, under normal circumstances, be incited to respond painfully to any form of stimulation. (16) However, in the presence of inflammation, the same stimuli may give rise to pain in these areas. The relationship between the end organs or nerves ordinarily concerned with the reception of other forms of sensation and those of pain has been considered by several authors. Weddell (17) has demonstrated that the various complex end organ structures are supplied with accessory fibers, unmyelinated and beaded, analogous to those considered to be pain receptors. Head (18) has shown in experiments on the glans penis that there may be a fusion of various sensations into a single concept and that one sensation may inhibit another. According to Feng, (19) the balance in

excitability between touch and pain receptors may be upset peripherally by liberation of a chemical substance as the result of injury.

Considerable evidence exists to indicate that pain is the most primitive form of sensation. It is possible that in the presence of pathological disturbances, through dedifferentiation complex systems for the reception and transmission of other modalities of sensation come to act as pain receptors.

Whatever the exact mechanisms may be, the findings of different investigators have led them to the conclusion that abnormal chemical substances are released from pathologically affected tissues and that these chemical substances may play an important role in the production of pathological pain.

Chapter 4, Note 2. Blood Titrimetric Alkalinity and Urinary pH

The important role of the kidney in regulating the acid-base balance of the blood has been described (209) and a general relationship between daily acid excretion and plasma bicarbonate has been recognized. (210) However, a consistent relationship between blood acid-base variations and urine changes has not been clearly established.

The determinations of blood pH and CO₂ combining power are the most commonly employed methods for following acid-base changes. However, they indicate only certain distinctive factors intervening in acid-base balance. The pH is a measurement of the dissociated elements in the blood and is maintained within narrow limits by the buffer mechanisms, while the CO₂ combining power is a measurement of only one of the multiple factors in the buffer system, the bicarbonate group. (211) The inconsistent relationship between variations in urinary pH and blood values indicate that changes in urinary pH depend upon factors other than the blood's dissociated substances and bicarbonate-carbonic acid buffer mechanism. The phosphates, proteins and hemoglobin are among the members of other important buffer systems that have a role in the control of the acid-base balance of the blood. (*Figs. 203, 204*)

The titrimetric alkalinity of the blood represents a measurement of the totality (reserve supply) of the substances, both dissociated and non-dissociated, that are involved in the maintenance of the acid-base balance of the blood. (212) We considered it interesting to examine the relationship between blood titrimetric alkalinity and urinary pH. Concomitant variations were compared.

Human subjects and dogs without apparent kidney dysfunction were used. Blood was obtained by venipuncture with an accurately calibrated dry syringe. After the needle was introduced into the vein and before withdrawing blood, the tourniquet was released for several minutes to avoid changes due to stasis.

Exactly 5 cc. of blood was introduced directly into a flask containing 30 cc. of a 0.001N sodium hydroxide solution. The flask was immediately closed with a rubber stopper and the mixture agitated sufficiently to assure homogeneity. If determinations were not carried out at once, flasks were

stored at 5°C and then brought to room temperature (20°C) before analysis. Fifty cc. of distilled water was used as a wash when flask contents were transferred to a beaker for titration. The total alkalinity was determined by electrometric titration to pH 7.0 against a .01N hydrochloric acid solu-

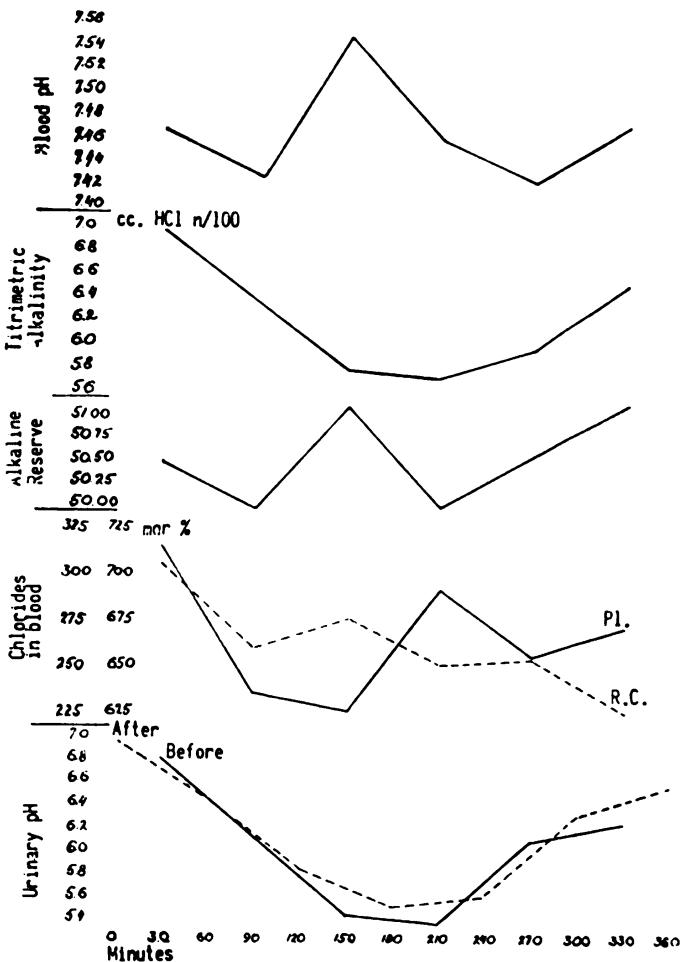


FIG. 203. The comparison between the concomitant changes seen in various blood and urine analyses concerning the *acid-base balance* of the body. It shows that the variations of the titrimetric alkalinity of the total blood are the only values which constantly parallel those of the urinary pH. (Patient with breast cancer)

tion using a Beckman pH meter, Model G, and a mechanical stirrer. Blanks of 30 cc. of the sodium hydroxide solution were stored and treated in the same manner.

Urine specimens from the human subjects were obtained through complete emptying of bladder contents by voluntary micturition. An indwelling catheter was used for dogs and some humans. Specimens were placed in containers closed with rubber stoppers and stored at 5°C. They

were brought to room temperature before tests. The pH values were determined electrometrically.

Blood specimens were collected each hour for at least five consecutive hours. Urine samples were obtained every thirty minutes, as specimens ac-

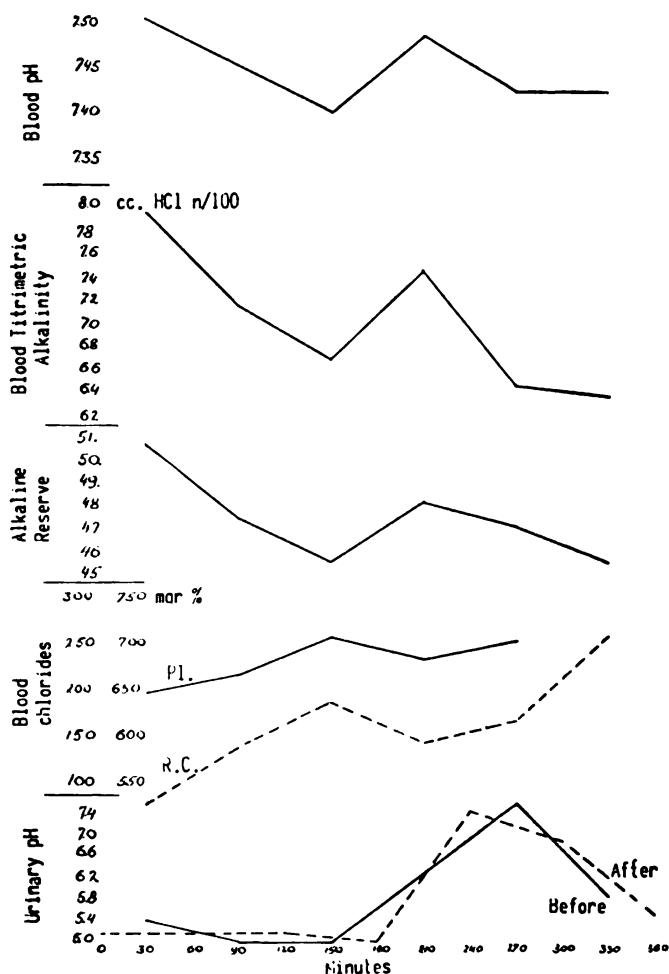


FIG. 204. In some cases the changes in several blood analyses parallel the changes of urinary pH. (Subject with metastatic melanoma)

cumulated in the bladder during the half hour preceding bleeding and again during the half hour following bleeding. The pH values of the urine specimens were plotted separately on graphs as A and B curves. Comparison was then made between the two urine curves and the curve representing the values of the blood titrimetric alkalinity, using time as abscissa. In another group of experiments using dogs, the bladder contents were drained at intervals of from five to ten minutes and blood specimens were obtained every thirty minutes.

Several preliminary tests were carried out to determine the degree of accuracy of the methods used. By employing a 0.001N NaOH solution, a probable error of no more than 0.1 cc. was found.

Comparisons between titrimetric alkalinity of hourly blood specimens and half-hourly urine specimens were made in thirty human and seven

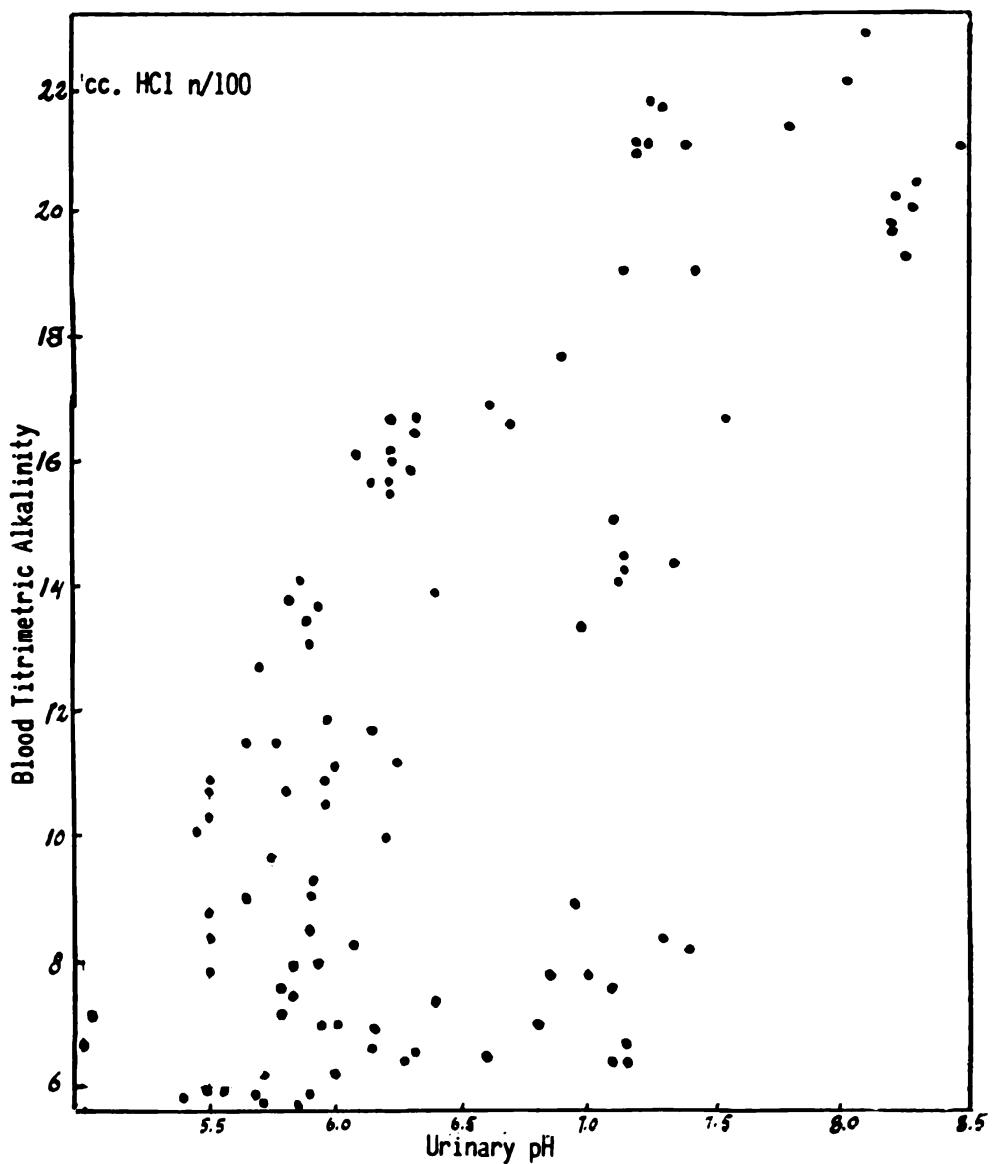


FIG. 205. The comparison between the concomitant values of urinary pH and of the titrimetric alkalinity of the blood shows that this relationship concerns more the occurring changes and less the absolute values of the findings. Urine samples with the same pH, in different subjects, are seen to correspond to blood samples with different titrimetric alkalinity.

canine subjects. In all cases, the curves of blood titrimetric alkalinity values showed a consistent parallelism to the pH curves of urine specimens that accumulated in the bladder during the thirty minutes preceding the bleeding and were collected at the time of venipuncture. (Fig. 206) The curves representing the pH values of urine specimens accumulated in the bladder dur-

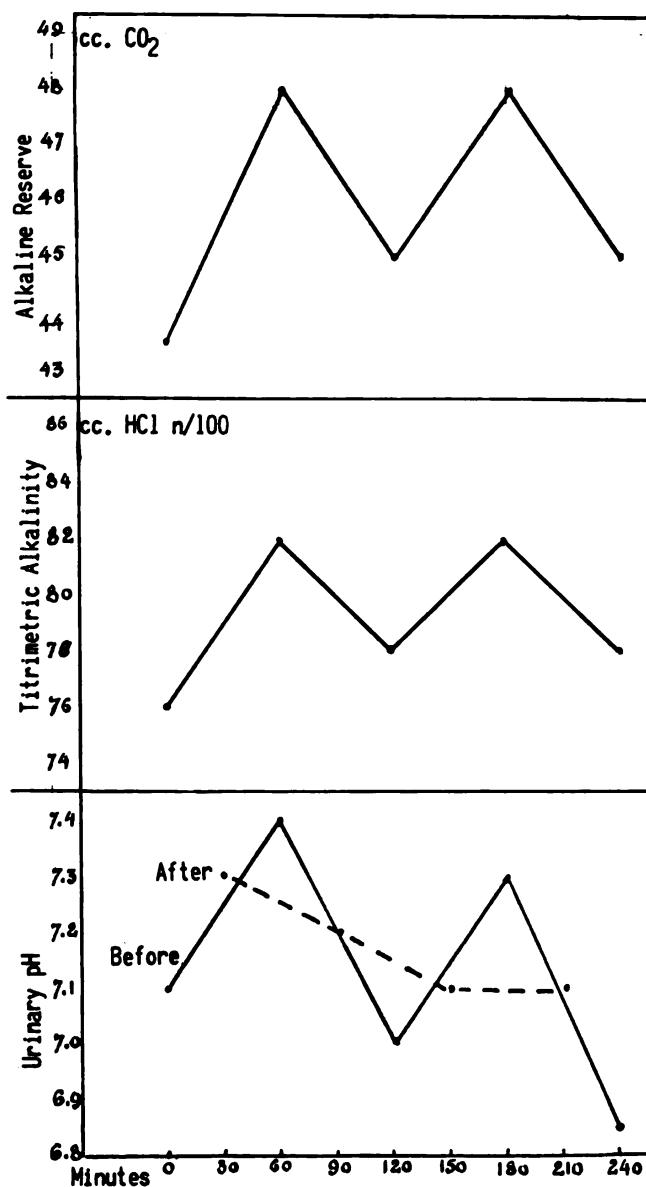


FIG. 206. Comparison of the hourly blood titratable alkalinity (in terms of cc. of .0IN HCl) and half hourly pH curves in a human subject. Urine curves A and B represent the before and after bleeding specimens. The parallelism between the titratable alkalinity of blood and the urine curve A is clearly shown. Curve B shows no correlation.

ing the thirty minutes after each bleeding (B curves) did not show the same consistent correlation to the blood titrimetric alkalinity. (Fig. 207)

In several tests, collections of half-hourly urine specimens were made fifteen minutes before and fifteen minutes after each bleeding. The same parallelism was found between the pH of urine accumulated in the bladder during the period from fifteen minutes before to fifteen minutes after bleeding, and the titrimetric alkalinity of blood specimens drawn in the middle

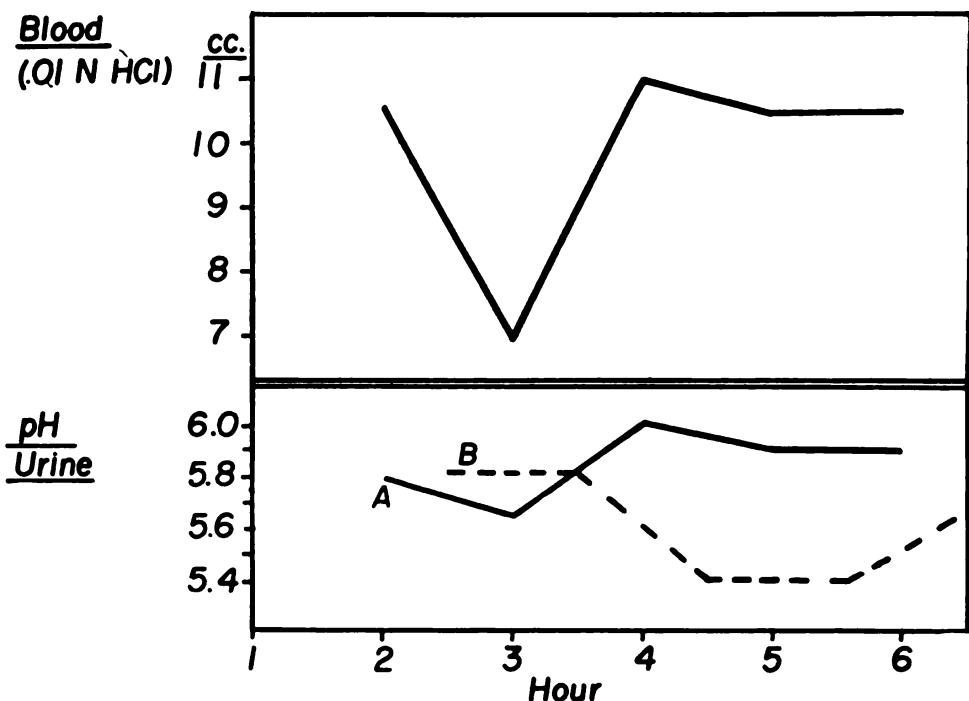


FIG. 207. Comparison of the curves representing hourly blood titratable alkalinity (in terms of cc. of .01N HCl) and urine pH in a human subject. Urine pH curve "Before" shows values of specimens accumulated in bladder during the half hour immediately preceding and collected at the time of bleeding, while urine pH curve "After" is for specimens collected one half hour after bleeding. There is a definite parallelism only between the curves of the titratable alkalinity of blood and the urine pH curve A.

of the urine collection period. The pH curves of urine specimens accumulated during the period from fifteen to forty-five minutes after each bleeding showed no consistent correlation.

When urine and blood specimens were obtained at shorter intervals, the same tendency of urine pH changes to precede the changes in blood titrimetric alkalinity was observed. In Figure 208 a rapid rise in urine pH is seen to begin within twenty minutes of the time of administration of sodium bicarbonate. The blood titrimetric alkalinity does not show an elevation for at least forty-five minutes.

These studies have shown that urinary pH variations correspond closely to changes in the values of an important factor reflecting acid-base balance changes of the blood, the titrimetric alkalinity. As a result, it has been possible to employ variations in urinary pH as indications of qualitative changes in acid-base balance of the blood for other studies.

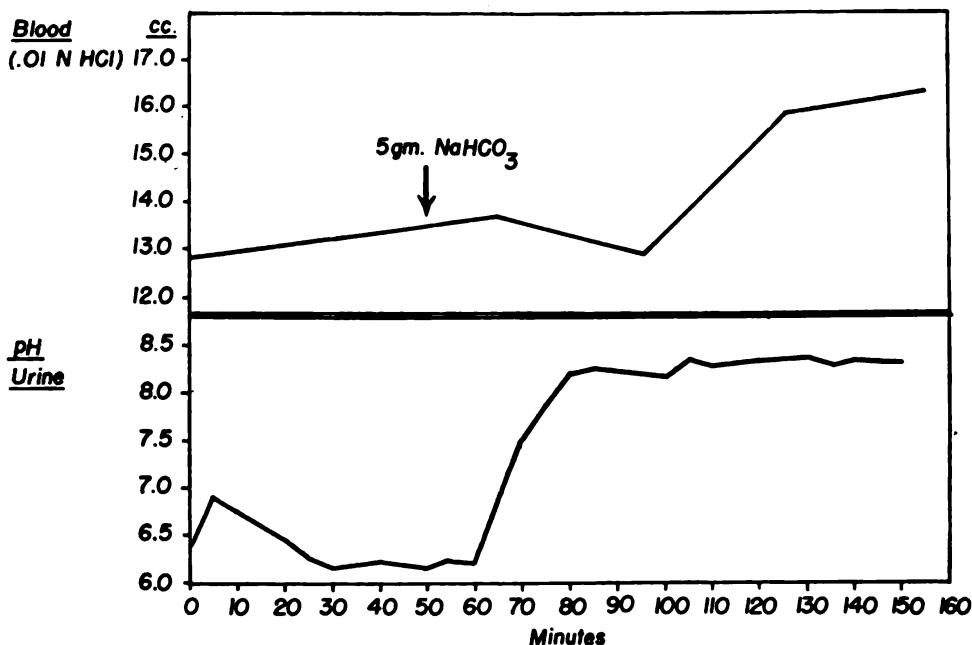


FIG. 208. The effect of the administration of 5 grams of sodium bicarbonate upon the *blood titratable alkalinity and urine pH curves* in dog. The elevation in urine pH is seen to precede by twenty-five minutes that of the titratable alkalinity of the blood.

Chapter 4, Note 3. Acid Pattern of Pain and Lactic Acid

We have investigated the relationship between pain of acid pattern and the appearance of lactic acid resulting from abnormal metabolism of carbohydrates. For this purpose we used the technique of Friedemann, Cotonio and Shaffer. In several patients it appeared possible to establish this relationship by measuring the lactic acid content of efferent blood from tumors during intensive pain of acid pattern. In a young man with a huge sarcoma of the knee, for whom the acid pattern of the pain had been established through its relationship to the changes in the urinary pH, such analyses could be carried out in the blood obtained from the big easily accessible efferent veins. At the moments of very severe pain, the amount of lactic acid had markedly increased. Values as high as 128 mg./100 cc. blood were found during these painful periods, contrasting with values around 30 mg./100 cc. blood in the period of calm.

Chapter 4, Note 4. Itching

Although we were especially concerned with pathological itching, we also became interested in physiological itching, especially in its relationship to sensorial sensations in general. Physiological itching can be regarded as a distinct sensation, not just a gradation of another sensation. Itching seems to have its own end organs. The propensity of certain regions of the body—such as the nasal mucous membranes, the skin near the nostrils, and the perioral and perianal skin—to itch in response to external stimuli can be correlated with the presence of such end organs. The proteopathic character of itching would also prompt us to consider less myelinized or even nonmyelinized nerves as its conductors. By analogy with pain, the existence of proper central centers could be conceived.

The most important characteristic of itching is that it can be induced by stimuli which, at other intensities, result in a different sensation such as touch, for instance. Although less manifest, other stimuli, such as heat and cold, also can induce itching. We have seen that stimuli which usually induce other sensations can produce physiological pain if they have an intensity above a threshold level. It is the intensity of the stimulus which determines whether it causes pain or a sensation of touch, heat or cold. Since pain appears if the stimulus is above the threshold level, it serves as a warning of a damaging incitation.

In studying itching under a similar aspect, it can be seen that it, too, is induced by nonspecific stimuli. But, for itching, the intensity of the stimuli is low. Everybody knows that an essential condition for the induction of itching is that the incitation be slight. This is easily seen for the skin, and especially the nasal mucous membrane, where a stronger stimulation will not induce itching but a touch sensation. Just as the intensity of a stimulus determines whether pain or touch is produced, so the intensity also determines whether itching or touch is felt. While the sensorial sensation of touch is induced by stimuli with intensities below those required for pain, itching is induced if intensities are below those required for touch.

The relationship of intensity of stimulus to itching, sensorial sensation of touch, and pain is shown in Figure 208 bis. This correlation explains why itching is present sometimes for a brief period when skin or mucous membrane sensorial sensation or even pain is induced. Immediately after an injury, for example, itching may be felt for a short time only to disappear just prior to the development of pain. The low intensity of the stimulus required explains a striking characteristic of itching: its disappearance when a stronger stimulus is applied. Thus scratching, which adds more intensive stimulation, makes itching disappear. The more violent the scratching, even to the point of inducing pain, the more effective it can be in eliminating itching.

The general reaction toward itching also appears related to the character of its induction. The individual responds to pain by fleeing or fighting in order to escape the intensive noxious incitation. As the incitation that produces itching is minimal by definition—the presence on the skin of a

minor irritant such as a fly or mosquito, for instance—scratching is sufficient to eliminate it. With a fly on his skin, the individual need not flee or fight, but only scratch. With the concept that itching can result from exactly the same type of stimuli as pain and touch, we integrate it in the group of sensorial sensations. We can then establish a separate sensorial subgroup for itching and pain. While other sensorial sensations inform us of the nature of the excitation—heat, cold, sound, taste, etc.—itching and pain inform us only about the intensity of the stimulus, not its nature.

Pathological itching, like pathological pain, is related to the existence of abnormalities. In addition to the differences in stimulus intensity required to induce itching and pain, their different nervous formations help explain their clinical separation. No patient we have studied has ever indicated any confusion as to whether his discomfort was due to severe itching or pain. The two sensations are seldom concomitant; usually they succeed one another. The fact that proteopathic pain and itching both seem to be conducted through unmyelinated nerves indicates why they can appear under similar conditions, as in nerve regeneration. This seems to have led to confusion between itching and pain. However, itching and pain observed during nerve regeneration can be clearly differentiated by the patient. The fact that the itching sensation is produced by stimuli of low intensity also explains why itching is so often present on skin or mucous membranes without appreciable pathology. Minimal changes appear sufficient to induce the sensation.

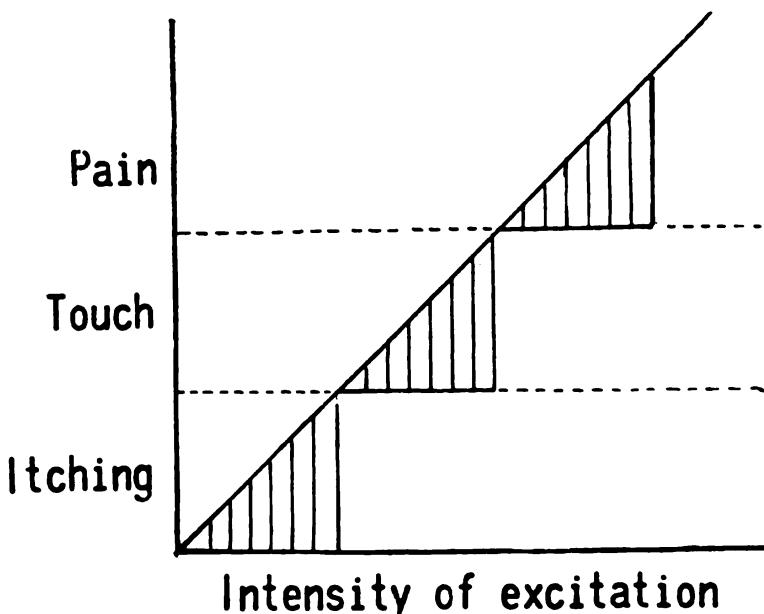


FIG. 208 bis. Similar to pain, *itching* represents a special kind of sensation, with the aim to inform about the intensity of the excitation. If this is very slight, it induces *itching*. If above the threshold, at which the incitation acquires a noxious character, it induces pain.

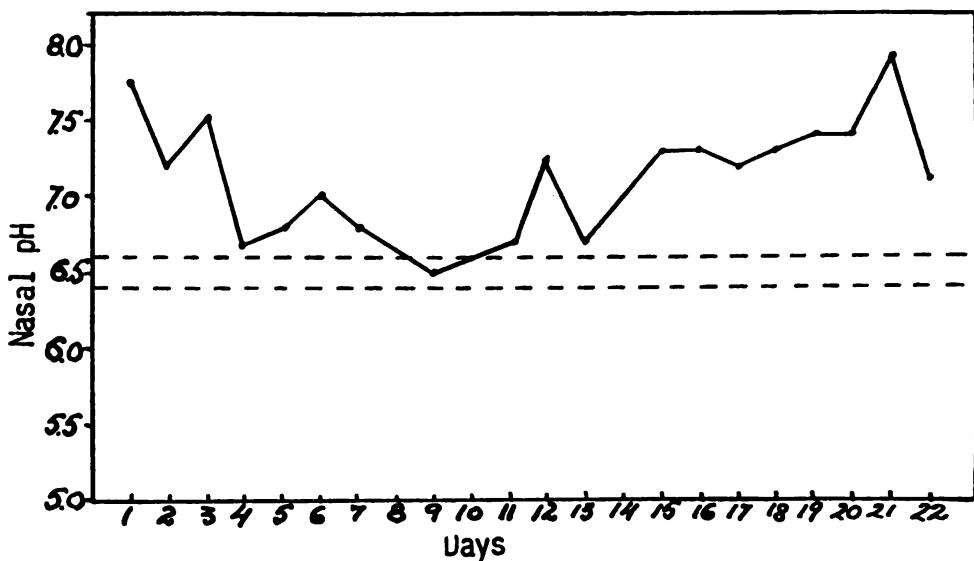


FIG. 209. The nasal pH measured with a glass electrode introduced deep in the nose shows the same dualism as the other analyses. With 6.5 as the average value, the curves of the nasal pH has more rapid and broader variations than other analyses. Curve of daily analyses shows values above the average line in a case of generalized melanoma.

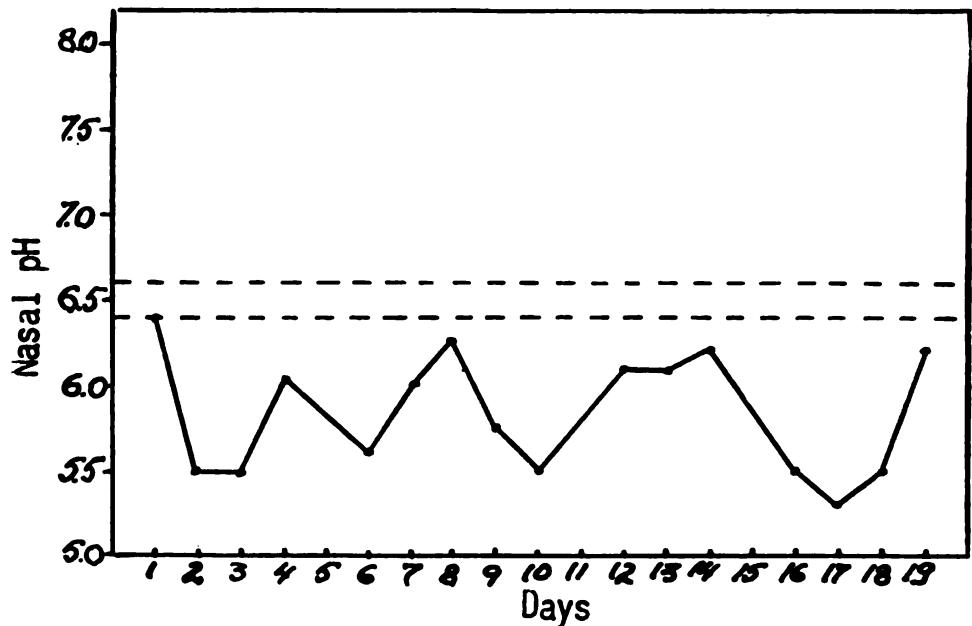


FIG. 210. The nasal pH shows persistent low values in a case of cancer of the liver.

Chapter 4, Note 5. Nasal pH

Nasal pH was measured using a portable Beckman pH meter and a glass electrode small enough to penetrate deep into the nose. In a research made with N. Buchanan it was found that valid data could be obtained only if the electrode touched the turbinate, otherwise marked differences in values were noted.

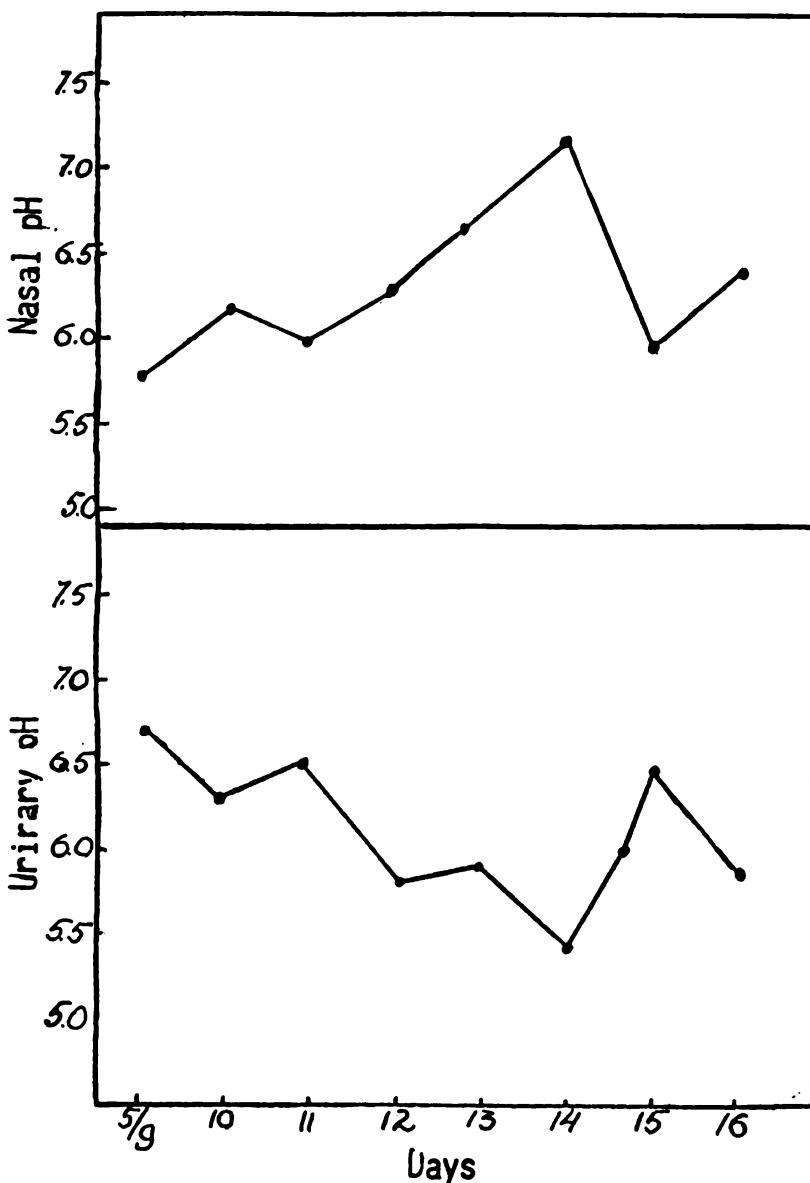


FIG. 211. The relationship between daily changes of the nasal and urinary pH shows opposite variations.

In a simplified method, cotton applicators were soaked in Guillaumin indicator solution with methyl red and bromothymol blue and left to dry. They were easily introduced to sufficient depth in the nose and left in place for at least two minutes. Color of the wet spots was checked with a colorimetric scale. Data obtained with glass electrode and colormetric applicator were found to coincide closely.

Two offbalances could be seen, one with the pH elevated sometimes even above 8. Figures 209 and 210 show curves of the offbalance in two patients. It is interesting to note that the changes in the nasal pH values parallel those seen at the level of lesions and are opposite to those concomitantly occurring in the urinary pH (*Fig. 211*) which parallel those of the titrimetric alkalinity of the blood.

Chapter 4, Note 6. Wheal Resorption

Interesting information could be obtained by analyzing the absorption of fluid injected intradermically in various subjects, and correlating the results with the existence of metabolic offbalances. We used the technique proposed by McClure and Aldrich, in which they measured the time needed for the disappearance of a wheal, resulting from an intracutaneous injection of a saline solution. A relatively extensive study of absorption was made in more than 500 subjects—both normal and abnormal. We present here a few of the conclusions from this study.

The average time necessary for resolution of the wheal obtained by the injection of .2 cc. of 7% NaCl solution, in normal subjects, was 23 minutes; the range was from 15 to 30 minutes. When deviations from these values were observed, they were consistent, in the sense that tests repeated at short intervals in the same general area in the same subject gave values in the same abnormal range. Abnormal values occurred in two directions. Resorption time was shortened in some cases and values as low as 1 to 2 minutes were noted. In the opposite direction, values as high as 90 minutes were observed. These deviations from normal time could be related to local and general conditions. The presence of local or regional edema shortens the resorption time so much, that in some cases with massive edema, no wheal could even be realized. Shortening of time was found to be true for an edema, regardless of cause—inflammation, impaired local circulation as in phlebitis, impaired general circulation as in cardiacs or in renal failure. Lengthening of resorption time in cases of phlebitis provides valuable information on the evolution of the condition. The return of resorption time to normal values seems to indicate sufficient improvement to permit mobilization of the patient.

In subjects in whom no local factor could be considered to be responsible for changes in the resorption time of the wheal, we could see that abnormal variations had a direct relationship with the general offbalance present. In some subjects with a manifest offbalance of type D, wheal resorption time was shortened. Values as low as 4 to 5 minutes were obtained. Analysis of a number of cases indicated that this shortening of

resorption time meant a bad prognosis. A few patients with only values of 2 to 4 minutes died within a few days although other symptoms gave no indication of a fatal outcome within a short time.

Extended resorption time has been found in subjects with an offbalance of type A. Values as high as 60 to 90 minutes were found in subjects in whom all other analyses indicated this offbalance. It is also interesting to note the existence of slow resorption time for aged subjects. In a group of 80 patients ranging from 70 to 90 years of age, an average resorption time of 90 minutes was found. (*Fig. 68*)

Chapter 4, Note 7. Eosinophiles

The role of the blood as the secondary part of the entity organism has explained many of the peculiarities of its cells. Aside from the phagocytary functions that can be considered as a particular form of capturation, the leucocytes have to be recognized as acting as holocrine monocellular formations whose specific constituents are liberated by cellular lysis. We have seen that in the case of the neutrophilic granulocytes, the hydrolytic enzymes so liberated, strongly resemble the external secretion of the pancreas. Under this aspect, we have investigated the blood eosinophiles with a role similar to the Paneth cells of the duodenum.

The physiology of these leucocytes has to be sought in the acidophilic character of their granules. Morphological analysis of the eosinophile granule shows that it is formed by a content and a membrane, the last clearly seen in preparations in which the granules have lost their content. Like many other membranes, that of the eosinophile granule can be easily identified as being made partially at least by lipids being stained with dyes dissolving in lipids, such as black Sudan or Scharlach. However, it is the content of the granule with its ability to combine with acid dyes that indicates its specific characters. Under certain circumstances, when the blood is maintained *in vitro* between the slide and cover object for a certain time, the membrane and granule content are seen to separate. Before this occurrence, a lysis of the eosinophile leucocyte itself takes place. This is manifested through the breakdown of the cellular membrane with the lysis of the nucleus. It is in a second step that the eosinophile granule loses its content. Following it, besides the empty granules and lysed eosinophiles, characteristic Charcot-Leyden crystals appear. The correlation between these crystals and eosinophiles has been recognized and is generally accepted as occurring *in vivo* and *in vitro*.

Ayer (215) has shortened the process of lysis of the eosinophiles *in vitro* by treating the blood preparations with a detergent, aerosol. By repeating Ayer's experiments, the relationship between the appearance of the Charcot-Leyden crystals and the more complex process of lysis of the eosinophiles has become apparent. It could be seen that the crystals would appear at the site where the nuclei of the eosinophiles disappeared through lysis, and where careful observation of the granules reveals the loss of their eosinophilic content. The presence of empty granule membranes

stained by the fatty dyes, in addition to the lysed nuclei, would indicate the conditions under which Charcot-Leyden crystals appear. The eosinophilic content of the granule and products induced by the nuclear lysis represent the two factors that together result in these crystals.

Concerning the relationship between Charcot-Leyden crystals and eosinophiles, it is interesting to note the difference that exists between eosinophilic granules in various animals. Besides the morphological aspect which can be very different, apparently no Charcot-Leyden crystals are obtained from species other than humans and certain simians. This indicates that when the biological role of the eosinophiles is considered, we have to seek another common factor in addition to the morphological and chemical ones. It would seem that it is in their basic reactivity, *i.e.* in their capacity to bind substances of acid character, that the common character of all eosinophile granules has to be sought. This is also true for the duodenal cells.

Following this view, we initially tended to accord more importance to an antacid property than to any other, seemingly agreeing with other data obtained from this study. Among the substances found to be the principal constituents of these eosinophile granules, the alkaline amino acids, of which arginine is the the principal one, assume a very important role. According to the hypothesis we advance, these alkaline amino acids would represent the active factor of these granules and would be liberated by the eosinophiles when they disintegrate. The eosinophiles would intervene in physiology for the specific purpose of furnishing certain alkaline compounds in whose constitution the alkaline amino acids enter. The solubility of the granule content, when liberated, and the Charcot-Leyden crystals indicate, according to this view, that the main character of the eosinophile granule is its capacity to furnish alkaline compounds. Under special circumstances, they are able to act against substances with acid properties that result from the lysis of the nuclei, and together to form the Charcot-Leyden crystals.

The relationship of the disintegration of the eosinophile to the surface tension lowering agents is also interesting for the further liberation of the content of these granules. Just as for other granulocytes and lymphocytes, lysis is the characteristic fate of these cells and would constitute their most important character. As seen above, it can be related to the role of blood in the organization, *i.e.* as the secondary part of the organism level.

As for the other leucocytes, an important factor in the holocrin role of the eosinophiles has been seen in the necessity of a maturation of these granules for their active intervention. When lysis was induced, it was seen to affect only the cells that had reached a certain degree of maturity, not only for the cells themselves but also for the granules. Young cells, recognized by more intense basophilic of the cytoplasm, by lack of, or reduced lobulation of the nucleus, and especially by a neutrophilic or even basophilic character of the granules, do not break down. As in the circulating blood, immature elements are seen, the delay observed in inducing eosinopenia by various agents can be interpreted as corresponding to the time needed for the circulating eosinophiles to reach maturity, as an essen-

tial condition for their lysis. This situation was apparent in a particular case, that of eosinopenia induced through administration of the adrenal corticoids. Although this appears to have a direct effect upon the eosinophiles, a definite time, often even 24 hours, is seen essential in order to achieve the disappearance of the eosinophiles from the circulating blood. This delay has been related to the presence of eosinophiles, allegedly "resistant" to the corticoids. In fact, in studying the eosinophiles which persist after the administration of these hormones, we could see that they represent only immature elements, probably prematurely liberated in larger amounts from the bone marrow. The cells from which some will persist even for 24 hours after administration of corticoneoglucogenic hormones, do not show lysis in vitro nor the appearance of Charcot-Leyden crystals, and they present the tinctorial characters of immaturity for the cytoplasm and especially for the granules.

Granule maturation, which corresponds to the acidophilic character, seems to be the essential condition for the lytic intervention of these cells. In the physiological role of eosinophiles, an important aspect of maturation was seen in the relationship between the richness of these elements in the circulating blood and the processes in which a manifest local eosinophilia is induced, as through injection of parasite larvae or vegetal oils. A direct relationship between local and sanguine eosinophilia was apparent, the value of the former being the function of the latter. The ability of bone marrow to rapidly compensate the transitory eosinopenia following the passage of these cells into the tissues, has further directly connected the local richness in eosinophiles to the bone marrow's capacity to send new cells into the circulation. In all these changes, the prevailing factor has apparently been the degree of maturity of the eosinophile granulae, which seems to require a certain time to reach the desired degree which is the principal condition also for their physiological intervention.

Correlation between the biological intervention of the eosinophiles and the acidophilic character of the granules and their richness in alkaline amino acids has been confirmed in a study of the basophiles of the blood cells with granules having an opposite character. These granules have an acid content, as seen by their tinctorial affinity for alkaline dyes. They were also observed to contain heparine, a polysulfonated mucoid of frank acid character. The biological antagonism between heparine and alkaline proteins is well known. Protamines, the correspondent of histones for fish, are used to correct the excesses of heparin in the body, especially as therapeutic measures. Therefore, the antagonism between eosinophilic and basophilic granulocytes goes beyond their tinctorial characters.

Through the alkaline reactivity of eosinophiles as related to the fundamental separation of intervening constituents according to their positive or negative character, the antacid eosinophiles could be considered to be in the former group, while the basophiles, rich in lytic heparin, are in the latter.

We shall more fully discuss below the nature of the intervention of the eosinophiles after studying the role of a special group of constituents. For

the present, it seems that under abnormal circumstances, exaggeration in the amount of eosinophiles would indicate an existing predominance of conditions that correspond to agents of positive character, *i.e.*, with heterotrophic tendency. The more precise antacid character of these cells further indicates the place that has to be reserved for the eosinophiles in the group of heterotrophic agents. Under this aspect, the eosinophile would be seen as an agent of anti-acid character in the blood and tissues, conceived to act as a holocrin cellular gland, *i.e.*, through the lysis of the corresponding cell. Therefore, the richness of the blood and tissues in eosinophiles would indicate a predominance of heterotrophic tendency, while paucity in eosinophiles or their absence would indicate a homotropic trend.

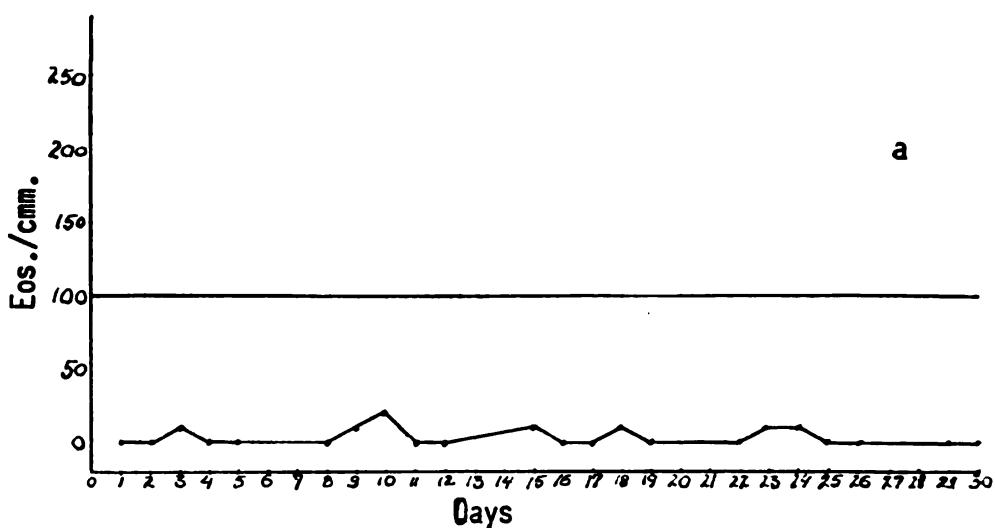


FIG. 212. Curve of blood eosinophiles in a case of breast adenocarcinoma with multiple metastases, showing values persistently below the average line of 100.

In order to understand this aspect of the eosinophiles, we tried to follow the changes in their amount in the blood in relation to normal and abnormal physiology. Study of the changes in the number of eosinophiles in the circulating blood under physiological conditions has indicated the existence of the same 24-hour oscillations as seen for many other constituents of the blood. A relationship is apparent between the periods corresponding to higher or lower quantities of circulating eosinophiles and the degree of activity of the individual. This appears to be opposite in humans who show diurnal activity, and mice and rats that show nocturnal activity. By experimentally changing the hours of light and dark for mice and rats, and through it the time of rest and of activity, the rhythm of change was reversed.

Following the concept of the intervention of eosinophiles in biological balance, we further investigated this aspect of the problem in relation to the dualism in abnormal conditions. Just as for other tests, we obtained an

average value in a large series of normal human subjects. Utilizing the Dungar technique for a direct count of eosinophiles, the value of 100 cells/1 cmm. was found to be the average value. An impressive direct correlation could be found between the amount of circulating eosinophiles and the two patterns of abnormality. In one group, that corresponding to type A, the number of eosinophiles appeared not only high but with their values fixed above the average value. High values were observed to persist for long periods of time. Figure 213 shows such a case. For the opposite pattern, corresponding to the fundamental type D, these values appeared to be below 100 and very often 0, persisting for a long period of time. (Fig. 212) In these dual patterns, the degree of abnormality could be related to the deviation in the number of these elements from the average value of 100 elements/1 cmm.

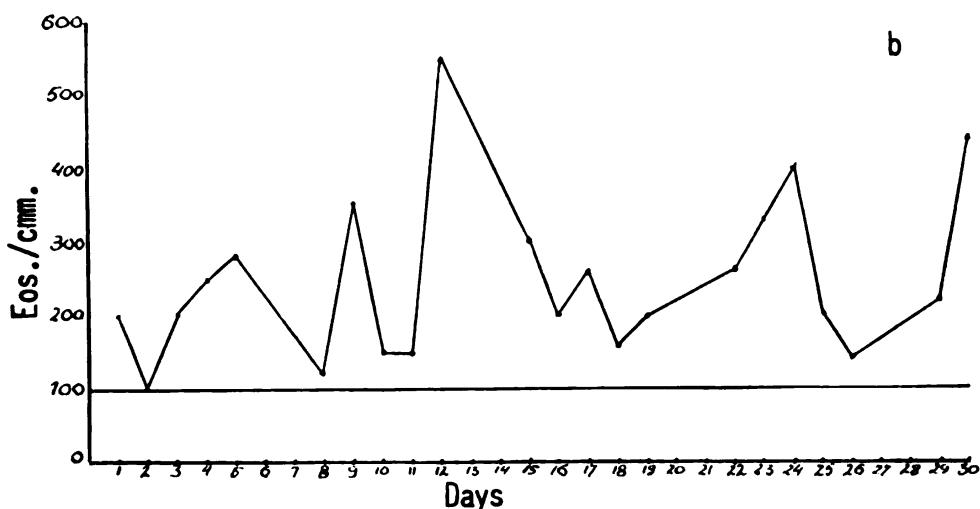


FIG. 213. Curve of the blood eosinophiles in a case of generalized melanoma showing values persistently above the average line of 100.

The relative facility with which the number of eosinophiles in the blood of an individual can be determined, has made it an important research tool for information regarding the balance between the two fundamental biological tendencies.

Chapter 4, Note 8. Total Blood Potassium

For a large-scale investigation—requiring as many as one hundred determinations a day—the technique of separating red cells from plasma appeared to be impractical. In view of the relatively minute amounts of potassium in plasma as compared to cells, we could utilize total blood instead of the cells. It was also found that by diluting the blood 1/10, the values obtained were in the same range as for serum potassium, a fact which permitted the use of the flame photometer without any change in the

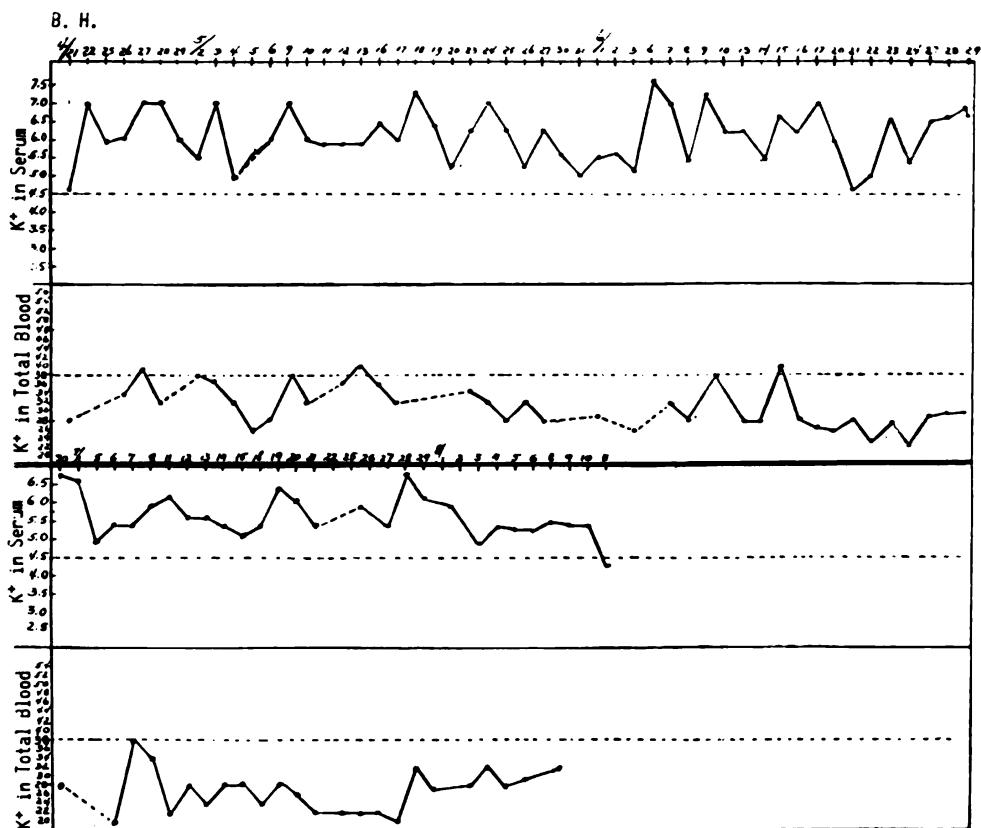


FIG. 214. Relationship between serum K⁺ and total blood K⁺ permits to recognize the nature of the changes concerning the intervention of this element. In a case of periarteritis nodosa, the high values of serum potassium and low values of the total blood potassium indicate an offbalance type D.

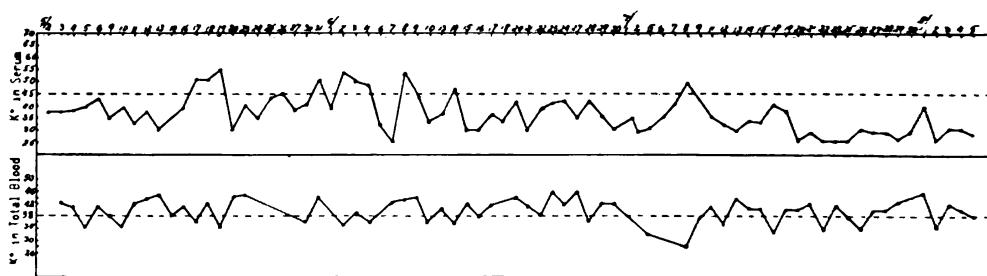


FIG. 215. Low values of serum potassium and high values of total blood potassium indicate an offbalance type A in a case of cancer of the gallbladder.

set-up of the apparatus. The blood was diluted with a 1% acetic acid solution in the pipette used for counting white cells. The pipette was shaken as for the count of cells, and the necessary amount taken from the diluted content. The potassium amount was determined and the result multiplied by 10. While the average value for the total blood was found to be around 38 mEq., values as low as 20 or as high as 60 were seen. (Figs. 214, 215, and 216)

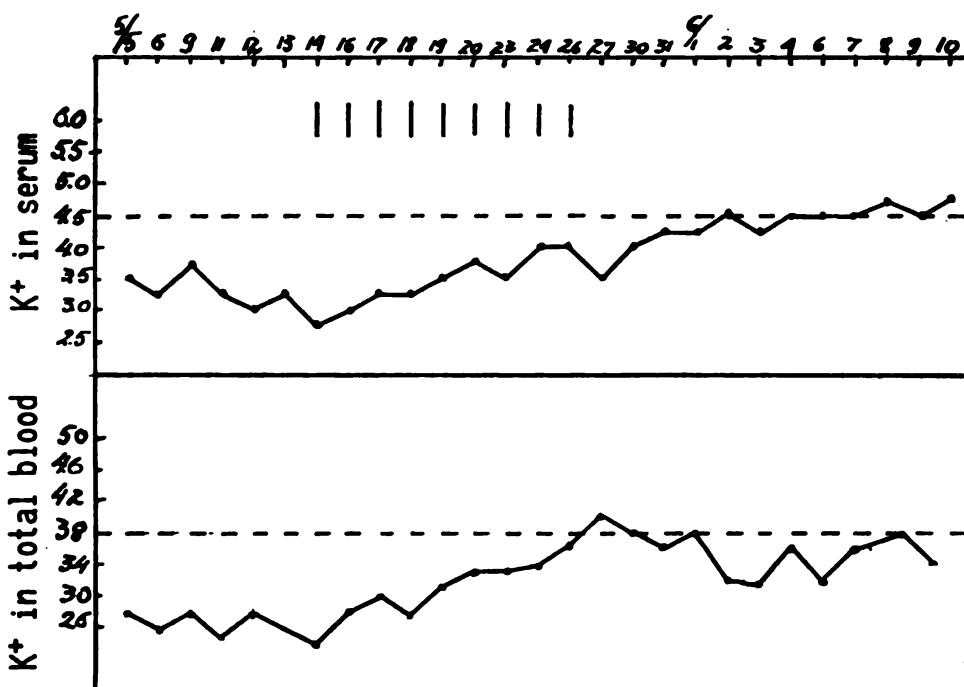


FIG. 216. Low values of potassium in serum and in total blood indicate a quantitative deficiency, in a subject with a liver adenocarcinoma. The administration of 40 m Eq KCl daily, for 9 days brought the two curves to normal.

Chapter 4, Note 9. Sulphydryl Determination

The catalytic effect of sulphydryl groups on the oxidation of sodium azide by iodine was first described by F. Raschig (214), and F. Feigl (217) utilized it to develop the most sensitive qualitative test for the presence of sulphydryl containing compounds. The reaction, initiated by mercaptans, sulfides, thiosulfates and thiocyanates, takes place as follows



While this equation implies that the sulphydryl compounds do not take part in the reaction, this is not entirely correct, because simultaneously, the sulphydryl groups are oxidized by the free iodine. Accordingly, the reaction is carried out in a Warburg apparatus, where 1 ml. of 0.2 M sodium azide

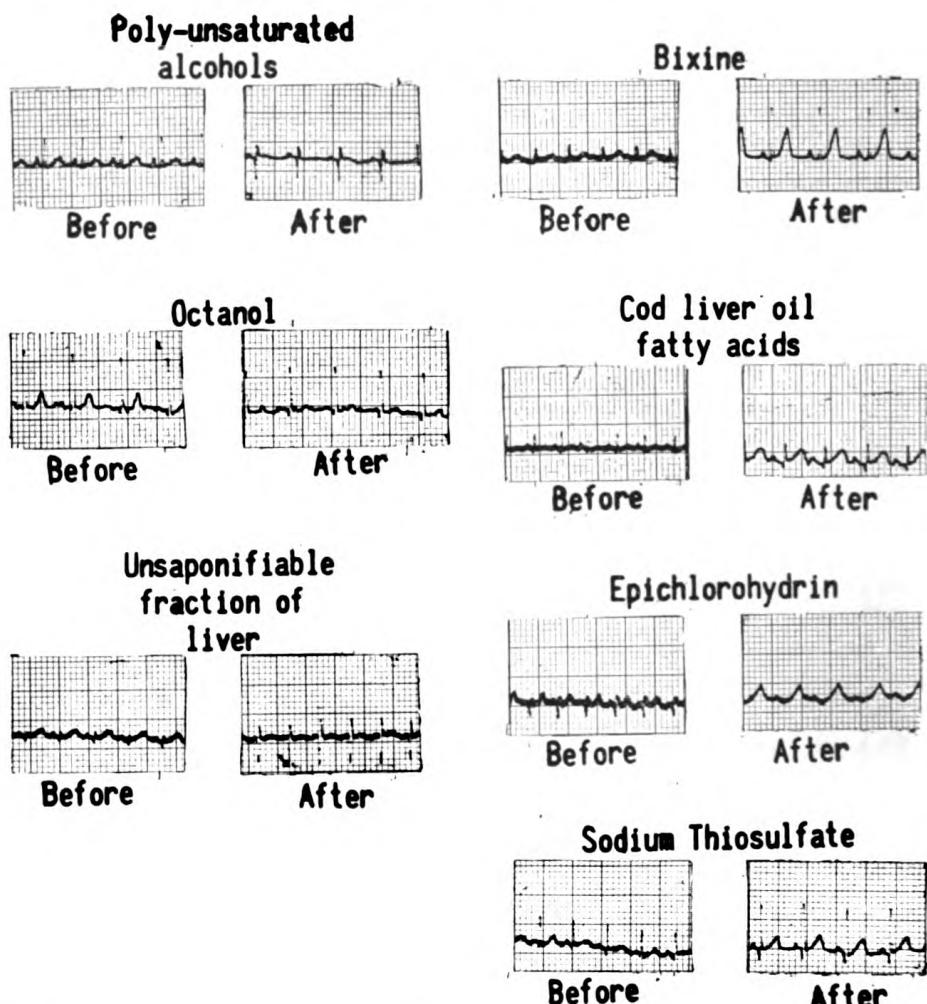


FIG. 216B. Electrocardiograms, in first lead in rabbits injected intraperitoneally with sublethal doses of different agents. In the group with lipoids with positive polar groups besides other changes, a flattening of the wave T is induced, which contrasts with a more elevated T for the group of lipoids with negative character.

and 1 ml. of 0.1 M iodine-potassium iodide solutions are mixed, while the sulphydryl containing solution is kept in the sidearm, and then added to the reagents after temperature equilibrium is reached. Upon complete mixing, there is rapid nitrogen evolution, which, however, ceases within 13 minutes. The amount of nitrogen evolved is found to be linearly proportional to the content of the sulphydryl groups, and on the average, 1 ml. of urine, 0.05 ml. of blood or 1 ml. of a 3×10^{-4} M sulphydryl containing solution are amply sufficient for an assay. The method is thus well applicable to the determination of sulphydryl levels, provided the method is standardized with the appropriate compound to be tested, as the catalytic effect of all mercaptans is not the same. (218)

Chapter 4, Note 10. Calcium in Urine

1 cc. of the urine was diluted in a test tube with 8 cc. of distilled water and the optical density of the mixture was determined. To the mixture, 1 cc. of a 1% solution of potassium oxalate and 3% of oxalic acid was added. After standing for 5 minutes, the tube was shaken and the optical density again was read. The difference, multiplied by 10, was divided by the two figures of the specific gravity of the sample. The value obtained was called the calcium index.

Chapter 4, Note 11. Urinary Surface Tension (ST)

The role of changes in the surface tension of various body fluids in normal and abnormal physiology has become of increasing interest. Some authors have gone so far as to consider the surface tension forces present at the interfaces separating entities, to be the most important factors in the boundary formations which serve to individualize these entities.

Considering multiple aspects of the problem, it appeared interesting to attempt, as a first step, to obtain information about the surface tension of different body fluids. It was as part of this program that urinary surface tension was investigated with the intention of utilizing the data to gain insight into changes related to the dualistic offbalances. Before we could proceed, it was necessary to resolve several problems, including the technical difficulties in measuring surface tension that result from the special constitution of the urine.

Technical Problems

Successive measurements of surface tension, when made on fluids formed by a single substance, consistently furnish the same value. But for fluids composed of two or more constituents, values vary from one moment to the next. This is explained by the fact that molecules of constituent substances have a tendency to migrate in the fluid, some accumulating at the surface, others concentrating in the bulk. (Gibbs dictum) The surface tension of different complex fluids has been found to vary according to the nature and amount of tensio-active substances present. And, study of the variations has furnished information about the nature of these substances.

In a fluid such as urine, containing many different substances, the problem of variations in surface tension is a major one. ST measurements, made without considering these variations, would be subject to serious errors. Examination of different samples of urine has shown great differences between values obtained at different times. Using Lecomte du Noüy's tensiometer (215) it could be seen that, for the same urine sample, values vary according to the length of time the sample is left to stand. Values progressively decrease as standing time increases. Similar changes are seen when the pendant drop method is used. (216)

Because of the fact that a certain time is needed for changes to take

place, the relationship between change and time was investigated. The study of various urine samples has emphasized the inequality which exists between them not only in the intensity of changes but also in the time necessary for the changes to take place. This fact has rendered useless the measurement of the surface tension of different samples if all are made at some given moment. Except for measurements made at frequent intervals, use of du Noüy's tensiometer has appeared to be inadequate for urine. Traube's stalagmometer also is unable to furnish values that take these changes into account.

Theoretically, it would appear possible to obtain measurements that would correspond to the surface tension for each drop at a desired moment by changing the rate of flow of the urine through the apparatus. But the differences between urines, related to changes in distribution of components, have made this inadequate.

With the pendant drop method, progressive changes which occur in the shape of the drop would appear to indicate the changes in surface tension. (216) Technically, it would appear necessary to obtain data as frequently as possible in order to follow changes which occur at various times. By using serial pictures, the changes, the moment of their occurrence, and their intensity can be studied accurately. Unfortunately, the complexity of the method, with the need for frequent pictures and involved calculations, prohibits its use for routine measurements and, consequently, for any broad clinical and experimental research.

It was under these circumstances that we returned to the capillary method which we considered capable of furnishing the desired data. Classically, the height of the ascending column in a calibrated capillary is used to calculate the surface tension. Height alone, however, is unsatisfactory, since it does not reveal the changes that take place. It was by studying the descent of the column in a capillary that we were able to obtain the data which we were seeking. We could show that the column does not descend with uniform velocity. It stops or slows down perceptively several times before it comes to rest at a fixed value. We could recognize that, for most urine samples, there is a first stop usually of several seconds duration. In some urines, this first stop is replaced by a marked slowdown in velocity of descent. The stop or slowdown is followed by renewed but slower descent and a second stop somewhat longer than the first. After another descent, often lasting more than 20 minutes, a new stop occurs.

The time of descent, the duration of the stops, and especially the heights of the column at which the stops occur, while reproducible for the same urine, vary widely with different samples. They would thus indicate different repartitions and the times when they occur. This technique of using the capillary consequently appears to be adequate for the study of the surface tension of complex solutions and particularly for the study of urine.

Each of the heights at which the descending column stops would indicate the surface tension for a particular stage in the repartition of the constituents. In studying this problem further, it appeared advisable to try to

have the capillary so calibrated as to permit a direct reading of surface tension values at these stops. The study of the relationship between the surface tension of a fluid and the height of the column has indicated the nature of intervening factors, their values, and under what conditions a direct reading is possible.

The fluid column remains stationary in a capillary tube when the surface forces which bind the column of fluid to the wall of the capillary are equal to the weight of the fluid column.

With σ representing the surface tension; r , the radius of the capillary tube; h , the height of the column; Δ , the specific gravity of the fluid; and g , the acceleration of gravity, we have $2 \pi r \sigma = \pi r^2 hg \Delta$. It can be seen that the specific gravity is the only factor related to the sample, other than the surface tension, which intervenes in determining the height of the fluid column.

According to this formula, the relationship between the surface tension and the specific gravity of the specimen is: $\sigma = \frac{\Delta rhg}{2}$. The same height of the column is obtained if the relationship between surface tension σ and σ' of two different liquids with the specific gravities Δ and Δ' fulfills the condition: $\sigma = \frac{\sigma' \Delta'}{\Delta}$.

If measurements with a capillary tube having a bore radius of 0.5 mm. are made in New York City, where the acceleration of gravity is 981 upon water which at 18°C has a surface tension 73 dynes/cm., the height of the fluid column is found to be 6.0 cm. and the relationship between σ and Δ , expressed in the cgs. system is $\sigma = 73 \Delta$.

A capillary tube thus can be calibrated to permit the direct reading of the surface tension in dynes/cm. for any liquid having the same specific gravity. For fluids of different specific gravity, the same capillary tube can be used if a correction of 0.073 dynes/cm., is made for each 0.001 increment of the specific gravity.

Urinary specific gravity values encountered clinically range between 1.001 and 1.035, with an average value around 1.015. Tubes calibrated to measure urine specimens with specific gravity values at either extreme can yield errors in the surface tension of as much as 2 dynes/cm. In order to minimize the degree of error for routine laboratory use, the capillary tube has been calibrated to correspond to a fluid with a specific gravity of 1.015. The maximum error of the surface tension values for the extremes of specific gravity clinically observed will be reduced to approximately ± 1 dyne/cm. in this way. Furthermore, the fact that sodium chloride concentration is one of the important factors inducing different values for urinary specific gravity reduces the influence exercised by specific gravity upon the height of the column. Sodium chloride represents a negative surface active substance. It will raise the surface tension values as its concentration increases because of its tendency to migrate from the surface toward the bulk of the fluid. This will partially decrease the influence exerted by the specific gravity of the urine. Since the surface tension values of human urine speci-

mens measured by this method have been found to vary between 73 and 50 dynes/cm., the error for extreme values of specific gravity is less than 5% and is not clinically significant. If more precise values are desired, the necessary correction can be made by adding or subtracting 0.073 dynes/cm. for every .001 difference in the sample above or below the specific gravity for which the tube is calibrated (*i.e.* 1.015).

The temperature of the urine to be tested is another factor which intervenes. Although the fluid rapidly attains the same temperature as the capillary walls, it is advisable to perform measurements when the temperature of the fluid is around 18°C, since the surface tension of a liquid decreases as its temperature increases. For clinical use, corrections for differences in temperature are not considered necessary.

Design and Calibration of the Urotensiometer

In order to obtain direct readings of the urinary surface tension values with a maximum error of ± 1 dyne/cm., the previously discussed factors were taken into consideration in designing and calibrating the urotensiometer which we conceived. (Fig. 217) The glass capillary tube has a bore

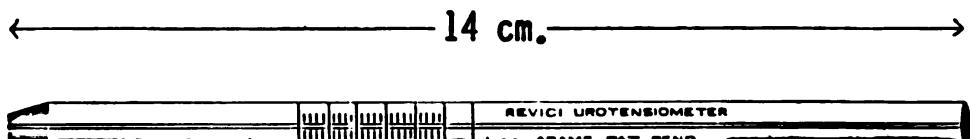


FIG. 217. Urotensiometer—calibrated to indicate by direct readings in dyne/cm the surface tension of fluids having a specific gravity of 1.015.

diameter of 0.5 mm. and is approximately 14 cm. in length. It is calibrated to indicate the surface tension of a fluid with a specific gravity of 1.015 directly in terms of dynes/cm. in the following manner: a continuous column of distilled water at 18°C is drawn up to about three-fourths of the height of the tube and allowed to descend with the tube maintained vertically. The point at which the top of the column stops is marked. It represents a surface tension of 73 dynes/cm. for water having a specific gravity of 1.000. In order to make the necessary correction for a fluid having a specific gravity of 1.015, the distance between this point and the tip of the tube is divided into 74 (instead of 73) equal parts. The tube is calibrated down to 50 dynes/cm. since lower values have not been encountered. In the tubes manufactured by Clay Adams, New York, the markings are permanent. The split line feature of the scale permits easy visualization of the meniscus. The encircling lines help in maintaining the tube in the vertical position.

The Measurement of Urinary Surface Tension with the Urotensiometer

To determine surface tension by means of the Urotensiometer, the tapered end of the tube is introduced into the bulk of the urine specimen.

The fluid is drawn slightly above the highest mark by mouth suction and evacuated several times by positive pressure. The tube is again filled to the same point, care being taken this time that no air bubbles interrupt the continuity of the fluid column. The tube is removed from between the lips, and the tip of the capillary is then gradually raised toward the surface of the fluid. When the top of the column descends to the top line (T) of the scale, the tip of the tube is removed from the fluid and maintained in a vertical position at eye level. The descent of the top of the column can best be observed by viewing the meniscus between the ends of the split line calibration markings. The top of the column descends within one or two seconds to an initial point (P_1) where it comes to a temporary halt or its rate of descent suddenly slows perceptibly. The column again slowly descends, coming to rest after several minutes at a second point (P_2). After some time, the descent may again be resumed at a much slower rate until a third and final stopping point (P_3) is reached after more than fifteen minutes. For routine measurements, the first reading (P_1) is considered as the surface tension value of the urine. This corresponds roughly to the surface tension value of the specimen before any important secondary redistribution of molecules has taken place.

The capillary tube should be thoroughly cleaned with distilled water after use. It is well to check the tube before each series of measurements, using distilled water at room temperature. If the check readings are above 74 or below 73 on the scale, the tube must be carefully flushed through with distilled water by means of a suction pump. Occasionally, water alone may not be sufficient and it will be necessary to clean the tube with sulfuricochromic cleaning solution, followed by thorough flushing with water, in order to obtain correct check readings. When the tube is not in use, it is best left standing in a glass beaker containing distilled water.

Surface Tension in Clinical and Experimental Research

The Urotensiometer for the first time makes possible determinations of the surface tension of urine and other physiological solutions as a routine laboratory procedure. The highest surface tension value for urine encountered clinically is 73 dynes/cm., and this is correlated with a minimal quantity of surface-active substances. The lower the surface tension of the urine in dynes/cm., the greater the amount of tensio-active agents present in the specimen. A surface tension of 52 dynes/cm. is the lowest clinical value that we have found by this method in more than 100,000 measurements made during the last 12 years.

The first problem concerning the meaning of the different values of urinary surface tension arose when it was observed that usually the urines with low specific gravity have high surface tension, while those with high specific gravity have low surface tension. The direct correlation between the values of surface tension and specific gravity of the samples thus had to be investigated with the supposition that the amount of water in the urine will have a great influence, by itself, on surface tension. While a correlation between surface tension and water content is often observed,

it is not a cause and effect one. Urinary samples with a specific gravity as low as 1.003 were seen with a surface tension of 58 dynes/cm. while samples with a specific gravity as high as 1.030 had a surface tension of 70. Although very seldom encountered, these values have invalidated the supposition that it is the amount of water in the urine which determines the value of the surface tension, so that from the analytical point of view determination cannot be substituted.

The Nature of the Intervening Substances

The existence of several values for the surface tension of urine has suggested the intervention of different substances in the determination of surface tension. We used the study of the changes induced in the three values of P obtained for a sample. Different repartition capacities were considered as corresponding to different groups of substances. Several methods were used in order to identify these substances. In one method, different constituents of the urine were separated by using solvents or absorbents, or by allowing the constituents to assemble at the surface.

The fact that the solvents, if they remain in the fluid even in very minute amounts, influence the surface tension, has largely handicapped their use. However, when lipid solvents were used and could be thoroughly eliminated, the treated urine showed a change in surface tension, especially in P_1 values. With the use of activated animal charcoal absorption, all the P values were changed toward higher values.

M. Bier in our laboratories has studied the nature of the surface-active constituents, separating them from urine by using the fact that they assemble at the surface. Urine was made to foam by passing an inert gas through it. The foam—and, with it, a high proportion of surface-active substances was separated. By repeating the procedure, the separation could be pushed far enough so that it could be seen that the ST values, especially those of P_3 , were influenced. Analyses of the fractions obtained indicated that lipids would intervene in determining the surface tension revealed by the P_1 value, while proteins would intervene for the P_3 , i.e., after a repartition requiring a specific time. We have tried to confirm these preliminary data by adding the agents to urine and following the changes induced.

The addition of minimal amounts of soaps to urine has been found to induce a change in all P values and especially in P_1 . The addition of billiar salts changed P_2 values, while the addition of proteins, such as albumin, influenced the values of P_3 . It would appear from this preliminary research that while P_1 changes are related to an increase in fatty acid derivatives, P_2 changes are related more to the intervention of billiar acids, while proteins and amino acids exert greater influence on the values of P_3 .

This explains why surface tension, corresponding to P_1 , is still high in urines rich in albumin, and sometimes also in those with billiar acids. It would also explain the observation in the Hay's Test with sulfur flower in urine, that the sulfur starts to fall quickly if the urine is left standing for a while, but for the same urine this fall occurs only after a certain time if the sulfur is added to urine immediately after stirring. With surface tension

affected by fatty acids even in minimal amounts, ST changes in relationship to conditions where these substances intervene are particularly interesting. It is chiefly with these data in mind that we tried to investigate surface tension in relation to normal and abnormal physiology.

Surface Tension and Normal and Abnormal Physiology

The ability to measure surface tension rapidly and accurately enough, even for very small amounts of fluid, has made it a preferred method for many investigations. In addition to clinical applications, where the information furnished has been especially valuable, we have utilized this method in experiments in animals.

Time of the Day and Urinary Surface Tension

Urinary surface tension measurements were made in several normal subjects at hourly intervals. In order to eliminate the influence exerted by exercise and food, the subjects were kept in bed for a few hours preceding

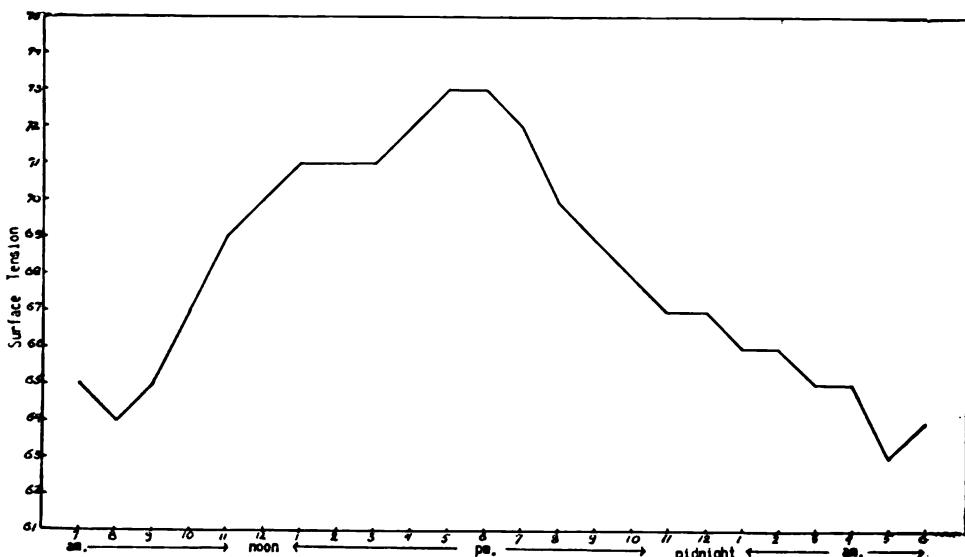


FIG. 218. 24-hours hourly urinary surface tension value of a 30-year old normal male, kept resting and with a constant hourly food intake, showing a maximum in the afternoon and a minimum around 5 a.m.

measurements. During this period, and the entire period of the experiment, the subjects were permitted to leave their beds each hour to void. Throughout the experiment, they were given the same kind and amount of food each hour. This eliminated as variable the influence of food and activity. Figures 218, 219 and 220 and Table XXVI show samples of the curves of surface tension in such cases. A 24-hour diphasic curve can be noted.

Surface tension in mice under similar conditions, however, shows differences. A group of 20 mice kept in cages were used. By slight squeezing of

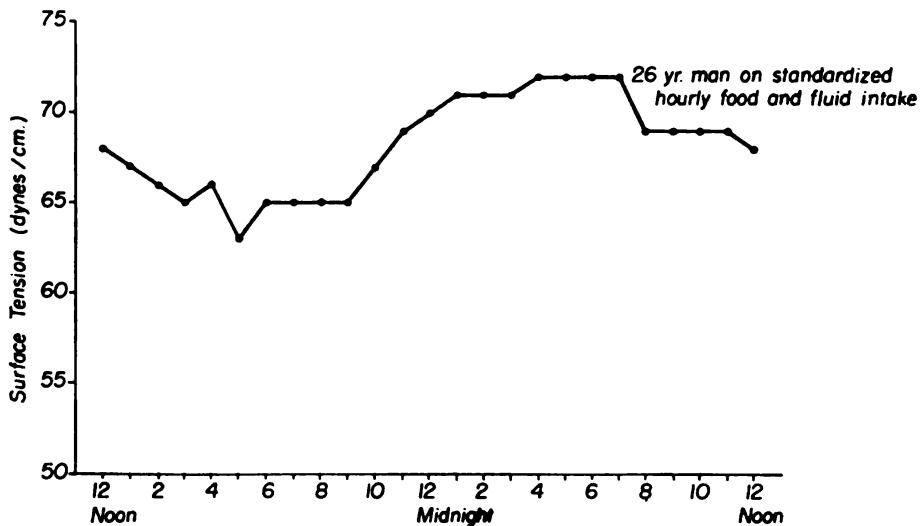


FIG. 219. Curve of the urinary surface tension in a 26-year old male on standardized hourly food and fluid intake showing a maximum toward the early morning hours and a minimum toward the evening.

the lower abdomen, a few drops of urine were obtained in a little cup and used for surface tension measurement. Changes seen in Figure 221 show that with the passage of time, there is a dampening effect on the curve. This has made us doubt that the intervention of stress in these cases can be responsible for the changes. In order to eliminate stress as a factor, a second group of experiments was done in which urinary samples were obtained

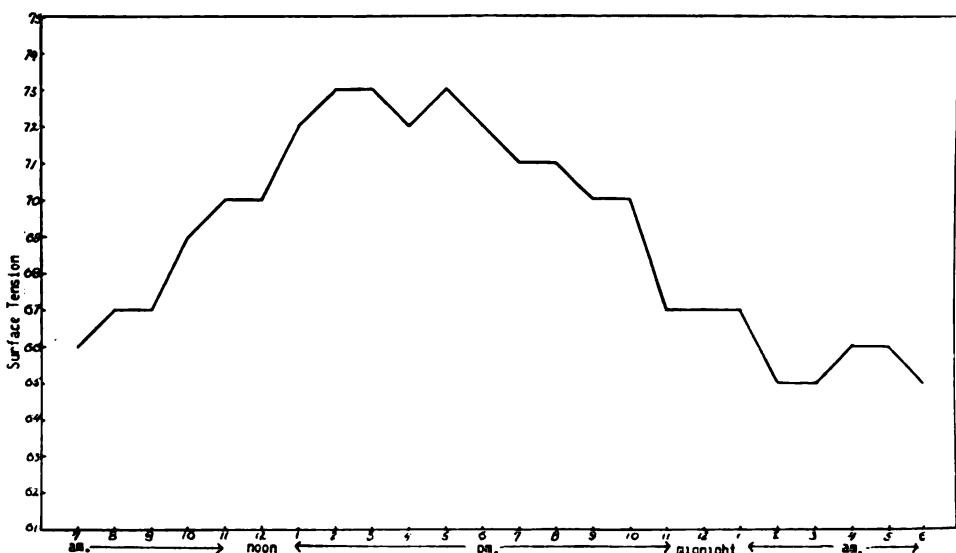


FIG. 220. Curve of the urinary surface tension in a 27-year old female on standard hourly feeding, with a maximum in the afternoon and a minimum in the morning.

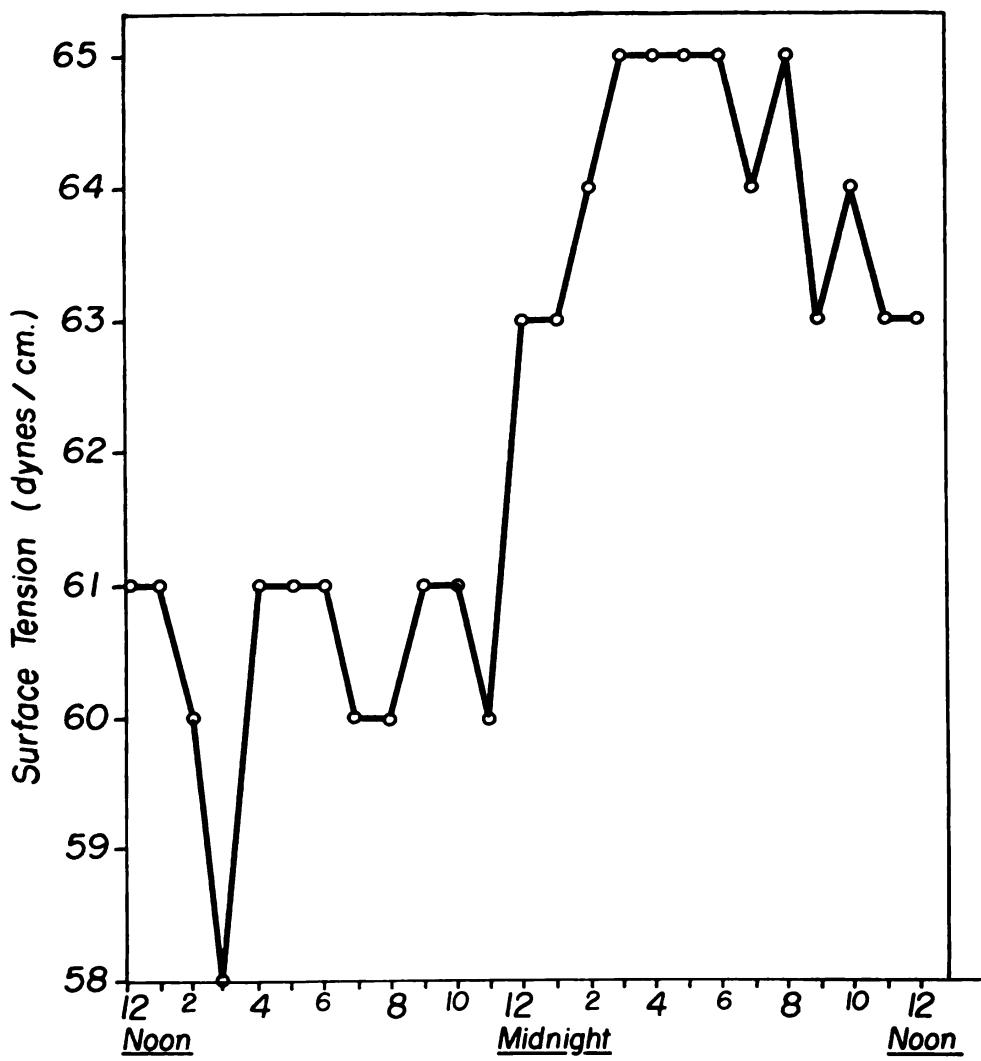


FIG. 221. Average value of surface tension in the urine of 20 mice, obtained every hour, showing variations with a dampening character.

each hour from a different animal. Thus, each animal provided a sample of urine only once and was not under stress. Under these conditions, the dampening effect was not present. A curve showing only two phases in 24 hours was obtained. The ST curves for humans and mice are opposite. During the period when high values occur in humans, low values occur in mice, and vice-versa. Because such opposite variations between humans and mice were found for many other analytical data and were considered related to the nocturnal activity of mice, a third group of experiments was performed in which the mice were kept in darkness during the day and under light during the night in an attempt to change the rhythm of their activity. After three weeks, there were no marked changes in analytical

data obtained in these mice. It is possible, however, that more time is required to induce changes in surface tension by altering the rhythm of mouse activity. (Fig. 222)

TABLE XXVI
SURFACE TENSION IN NORMAL HEALTHY 32 YEAR OLD MAN AND
27 YEAR OLD FEMALE ON STANDARD HOURLY FEEDING

Hour	Male S.T. in Dynes/cm.	Female S.T. in Dynes/cm.
7 a.m.	65	66
8 a.m.	64	67
9 a.m.	65	67
10 a.m.	67	69
11 a.m.	69	70
12 Noon	70	70
1 p.m.	71	72
2 p.m.	71	73
3 p.m.	71	73
4 p.m.	72	72
5 p.m.	73	73
6 p.m.	73	72
7 p.m.	72	71
8 p.m.	70	71
9 p.m.	69	70
10 p.m.	68	70
11 p.m.	67	67
12 Midnight	67	67
1 a.m.	66	67
2 a.m.	66	65
3 a.m.	65	65
4 a.m.	65	66
5 a.m.	63	66
6 a.m.	64	65

Surface Tension in Normal Humans and Animals

From the first analyses of urinary surface tension in groups of individuals it could be seen that certain changes common for all were taking place. There were days when all subjects had higher relative values and other days when lower values prevailed. Since there was no common dietetic or habit factor for all the subjects studied, we searched for environmental changes that might be the immediate cause of these variations. In collaboration with P. Teitelbaum we made the following experiment, using 80 rats divided into four groups. One group consisted of females of the Wistar strain and a second consisted of males of the same strain. The remaining two groups were composed of 20 females and 20 males of a black hooded strain. The animals were maintained in groups of five in separate cages on Purina and water ad lib. They were kept in a nonconditioned room. The experiment was conducted for one month, from May to June.

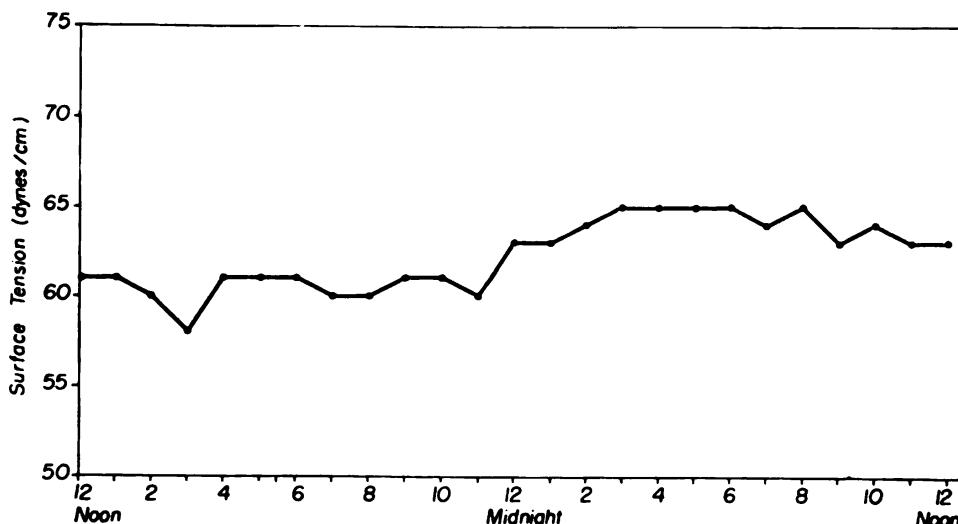


FIG. 222. Average hourly values in the urinary surface tension of groups of 5 mice, the group being changed each hour.

Urine was collected in a small vessel by keeping the animal firm and pinching the lower abdominal skin. Surface tension was measured a few minutes later. In each group, animals which did not give urine under this procedure for several consecutive days were replaced by others.

Samples of urine were obtained 6 days a week, between 9 and 10:30 in the morning. From the data obtained, an average value was calculated for each group, and the respective values were plotted in curves having the days as abscissae. The values for the female group were higher than for males. No differences were seen between the two strains. And all four curves showed the same variations at the same times. Thus it appeared clear that the variations were related to some external factor acting upon all the animals. We compared the ST curves with others traced for different environmental values present at the time of observation. Such values—barometric pressure, electrostatic value and temperature—were obtained from the Weather Bureau and the curves for the area at the hour of the experiment traced. Of them all, only the curve for temperature change was significant. The ST was seen to rise each time that the temperature fell and fall when the temperature rose. (Fig. 223)

This correlation was further studied by using induced rather than natural temperature changes in the following experiment which was made in collaboration with E. F. Taskier.

Adult female CF₁ strain mice were divided into three groups of 20 mice each. They had ad lib access to food and water. One group was placed in an incubator in which the temperature was maintained at 37°C. A second group was kept in a refrigerator at 8°C. The third group served for control and was maintained at ordinary laboratory temperature which ranged between 20-25°C.

Because of the diurnal pattern of surface tension variations, urine specimens were collected at the same hour every day. During a period of 22 days, daily urine specimens were obtained between 9 and 11 A.M. This was easily accomplished by firmly gripping a mouse in one hand by the scruff of the neck and tail. With the finger of the other hand, the lower

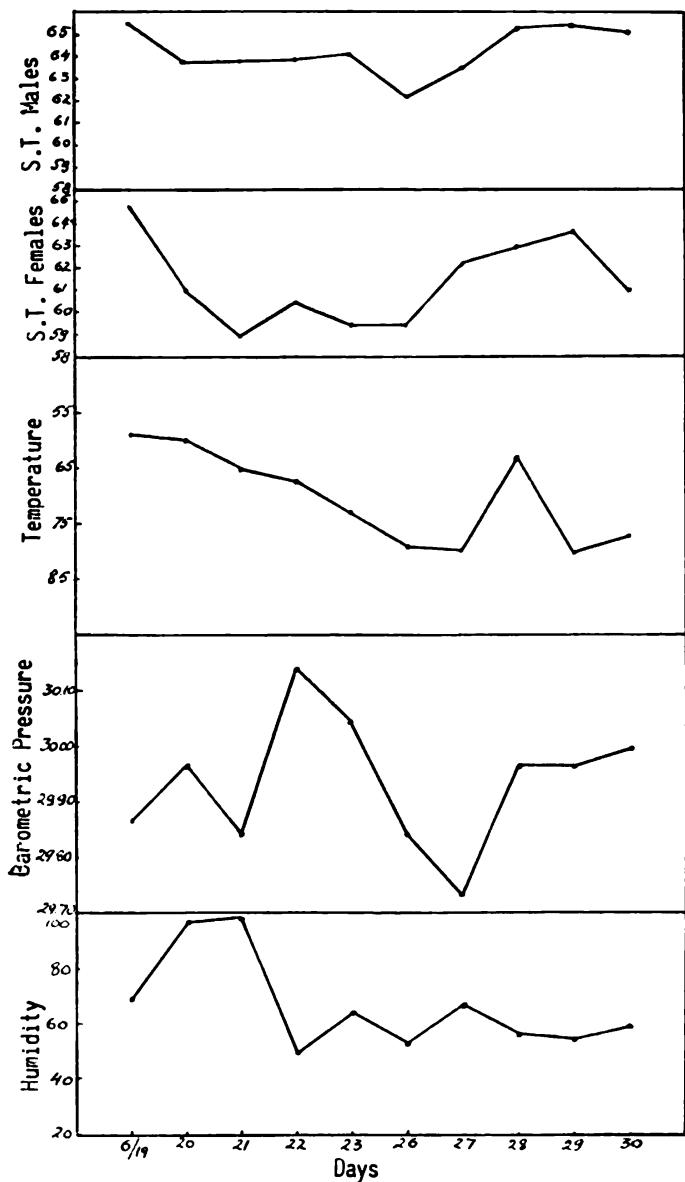


FIG. 223. Comparison between weather data and the average value of the surface tension in 40 male rats and 40 female rats. It shows a relative parallelism with the curve of the barometric pressure and a more consistent relationship with the inverse curve of the temperature.

abdomen was gently massaged, causing the animal to void 2-5 drops of urine into a small glass cup.

The surface tension of each specimen was determined within a few minutes after it was obtained.

The values obtained from day to day were charted for each individual mouse; the average for each group maintained under different temperature conditions also was determined.

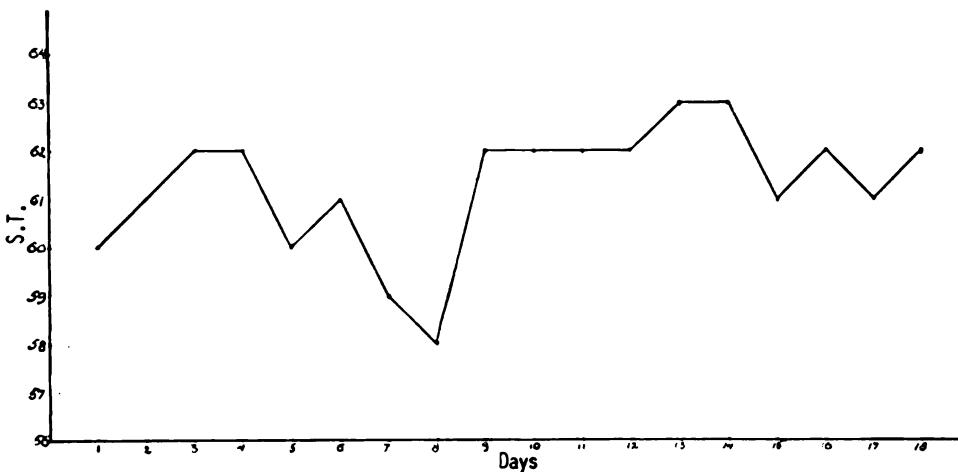


FIG. 224. Average value of surface tension of the urine in control mice over a period of 3 weeks.

The average surface tension readings in the control groups are shown in Figure 224. It can be seen that these values fluctuated in an irregular fashion between 58 and 63 dynes/cm. The surface tension values of the group maintained at 37°C show a steady sustained rise from 61 to 65 dynes/cm. (Fig. 225) The mice kept at 8°C showed an initial slight fall in surface tension, with a gradual return to the original levels. (225)

After several days in the incubator, the mice began to lose weight, their fur became sparse, and snout areas were constantly wet. The urinary output was scanty as compared with the two other groups. Death began to occur in the mice kept at a high temperature on the 12th day. The animals in the refrigerator developed thick luxuriant coats and huddled closely together at most times. None died from exposure to this temperature.

The fact that the animals maintained at 37°C showed a steady rise in urinary surface tension was especially significant. As expected, the urine of these animals was scanty and more concentrated than for the other groups. With this diminution in volume, it would be expected that the concentration of surface-active substances would rise and the surface tension would be lowered. The fact that the exact opposite occurred indicates that the observed change has to be considered as an effect of the high temperature.

This appears especially interesting for the relationship between temperature and the two ST patterns. While higher temperature induces one

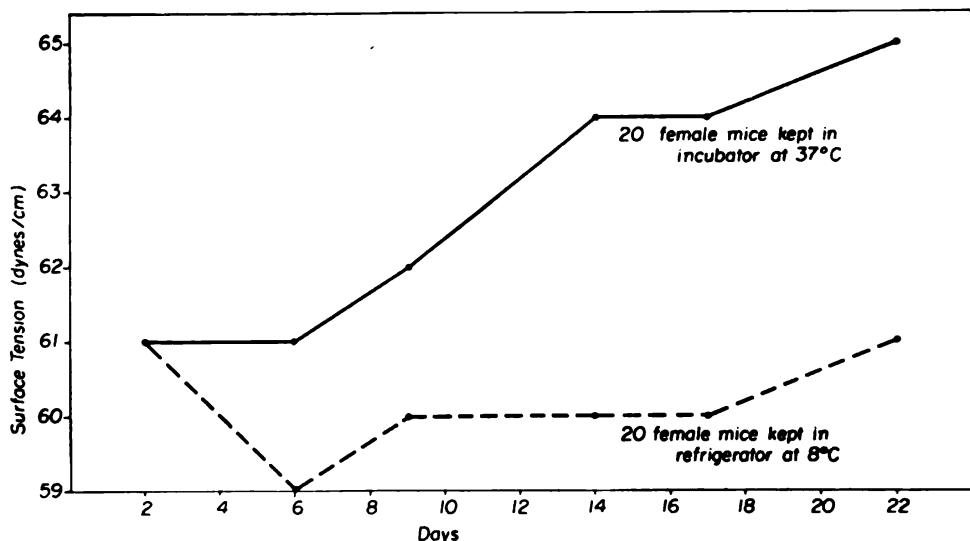


FIG. 225. The average values of the urinary surface tension of 20 female mice kept in the incubator at 37°C. and of 20 female mice kept in refrigerator at 8°C. The values are progressively increasing for the animals kept in the incubator until the animal died. For the animals kept in refrigerator, after an initial descent, the values ascend toward normal.

pattern, cold induces the other. It must be noted that the organism cannot defend itself against the pattern induced by higher temperature and the animal dies after a certain time, but for the pattern induced by cold, defense is possible. The body seems to be able to overcome the change. The surface tension returns to normal and the animal becomes adapted to the temperature. Not one of the animals kept in the refrigerator died, while all in the incubator were dead after a month. Adrenalectomy induces an immediate increase in the surface tension of the urine. (Fig. 226)

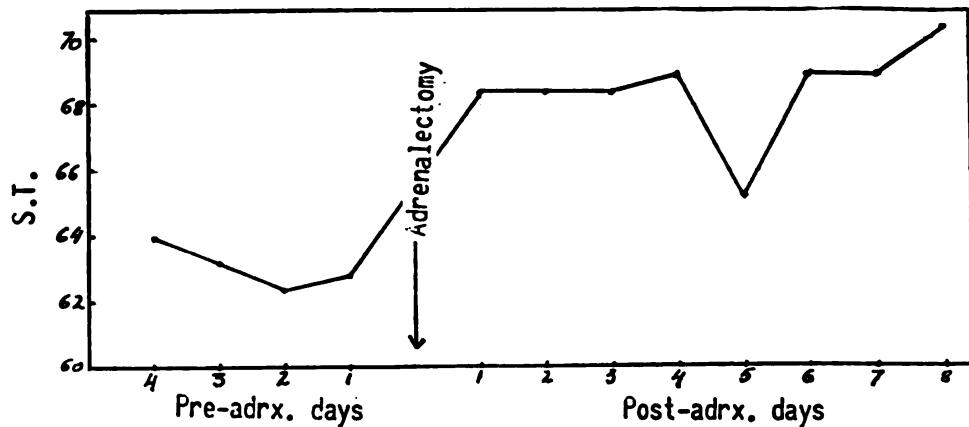


FIG. 226. The surface tension of the urine increases to high values after adrenalectomy.

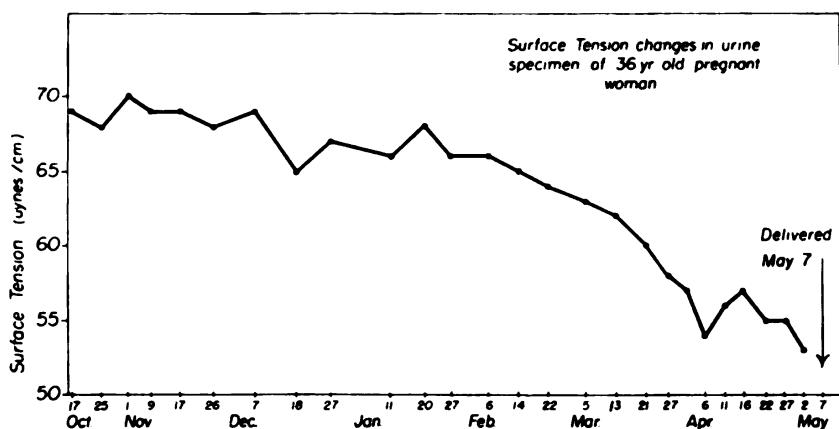


FIG. 227. Curve of the surface tension changes in urine specimens of a 36-yr. old pregnant woman shows a manifest change toward low values, starting with the 4th month, and becoming especially low in the last three months.

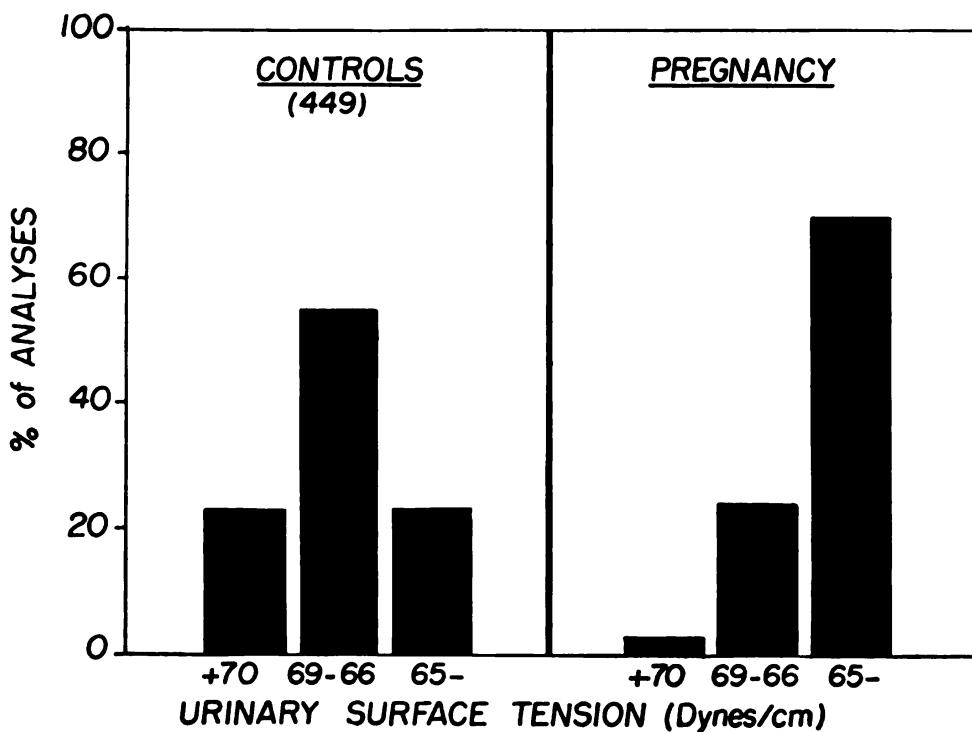


FIG. 228. The average value of the surface tension in pregnant women shows a manifest change toward low values.

Colloids in Urine and Surface Tension

An interesting relationship between urinary surface tension and the presence of "colloids" in the urine was noted by Butt and his associates. (240) Using the pendant drop method for the ST, direct examination in dark field for the presence of colloids in the urine, electrophoresis for determination of electrical charges, and the study of evaporated urine smears, they showed that urines with a high content of colloids, have a low ST; those with a low colloid content, a high ST. They have further correlated a low amount of colloids with a tendency of urine to precipitate and form stones. (242) They examined the colloid particles in the urine of different groups of individuals and found them high in Negroes, and especially high in pregnant women, which is in accord with the low surface tension of the urine which we found in these cases (*Figs. 227 and 228*) and the low tendency of both groups to form urinary stones.

We were interested in the relationship between variations in the urinary content of colloids and systemic patterns corresponding to high and low ST. R. Ravich in our laboratory has confirmed the correlation between presence of colloids and ST by using our urotensiometer. (219)

Chapter 4, Note 12. Urinary Oxidoreduction Potential

For the study of the *oxidoreduction potential* of the urine, we used a Beckman pH meter with platinum electrodes. We measured the potential at the pH of the sample and also at pH 7. For this purpose, the platinum and the respective calomel electrodes used for these measurements were introduced into the beaker of the Fisher titrimeter together with the electrodes of the potentiometer. After stirring, the pH of the sample and its oxidoreduction values were measured. The pH then was brought to 7 with HCl or NaOH solution, and the value of the oxidoreduction potential was again measured. Four values were thus obtained: the original pH, the titrimetric acidity or alkalinity, and the oxidoreduction values at the original pH and at pH 7. Figures 229 and 230 show a sample of such curves.

Chapter 4, Note 13. Oxidoreduction Potential of the Urine

We have tried to determine the oxidoreduction potential of urine samples by using the change of a color indicator in its leuco base. We chose toluidine blue which, with a rH_2 of 14, is at the middle of the scale of the rH_2 values. In order to eliminate two of the important factors which intervene in the oxidoreduction potential—differences in pH and temperature—we used a fixed temperature and very low pH. The degree of oxidoreduction potential was determined by the time necessary to obtain the discoloration for a standard amount of the color indicator. The reactive used was a solution of toluidine blue in a normal solution of hydrochloric acid. The amount was chosen so as to give a discoloration at 100 seconds for the normal individual. 1.5 cc. of a saturated solution of toluidine blue in al-

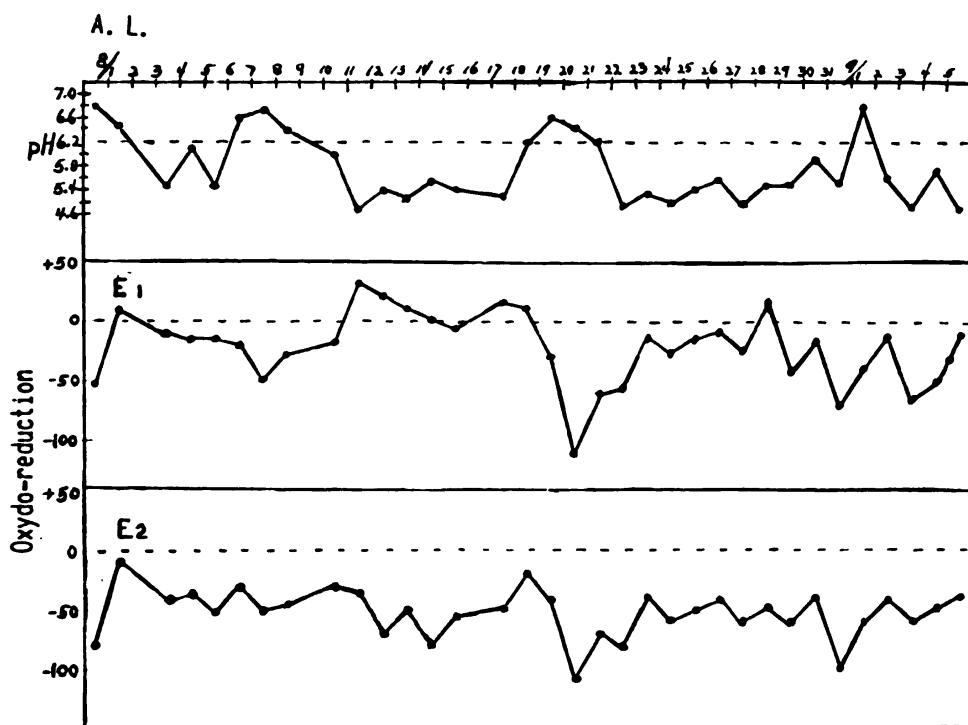


FIG. 229. The curve of the oxidoreduction potential of the urine measured electrically. The curve of the measurements made directly on the urine (E1) show big variations which are smaller if the pH of the sample is brought to 7 (E2). In a case of cancer of the breast, the curve remains constantly below the 0 value.

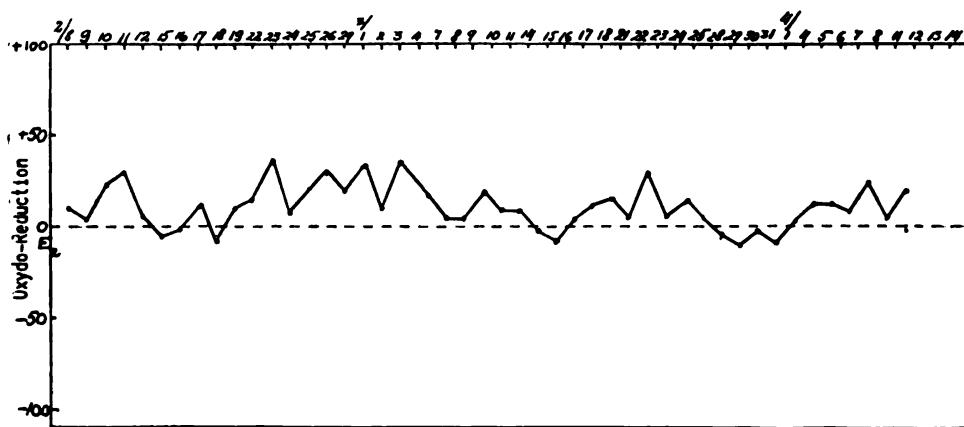


FIG. 230. Curve of the oxidoreduction potential values of the curve brought to pH 7 of a case of cancer of the breast, shows values around or above the 0 value.

cohol was added to 100 cc. of n/10 hydrochloric acid. 1 cc. of this reagent was added to 4 cc. of urine in a test tube kept for a while in boiling water. The time necessary for the discoloration was marked. Values as low as 3-4 seconds or as high as above 420 seconds were seen. A high oxidoreduction potential inducing a rapid discoloration was found to correspond to a pattern of the offbalance type A while a low discoloration was seen to corre-

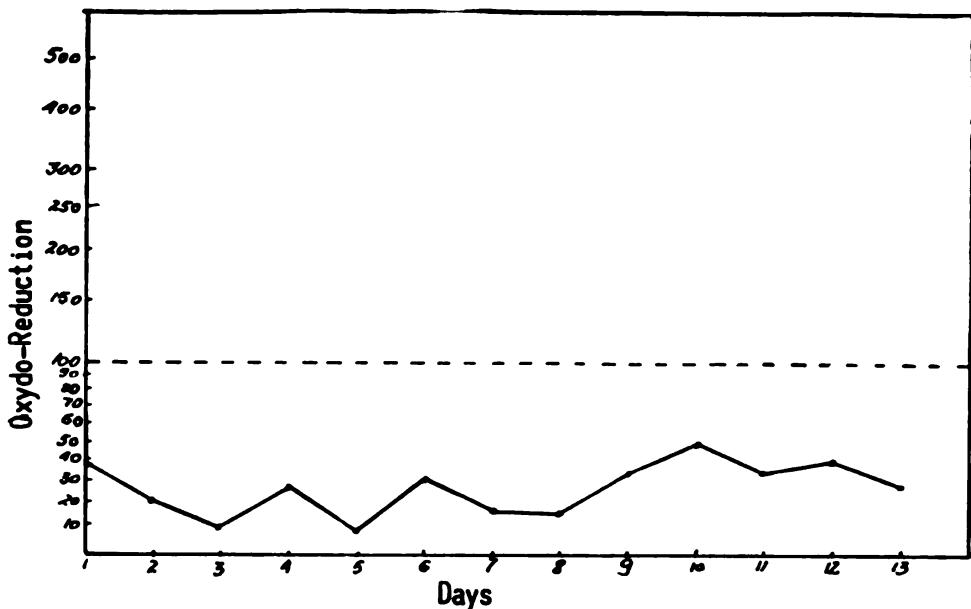


FIG. 231. Curve of urinary oxidoreduction values in a case of carcinoma of the breast with multiple bone metastases. The values are established as the time necessary to obtain the reduction at 100°C and with a pH around 2, of a solution of toluidine blue so chosen as to have 100 seconds as the average value for groups of normal individuals. In this case the values remain fixed low below 100 seconds, corresponding to a pattern of the offbalance type A.

spond to the pattern of type D. Figures 231 and 232 show two such curves. We used this test for many years as main analyses to determine the existing offbalances. (220)

Chapter 4, Note 14. Peroxides in the Urine

The hypothesis of the existence of a phase "oxygen" of offbalance D led us to study the appearance in urine of products resulting from abnormal oxidation. We were especially interested in the existence of substances having peroxide properties. We found that addition of sulfuric acid to urine of certain subjects induced appearance of indigo-tin and indigo-rubin. In order to investigate the reaction, we have utilized the solubility of indigo-tin and indigo-rubin in neutral solvents. Through their extraction it appears possible first to prevent their transformation in colorless isatin and, second,

to evaluate the relative amounts when they appear during the reaction. To 4 cc. of urine, one centimeter of toluene was added. After shaking the mixture, 1 cc. of pure sulfuric acid was added and the mixture was immediately shaken again. When the mixture was allowed to stand, the toluene separated and its color, blue or violet, indicated the presence and also the relative amounts of indigotin and indigo-rubin.

Another method used to detect peroxides was the acidification of urine followed by addition of potassium iodide. For iodometric evaluation, starch

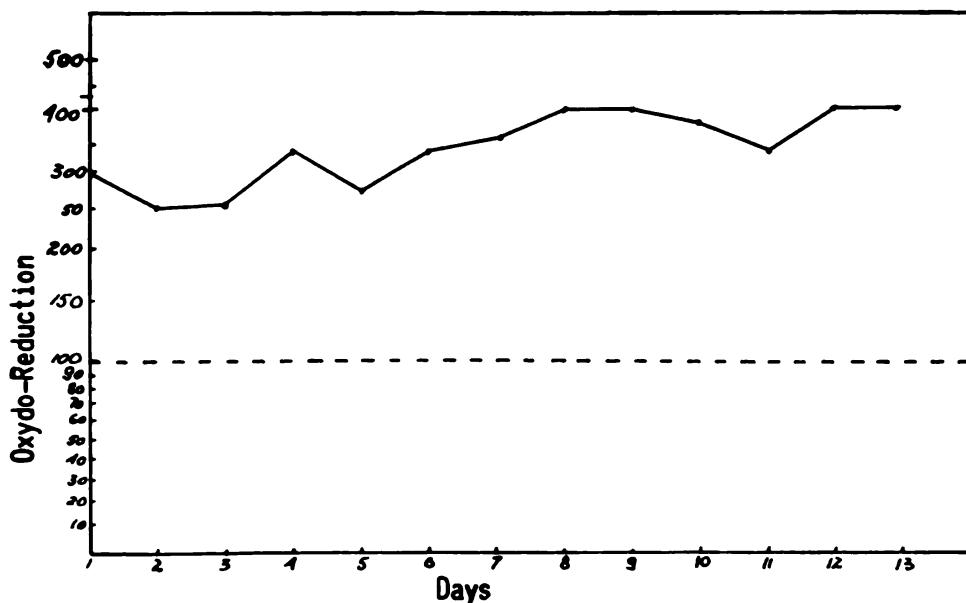


FIG. 232. The urinary oxidoreduction values in a case of cancer of the colon with abdominal metastases. The values remain the whole time above 100 seconds, corresponding to the pattern present in the offbalance type D.

solution was added. The amount of iodine liberated could be determined titrimetrically.

The form in which peroxides are present in the urine is not clearly established. Although the distillation of the urine gives peroxides in the first distillate, these are not in the form of hydrogen peroxide, since catalase does not induce their disappearance. The values obtained with both methods, sulfuric acid and iodometric, are relatively parallel. The sulfuric acid method, however, produced a higher percentage of positive results.

The presence of slight amounts of peroxide in the urine has been found in about 3% of normal subjects. In contrast, we found peroxide in the urine of 87% of a group of 27 schizophrenics studied through daily analyses over a period of three years. In some of the subjects, throughout the entire three-year period with more than 1,000 analyses, not a single negative reaction was seen. (Fig. 233) (221, 222)

We have also found positive reactions during streptococcic infection,

erysipelas or tonsillitis. In radiation sickness the reaction is positive especially when tissue lesions are manifest such as mucositis or epidermitis. In general, treatment with selenium has given a relatively high proportion of positive results, especially at the beginning of treatment. While positive reaction appeared to be consistent with a favorable evolution of tumors, an extremely intensive reaction appeared related to a bad prognosis. The following observation is characteristic.

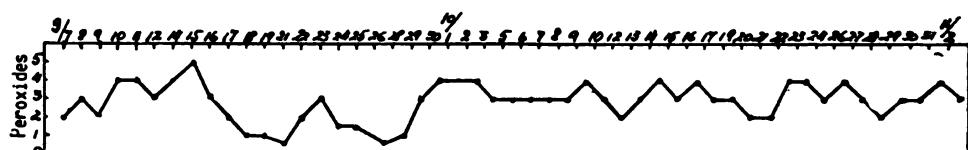


FIG. 233. The reaction for peroxides remains consistently positive in the urine of a schizophrenic in daily analyses during a period of 3 years. (Part of the curve.)

Mrs. N. C., 28 years old, with Hodgkin's disease, had received three treatments of teleradiotherapy, with the general condition completely unchanged. The patient presented an extremely intensive urine peroxide reaction. Dilution of the urine to 1/50 still showed a marked blue color after treatment with sulfuric acid and toluene. We informed the attending physician about the finding and advised the discontinuance, at least for the moment, of the treatment. The perfect general condition of the patient induced the radiologist to disregard our advice. A new treatment of teleradiotherapy was administered. Three hours later the patient, who only that morning had been shopping, went into a shock and died during the night.

We want to emphasize, however, the correlation between the lack of peroxides in the urine and a poor prognosis during radiotherapy. The cases in which a positive reaction disappeared, were always followed by a change for the worse in the general condition. The persistence of this lack of peroxides was seen in the cases with a rapid lethal termination.

Chapter 4, Note 15. Index of Excretion and of Retention

One of the most important aspects of the relationship of an entity to its environment is given by its intake and output. The concept of hierarchic organization with emphasis on the individuality of the entities, has given a special meaning to the study of these processes. For each entity its proper environment is represented by the secondary part of the entity immediately superior to it. The nuclear sap represents thus the environment from which the chromosomes take the material necessary for their metabolism and where they reject these substances which are no longer needed. Similarly, the cytoplasm represents the environment for the nucleus, the interstitial fluid for cells, the lymph for tissues, the blood for organs and the actual environment for the organism. This systematization, based on the organizational individuality of the entities, has guided the study of the relationship

between entities and their environments under normal and abnormal conditions. It is under this aspect that we have investigated the renal excretion, which according to the hierarchic organization, corresponds to the relationship between the organism as an entity and its environment.

Some of the substances excreted come from the metabolism of lower entities. Related to blood, they would represent often noxious undesirable substances, if the higher mechanism of the blood would not intervene. Although that which we see as urine is the result of the relationship between the organism as an entity and its environment, the origin of the different substances forming it, as related to the different other levels, has to be considered. While for certain elements this origin is evident, for many substances only suppositions are available today.

When a systematic analysis of these constituents was attempted, other difficulties arose. The isolated urine samples easily available, have only a very relative value for many of these investigations. The titrimetric data expressed as concentration of various substances are all functions of the amount of water eliminated in the sample. As this often varies widely, the informations obtained are only relative. Balance analyses concerning entire intakes and outputs represent such technical difficulties as to make them unavailable for routine investigation, in which hundreds of subjects are daily studied. We tried to bypass this difficulty by eliminating the factor water excretion, from the considered data. The fact that the concentration of a substance and the specific gravity of the urine, are both direct function of the amount of water present, has permitted to eliminate this factor. The ratio between them appears thus independent of the amount of water present. It relates the amount of a substance to that of the bulk of the substances eliminated through the kidney. An index of excretion was thus obtained by dividing the concentration of the substance by the specific gravity. The opposite ratio would correspond to an index of retention.

From the physiological point of view, these ratios are not affected by the factors which govern the glomerular filtration, which are acting similarly for all the substances present. They are little or not affected also by the back resorption, where the differences between the various substances are reduced. They will show consequently, big variations as resulting from the active reabsorption, which takes place in the distal portions of the convoluted tubes. It is this character which gives the indexes of excretion or retention, as we calculate them, their value. We have utilized for years these indexes for chlorides, sodium, potassium, phosphoric ion, sulfhydryl, calcium, to obtain valuable information which otherwise could not be furnished by the simple analysis of the isolated urine samples. We will come back to these indices during further analyses.

Chapter 4, Note 16. Water and Nitrogen Metabolism

The analyses of different urine samples have shown that the amount of water present in urine seems to influence indirectly its constitution. It could thus be seen that while very diluted urine, corresponding to a large

amount of water excreted, is usually alkaline, concentrated urine, corresponding to small amounts of water excreted, is generally acid. Furthermore, it could be seen that these changes are related to more profound metabolic differences. When related to the nitrogen metabolism, it could be seen that diluted urines are rich in free ammonia while concentrated urines in uric acid. We have thus investigated the relationship between these two factors, the amount of water excreted and the form under which the nitrogen is eliminated.

The comparative physiology shows us that the manner under which nitrogen is excreted varies for different animals according to the amount of water available in the surrounding environment. In fish, with water almost unlimited, nitrogen is excreted in the form of ammonia. The high toxicity in this form of nitrogen excretion is counter-balanced by the amount of water in which the excreta are diluted. Fish are ammonioselcic. In terrestrial mammals, where the amount of water available is more limited, the excretion of nitrogen is made in the form of urea which is much less toxic than ammonia. The danger of poisoning the drinking water through excreta is thus reduced. Mammals are ureoselcic animals. For birds, for whom water is scarce, the form of nitrogen excretion is of uric acid, which through its low solubility in water, has little chance to contaminate the drinking water. Birds are uricoselcic. Based on this relationship between water availability and the type of nitrogen excretion, we looked for a similar relationship in humans between the excretal amount of water and the type of nitrogen metabolized under normal and abnormal conditions. An immediate confirmation was obtained in those abnormal conditions where the amount of water excreted is abnormal. As already seen above, in subjects having a high diuresis, which in general would correspond to a high amount of water available to be excreted from the body, the urine is usually alkaline, the alkalinity due to ammonia. On the opposite side of the normal, there are subjects with a very reduced urinary excretion. In patients eliminating only two to three hundred cc. in 24 hours, the amount of uric acid in the urine is manifestly increased. Upon standing, these urines always show a reddish deposit formed mostly by uric acid. Relating this to comparative physiology, while the normal subjects would appear ureoselcic, those with polyuria can be considered as ammonioselcic and those with oliguria, uricoselcic.

We tried to see if a change in the form under which the nitrogen is eliminated can be induced by changing the amount of water available to be excreted. Normal subjects whose urines were tested for certain periods of time for their content of ammonia, urea and uric acid, were given 1 to 2 liters of water to drink. The highly diluted urine which was subsequently excreted, became alkaline. The total amount of ammonia increased while urea and uric acid were slightly reduced. The same subjects were later given a dry diet for 12 hours or more. The specific gravity of the urine in most of these cases was above 1.026, indicating a kidney with normal concentration capacity. Although the content in ammonia decreased manifestly in these urines, the increase in uric acid excreted was minimal. We sub-

mitted the same subjects to a diet with fluids reduced to a minimum, for 3 to 4 days. Under these conditions, the excretion of uric acid started to increase. Great intake of water would thus transform a normal ureoselic individual into an ammonioselic in only a few minutes. A similar passage into uricosellic was seen to need days, and even then it showed only minimal changes. This can be explained by the fact that while ammonia in urine appears largely through changes taking place in the kidney cells themselves, uric acid results from more profound metabolic changes, which concern especially the nitrogenous bases, particularly the purines.

Chapter 5, Note 1. Second Day Wound Crust pH

The metabolic processes characterizing abnormal foci have been investigated in various ways. One method was devised in order to study the acid-base changes which take place within abnormal foci created by surgically produced wounds under the influence of various chemical, physical and biological factors. This research was made in collaboration with C. Huesca-Mejia. (212)

Adult Carworth Farm female and male albino rats weighing 150 to 200 grams were employed. The animals were separated according to sex and all received a standard diet of Purina Chow and water ad lib. Tests were run on groups of twenty animals, with from two to four control animals in each group.

The animals were divided into groups that were subjected to various experimental conditions for three days, after which time, under ether anesthesia, a wide area of the back of each animal was carefully depilated by hand. A 1 square cm. wound was then produced in the depilated area down to the dorsal aponeurosis. The wound was kept free of blood with dry gauze until bleeding had entirely ceased, and was then left uncovered. All wounds were made between 8:00 a.m. and 10:00 a.m.

A glass electrode made according to the specifications of McInnes and Dole (223) was used. As reference, an electrode containing normal saline was employed. (224) The electrodes were mounted on a stand in a fixed position. A model H Beckman pH meter, whose signal was amplified by a 6H6 and 6SN7 push-pull, was used as the first amplifier. The readings were made on a 200 micro-ampermeter adjusted so as to have the full scale represent one pH unit. The experimental error using this apparatus was found to be less than $\pm .01$.

pH determinations were carried out by bringing the wound area into contact with the tips of the electrodes. The animals were held gently but firmly in one hand until they were completely immobile, at which time a firm contact was established between the center of the wound area and the tip of the glass electrode, and between the reference electrode and the wound periphery. Readings were carried out on the surface of the freshly exposed aponeurosis within ten to fifteen minutes after the surgical procedure. Subsequent readings were made every twenty-four hours upon the wound crust, which was washed and then moistened with a drop of an

0.1% sodium chloride solution freshly adjusted to pH 5 as suggested by Blank for use in skin pH determinations. (225) The electrodes were standardized with buffers of different pH before and during each period of testing.

The effect of various chemical agents upon the wound pH was studied by daily oral administration for three days preceding the operation and thereafter until the conclusion of the series of pH determinations. In general, the substances were administered according to weight in quantities corresponding to the therapeutic doses accepted for humans or in amounts equal to 10% of the daily toxic dose. Water soluble substances were administered in drinking water. Oil soluble substances were administered on pieces of bread. In some cases, substances were introduced directly into the stomach through a catheter. In a few cases, substances were injected subcutaneously.

In the 164 control animals, the pH of the wound surface varied between 7.30 and 7.33 when measured ten to fifteen minutes after the completion of the surgical procedure. The pH remained at this level for at least three hours.

Twenty-four hours after the wounds were produced, the pH of the moist crust covering the area was found to be between 7.72 and 7.76 in all untreated animals. (*Fig. 234*)

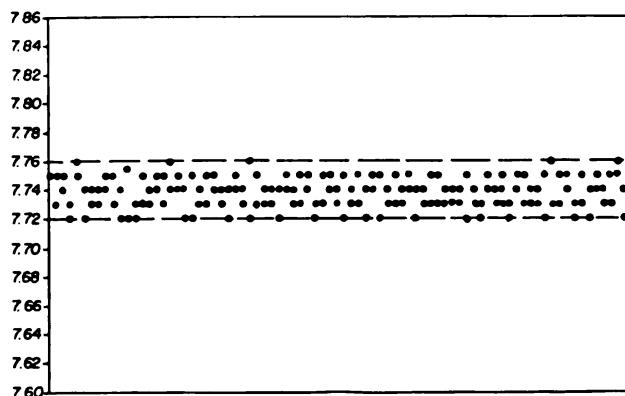


FIG. 234. Second day wound crust pH values remain in a range from 7.72 and 7.76 in normal rats as seen in the animals which served as controls for the multiple experiments.

At forty-eight hours, the pH of the crust was found to be between 7.42 and 7.60, this being the period of greatest variability. At seventy-two and ninety-six hours, all the readings were between 7.28 and 7.32. After the fifth day, similar values were found and further readings were not taken.

No consistent correlation was found between the values on different days for individual animals. For example, in some animals with a wound pH of 7.30—the lowest value—immediately after the operation, pH reached 7.76, the highest value for the normal range, in twenty-four hours. In males, values appeared to be slightly higher than in females.

In 410 of the 860 animals exposed to various chemicals pH value of the wounds was determined several minutes after the operation and found to be between 7.28 and 7.35. Only 22 showed a deviation from the control range of 7.30 to 7.33. No correlation was observed between the minimal changes in these animals and the type of treatment administered.

While the pH values found several minutes after wound induction showed very little or no variation from the control range and no correlation with the various experimental conditions employed, the findings at twenty-four hours showed considerable significance for the agents used. At forty-eight hours and thereafter, no important differences were observed between values for controls and those for groups treated in different ways. In continuing these studies therefore, determinations were carried out only several minutes after the production of the experimental wounds and then again 24 hours later. Actually, only values for the twenty-four hour reading appeared significant and will be discussed. We made 24 hour measurements for all of the 860 animals treated with different agents. For convenience, we refer to the pH value of the crust at twenty-four hours after creation of the experimental wound as the s.d.c. pH (second day crust pH).

From four to twelve animals were employed in the assays of the activity of each agent. By applying the same experimental conditions to animals in groups tested at different times, it was possible to determine whether the changes observed were due to some external factor such as temperature, humidity, etc., or were actually due to the imposed experimental conditions. The s.d.c. pH has proven to be of considerable interest because consistently similar changes have been found to be produced by the same agents when applied to animals in different groups tested weeks or months apart.

Considering all the animals treated with various agents, three possibilities have been found to exist: 1) There may be no effect upon the s.d.c. pH, in which case the values will all fall within the control range of 7.72 and 7.76 found in untreated animals; 2) s.d.c. pH may be elevated to values between 7.77 to 7.85; or 3) the s.d.c. pH may be reduced to values of 7.70 to 7.60.

We will present here only the conclusions of these studies as related to the various agents investigated.

CHEMICAL FACTORS

Cations and Anions

It was interesting to investigate the influence exerted by some cations and anions by first using the same anion with various cations and then using different anions with the same cation. It was apparent in all the experiments that the immediate pH of the wound does not differ from that of the untreated animals, and that the s.d.c. pH data obtained are concordant.

We studied the influence exerted by anions first by investigating the effects of administration of acids. With even strong inorganic acids, no

changes were seen in the normal tissues. Definite and opposite effects were thus obtained with acids corresponding to the elements of the VIIth series and with those of the VIth series. The first group of acids, especially HCl, induced a frank acidifying effect while the second produced an alkalizing effect. The phosphate and nitric ions showed strong acidification, the iodide and bromide ions were weaker than the chloride ion. The bicarbonate ion clearly alkalinized, as did sulfate and selenate. The thiosulfate ion had an obvious alkalinizing effect, with pH values as high as 7.85. Among the organic acids, citric acid produced one of the strongest effects, even stronger than that of the inorganic acids. The fact that citric acid is only slightly metabolized could explain its strength. The gluconate ion induced a slight acidification. The cacodylate ion seemed to induce no changes although not enough animals were utilized to judge its effect thoroughly.

In studying the effects of salts, the roles of both cation and anion were considered. Using different anions for the same cation, it was possible to judge the effect of the cation.

Sodium and lithium produced relatively slight acidification. Potassium manifestly acidified as did ammonium, the latter, however, to a less marked extent. Marked acidifying effects were seen for iron, mercury and bismuth. A lesser effect was obtained with molybdenum and aluminum. On the other hand, a manifest alkalinization was found for bivalent calcium, strontium, copper, barium and cobalt. Manganese and silver cations seemed to influence the second day wound pH only slightly toward alkalinization. It seems that there is an additive effect for the different elements in their acidifying or alkalinizing influence. This has permitted us to judge the effects of the different ions. Potassium induces greater acidification than sodium, and still greater acidification than ammonium. Potassium chloride, in which the two ions have an additive effect, is thus more frankly acidifying. The same is true for the acid phosphate. The alkalinizing tendency of sulfate ion opposes the acidifying effect of potassium and explains the slight acidifying influence of potassium sulfate. We must arrive at the relatively strong alkalinizing tendency of the carbonate ion to find an anion able to counteract the acidifying effect of potassium. The s.d.c. pH effect of potassium carbonate is in the normal range. The data obtained through this study led to the research on the intervention of the elements in biology, which is the subject of Chapter 5. Information concerning the effect upon the s.d.c. pH of these elements as well as the relationship between this effect and the structure of the elements is discussed in this chapter.

Calcium ion has an alkalinizing influence strong enough to counteract the acidifying tendencies of such anions as chloride and phosphate. Weaker acidifying anions, such as lactate and gluconate, are not sufficiently strong to counteract the alkalinizing tendency of calcium, and calcium salts of these acids have a strong alkalinizing effect.

This analysis indicates that the effect of a salt upon the local pH of abnormal tissues can be judged by considering the additive influence exerted by anion and cation, the effect increasing if both have tendencies in the same direction, and decreasing if the tendencies are opposed. We will not

emphasize here the other antagonistic biological effects of cations and anions, as they pass from acidifying (citric acid and potassium) to alkalinizing (calcium and thiosulfate). We will discuss these effects in connection with the pharmacological studies of these agents. For the moment, we want only to emphasize the value of this investigational method.

Acid Lipoids

The next step in the use of the s.d.c. pH was the study of the effects of a special group of acids in which we were interested, the fatty acids. Analysis of the results obtained clearly shows the importance of the nonpolar group. A carboxyl, when bound to a long chain as in the fatty acids, does not by itself seem able to induce a change in the second day wound crust pH, the values remaining in the normal range. The substance appears inactive when the nonpolar group does not have its own energetic center or formation. The saturated fatty acids with 10-16 carbons do not influence the s.d.c. pH. The presence of double bonds in the nonpolar group changes the influence exerted. All the nonsaturated fatty acids studied show an alkalizing effect, with relatively slight differences for the higher desaturated members such as linolenic or arachidonic fatty acids, or for the fatty acid mixture obtained from cod liver oil. However, the conjugated fatty acids studied, such as eleostearic acid, or the mixture of acids starting from cod liver oil, showed the highest values in this group, even for so small an amount as 5 mgr. per animal per day. These data indicate the role of energetic formations in the nonpolar groups of fatty acids.

We must emphasize the difference between the hydrosoluble organic acids and the group of fatty acids mentioned above. In the former, the action seems due to the intervention of the carboxyl, probably explaining the strong activity of tricarboxylic citric acid. With an important nonpolar group, this carboxyl seems to be unable to carry the molecule, and therefore cannot act. The intervention of a double bond could serve in two ways: 1) by bending the molecule, thus increasing its mobility as it also reduces the melting point; 2) as an energetic center where reactions take place. The fact that the 10 carbon lauric acid is inactive would indicate the slight influence to be expected from the bending of the molecule alone, as in oleic acid. The influence exerted by metabolic changes in which fatty acids intervene through the energetic centers in the nonpolar group explains the fact that they have a local alkalizing effect instead of the acidifying effect of many other organic acids. (*Fig. 235*)

Alcohols

The role of the relationship between polar and nonpolar groups appeared very clear in the study of the series of aliphatic alcohols.

We have considered separately a group of agents extensively used in our research. These are organic substances that have a radical with a bivalent sulfur as the polar group. We have mentioned above that an inorganic substance with similar constitution, sodium thiosulfate, has a strong alkalizing effect upon the s.d.c. pH. Such an effect upon the s.d.c.

pH has been obtained with all preparations containing a polar thiol group when administered orally or parenterally. We must emphasize the unusual uniformity of results, rarely encountered in other biological experiments. It appears that the alkalinizing effect of these preparations is sufficient to override the individual differences in subjects receiving them. The same alkalinizing effect has been apparent for sodium thiosulfate, and seems to be a common characteristic for substances having a bivalent sulfur in their polar group.

These alcohols exert no influence upon the pH of normal tissues, as seen in measurements taken immediately after the skin is cut. The first members of the series, the methyl to propyl alcohols, do not influence the

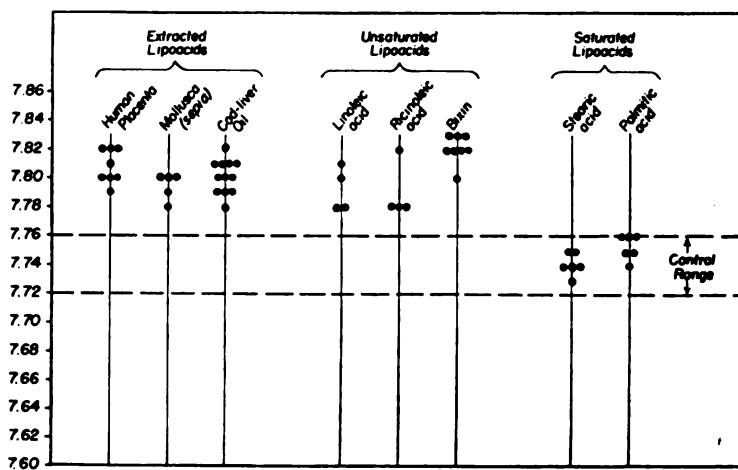


FIG. 235. The administration of various lipoacids upon the s.d.c. pH shows that while the saturated fatty acids do not influence it, the non-saturated fatty acids as well as the lipoacid preparations obtained from different sources induce an elevation of the local pH.

s.d.c. pH. From butyl alcohol to nonyl alcohol, the members of the series show a consistent acidifying influence. However, a very important observation was made when the four isomers of butyl alcohol were studied. Three showed the acidifying effect while one, the tertiary isomer, like the lower alcohols, did not influence the second day wound crust pH. This could be correlated to a special characteristic of these substances, their relative solubility in water and in neutral solvents. Just as do the lower alcohols, tertiary butyl alcohol mixes with water and neutral solvents, while the other three butyl alcohols, like the higher members of the series, are more soluble in neutral solvents than in water. (Fig. 236) This characteristic, which was used in systematizing the polar-nonpolar substances, appears to determine the activity of the aliphatic alcohols upon the s.d.c. pH. We must again emphasize that this activity is not direct but influences certain metabolic processes, since the active alcohol induces local acidification rather than the alkalinization to be expected with a direct effect.

Another factor which seems to influence the activity of this alcohol series is the length of the carbon chain. While heptanol induces the characteristic acidification, octyl alcohol does so in only some animals. Nonyl and decyl alcohols appear inactive.

The possibility of a biological competition between these agents and fatty acids has led to research with other alcohols capable of combining with fatty acids, particularly *in vivo*. We studied glycerol, glycerophosphoric ion and sterols, which are frequently found combined with fatty acids. We added glucose to this group because of its metabolic relationship to glycerol derivatives, although it is apparently not related to fatty acids.

It is interesting to note that glycerol produced only minimal influence upon the immediate pH. The highest values were still in the normal range. The acidifying effect of glycerol upon the s.d.c. pH, seemed to be subject

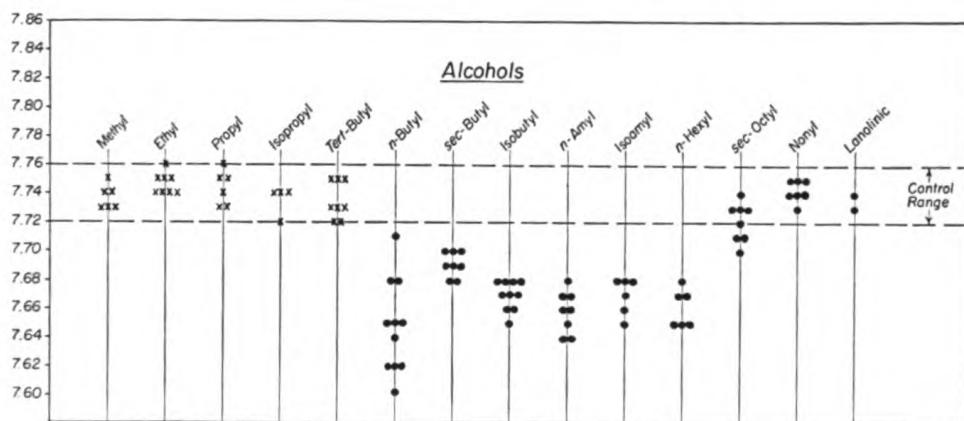


FIG. 236. Second day wound crust pH values for various alcohols shows the relationship between activity and lipoidic property. All the alcohols below butanol and tert. butanol, which are not lipoids, are inactive. This also holds true for alcohols with chains longer than eight carbons.

to individual variations but also with values at the lower limit of normal, such as 7.72. Glucose showed a slight acidifying effect, while cholesterol showed definite acidifying activity upon the s.d.c. pH, as did all the preparations of the insaponifiable fraction of various organs or tissues. The acidification produced by the insaponifiable fraction preparations is more intense than for cholesterol alone, indicating that the other constituents of these preparations also have an acidifying influence.

Other Agents

We investigated some of the hormones that are often used clinically and found that the biological antagonism between male and female sex hormones is also apparent in their influence upon the s.d.c. pH, the male hormone having an alkalizing effect, the female hormone, an acidifying one. We must note here that the acidifying effect is also apparent for pro-

gestosterol. This effect is opposite to that of the male hormone and similar to that of stilbestrol, contrary to what we expected. There is similar antagonism between desoxycorticosterol and suprarenin, the former being acidifying and the latter slightly alkalizing. The liver antianemic extract also has an alkalizing effect.

Several vitamins also were investigated. We were able to see that while vitamins B₁, B₂, E and K have an acidifying effect, vitamins A, D and B₆ have an alkalizing action. Ascorbic acid seems to have no effect upon the s.d.c. pH.

A few alkaloids and glucosides, important for their pharmacodynamic activity, were studied. The two opium alkaloids and a similar synthetic agent are moderately acidifying. Atropine, caffeine, and quinine have a slight alkalizing effect. The different effects of digitaline and saponine were unexpected; both show slight but opposed action, the first acidifying and the second alkalizing.

Because of their effect upon the central nervous system, narcotics and hypnotics were studied. While ether and chloroform are slight alkalizing, the two barbiturates which we tested showed an acidifying effect. This is interesting, especially when related to the opium alkaloids and demerol which also induce acidification, although to a lesser extent than the barbiturates.

Various other agents were studied. Among pyretogenics and antipyretics, there is an obvious antagonism in influence upon the s.d.c. pH. While the pyretogenic, methylene blue, induces a frank acidification, the three antipyretics we examined produced alkalization. However, acetylsalicylic acid does not follow this rule—it has an acidifying effect.

Among antimicrobial agents, a parallel action was apparent for the three antibiotics of fungal origin and the two sulfa drugs studied. All have an alkalizing effect, like the antipyretics. The acidifying effect noted for benzedrine does not accord with a similar effect observed for substances with hypnotic and sedative activity. This discordance between principal pharmacological activity and effect upon the s.d.c. pH indicates that the latter must, on many occasions, be considered to be due to a secondary influence exerted at the interstitial level.

The antagonistic biological activity of anti-anemic liver extract and iron preparation, the former favorably influencing the hyperchromic anemias, the latter favorably influencing the hypochromic forms, is reflected in their opposite effect upon the s.d.c. pH. The anti-anemic liver extract induces alkalization, while iron induces acidification. The same antagonism is seen with two agents having an opposite action on blood coagulation. While vitamin K induces acidification, dicumarol is alkalizing. Although rutin acts upon other factors when it intervenes in bleeding, it has the same effect as vitamin K on the s.d.c. pH.

Aminophyllin induces acidification in contrast to caffeine, which produces slight alkalization. On the other hand, procaine's effect is like that of the opium alkaloids and barbiturates, all inducing acidification. This can be

related to the effect of higher alcohols which also have narcotic activity and, as seen above, also induce acidification.

TABLE XXVII shows the effects upon the s.d.c. pH of all of the substances examined.

TABLE XXVII
THE EFFECT OF VARIOUS SUBSTANCES UPON THE S.D.C. pH.

	Elevate	Reduce	No Effect
Vitamins	A, D, B ₁ , B ₂ , K, E		C
Hormones	Testosterone, Epinephrine	Stilbestrol Progesterone, Desoxycorticosterone	
Alkaloids	Atropine, Quinine	Codeine, Morphine	
Antibiotics	Penicillin, Streptomycin, Aureomycin		
Sulfonamides ..	Sulfathiazole, Sulfamerazine		
Antipyretics and analgesics	Acetophenetidin, Aminopyrine Antipyrine	Acetyl-salicylic acid	
Narcotics	Chloroform	Phenobarbital, Pentobarbital	
Anti-anemics ..	Liver extract	Iron (reduced)	
Xanthines	Caffeine	Aminophylline	
Miscellaneous ..	Dicoumerol, Benzene, Toluol, Saponin, Pteryl-glutamic acid Teropterin)	Procaine, Benadryl, Coramine, Glucose, Glycerine, Sodium gluconate, Rutin, Methylene blue, Mercuryhydrin, Benzedrine, Demerol	

Based upon the findings for such different groups of agents, the s.d.c. pH method appears to be an interesting tool for the study of pharmacological activity. We must emphasize that not one of these substances, at least in the dosage given, has had any influence in changing the pH of normal tissues from normal range, as indicated by pH values obtained immediately after wound production. As our research concerns only ab-

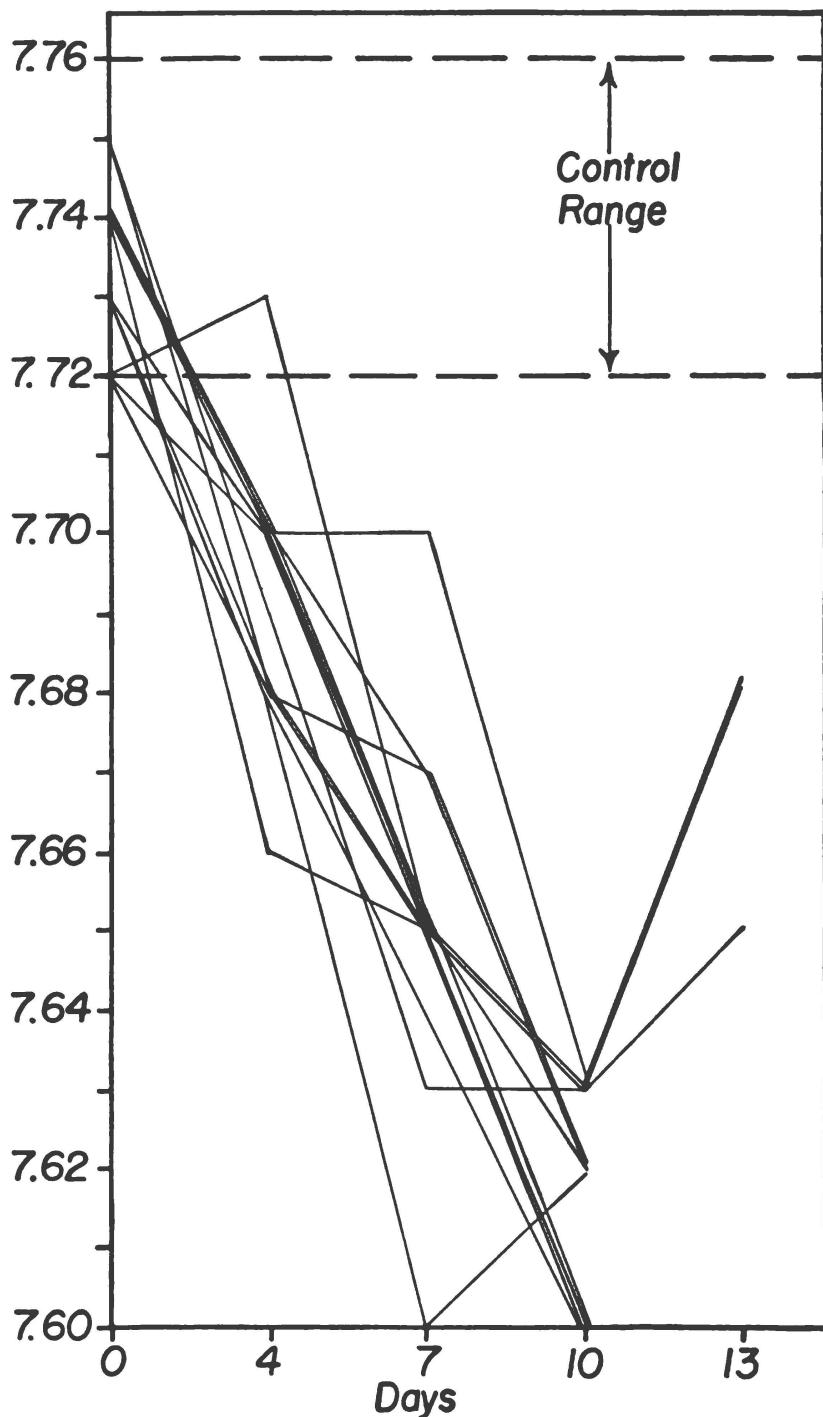


FIG. 238. The effect of a growing transplanted Walker tumor on the s.d.c. pH determinations in surgical wounds produced serially on the day of transplant and every three days thereafter. A marked reduction in the s.d.c. pH was observed in all animals.

normal tissues, the s.d.c. pH is of use. However, in integrating the pH values into the general picture of the offbalances, it must not be forgotten that they represent changes in the interstitial fluids, which correspond to the secondary part of the tissue level.

PHYSICAL AGENTS

Animals kept in an incubator at 38° C. showed a definite tendency toward a lowering of the s.d.c. pH. The effect of cold upon animals maintained in a refrigerator for forty-eight hours before operation was less apparent.

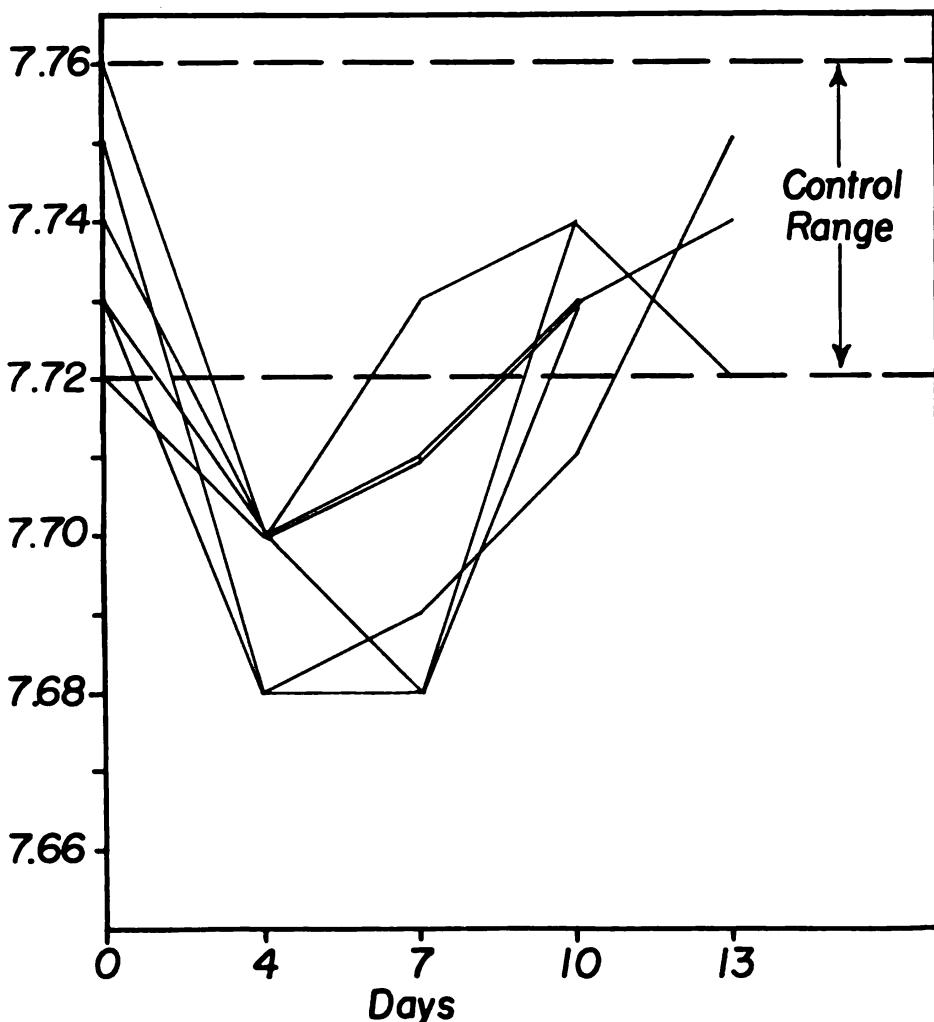


FIG. 239. Serial s.d.c. pH changes in animals with transplanted tumors that regressed rapidly or failed to grow. A slight fall is seen to occur in the fourth and seventh post-transplant day determinations, with a return to the control range in all the animals by the tenth day.

BIOLOGICAL FACTORS

Transplanted Tumors. Preliminary studies regarding the effect of a transplanted Walker tumor upon the s.d.c. pH of an experimental wound have been carried out. Prior to transplantation, the control s.d.c. pH was determined for each animal. Tumors were then transplanted subcutaneously to the left flank region by the trocar method. After the tumor had been transplanted to the animals, experimental operative wounds were produced at intervals of three days and the serial s.d.c. pH values on the

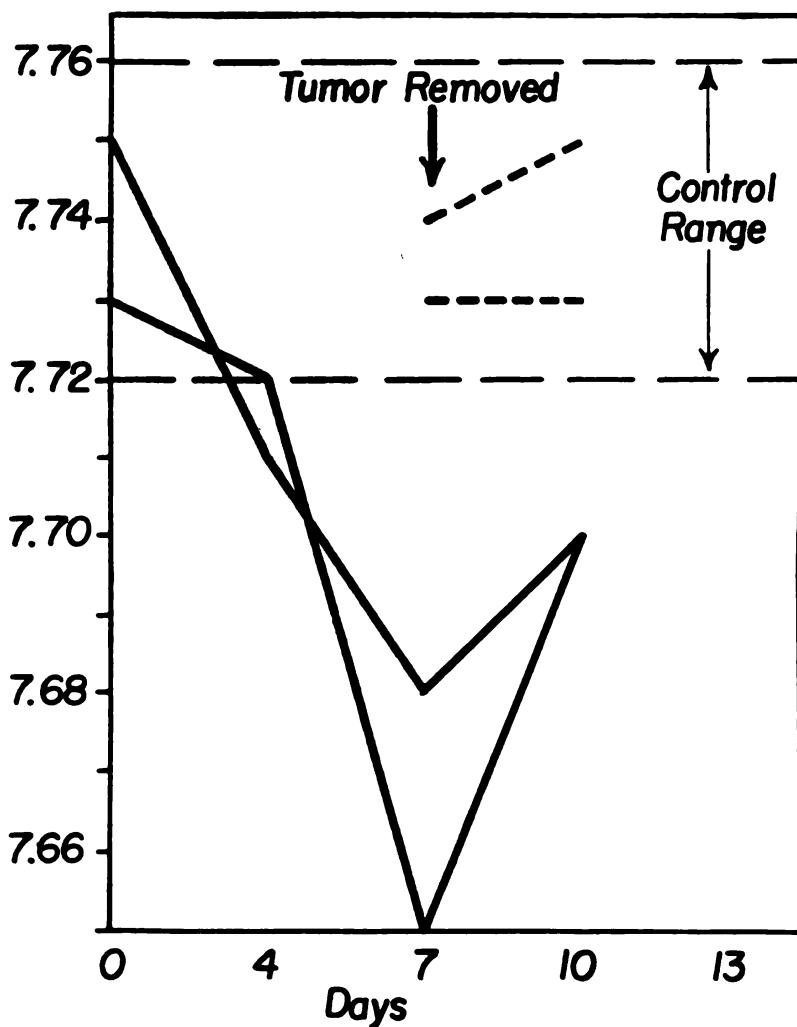


FIG. 240. The s.d.c. pH changes in two rats with growing Walker tumors (—), from which the tumor was removed on the seventh post-transplant day. Following the removal, the s.d.c. pH began to return towards the control range. A similar large incision was made in two animals without tumors and no effect was observed upon the s.d.c. pH in these animals (....).

fourth, seventh, tenth and thirteenth post-transplant days determined. The pH values determined each time immediately after the wound was produced, did not differ from the values found in untreated controls. As in all other animals, the s.d.c. pH only showed significant changes.

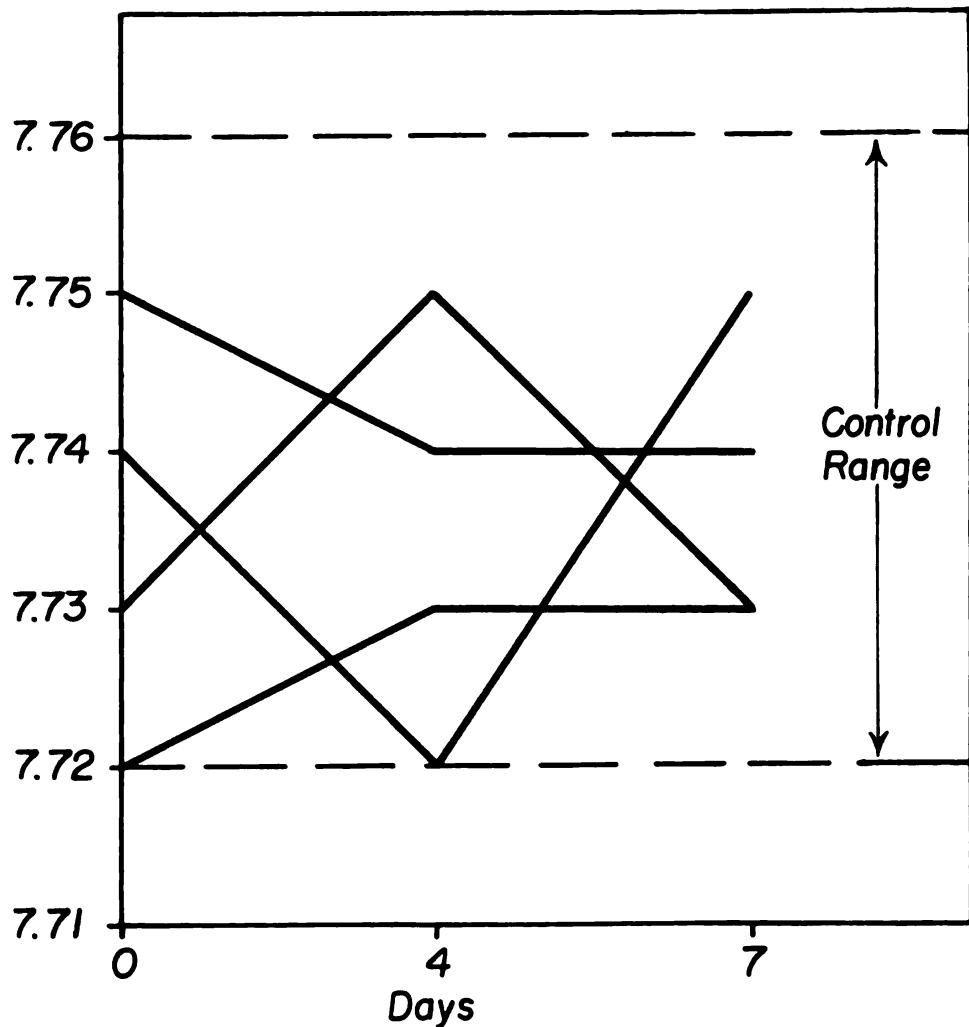


FIG. 241. Serial s.d.c. pH determinations in rats without transplanted tumors. The serial small surgical procedures did not alter the s.d.c. pH from the control range.

Figure 238 illustrates graphically the changes in the s.d.c. pH in a group of twelve animals with successful tumor transplants. A steady and maintained lowering of the s.d.c. pH was observed in each animal, with a tendency for it to rise slightly in those animals surviving thirteen days.

In some of our animals the tumor has shown a tendency to regress spontaneously or fail to take. In six animals in which the tumor failed to take or underwent early regression and disappeared, the s.d.c. pH in the

fourth and seventh post-transplant day tests fell slightly, but thereafter returned to control levels. (*Fig. 239*)

When transplanted tumors that were growing well, were surgically removed following the second post-transplant determination on the 7th day, the s.d.c. pH was seen to increase at the next test. (*Fig. 240*)

Surgical wounds. The same incision as for the removal of the tumors was produced in control animals, but the incision alone did not affect the s.d.c. pH. (*Fig. 240*)

In order to further control these experiments, repeated s.d.c. pH determinations were carried out upon wounds produced serially as in the tumor bearing animals. When no tumor was present the serial s.d.c. pH values did not vary from one examination to the next. (*Fig. 241*)

The serial small surgical procedures did not alter the s.d.c. pH from the control range.

Chapter 5, Note 2. Potassium

We have seen in Chapter 2, Note 1 how the place occupied in nature by sodium and potassium can explain their peculiar distribution in the body. Proper to the earth crust, one of the environments through which the complex individual has passed during its phylogenetic evolution, potassium appears as an element of the secondary part of the cellular compartment in the hierarchic organization. As a monovalent heterotropic element, potassium represents the principal organizational cation of this compartment. Its influence exerted in normal and abnormal physiology can be understood through its specific intervention at the cellular level, the changes in potassium content of the other compartments of the hierarchic organization being secondary to those occurring at the cellular level.

Potassium, absent at the nuclear compartment, is thus found in the nuclear or chromosomal sap, only in minimal amounts. We have no direct information about a passage of potassium from cytoplasm into the nuclear compartment under abnormal conditions. Judging by analogy, it appears probable that such a passage would occur and result in the appearance of nuclear vacuoles.

Ample data are available concerning the relationship between potassium of the cellular and of the metazoic compartments. This information receives a special interpretation when related to the above mentioned hierarchic distribution of the elements.

The cells maintain a proper amount of potassium in the cytoplasm which corresponds to a cellular primary constant. This constant insures a normal cellular metabolism and is controlled in part by the selective intervention of the cellular membrane. Under normal conditions, only a slow passage of potassium through this membrane takes place as compared to other constituents, such as of water, for example. On using radioactive potassium, Moore has shown that it takes about fifteen hours to bring it into balance with the intracellular potassium, while for heavy water, such an equilibrium was reached in less than two hours. (254) Due to the

intervention of the membrane, only minimal changes result in the cellular potassium even though rapid potassium variations take place in the extracellular compartment. Under normal circumstances, the body is insured through a regulatory mechanism against too strong or too rapid systemic changes. Normally, no potassium is stored in the body beyond that which is contained in the cell and metazoic compartment. Following a high intake, potassium is rapidly excreted. A very small amount is lost through perspiration, an additional amount, of around 10% is lost in the stool (252), and the remainder is lost through the kidney. (253) For insufficient intake, or an abnormal loss through excessive diuresis, prolonged diarrhea or vomiting, the organism tries to reduce this loss of potassium to a minimum. A prolonged systemic potassium deficiency brings about an increase in the weight of the kidney with tubular hyperplasia, which can be interpreted as a compensatory hypertrophy, in order to insure the reabsorption of very small amounts of potassium from the glomerular filtrate, and thus save this important element for the organism. Consequently, a quantitative abnormal intake or loss of potassium will result in an abnormal amount, either too high or too low in the metazoic fluids, only in case of a concomitant deficiency of the regulatory system. Prolonged quantitative changes in the amount of potassium available to the total body, influence only up to a certain point, the potassium content of the cells. Thus when the total body potassium remains low over a period of time, the cells too lose potassium. (250) On the other hand, the potassium in the cells increases after an abundant, prolonged administration of the cation. (251)

Besides these quantitative changes we have to consider abnormal conditions as affecting the specific intervention of this element. The proper level to which potassium belongs and the characteristic changes which take place at the cellular level, could be translated in too high or too low values. We tried to further interpret its intervention through the heterotropic character of this element.

The changes seen to occur in muscles have permitted us to relate this dual occurrence observed for the potassium content of the cells to the two abnormal metabolic conditions which take place in the cells. A muscle in anabolic metabolism and characterized by a process of glycogenogenesis, shows an increase of its potassium content. On the contrary, catabolic processes such as those occurring during muscular exercise (255) or in tissues in agonic states, are related to a loss of potassium from the cells. This metabolic loss of potassium is different from that seen to result from the death of the cells.

The destruction of cells in general, results in a liberation of their potassium into the interstitial fluids. However, such a destruction explains only in part the progressive loss of potassium encountered in abnormal conditions. The erythrocytes of stored blood lose their potassium to plasma through another process than their destruction. The same is true for patients undergoing surgery. They experience a constant loss of cellular potassium into the metazoic fluids, for which the process of destruction is only partially responsible. The breakdown of cells as it occurs in starvation or dehydration

for instance, releases a proportion of 2.4 gm. of potassium for every gram of nitrogen which is liberated under these conditions. (256) However, in surgical patients, the potassium loss was found to be two or three times higher than expected, considering the nitrogen loss. (257) This indicates a passage of potassium out of the cells without cellular destruction.

The study of the red cell potassium changes in stored blood and in the diphasic biological phenomenon such as in hemo-shock, has increased our knowledge of the conditions which correspond to these changes. We found thus that in the first phase of hemo-shock there are lower amounts of potassium in the red cells, followed by a second phase where this cellular potassium content increases. These two changes were thus connected with the characteristic offbalances occurring in hemo-shock: type D in the first phase, and type A in the second. Further studies of tissues in offbalance A or D in pathological conditions have confirmed this correlation between the two fundamental offbalances and the two opposite variations in cellular potassium.

The heterotropic character of potassium as seen through the test of the second day wound crust pH, (*See Note 1, Chapter 5*), has correlated the changes in potassium distribution to the heterotropic or homotropic character of the occurring processes. A low cellular content in potassium would correspond thus to the homotropic character of the processes characterizing the offbalance D. Those corresponding to a high cellular content concord with the group of heterotropic processes. Increased intracellular potassium results in a heterotropic anabolic effect, while loss of cellular potassium corresponds to catabolic metabolism.

The relationship between the hierarchic compartments explains the changes under abnormal conditions in the amount of potassium present at the different compartments. The study of the diphasic phenomenon of hemo-shock has permitted us to follow this relationship between cellular and plasmatic potassium. In the first phase of a hemo-shock, to the low potassium of the cells corresponds a hyperkalemia, while in the second phase, to a higher cellular potassium corresponds a hypokalemia.

With potassium, a cellular element, the changes seen in the metazoic compartment can thus be considered to be secondary to those occurring at the cellular level. An abnormal cellular condition, with an increase in the cellular potassium content, would thus have an opposite effect upon the amount of potassium present in the metazoic fluids. The fact that plasma potassium values are kept below normal, has to be interpreted as a means of compensating the high values present in the cells. These low values in plasma would result in a reduction of the cellular potassium and favor its return to normal values when possible. It is especially through changes in the urinary excretion that the respective hypokalemia are induced and maintained. On the other hand, an abnormal change in the cellular condition resulting in a low amount of potassium in the cells is seen to induce a prolonged compensatory increase in the potassium content of the metazoic fluids. Oliguria with reduced loss of urinary potassium, permits the creation and maintenance of this secondary hyperkalemia.

Potassium and Offbalances

We tried to utilize the information concerning the relationship between the two types of offbalances and the changes in potassium distribution between cells and metazoic fluids, in order to recognize the existence and nature of these offbalances. This led us to compare the amount of potassium present in red cells and in serum as an indication of this distribution between cells and metazoic fluids. From a practical point of view, we utilized the total blood instead of the separated red cells. (*Chapter 4, Note 5*) From the relationship between the two values, we could interpret the nature of the occurring changes as corresponding either to a quantitative abnormality excess of deficiency—or to a qualitative abnormality due to an offbalance A or D. Fig. 127 shows this correlation. With the values of serum potassium around 4.5 mEq and of total blood around 38 mEq, the condition is considered normal from the point of view of the potassium intervention. Low values in serum and total blood correspond to a quantitative deficiency, while high values for both, a quantitative excess. High serum with low total blood potassium correspond to the offbalance type D, while low serum potassium and high values in total blood to the offbalance type A.

In chronic conditions, such offbalances are seen to persist over long periods of time. Fig. 214 shows an example of a typical D offbalance, with the serum potassium high and the total blood potassium low. Fig. 215 shows an offbalance type A, with low serum potassium and relatively high total blood potassium. Fig. 216 shows an example of lack of potassium, with low values in both serum and total blood.

Potassium and Lipids

The correlation between potassium distribution and the offbalances A and D, has linked this information to the lipids and lipoids. The administration of agents of one or the other of the two groups, positive or negative, has produced opposite changes in the distribution of potassium total blood and serum. These experiments were made in rabbits in collaboration with Ismail Eroglu, Patricia McLachlan and Lee Weston. Administered in large amounts, all the positive lipoids and especially heptanol were seen able to reduce potassium in blood and increase it in the red cells. The negative lipoids have an opposite effect. Administered in reduced amounts, big differences could be seen between the agents of the same group, many having no influence on the potassium and only few showing manifest effects. Among the negative lipoids, the most active agents have appeared to be heptyldisenide, sulfur and selenium tetra-hydronaphthalene and epichlorohydrin.

Potassium and Sodium

The changes in the amount of potassium could be connected to its relationship to sodium, and further interpreted in the frame of the hierarchic organization. Both are members of the same series in the periodic chart,

but they correspond to different compartments of the organization. Sodium is the cation of the metazoic compartment, and potassium that of the cellular. They would consequently act differently towards cells, for instance. Under abnormal conditions, sodium is able to enter the cells. For example, following an injury, the cellular membrane which is almost impermeable to sodium, lets it pass through. A penetration of sodium into cells occurs, as demonstrated by the use of radioactive sodium. (42) As a consequence, potassium is released from the cells in order to maintain the necessary osmotic pressure constant. Many abnormal processes occur following the penetration of the sodium in the cells and others occur due to the release of potassium. The sodium which entered in the cells is partly isolated together with water, to form cellular vacuoles. The response of the metazoic compartment to those changes are interesting. Hyperkalemia, with a simultaneous hyponatremia occurs in the first phase of the diphasic phenomenon. The opposite happens in the second phase, when hypokalemia coincides with a hypernatruria.

This antagonism between sodium and potassium is seen further in the pharmacologic activity of these elements. The administration of potassium salts induces a greater elimination of sodium and water, which explains its diuretic action with dehydration of tissues and progressive alkalinization of the urine. The effect upon the different organs is also antagonistic. For instance, in their effect upon the heart, as Merrill and co-workers have shown, the characteristic electrocardiographic effects of hyperkalemia appear only when the sodium level of blood is lowered. (259) A high sodium content prevents the cardiac effects of hyperkalemia.

In opposition to the atrophy of the adrenal glomerulosa induced by an excess of sodium administration, a potassium overdose can cause an enlargement of these zones in rat adrenals.

The hyperkalemia due to external intake such as in potassium poisoning, or due to systemic response to low cellular potassium, specifically influences certain functions. The peripheral vascular collapse with lowered blood pressure, cold clammy skin, pallor, listlessness and mental confusion, are symptoms related to the offbalance D, and encountered in high plasma potassium. They appear in conditions such as shock and burns which correspond to a prolonged first phase of the diphasic phenomenon. Pares-thesis and flaccid paralysis (260) (261) are also important results of hyperkalemia. The most important changes appear in cardiac physiology and can be interpreted to correspond to low cellular automatism. Hyperkalemia will thus induce dromotropic positive changes, such as the increase of the duration of the Q R S complex, or that of the P-R interval, with a delay in the ventricular contraction, or a block which can lead to the arrest of the heart in diastole. (262), (263) Characteristically appears the changes in wave T, which increased became even angular. The change seen in offbalance D, are similar to those induced by a prolonged administration of potassium. In a study of the pharmacological activity of various agents upon the heart, made in collaboration with I. Eroglu, we used these changes as an indication of the type of offbalance they induce.

Fig. 216B shows some examples of this effect on rabbits. One group comprises agents considered as able to induce an offbalance D. They induce changes in the T wave which high amplitude is characteristic for a hyperkalemia. An opposite effect is seen in the other group, formed by agents able to induce an offbalance A, and where the T waves are remarkably depressed. (See page 574)

Potassium and Therapy

All these considerations have led to a more precise use in therapy of the information furnished by the study of potassium distribution. The quantitative deficiency—recognized by low potassium in total blood and serum—is controlled by oral and parenteral administration of potassium. A quantitative excess—with high values in serum and total blood—is treated with the administration of ionic exchange compounds, diet, laxatives and more sodium intake. For the offbalances, the treatment is especially directed to those agents which seem to influence more strongly the cellular potassium metabolism. Selenium lipoids and sulfurized tetra-hydronaphthalene are used for offbalance A, and heptanol for offbalance D.

The fact that the cellular membrane plays an important role in the metabolism through which the abnormal changes in potassium occur, has led us to use agents acting upon these membranes. Adrenaline has been seen to favor the discharge of potassium from the liver cells, simultaneously with glycogen. We thus used adrenaline for cases of offbalance A in which an especially high cellular potassium was present. On the opposite side, the administration of insulin together with an increased intake of glucose was seen to increase the cellular potassium. (258) A similar effect was seen for cortisone, and ACTH, for heptanol, cholesterol and also for the unsaponifiable fractions of organs. These agents have been used in the treatment of offbalance D at the cellular level, where abnormal high values of serum potassium are present.

Chapter 6, Note 1. Definition of the Lipids

According to Bloor: "Lipids may be defined as a group of naturally occurring substances consisting of the higher fatty acids, their naturally occurring compounds and substances found naturally in chemical association with them. The group is characterized in general by insolubility in water and solubility in "fat solvents," e.g., ether, chloroform, benzene, etc." (226)

Chapter 6, Note 2. Definition of Lipoids (227)

From a physico mathematical analysis of our definition of lipoids, J. Mariani arrives to the following conclusions:

Any substance will behave like a lipoid with respect to a polar solvent when in:

$$\varphi = \frac{1}{2} \sum_{\text{surface}} a_{11}^2 - \sum a \cdot a_{22} + \frac{1}{2} \sum_{\text{surface}} a_{22}^2$$

φ will be positive. The electrical attractions given by:

$$\Sigma a_{11} a_{22}$$

will not be able to balance the attractive forces:

$$\frac{1}{2} \Sigma a_{11}^2 + \frac{1}{2} \Sigma a_{22}^2$$

due to van der Waals forces (and to the polar groups of A). But we can simplify this definition. Let us consider a solvent the molecules of which are dipolar and form polar groups similar to those contained in the solute. We can call those substances *lipoids* for which the dipole interaction energy in the cavity occupied by the molecule is less than the energy arising from van der Waals forces.

We get:

$$\Psi = \frac{1}{2} \sum_{\text{surface}} (a_{11} - \rho) - \sum (a_{11} a_{22} + \rho) = \frac{1}{2} \sigma - \xi$$

where now σ represents van der Waals forces in a cavity occupied by the molecule and ξ the dipolar forces at the surface of the cavity.

Chapter 6, Note 3. Separating Membrane Between Aqueous Media

The possibility of having a membrane with polar groups at both surfaces, as shown in mitochondria, represents one of the most important means used in biology to separate two aqueous solutions. (228) One of the characteristics of such a membrane is that often it represents an isolating boundary rather than a membrane with functional activity. This is seen in the fact that very often such a membrane breaks when the two separated aqueous media have to mix.

Chapter 6, Note 4. Fatty Acids Break Down

In a work hypothesis of the biological breakdown of a long chain molecule for caloric metabolism, we consider two factors as being of capital importance: 1) that the result be an even carbon-number chain molecule, since the Knoop oxidation leads to complete caloric utilization only for such molecules, and 2) that the length of the chain of carbons bound to the carboxyl in the new molecule be not higher than 11 carbons in order to permit direct beta oxidation. To fulfill the first condition, the breakdown process does not occur between the carbons of the double bonds themselves, since in natural fatty acids the double bonds separate portions of the chains, usually with odd number of carbons. Such a breakdown would lead to molecules resulting in further incomplete Knoop oxidation.

According to the hypothesis the breakdown of the molecule occurs at the carbon nearest the double bond. Through the energetic influence exerted by the double bond, the even numbered carbon nearby appears strongly positive. As a first step, this carbon was seen to fix a molecule of

oxygen resulting in a hydroperoxide as shown by Farmer and co-workers first for rubber (30) and later for fats in vitro. (31) It is with the passage, in a second step, of this hydroperoxide group into a carboxyl that the breakdown of the molecule occurs at this level, as shown in Figure 242.

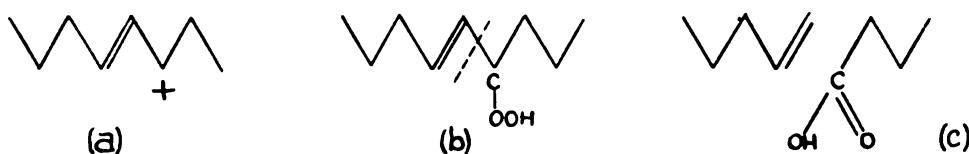


FIG. 242. The oxidative breaking down of a fatty acid molecule (a) occurs in vivo taking place through the appearance of a hydroperoxide at the carbon adjacent to the double bond (b). It leads ultimately to a carboxyl formation (c) at this adjacent carbon and results thus in chains with even number of carbons.

If the even numbered carbon near the double bond is toward the terminal methyl group, a monocarboxylic acid will result. A similar process taking place at the other carbon adjacent to the double bond toward the carboxyl will lead to a dicarboxylic molecule. The metabolic changes in vitro—and also in vivo—have shown the appearance of these two groups of even carbon mono and dibasic fatty acids. By binding two molecules of water the remaining 2-carbon chain linked by the double bond would result in acetic acid molecule. Such changes occurring in the caloric monoethenoids permitted us to explain one of the baffling peculiarities seen in the constitution of the monoethenic fatty acids.

In Note 5, we discuss the position of the double bond in the principal naturally occurring monoethenoids as it follows a characteristic pattern. In molecules with 16 or less carbons the double bond is more often placed so as to separate a group with 9 carbons toward the carboxyl end, while in molecules of 18 carbons or more, the double bond separates almost constantly a group of 9 carbons toward the methyl end. Figure 243 shows two characteristic examples.

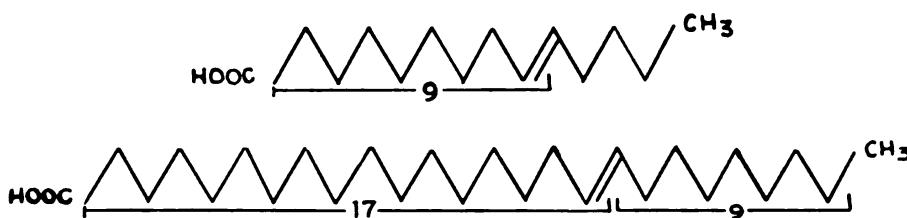


FIG. 243. Characteristic emplacements are seen for the double bond in two monoethenoids. The double bond separates two groups with odd number of carbons. For the myristoleic acid, it separates a group of 9 carbons toward the carboxylic end, and a group with a short 5 carbon chain toward the methyl end. For hexacosenoic acid (26C) a chain with 17 carbons is separated toward the carboxyl end and one of 9 carbons toward the methyl end.

The breakdown of the molecule according to the process mentioned above explains the peculiarity. For the chain with 18 or less carbons, biological fission would result in a diacid with 8 carbons and another monoacid with 8 or less carbons, both subject to Knoop oxidation. In the long chain fatty acid the double bond separating a 9 carbon fraction toward the methyl-end of the molecule will result in an 8-carbon chain as monoacid having the methyl group at the other end. The other part of the molecule, with more than 8 carbons and which corresponds to the long fraction hav-

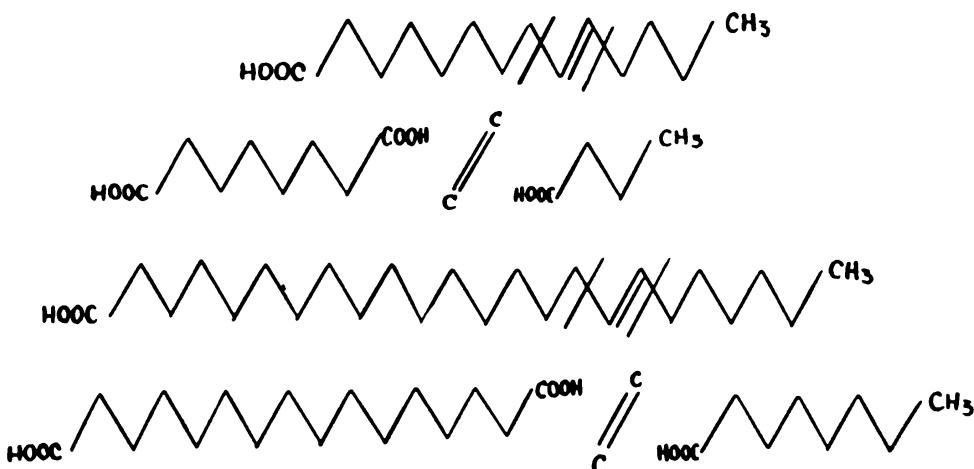


FIG. 244. Through the emplacement of the double bond in the molecules of the monoethenoids, the breaking down of the molecules of fatty acids at the carbons adjacent to the double bond leads to the appearance of a dicarboxylic acid for the part of the chain having more than 10 carbons.

ing the carboxyl at its end, results in a diacid. (Fig. 244) This also will be subject to Knoop oxidation, even with a long chain. In the diacid, this process, which is related to the intervention of the carboxyl, can take place at both ends where carboxyls are present.

Chapter 6, Note 5. Position of the Double Bond in Monoethenoids

The comparative analysis of the principal naturally occurring monoethenic fatty acids has shown a curious configuration due to the peculiar relative position of the double bond in these molecules. Figure 245 shows the position of this double bond in the principal monoethenoids. (267) Andre has shown that the double bond is more often placed so as to separate a group of 9 carbons ending with the carboxyl. We could show that this group is present toward the extremity ending with the carboxyl especially if the chain has 18 carbons or less. For the longer chain, this group of 9 carbons is present but usually toward the extremity, ending with the methyl. The importance of this configuration for the Knoop beta-oxidation of these acids is discussed in the previous Note.

Similar separation in groups of carbons totaling 9 carbons, are seen in polyunsaturated fatty acids. (Fig. 246)

Chapter 6, Note 6. Saturation-Desaturation Balance in the Liver

The total number of double bonds does not change in the simultaneous processes of saturation and desaturation occurring in the liver. To a preparation of liver cells, saturated and polyunsaturated fatty acids were added. The iodine number of the fatty acid mixture present was determined, as

Position of the Double Bond in Monoethenoid Fatty Acids

<u>Common Name</u>	<u>Systematic Name</u>	<u>Formula</u>
Obtusilic	Δ 4,5 Decenoic	
Caproleic	Δ 9,10 Decenoic	
Lainoleic	Δ 9,10 Dodecenoic	
-----	Δ 5,6 Tetradecenoic	
Myristoleic	Δ 9,10 Tetradecenoic	
Palmitoleic	Δ 9,10 Hexadecenoic	
Petroselinic	Δ 6,7 Octadecenoic	
Oleic	Δ 9,10 Octadecenoic	
Vaccenic	Δ 11,12 Octadecenoic	
Gadoleic	Δ 9,10 Eicosenoic	
-----	Δ 11,12 Eicosenoic	
Cetroleic	Δ 11,12 Docosenoic	
Eonicic	Δ 13,14 Docosenoic	
Selacholeic	Δ 15,16 Tetracosenoic	
-----	Δ 17,18 Hexacosenoic	
-----	Δ 21,22 Tricosenoic	

FIG. 245. Principal naturally existing monoethenoids show that for the members with a short carbon chain the double bond separates a group of 9 carbons toward the end of the molecule having the carboxyl. For fatty acids with more than 18 carbons, the group of 9 carbons separated is toward the end with the methyl group.

well as the quantity of mono-unsaturated members. After incubation at 37°C, the amount of monoethenic members was seen to have increased greatly. Analysis of the total fatty acids present in the preparation, however,

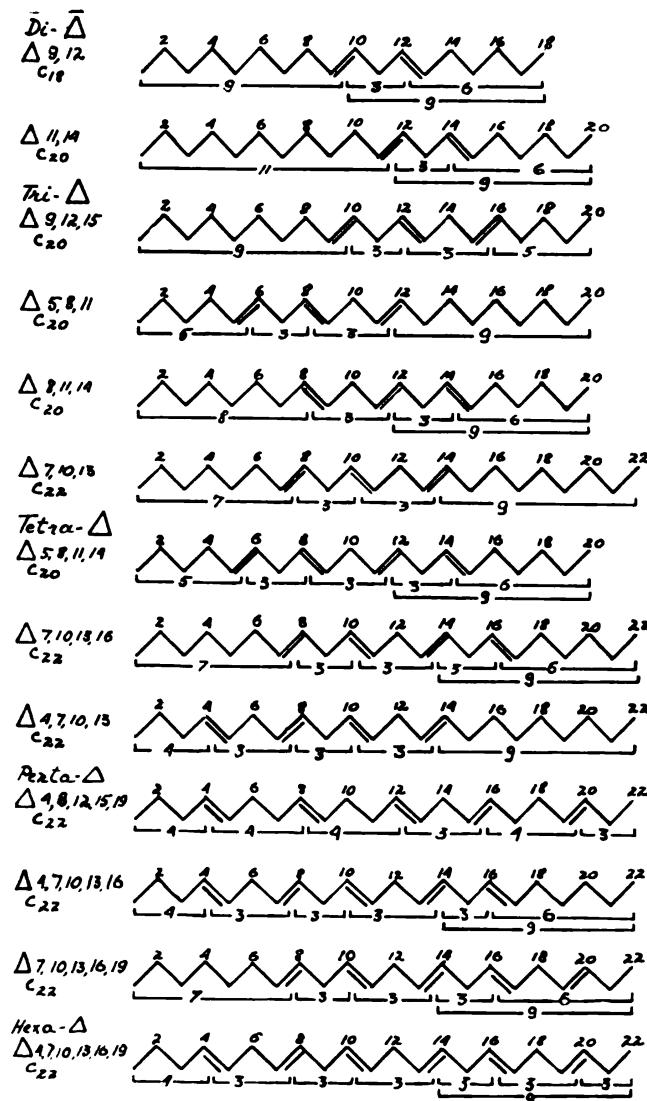


FIG. 246. In the polyethenoids, groups of 9 carbons are recognized, formed usually by the sum of two or three groups, multiple of 3 (3 or 6). The group of 9 carbons is usually placed toward the methyl end of the molecule.

showed the same iodine number, indicating that in the changes which had taken place, the saturation and desaturation processes had compensated each other through a transfer of double bonds from the polyunsaturated to the saturated members.

Chapter 6, Note 7. Essential Fatty Acids

A strong positive character of the carbon of the carboxyl results from its bond to two oxygens. This induces an alternating polarity, with the odd carbons positive in the chain. On the other hand, the influence exerted by a double bond in the molecule corresponds to an enhancement of the proper charge of the adjacent carbons. When a carbon is situated in an intermediary position between two double bonds, the influence resulting from the two double bonds is highly increased. These two factors—positive character as an odd carbon and intermediary position between two double bonds—make C₁₁ of linoleic acid a particularly strong positive carbon which appears to be especially able to bond a negative oxygen. We consider this strongly positive methylenic carbon to be the condition which determines whether a fatty acid has the character of an “essential” fatty acid.

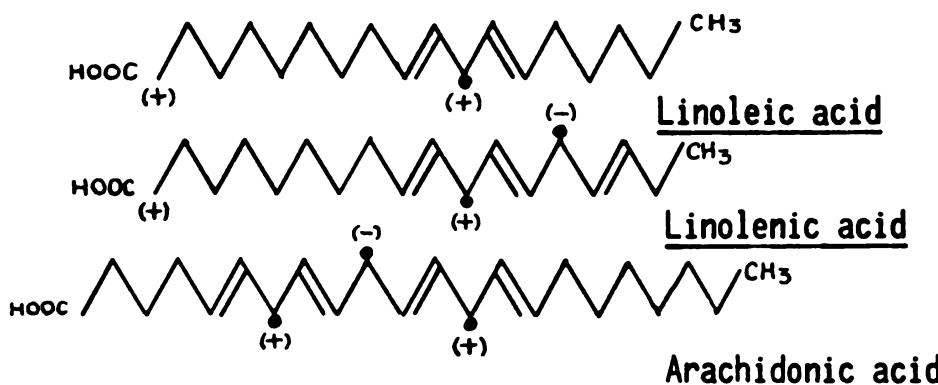


FIG. 247. Relationship between the positive charge of the methylenic carbon and the character of essential fatty acid. The similarity which exists between linoleic and linolenic fatty acids as essential fatty acids, can be explained by the fact that both have only one positive methylenic carbon. Arachidonic acid, with two such positively charged carbons, has this character of essential fatty acids markedly increased.

This explains further a peculiar relationship between the three important essential fatty acids. No differences are seen, from the point of view of activity as essential fatty acid, between linoleic and linolenic acid, although the last has 2 intermediary carbons, one at C₁₁ and another at C₁₄. This can be explained by the fact that the second intermediary carbon, the C₁₄, as an even carbon, has a negative electrical character. From this point of view of strongly positive methylenic carbon, there is no difference between linoleic and linolenic acids. The relationship between the character of essential fatty acid and intermediary positive carbon is further confirmed by the fact that arachidonic acid, with two positive and one negative intermediary carbons, is also a more active essential fatty acid than linoleic and linolenic acids, each of which has only one positive intermediary carbon. (Fig. 247)

Chapter 6, Note 8D. Fixation of Halogens on Conjugated Double Bonds

The fact that the fixation of halogens at the conjugated double bonds occurs in two steps would explain the relative difficulty in a reverse reaction. As seen for butanediene, the halogen ions are first attached to the external carbons of the conjugated formation with the appearance of a double bond between the central carbons. It is only in a second step that two other halogens are bound to these central carbons too, thus completing the bond of halogens to all the carbons of the conjugated formation. (Fig. 248)



FIG. 248. Fixation of halogens at the conjugated double bonds takes place in two steps, with a displacement of the double bond in an intermediary position in the first phase, a fact which explains the nonreversibility of the process.

Chapter 6, Note 8A. Solvent Fractionation of the Lipidic Constituents

In studying the biological role of the lipids, we recognized the importance of the forms under which the different lipids are present in the body, forms which seem to determine greatly their activity. A lipid changes its reactivity when it passes from the free form to one bound to other constituents. A first step in this study was the analytical separation of these forms. We distinguished thus four fundamental forms for acid lipids as well as for unsaponifiable fractions:

- I. Free lipids, or those bound in such a labile physical form as to be able to take part directly in different reactions through their polar groups.
- II. Lipids bound in cenapse with other constituents, that is, in a relatively labile form.

III. Lipids in combinations through their polar groups as esters or fats. This form represents usually a reserve or an inactive circulating form which can become active through hydrolysis.

IV. Lipids bound so firmly to other constituents as to be inseparable through solvents and to need saponification of the material in order to be liberated.

The first form would represent the functional form, the second a rapid available functional reserve, and the third, a reserve. The last would represent a stable combined form as part of the building of entities themselves.

We have utilized the differences in solubility of these various forms of lipids in order to separate them from the material to be studied, and thus to study their intervention in different normal and abnormal conditions. It must be emphasized that this separation concerns only the form under which the lipids are present in the organism and not their chemical constitution.

In spite of only a relative degree of accuracy in some separations, the

differences noted from one sample to another are so manifest and so consistent that this method can be considered as an interesting and reliable source of information. We have, therefore, used this technique of separation for thousands of samples through the years.

According to the technique we devised, the material to be analyzed—tissues, organs, entire organisms or only biological products—is finely divided in a blender. It is then extracted several times with ether under stirring, or in a Soxhlet apparatus. Under these conditions, ether removes the lipids present in the form of free lipids and neutral fats. This represents a mixture of the fractions I + II. The residue is again extracted, this time with a mixture of 10% ethyl alcohol in ether which breaks the cenapses and separates the lipids previously bound in cenapse. The result represents fraction III. The residue is saponified with 10% KOH and extracted with ether. This represents fraction IV US or the unsaponifiable lipids of fraction IV. After acidification with tartaric acid, a new extraction is made which represents fraction IV LA or the acid lipids of this fraction.

The ether of fraction I + II is distilled and the residue treated with 85-90% alcohol concentration which dissolves selectively the free lipids as fraction I, and leaves the part formed by neutral fats as fraction II.

Fractions I, II and III are then saponified separately. The unsaponifiable fractions are extracted with ether, giving respectively the fractions I US, II US and III US. After acidification with tartaric acid, other extractions with ether are made which respectively represent fractions I LA, II LA and III LA. Each of these fractions is washed with distilled water, dried with anhydrous sodium sulfate and, after the ether is distilled off, the fractions are weighed.

For each material, we obtained thus four different fractions for the unsaponifiable part which we called the US fractions, and four for the saponifiable called the LA fractions. They correspond respectively to: I, free lipids; II, lipids present as esters or fats; III, lipids bound in cenapse; IV, lipids fixed in combinations which are liberated only through saponification. In the following examples, we chose different materials to illustrate the kind of information obtained through this method. Fig. 249 represents the results obtained in normal rats, in rats under abnormal conditions, as well as in tumors, all expressed as the 8 lipidic fractions.

In order to facilitate the comparison between normal and abnormal conditions, we chose for this example, rats between 180 and 200 gms. of weight, all males with one exception (case b). The data obtained were thus compared with that obtained for case (a) which corresponds to a normal male rat killed by ether. The analysis for the lipoacid fractions of this case shows a fraction (IV) at 3.5 mg./per gram of animal, the fraction III in cenapse at .2 mg./per gram, II corresponding to fats at 1.2 mg./per gram and the fraction I as free lipids at 8.1 mg./per gram of animal. For the unsaponifiable fraction, the fixed part IV is at 0.8 mg./per gram of animal, the III in cenapse at 2 mg., the II as fats at 0.8 mg., and the free part I at 7 mg./gram of animal.

The female rat (b) shows in general, lower values for the acid frac-

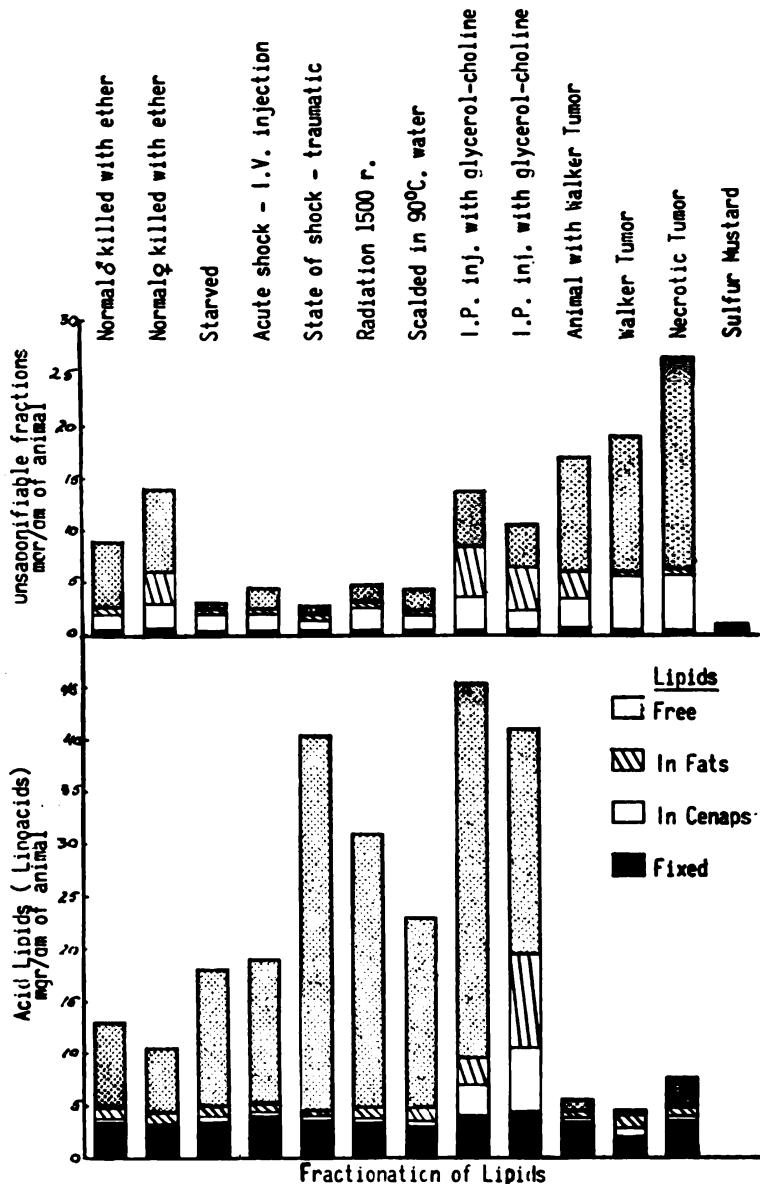


FIG. 249. Solvent separation between different lipoacids and unsaponifiable fractions in various animals and tumors. The values expressed in mgr/gr of animal show big variations concerning especially the free lipids. (Top fraction)

tions and higher for each fraction of the unsaponifiable part. It is interesting to note that the fixed part (IV) in acid lipids is lower than in any male, a fact confirmed by other analyses. The increase in unsaponifiable part concerns especially the cenapse, fats and free lipid fractions. These differences are in agreement with the data concerning the relationship between lipids in males and females, as discussed in Chapter 6.

It is interesting to note the changes in starved animals as shown in case

(c) where a marked decrease in the unsaponifiable fractions is seen, but with an increase however, in the acid part, especially in the free fraction (which reaches values of 13 mg./1 gm.). Similar changes are seen in acute shock (d) induced by the intravenous injection of a rich culture of *Esch. coli* in broth. Although death occurred in less than 40 minutes, a marked increase in free fatty acids is seen with a corresponding decrease in the unsaponifiable fractions. The deviation from normal in the same direction appears still more manifest for the animal in a traumatic state of shock (induced by 700 falls in the Collip-Noble drum), (e). The dying animal showed a marked increase in free fatty acid (37mg./1 gm.) with a notable reduction in the unsaponifiable fractions. Cases with over 70 mg./1 gm. of free fatty acid were also found. A lethal dose of radiation (1500r) (f), and caloric burns (g), were seen to induce similar changes.

Also of interest was the influence exerted by the intraperitoneal injection of glycerol and choline, with an impressing increase in all the fractions of the acid and unsaponifiable lipids, and of those in cenapse and fat fractions in (h). We chose example (i) of another animal submitted to the same treatment to show the extent of the concordance of the information furnished by this method.

It can be seen that almost under all conditions, the amount of fraction IV as obtained through saponification, changes very little. The amount present in cenapse and as neutral fats show more variations. Varying considerably from one case to the other, are the free lipids as fraction I which thus could be connected more directly with a pathogenic intervention of the lipids.

Another conclusion can be drawn from the analysis of these results. The high amount of free lipids often obtained under abnormal conditions cannot be considered to result only from a liberation of these lipids from the pre-existing reserve as neutral fats or from the more labile cenapse form, since the total amount of the free lipids found is much greater than the sum of these forms. An appearance of new lipids, through synthesis, has become evident in these conditions.

Example (j) is the analysis of an animal with a 12-day old Walker tumor. Comparison with the normal controls shows a reduction of the acid lipidic part, with an increase in all the four fractions. The study of the tumor itself (k) shows still greater differences. The fixed fraction is smaller than in the total body of the animals, while the cenapse part is greatly increased. The free lipoacidic fraction is almost nil. For the unsaponifiable fractions, that in cenapse, and especially that corresponding to free lipids, is greatly increased. In the necrotic tumor all four lipoacid and unsaponifiable fractions are increased. The latter however, to a greater degree. Though the cenapse amount is high, an increase is most evident in the free fraction. Case (m) of an animal treated with sulfur mustard applied on the skin, appears particularly interesting. The animal, dying the 14th day, has almost no unsaponifiable fractions left (less than 1 mg./1 gm.).

We used this method extensively throughout the years, in spite of its one limitation—the imperfect separation of neutral fats from free lipids.

The great concordance for all variations however, has practically overruled any objection of a failure of the separation of these free lipidic fractions.

Chapter 6, Note 8B. Spectral Analysis

In collaboration with Carlos Huesca-Mejia and Priscilla Teitelbaum, we studied several thousand samples of lipid preparations through spectral analysis, in ultraviolet and first portion of visible light, and more rarely in infrared, using the Beckman spectrophotometer. We will limit ourselves to note here only some of the principal conclusions reached.

1) Concerning the chemical isomerization procedures, we could show the importance of the temperature used when a mixture of fatty acids is treated. The conjugation in vitro as it is usually carried out, with ethylene-glycol or glycerol as solvents, was seen to result in preparations with too low amounts of tetra-, penta- and hexaenic conjugated members. A relatively rapid disappearance of the conjugated formations with 4, 5 and 6 double bonds was seen to be induced by the high temperature used. This led us to utilize a new method of conjugation, at lower temperatures. Using ethyl alcohol as solvent, preparations with high conjugated formations were obtained.

2) We utilized the spectral analysis for quantitative determination not only for di-, tri- and tetraenes as usually employed, but also for pentaenes and hexaenes. For this purpose we determined the extinction coefficient corresponding to these pentaene and hexaene formations. This was made possible by isolating the respective pentaenic and hexaenic conjugated members through appropriate solvents.

3) We studied various materials and especially different organs in order to correlate their richness in different fatty acids to their biological activity, by using the spectral analysis of the in-vitro conjugated fatty acids, as mentioned above.

4) Similarly, we tried to correlate the existence of characteristic peaks in the spectral analysis curve of unsaponifiable fractions of organs to their biological activity.

5) We utilized spectral analysis for the study of the effects of various agents such as chlorine, sulfur, sulfuric acid or oxygen upon the conjugated fatty acids.

6) We showed that minimal changes are induced in the nonpolar groups of conjugated fatty acids by changing their polar group from carboxyl into a primary alcohol, by treatment with lithium-aluminum hydride.

7) We studied the influence exerted by conjugated fatty acids upon carcinogens. This can be partially revealed by the quenching action induced upon the fluorescence of these latter agents.

8) In an extensive study we investigated the influence exerted by radiation upon fatty acids in-vitro and in-vivo. This influence was characterized by the appearance of conjugated trienes, and is presented in Chapter "Radiation," and in other Notes.

Chapter 6, Note 8C. Vapor Fractionation of Fatty Acids

In a group of experiments we applied the gas chromatography method to the study of fatty acids. The principal aim was to investigate the value of the information furnished by this method concerning the presence of conjugated fatty acids. This study was made in collaboration with Ivan Bier and with Winston Dindial who prepared the samples.

Methyl esters of eleostearic acid, linoleic acid and its conjugated isomers; of linseed oil fatty acids and the conjugated preparation; of cod liver oil fatty acids and the conjugated preparations; and of samples of fatty acids obtained from animals and tissues under normal and abnormal conditions were obtained. We analyzed through vapor fractionation, these different preparations as such, the preparations obtained through condensation on cold fingers during distillation in vacuum at different temperatures, and the different fractions obtained through distillation in vacuum. For all these tests we used the Perkin-Elmer vapor fractioner with a column of succinyl polymers heated up to 235°C. Under these conditions, no differences could be seen between the respective conjugated and nonconjugated samples. Figs. 250 and 251 show examples of such analyses of a cod liver oil fatty acid preparation and of a preparation obtained after treatment with KOH in butyl alcohol. Fig. 252 shows the spectral analysis of this last product.

Under the condition of analysis used, the gas chromatography method does not permit the identification of the conjugated isomers present. This is the reason why the analytical method could not indicate the presence of such members in materials obtained during abnormal conditions. The conjugated isomers can be identified by other methods—such as spectral analysis and especially oxalic index—after oxidative fission.

We are now trying to obtain columns that would permit working at much higher temperatures and would permit us to identify these conjugated fatty acids. In view of the minimal amount of material needed for analyses and the precision of the results usually obtained, an adaptation of this method for the identification of conjugated members would be of especial great value.

Chapter 6, Note 9. Twin Formation

The odd number of carbons in a cyclic molecule represents one of the conditions which always would result in the appearance of a twin formation, since the alternation between positive and negative signs gives the same electrical charge to two nearby carbons. (Fig. 254) The correlation of the positive and negative charges of nearby carbons to the fact that acetic acid molecules have been utilized in the synthesis of the molecule represents an additional factor for inducing twin formations even in cycles with an even number of carbons.

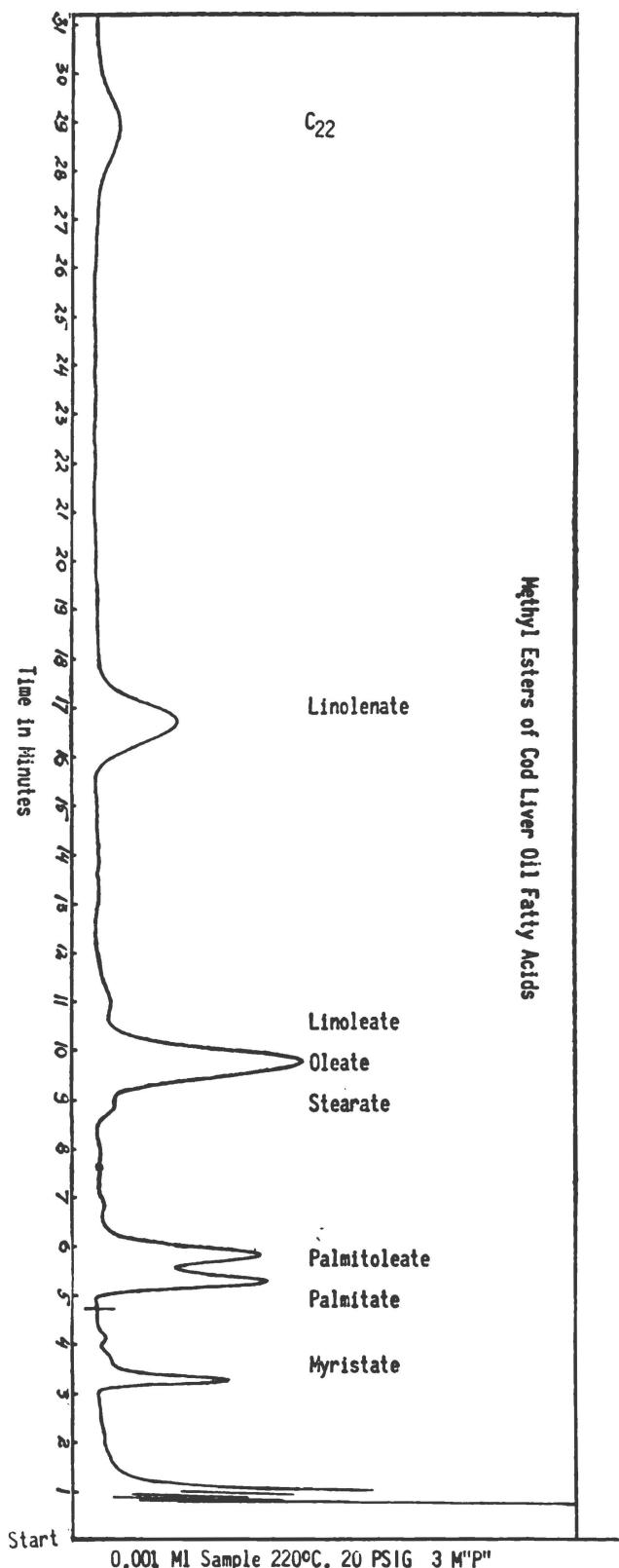


FIG. 250. Gas chromatographic analysis of a sample of cod liver oil fatty acids showing some of the different constituents.

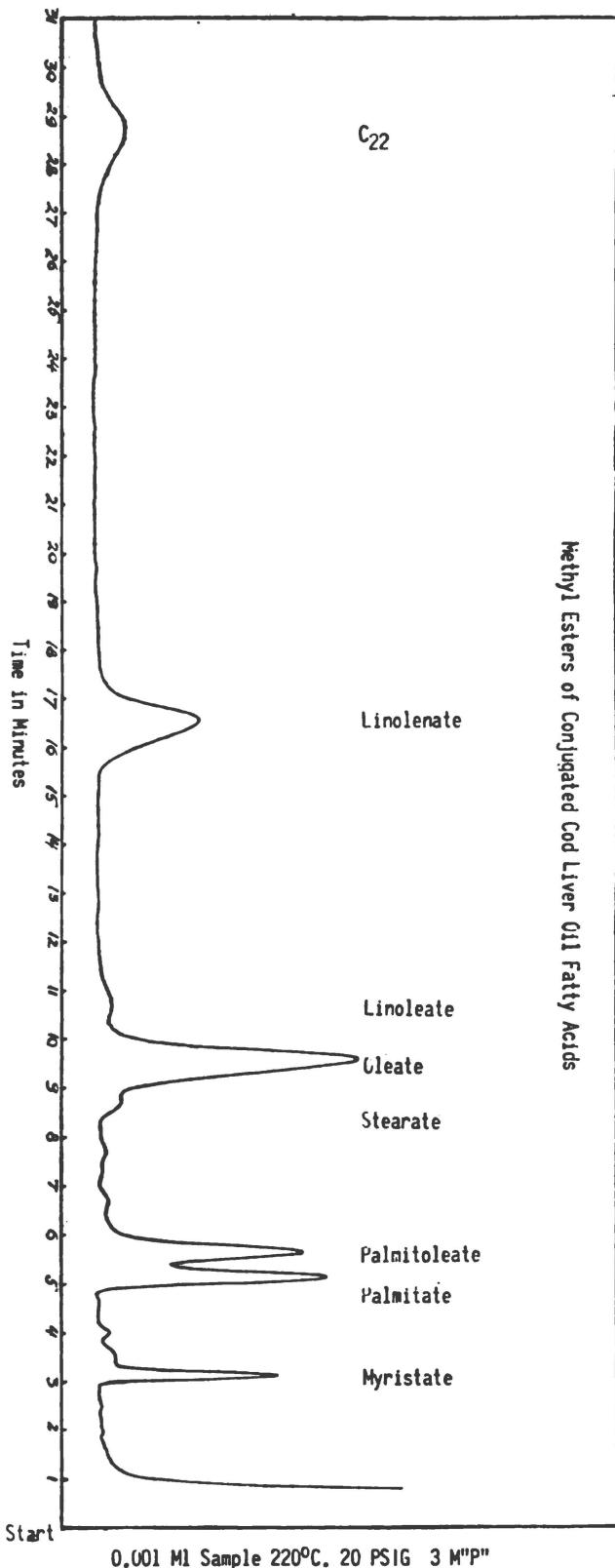


FIG. 251. Gas chromatographic analysis of a sample of the fatty acids of cod liver oil after chemical conjugation. No differences are seen between the curve of Fig. 250 and this curve, indicating that in the conditions under which the analysis was made, the conjugated members are not detected.

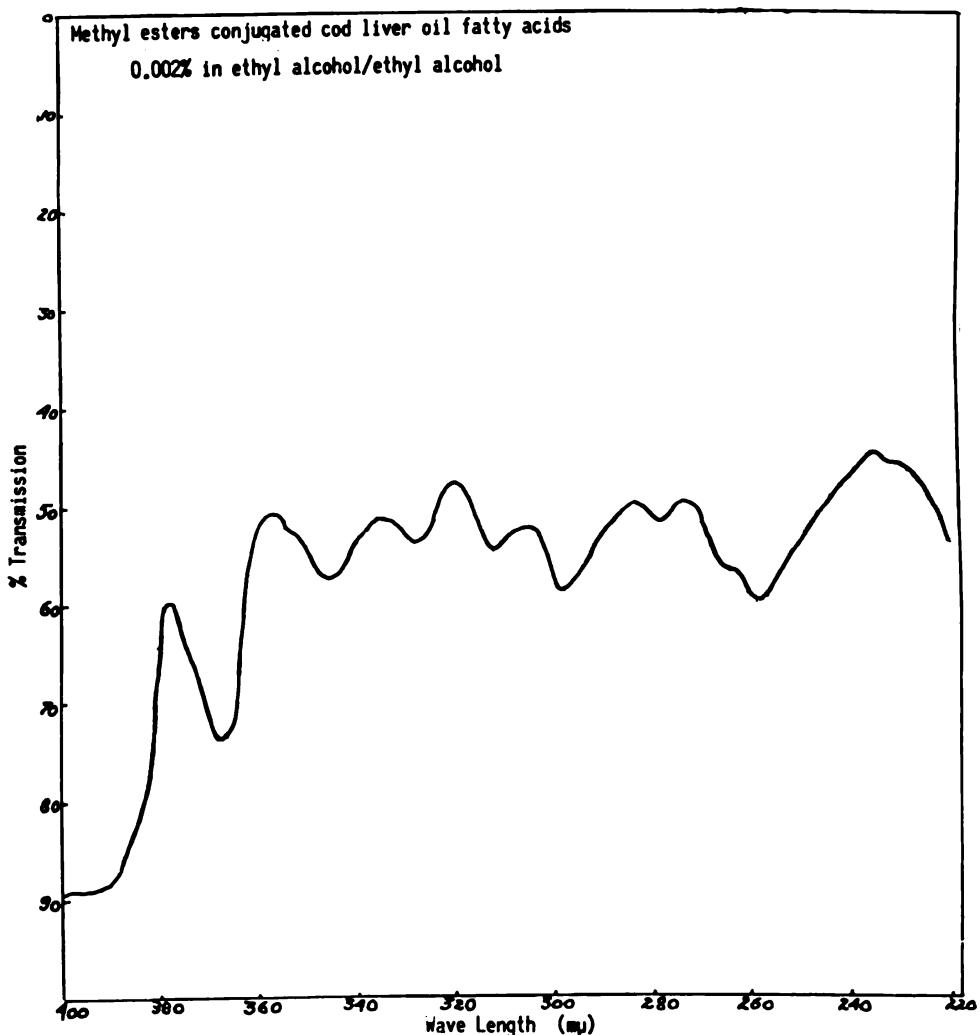


FIG. 252. Spectral analysis of the sample used for gas chromatography, shown in FIG. 251, indicating the presence of di-, tri-, tetra-, penta- and hexaenic conjugated members.

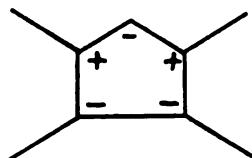


FIG. 254. A twin formation appears in cyclopentane as a result of the alternate sign of adjacent carbons.

Chapter 6, Note 10. Arachidonic Acid—Sterol Relationship

The relationship between arachidonic acid and sterols, both of which are present in the adrenals, has been followed through the changes which occur in the amount of these substances in the adrenals during certain pathological processes. Rabbits were slowly injected intravenously with a suspension of microbes until symptoms of deep acute shock appeared. The animals were sacrificed immediately by bleeding them and the adrenals and blood analyzed. The amount of polyunsaturated fatty acids of the adrenals had markedly decreased, and, in some animals, had almost disappeared from these organs. At the same time, the amount of the same fatty acids of the circulating blood had manifestly increased. The amount of insaponifiable fractions and sterols in the adrenals was unchanged as compared with controls. It seems that fatty acids have passed from the adrenals into the general circulation as a first response to the noxious intervention.

Chapter 6, Note 11. Steroids Deriving from Arachidonic Acid

The study of the relationship between steroids and certain fatty acids led us to the hypothesis, according to which some steroids are derived from fatty acids themselves. Although the synthesis of cholesterol from scalene (230) is highly plausible, it would not represent the only origin for all the steroids. The members with a two carbon chain would derive from other substances. We present this hypothesis here because it also represents an example of another important role played by the double bonds which appear to intervene in the process of cyclization in the organism.

According to the hypothesis which we have advanced, polyunsaturated fatty acids lead to allopregnane, the parent steroid with a two carbon lateral chain. Figure 255 shows the different phases of such a transformation starting from arachidonic acid. The presence of a carboxyl and four double bonds in the arachidonic acid molecule results in some of its carbons having a particularly strong energetic value. The strongly charged carbons are: 1) C₁, through its bond to = O and — OH; 2) C₂ and C₃, through an induction process since they are close to the carboxyl; and 3) C₅ and C₆, C₈ and C₉, C₁₂ and C₁₃, and C₁₆ and C₁₇, respectively bound by double bonds.

Due to alternate induction, all the odd-numbered carbons have a positive sign and the even-numbered have a negative one, as indicated in Figure 255a. Because of the high flexibility of the aliphatic chain, and the presence of carbons with strong positive and negative character in the same molecule, attractions between the strongly charged carbons with opposite electrical signs in the same molecule would occur. This would lead, as a first step, to a bending of the chain so that the strongly charged carbons with opposite signs would face one another. (Fig. 255b)

In a second step, as these carbons are bound by double bonds, the respective π electrons of the double bonds would serve to form a new

bond between the facing carbons and thus to close cycles. This would occur without any loss or gain of electrons. The three double bonds between C₅ and C₆, C₁₁ and C₁₂, and C₁₄ and C₁₅ would serve to close the three cycles. (Fig. 255c) The double bond between C₈ and C₉ would serve to make C₉ highly reactive. It can be seen that C₉ of arachidonic acid corresponds to C₃ of cyclopentanophenanthrene, which explains why this carbon has a high positive character, with an oxygen fixed on it. The carbon of the carboxyl could be used either to form the methyl group at C₁₈ or, more plausibly, could be lost in a process of decarboxylation, which by itself, in this case, would induce the bond between C₂ and C₁₈ of the arachidonic molecule. Through the intervention of the strong energetic center, the pentanic cycle would be closed and would have two carbons with the same charge. (Fig. 255d) The two methyls, corresponding to C₁₈ and C₁₉ of steroid molecules, would result from a further process of methylation after the polycyclic molecule was formed. C₂ and C₆ of arachidonic acid would be especially likely to have a methyl group fixed on them through their electronic displacement due to the new bond.

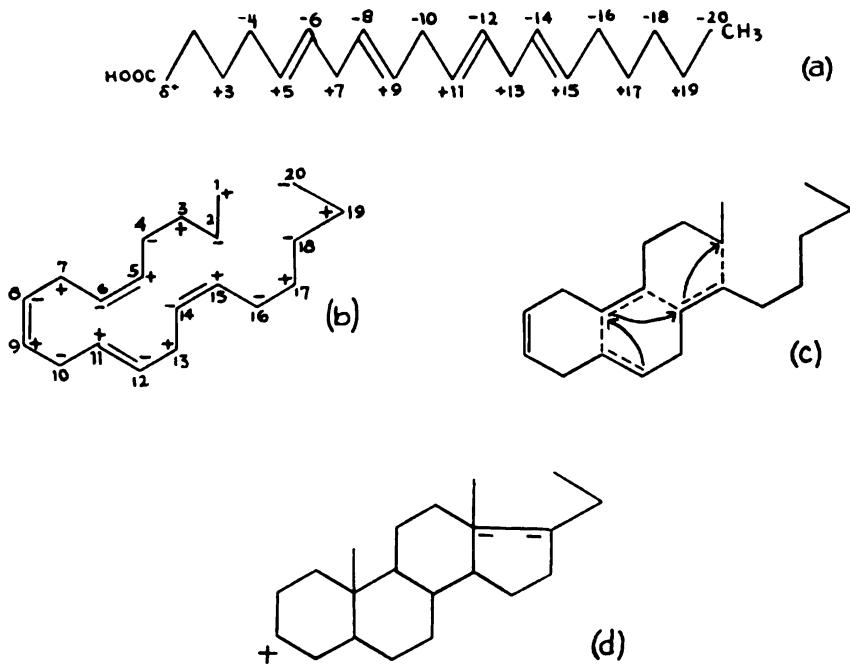


FIG. 255. Hypothesis of the synthesis of the allopregnane radical from arachidonic acid. Fig. (a) shows the relative position of the double bonds in the arachidonic acid molecule. Fig. (b) shows how the molecule bends, due to the attraction between the energetically oppositely charged C₂ and C₁₈, C₅, and C₁₄, and C₈ and C₁₁. In Fig. (c) the cycles are closed with the electrons furnished by the double bonds. (d) The closing of the cyclopentane occurs with the appearance of a twin formation. The electrons of the double bond between C₈ and C₉ become available at C₉ (C₉ of allopregnane) to realize the bond with an oxygen at this carbon.

This hypothesis explains the biological relationship between arachidonic acid and corticoids. The adrenals are especially rich in both. The synthesis, which occurs with a minimum change in the richness in electrons or atoms, explains two of the most important characteristics of these corticoids, the high energetic value of C₈ and of the cyclopentane with its twin formation. Preliminary experiments seem to show that, in a preparation of adrenal tissue, arachidonic acid can be transformed to corticoids under the influence of the adrenocorticotrophic hormone of the hypophysis (ACTH).

Chapter 6, Note 12. Steric Coupling

Through reciprocal influence, the energetic centers present in the non-polar parts of two molecules which combine, largely lose their activity. Steric coupling is possible if the two opposite molecules, once bound through the combination of their polar groups, also adhere together through their nonpolar groups via the opposing energetic centers or formations present in the two molecules. In this way, steric coupling completes the partial reciprocal neutralization of the molecules obtained through the combination of polar groups. The bond between the polar groups that keeps the two molecules in a reciprocal position is thus an important condition for steric coupling.

Chapter 6, Note 13. Luteoid Function

It appears that the luteoid pattern can be correlated to a specific aspect of the energetic picture of this substance—the presence of two relatively strong nucleophilic centers in the characteristic opposite positions, one at C₈ and the other at C₂₀, as in progesterone. In fact, any change in the energetic picture of this steroid will decrease luteoid properties. The relationship of the luteoid property to the nucleophilicity at C₈ is easily seen. The lack of the nucleophilic center at the carbon 3, as in pregnane one 20, (*Fig. 256a*) leads to an inactive substance, the energetic picture being entirely changed. A single nucleophilic center thus appears to be insufficient for the luteoid property.

The substance becomes inactive if, instead of a nucleophilic center through = O at the carbon 3, an electrophilic center is present, as in the pregnane ol 3 one 20, so that the energetic picture no longer corresponds to the luteoid pattern. (*Fig. 256b*)

Any change in the nucleophilicity of the C = O center at C₈ will decrease the luteoid properties of the substance. The presence of a second double bond between carbons 6 and 7, as in the 4, 6 pregnadiene-dione 3-20, (*Fig. 256c*) in spite of the fact that it will increase the value of the nucleophilicity of the carbonyl at the carbon 3, will change the energetic pattern and thus also decrease the luteoid character. On the other hand, the reduction of the nucleophilicity of the center produces, through a similar influence upon the energetic pattern, a decrease in the luteoid property. In the case of pregnane-trione 3-6-20, (*Fig. 256d*) the lack of a double bond

in the cycle and the reciprocal induction realized by the parallel double bonds of the two carbonyl, will reduce the nucleophilicity of the C₃ center, and with it, the luteoid properties. This also is true for Δ₅ pregnene-dione-3-20-ol 6, (Fig. 256e) where the presence of the hydroxyl at the C₆, as well as a nonparallel double bond between C₅ and C₆, decreases the ionic character of the C = O at the carbon 3. In Δ₅ pregnene-dione 3-20, (Fig. 256f) where only the double bond between carbons 5 and 6 is changed, being opposite to that of the carbonyl at the carbon 3, the ionic value of C = O at C₃ is decreased instead of increased. The luteoid property seems to have almost disappeared.

The second condition for the luteoid property is the nucleophilic center at C₂₀. Any change in its character or value will influence the luteoid property of the substance. Androstandione, (Fig. 256g) with a nucleophilic center at carbon 3 similar to that of progesterone, but with an = O directly at-

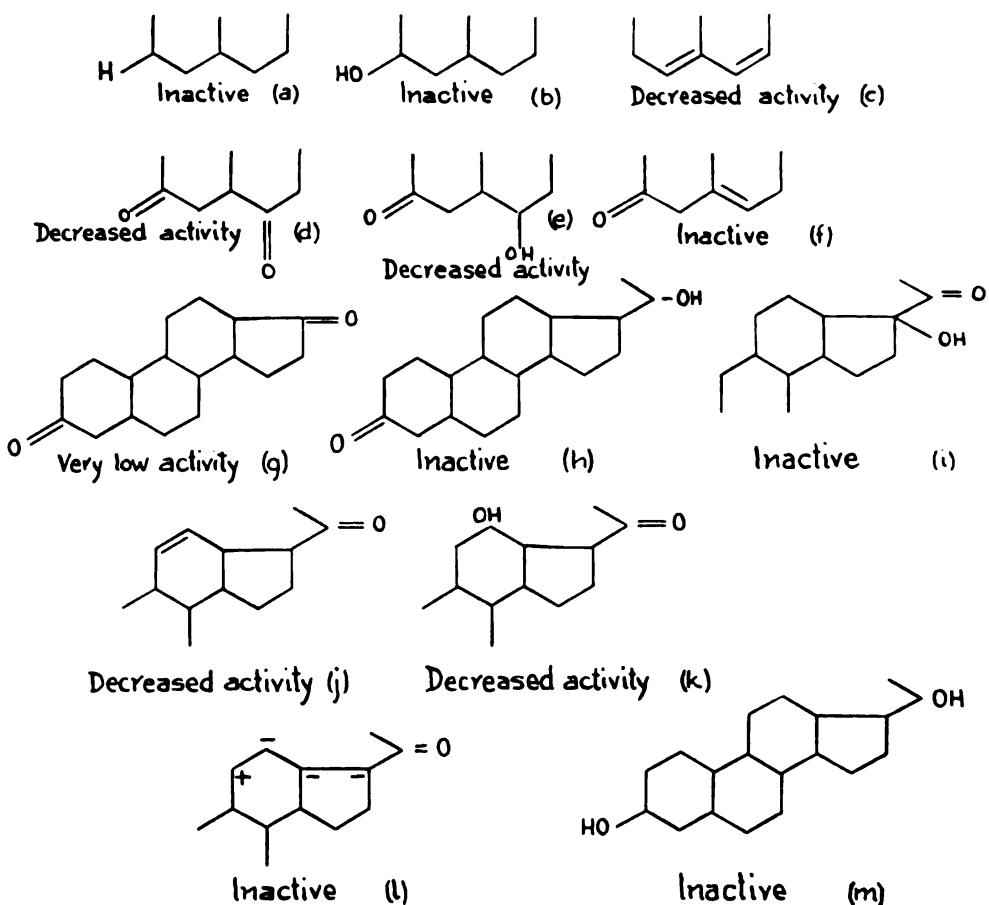


FIG. 256. Any change in the energetic characteristics of the centers which appear related to the specific luteoid activity leads to a decrease or disappearance of this activity.

tached to carbon 17 and without any side chain, presents an energetic picture with two nucleophilic centers. But the nucleophilic center = O attached directly at carbon 17, gives a different character to this part of the molecule. The substance thus has only some luteoid properties. Δ 4 pregnene-one 3-ol-20, (*Fig. 256h*) in which the carbonyl at carbon 20 is changed by a hydroxyl, is, on the other hand, inactive, the substance having a picture different from that of the dinucleophilic pattern. Also inactive is Δ 5 pregnone—dione-3-20-ol 17, (*Fig. 256i*) in which the presence of the hydroxyl at carbon 17 changes the energetic picture at this extremity of the steroid.

The presence of a double bond between carbons 11 and 12 (*Fig. 256j*) will decrease luteoid activity through an induction and field influence between the nonparallel double bonds and a decrease of the ionic character of C_{20} bound to oxygen. The influence exerted by an hydroxyl in the neighborhood of carbon 17 is interesting. Through an inductive effect, the OH attached at carbon 12 (*Fig. 256k*) changes the ionic value of the $C = O$ attached at carbon 20 and thus decreases luteoid activity. An hydroxyl bond to carbon 11 has a much more intensive effect, changing the ionic character of the bonds between it and the = O and thus inactivating the luteoid property. (*Fig. 256l*)

For most of the steroids with = O centers, metabolism leads to a change of this center in an hydroxyl. The product of the change of both $C = O$ into $C = OH$, which represents the form in which this hormone is excreted, is inactive. Pregnanediol 3-20, (*Fig. 256m*) with a structure far removed from the pattern required for luteoid activity, has no luteoid properties.

Chapter 6, Note 14. Energetic Center in Steroids

Energetically, as a consequence of the presence of a nucleophilic center in this situation, there is a strong positive charge for the carbon 20 of the chain itself. Consequently, a strong negative charge appears for carbon 21. The ionic value of the bond linking OH to C_{21} is thus increased. With the reactivity of this radical increased, the electrophilic character of this OH center becomes still stronger. The respective activity of two opposed energetic centers near to each other is increased inductively.

Chapter 6, Note 15. Relationship Between Corticoids

Corticoids can be viewed in terms of changes taking place in the general metabolism in the organism. In the evolution of these steroids, a process of oxidation would intervene at the principal energetic centers. To the hydrocarbon, first an hydroxyl and then an oxygen would be attached. An inverse process of reduction would take place as a metabolic process, the OH being the usual form through which steroids are eliminated, often bound to glucuronic acid. This process of oxidation would occur at C_{11} .

Attaching an OH to desoxycorticosterone (Δ 4 pregnene-21-ol-3:20-

dione), would lead to the appearance of corticosterone (Δ 4-pregnene-11:21-diol-3:20-dione). A further oxidation would change this OH into an O, resulting in dehydrocorticosterone (Δ pregnene-21-ol-3:11:20-trione). All these are mineralocorticoid compounds.

With a further change, this time at C₁₇, where an hydroxyl would be attached, all three compounds—desoxycorticosterone, corticosterone and dehydrocorticosterone—display neoglucogenic properties. They represent 17 hydroxy derivatives as 17 hydroxy-desoxycorticosterone (Δ 4-pregnene-17-9 (beta):21-diol-3:20-dione); 17 hydroxycorticosterone (Δ 4-pregnene-11 (beta):17 (beta):21-triol-3:20-dione), or compound F; and 17 hydroxy-11 dehydrocorticosterone (Δ 4-pregnene-17:21-diol-3:11:20-trione), or compound E or cortisone.

The fact that the presence of an hydroxyl at C₁₇ greatly changes the properties of the entire group makes it likely that the energetic formation of which C₁₇ is a part intervenes in the specific activity of these substances.

The analysis of the constitution of the corticoids has further shown that they have the characters of lipoids—being polar-nonpolar substances—with the nonpolar group predominant. The members studied, mineralo- as well as neoglucogenic corticoids, have been shown to induce a change toward lower values in the second day wound crust pH, indicating thus a tendency to induce an offbalance of the type A.

Chapter 6, Note 16. The Template Hypothesis

Figure 257 shows the template formation in cortisone, which extends from C₁₁ to C₂₁. Each one of these six carbons will attract a carbon from the radical in front of it. The energetic character of each of the six carbons of the template will determine the electrophilic or nucleophilic character of the carbon so attracted. This attraction is easily induced when acetic radicals, with an electrophilic and a nucleophilic carbon, form these groups. Furthermore, the value of the carbons of the template also will determine which polar radical will be bound to the respective carbon kept in front of it. In general, the carbon kept in front of a carbon of the template will have an opposite electrical sign. The polar group bound to the carbon kept in place will be opposite in sign to the polar group bound to the carbon of the template. When the first polar group takes a position parallel to that of the polar group of the template, both will have the same electrical sign.

It can be seen that C₂₁ within the template has an OH, the group being electrophilic. This will cause the carbon kept in front to preferably bind an oxygen, realizing a nucleophilic center. C₂₀ of the template, which corresponds to a carbonyl, represents a nucleophilic center. With an oxygen bound through a double bond, it has strong reactivity. The positivity of C₂₀ also is highly enhanced through its bond to the two strongly negative carbons, C₂₀ and C₁₇, each being bound respectively to a hydroxyl. With this high positivity, C₂₀ will induce a strong reactivity in the carbon kept in front of it. This will be strong enough to bind a radical energetically opposite to oxygen and stronger than the hydroxyl; that is, an amino group.

The special position of the OH bound to C₁₇ as related to the template will induce the carbon kept in front of it to bind another hydroxyl. The same applies to carbon 13. This is the result of the relatively strong molecular reactivity of these two carbons, due to the twin formation which they

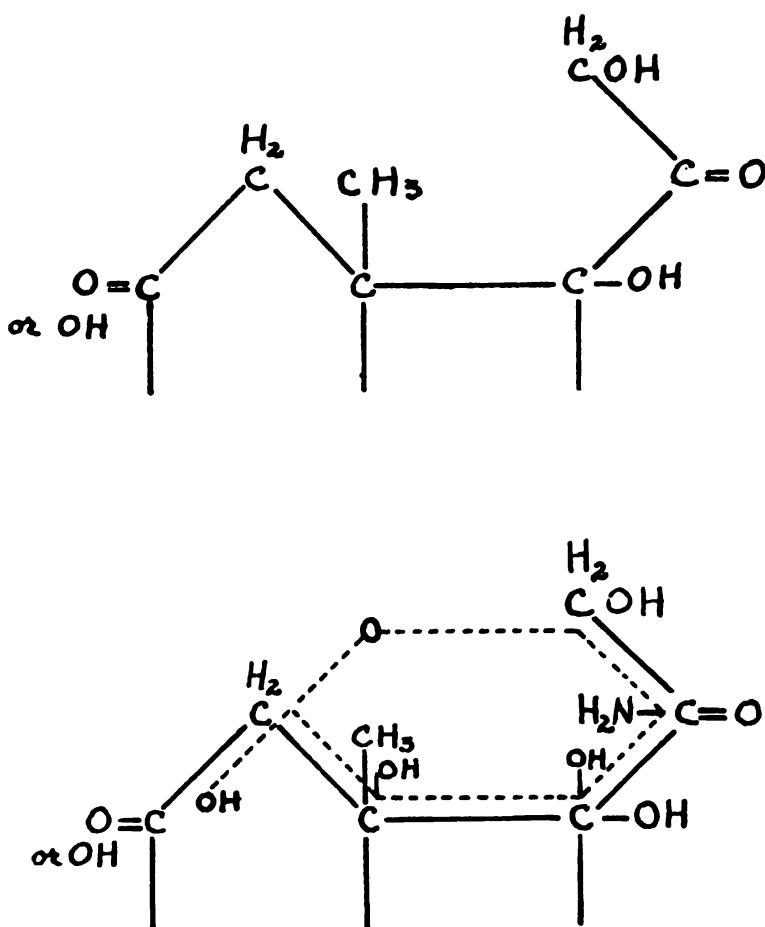


FIG. 257. Hypothetical view of the template formation between carbon 11 and carbon 21 of the corticoid molecule. The groups kept in front of the different carbons of the formation serve to synthesize new substances. In the example presented above, the template of the cortisone molecule would lead to the appearance of a glucosamine molecule.

realize. The methyl bound to C₁₃ will determine the steric position of the hydroxyl bound to the respective carbon kept in front of this carbon.

The effect of carbon 12 is different. It is highly influenced by the twin formation and has an opposite energetic character to carbons 13 and 17, consequently, it will favor the binding of the carbon kept in front of it to an oxygen which is the same which binds the carbon in front of C₂₁. Positionally, the carbon in front of C₁₂ also will be nearest to the C kept in

front of C₂₁, which also was induced to bind an oxygen as seen above. This will make it possible for a similar oxygen to be bound by the two carbons kept in front of C₁₂ and C₂₁, thus closing an hexagonal cycle. The radical bound to carbon 11 will cause the carbon in front of it to bind an opposite radical. In the cases in which C₁₁ has an oxygen bound to it, as in cortisone, the carbon kept in front of it will bind a hydroxyl. The presence of an hydroxyl bound to C₁₁, as in hydrocortisone, will cause a carboxyl to be bound to the respective carbon kept in front of it. It is thus seen that the synthesis induced by the template formation of cortisone will result in a molecule of glucosamine, while for hydrocortisone, the synthesized molecule will correspond to glucosaminic acid.

Chapter 6, Note 17. Adrenal Defense Index

E. F. Taskier in collaboration with the author, has studied the intervention of adrenals in the defense against various fatty acids. (231)

It is known that the adrenals have an important role in the defense of the organism against noxious agents, a normal animal being more resistant to toxic effects than an adrenalectomized one. Systematic study of adrenal intervention has shown that it occurs with a certain degree of specificity.

The method of investigation used was the following: inbred Wistar rats of the same sex and approximate body weight of 150 gr. were adrenalectomized. The surgical procedure was carefully standardized, lasting less than two minutes, and producing a minimum of trauma. This was made possible by the Noyes Fixation Forceps which is utilized in ocular surgery and is well suited to pick out the adrenal gland without damage to it or to neighboring tissue. As controls, animals of the same weight and sex were sham-operated. On the third postoperative day, the agent to be tested was injected intraperitoneally. At this time, the organism had recovered from the immediate traumatic effects of the operation and no manifest adrenal deficiency symptoms were as yet present. Only those deaths occurring up to 48 hours postchallenge were considered to be due to the direct toxic effects of the substance. Deaths occurring more than 5 days after adrenalectomy could be attributed to effects of the adrenal deficiency itself, and for this reason, were not included in the experiment.

The minimal Lethal Dose for each agent was determined by using progressively increasing doses. This was done separately for adrenalectomized and for control animals. It was the difference in the toxicity in sham-operated and in adrenalectomized animals that was considered rather than the toxicity of the substance itself.

The ratio of the Minimal Lethal Dose for the two groups of rats, sham-operated and adrenalectomized, was calculated. It furnished a numerical representation of the degree of adrenal intervention and was called the "Adrenal Defense Index" or A.D.I. We utilized this index,

MLD Sham-operated / MLD Adrenalectomized, as a measure of the relative adrenal participation in the body defense against different substances. A low index points to a

general nonspecific response; a higher index indicates a more significant intervention.

We obtained the adrenal defense index for various groups of fatty acids. They included homologous series of saturated, unsaturated, alpha-OH and conjugated fatty acids. Over 900 rats were used in this study.

For the saturated fatty acids we found:

FATTY ACID	A.D.I.
Caproic Acid	2.5
Caprylic Acid	5
Capric Acid	5
Lauric Acid	1.5
Myristic Acid	2
Palmitic Acid	12
Stearic Acid	6

We interpret this to mean that the defense capacity of the organism against these fatty acids was only slightly more effective in the sham-operated than in the adrenalectomized animals. The adrenals did not seem to be especially active in the defense against these substances.

For the unsaturated fatty acids we found:

FATTY ACID	A.D.I.
Oleic	6
Linoleic	9
Linolenic	5

These values indicate more active adrenal intervention against these fatty acids.

For the saturated alpha-OH fatty acids the results were:

FATTY ACID	A.D.I.
alpha-OH Caproic Acid	4.5
alpha-OH Caprylic Acid	4
alpha-OH Capric Acid	3
alpha-OH Lauric Acid	20
alpha-OH Myristic Acid	9
alpha-OH Palmitic Acid	3
alpha-OH Stearic Acid	50

We choose this series of substances because Camien and Dunn, and others, have shown the importance of these fatty acids for bacterial growth, as well as the presence of alpha-OH lauric and alpha-OH myristic acids as part of the lipido-polysaccharide fraction of bacteria.

Through related research, we were particularly interested in the A.D.I. value of fatty acid molecules with conjugated double bonds. As conjugated diene, we administered conjugated linoleic acid. The A.D.I. of this substance was similar in value to that of its nonconjugated isomer. The index was 5. For the conjugated triene, we used eleostearic acid obtained from tung oil. The results were striking. We found an A.D.I. value of 120. The

A.D.I. of this acid thus showed a 24-fold increase over the index of its nonconjugated isomer.

The data indicate a degree of specificity for the adrenal defense mechanism. A.D.I. values of 3 or less could be interpreted to correspond to a nonspecific adrenal intervention toward fatty acids in general, while higher values indicate a larger, probably specific adrenal activity. In the case of alpha-OH lauric and alpha-OH stearic acids, the high A.D.I. values could be related to the fact that these fatty acids have been found to be part of the constitution of bacteria.

The intensive defense evidenced by the high A.D.I. value for eleostearic acid is related to the appearance of conjugated trienes during the course of certain pathological conditions, especially trauma. The continuous increase, especially of conjugated trienes, in the adrenalectomized animals suggests that these fatty acids appear in the organism but are destroyed under normal conditions. They accumulate, however, in adrenalectomized animals and may contribute to death when they reach a certain critical concentration. It is possible that with additional administration of these conjugated trienes their critical concentration would be reached and the animals would die.

In the light of these data, we investigated the role of different adrenal factors in these responses. Cortisone, desoxycorticosterone acetate (DOCA), and sodium chloride were tested for their protective action against the toxic effects of oleic and eleostearic acids. Groups of rats were treated immediately after adrenalectomy with daily doses of 1 mg. of cortisone, two-tenths of a cc. of DOCA, or 1% NaCl drinking water ad libitum. Control adrenalectomized rats were given no sustaining therapy. Three days after adrenalectomy, a challenging intraperitoneal dose of 1 cc. of 10% oleic acid per 150 gr. of body weight was administered.

Whereas the mortality of the control rats was 90%, it was 25% in the cortisone treated animals. DOCA administration decreased the mortality only to 65% and NaCl had little effect, decreasing it only to 85%.

The protective effect against oleic acid in adrenalectomy is seen in the following table:

AGENT	% MORTALITY
Control	90
Cortisone	25
DOCA	65
NaCl	85

This suggests that the neoglucogenic hormone plays a significant protective role in the defense of the organism against the noxious effects of fatty acid. The mineralocorticoid, on the other hand, seems to play a lesser role in this mechanism, a fact which is confirmed by the ineffectiveness of sodium administration.

A similar preliminary experiment carried out for eleostearic acid shows the same protective effectiveness of cortisone, lesser effectiveness of DOCA and almost no effect at all of sodium chloride.

Chapter 6, Note 19. Bonds of Glucuronic Acid

The study of the detoxifying-excretion of different agents led to the following conclusions. Primary aliphatic alcohols—except methyl alcohol—are eliminated coupled by glucuronic acid; the same for the secondary aliphatic alcohols; tertiary aliphatic alcohols and glycols from propylene glycol up. Of the aliphatic aldehydes only a few are coupled and only after transformation in vivo. While most of the aliphatic ketones are coupled, phenol and more emphatically the cresols and salicylic acid are in part excreted as sulfo-coupled, and only in part as glucurono-coupled. Resorcinol, catechol, orcinol, phenolphthaleine, phloridzin are mostly eliminated as glucurono-coupled, while adrenaline almost solely as sulfate. Most of the aromatic hydrocarbons are also bound to glucuronic acid, but only after having been changed in vivo.

For the aromatic acids, the bond to glucuronic acid is conditioned by the presence of second polar groups, usually one or more hydroxyls. The aromatic nitrogen compounds are first changed into amino groups before they are coupled with the glucuronic acid. Only relatively small amounts of sulfonamides were excreted bound to glucuronic acid.

Many of the heterocyclic compounds are bound to glucuronic acid with the condition to have amino or hydroxyl groups; the same for the sex hormones, the estrogens being the group especially excreted as such.

The general characteristic of the substances excreted as coupled with glucuronic acid is the presence in their molecule of one or more positive polar groups hydroxyl or amino. As with few exceptions all these substances have also lipoidic properties, the excretion coupled to glucuronic acid appears as a means to eliminate positive lipoids.

Chapter 6, Note 20. Glucuronic Acid—Mechanism of Coupling

The analysis of urine specimens containing peroxides, also has revealed significant amounts of glucuronic acid compounds. We utilized a slightly changed Tollens technique for the dosification of glucuronic acid in the urine, based on the reaction of this acid with naphthoresorcine in an acid medium. To 5 cc. of urine, 0.5 cc. of a 1% solution in alcohol of naphthoresorcine (1.3 dioxyphthalene) and 5 cc. of concentrated hydrochloric acid were added. The mixture was boiled for one minute, allowed to stand for another five minutes, then cooled, preferably in an ice water bath. When cold, a mixture of 90% ether and 10% alcohol was added, agitated, and the blue-violet color of the ether-alcohol measured, using a spectrophotometer. The values obtained in different subjects have shown a definite increase in the amount of glucuronic acid in the urines containing peroxides, indicating a probable relation between them. Based on this correlation, we investigated one of the roles of glucuronic acid in the organism, that of detoxifying agent.

Glucuronic acid could be considered to result, at least in part, from oxidation of glucose. Usually oxygen intervenes in glucose metabolism

only after the desmolyse processes * (232), corresponding to the fermentative phase has progressed to the appearance of pyruvic acid. Glucuronic acid could be considered to appear from a more direct fixation of oxygen to the glucose molecule. This fixation has to take place upon C₆ in order to lead to the appearance of glucuronic acid. Theoretically, the oxygen fixation might be expected to occur at C₁ in view of the aldehyde group present at that carbon. This takes place in vitro. It would lead to the appearance of gluconic acid. However, if a phosphoric or other radical is bound to C₁ in vivo, fixation of the oxygen at this carbon is prevented. Oxygen attaches itself then at C₆ which is the next most reactive carbon in the molecule. This reactivity at C₆ is seen when oxidation in vitro is continued beyond gluconic acid, leading to saccharic acid, a dicarboxylic acid with one carboxyl at C₁ and another at C₆.

Glucuronic acid intervenes in the physiological defense processes by combining with certain noxious products and helping to eliminate them in non-toxic forms. The resulting compound between glucuronic acid radicals and various substances—and for sulfuric acid radicals as well—has been called "conjugation," the substances being sulfo- or glucurono conjugated compounds. Because of the special attention given in this publication to the conjugation of the double bonds, we will use the term "coupled" for this bond to sulfuric or glucuronic acid.

A certain parallelism exists and has always been emphasized between the detoxifying and eliminating function exerted by the sulfuric and glucuronic radicals. Not only the two derivatives appear in the urine, but it is often noted that glucuronic acid intervenes when large amounts of certain substances, such as menthol or phenol, are present and the sulfuric acid radicals are not in sufficient quantity to insure detoxification and elimination. By administering mineral sulfates to the subject, the proportion of sulfo-derivatives is seen to increase. This parallelism appears especially interesting when we recognize that sulfuric acid represents the final stage of the oxidation of sulfur introduced into the organism in combinations in which it enters as a bivalent negative element. Both sulfuric and glucuronic acid result from oxidative processes, one involving the thiol group and the other, glucose.

While glucuronic acid often substitutes for the sulfuric radical in the excreted substances, qualitative differences exist. For example, phenol as well as indoxyl is bound to the sulfurous radical, while the higher alcohols and especially the cyclic oxyacids do not combine with this acid. For many substances the parallelism that exists in the bond to sulfuric and glucuronic radicals extends only up to a certain point, after which the amount of the sulfuric ester no longer increases. This fact has raised the problem of the action of these two radicals and the differences between them, and can be

* The term "desmolyse," widely used by some authors abroad, corresponds to the changes occurring in metabolites under the action of hydrolases, which, when completed, result in a splitting apart of the molecules and the liberation of the energy they possess.

understood only by considering the substances that are bound by these acids and eliminated as sulfo- or glucurono-coupled derivatives.

We have seen in Note 19 that, with very few exceptions, such as benzoic acid, almost all the substances excreted or bound to glucuronic acid have an OH as polar group. However, many have more than one polar group.

The bonding with the sulfuric radical, as it occurs in the organism, follows a relatively simple pattern. (Fig. 258a) In a first step, the bonding of one acid function of the sulfuric radical to the substance produces an acid sulfuric ester. This is further changed by combination with a metal, usually potassium, which produces a highly hydrosoluble salt and represents an excremental derivative.

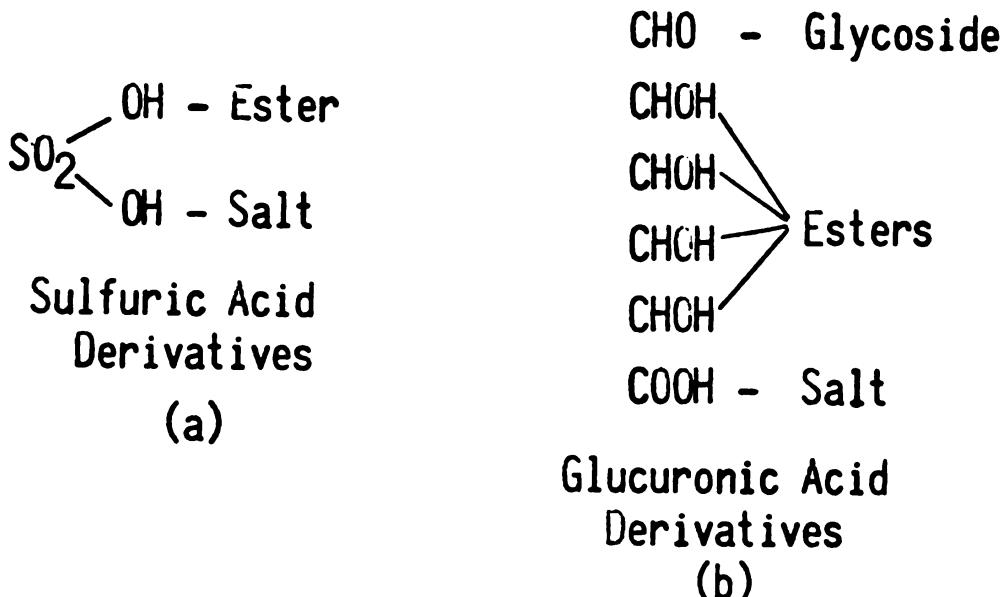


FIG. 258a. The bond of sulfuric acid in the organism can result in an ester and a salt.
FIG. 258b. The bond of glucuronic acid can result in a glucoside, an ester or a salt combination.

The binding is more complicated for glucuronic acid. Glucuronic acid does not realize the bonds, since it is eliminated as such if administered. The bond occurs at the aldehyde group of glucose, forming a glucoside, which is passed further in glucuronic acid. As is seen in Figure 258b, glucose can realize different bonds. It can bind a radical either to the aldehyde group at C_1 to form a glucoside, or to its alcoholic hydroxyls to form an ester. The glucuronic acid can bind a metal to its carboxyl to form a salt. The possibility of realizing a glucosidic coupling at C_1 or an esteric at C_2 has been revealed by Quick. (233) The ability to realize concomitantly a multiple coupling, for which glucuronic acid seems to be highly suitable through its multiple and various functions, appears more interesting. The study of elimination of different oxybenzoic acids shows that it is bound to gly-

cocoll, (*Fig 259a*) while the para isomer is eliminated coupled by the glucuronic radical. (*Fig. 259b*)

The difference between sulfuric and glucuronic acid thus appears to be related to double coupling especially if the second function is acid. The presence of two opposite polar groups will thus prevent the bond to sulfuric acid and favor the bond with the glucuronic radical. Organic oxyacids are thus not bound by sulfuric radical as long as their carboxyls are free. The amides, such as salicyl amides—and, the esters such as methyl salicylates—are coupled by the sulfuric radical, while the acids are not. It is through these multiple coupling possibilities—and, in addition the possibility of forming a salt especially with potassium—that glucuronic acid corresponds to a broader activity as a detoxifying and eliminating agent than sulfuric acid.

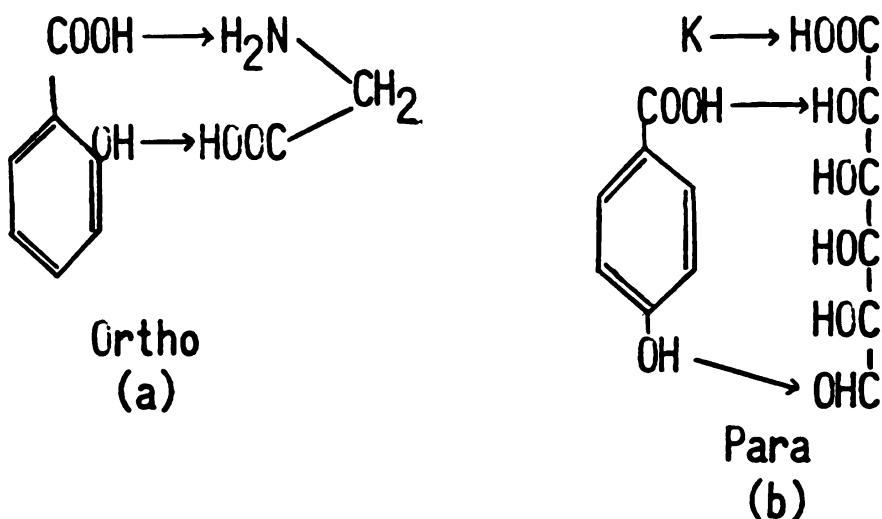


FIG. 259. The ortho-oxybenzoic acid (salicylic acid) is bound in the organism to glycocoll, (a) while the para-isomer is coupled by the glucuronic radical. (b).

The capacity to utilize glucuronic acid for excretion of noxious substances varies widely between species. Benzoic acid has been shown to appear as a glucuronic derivative in animals by Csouka, Brakefield, and Schmidt (234), but never in humans, a fact that is not confirmed by Quick. (235) Rabbits eliminate the tertiary alcohols as glucuronic derivatives more completely than dogs. While thyroidectomy in rabbits decreases the synthesis of camphoroglucuronic acid (236), the administration of thyroid extract increases it.

Chapter 6, Note 21. Paraplegia Induced by Cholesterol

Male and female rats, weighing approximately 250 grams, were injected with 5 cc. of 10% cholesterol only partially dissolved in a mixture of 3 parts oil and 1 part ether. The next day paraplegia with some ulcer-

tion of the hind legs was seen. Ulceration became accentuated in the following days. Curiously enough, this occurred only in the females. In repeated experiments in rats, and also in rabbits, guinea pigs and even mice, the same sex differentiation has been observed. Most of the female animals died from retention of urine as a principal complication of the paraplegia.

To investigate the nature of this sex difference, groups of males and females were castrated and spayed, then tested with the same cholesterol injection at various times from a few days to four months afterward. Castration had no influence in male rats; nor did the spaying of females reduce the incidence of paraplegia. The administration of 5 mgm. of testosterone daily for ten days to female rats, spayed or unspayed, also did not prevent the appearance of paraplegia after the injection of cholesterol in ether-oil. The administration of $\frac{1}{2}$ mgm. of stilbestrol daily for 10 days to males, castrated or not, did not break down their resistance to the cholesterol injection. However, the administration of the insaponifiable fraction of placenta or of the total body of rats, in doses of 2 cc. of a 5% solution in oil daily for ten days, made the males respond with paraplegia to the injection of cholesterol in ether-oil solution. The administration to females, spayed or unspayed, of a 10% solution in oil of the acid-lipid fraction of the same origin in doses of 2 cc. daily for ten days prevented this effect.

Chapter 6, Note 22. Adipose Cells and Sulfur Mustard

In collaboration with the late Prof. R. Leroux, Professor of Pathology of the Faculty of Medicine in Paris, we studied the influence exerted by sulfur mustard applied on the skin. The following technique was used: One drop of the pure substance was deposited on the external side of the ear pavilion of adult white rats, left in place for 10 minutes, and wiped off. A part of their ear pavilion was excised in a V form. The fragment so obtained was immediately processed through the special technique used for the extemporaneous examination of operatory biopsies. A longitudinal section of the pavilion was made in the frozen fresh material, and this surface treated first with formalin and then stained with scarlet red or black Sudan and hematein. The section was kept in water covered with a glass cover and immediately examined under incident light, using the Zeiss "Ultropak" dispositive. A water immersion objective was used for higher amplification. From the same material, fixed in formaldehyde at 10%, frozen and paraffin imbedded sections were also obtained.

In nontreated controls, except for the small fatty droplets in the cartilage cells themselves, the only fatty cells found were at the base of the pavilion. In the mustard treated animals, two or three layers of adipose cells were seen to appear beneath the treated skin, near the cartilage, around 20 minutes after the application of the sulfur mustard.

In our group of experiments, curiously enough, this phenomenon was seen to occur only in the treated female rats and not in the males. This difference in the response between males and females was not influenced either by castration or by treatment of the animals with sex hormones. It

was induced in males however, by the administration of unsaponifiable fractions obtained from the total body of rats, or from human or cow placenta, and administered in doses of 1 cc. of a 10% solution in oil for at least one week prior to the application of sulfur mustard. In females, the appearance of adipose cells after the sulfur mustard applications was seen to be prevented, if the animals were treated daily for 10 days prior to this application with 2 cc. of a 10% solution in oil of lipidic acid fraction of human or cow placenta or of cod liver oil fatty acids.

Chapter 6, Note 23. Fatty Acids and Old Tetrahymena

Cultures of different ages of tetrahymena pyriformis in proteose peptone medium, were analyzed for the fatty acids present. The richness in fatty acids was seen to increase with the age of the culture, the old cultures being the richest.

Chapter 6, Note 24. Lipids and Old Age

In the changes related to old age, we have recognized a relationship between the amount of insaponifiable fractions and fatty acids present at different levels of the organization. A certain degree of opposite changes in two successive hierarchic levels can explain certain peculiarities found in the variations of sterols and fatty acids in old age.

In old rats we could establish that changes occur in two opposite directions between the cells and the metazoic compartment. Serum cholesterol amounts increase, as an increase of fatty acids and particularly polyunsaturated members occurs in the cells. The contrast between the two compartments becomes progressively more evident with old age. We tried to apply this data to the study of the relationship between the same compartments in humans. Instead of other cells, we analyzed red blood cells. In rats, rabbits and humans, the cholesterol in serum increases progressively with age but does not do so in the red cells. The amount of fatty acids in red cells is relatively increased with age at the same time as cholesterol content is decreased. The comparison between the cholesterol of serum and cholesterol of the red cells as well as fatty acid content of red cells seems to furnish information related to the progression of abnormalities of old age. This preliminary research, however, needs more confirmation.

Chapter 6, Note 25. Surface Tension of Urine in Old Age (237)

During the course of a study of the biological changes in old age, the renal excretion of surface-active substances was found to be considerably below the levels seen in younger age groups. These findings were considered important since (1) they were observed in individuals with no evidence of significant renal impairment, (2) the same decrease was observed in all subjects examined, and (3) information on this subject is lacking in the absence of a suitable analytic method.

The capillary device has made it possible to determine surface tension within a few seconds, as part of routine urinalysis, without need for complicated apparatus or involved formulas.

A group of 23 inmates of an old age institution were studied. Ages of these 14 men and 9 women ranged from 70 to 87 years. All appeared in good general condition and showed no evidence of significant renal pathology. Morning urine specimens were obtained from each patient for several weeks. These were examined within several hours after voiding.

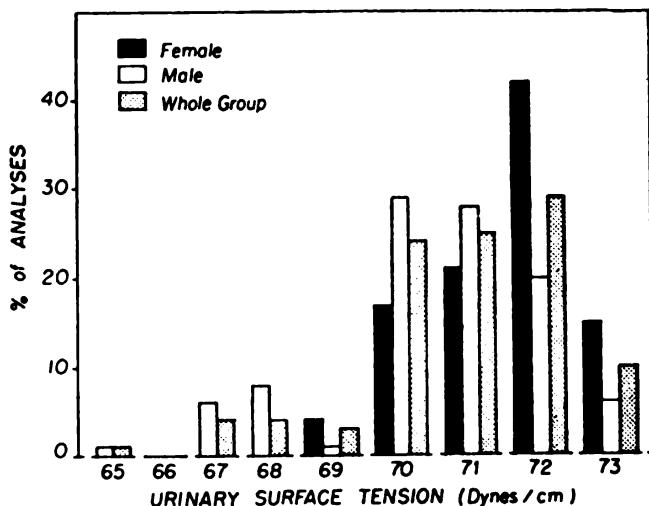


FIG. 260. Distribution of urinary surface tension values in the group of old people studied.

Surface tension values for the entire group averaged 70.9 dynes/cm. The average value for the women was 71.5 and, for the males, 70.4 dynes/cm. When surface tension values were determined in serial samples from individual subjects, variations of only 2 to 6 dynes/cm. were found. None of the samples from the women showed a surface tension below 69 dynes/cm. All but 3 of the specimens from the men had surface tension values of 68 dynes/cm. or higher. (Fig. 260) Specific gravity varied from 1.006 to 1.030, with an average of 1.017 for the group.

In control groups, the average surface tension is 67 to 68 dynes/cm. Diurnal and day-to-day variations of 5 to 11 dynes/cm., with values ranging between 72 to 62 dynes/cm., are found characteristically in healthy subjects.

It is evident that the pattern of excretion of surface-active material in old age is quite different. This is indicated by (1) the high average surface tension value of 71 dynes/cm. for the group, which is at least 3 dynes/cm. higher than for the controls; (2) the limited day-to-day range of variations, which in no case was greater than 6 dynes/cm.; and (3) the striking disparity in the distribution of values in healthy young adults and in old age. (Fig. 261) Eighty-eight percent of the urine specimens from

old individuals had surface tension values of 70 dynes/cm. or higher, and only 1 percent were below 66 dynes/cm. In contrast, 23 percent of the specimens from the control group had a surface tension of 70 dynes/cm. or higher, and 23 percent were below 66 dynes/cm. These findings indicate that the amount of surface-active substances excreted in the urine of old people of 70 to 87 years is greatly reduced as compared with that in healthy young adults. The fact that specific gravity values for the old age

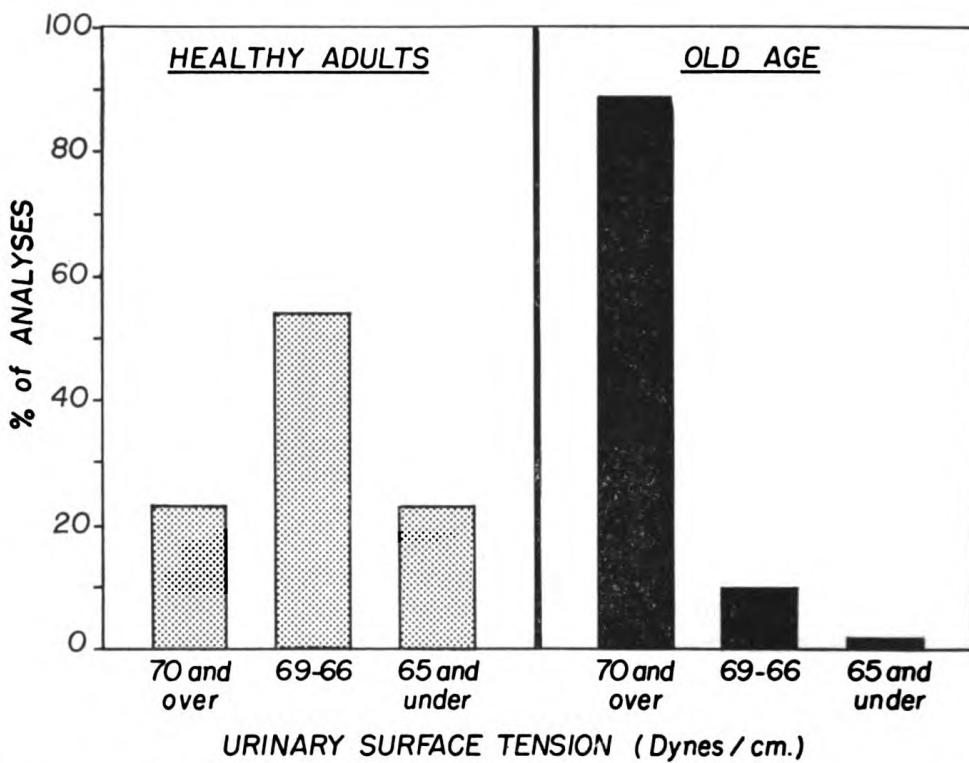


FIG. 261. Distribution of surface tension values in healthy adults and in old age, shows the high proportion of values of 70 dyne/cm. and above for the last group.

group were not significantly different from the controls indicates that this change cannot be explained on the basis of a failure of the kidneys to concentrate by reabsorbing water.

The average surface tension value for the females was 1 dyne/cm. higher than for males. The same sex difference has been noted in studies of healthy young adults and in groups of patients with a variety of diseases. It is of interest to find the difference persisting in these old people.

In the light of current concepts of the possible role of surface-active "colloidal" substances in the urine, it is interesting to speculate on the possible significance of the observed decreased excretion in terms of renal physiopathology. Various authors have contended that the crystalloids of the urine are maintained in solution by the colloids, which prevent ag-

glomeration and precipitation of salts. (238, 239) Some such mechanism is undoubtedly present in the complex physiological solution finally excreted by the kidneys, since most of the urinary salts are present in proportions far exceeding their ordinary limits of solubility in aqueous solution. Low levels of colloidal activity and high surface tension values have recently been correlated with an increased tendency to stone formation in the urinary tract. (240) It is possible that this reduction in surface-active substances in the urine of old people, combined with the obstruction at the vesical neck due to prostatic hypertrophy, may lead to retention of precipitated material in the bladder, accounting for the increased incidence of vesical calculi during the later decades. In women, incidence of bladder stone is relatively low, even though the quantity of excreted surface-active substances is small, because there is no anatomical obstruction to urinary flow.

Administration of hyaluronidase raises the level of colloid activity (241) and lowers the surface tension of the urine to a slight degree. This enzyme has been utilized in the treatment of chronic recurrent stone formers with apparent effectiveness. (242) The reduced excretion of tensio-active substances in the urine may be related to the decreased enzymatic activity within the tissues.

Chapter 6, Note 26. Environmental Influences

In most of the experiments in animals, the analytical data followed over a certain length of time show variations which cannot be explained by the experiment itself. A direct relationship of such variations to changes occurring in the environment could be seen in the following experiment.

Six groups of 20 female white Wistar rats each, were injected on the same days, each group with a different agent. One group of animals received neutral oil as control while the others received different fatty acid preparations. The urinary surface tension was measured daily at approximately the same time of day for all the animals. The average value of these daily data was obtained for each group and used to trace the respective curves.

By comparing these curves, two characters were recognized. One was seen to concern differences from one curve to another, and consequently, would be considered as resulting from differences in the direct effect of the medications used. The other group of changes concerning variations from one day to the other, was seen to exist in all the curves, the curves having thus parallel variations. (*Fig. 262*) These variations, common to all the curves, were considered induced by a general influence. The analysis of these curves shows that the first kind of changes, related to the agents used, concerns differences in the levels of the curves themselves, when compared with that of the control. Treatment with stearic acid does not influence the level, and treatment with oleic acid has only a slight influence. A manifest change is seen for the other curves. The urinary surface values correspond to lowest values for linoleic acid (d) and for cod liver oil fatty

acid (e). Some less marked differences from the control curve is seen for the fatty acid preparation obtained from cow spleen (f).

Independent of these level differences, all the curves of the controls as well as of the treated groups show parallel daily changes. An exception

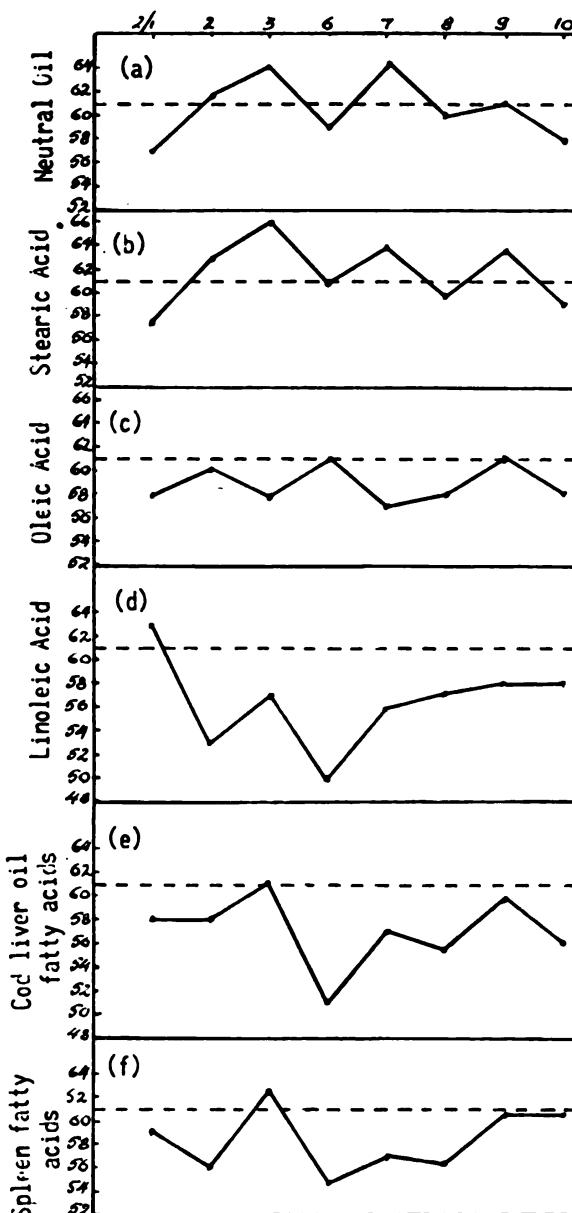


FIG. 262. Curves of the average value of the urinary surface tension in groups of rats treated with different fatty acid preparations (1 cc of 10% in oil daily). The parallel changes in the curves, except for oleic acid when the variations are opposite—indicate a common external influence. The differences in the relationship of the curves to the average value line, corresponds to the direct influence exerted by the agents.

is seen for the curve for oleic acid, which shows opposite variations. We do not have an explanation for this discrepancy. The parallel change would indicate the intervention of a common factor, independent of the experiment itself. By relating these changes to those taking place in the environment, the common variations in the curves could be recognized to follow—in an opposite sense—those of the environmental temperature.

It should also be noted that this environmental influence is progressively more accentuated for the curves where the level of the surface tension is lowered as a result of the agent administered. This correlation would suggest the possibility that the action exerted by the environment would take place largely through changes in the intervention of the fatty acids of the organisms themselves.

Chapter 6, Note 27. Environmental Influences Upon Urinary Surface Tension

The morning urinary surface tension was measured in four groups of 40 rats each: one group of 20 males, one of 20 females of the white Wistar strain, one group of 20 males and one of 20 females of a black "hooded" strain. From the data obtained on each group, the average value was calculated and its respective curve traced. The four curves were seen to be parallel, suggesting that the variations noted result from the intervention of a common external factor. We sought this factor in the changes occurring in the environment. For this reason, we compared the variations present in the surface tension curves with the meteorological data, furnished by the U. S. Weather Bureau, corresponding to the time of this experiment. Such a relationship was seen to only partially parallel the barometric changes, but appeared more closely related to the temperature changes. The observed relationship however, is inverse, that is, for higher environmental temperatures the surface tension values are low whereas for lower environmental temperatures, the surface tension values are high, as seen in Fig. 223. (Page 586)

This correlation appears still more interesting when it is compared to that induced by keeping animals at a constant temperature, as in an incubator or in a refrigerator. The effect of such an induced temperature is opposite to that caused by the environment. The high temperature of the incubator induces progressively higher surface tension values while the low temperature of the refrigerator lowers the surface tension, at least at first. Fig. 224 shows the average value of the urinary surface tension in controlled animals, while Fig. 225 that of the animals kept in an incubator at a temperature of 37°C and in a refrigerator at 8°C. (Page 588)

We tried to explain this discordant influence between the induced and natural temperatures, through the fundamental characters of these two factors. In the influence exerted by the environment, temperature with its variations, represents a factor which has acted upon organisms with the same rhythmic characters for many millions of years, while in the experiments, the constancy of the temperature represents its main character.

The influence exerted by the rhythmic environmental changes in air temperature is reflected in the parallel body temperature. The organism still tries to control this influence as exerted upon the lower levels. This is seen in rats in the opposite rhythmic changes of the urinary surface tension values. The organisms appear sufficiently sensitive to changes in temperature occurring in the environment. The body responses oppose these changes as shown in the variations in urinary surface tension. This rhythmic response, as well as that opposing the variations in the environmental

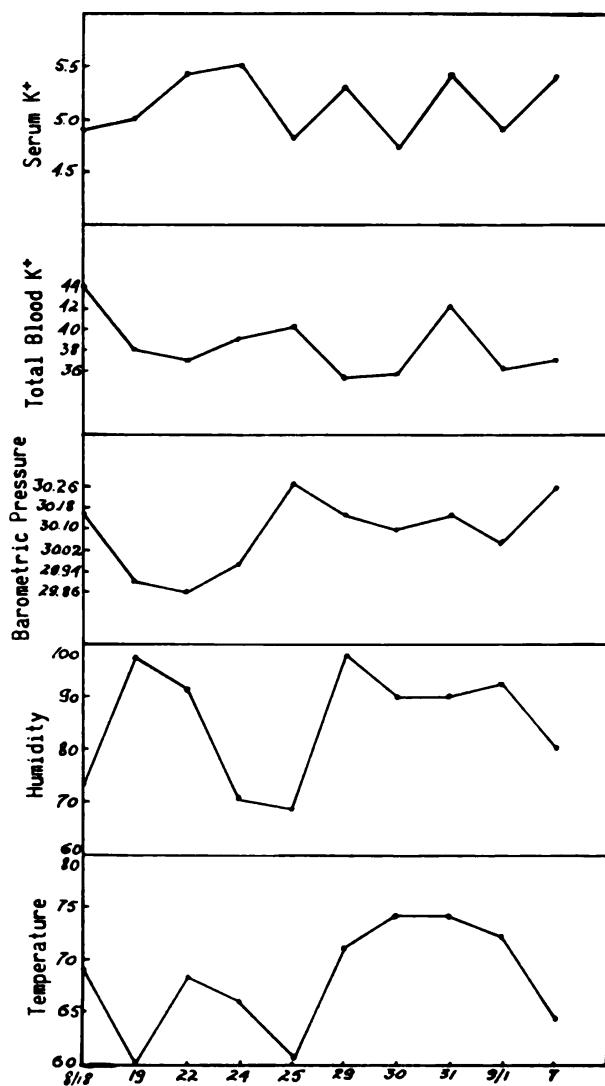


FIG. 263. Relationship between the curves of serum and total blood potassium of a group of 20 subjects and the atmospheric temperature, humidity and barometric pressure. Parallel variations are seen between the curves of total blood potassium and of barometric pressure. An inverse relationship is seen with the curve of humidity, and only partial relationship to the temperature.

temperature, are broken when a continuously unchanged high or low temperature is applied. With the reactional response exhausted after a certain time, the influence exerted appears a direct one, the high temperature inducing a high urinary surface tension and the low temperature a low one.

However, a difference is seen between the influence exerted by the high and low temperature. After some time, the animals kept in a refrigerator usually recover the capacity to fight the persistently low temperature, and the surface tension returns to normal values. This does not occur for the persistingly high temperature. The animals die with a progressively high surface tension as if the defensive response seen for low temperatures would not intervene for high temperatures.

This difference in the response of an animal toward a persistently high or low temperature can be explained by the fundamental difference which exists between these two factors from the point of view of homo- or heterotropy. While the low temperature has a homotropic character, the high temperature has a heterotropic one. The bodies are basically more prepared to react successfully toward homotropic influences as they have done it for millions of years in the past, than to an entirely unusual heterotropic influence.

Chapter 6, Note 28. Barometric Influence

For several years, we studied the potassium content of blood red cells in subjects with various abnormal conditions, usually following it daily for the same patient for weeks or months. Besides other informations which this study furnished and which are discussed above, we want to emphasize now the relationship with barometric pressure which we were able to establish during repeated analyses on the same group of subjects. We were impressed by the parallel variations seen in the amount of potassium in the total blood of different patients on different days regardless of medication. The variations could be correlated with changes in the barometric pressures. With lower pressures, a decrease in the amount of potassium in total blood (*Fig. 263*) was noted. Less manifest changes were seen with the opposite variations in atmospheric humidity.

Chapter 6, Note 29. Age, Lipoids and Tumor Transplants

The influence of age of a host upon the different manifestations through the intervention of sterols and lipoacids can be seen in the following experiments.

Transplants from the same Walker tumor were grafted at the same time in animals of different ages, such as newborn, weanlings, young animals, adults and aged. The difference between transplants was evident even from the beginning but was still more enhanced if further transplants were made in animals under similar conditions. After one transplant and especially after several transplants, the following changes could be seen. In the newborn, the tumor took on the aspect of an hemangiomatous lesion. There was

no massive tumor and the amount of blood present gave the tumor the appearance of a piece of liver. In weanlings, the character was opposite. Massive tumors without necrosis and with the aspect of fish meat were seen. In youngsters the same character was obtained. In adult animals, the tumor had a large portion of necrosis with predominance of hemorrhagic fluid. In very old animals, this character was still more accentuated, and the tumor showed big cavities with hemorrhagic fluid and very little tumor substance between.

Similar changes were obtained by changing the site of the graft. By grafting a portion of the same tumor, intramuscularly, we obtained a massive whitish, nonnecrotic tumor, while subcutaneous injection even in the same animal led to the appearance of a tumor with multiple necrotic areas and cavities filled with fluid.

We tried to correlate these changes with the nature of different lipids predominant at different ages. The administration of fatty acids tends to promote necrosis, edema, and formation of cavities filled with fluid, while administration of insaponifiable fractions, especially from placenta or liver, tends to produce a type of tumor with whitish, nonnecrotic masses.

Chapter 6, Note 30. Temperature, Lipids and Viral Infection

The relationship between epidemics and seasons is a well-established concept. In an attempt to explain this correlation, we considered, as one of the intervening factors, the seasonal changes in lipids, in view of the influence exerted by the two opposite groups of lipids upon the receptivity and manifestations of infectious disease.

As we have mentioned, viral infections are especially influenced by the predominance of one or the other group of lipids in the body. Among other factors, temperature changes were found related to this predominance. A relatively direct correlation was found for polio, for instance. The appearance of neurological symptoms such as paralysis was seen to increase on days with high temperature. Such a correlation could be established experimentally. Among mice injected subcutaneously with smallpox vaccine and kept in an incubator at 35° C, the incidence of encephalitis rose to more than 90% as compared to 10% in controls kept in an air-conditioned room. This correlation was further explained by the predominance of sterols in the organism under the influence exerted by high temperature. This predominance was further seen to induce a higher receptivity of the organism to viral infection, and a change in the virus virulence itself, both of which increase with temperature and with the richness of sterols.

Chapter 6, Note 31. Youth and Viruses

Another interesting aspect of the changes induced in viruses by lipoids with an alcoholic polar group, especially sterols, is the relationship to age of the host. The great amount of insaponifiable fraction present in youth—

which appears to be capable, in itself, of increasing virus virulence—has been discussed previously.

In an experiment, smallpox vaccine was inoculated in groups of very young, adult and old rabbits. The young animals reacted much more intensively than did the adults, with confluent pustulae; in the old animals, only a few small pustulae appeared. After several passages in animals of the same age, we tested the virus on mouse skin and found the virulence increased by each passage in young rabbits. On the other hand, virulence was decreased by passage in old animals, becoming negative with the fourth passage. After the third passage, virus obtained from young rabbits induced a strong response in mice, while no pustulae were obtained with virus from old animals. The latter was still able to induce some response in young mice, but only few small pustulae. The virus obtained from the third passage in young rabbits induced a frank response even in old mice. This indicates that opposite changes in virus virulence are induced simply by passage through young and old animals.

This experiment appears especially interesting in connection with childhood colds. It is known that children catch colds frequently and that this susceptibility disappears as they approach puberty. From a pathogenic point of view, it is an interesting fact, however, that older siblings, parents, and even grandparents are still infected if the cold comes from a child in the family. The virus itself seems to be changed by passage through the child so that it becomes virulent not only for teenagers and parents but for older people, all of whom previously may have been free of colds for years.

Chapter 6, Note 32. Changes in the Viruses Induced by Lipids

The effect of lipids upon viruses seems not to consist exclusively in an alteration of the host's response but also includes a change in the viruses' virulence. We have noted previously the big difference in the response of the skin of an animal when, prior to inoculation with smallpox virus, a lipoid with a positive or negative polar group was injected subcutaneously. The lipoid with a positive polar group induces an exaggerated response; the lipoid with a negative polar group, a reduced one.

We used viruses obtained from both types of lesions for inoculations in new animals. Viruses from lesions in which the response had been exaggerated produced again an exaggerated response, while those obtained from a small pustule induced a reduced response. The effect, in one or the other direction, was enhanced by successive passages on skin pretreated by subcutaneous injections. The changes in virulence also were confirmed in mice tests.

The importance of this experiment lies in the fact that, by treatment of the host with members of one or the other group of lipoids, we can obtain a desired increase or decrease in virus virulence. In several other experiments, we found the method applicable to other viruses, generally making the virus more or less virulent even for intracerebral inoculation. Interesting changes are produced by the polyunsaturated fatty acids and the alcohols

obtained from these acids by changing the carboxyl into a primary alcohol through treatment with lithium aluminum hydride. Especially effective influences are obtained using acid lipidic fractions of refractive species in order to reduce the virulence, or insaponifiable fractions of especially sensitive species or of chicken embryos to enhance virulence. Treatment of the host with lipoacid preparations, repeated for several passages of the virus, has reduced virulence to the point where the virus no longer is pathogenic. This treatment seems to provide a method for reducing virulence and obtaining nonpathogenic live virus vaccines.

Chapter 6, Note 33. Microbes, Phages and Lipids

We have investigated the influence of various insaponifiable fractions upon the relationship between microbes and bacteriophages. *Coli bacilli*, of a strain which has shown considerable resistance to phage, were grown in broth to which insaponifiable fractions from different sources—such as human placenta, eggs, butter—were added in colloidal suspensions. While in these media the microbes showed a higher susceptibility to being attacked by phage than the controls grown in simple broth, only very few microbes if any remained resistant. In another experiment, this same strain of *coli* resistant to phage was grown in successive passages in broth containing insaponifiable fractions. After several such passages, the microbe was grown in simple broth and its sensitivity to phage was tested in this medium. While the phage used appeared unable to attack the microbes of the untreated cultures, lysis was manifest in the treated *coli*. Thus, insaponifiable fractions increased the sensitivity of the microbes to bacteriophage, not only as an immediate effect, but also as a transmissible character.

Chapter 6, Note 34. Lipids and Survival Time of *Tetrahymena*

Various fatty acids have shown a special influence on survival time of *tetrahymena pyriformis*. Survival time was determined by keeping a few drops of culture in a capillary closed at one end and by daily examination of the mobility of the *tetrahymena*. By adding progressive amounts of an agent and withdrawing such a capillary after each addition, we also were able to determine the influence exerted by different concentrations.

In general, addition of very small amounts of fatty acids was found to prolong survival. Similar effects were obtained with hydrosulfide, and even with a solution of saponines which are known to bind sterols in insoluble combinations. The most marked effect was obtained with a fatty acid having a relatively low number of carbons. The addition of heptanoic acid in progressive amounts greatly increased longevity. The longevity increased as the concentration increased up to a point, after which it declined rapidly when greater concentrations were used. Of all the substances studied, heptanoic acid appeared to be most effective in increasing the longevity of *tetrahymena pyriformis*.

Chapter 6, Note 35. Lipids, Temperature and Tetrahymena

We have investigated the influence exerted by different lipids upon the capacity of tetrahymena pyriformis to resist increased temperature. In general, exposure of a culture of tetrahymena pyriformis to increased temperature induces rapid death. Kept in capillaries so a temperature of 37°C could be quickly attained, tetrahymena died in about 15 minutes. By growing tetrahymena for several successive generations in a medium containing insaponifiable fractions, increased sensitivity to temperature was obtained. In some experiments, death occurred within five minutes after exposure to 37°C. On the other hand, growth in media to which fatty acids were added markedly increased resistance, and survival after more than ½ hour of exposure to 37°C was seen.

Chapter 6, Note 36. Pain Induced by Lipids

During treatment of cancer patients with lipids, especially in exceptionally large amounts, some who had been pain-free before, experienced pain. Correlation could be established between the appearance of pain and the administration of lipids since pain increased with each new injection. Furthermore, a difference was seen to exist between the pain induced by the administration of fatty acids and insaponifiable fractions. While the former had an alkaline pattern, the second had an acid one. In several cases, pain subsided with discontinuation of the medication; in others, it persisted. The pain always was controlled by the administration of the opposite lipids, fatty acids for the acid type of pain and sterols for the alkaline.

Chapter 6, Note 37. Lipids and Wound Healing

The influence exerted by lipids upon wounds was followed by the changes induced in the healing processes. In order to compare the wounds, one square centimeter of the skin was excised down to the aponeurosis on the back of rats and rabbits after mechanical epilation of the skin, and the surface measured daily. We used transparent cellophane on which the exact dimensions of the wounds were drawn. The outlines which corresponded to the surface of the wounds were then passed on paper and cut out. The paper outlines were then weighed to give us a means of comparing the changes in the actual surfaces of these wounds during healing.

While lipid acids in general induce a retardation in the healing of the wound, the administration of lipoids with a positive character were seen to have an opposite effect. It is worthwhile noting that the naturally occurring sterols—cholesterol and insaponifiable fractions—are much more active than the synthetic in inducing rapid wound healing.

We studied the histological and cytological changes in parallel incisions made on the back of rats and rabbits and excised at intervals. In animals treated with sterol preparations, a difference was seen between the healing

of epithelial and connective tissue wounds. While healing of the former was enhanced, healing of the latter was not influenced. On the other hand, the higher alcohols, such as polyunsaturated alcohols or butanol, were more active in increasing healing of connective tissue wounds than of epithelial. It is interesting to note also that in the wounds treated with the sterol preparations, the scar showed an epithelium with many more layers than that of the normal surrounding skin. In rabbits, instead of two or three layers, there were more than ten.

Chapter 6, Note 38. Liver Regeneration

The ability of rats to regenerate almost $\frac{3}{4}$ of their liver in a short time has made them valuable for the study of the factors which intervene in cellular multiplication and differentiation. A study which we made in collaboration with E. F. Taskier has shown the importance of biological age of the individual in these processes. Regeneration is rapidly completed in young animals; much more time is required in the old. Liver regeneration has been seen to be related to the appearance of fats in the form of droplets filling up liver cells. Regeneration follows this first phase. The appearance of the fatty droplets provides a means of judging the velocity of regeneration. The importance of age is shown by the fact that fatty droplets appear early in the liver cells of very young animals, filling up the cells, in the first 24 hours. They appear later—in about two days—in young adults; in three days for middle-aged rats; after the fourth day in old animals.

The influence exerted by administration of different agents upon regeneration could also be judged through the changes induced in the appearance of the fatty droplets. The administration of insaponifiable fractions in general has induced an earlier appearance of the fatty cells. Injection of 2 cc. of a 10% solution of the insaponifiable fraction of human or even cow placenta was seen to induce an early appearance of the fatty droplets and a filling up of the liver cells. Even in old rats such changes occurred on the second day, contrasting markedly with control rats of the same age in which this would happen on the fourth day or later. Under the influence of these insaponifiable fractions, the old animals behave like youngsters, from the point of view of liver regeneration.

The opposite effect was exerted by the administration of 1 cc. of a 10% solution in oil of the lipoacids of human placenta or of a 10% solution of cod liver oil fatty acids. Appearance of fatty droplets was delayed. In young animals, the droplets were not seen until the third or even the fourth day. With a high dose such as 2 cc. twice a day, of the same preparation in animals of 150 grams, no fatty droplets appeared at all. It is interesting to note that in animals treated with such large doses of lipoacids, regeneration still takes place even without the appearance of the fatty droplets. In these cases the liver cells are comparatively very small and have compact nuclei, instead of the reticular aspect of the nuclei in the controls.

It is also to be noted that a parallelism was seen between the appearance of fatty droplets in liver cells and the richness of adrenals in sudano-

phil granules. In cases in which the administration of large amounts of lipoacids was followed by nonappearance of fatty droplets in the liver, the adrenals were found to be entirely depleted of fats.

The influence exerted by the lipids upon liver regeneration confirms the antagonistic role of the two groups of lipids in aging processes. The administration of insaponifiable fractions produces a regenerative response characteristic of young animals, while lipoacids produce the response of aged animals. We have applied these findings to other processes in which age is known to be a major factor—such as in the healing of wounds, and especially of fractures, in older people, where administration of insaponifiable fractions has been seen to change an atonic lesion into a rapidly healing one.

The study of liver regeneration has also indicated qualitative differences between various preparations. It is thus interesting to note that, of all the insaponifiable fractions used, the most active were those from placenta and embryos. The insaponifiable fraction of liver also has shown a special capacity to induce rapid regeneration especially of liver tissue. Higher alcohols have shown much less regenerative effect than the insaponifiable fractions.

Chapter 6, Note 39. Lipids and Convulsions

The administration of insaponifiable fractions of placenta or organs sometimes produces no observable manifestations in rats and mice, except an exophthalmia. However, more profound changes occur, since injections of thiamine in doses otherwise harmless are followed by lethal convulsions in these animals. 80 to 100 milligrams of thiamine/100 gr. of body weight produce lethal convulsions in rats or mice who received 1 cc. of 5% insaponifiable fractions of placenta per 100 gr. of body weight in daily injections for a week. In controls, 150 milligrams of thiamine per 100 gr. of body weight were necessary to induce fatal convulsions.

High doses of insaponifiable fraction of organs alone, also produced convulsions. The injection twice a day of a 5% oily solution of insaponifiable fraction of placenta in doses of 2 cc./100 gr. of body weight was seen to induce lethal convulsions after less than a week of treatment.

Chapter 6, Note 40. Lipoids and Coma

The administration of heptanol even in larger doses was not seen to induce somnolence or coma. Intravenous injection of a saline solution of 1 milligram of heptanol per cc. induced death in mice in doses above 0.5 cc. With 0.3 cc., the mice remained in deep sleep, sometimes with respiratory arrest. Most of the animals, however, recovered, starting to breathe in less than half a minute and awakening in about ten minutes. A dose as high as 10 cc. of the same solution, containing 10 milligrams, injected intravenously in rabbits, produced no more than a very short period of inactivity, without inducing sleep. Intramuscular doses as high as 500 mil-

igrams of heptanol in oil in humans did not produce somnolence. However, after several days of concomitant administration of heptanol and cortisone, even in reduced amounts such as 50 milligrams of heptanol and of cortisone daily, deep somnolence was seen to appear in some patients, and coma in two cases. In one, a man of 85, we were unable to overcome the coma. In the other, administration of cod liver oil fatty acids, sodium thiosulfate, and especially $\frac{1}{2}$ cc. of DOCA (desoxycorticosterol acetate), brought the patient back to normal state.

Chapter 6, Note 41. Cardiac Rhythm

The antagonistic influence exerted by the two groups of lipoids was seen to have an especially interesting effect upon the cardiac cells. The importance for the pharmacological study of the lipoids, as well as for the cardiac physiology and pathology of the changes induced, has urged us to study them in more detail.

The principal physiological property of the cardiac cell is its automatism, that is, its capacity to produce the proper energetic influx which when discharged, will induce the contraction of the myofibrils. Through the cytoplasmatic bond formations characteristic of the myocardial cells, the discharged influx passes also into the nearby cells where it acts as an external incitation which, in turn, induces the discharge of the influxes produced by these cells. It is through this progressive discharge of contiguous cardiac cells that the contraction progresses in a centrifugal manner through the heart.

Each cell needs a definite time to "mature" its own influx, a negative period following each discharge. During this refractory period, the cell does not respond to any influx, either from nearby cells or from any external excitation. On the other hand, due to the same progressive maturation of its proper energetic influx, if within a certain time this influx incitation produced in the cell has not been discharged by an influx coming from a nearby cell, the cell itself discharges it. This automatism is common to all cardiac cells. It differs however, from one cell to another in the time necessary for the influx to mature, that is, in the time necessary to bring the cell out of its negative refractory period or to discharge its own influx, if not discharged by an external incitation to the cell. A cell has a high automatism if it has a short refractory period, if it rapidly produces its influx, and if it discharges it early. A cell has a low automatism if its negative period is long and if it requires a long time to discharge its own influx if not discharged by an influx coming from the nearby cells. The rhythm of the contractions of the entire heart will be given by the discharge time of the cells with the highest automatism.

If groups of cells have an abnormally low automatism and their negative period is so long that these cells will still be in the refractive negative period when the influx from nearby cells arrives to them, they will not be discharged by this flux. If the group of cells represents a part of the heart

through which the influx has to pass in order to attain the entire heart, it will block its propagation.

The normal cardiac physiology results from the inequality of the automatism of the different cardiac cells. Those with the highest automatism will represent the pacemaker for the entire heart contraction. Under normal conditions the cells of the sino-auricular node show this highest automatism. Other cells with an automatism lower than that of the pacemaker, but still sufficiently high to be out of their refractive period, will respond when the influx started by the sino-auricular node arrives to them. The automatism of the other centers present in the heart—Aschoff-Tawara's node, Hiss's band, its branches, Purkinge's cells—progressively lower than that of the sino-auricular node, will supply an influx if that of the sino-auricular fails to reach them in due time.

Under abnormal conditions, this automatism is influenced. It can be either increased or decreased. In general, if the automatism of cells other than those of the sino-auricular node, is increased above that corresponding to the rhythm of this node, their influx will be prematurely discharged. If the cells around it are out of their refractive period, this influx will propagate and induce a contraction. They appear as abnormal pacemaker centers due to their premature discharge and also to their ectopic position. The resulting contractions will be manifested as extrasystoles, if the abnormal discharge appears as an isolated event, or as paroxysmal tachycardia if the abnormality persists. In auricular fibrillation, this abnormality takes place in a larger group of cells. Oppositely, a lowered automatism affecting an entire group of cells will result in a blockage of the passage of the normal influx due to this lengthened negative period.

The factor which appears to govern the differences seen in automatism of the various centers in the heart, is the degree of differentiation of the respective cells. As a general rule, a less differentiated cell has a higher automatism, while a more differentiated cell has a lower automatism.

We have seen that up to a certain point, the properties related to the degree of the differentiation of these cells can be connected with youth characters. The changes seen in heart cellular physiology, and especially those which appear under abnormal conditions, can be conceived as taking place through changes in the degree of the differentiation of the cells. We have seen above, in the study of the influence exerted by lipids, that while the unsaponifiable fraction induces a "prolonged youth" with a degree of the dedifferentiation of the cells, the acid lipid fractions induce a process similar to a more rapid aging, respectively a more advanced differentiation. This effect was seen also to be general for the respective positive and negative lipoids. While for other cells such a change may be uneventful, for the cardiac cell it will be marked by a change in automatism.

From this specific point of view, we have studied the influence exerted by different agents upon the heart, seeking in the changes induced, modification corresponding to an increased or decreased automatism. Clinical observations have shown such correlation. Extrasystoles were seen to appear

in subjects who had previous extrasystoles, when lipoids with positive polar groups were administered in high doses. They disappeared when the medication was stopped and reappeared when medication was resumed. In cases with previous auricular fibrillation we have seen it reappear with high doses of positive lipoids, disappear with cessation of the medication and reappear when medication was resumed for even a short time. This was fully controlled by the administration of lipoids with negative polar groups.

In hundreds of electrocardiograms taken of experimental animals, such a correlation between the administration of lipoids and induced arrhythmias was investigated in collaboration with I. Eroglu. We studied thus various substances, lipoids with positive or negative characters administered intraperitoneally or intravenously in rabbits. An extremely high amount of the agent was necessary to influence the cardiac rhythm in normal animals. It was usually near a lethal dose and in general, proportionately many hundred times that used therapeutically in humans. In repeated injections however, changes could be induced with relatively smaller doses. In sufficient doses, the positive lipoids were seen to induce extrasystoles. Figures 292 and 293 show such changes obtained with huge doses of butanol and glycerol administered intravenously. (Page 714)

In animals, the negative lipoids induce a dromotropic negative effect, leading to auricular contractions not passing to the ventricles. Huge doses were seen to induce a bigeminated pulse.

The study of the intervention of lipoids has led to a new therapeutic approach. Extrasystoles, paroxysmal tachycardia and auricular fibrillation were seen to respond well to the administration of lipoacids and lipoids with negative polar groups, while partial blocks were influenced by lipoids with positive polar groups.

Chapter 6, Note 42. Some General Considerations of the Role of Lipids in Blood Physiology *

Lipids and Red Cells "In Vitro"

Among the first experiments concerning the influence exerted by sterols and polyunsaturated fatty acids in vitro, were those concerned with the effects upon red cells. We have noted that when citrated blood is kept for two hours at 37°C in a test tube, the walls of which have been coated with crystals of cholesterol or with nonsaponifiable fractions obtained from various natural sources, the red cells become more swollen and turgescent, and less crenated than those not treated. Seen under the dark field microscope, the cell crown appeared uniformly more refringent. It was also noted that the treated red cells failed to form rouleaux or conglomerates similar to those seen as sludge in vivo. At the same time, the cells appeared richer in their sterol content. None of these changes were observed when the red cells were separated from their plasma and washed with saline and kept in a saline solution when treated with sterols, in the manner mentioned above.

* Delivered at Gordon Research Conferences, Kimball Academy, Meriden, New Hampshire. 1955

Opposite effects were observed when fatty acids were added to blood. As the direct contact with the red cells produces hemolysis, the following technique was used. Fatty acid preparations especially as mixtures obtained from blood or cod liver oil, were added to heparinized or citrated plasma, thoroughly agitated and the excess separated by centrifugation. The plasma so treated was then added in various proportions to citrated or heparinized blood from the same subject. This portion of this blood was centrifuged and the treated plasma added to the supernatant plasma from which the same amount was withdrawn. The added plasma was mixed with the supernatant plasma and then this was mixed with the red cells. In this way hemolysis was prevented. Small amounts of treated plasma caused the red cells to shrink in size and frequently become crenated. In addition, a strong tendency to conglutinate which exceeded that noted in corresponding control specimens, was observed. When the quantity of fatty acid-treated plasma exceeded a certain amount, hemolysis was induced. The addition of these two groups of lipids to red blood cells, have appeared to exert frank antagonistic effects.

Sedimentation Rate

The two groups of lipids were also found to influence oppositely the red cell sedimentation rate in citrated blood. When citrated blood samples having high sedimentation velocities, were treated with cholesterol or an unsaponifiable fraction in the manner described above, the speed of sedimentation was markedly reduced. TABLE XXVIII shows results obtained

TABLE XXVIII

RED CELL SEDIMENTATION RATE (mm./hr.) SAMPLES TREATED
WITH UNSAPONIFIABLE FRACTION OF BLOOD LIPIDS

Control	Treated
110	12
96	19
81	18
48	15
18	10
12	6
9	7
8	8
6	5

mentation rate tended to increase to abnormal values. This varied with the amount of treated plasma added. (TABLE XXIX) in different blood samples in which the sedimentation rate during one hour, was measured by the Westergren method. In general, it can be seen that the higher the sedimentation rate of the untreated sample, the greater was the effect of adding sterols.

On the other hand, when polyunsaturated fatty acids were added in the manner already described, to citrated blood from healthy subjects, the sedi-

TABLE XXIX
RED CELLS SEDIMENTATION RATE—mm./1st hour

Fatty Acid Used	Control	Quantity of Treated Plasma Added to 5 cc. Citrated Blood		
		1/4 cc.	1/2 cc.	1 cc.
Stearic	9	8	9	9
Palmitic	9	10	9	10
Linoleic	9	15	18	22
Linolenic	9	15	21	25
Cod Liver Oil	9	20	36	Hemolysis

Red Cell Volume

The same opposite effects of sterols and fatty acids were further observed upon the volume of red cells, as determined by the hematocrit, or also when the sedimentation in tubes was observed over a 24 hour period. (TABLE XXX) Sterol-treated blood showed a significant increase in red

TABLE XXX
CHANGES IN VOLUME OF RED CELLS IN CITRATED BLOOD TREATED IN VITRO
(Sedimentation After 24 Hours)

Substance Used	Control	Treated
Unsaponifiable fraction of blood	53	66
Stearic Acid	53	54
Saponifiable fraction of blood	53	50

cell volume, while on the other hand, with the addition of polyunsaturated fatty acids, the red cell volume decreased. This agrees with a retention of water by the cells in general when richer in sterols, which Schaeffer has described as the lipocytic index.

On further analysis, these effects of fatty acids upon red cells mentioned above could be related to the polyunsaturation of these acids, since by treating the blood under the same conditions with saturated members, such as palmitic or stearic, these changes were not obtained.

The treatment of red cells with conjugated fatty acids, especially trienes, induces a marked vacuolization. This is seen to occur through an accumulation of part of the content of the red cell in droplets, strongly stained with eosin. (Fig. 264a) Similar changes are seen to occur *in vivo*. In lesions characterized by a predominance of fatty acids or induced by the administration of conjugated fatty acids, such vacuolated red cells are often seen. We used their presence, together with other characters, for the pathological diagnosis of the type D present in a lesion. (Fig. 264b)

Red Cells, Plasma and Lipids

As most of these changes did not occur with the red cells in suspension in different isotonic saline solutions, we have attempted to explore the re-

lationship of plasma to red cells and lipids. This was done in the following manner. The cholesterol content of red cells was seen to be progressively lowered by repeated washings with isotonic saline. When the amount of cholesterol is reduced below a certain level, hemolysis ensues. Standardizing these washings by replacing the plasma with an equal amount of saline, hemolysis is usually obtained in some bloods after 1 or 2 washings while in most after more than 10 washings. This occurs when the cholesterol con-

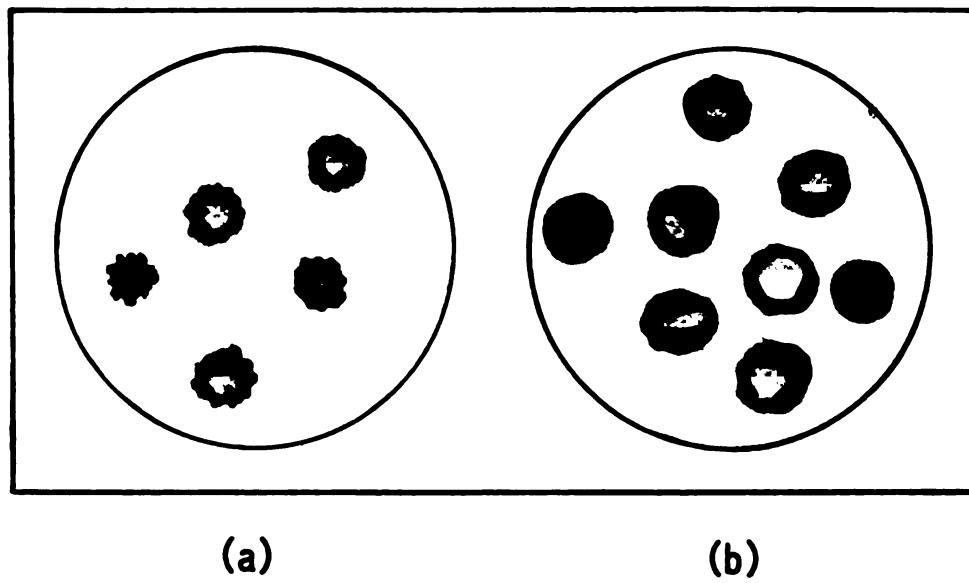


FIG. 264. The treatment of red cells *in vitro* with conjugated fatty acids (trienes) induces the appearance of vacuoles. (a) In spontaneous lesions characterized by an off-balance type D with a predominance of fatty acids or in lesions induced by the administration of conjugated trienic fatty acids, vacuolated red cells are seen. (b)

tent of the red cells falls below 58 mgm. %. When the separated plasma is again added to these repeatedly washed and consequently cholesterol-impoverished blood cells, their cholesterol rises. (TABLE XXXI) By further additions of new portions of the same plasma, the cholesterol content of the red cells can be progressively elevated to the original values. Apparently cholesterol passes readily from the plasma to the red cells. This

TABLE XXXI
INFLUENCE OF CITRATED PLASMA UPON RED CELL CHOLESTEROL

Before Treatment	186	mgr.%
After 10 Washings	62	"
After First Treatment with Plasma	111	"
After Second Treatment with Plasma	142	"

was confirmed by measuring the cholesterol content of the plasma before and after it was mixed with cholesterol-impoverished cells. After treating a portion of citrated plasma several times with other portions of cholesterol-impoverished red cells, the amount of cholesterol in the plasma was markedly reduced. The addition of the unwashed red cells to this cholesterol-impoverished plasma raised back its cholesterol content. The content could be increased still further almost back to the previous values by repeating this procedure. (TABLE XXXII) It was clear that a balance in

TABLE XXXII
INFLUENCE OF RED CELLS UPON PLASMA CHOLESTEROL

Before Treatment	226	mgr.%
After Fifth Treatment with		
Washed Red Cells	96	"
After First Addition of		
Unwashed Red Cells	163	"
After Second Addition of		
Unwashed Red Cells	180	"

the cholesterol content seems to be realized between plasma and red cells through possible passages in both directions. This fact would imply that the red cells may serve as a buffer reserve for rapid changes occurring in the plasma sterols.

The role of red cells in the transportation and distribution of fatty acids through the blood not only appears more evident, but also indicates a selective intervention. When plasma was treated as mentioned above with fatty acids that were easily identifiable, and then mixed with the red cells, an unequal distribution between plasma and red cells was seen. The different influence of the different fatty acids became obvious. Saturated fatty acids could not be found in the red cells when these acids were used, while unsaturated fatty acids seemed to be selectively retained. Fatty acids such as oleic, linolenic, eleostearic, or norbixine were found convenient to be used for this purpose. They were easily identified, the first through its chemical character, the second after conjugation, and spectral analysis, the third through its characteristic absorption in ultraviolet light, and the last through its color in chromatographic column. After mixing with the red cells, they were found unequally and selectively fixed to the red cells: least for oleic, fixation was found to increase with the degree of desaturation. The same fixation was seen to occur in vivo. When animals were treated with saturated fatty acids, these substances did not appear in the red cells. When treated with oleic, linolenic, eleostearic acid or norbixine, the content of these acids in the red cells was found to be for the last acids as much as five times higher than in plasma. A selective fixation of those fatty acids on the red cells could thus be recognized. This appeared still more striking when compared with that of cholesterol. In animals treated with cholesterol, the relative proportions between the proportion in plasma and cells

was not seen to be altered by a total increase in cholesterol. It appears that the red cells have the capacity of selectively fixing from the plasma certain fatty acids, particularly the more unsaturated ones.

Lipids and Blood Oxygen Transport

One of the most interesting observations and one of the simplest *in vitro* experiments that indicates these opposite effects of sterols and fatty acids is their influence upon the oxidation processes in which red cells intervene. When a sample of ordinary venous blood is treated with a preparation of cholesterol or nonsaponifiable fractions, using the above mentioned technique and after its separation from the cholesterol it is agitated with air or oxygen which is passed through these samples, the color becomes a bright vermillion red, and this persists for a long time. When the same venous blood is treated with a preparation of polyunsaturated fatty acids as mentioned above, the color becomes very dark, almost black purple. When air or oxygen is passed through these samples, the blood becomes lighter in color for only a short time, the darker color reappearing within a few minutes. One is immediately impressed by the similarity of the cholesterol-treated blood to arterial blood, while the fatty acid-treated blood is similar to venous blood, and especially to the color of venous blood in cases of shock.

We tried to tie in these findings with the observation of Binet concerning the changes in blood fatty acids when passing through the lungs. He has been able to show that the amount of the polyunsaturated members appears to be reduced by the passage of blood through the lungs. We could show that the red cells leaving the pulmonary vascular bed are somewhat richer in the unbound cholesterol than they have been in the blood which entered the lungs. The lipid content is altered in an opposite way as the blood travels through the general circulation. That is, the polyunsaturated fatty acid content is increased in the red cells while the quantity of free cholesterol seems to be diminished. The sterol-richer red cells appear capable of retaining for a longer period of time, the amount of oxygen which hemoglobin has fixed, while a rapid reduction of oxyhemoglobin is seen in the red cells when the polyunsaturated fatty acids intervene. This led us to consider an intervention of these two groups of lipids in relation to the oxygen transportation by the red cells. Bearing in mind the fact that while cholesterol reduces cell permeability and polyunsaturated fatty acids increase it, an alternating intervention of these lipids seems to play a role in a better distribution of oxygen. The oxygen which is fixed by hemoglobin when the red cells have passed the lungs, is largely retained as such by the intervention of the sterols until they reach the point in the tissues where liberation of oxygen is necessary, this being favored now by the intervention of the fatty acids.

Lipids and State of Shock

The abnormally dark color of the blood resulting from its treatment *in vitro* with polyunsaturated fatty acids has suggested the intervention of such

substances in those clinical conditions in which similar color changes are noted in the blood as in shock. We will present our studies in shock below. For the moment we will only note that in the state of shock experimentally induced by trauma, burns or irradiation, or found in terminally ill adrenalectomized animals, these animals have not only a high fatty acid content, but that the kind of fatty acids encountered are not the same as in normal animals. We have discussed these abnormal fatty acids above. The existing differences have been shown by measuring the quantity of oxalic acid that is produced when these fatty acids are submitted to a careful standardized oxidative fission. The oxidative fission of the fatty acids not only from their entire body but even from their blood has shown that for normal animals, no oxalic acid could be found, leading to the assumption that no conjugated fatty acids are present. On the other hand, oxalic acid appeared when fatty acids obtained from animals in shock or from their blood were broken down with the analytical method utilized.

Of particular significance for the pathogenic role of these fatty acids is the fact which we will discuss again below, that death appears to ensue when the conjugation of fatty acids reaches a certain value, which is approximately the same whether the animal has been traumatized, burned, irradiated or adrenalectomized, and independent of the fact that death occurs in a short time or several days. It corresponds to 14-17 mgm. of oxalic acid per gram of fatty acids. It is also interesting to note that these abnormal fatty acids were found to be more abundant in the red cells than in the plasma.

Effect upon Leucocytes

The biological antagonism between sterols and fatty acids has appeared in the influence upon other blood constituents. We have observed that the administration of sterols tends to elevate the total white blood cell count and especially the number of neutrophilic granulocytes. Polyunsaturated fatty acids, on the other hand, produce rapid leucopenia, and again it is the neutrophile elements that are first affected. A hyperleucocytosis often was seen following the neutropenia induced by polyunsaturated fatty acids if small amounts are administered. This effect could be considered as being reactional to the first leucopenia, since it is retarded or even prevented if large doses of these fatty acids are injected. (TABLE XXXIII) It is also interesting to note that a deviation to the right, in Arneth's formula, was seen after the treatment with fatty acids; and to the left after treatment with sterols. Thus, this concords well with the antagonistic effects upon the aging process seen for these lipids and which is discussed below.

Lipids and Blood Serum Cholesterol

Further study of the relationship between blood and lipids has permitted the recognition of several peculiarities concerning the blood serum which when related to abnormal conditions, acquires a special significance. Policard has observed that when crystals of cholesterol are added to blood sera, two opposite changes can ensue. In one, a precipitate appears while

TABLE XXXIII

EFFECT OF LIPIDS ADMINISTERED IN VIVO UPON THE TOTAL NUMBER OF LEUCOCYTES

Unsaponifiable Fraction of Blood—10% Solution—5 cc. I.P.

Before Administration	14,600	12,000
2 Hours Later	18,400	19,000
7½ Hours Later	26,000	22,600

Saponifiable Fraction of Blood—10% Solution—5 cc. I.P.

Before Administration	13,200	16,200
2 Hours Later	11,000	6,800
7½ Hours Later	6,000	5,100

Stearic Acid—10% Solution—5 cc. I.P.

Before Administration	16,100	14,200
2 Hours Later	12,800	15,100
7½ Hours Later	15,000	12,000

the serum cholesterol content decreases. On the contrary in the other, a part of the added cholesterol passes in solution into the serum, thereby causing an increase in cholesterol content. When animals were treated with large amounts of sterols for a long time, their sera showed this tendency to precipitation when in contact with cholesterol in vitro, while the sera of animals treated with large amounts of fatty acids showed the capacity of dissolving more cholesterol. We believe that this capacity of sera to precipitate in the presence of cholesterol may be correlated with the clinical conditions present in arteriosclerosis when acute episodes occur.

These studies of the role of lipids in blood physiology suggest that the general antagonism between sterols and polyunsaturated fatty acids also intervenes in other important processes of blood physiology. It has thus raised the question of the role these lipids may play, through their opposite effects, in different metabolic balances of the body governed by blood changes.

Chapter 7, Note 1. Analyses Used for the Study of Hemoshock

Besides body temperature and blood pressure, the following blood analyses were made: complete blood count; coagulation time; clot retractability; values of albumin and globulin, total and free cholesterol, free fatty acids, degraded proteins, antitryptic power of the serum, esterase amylase, potassium, sodium, calcium and glucose. Most of these analyses were made in venous blood samples obtained every five minutes, during the fifteen minutes preceding and the half hour following the noxious intervention.

Chapter 7, Note 2. Morphine and Shock

The possibility of inducing hemoshock simply through an intravenous injection of colloidal metals has provided a useful method to study the conditions under which hemoshock can be induced or suppressed. We have investigated a series of agents to determine their influence upon shock. Adrenalin, quinine, ephedrine and atropine have not changed the course of clinical and hematic manifestations. On the other hand, morphine, as well as other opium derivatives, completely prevented the development of these manifestations. Subcutaneous injection of 2 centigrams of morphine

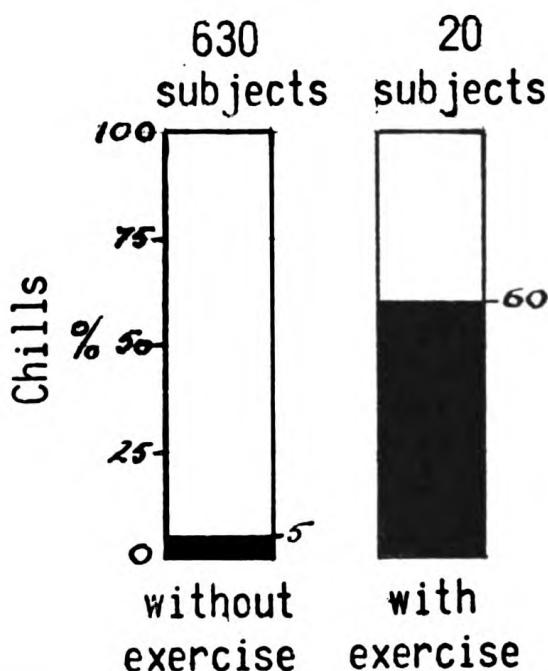


FIG. 265. The proportion of chills appearing after direct transfusion (with the Jubé syringe, injecting 500 cc in less than 10 minutes) increases manifestly after exercise (walking in the room).

sulfate fifteen minutes before the intravenous injection of the metal always suppressed the manifestations as did an intravenous injection of 5 milligrams of morphine sulfate. Analyses have shown no leucopenia, to occur. Similar effects were obtained, with none of the changes characteristic of hemoshock, for instance in transfusions where 500 cc. were injected in less than ten minutes and where morphine prevented the frequently observed chills. The influence of morphine upon the leucocytes has been confirmed in the following experiment.

In rabbits, a pleural exudate was obtained by an intrapleural injection of broth, 16 hours later, pleural punctures furnished fluid rich in leucocytes. As we have noted above, the addition of a colloidal suspension of silver

proteinate (collargol) was followed by the appearance of rapidly growing vacuoles which led to bursting of leucocytes. (*Fig. 76*)

The addition of even minimal amounts of morphine or other opium derivatives entirely prevented these changes in leucocytes. No lysis nor vacuoles were seen.

Chapter 7, Note 3. Physical Exercise and Shock

The study of hemoshock has shown the influence exerted by physical exercise upon shock. A leucopenia was observed in normal subjects after an intensely sustained physical effort, such as after running for five minutes, a fact which led us to try to see what influence exercise would have on the shock induced by the intravenous injection of colloidal metal. The shock was seen to be much stronger than usual. The chill which followed 25 minutes later was also proportionately severe. We have since correlated the appearance of hemoshock with exercise in patients having direct transfusion. If the patient exercised immediately after the transfusion, a chill consistently followed about a half hour later. (*Fig. 265*)

Chapter 7, Note 4. Lymphocytes and Effects in Vitro

The capacity of lymphocytes to hydrolyze even higher esters can be demonstrated by having lymphocytes separated and their activity tested. Fluid obtained from tuberculous pleural effusion rich in lymphocytes was centrifuged and the fluid decanted. The centrifugate was then put on a plate of beeswax, covered with a cup and left for several hours at 37°C. A clearly visible depression appeared where the lymphocyte preparation had been added.

Chapter 7, Note 5. Lipids and Immunity

Three groups of five rabbits each, of the same sex and weight, were injected intravenously on two consecutive days with the same amount of a suspension of killed *Eb. Thyphi*. One group was kept as control, receiving daily injections of 1 cc. of cottonseed oil. Of the other groups, one was injected subcutaneously daily with 1 cc. of a 5% solution in cottonseed oil of the acid lipids mixture obtained from human placenta. The third group received daily 1 cc. of a 5% solution in cottonseed oil of the insaponifiable fraction of the same origin. Every second day, venous blood was obtained and the agglutinating power of the serum determined. Figure 265A shows the average values for each group.

Chapter 7, Note 6. Microbes Treated with Lipids

The injection of microbes killed by heat and treated in vitro with various lipids and lipoids had an interesting effect on the appearance of antibodies. *Eb. typhi*, cultivated on agar and suspended in saline so as to give

nephelometrical values corresponding to 30 mil. per cc., were killed by heating for 1 hour at 62°C. Different portions of this suspension were treated by mixing them with preparations of the acid lipidic or insaponifiable fractions of various origins, such as human placenta, cow, carp or rabbit organs; entire bodies of guinea pigs or rats; entire bodies of squid; seeds of *Bixa orellana*; microbes such as *Esch. coli*, *B. subtilis*, and tubercle bacilli. Fatty acids such as oleic, linoleic, eleostearic or mixtures of acids obtained from

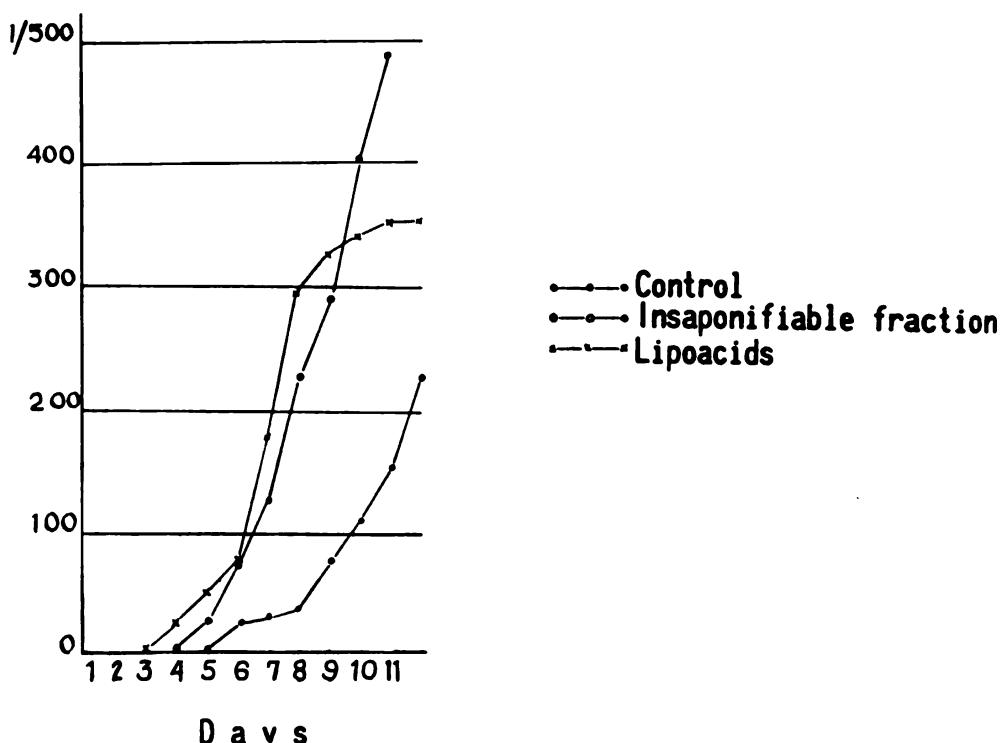


FIG. 265A. *Lipids and agglutinines.* Influence exerted by the administration of unsaponifiable lipid fraction and the acid lipid fraction of human placenta upon the appearance of agglutinines against *Eb. typhi* vaccines injected intravenously. Each curve corresponds to the average value of 5 rabbits. The agglutinines appear earlier and their amount increases more rapidly in the animals treated with lipids than in the controls.

cod liver oil or butanol or heptanol also were used. 5 cc. of a 2% alcoholic solution of the lipoids were introduced in 110 cc. distilled water and the solvent eliminated by boiling the mixture to bring the amount to 100 cc. 1-5 cc. of these milky preparations were added to 5 cc. of the microbe suspension and incubated at 37°C for 24 hours. The treated microbes were then separated by centrifugation, the fluid decanted and the microbes resuspended in the same amount of saline. They were used in doses from 1/10-1 cc. for subcutaneous injections in rabbits, repeated every third day for two weeks. As controls, animals were injected with the same amounts of suspension of untreated microbes.

For the first two weeks small amounts of blood were obtained every third day from the ear veins of the treated animals. Blood was obtained from the heart every second week for 8 weeks. The agglutinins were determined for all the samples. In the two-week samples, the presence of immune antibodies was determined by the capacity of different amounts of the sera to prevent a lethal infection in mice given intraperitoneal injections of standard amounts of living microbes. The agglutinins were seen to appear earlier than in controls—in a manner similar to that seen when lipids were injected in the animal as related in Chapter 7 Note 5. It was especially in the immune protective antibodies where the difference was manifest. It was not only an earlier appearance of these antibodies, but the protection against a lethal injection was obtained with much smaller amounts of serum.

Chapter 7, Note 7. Skin Allergy

An aqueous extract of squid body was prepared by blending it and extracting with saline in a proportion of 1/20. After filtration, the mixture was centrifuged and the supernatant fluid put in ampules with methiolate added as a preservative. Some of the ampules were tyndallized by heating them at 56°C one hour daily for four consecutive days.

1/10 cc. of these preparations was injected intradermically in various subjects who also received control injections of saline. Immediate and 24 hour reactions were noted. An induration present the second day was considered as a positive cellular response, while the immediate appearance of a hive was considered as a reaction taking place in the metazoic compartment.

Twelve days after a first injection in exactly the same place, a second injection was given with the same material. The immediate and the 24 hour reactions were judged. If the reaction was negative, a third injection was made, ten days after the second, in the same place. It could be seen that in normal individuals, the second sometimes, and the third injection always induced second day induration which persisted for several days. In cancer patients, including those in terminal condition, the injection of this antigen induced virtually the same reaction as in normal individuals.

Chapter 9, Note 1. Hemoglobinuria a frigore (244)

Certain information about shock which has emerged from the study of a rare condition is worth noting here. When a patient with paroxysmal hemoglobinuria—also known as both hemoglobinuria a frigore and cold hemoglobinuria, immerses his hand in icy water, he experiences a chill a half-hour later which is followed by the appearance of hemoglobin in the urine. Classically, this phenomenon was considered to result from the intervention of a hemoshock. We investigated such "attacks" of hemoglobinuria in three cases, inducing and studying the phenomenon several times in each subject. Usually, observations were carried out during a three hour

period after the immersion of the patient's hands for ten minutes in icy water and included the following procedures:

- 1) Measurements of blood pressure and temperature every 5 minutes;
- 2) Determinations at ten minute intervals, of coagulation time, clot retraction, white cell count and differential; serum hemoglobin content, serum proteins, antitryptic power and esterase—all measurements being made on venous blood.
- 3) White cell count and differential measured on capillary blood obtained every 10 minutes by finger puncture at 5 minute intervals after withdrawals of venous samples.
- 4) Tests for the presence and amount of hemoglobin in the urine at 15-minute intervals.

Coagulation time was established in the centrifuge tube and was related to the moment when blood ceased to flow if the vertical position of the tube was changed. Clot retraction was determined by centrifuging the coagulated blood after 2 hours at room temperature and measuring the amount of serum obtained from 15 cc. of blood. The serum and urine hemoglobin content was determined photometrically. For total protein content, we used both the refractometric index of the serum and gravimetric measurements after adequate precipitation. For antitryptic power we determined the inhibitory effect of the serum upon the digestion of a solution of casein by trypsin. The quantity of esterase present was determined by the changes upon ethyl-butyrate.

The data obtained were plotted as curves with time as common abscissa. Parallel variations were observed in all three patients during repeatedly induced attacks.

For almost all analyses, except for the presence of hemoglobin in urine, the variations indicated a diphasic phenomenon. (*Fig. 266*) The first phase was characterized clinically by hypotension and slight hypothermia. The characteristic analytical changes were leucopenia, prolonged coagulation time, reduction in clot retraction, lower refractometric serum value, lower antitryptic serum power and increased serum esterase. During this first phase of the diphasic phenomenon, hemoglobin also appeared in the serum and, when abundant in the serum, also was found in the urine. The first phase was followed by a second 5 to 10 minutes later. The clinical manifestations were a sensation of chill, varying from very slight to severe, followed by temperature elevation and slight hypertension. Analytical changes in the opposite direction from those noted during the first phase could be seen. Hyperleucocytosis, reduced coagulation time, higher retraction of the clots, elevated refractometric value and antitryptic power, and reduced esterase content were characteristic of the second phase. Hemoglobin present in the serum in the previous phase disappeared at this time.

The most interesting finding in paroxysmal hemoglobinuria was that two or three such distinct diphasic episodes followed each immersion. In all cases, the first diphasic complex appeared in about 10 minutes after

immersion. It was relatively mild and lasted in general about 10 minutes, after which all values returned to pre-attack.

About a half-hour later, however, a second diphasic complex, much more intense in its manifestations, was noted. Hypothermia and hypotension were more marked. Leucopenia was more intense, the number of leucocytes falling to as low as 200 per cubic mm. The quantity of hemoglobin in the serum was very high, with hemoglobin spilling over into the urine in large amounts. Serum antitryptic power decreased to much lower levels than during the first diphasic complex. In some cases the coagulation time increased to 15 minutes and the clot almost failed to retract. These changes

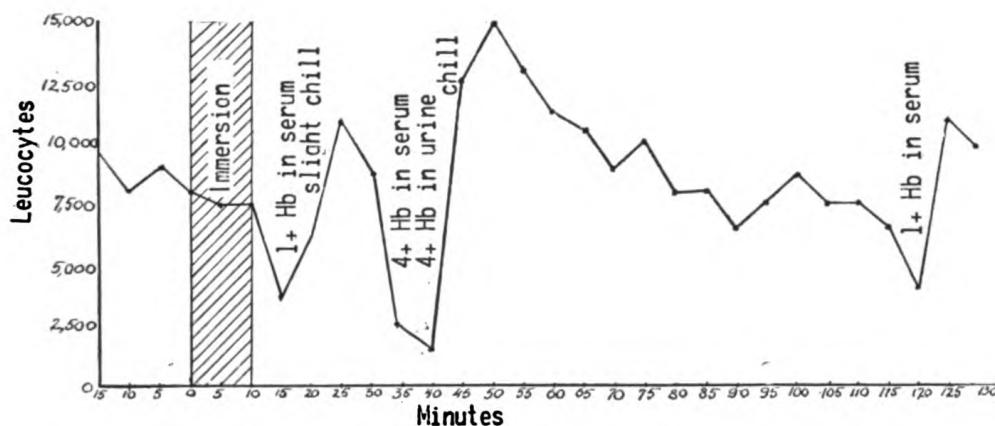


FIG. 266. The clinical data and analytical changes of blood and urine following the immersion in icy water of the hand of a subject with hemoglobinuria a frigore reveal three distinct diphasic phenomena corresponding respectively to three separate hemo shocks. Their intensity appears correlated to the degree of the occurring leucopenia.

were followed by the second phase of the complex, with severe chill and manifest changes in the analyses in the opposite direction. It is the second phase of the diphasic complex that is recognized clinically as the "attack" of hemoglobinuria. The chill was often very intense, followed by a temperature above 39°C, and hypertension. Leucocytes increased to as much as 20,000 per cmm., blood coagulation time was abnormally shortened and clot retraction increased. Hemoglobin disappeared rapidly from the serum. The albumin content as well as the antitryptic power of the serum increased, while the esterase content fell. In about 30 minutes, however, all these changes were damped and the blood slowly regained its normal characteristics. This period, with almost all manifestations slowly returning to normal often was followed by a third diphasic complex, not clinically evident but revealed by hematological findings. In most cases, it appeared about two hours after immersion. While it was much milder than the first two, its diphasic character was quite clear. Occasionally the patient reported a slight sensation of cold. The amount of hemoglobin in the serum was less

than during the first complex, and hemoglobinuria was never seen. Figure 266 shows these findings in a typical succession of these complexes.

An analysis of these cases indicated two striking characteristics. One was the time sequence of the three diphasic changes which was uniform for all attacks in all subjects. The second was that although they differed in intensity, all the attacks were qualitatively alike.

In further studies we tried to understand the meaning of these changes. Similar changes, but without hemoglobin in serum and urine, and with only slight chill as a clinical manifestation, could be observed when the hands of normal individuals were placed in icy water for 10 minutes. Two similar diphasic phenomena were seen to appear. While their intensity was greatly reduced, the time of appearance of the two diphasic phenomena observed

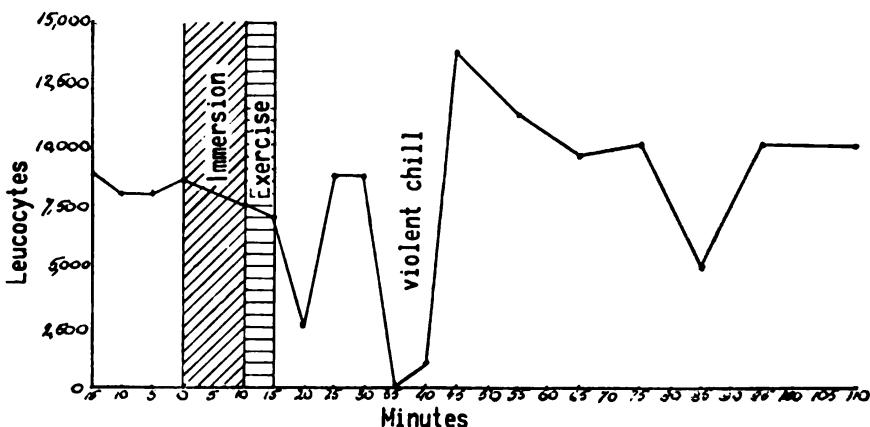


FIG. 267. In the patient with hemoglobinuria a frigore, exercise after immersion of the hand in icy water induces a violent chill with abundant hemoglobinuria.

was basically the same as in patients with cold hemoglobinuria. This would indicate that the leucolysis in hemoglobinuria a frigore corresponds to a physiological response which induces hemolysis because the red cells have been sensitized by cold. This sensitization is recognized in the Donath-Landsteiner reaction. It appears probable that leucolysis liberates the complement necessary to induce the hemolysis of the sensitized red cells.

We have tried to correlate these changes with other processes encountered in normal and abnormal physiology. In differential studies of blood smears obtained during an induced attack of hemoglobinuria, nuclear shadows were observed in the smears at the time of the leucopenia of the first phase. This was exceptionally manifest for the second diphasic complex, when a marked leucopenia occurs. We have seen above that this leucopenia has been correlated to the lysis of the leucocytes.

In patients with cold hemoglobinuria, we were able to show that, if with any physical exercise after the hands were out of the icy water, such as even walking in the room, the severity of the attack induced was much greater than when they were allowed to rest quietly. Not only was the

severity of the chill and the degree of hemoglobinuria corresponding to the second diphasic phenomenon greatly increased, but all other changes were similarly intensified. The number of leucocytes decreased to less than 200 per cubic mm. Blood coagulation time went to values as high as over $\frac{1}{2}$ hour with almost no retraction of the clots. The antitryptic power of the serum, obtained only after centrifugation, reached the lowest values observed. Figs. 266 and 267 show the changes for the same subject with and without physical exercise. These findings could explain observations indicating the importance of rest, after blood transfusions. With the direct transfusion method, where 500 cc. of blood was administered in less than 10 minutes, chill was seldom seen in patients resting quietly, while it was constantly seen to appear in subjects taking any exercise immediately following the transfusion. (Fig. 265)

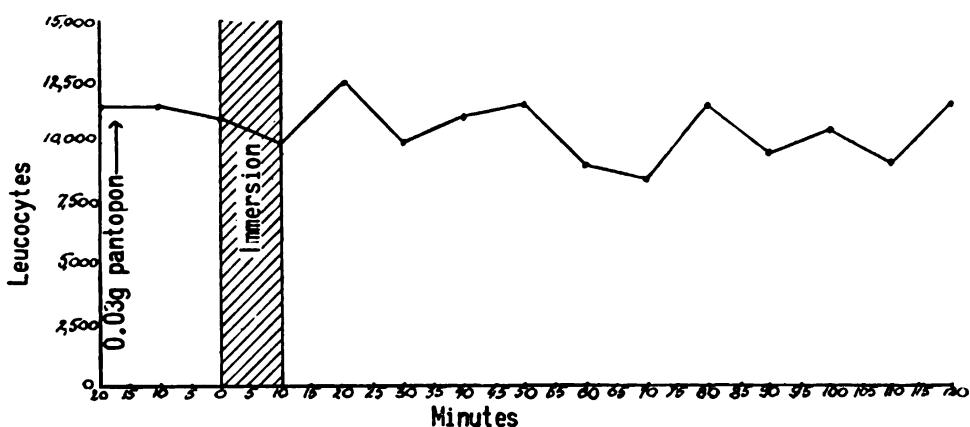


FIG. 268. The administration of 0.03 gm. Pantopon prevents the appearance of manifestations after the immersion of the hand in icy water, in the subject with hemoglobinuria a frigore.

This relationship of leucolysis to the pathogenesis of the diphasic complex phenomenon in hemoglobinuria a frigore was confirmed when the attack could be prevented by a pretreatment which influenced the leucocytes. The study of the influence exercised by various agents in vitro upon the leucocytes of a pleural effusion preparation from rabbits, when collargol solutions were added, has shown that only morphine and other opium alkaloids were able to prevent lysis. Adrenaline and quinine—among others—appeared inactive. Administered to patients with paroxysmal hemoglobinuria, these last substances did not prevent attacks. However, morphine in minimal doses, such as 5 mgr. ($1/12$ g.) or less, by intravenous injection, entirely prevented the development of any clinical manifestations as well as analytic changes. Minimal or no leucopenia was seen, and there were no changes in coagulation time, retraction of the clot, antitryptic power, refraction index of the serum, esterase, etc. (Fig. 268)

In addition to confirming the hypothesis that leucolysis intervenes in the

pathogenesis of the diphasic complex, the influence of morphine has furnished a means of preventing the attacks.

The presence of two or three successive diphasic phenomena in the case of paroxysmic hemoglobinuria attack has revealed another important relationship. As mentioned above, hemolysis has been seen to occur only in the first phase of the diphasic phenomenon. Hemoglobin disappears from the serum between the complexes. It is classically accepted that in cold hemoglobinuria two factors must intervene to produce hemolysis. One is sensitization of the red cells which results from the influence of cold; the other is the presence of the complement. It appears that while sensitization persists for a while after exposure to cold, hemolysis will occur when the second condition is also fulfilled. Complement is released in the first phase of the diphasic phenomena since it is during the first phase that hemolysis occurs through the changes induced by the lysis of the leucocytes. The correlation between these two processes is clear from the fact that, under the influence of morphine, leucolysis does not take place and consequently hemolysis is prevented.

Chapter 9, Note 2. Lipids and Rouleaux and Sludge Formation

Human blood obtained through venous puncture was mixed in the syringa with 1/10 of its volume of 1.5% sodium citrate, passed into several centrifuge tubes and separated into plasma and cells. The plasma from the various centrifuge tubes was placed in separate test tubes. One of these samples of plasma was treated with a mixture of conjugated fatty acids of cod liver oil, another with a lipoacid preparation of human placenta, a third with a preparation of the insaponifiable fraction of human placenta. As control, the plasma was treated with liquid paraffin. The mixtures, frequently shaken, were kept in a warm bath at 37°C for one hour, after which they were centrifuged and the oily material separated. The plasma treated with the lipoacids was then added in proportion of 10% to untreated plasmas which then were reunited with their red cells. The plasma treated with the insaponifiable fraction was mixed directly with its respective red cells. Plasma and red cells were shaken for five minutes and left at room temperature for another ten minutes. One small drop of this blood obtained with a platinum loop or a capillary pipette was mixed with two drops of saline on a slide covered by glass and examined under the microscope. While the controls showed few short rouleaux, the blood treated with insaponifiable fraction showed isolated cells. In the blood treated with placenta lipoacids, almost all of the red cells formed rouleaux; in blood treated with conjugated fatty acid, almost all cells formed sludges.

Chapter 9, Note 3. Dark Color of the Blood in Shock

In order to determine why dark-colored blood is seen in shock and the role of fatty acids, the following experiments were performed.

Venous blood of patients in severe state of shock was drawn and mixed

with 1/10 of its volume of a citrate solution. The hematocrit value was determined and saline added in order to bring it to the value of the normal blood. Through these blood samples, oxygen was passed for five minutes at the rate of 50 cc. per minute. At the same time, blood from a normal person was obtained and similarly treated. The changes in color of the two samples after cessation of oxygenation were compared. While the normal blood needed almost ten minutes to return to the previous color, the blood from the patient in shock was back to the deep dark color in less than three minutes.

Blood samples from subjects in shock were treated in vitro with unsaponifiable fractions and subsequently with oxygen for five minutes at the rate of 50 cc. per minute. While the color became red immediately, the time required for it to return to the previous dark color was entirely different from that of controls. As against a few minutes for controls, more than twelve minutes were required for the blood treated with insaponifiable fraction. From these experiments it appears that the dark color of blood in shock results from changes in the red cells and not because of impaired circulation, and that these changes can be related to the intervention of fatty acids. This was confirmed by the fact that the dark blood of shock patients, if treated in vitro with insaponifiable fractions of placenta, for instance, loses its characteristic color.

Chapter 9, Note 4. Induction of Acute Shock

Acute shock can be induced by intraperitoneal injections of mixtures of conjugated fatty acids. The preparation largely used was a 10% solution in oil of fatty acid of cod liver oil conjugated through treatment with KOH in ethylene glycol or in ethyl alcohol. For a rat of 200 grams, 8 cc. of this preparation injected at once was able to induce an acute shock.

Chapter 9, Note 5. Induction of State of Shock

State of shock was induced by the repeated administration of a mixture of conjugated fatty acids obtained from cod liver oil. To insure a progressive systemic absorption, the preparation was injected subcutaneously. The injection in rats of 1 cc. per 100 gram of body weight of the 10% solution of these fatty acids in oil, repeated every hour was seen to induce after 3-5 injections a state of shock. The addition of 4% of sodium thiosulfate in a dose of 5 cc. per 100 gram of body weight in rats, was seen to favorize the appearance of this state of shock.

Chapter 9, Note 6. Influence of Fatty Acids Upon Traumatic Shock

Rats of 250 grams were introduced in the Collip-Noble Drum with their forepaws taped with adhesive and submitted to 500 falls at a rate of 40 per minute. 50% died of acute shock in less than two hours. If 2 cc. of a preparation of 10% cod liver oil fatty acids in oil per 100 grams of body

weight was injected intraperitoneally or even subcutaneously $\frac{1}{2}$ hour before the animals were placed in the drum, more than 50% of the animals died during the trauma itself and the fatality rate in some experiments approached 100%. Bleeding from the nose and mouth was dark in color and smaller in quantity than in untreated animals. If the same amount of the lipoacid preparation was injected immediately after the animal came out of the drum, it also increased mortality within the first two hours. For some animals, death occurred in a few minutes after the injection.

Chapter 9, Note 7. Influence of Unsaponifiable Fractions Upon Traumatic Shock

The influence exerted by the unsaponifiable fractions upon traumatic shock appeared evident in rats submitted to 5-700 falls in the Collip-Noble drum. 10% solutions in sesame oil of the unsaponifiable fraction of human or cow placenta, of eggs or of butter, were used in these experiments. 1 to 5 cc. of these solutions were injected intraperitoneally at different intervals before or after trauma. The injection of 2 cc., $\frac{1}{2}$ hour before trauma, was seen to entirely prevent lethal shock (0/20) in a group of experiments where the mortality of controls was 18/20. The same results were obtained with 2-3 cc. of the preparations injected immediately after the animals were taken out of the drum. Doses as high as 5 cc. injected one hour after the animal was removed from the drum, protected only a few animals (11/20) and generally only those without symptoms of shock. Once the symptoms of shock were present, the effect of the unsaponifiable fractions was greatly reduced. (From 2/20 to 5/20 in different experiments.)

Chapter 10, Note 1. Oxalic Index

The need to have quantitative information about the amount of double bonds present in the organism or in its lipoacids has led to a method of analysis based on the fact that molecular breakdown or fission will furnish characteristic components. With fission occurring at the level of the double bonds, the fraction corresponding to a conjugated double bond will appear as oxalic acid. The problem was to obtain this fission with a carboxyl corresponding to each carbon and without having artificially induced displacements of the double bond which is a frequent result of treatment.

We employed the following technique. Fatty acids from an organism or any other preparation were neutralized with the exact amount of sodium carbonate necessary. This amount was established through the neutralization index of the substances to be treated. After sufficient dilution, an excess of sodium carbonate was added with the aim of obtaining an alkaline medium. After bringing the solution to 4°C potassium permanganate was added until further discoloration of the permanganate stopped, after which 20% more of the amount already used was added. The mixture was kept refrigerated at 4°C for 16 hours, after which the excess of permanganate was reduced by sodium bisulfide. The liquid obtained was filtered and the

precipitate washed. The liquid was extracted first with ether to eliminate the higher fatty acids, after which it was submitted to distillation in order to eliminate the volatile fatty acids. In the remaining part, the oxalic acid was precipitated with calcium chloride. From the precipitate, the part corresponding to calcium malonate was separated from calcium oxalate, by using the difference in solubility at the boiling temperature. The oxalic acid was then titrated in the usual manner. The amount of oxalic acid divided by the quantity of lipoacids used represents the oxalic index of the preparation. Carlos Huesca Mejia and Daisy Franco have widely studied the changes of this oxalic acid index in our laboratories.

Pure nonconjugated fatty acids treated in this manner yield no oxalic acid. When linoleic acid is conjugated (*e.g.* by treating with KOH in ethylene glycol) oxalic acid is found in the fission products in amounts that gradually change as the treatment continues. (TABLE XXXIV)

TABLE XXXIV

THE QUANTITY OF OXALIC ACID PRESENT AFTER OXIDATIVE FISSION AND IODINE NUMBER OF SAMPLES OF LINOLEIC ACID CONJUGATED FOR DIFFERENT PERIODS OF TIME. (LINOLEIC ACID MIXED WITH EQUAL QUANTITIES OF KOH: DIS- SOLVED 5% IN ETHYLENE GLYCOL; CONTINUOUS TREATMENT IN REFLUX.)

Time	Oxalic Acid mg/gm of Fatty Acid	Iodine Number
Before treatment	0	180
After 30 minutes	117	119.8
" 1 hour	205	115.1
" 2 hours	114.2	94
" 4 hours	119.4	96
" 8 hours	99.9	91
" 12 hours	92	86
" 24 hours	85	81.7
" 36 hours	80	76
" 48 hours	77.3	76.5
" 144 hours	40.8	57.3

The quantitative relationship between known proportions of conjugated fatty acids and the oxalic acid obtained through their oxidative fission has been studied.

Autolytic changes have been found to influence the nature of the fatty acids extracted from tissues. Studies have shown that formalin fixation does not significantly change the fatty acids present. (TABLE XXXV)

It has been noted that relatively stronger methods of conjugation, utilizing KOH at higher concentration and higher temperature, are necessary to conjugate the fatty acids of normal tissues, than are needed for the fatty acids extracted from pathological tissues (burn, shock, adrenalectomy, tumor necrosis) which can readily be conjugated by much milder procedures.

TABLE XXXV

EFFECT OF FORMALIN UPON THE QUANTITY OF OXALIC ACID PRODUCED BY OXIDATIVE FISSION OF A MIXTURE OF CONJUGATED FATTY ACIDS. (5 CC. ALIQUOTS OF A MIXTURE OF CONJUGATED FATTY ACIDS WERE MIXED IN A STOPPERED CYLINDER WITH 10 CC. OF A 20% FORMOL SOLUTION. THE CYLINDER WAS SHAKEN AND SAMPLES WERE REMOVED AT FREQUENT INTERVALS FOR DETERMINATION OF OXALIC ACID.)

Time	Mgm Oxalic Acid/gm Fatty Acids
Before mixing	121.7
After 1 hour	121.8
" 24 hours	120.5
" 48 hours	120.9
" 72 hours	118.9
" 144 hours	121.9

Chapter 10, Note 2. Irradiation and Oxalic Index

Forty male albino rats separated in groups of 10 were irradiated with nonfiltered X-rays from a 200,000 v. machine, receiving in one session a dose of 1500 r. which is considered a lethal dose. Similar experiments were repeated several times, some with the radiation dose obtained from radioactive cobalt. The four groups of animals were first mixed together and then separated in four big cages. The animals and controls were kept on Purina Chow and water ad libitum. Two of the controls, two of the treated animals, and any others approaching death were sacrificed daily.

Each dead animal was saponified separately. The total amount of acid lipids extracted was analyzed for conjugated fatty acids, using the method indicated previously in which the amount of oxalic acid which appears as the result of oxidative fission is measured. The values were expressed in oxalic index, which corresponds to the amount of oxalic acid in milligrams per gram of fatty acids. Figure 85 shows the results of two such experiments.

Values found for normals were zero or less than 0.5, but a constant increase of the oxalic index was noted after irradiation. While irregular values were still seen during the first three days, all were above 3 on the fourth day and continued to increase constantly afterward. Death occurred when the oxalic index reached a critical point which was found to correspond to 14-17 mgr. of oxalic acid per gram of the fatty acids of the entire body.

Chapter 10, Note 3. Oxalic Index in Sublethal Irradiation

We studied the changes in oxalic acid in animals treated with an amount of radiation below the lethal doses. The oxalic acid rose after treatment with values much lower than those seen when lethal doses were used. Three groups of ten animals each were radiated with 600 r., and every few days

two of the animals were sacrificed along with two of the controls. Figure 91 shows the values obtained for these animals. It could be seen that although indices of 6 and 7 were found, these never reached the critical point of 14, and that the indices went down in an irregular fashion. While some animals had values of around 4 and 5 after the tenth day, others had high values at the same time.

The administration of polyunsaturated conjugated fatty acids (1-2 cc. daily of a 5% oily solution of conjugated cod liver oil fatty acid) induced death in a high proportion (16/20) of animals irradiated with otherwise nonlethal doses such as 800 r. In these animals the oxalic index was high, often showing values above 17.

Chapter 10, Note 4. Radiation-induced Offbalances

Through the routine analytical technique used for the study of the offbalances, we studied the changes occurring in a group of 56 subjects with different conditions, submitted to radiotherapy alone. We tried to correlate the clinical noxious effects of radiation to these offbalances.

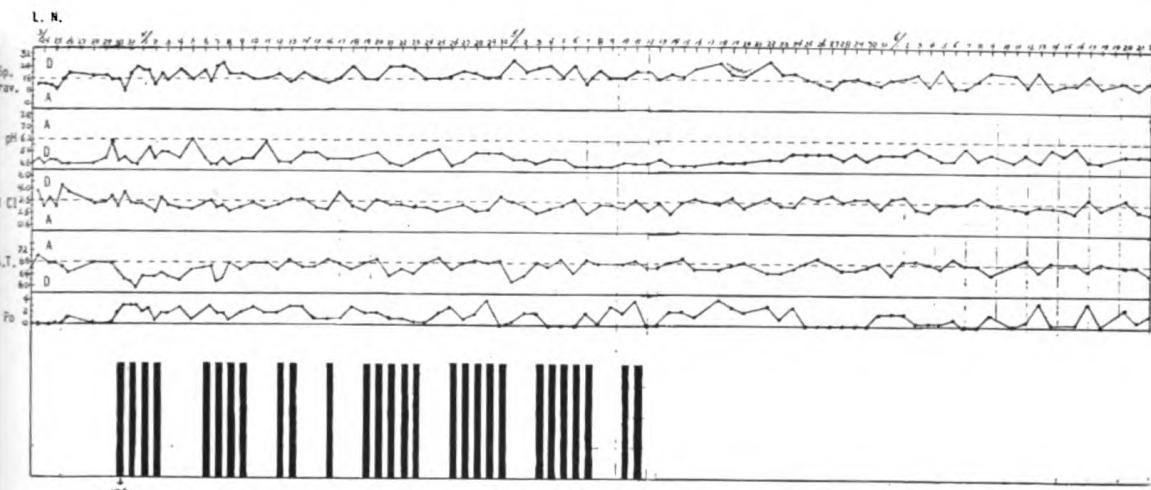


FIG. 270. L.N., 59 years old. Cancer of the breast. Mastectomy two months earlier. No recurrence. Treated with radiation, 125r per seance. Entirely uneventful except moderate erythema. Minimal or no changes in the analyses.

In this part of the study, we limited ourselves to the consideration of the immediate changes. Figs. 270 and 271 show the analyses in two cases in which no clinical, local or systemic changes were observed during and after the radiation. It can also be seen that no changes occurred in the analyses; they remained within normal limits during the entire period of observation. It is especially noted that the peroxides were present, almost constantly in high amounts in the urine.

Fig. 272 shows a case who died during the radiotherapeutic treatment, probably directly influenced by it. All the analyses show that a change to-

ward offbalance type D had occurred. Among these, we want to single out the urinary surface tension and the urinary chloride index, both evidencing very manifest changes toward the patterns corresponding to type D. The peroxides were on the contrary almost completely missing from the urine.

Another case (*Fig. 273*) had severe clinical reaction to radiation which persisted until the death of the patient. Again, we judged these effects of radiation mainly through the changes occurring in the two analyses, urinary surface tension and chloride index, which show the same shift toward a strong offbalance type D. The peroxide reaction became negative at the end.

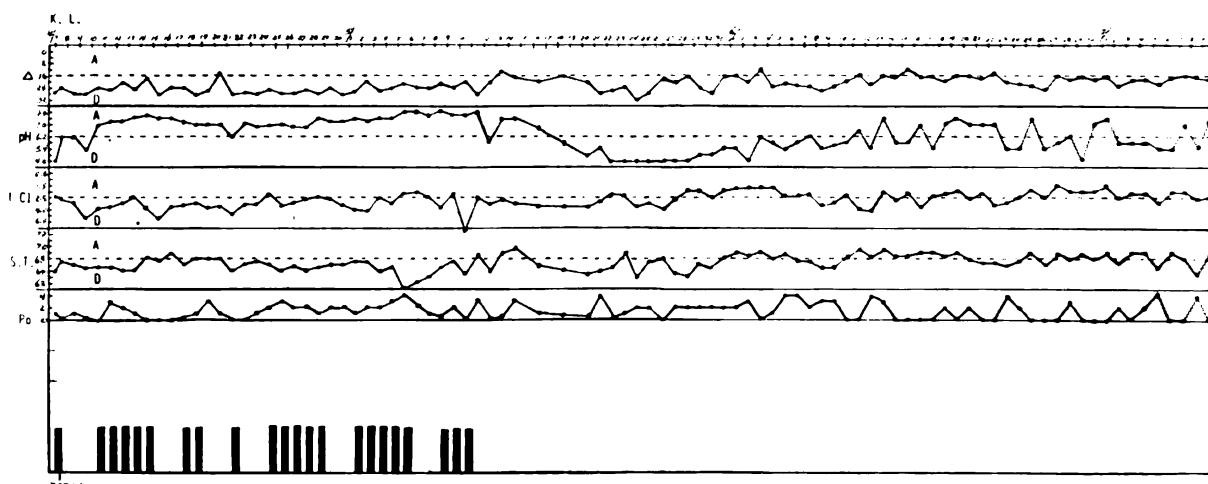


FIG. 271. K.L. 52 years old. Cancer of the hypopharynx. Treated with 300r for each session. The high specific gravity shows a tendency toward the type A, a month after the completion of the treatment.

While specific gravity and ST show a slight offbalance D during the treatment and the pH a manifest change toward the type D, following the radiation all the analyses show a change toward the type A, one month after completion of the radiation. Peroxides persisted in the urine. Clinically the entire evolution was eventless.

When the different tests were discussed, we mentioned that each one of the analyses used furnished information concerning changes which take place at a specific level of the organization. This would explain why the noxious effects are seen to be serious when the changes take place concomitantly in different analyses, that is, at different levels, indicating thus a more complete offbalance. When this concomitance does not exist, when the abnormal patterns concern only one analysis, the clinical manifestations are seen to be less serious. This was seen true in the case shown in *Fig. 274*. The importance of this concomitance in the changes present is seen in *Fig. 275*. In this case, although manifest changes corresponding to type D are seen for some analysis, they do not coincide. The peroxide reaction in the urine is constantly positive. This seems to permit the patient to withstand the noxious effect of radiation. The evolution of the changes induced by radium application is shown in *Fig. 276*.

The possibility to evaluate through urine analyses the noxious effects of radiations, has appeared especially important for the prevention and even treatment of the serious inconveniences during radiation therapy. By observing the changes in the analyses, particularly of the urinary surface tension and chloride index, valuable information can be obtained, permitting one to guide the application of these therapeutic agents. By being easily and continuously informed about the occurring changes, we need no longer consider the amounts of radiation to be administered as standard values for each patient. Through analytically guided radiotherapy one can replace the common pattern presently used for all patients by

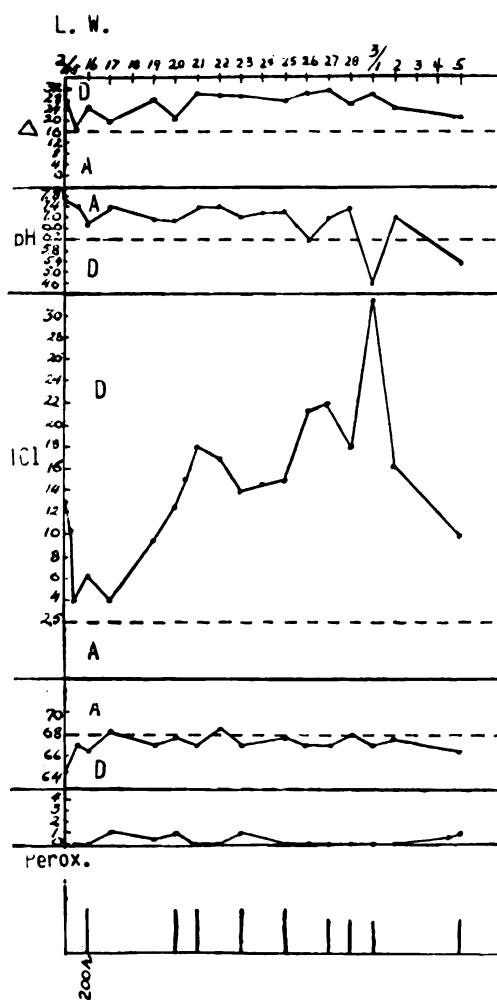


FIG. 272. L.W., 58 years old. Cancer of the lung. After receiving only 1000r, very weak. Much worse after, with very rapid downhill course. After the treatment on 3/5, the patient entered into coma and died 3/7. The extremely high values for Cl I and high specific gravity characterize the analytical changes. It is interesting to note the negative reaction for peroxides for almost the entire time.

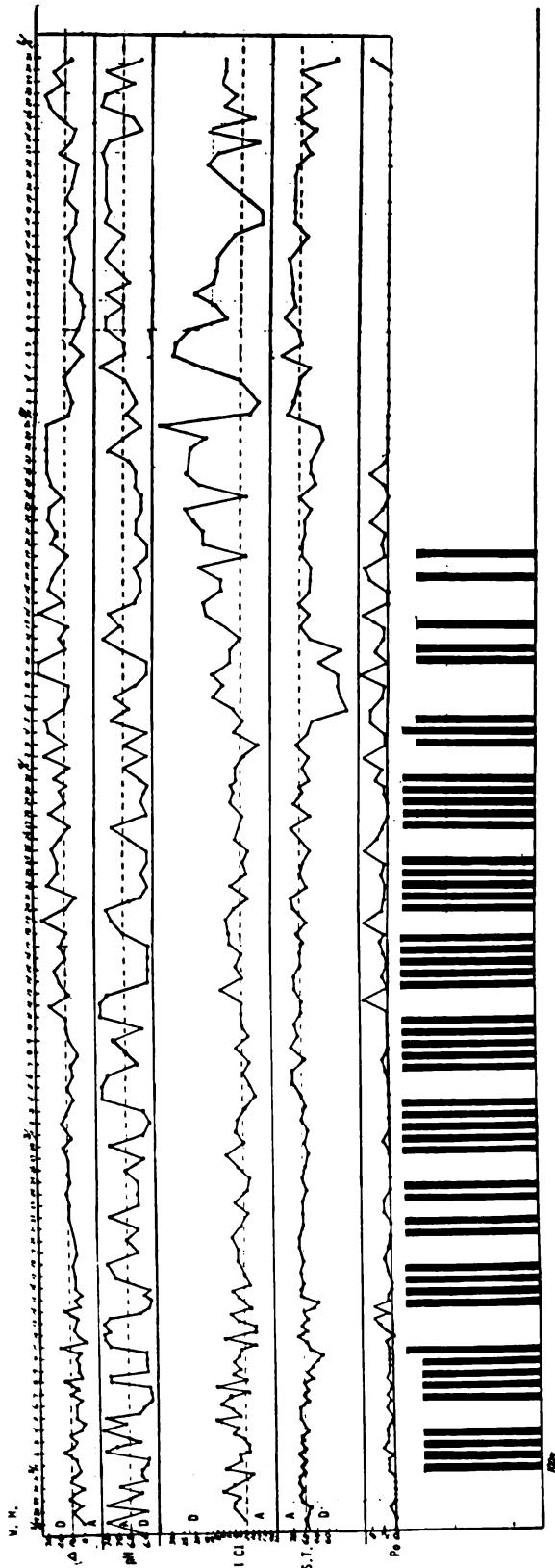


FIG. 273. W.M., 50 years old. Cancer of the ovary with abdominal metastases. Repeated doses of 100 and 120r. Almost uneventful for more than 1½ months of treatment after which the specific gravity analyses pass into the D pattern. After two months, the Cl index and ST show manifest concomitant changes toward the D pattern, a fact which coincided with a worsening of the general condition. In spite of changes in these analyses toward more normal values, the condition worsened with a lethal issue. The peroxide negative reaction—of bad prognostic in radiotherapy—is to be noted.

individualized treatments adapted to the need and the response of each subject.

Continuously followed analyses permit their utilization as a guide for more general application of radiotherapy. When the surface tension and chloride index remain within normal values, treatment can be continued with administration of doses above those originally intended, without any danger of serious noxious effects. A change toward low surface tension

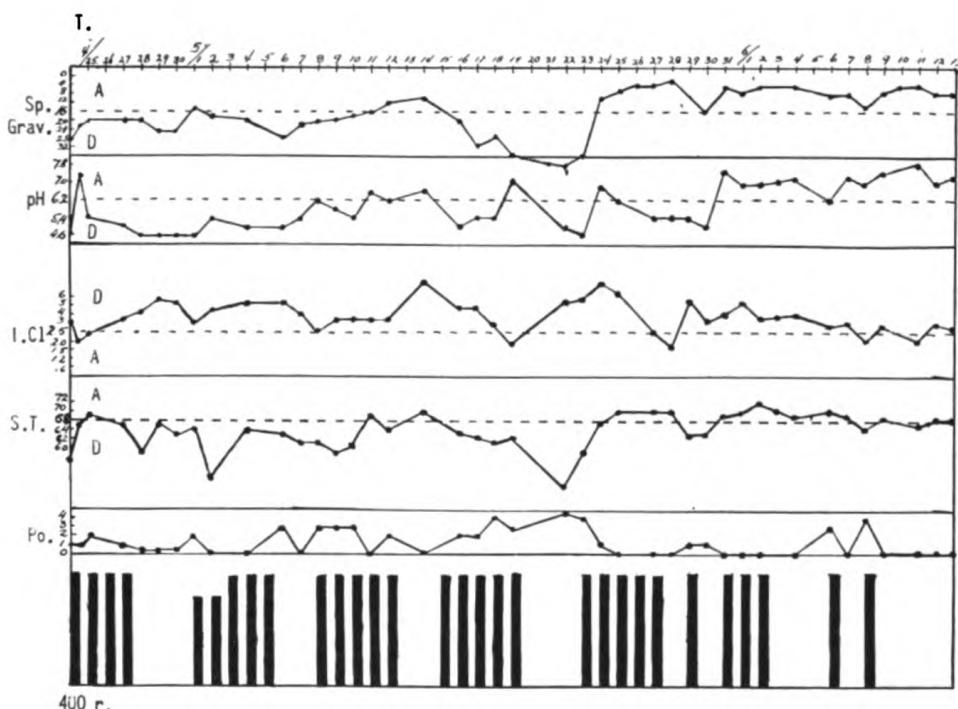


FIG. 274. Cancer of the cervix intensively irradiated. Although changes occur in the analyses, the passages in offbalance D do not coincide in the different tests. With the continuation of the treatment, the patient passes in offbalance A. No clinical, noxious manifestations were seen.

below 65 dynes/cm. or high chloride index above 5, represents a warning which should not be ignored. The treatment should be discontinued, the dosage reduced or the sessions more widely spaced, even if the desired radiation dose has not yet been attained. Concomitant changes of the analyses should constitute a serious warning even when the general clinical condition does not indicate any abnormality. The bad prognosis of persistent strong "D" offbalance during radiation is related to the progression of the anomaly as described in the experimental studies. For this reason, a persistent strong offbalance D seen for the urinary surface tension and chloride index indicates the need for the administration of lipoids with positive character, even if the clinical manifestations are not too serious.

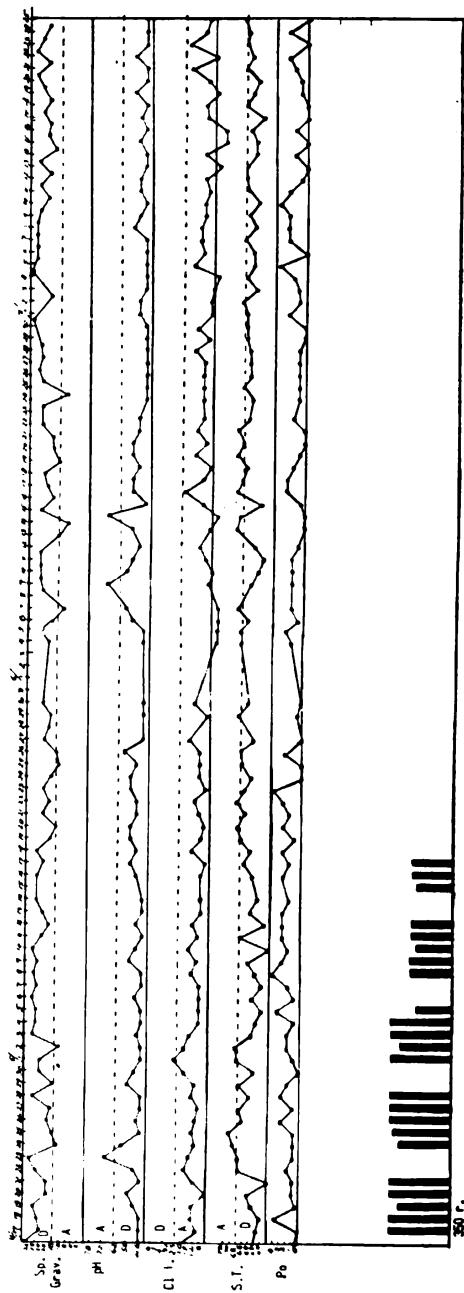


Fig. 275. Cancer of the larynx. No changes in the analyses. Entirely without clinical noxious reactions. (The Cl Index values are inverted.)

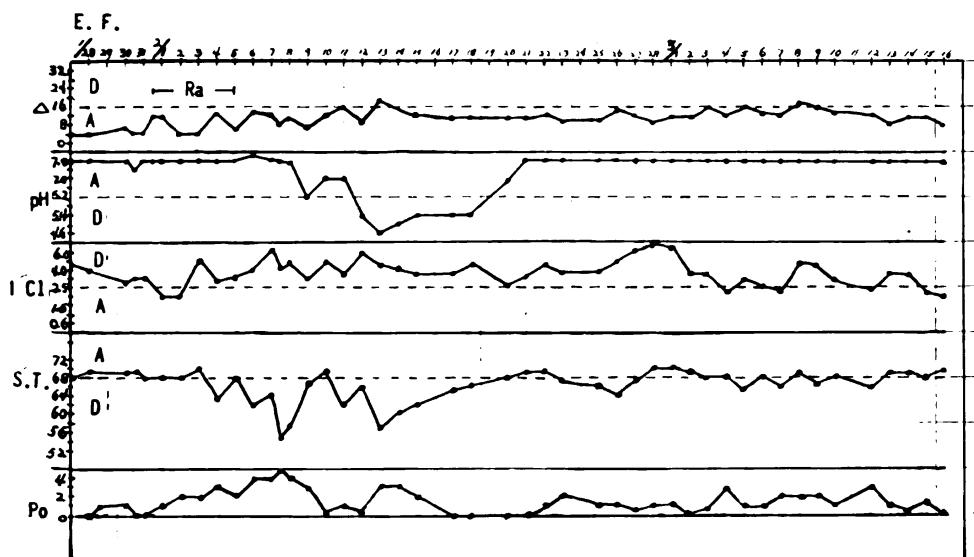


FIG. 276. F.E., 64 years old. Cancer of the fundus of the uterus, treated with 45 mgr. radium for 122 hours in situ, with a total of 5490 mgr. hours. Felt subjectively well after radiation without any complaint. The analyses show a manifest change for almost one week from the type A to type D. For the S.T. it starts 2 days after the insertion of radium with the values dropping from 70 to 55. For the pH, the change started a few days later with the values passing from 7.8 to 4.6. The changes of Cl I show values as high as 7 without however, having the changes coincide with the pH and S.T. 16 days after radium was taken out, the analyses went to previous values, except the peroxides, which remain present.

Chapter 11, Note 1. Carcinogenic Activity of Urethane

The interesting research of Berenblum has brought an important contribution not only for the largely debated role of urethane as carcinogen, but also for the problem of carcinogenesis in general. The fact that croton oil, applied to the skin, induces the appearance of malignant tumors in animals previously fed with urethane, concords largely with the concept of plural changes taking place in carcinogenesis. The analysis of the influence exerted by carbamic acid upon amino-acids would place the intervention of this agent at the first members of the biological realm. It can thus be seen that the bond between the amino-acid group and the carboxyl and amine groups of carbamic acid occur in a way similar to that which occurs between two amino-acids with the big difference that in the first case it would result in the appearance of the CNCN formation. (Fig. 277) As mentioned above, this CNCN formation represents the group which characterizes the first biological entity. The place of this CNCN group, not at the end of the molecule opposed to the carboxyl as in the alkaline amino-acids, but as corresponding to the bond which results in polymers, represents the anomaly, which according to the work hypothesis we advance, would correspond to the first cancerous entity. The fact that the carcino-

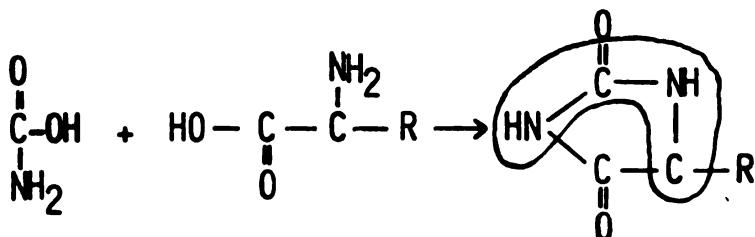


FIG. 277. The bond between the carbamic acid and an amino-acid group leads to the formation of the NCNC group.

genic activity of urethane takes place at the lowest levels of organization, explains the necessity that a certain time separates its intervention from that of croton oil, which would act only at the higher levels, probably inducing the passage from noninvasive to invasive phase. This time is necessary for the first cancerous changes to build up the series of cancerous hierarchic entities since the cocarcinogen, croton oil, would act only in those more evoluted cancerous entities. In experiments in course, the passage of the noninvasive urethane-induced carcinogenesis entities into invasive cancer, is successfully induced by preparations of unsaponifiable fractions.

Chapter 11, Note 2. Constitution of Viruses

It is superfluous to emphasize the interest with which viruses are being studied from all points of view. Their role in carcinogenesis has placed them in the limelight of cancer research, and any contribution concerning their constitution or activity is of great interest. A much debated initial problem concerns the nature of viruses and their place among the other entities. (293)

Two fundamental groups of constituents—DNA and proteins—(301, 289) have been recognized to take part in the formation of the viruses. These two groups could be separated and reunited, reproducing the original virus with all its characters. Furthermore, new viruses could be created when fractions resulting from different viruses were bound together. (289) However, the fact that a part of the virus, the DNA fraction, was seen to be furnished by the constituents of the host cell, and the protein directly by it (312), has raised the question of the nature of the virus itself. Some workers have gone so far as to see the viruses as parts of the constituents of the cells. By considering the viruses in the concept of the hierarchic organization presented above, a new aspect emerges.

According to this organizational concept, a virus represents an entity that has reached a certain step in the hierarchic evolution, and remained there throughout its individualization. Like all entities, a virus can be conceived to be formed by a principal part bound to a secondary part, the ensuing entity limited by a boundary formation. The principal part would be formed by a group of immediately inferior entities in the hierarchic

scale. In the case of viruses, such inferior entities would be formed by what we could call "proviruses," which correspond to characteristic DNA formations. The principal part of the virus would be formed by the grouping together of provirus entities, proper to each virus. The secondary part is conceived to result from the immediate environment of these entities, taken directly from the host's own protoplasmatic or nuclear formation in which the principal parts are present as free entities. This secondary part is represented by the protein fraction furnished as such by the invaded nucleus or cell protoplasmatic formations. This protein fraction conserving its characteristics can be recognized and identified.

Having nuclear formations, nuclei and protoplasmatic formations as their environment, the principal part of the virus, the proviruses, multiply as proper hierarchic entities. These proviruses will leave the host usually when the cell bursts, bound this time to the proper secondary parts directly furnished by the host entity. Under these conditions, the principal part multiplied in the protoplasmatic formations or in the nucleus and the secondary parts, furnished as such by the host, would form an immediately higher entity, the virus. In the multiplication of the virus (299), the pattern followed is the same as that of other hierarchic entities. This has to occur in the proper environment which, for the viruses, is the immediately higher hierarchic entity, the nuclear level. This is represented in microbes by the individual itself, and in cells by the nucleus or by the protoplasmatic formations, which we consider to belong to the nuclear level, due to their ribonucleo-proteins. It is in these nuclei or protoplasmatic formations that the viruses multiply. This explains the development of viruses in cells in compact groups, which would correspond to parasitized protoplasmatic formations and not in a diffuse form in the cytoplasma. The virus loses its secondary part upon entering the entity where it will multiply, but will take it back when it leaves its host, becoming again the entity, virus. The parasitized entity has contributed two parts to the multiplication of the virus—one, indirectly, by furnishing material which will be utilized by the provirus and transformed into its specific DNA, and two, the secondary part, directly formed by its own proteins. Like all the secondary parts, that of the virus directly furnished by the host will surround the group of proviruses forming the principal part.

Chapter 12, Note 1. Lipids and Cytolytic Activity of Sera (245, 246)

The fact that cancer cells are found circulating in the blood without inducing metastases throughout the body, as might be expected, has made various workers investigate the means by which the organism rids itself of these cells and which might represent part of a general process through which the organism could fight cancer. Research to confirm the capacity of blood sera for influencing the lysis of cancer cells has been carried on in our laboratories by Robert Willheim and co-workers. Sera of both normal individuals and cancer patients were found to induce lysis of cancer cells in vitro. Ehrlich, Krebs and Sa 180 ascites in mice were used, as well

as cells obtained from solid tumors with encephaloid character. Tumors obtained from biopsies or autopsies of human beings also were used. Lytic activity was determined through the dilution of sera which later was mixed with a suspension of a known number of cells. After incubation for one hour

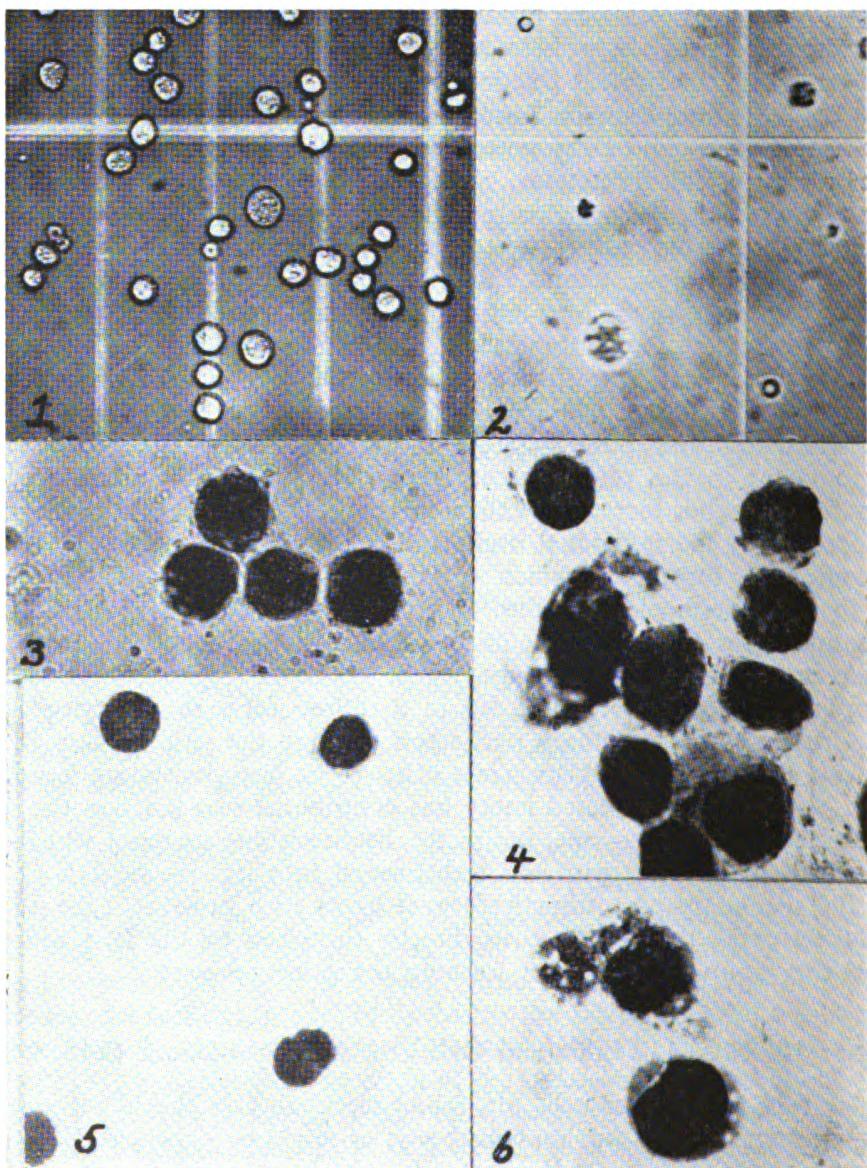


FIG. 278. Lytic action of human serum upon Sa 180 ascites cells.

- (1) Normal SA 180 Cells X 300, not stained.
- (2) Lytic effect of Serum on SA 180 X 300, not stained.
- (3) Normal SA 180 Cells X 1000, Giemsa stain.
- (4) Lytic effect of Serum on SA 180 Cells X 1000, Giemsa stain.
- (5) Normal Krebs Cells X 1000, Giemsa stain.
- (6) Lytic effect of Serum on Krebs Cells X 1000, Giemsa stain.

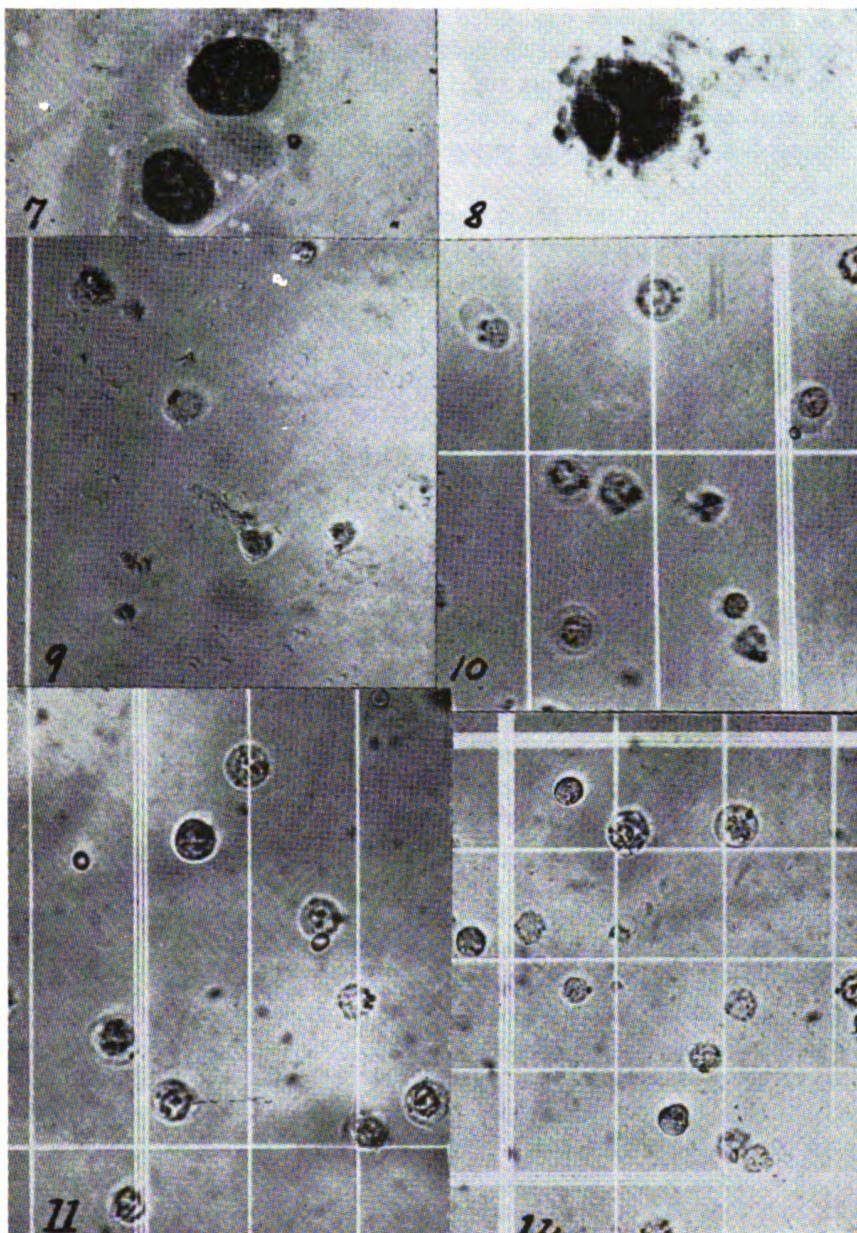


FIG. 279. Continuation—

- (7) Normal SA 180 Cells X 1800, Giemsa stain.
- (8) Carcinolytic effect of Serum on SA cells X 1800, Giemsa stain.
- (9) Normal Lysis of SA 180 Cells as control in Polysaccharides Inhibition-Studies on Carcinolysis X 300, not stained.
- (10) Effect on Carcinolysis by the Polysaccharide Dextran X 300, not stained.
- (11) Effect of Levan on Carcinolysis. Note the clearly distinct outline of unaffected cells X 300, not stained.
- (12) Effect of Mucin on Carcinolysis X 300, not stained (evidence of inhibited Lysis).

at 37°C the product was examined and the cells counted in an adequate chamber. (*Fig. 278*) Relatively big variations were seen in different individuals, the results being negative for 1:1 dilution in some cases and positive in 1:32 and even higher for other individuals. No correlation could be established between this lytic power and the clinical condition of an individual.

With R. Willheim and M. Auber (247) we showed that the addition of a suspension of unsaturated fatty acids is able to induce such a lytic property in sera without this capacity, while the addition of insaponifiable fractions or of cholesterol inhibited lytic activity. With R. Willheim and M. Auber we (248) have investigated the problem of the correlation between the lytic activity and the structure of different fatty acids and their sodium soaps. The higher members of the fatty acid series have shown intense lytic activity. It could be shown that the lipoidic character of the fatty acids increases their lytic capacity. The value of the lipoidic character of the fatty acids used has appeared when members with the same number of carbons but having an hydroxyl or carboxyl in their molecule, were tested. With disappearance of lipoidic character, lytic property disappeared. The research covered not only cancer cells but also liver, red blood cells and lymphocytes. It could be seen that a correlation exists between the rapid growth character of these cells and the capacity of the fatty acids to attack them. It is interesting to note that cancer cells treated *in vitro* with fatty acids having lytic activity no longer produced cancerous growths when transplanted to animals. (249)

Chapter 12, Note 2. Fatty Acids Transportation in the Blood

It appeared especially interesting to study the distribution and transport of polyunsaturated fatty acids in the body in view of the fact that parenteral administration of the acid lipidic fraction of various organs to subjects with acute pain had an effect within only a few minutes. The rapid action was independent of the nature of the induced change, that is, decrease of the intensity of pain of acid pattern, and increase of pain of an alkaline pattern. This effect in opposite directions, occurring after the same short interval and seen in hundreds of cases, eliminated the possibility of a psychological factor, as suspected at the beginning.

The fact that the change occurs at the level of the painful lesion itself raised the question of rapid transport between the injection site and the lesion. In order to investigate it, we used two fatty acid preparations containing easily identifiable substances, norbixine and polyconjugated fatty acids. Norbixine could be identified by its characteristic color while the polyconjugated fatty acids were identified by their specific curves in spectral analysis in ultraviolet light.

Adult New Zealand rabbits were injected intraperitoneally with 8 cc. of 0.3% solution of bixine in sesame oil. The injected animals were bled at different intervals by heart puncture. The red cells were separated from the plasma by immediate rapid centrifugation. Each fraction, plasma and red

cells, was hydrolyzed separately with 5% KOH. The acid lipidic fractions obtained as a solution in benzene was passed through a chromatographic column with alumina. Bixine was easily recognized because of its red color. After elution with chloroform the amount present was determined photometrically. Similar experiments were made by using a 10% solution of eleostearic acid in oil. The acid lipidic fractions obtained separately from the red cells and the plasma were submitted to spectral analysis and the presence and amount determined by the characteristic peaks. Both nor-bixine and eleostearic acid were seen to appear in the red cells in less than two minutes, the amount increasing rapidly.

A marked difference was found between the amount of these fatty acids in the red cells and in the plasma of the same blood, for all the samples. The red cells contained 5 to 6 times as much of the injected lipids as the plasma. This unequal distribution, also seen for other highly unsaturated fatty acids, indicates an important physiological role for the red cells which has not been recognized before. Red cells appear to be preferred vehicles for transporting polyunsaturated fatty acids through the blood.

Chapter 12, Note 3. Conjugation Method

Spectral analysis of a mixture of fatty acids such as obtained from cod liver oil has shown that prolonged conjugation sometimes is detrimental for some members. Prolongation of conjugation was found necessary, however, since the members with a lower number of conjugated double bonds needed more time to appear. We investigated the factors which would intervene in these changes. High temperature was seen to affect the polyconjugated formations. With ethylene glycol or glycerol as solvent, conjugation took place rapidly, but the peaks of tetraenes and especially pentaenes and hexaenes were seen to go down rapidly. This was not seen to occur if the temperature of conjugation was lower. In this last case, the conjugation was seen to take much more time. This study led us to use ethyl alcohol as a solvent. Maximum conjugation however, required a longer time, usually around 100 hours. With this method we could obtain from the same preparations much higher amounts not only of conjugated pentane and hexane but also diene and triene. (Fig. 280) We also utilized the same method for analytical purposes with the same good results.

Chapter 12, Note 4. Quenching Action and Anti-Carcinogenic Effect of Conjugated Fatty Acids

We have investigated the influence exerted by different fatty acids, conjugated and nonconjugated, upon various carcinogens. In a first group of experiments we studied this influence *in vitro*, and chose the quenching of the fluorescence of the carcinogen as criterion of activity.

This part of the research was made in collaboration with C. Huesca-Mejia and P. Teitelbaum.

The quenching effect has been studied as follows:

The fluorescence of carcinogenic hydrocarbons is measured by means of the fluorescent attachment to the Beckman spectrophotometer using a wave length around $365 \text{ m}\mu$. The sensitivity of the apparatus is adjusted to show a value of 100 for the fluorescent light utilizing the concentration of the hydrocarbon having the maximum fluorescence. The carcinogenic hydrocarbons are dissolved in alcohol, iso-octane or cyclohexane, the last two being purified and thus rendered optically inactive by passage through a silica column.

The fatty acids are dissolved in varying dilutions in the same solvents and added to the solution of hydrocarbon carcinogen which has been previously chosen to give a fluorescent value of 100. The fluorescence of the mixture is immediately determined. The quenching effect of the fatty acid is shown by the percent of the residual fluorescence of the carcinogen when mixed with different solutions of a mixture of conjugated fish oil fatty acids. TABLE XXXVI shows the quenching effect of mixtures of fatty acids conjugated by treatment with KOH upon different carcinogenic and related hydrocarbons.

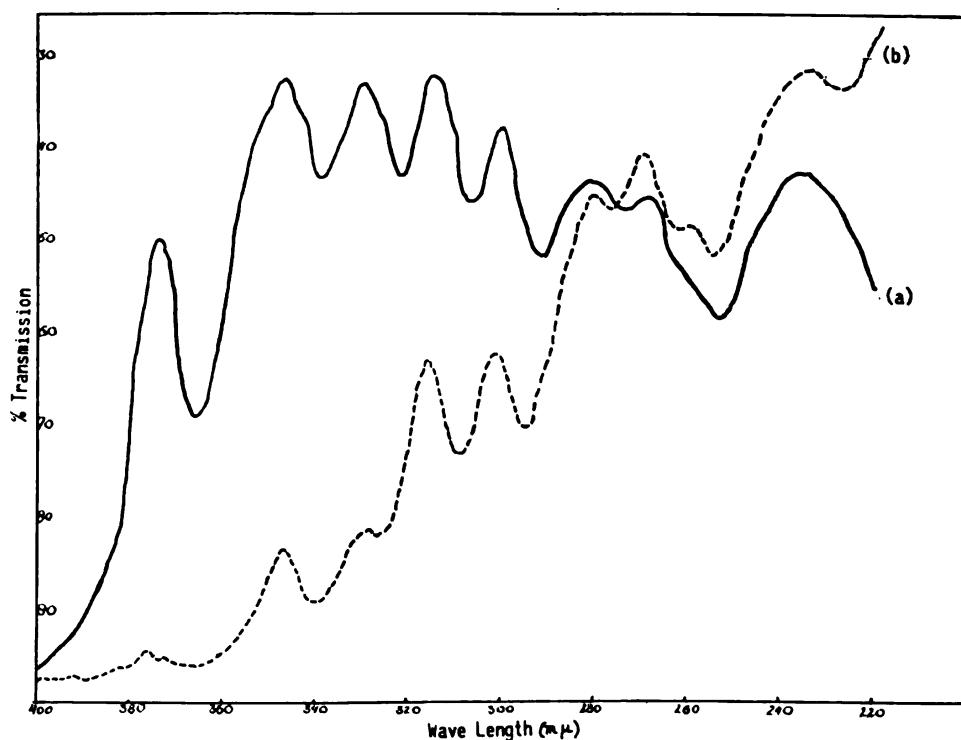


FIG. 280. Conjugation method. The use of ethylic alcohol as solvent for conjugation changes the amount of polyconjugated members obtained for cod liver oil fatty acids (a) as compared with those obtained when the conjugation is made at higher temperatures using ethyleneglycol, (b) glycerol or other solvents.

Dilution 0.002% in ethyl alcohol.

TABLE XXXVI

QUENCHING EFFECT OF CONJUGATED FATTY ACID MIXTURES UPON THE
FLUORESCENCE OF CARCINOGENIC AND RELATED HYDROCARBONS

Hydrocarbon	% Concentration	Quenching Agent	% Residual Fluorescence
Methylcholanthrene	0.0062	Mixture of conjugated fatty acids from cod liver oil (0.1% solution)	25.0
3, 4 Benzpyrene	0.0062	"	36.0
1, 2 Benzanthracene	0.0031	"	24.0
1, 2, 5, 6 Dibenzanthracene	Sat. Sol. (alcohol)	"	15.2
9, 10 Dimethyl -1, 2 Benzanthracene	0.0062	Mixture of conjugated fatty acids from fish oil (0.2% solution)	23.0
Benzanthracene 12 ol - 7 Methylacetate	0.005		5.7
7 Chloro-10 Methyl -1, 2 Benzanthracene	0.005		7.9
1 Cholanthrene -3 Methyl	0.005		10.0
Benzo (d) Pyrene -5 Methyl	0.005		11.2
3, 10 Dimethyl -1, 2 Benzanthracene	0.001		6.5
7 Cyano-10 Methyl -1, 2 Benzanthracene	0.0057		4.0
5 Chloro-10 Methyl -1, 2 Benzanthracene	0.0025		5.8
6 Chloro-10 Methyl -1, 2 Benzanthracene *	0.01		5.2
4 Methoxy-3, 4 Benzpyrene	0.005		13.0

* Fluorescence set at 45.

TABLE XXXVII
QUENCHING EFFECT OF VARIOUS NONCONJUGATED AND CONJUGATED FATTY
ACID MIXTURES UPON THE FLUORESCENCE OF CARCINOGENIC
AND RELATED HYDROCARBONS

Hydrocarbon	% Concentration	Conjugated Fatty Acids from Cod Liver Oil		B-Eleostearic Acid		Conj. Linoleic Acid		Oleic Acid
		0.1% 92.5	0.1% 25.0	0.1% 95.8	0.1% 95.0	0.1% 89.8	0.1% —	
Methylcholanthrene	0.0062	0.0062	0.0062	0.0062	0.0062	0.0062	0.0062	0.0062
3, 4 Benzpyrene	0.0031	0.0031	0.0031	0.0031	0.0031	0.0031	0.0031	0.0031
1, 2 Benzanthracene	Sat. Sol. (alcohol)	89.0	93.4	24.0	94.8	100.5	97.0	100.8
1, 2, 5, 6 Dibenzanthracene	0.0062	90.8	15.2	100.0	97.0	98.0	100.0	100.0
9, 10 Dimethyl-1, 2 Benzanthracene			23.0	97.8	93.5	100.0	96.0	96.0
				Fish Oil				
			0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Benzanthracene 12 ol -7 Methylacetate	0.005	66.0	5.7	89.0	87.0	80.0	92.1	
7 Chloro-10 Methyl -1, 2 Benzanthracene	0.005	67.0	7.9	84.0	85.0	87.0	87.0	
1 Cholanthrene -3 Methyl	0.005	110.0	10.0	110.0	110.0	110.0	110.0	
Benzo (d) Pyrene -5 Methyl	0.005	88.0	11.2	88.0	70.0	90.0	91.0	
3, 10 Dimethyl -1, 2 Benzanthracene	0.001	72.0	6.5	63.0	78.0	74.0	73.0	
7 Cyano-10 Methyl -1, 2 Benzanthracene	0.0057	83.5	4.0	91.0	90.2	88.5	91.0	
5 Chloro-10 Methyl -1, 2 Benzanthracene	0.0025	89.0	5.8	92.0	95.0	92.0	93.5	
6 Chloro-10 Methyl -1, 2 Benzanthracene *	0.01	47.0	5.2	43.0	45.0	42.0	45.0	
4 Methoxy -3, 4 Benzpyrene	0.005	88.0	13.0	86.0	90.0	89.0	91.0	

* Fluorescence set at 45.

Conjugated Fatty Acids and Quenching

Nonconjugated fatty acids such as linoleic acid, linolenic acid, arachidonic acid, mixed fatty acids from body liver oil and cod liver oil have a limited quenching action. Conjugated dienes such as isomers of linoleic acid or conjugated trienes such as eleostearic acid obtained through conjugation of linolenic acid or extracted from China wood oil also have a limited quenching effect upon hydrocarbon carcinogens and related compounds. (TABLE XXXVII) The same is true for mixtures of conjugated dienes and trienes.

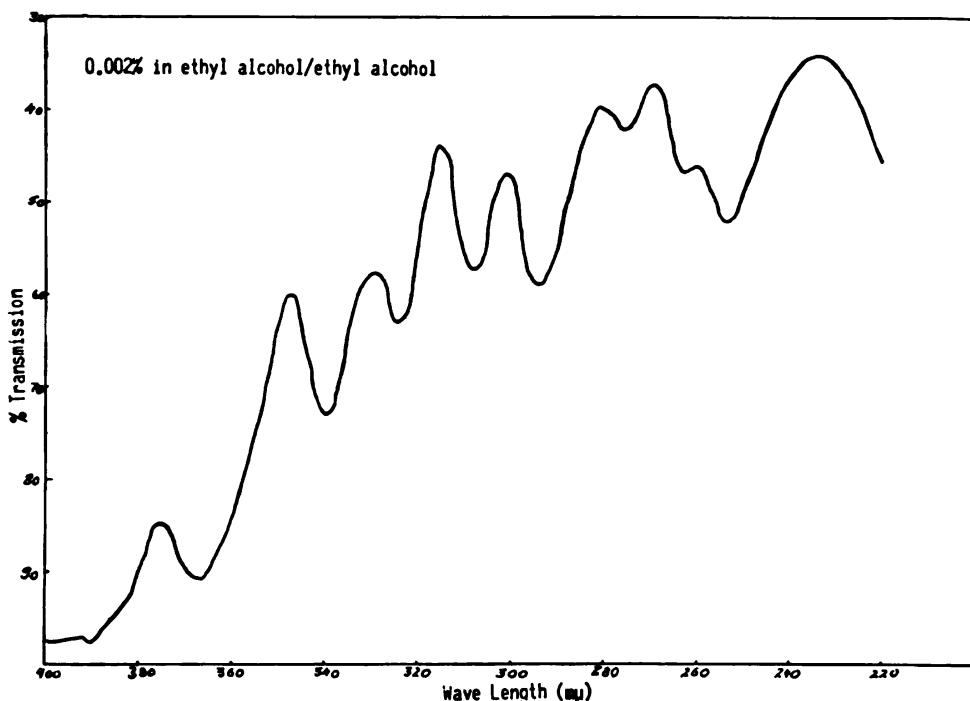


FIG. 281. Spectral analysis of conjugated cod liver oil fatty acids shows the presence of conjugated di-, tri-, tetra-, penta- and hexaenes.

Fatty acid mixtures having conjugated di-, tri-, tetra-, penta- and hexaenes as shown by spectral analysis (Fig. 281) have been found to exhibit a high degree of quenching activity (Fig. 282) (TABLE XXXVII) when mixed with hydrocarbon carcinogens.

The quenching action of fatty acids upon the fluorescence of hydrocarbon carcinogens appears to be nonadditive. When the incident ray is passed first through an 0.2% solution of conjugated fish oil fatty acids in alcohol and then through an 0.012% solution of methylcholanthrene in alcohol in separate vessels, the residual fluorescence is 81%. When the same two solutions are mixed together in one cell, the residual fluorescence is 11.2%.

The relationship of the different conjugated members to the quenching effect has been studied. Fatty acid mixtures having *different* proportions of isomers with 2, 3, 4, 5 and 6 conjugated double bonds were obtained by conjugation or by treatment of conjugated mixtures with heat, oxygen,

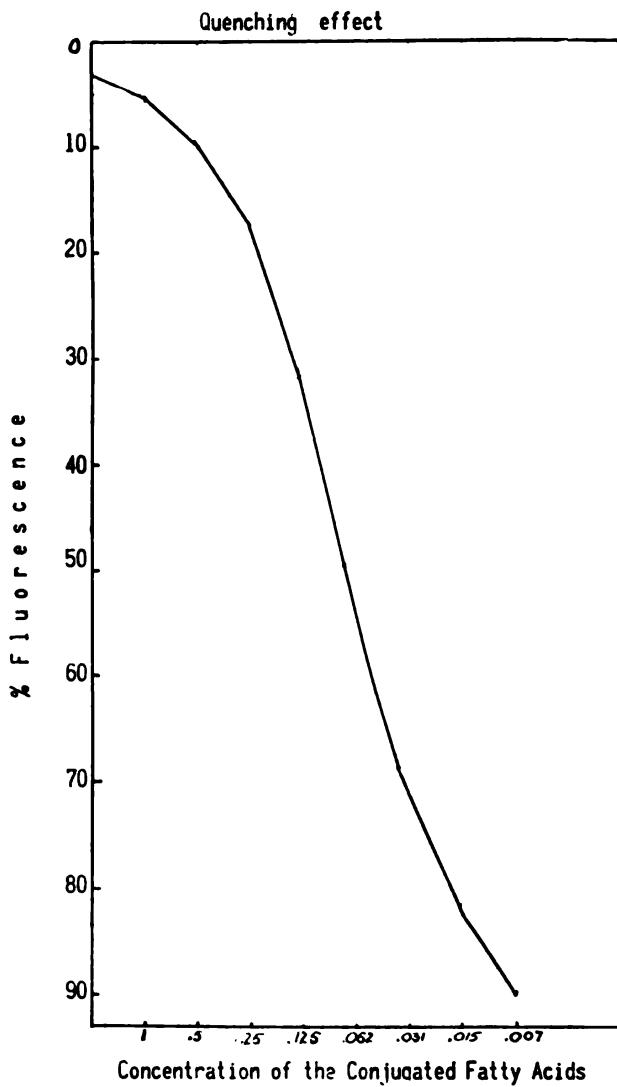


FIG. 282. Quenching of fluorescence of a methylcholanthrene (.0062%) solution in alcohol by different concentrations of conjugated fish oil fatty acids.

chlorine or sulfur. Changes in the proportions of the di-, tri-, tetra-, penta- and hexaenes were followed by means of spectral analyses. The changes in the height of the peaks in these curves corresponding to the different conjugated polyenes were then compared with the changes in the quenching effect of the corresponding fatty acid mixtures. Figure 283 shows the spec-

tral analysis of samples obtained at various intervals during the action of oxygen upon a mixture of conjugated fatty acids. As seen, oxygen induces unequal changes in the height of the peaks in the curves of which correspond to di-, tri-, tetra-, penta- and hexaenes. Fig. 284 shows the quenching activity of the mixtures. It can be seen that a parallelism exists between the relative proportions of the tetraenic component and the quenching activity of

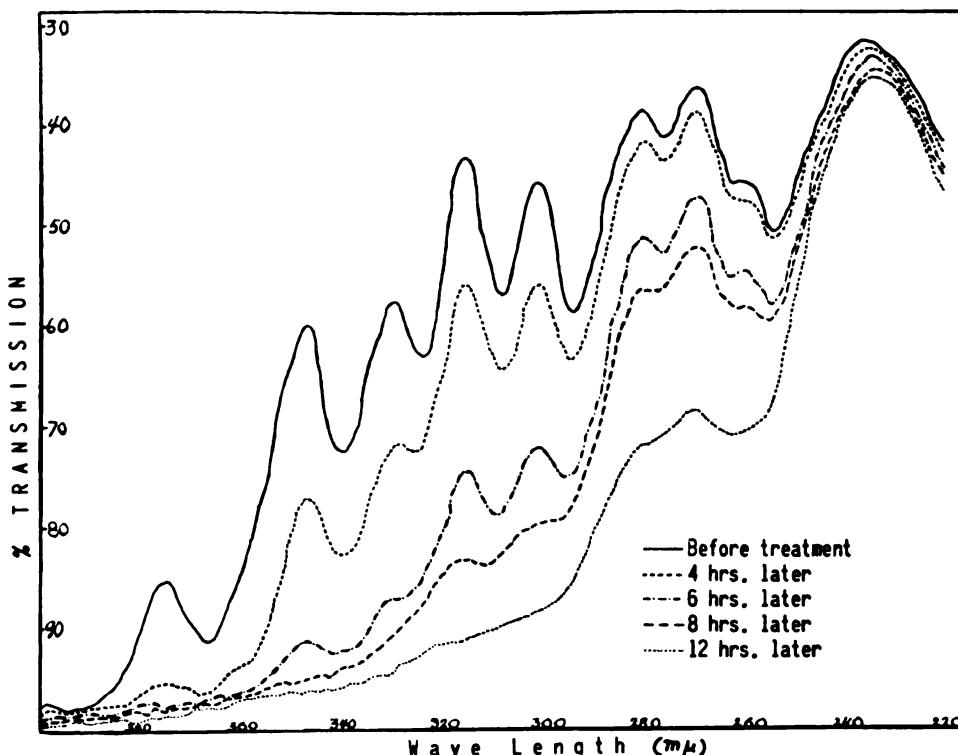


FIG. 283. Changes in the absorption spectra of a mixture of conjugated fish oil fatty acids induced by treatment with oxygen. The treatment has a greater effect on the higher unsaturated members, with the proportion of tri-, tetra-, penta-, and hexaenes decreasing as treatment continues, as seen by the reduction in the height or even disappearance of the peaks. After 12 hours of treatment, the conjugated dienes are the only ones not yet influenced.

Dilution 0.002% in ethyl alcohol.

the mixtures. In this experiment it appears that the quenching effect could also be related to the presence of conjugated pentaenes. Evidence available from other experiments do not, however, sufficiently support this.

We studied in a similar way the effect induced by the treatment—with sulfuric acid—of a mixture of conjugated fatty acids of cod liver oil. Fig. 285 shows part of the occurring changes and Fig. 286, the quenching effect.

Similarly, we studied the changes in the quenching effect during the conjugation with KOH of cod liver oil fatty acids in ethyl alcohol. Fig. 287

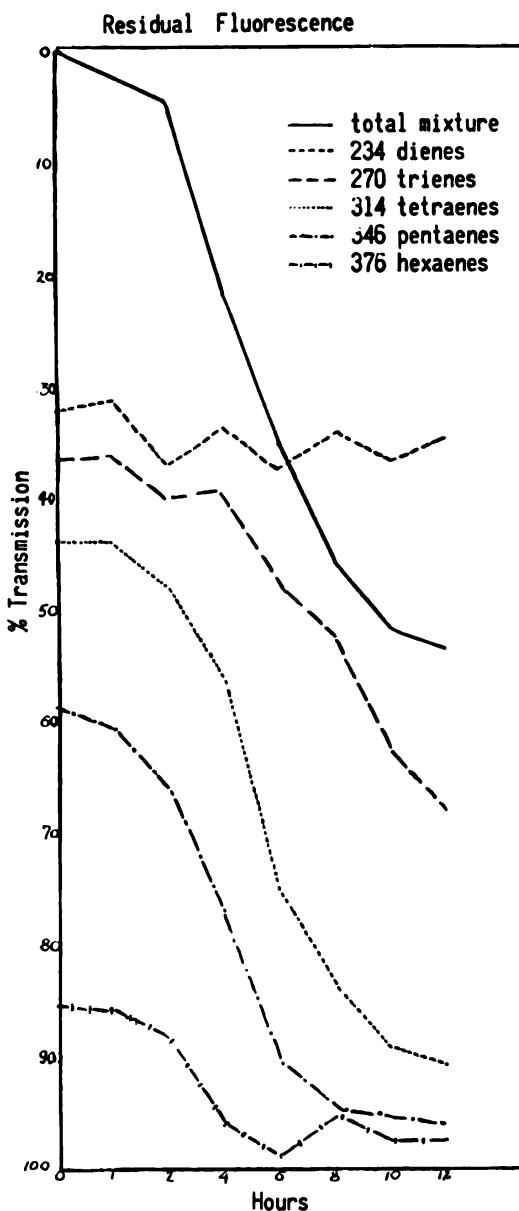


FIG. 284. The relationship between changes of the di-, tri-, tetra-, penta- and hexaenic peaks as found on spectral analysis of samples of conjugated fish oil fatty acids treated for different lengths of time with oxygen and the quenching effect of the same samples. A close parallelism exists between the decrease in the proportion of tetra- and pentaenic peaks and the quenching activity of the mixture.

shows the conjugation effect and 288, the quenching effect of the preparation at different moments, in various dilutions.

The entire problem was simplified by studying a pure conjugated tetraene. We have obtained pure tetraenic parinaric acid from akariton fat

of *Parinarium laurinum* seeds. In addition, we have prepared almost pure tetraenes utilizing the technique described by Maury, Brode and Brown. Unfortunately, with the last method, the results were less favorable, the proportion of tetraenes beginning to decrease long before the conjugated dienes and trienes have disappeared. Pure tetraenic conjugated acid has shown that the quenching action is related almost entirely to the tetraenic component alone, and in a mixture it is largely parallel to the content in conjugated tetraenic fatty acids. Fig. 289 shows the quenching curve induced by parinaric acid.

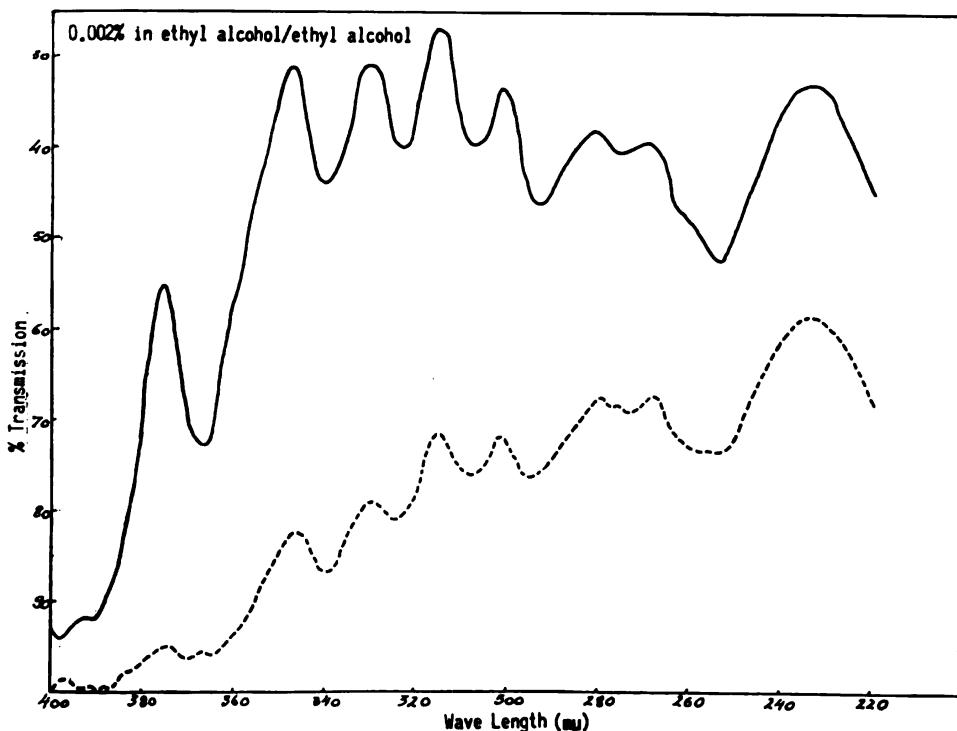


FIG. 285. The changes in the spectral analysis of a mixture of conjugated cod liver oil fatty acids, induced by the treatment with sulfuric acid. The treatment leads to unequal decrease in the amount of different conjugated members. Only two curves are shown: at the beginning of the treatment and at 260 minutes.

Conjugated Fatty Acids and Induced Carcinogenesis

We have investigated the influence exerted by the fatty acids—conjugated or not, and their mixture, upon the induction of tumors by carcinogens. From the various experiments, some were eliminated, either because the dose of methylcholanthrene employed did not produce tumors in a sufficient number in control animals to permit any conclusive comparison, or the death rate from intercurrent causes was abnormally high so that the entire experiments had to be discarded.

The experiments that were satisfactorily completed are summarized

in the following three tables. In the first group of experiments (TABLE XXXVIII), 4 groups each composed of 40 adult Swiss mice (20 male and 20 females in each group) were employed. Each animal received in the right flank a single subcutaneous injection 0.2 mg. of methylcholan-

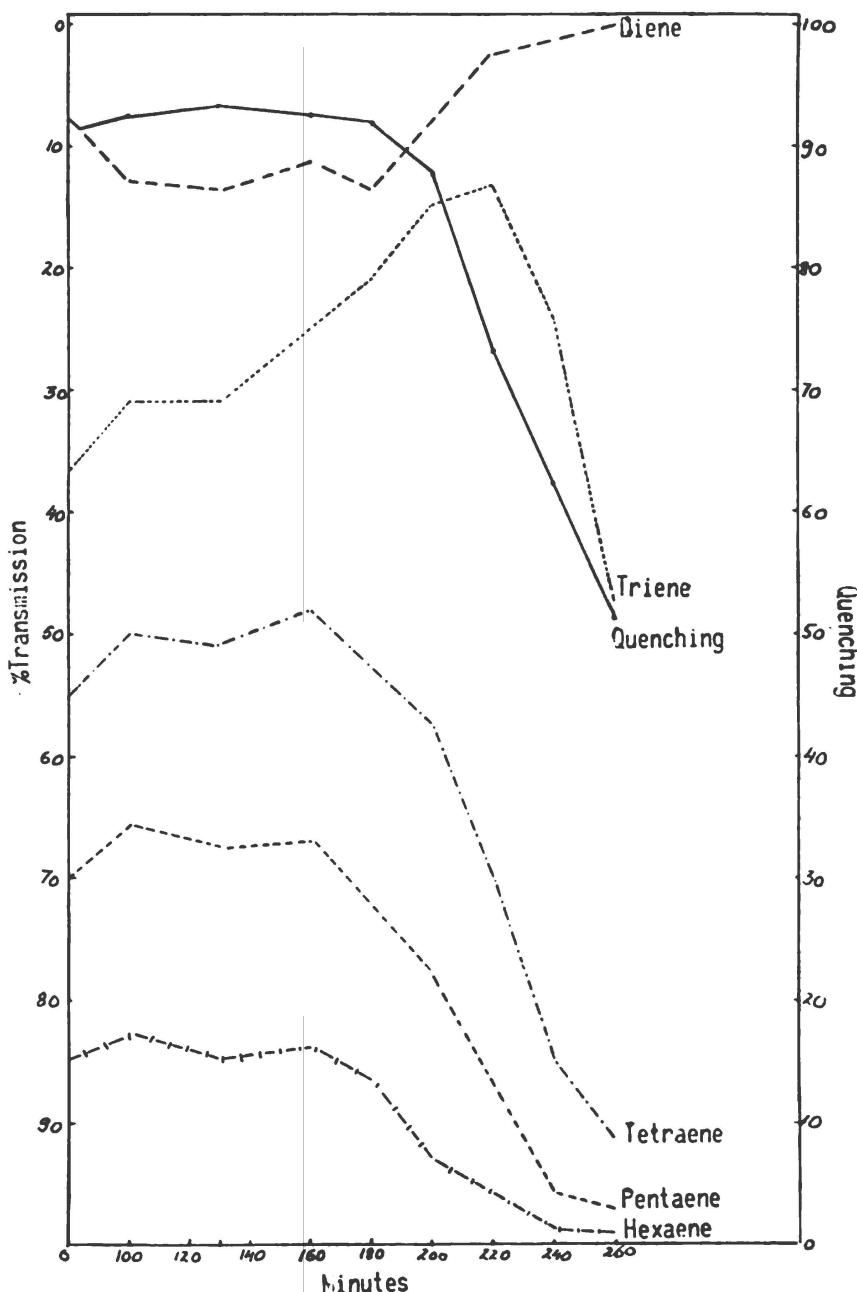


FIG. 286. The changes in the quenching of the treated mixture parallel the changes induced in the amounts of conjugated tetra-, penta- and hexaenes.

threne as a 0.2% solution in tricaprylin. These animals also received subcutaneous injections of a mixture of fatty acids extracted from cod liver oil, or a mixture of cod liver oil fatty acids conjugated by treating them with KOH. The fatty acids were administered as a 5% solution in cotton-

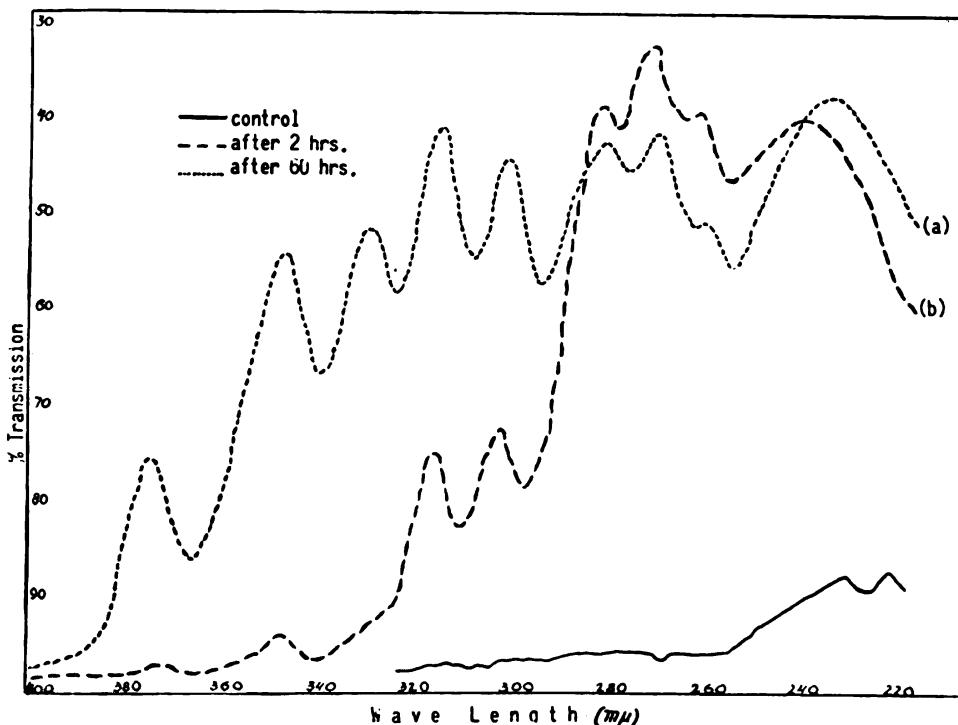


FIG. 287. Spectral analyses of fatty acids of cod liver oil, treated with KOH in ethyl alcohol. They show the appearance of high amounts of tetra-, penta- and hexaenes.

seed oil. Animals treated with fatty acids received 0.3 cc. of this solution in the contralateral side twice a week for three months. The control animals received the same volume of cottonseed oil in the same number of injections. In addition, one group of animals treated with the conjugated fatty acids received four injections during the two weeks preceding the

TABLE XXXVIII

Treatment	Died		% With Tumors
	Without Tumors	Tumors	
Cottonseed oil-controls	10	12/30	40
Fatty acids from cod liver oil	3	18/37	48
Conjugated fatty acids from cod liver oil	6	7/34	20
Conjugated fatty acids from cod liver oil (*)	14	3/26	11

* Received 4 injections of fatty acids before methylcholanthrene was administered.

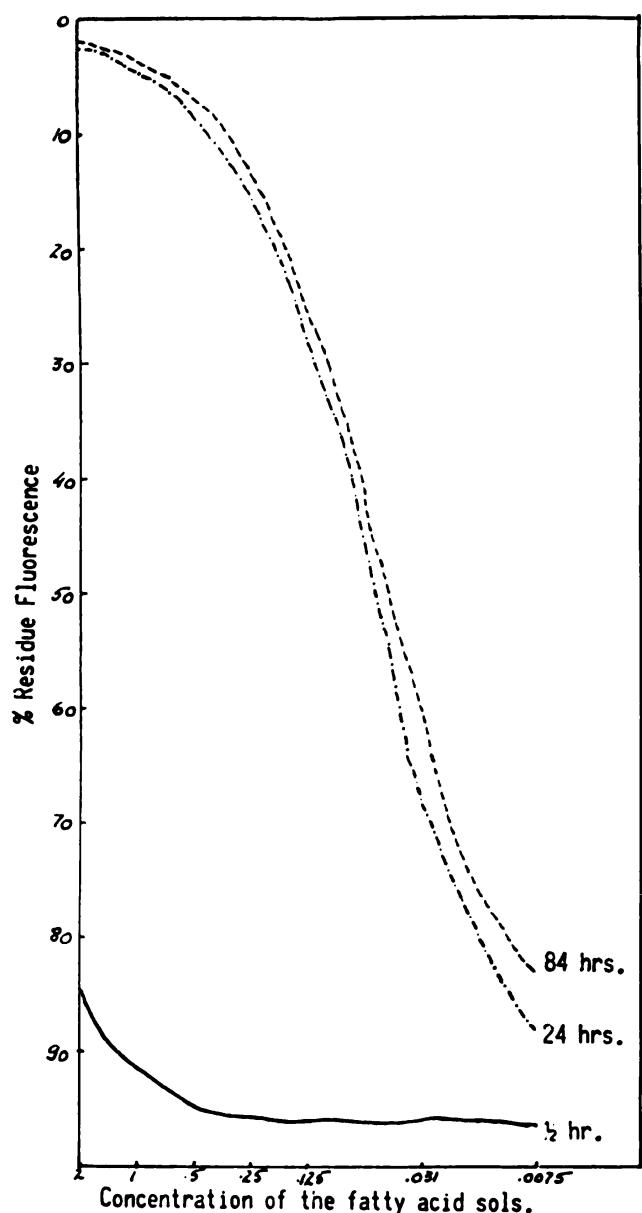


FIG. 288. Changes in the total quenching capacity of samples of cod liver oil during isomerization with KOH in ethyl alcohol. While the quenching effect is reduced—even for high concentration—for the sample having only $\frac{1}{2}$ hour of conjugation, it is high for that obtained after 24 hours. It remains almost the same for the sample after 84 hours of conjugation. The quenching appears related to the presence of conjugated isomers, with 4 or more double bonds.

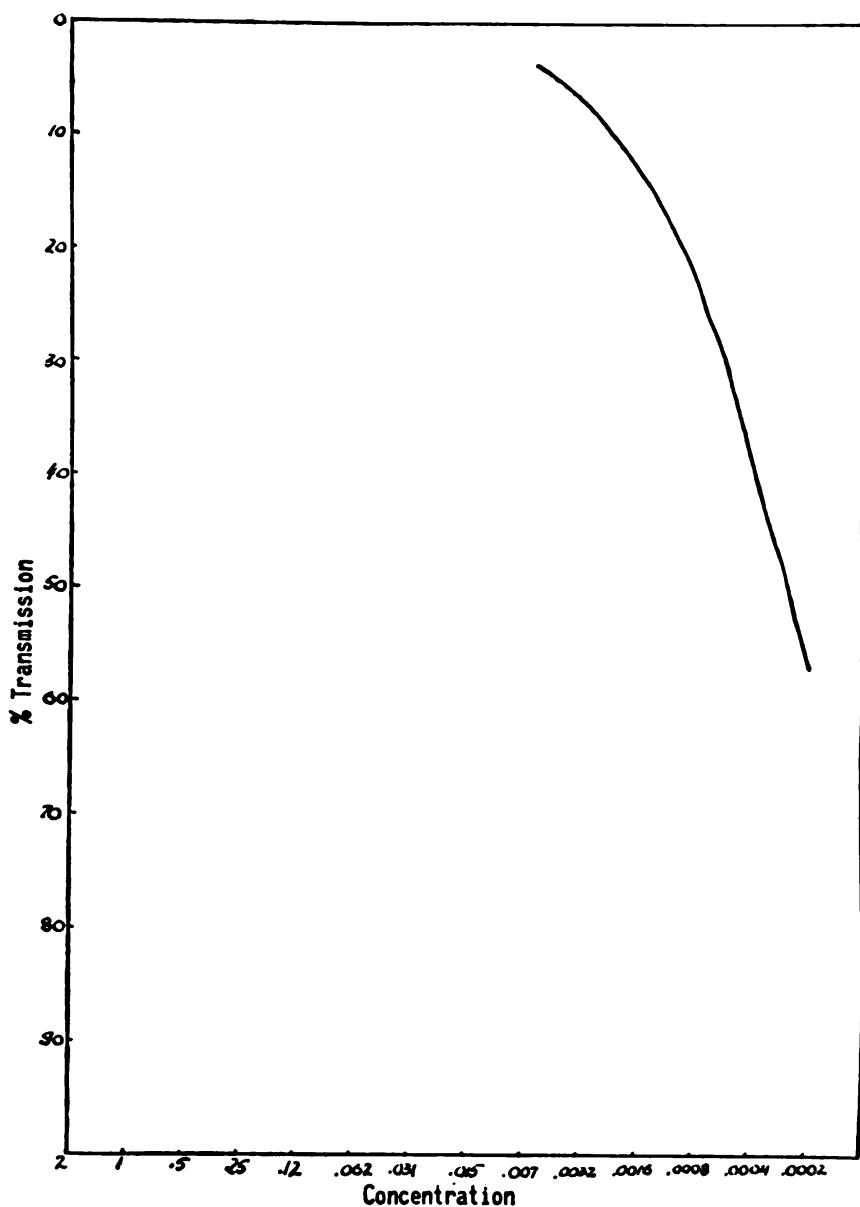


FIG. 289. Quenching effect of parinaric acid upon the fluorescence of methyl-cholanthrene. The relationship between the quenching effect and the presence of conjugated tetraenes is seen in the fact that parinaric acid has a quenching effect of 96.2 for a dilution of 0.006% and still one of 62% for a dilution of 0.0002%.

methylcholanthrene injection. Thirty-three animals died without tumors during the course of the experimental period. The number of animals surviving for five months plus the number in which tumors developed during this period of time are listed for each group.

In a second group of experiments (TABLE XXXIX) six groups of 40 mice each received one subcutaneous injection of 0.25 mgm. of methylcholanthrene of an 0.2% solution in tricaprylin and a second similar injection one week later. Groups were treated twice weekly for three months with 0.3 cc. of 5% solutions of the following fatty acids in cottonseed oil: Fatty acids from cod liver oil, conjugated fatty acids from cod liver oil, eleostearic acid, linoleic acid and conjugated linoleic acid. A control group of animals received 0.3 cc. of cottonseed oil in the contralateral flank twice weekly for three months.

TABLE XXXIX

Treatment	Died Without Tumors	Tumors	% With Tumors
Cottonseed oil—controls	4	31/36	86
Fatty acids from cod liver oil	3	33/37	89
Conjugated fatty acids from cod liver oil	6	15/34	44
Eleostearic acid	10	24/30	80
Linoleic acid	0	29/40	72
Conjugated linoleic acid	6	25/34	73

In the third group of experiments (TABLE XL), four groups of 30 mice each were employed. Mixtures of the methylcholanthrene and of the fatty acids used were prepared by adding 0.5 cc. of the 5% fatty acids in cottonseed oil solutions to 0.25 mg. of methylcholanthrene of 0.25% solution in tricaprylin. The injections were made subcutaneously immediately after mixing. Each animal received three injections at intervals of one week and

TABLE XL

Treatment	% Residual Fluorescence	Died With- out Tumors	Tumors	% With Tumors
Methylcholanthrene + cottonseed oil	95	6	33/44	86
Methylcholanthrene + fatty acids from fish oil	85	4	20/46	43
Methylcholanthrene + conjugated fatty acids from fish liver oil	19	4	6/46	13
Methylcholanthrene + eleostearic and conjugated linoleic acid	92	11	24/39	61

the observations on tumor incidence were followed for 5 months. The fatty acids employed were fatty acids from fish oil, conjugated fatty acids from fish oil, a mixture of equal parts of eleostearic and conjugated linoleic acids, and cottonseed oil as a control. The quenching effect is shown as the percent of residual fluorescence of methylcholanthrene when mixed with the fatty acid mixtures.

These results indicate a certain relationship between the quenching action of the conjugated fatty acids upon hydrocarbon carcinogens and the ability of fatty acids to reduce the carcinogenicity of these hydrocarbons. It is not sufficient to have a conjugated fatty acid present, in order to have the effect upon carcinogenesis. Eleostearic acid did not significantly reduce the incidence of tumors and conjugated linoleic acid was no more active than its nonconjugated isomer.

Conjugated fish oil fatty acids (which contain di-, tri-, tetra-, penta- and hexanes) when mixed with methylcholanthrene reduced the tumor incidence to 13%, while a mixture of eleostearic and conjugated linoleic acid (di-, and triene conjugated acids) which have a limited quenching action gave an incidence of 61%. Although the incidence of tumors was much lower in the group receiving conjugated fish oil fatty acids, the non-conjugated fatty acids from the same source has a limited influence upon the cancer inducing property of the hydrocarbon. When fatty acids were not mixed with the carcinogen, but were injected separately, the nonconjugated acids appeared without effect.

Statistical analysis of the data from these three experiments show the following: the results are significant for the group treated with conjugated fatty acids from cod liver oil before and after methylcholanthrene was administered as compared with control group treated with cottonseed oil in Experiment I ($\chi^2 = 6.65$ on basis of tumor/no tumor). In Experiment II,

TABLE XLI

QUENCHING OF METHYLCHOLANTHRENE 0.062% IN ETHYL ALCOHOL
BY SUBSTANCES OTHER THAN FATTY ACIDS

Substance	% Dilution Used	Fluorescence
Glycerol	5.0	106.8
n-Butanol	4.5	96.4
Butyl mercaptan	1.0	102.5
Hexyl mercaptan	2.0	92.0
Dodecyl mercaptan	2.0	82.0
Hexadecyl mercaptan	2.0	70.0
Na thiosulfate	.05 cc. from 50% solution	97.0
Ethyl sulfate	1.0	95.0
Nitrogen mustard	0.1	79.9
Allyl K xanthate	1.0	3.8
Nitromethane	1.0	7.4
Ethylene trithiocarbamate	1.0	.2
Cholesterol	1.0	93.0

the results are very significant for the group treated with conjugated fatty acids of cod liver oil as compared with the control group ($\chi^2 = 13.09$). In Experiment III, the results are very significant for all three groups in which fatty acids were added to the methylcholanthrene ($\chi^2 = 13.3, 41.56$ and 8.32 respectively).

When comparison is made on the basis of tumors/no tumors between groups receiving nonconjugated and conjugated isomers of the same fatty acid mixtures, the results were significant in all three experiments ($\chi^2 = 8$ in Experiment I, 22 in Experiment II, and 12 in Experiment III).

In the light of the relationship between quenching activity and the reduction of the carcinogenic activity, we are investigating different other agents. Table XLI shows the values of this effect.

Chapter 12, Note 5. Lipids and Tumor Chlorides

We submitted groups of mice grafted with DBA mammary adenocarcinoma, to treatment with various lipoacids or positive lipoids preparations. After ten days of treatment the tumors were removed and analyzed for their content of chlorides, using the Volhard technic, in which the titration of silver nitrate was made electrometrically. As lipoacids, we used for experiment cod liver oil fatty acids, lipoacids from human placenta and butyl-mercaptan; for lipids with a positive character, we used cholesterol, insaponifiable fractions of human placenta and butanol. In all cases treated with lipoacids, the amount of chlorides was higher than in untreated controls. With cod liver oil fatty acids, values as high as 135% above those of controls were found. With the lipoacids of human placenta, the average value was 114% above that of controls; with mercaptans, 78% above. The influence exerted by the opposite lipids was much less manifest. With cholesterol and insaponifiable fractions, chloride values were 20% below those of controls; with butanol, 33% below.

Chapter 12, Note 6. α -OH Fatty Acid and Experimental Tumors

We studied the influence exerted by the series of alpha OH fatty acids, saturated and unsaturated, upon the evolution of different tumors in mice and rats, to find that only one member has a manifest effect which is limited to a single tumor. Subcutaneous grafts of 6C₃HED lymphosarcoma in C₃H mice grew with abnormal rapidity. 48 hours after the transplant, the tumor could be felt. A very soft, highly edematous and, for this reason, diffuse tumor developed rapidly so that death occurred usually around the tenth day. This tumor was especially resistant to most of the chemotherapeutic agents tested. Daily administration of a 5% solution of alpha OH caprylic acid in a dose of 0.2-0.5 cc., started even the fifth day after the graft when the tumor was already well-developed, was followed by its rapid involution and disappearance in a high proportion of cases (55/60). In the few cases in which the tumor persisted, its evolution was very much changed. The animals remained alive for more than a month. If, after three

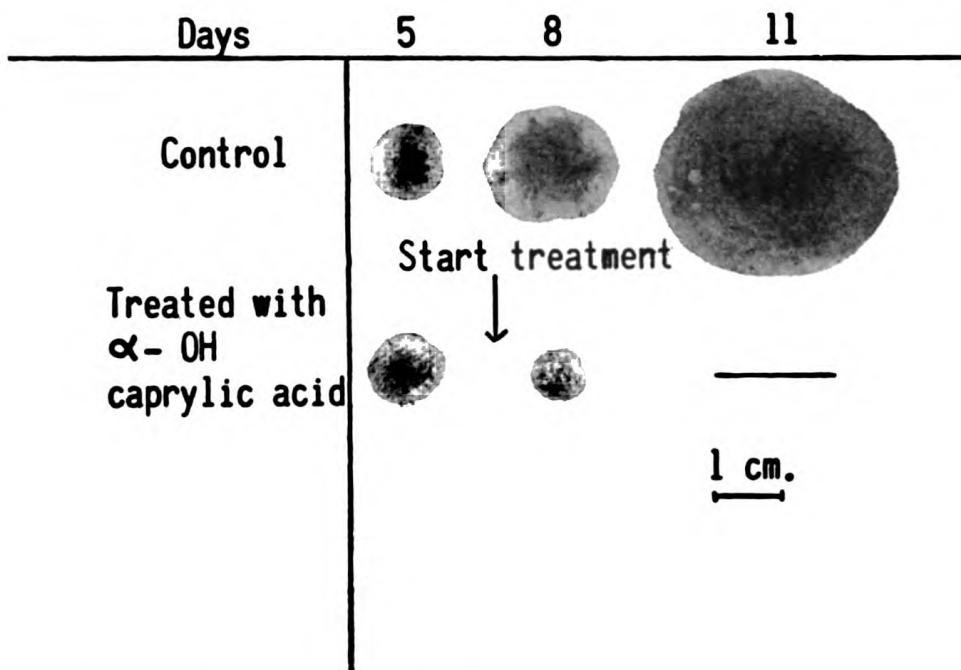


FIG. 290. The administration of alpha OH caprylic acid in mice bearing $6C_3HED$ lymphosarcoma tumor, induces the disappearance of the tumor in a high proportion of cases.

or four days, when the tumor was already highly reduced, the administration of the preparation was discontinued, the tumor began to grow again, but much more slowly than usual.

Chapter 12, Note 7. Hydrosulfides

The existence of different bonds between unsaturated fatty acids and oxygen led us to study different bonds similarly occurring between the same fatty acids and sulfur, the second member of the oxygen series. The treatment of polyunsaturated fatty acids or their triglycerides with sulfur has shown that two different formations can be obtained. By heating the mixtures above 110° but below $125^\circ C$, precipitated sulfur is incorporated without a manifest change in color or other properties. The iodine number is not changed. When conjugated fatty acids or their triglycerides are treated, no changes are seen in the spectral analysis. By heating above $130^\circ C$, the color of the preparation changed progressively reaching deep red-brown if the treatment is sufficiently prolonged. Concomitantly, the iodine number decreases progressively and eventually reaches zero. The spectral analysis of the conjugated fatty acids shows the peaks going progressively down until no more conjugated formations are present, indicating that these changes affect the double bond.

The analogy between the fixation effects of oxygen and sulfur has sug-

gested that the first bond corresponds to a hydopersulfide similar to a hydroperoxide. The second bond would represent a fixation of the sulfur at the level of the double bond itself, similar to a peroxide. Studies of similar bonds of sulfur were made in tetralin where hydopersulfides were obtained. The study of the properties of all these preparations seems to confirm the hypothesis that the compounds obtained are hydopersulfides.

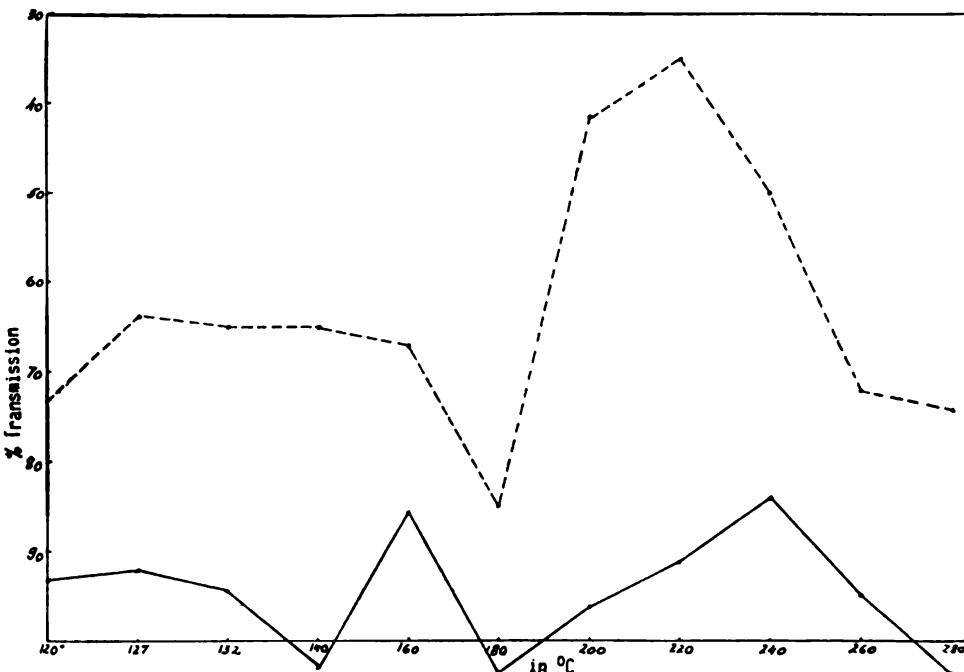


FIG. 291. Quenching of methylcholanthrene fluorescence in samples obtained when 0.5% sulfur in cottonseed oil was heated from 120°C to 280°C. The samples were dissolved in ether-alcohol mixture and mixed in equal part with .0125% methylcholanthrene. The existence of concomitant changes in the oil with sulfur and in the oil alone, indicates that the variations correspond primarily to changes which take place in the oil itself when heated.

We studied the changes in the quenching effect upon the fluorescence of methylcholanthrene which occur when sulfur added to a mixture of triglycerides is heated. This was compared with the effect of heating upon cottonseed oil alone. Fig. 291 shows the results of this analysis.

Chapter 12, Note 9. Magnesium and Adrenalectomy

The biological antagonism between homotropic magnesium and heterotropic sodium, both acting at the metazoic level, has led us to the study of the specific influence exerted by magnesium upon the recovery processes in adrenalectomized rats. It is known that, while an adrenalectomy is not always fatal in old rats—death occurs uniformly in younger animals weigh-

ing less than 150 grams. The administration of 1% sodium chloride as drinking water is known to protect the adrenalectomized animal and, if administered for a sufficient length of time, to prevent death. The administration of magnesium sulfate by repeated injections of .5 cc. of a 10% solution per 100 grams of body weight or even orally as .5—1% in drinking water has an antagonistic effect to that of sodium chloride. A 75% mortality rate in older animals receiving magnesium sulfate as compared to a 20% rate in the untreated was seen. Similarly, in young animals receiving magnesium sulfate in addition to salty drinking water, the mortality rate in some experiments was over 80%.

Chapter 13, Note 1. Glycerol and Chills

In one of a group of severely burned subjects, who were at that time under our care and had been experiencing several chills a day, an injection of glycerol solution had been given by coincidence just at the moment when a chill was starting. While such a chill always previously had lasted for more than ten minutes in this patient, it stopped almost immediately after the glycerol injection.

An experiment was set up to confirm or negate this correlation. As soon as any patient of this group felt the sensation or premonition of a chill, he was given either an intramuscular injection of 3-5 cc. of a 20% solution of glycerol in saline or 3 cc. of saline alone as placebo. In almost every case, the chill was cut short by the glycerol while the placebo had no effect. Less striking but still interesting effects were obtained when 20 to 30 drops of glycerol were given orally in 50 cc. of water against an oral solution of 1% sugar in water as a placebo.

Not one of the other substances used at this time, such as adrenalin, quinine, pilocarpine or pantopon, orally or parenterally influenced a chill once it had begun. Later, butanol also was found to have an effect similar to that of glycerol although less manifest.

Since the first experiment with glycerol, we have tried it in many patients subject to repeated chills and have frequently obtained the same results. We have tried to explain glycerol's effect upon chills by considering the role of chills in the defense mechanism. Chill would mark the beginning of the second phase of the diphasic defense phenomenon. (See Chapter 5.) It brings various constituents, especially those which have to replace constituents altered in the first hydrolytic phase. Among them are agents especially able to influence the free fatty acids liberated in the first phase. Apparently, the fact that it takes some time for the anti-fatty acid agents to pass into the circulation, most of them coming from the RES cells, makes the chill last so long. The immediate presence in the blood of a sufficient amount of glycerol, which is a relatively efficient anti-fatty acid agent, eliminates the need for liberation of body anti-fatty acid agents. Thus, with glycerol, the chill would no longer be required to induce liberation of such agents and would stop.

Chapter 13, Note 2. Influence of Glycerol Upon the Cardiac Rhythm

Figure 292 shows the electrocardiogram of a rabbit receiving a solution of 20% glycerol intravenously. Frequent extrasystoles appeared. It was interesting to note that, at the same time, the animal became somnolent.

Glycerol

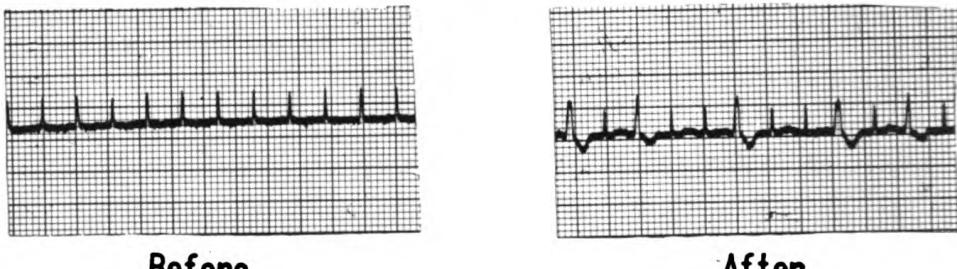


FIG. 292. Electrocardiogram of a rabbit receiving intravenous injections of a solution of glycerol 20%, characterized by the appearance of extrasystoles. (a) before treatment. (b) after 30 cc.

Butanol

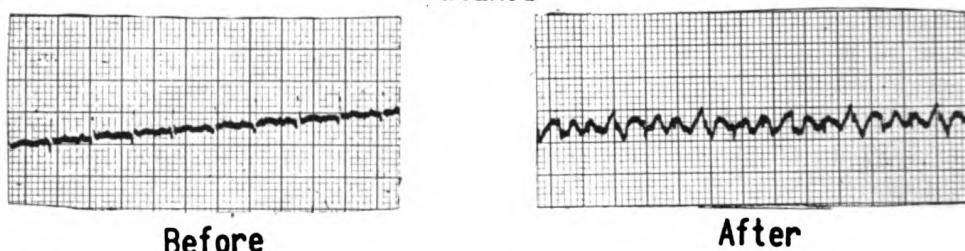


FIG. 293. Extrasystoles appear after the intraperitoneal injection of butanol in very high doses. (a) before treatment. (b) after 1.6 gram/1000 gr of animal.

Chapter 13, Note 3. Glycerol Induced Convulsions

Repeated injections of glycerol in rats were seen to induce convulsions. Using rats weighing 200-250 grams, 5 to 10 cc. of the 20% solution of glycerol in saline were injected intraperitoneally. The injections were repeated once or twice a day. After several days of treatment, usually from 3 to 5 days, one of the injections was followed within a few minutes by a severe convulsion, lethal for most of the animals. In the surviving animals, the next injection was always followed by a lethal seizure.

Chapter 13, Note 4. Suspensions of Lipoids

In order to obtain colloidal suspensions, various lipoids were dissolved in alcohol and a certain amount of the alcohol solution mixed with water, saline or isotonic solutions. From the resulting milky suspensions, the al-

cohol was eliminated by boiling under reduced pressure. To insure almost complete elimination of the solvent, an excess of water was added and the excess was then eliminated through boiling. The use of acetone or ether as solvent gave much less favorable results.

Relatively stable suspensions were obtained by mixing some lipid preparations such as mixtures of unsaturated fatty acids with a 0.5% solution of cellulose gum. Such stable suspensions could not be obtained with preparations of positive lipids.

Chapter 13, Note 5. Cholesterol Induced Convulsions

When relatively larger doses, such as 5 cc. of 2% cholesterol daily, were administered repeatedly to rats of around 250 gr. of weight, convulsions appeared after 4 to 8 days. They were induced earlier in females than males. The first convolution always was lethal. Convulsions also occurred in humans after repeated injections of cholesterol in doses as high as 20 cc. of the 2% solution in oil. Even small doses, such as 2 or 3 cc. of the same solution, induced convulsions in patients with brain metastases or in those who had had previous convulsions.

Chapter 13, Note 6. Treatments In Successive Generations

The relatively short survival time of animals bearing transplanted tumors has been a handicap for the study of the influence exerted by many agents. Effects requiring some time before they can be induced are thus missed. Changes which occur in tumors—such as the tendency to ulcerate after treatment with fatty acids—have been found to be transmitted in successive generations of the tumors. This has led us to carry on treatment beyond the survival time of one individual host in order to study the influence of various agents. In one group of experiments, this was done through treatment of the successive hosts of serial transplants. In another group of experiments, the treatment was applied to the transplants themselves in successive hosts.

Mice with grafted tumors were treated with the chosen agents. When the tumor in a treated host, or in a control, had grown to 1½ centimeter diameter, it was removed. Part of it was used for further transplants, part for microscopic studies. The rest of the animals were kept until death and the survival time was noted. Transplants of the tumor from treated animals as well as from controls were grafted in new animals and the treatment continued for the new hosts. This procedure was repeated for successive generations. In other experiments, the successive transplants were dipped, prior to grafting, in an oily solution or in a suspension in saline of the agent being tested. The procedure was repeated continuously for both treated animals and controls, and growth and survival time were noted. The following experiments are characteristic.

Using the insaponifiable fractions of human placenta in an oily solution

of 5%, or in a saline suspension corresponding to two milligrams of the material per cubic centimeter, the following results were observed in the case of Ehrlich mammary carcinoma in mice. No changes in survival time, evolution of the tumor, gross or microscopic character were seen in the first and in some experiments even in the second generation. Usually with the third generation, the survival time was reduced, the tumor growing much more rapidly and killing the animal in around 20 days. The malignant character of the tumor was seen to increase in the subsequent transplants and in the fifth generation in some experiments, killed the animal in

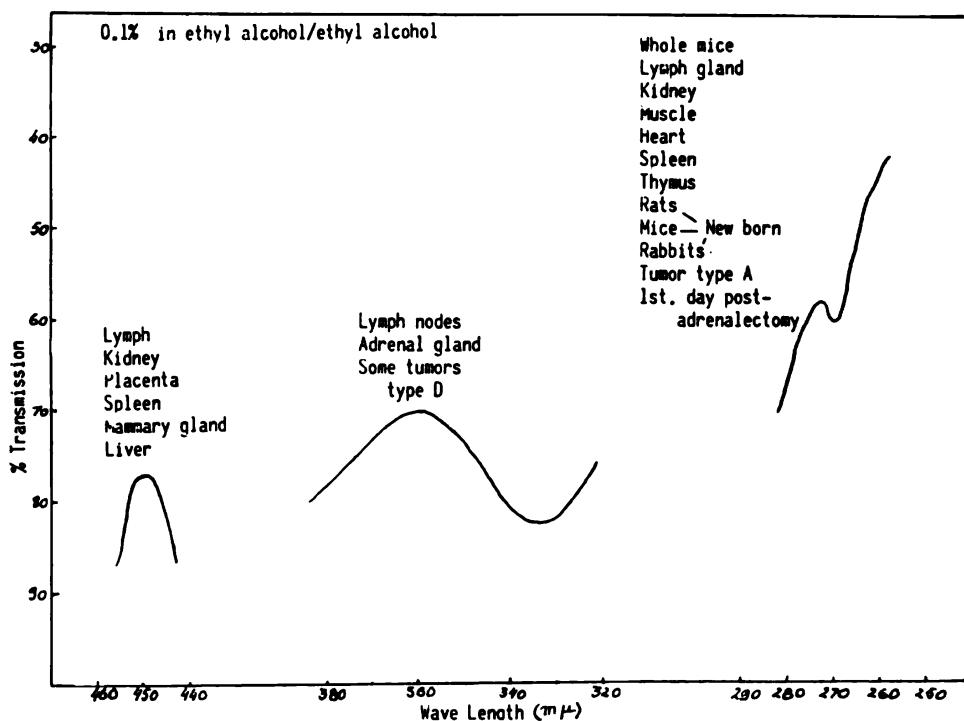


FIG. 294. Spectral analysis of the insaponifiable fraction of various origins. It shows a characteristic peak at 450 $m\mu$, another at 360 and another at 272. The organs in which they are especially present are indicated.

less than a week. The morphological change observed in these successive transplants were also characteristic. The tumor was seen to change from solid to encephaloid. The adenocarcinomatous character was thus altered and the degree of undifferentiation was increased by passing through the third, fourth and sixth generation. At the sixth generation in some experiments—and the fifth or eighth in others—microscopic examination showed that sarcomatoid portions were present in the tumor. The malignancy appeared to be at its maximum in these tumors. Transplants of tumors with sarcomatoid microscopic character, if treated in the same manner, gave negative grafts. Thus, it appears that the treatment with the insaponifiable

fractions has progressively increased the malignancy until the moment when sarcomatous character appeared after which negative transplants were observed.

The treatment of a tumor with lipoacid preparations of human placenta has produced opposite changes manifest even in the first transplants. These

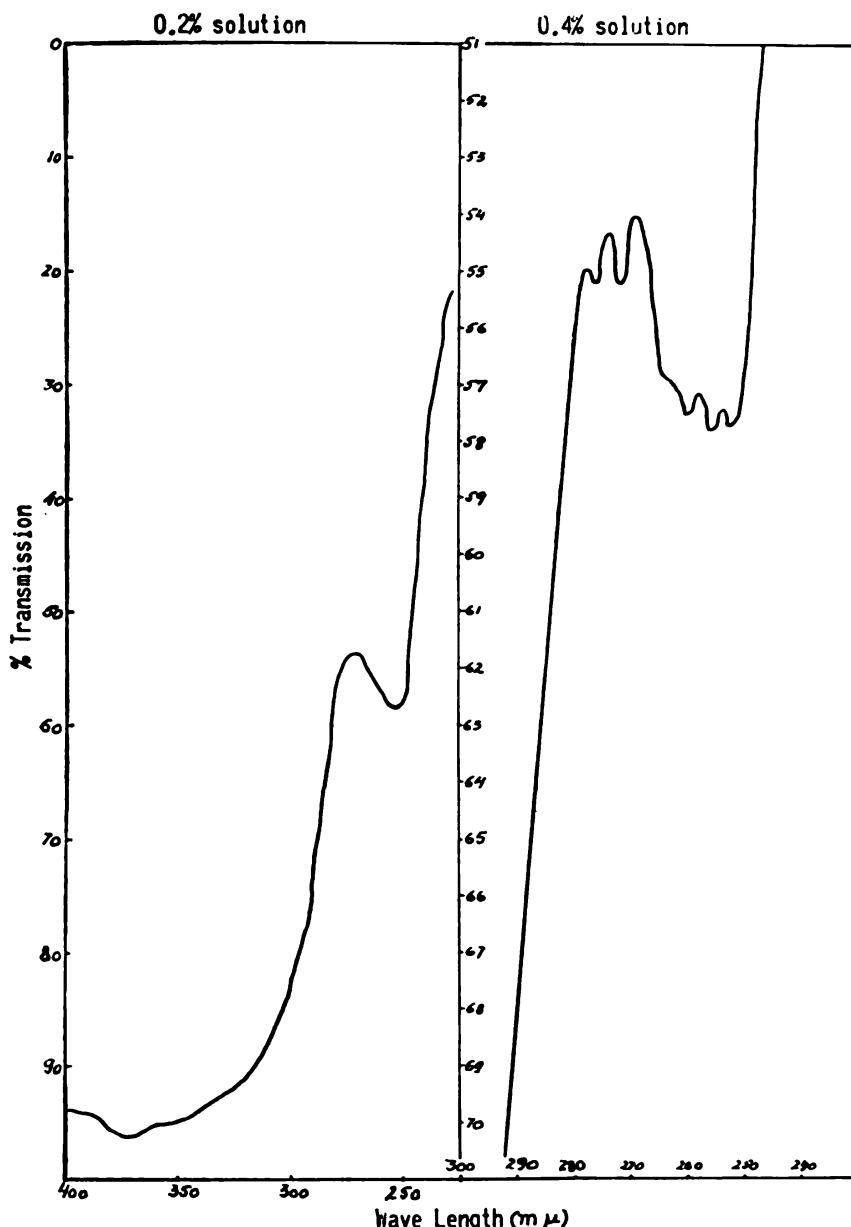


FIG. 295. Details of the spectral analysis of rat colostrum indicates the existence of a formation with three peaks in the region 290-250 m μ with some similarity to the conjugated trienes.

increased in the second generation. After the second and very rarely after the third grafts, negative transplants were obtained. We used this method of treating tumors through successive generations routinely. The results obtained for different agents are discussed in the text of this publication.

Chapter 13, Note 7. Conjugated Trienic Alcohols

Spectral analysis has permitted us to recognize the presence, in certain mixtures of the insaponifiable fraction, of several peaks, some especially interesting. Characteristic peaks were seen at 450, 360 and 272 m μ , as shown in Figure 294. In the first analysis, one was identified as corresponding to a peak of 2720 Angstroms. In more complete further spectral analyses, it could be seen to correspond to a conjugated triene with its characteristic three peaks. The fact that it corresponds to a substance with positive polar group explains why, compared with conjugated acids, the curve shows a marked displacement of the peaks toward higher wave lengths. (Fig. 295) This can be related to the different influence exerted by the electrically opposite polar groups. This compound was first found in the colostrum obtained from the stomach of newborn rats on the first day. In smaller amounts, it has been seen in other samples of milk or butter, and in pork kidneys. It has been found less frequently in growing tumors and is even rarer in growing animals.

The same spectral analysis has permitted us to recognize other peaks and relate them to the different sources from which the unsaponifiable fraction was obtained. Fig. 294 shows these peaks and indicates their correlation with the origin of the material.

Chapter 13, Note 8. Toxicity of Butanol in Humans

A group of advanced schizophrenic patients (221) were given 500 cc. of a 6% solution of n-butanol in saline intravenously, the entire amount being injected in 30 minutes. The only manifestation which could be considered to parallel the toxic effect in animals was a very short period of somnolence which, in only one or two cases, could be considered as sleep. Usually, even with doses of 500 cc. of a 6% solution administered intravenously in less than 25 minutes, it was not possible to obtain even this transitory somnolence. No toxic effect was noted when the same dose was again administered 24 to 48 hours later, and repeated several times. Except for an inflammation of the vein which appears only if hundreds of cc. of a solution above 6% is injected, no other noticeable effects are observed.

The intravenous administration, in postoperative cases, even of 15 gm. of butanol diluted in about 2-3 liters of saline per day, repeated for four and even five consecutive days, has been entirely free of any toxic effect.

Intramuscular administration was observed to be well tolerated even for higher concentrations of butanol. We obtained concentrated aqueous solutions by dissolving butanol in a 35% solution of sodium benzoate in water. Preparations containing more than 30% butanol seemed to induce

necrosis when administered intramuscularly in animals and to induce pain at the site of injection in humans. A 30% solution of butanol, however, was well tolerated. Daily administration of subnarcotic doses for long periods to mice caused no toxic effects. On the other hand, repeated injections with narcotizing doses were toxic and even led to death of the animals after several days.

Chapter 13, Note 9. Butanol and Leucocytes

The administration of butanol in solutions of 6.5% in saline intraperitoneally in rats was seen to induce a hyperleucocytosis. 5 cc. injected at once was seen to double the previous amount of leucocytes. This increase

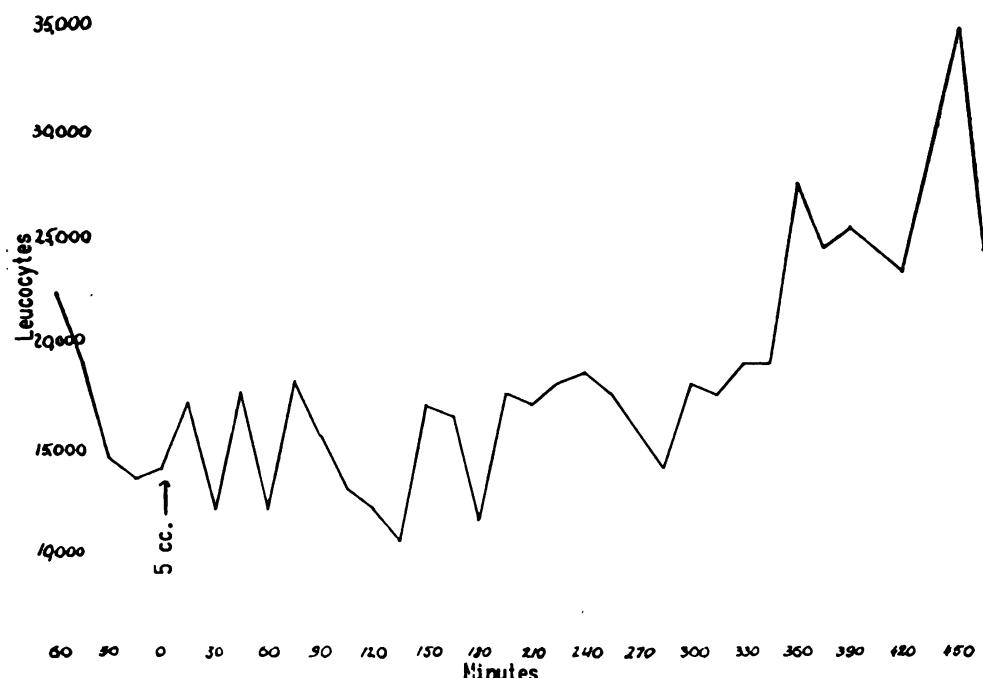


FIG. 296. The administration of 5 cc. of a solution of 6.5% n-Butanol intra-peritoneally to rats induces an increase in the leucocyte number.

started two hours after the beginning of the injections and continued progressively, to reach the value of 34,000, seven hours after the beginning of the injections. The hyperleucocytosis was seen to persist for more than 24 hours. The number of leucocytes was increased in the animal shown in Fig. 296.

A still more manifest effect was obtained with injections of 1 cc. of the same solution, repeated every hour during the day. It is interesting to note that this effect was manifested almost 6 hours after the injection with butanol. Fig. 297 shows an example of these experiments in which the number of leucocytes arrived at 42,500.

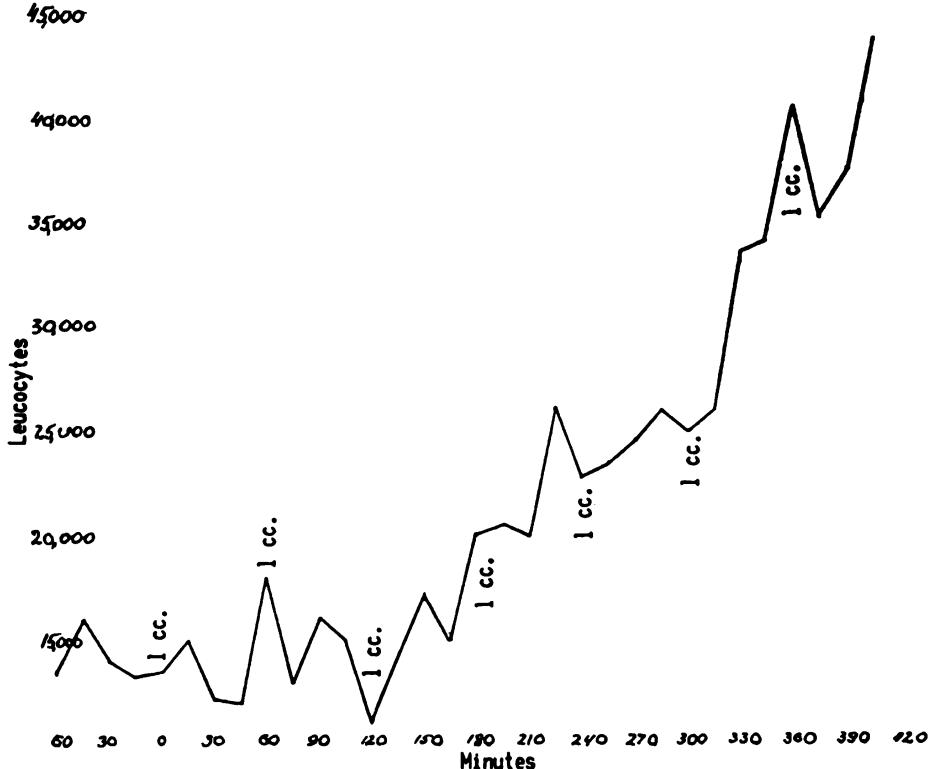


FIG. 297. The administration of 1 cc. of n-Butanol, repeated every hour, induces a sizable increase of leucocytes in rats.

Chapter 13, Note 10. Butanol-Sodium Lactate in Burns

In collaboration with R. Ravich and P. Teitelbaum we studied the effect of various agents upon the survival time of mice to which a severe caloric burn was inflicted. Under ether anesthesia, adult white female mice were scalded until the xyphoid, in water maintained at 90°C. When the duration of this treatment was 4 or more seconds, the animals died in a few minutes in superacute shock. With 3 seconds the animals survived the immediate effect of the burning.

They started to die several hours later, in 6 hours 40% of these animals had died, and at the 18th hour, 90% were dead.

The influence exerted by various agents was studied by injecting the respective solutions 2-3 times a day according to the experiment. The effect was judged according to the survival time. As the animals did not eat or drink and especially due to the local burns did not urinate or defecate, we considered the effects obtained during the first 18 hours, after which the animals were sacrificed and used for the study of the chemical changes. Fig. 138 shows the results of such an experiment with sodium chloride, isotonic solution—sodium lactate 6 M solution, butanol 6.5% in saline—and butanol 6.5% in the sodium lactate solution. While sodium lactate

alone seemed even to increase the mortality, and butanol in saline alone influence little this mortality, the effect of the butanol—sodium lactate solution was manifest. The mortality was reduced from beginning to end of the experiment. At the 18th hour it was of 30% instead of 90% for the controls. And of 95% for the animals treated with sodium lactate alone.

Chapter 13, Note 11. Effect of Heptanol

Adult rats and mice were injected subcutaneously in the back with 20 cc. and 3 cc. respectively of nitrogen which had been sterilized by being passed through sterile cotton plugs. Into the pouches so formed, a suspension of living coli bacilli—2 cc. for rats and $\frac{1}{4}$ cc. for mice—was injected. This suspension was obtained from a 24-hour culture on agar and was diluted to provide 10 million microbes per cc. One group of animals was treated by intraperitoneal injection of 1 cc. (for rats) and $\frac{1}{4}$ cc. (for mice) of sterile sesame oil. The other group received injections of similar doses of 2% heptanol in oil. In some experiments, only one injection with heptanol was given, while in others this was repeated daily or every second day. In the controls no special reaction was seen. In the heptanol-injected animals an exudate appeared in the infected pouch and led to rapid necrosis of the skin. A characteristic of the exudate was the presence of a small number of leucocytes.

Chapter 14, Note 1. Observations of Dr. E. Stoopen

From a series of observations published by E. Stoopen (184), we chose the following:

"Right trigeminal neuralgia for the past 10 years, with short, sharp pains. Neither food intake, nor time of day have ever influenced the pain. The patient was submitted to various treatments such as ultraviolet rays, quinine, neosalvarsan, cobra poison and vitamin B. First alcohol nerve block calmed the pain for 15 months. Second alcohol injection brought no relief. Third block calmed the pain for one year. Fourth alcohol injection calmed pain for three months. The last injection caused, however, trophic ulcerations of the throat and corneal ulcerations with ultimate loss of the sight.

In June 1942, a treatment with glycerin and the insaponifiable lipid fraction was begun. The pain which proved to be of an alkaline type, ceased in 3 days.

In July 1943, the patient had a lumbago attack, a condition from which she had often suffered and which had been both long-lasting and resistant to classical medication. Treatment with the insaponifiable lipid fraction made the pain subside within a few days.

In an attempt to modify the ulcerations in the throat, though the pain had not reappeared, the patient was continuously treated with the insaponifiable fraction and cholesterol, and with large doses of vitamin A. However, there was no effect on the ulcerations.

In February 1944, the pain reappeared. Study of the pain curve revealed it to be of the acid type since the paroxysmal pain corresponds to a very low pH. The patient was given ammonium acetate and lipoesters. Four days later the pain had considerably decreased, and fifteen days later, completely subsided."

"Mrs. W. For eleven years this patient has suffered of a left trigeminal neuralgia. Each year the crisis lasted four to five weeks, during which time the pain always appeared between four and seven in the morning, lasted for one to two hours and then disappeared. The pain was so severe, being almost unbearable. For the rest of the day, the patient only felt a slight sensitivity. Barbiturates taken even in large amounts had no influence on the pain. Removal of the Gasser ganglia was suggested as the sole possible cure by numerous doctors consulted in Mexico and the United States.

The patient came under our care on November 12, 1943. Her most recent crisis had started on October 31. Study of her pain showed it to be of an alkaline character, since pain is quite intense when the urinary pH is high. We recommended glycerine. The patient protested, feeling that a few drops of glycerine would not be able to help her, pointing out that intensive treatment, had given no results. However, on November 15th, when pain started at 5:00 A.M., the patient took the glycerine drops and to her amazement, the pain disappeared within two minutes. She asserted that no medicine had ever been able to stop the pain once it had started. At 7:30 A.M. the pain returned but again decreased after the patient had taken several glycerine drops.

On November 16th, the patient experienced pain at 1:30, 5:30 and 7:30 A.M. During the first two periods of pain, the pain was instantaneously calmed with glycerine and phosphoric acid; the third period of pain was decreased in intensity but lasted for 40 minutes.

On November 17th: pain appeared at 9:00 A.M. but disappeared three minutes after medication of glycerine and phosphoric acid.

On November 18th: pain which started at 8:00 A.M. could not be calmed with medication. Coramine, Cholesterol and insaponifiable fractions were then prescribed.

On November 19th: pain was experienced at 4:00 and 8:00 P.M. but subsided within three minutes after medication of glycerine, phosphoric acid and coramine was taken.

On November 20th: from this day through November 25th, the medication was unable to influence the pain, and consequently the patient became disheartened. A study of her pain pattern at this point indicated that it had changed to the opposite type—from alkaline to a definite acid pattern.

On November 26th: bicarbonate was given at the onset of pain resulting in a considerable decrease in its severity. No further pain was felt on the following days and the crisis was considered ended. In this instance, the crisis had lasted for 27 days (the length usually varied from 27 to 35

days). However, the treatment achieved what had been impossible for all previously tried treatments—the cessation of pain once it had started.

These observations led to the following pertinent conclusions: 1) they showed the existence of typical acid or alkaline pain; 2) the possibility of changing pain from one type to the opposite one, either during the course of the disease or due to medication; and 3) the possibility of eliminating pain with appropriate treatment."

Chapter 14, Note 2. Dr. Welt's Publication on Butanol—Conclusions

"n-Butanol was administered to a large number of patients with pain due to the trauma of various common otorhinolaryngological and ophthalmologic surgical procedures. Pain was relieved in approximately 90 per cent of the patients so treated.

These clinical results were considered in the light of studies by Revici and his co-workers regarding the physiopathology of wounds. The results indicate that the proposed concept of pain has significant practical clinical applications."

Chapter 14, Note 3. Dr. A. Ravich's Conclusions (189)

In his article concerning the post-operative care in prostatectomies, A. Ravich arrives at the following conclusions exemplified in Figure 298.

SUMMARY

"A new concept of the local physicochemical changes occurring within pathological foci as introduced by Revici, has been briefly described. According to this view, pain is the result of local pH changes brought about by the accumulation of acid or alkaline substances within disturbed tissues. Changes in the lipid balance are associated with and may account for these alterations.

"The possibility of correcting or neutralizing such lipid changes has been explored clinically in several series of urological cases. The favorable effects upon pain as well as upon bleeding, wound healing and other important postoperative problems and complications indicate the need for further study along these lines."

Chapter 14, Note 4. Treatment of Post-traumatic Conditions

Of special theoretical and practical interest has been the treatment of traumatic conditions, especially those following surgical procedures. The recognition of the role of fatty acids acting at different levels of the organization and inducing several different manifestations, has led to the concomitant use of various agents proper to the levels. From the various agents studied, heptanol was thus chosen as acting at the cellular level, glycerol, polyunsaturated alcohols and alkaline amino acids and butanol

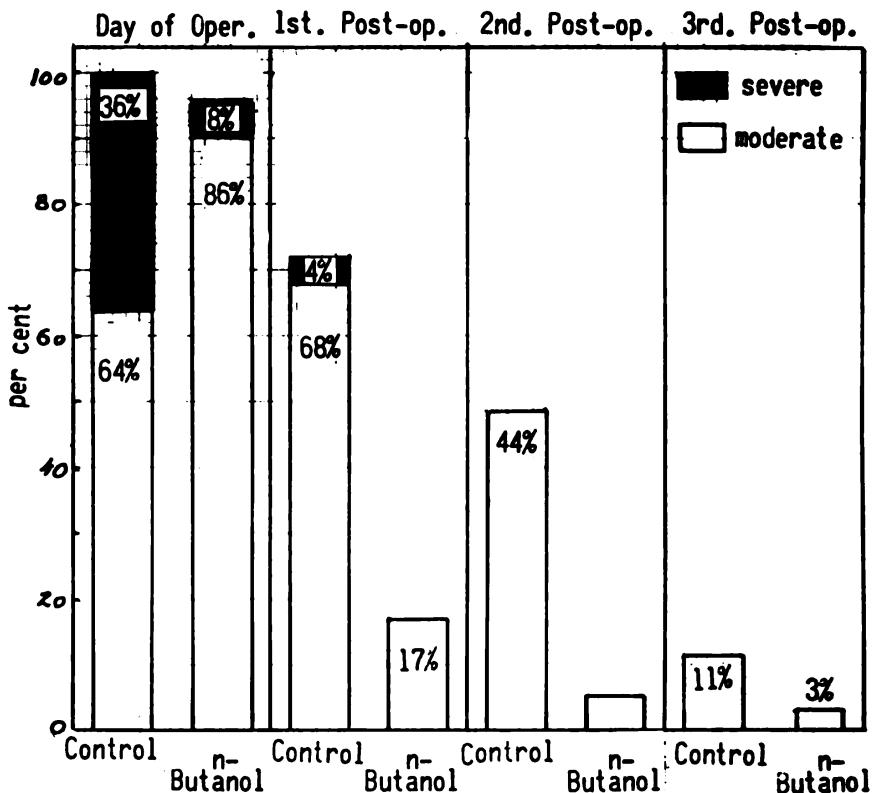


FIG. 298. The administration of n-Butanol after prostatectomy markedly reduces pain. (J. Urol. 62: 629, 1949.)

at the tissular level, glycerophosphoric and organic acids at the systemic level.

The various preparations, obtained by combining or mixing these agents, were administered by intravenous infusions together with glucose and saline or glucose and sodium lactate, in the more severe cases, and intramuscularly or orally in the milder cases. The results obtained with these preparations in hundreds of subjects have been highly satisfactory.

Chapter 14, Note 5. Dr. B. Welt's Conclusions on Hearing

In his studies on hearing, Welt arrives at the following conclusions, communicated at the Brooklyn Eye and Ear Society.

"1) The ideas, methods and substances devised by Revici have been applied to the problem of impaired hearing, and have shown significant results in improving that function.

2) The study has confirmed the dualistic concept about pathological foci, namely that a pathological focus may exist in two states of metabolic imbalance, leading either to a local alkaline or acid change.

3) The substances utilized in this study have been effective in influenc-

ing the symptom of impaired hearing, and it is reasonable to believe that the pathological structure has also been influenced to some degree.

4) Revici's ideas of the fatty acid-sterol imbalance have been confirmed by showing that other instances having similar biologic activities, act in the same manner. Their clinical application confirms this.

5) This study shows that both air conduction and bone conduction may be benefited. No method has been found to improve bone conduction up to the present time.

6) From the biochemical and chemotherapeutic point of view, this study indicates that the vestibular labyrinth and the cochlea should be viewed as one organ. Phylogenetic, histological, and this clinical study all tend to support this idea.

7) The study indicates that people up to 60 years of age may obtain normal audiometric hearing if treated early enough. Children and the younger age groups were those that had the highest incidence of good results.

8) Finally, this study has indicated a dual therapeutic attack on a hitherto insoluble problem. It can help us in this otologic problem at any age in life, the formative years, when hearing is vital to the education of the children.

9) It should not be construed or inferred, from this communication, that a cure for hearing is implied. The only conclusion to be drawn is that the author has beneficially influenced the impaired hearing function, or induced a remission for varying periods of time at an improved or normal functional level."

Chapter 14, Note 6. Butanol in Plastic Surgery

Following our indications, S. Sher has utilized butanol in post-operative cases. One of the most disagreeable complications seen in plastic surgery of the nose is seventh day bleeding, which while usually not severe, has been known to endanger the lives of several patients. Use of antibiotics has reduced remarkably both incidence and severity of the hemorrhage. However, the prevention of seventh day bleeding has remained a problem for the plastic surgeon. S. Sher has applied our treatment with butanol in almost 2,000 cases. Immediately following surgery, 10 cc. of a 6.5% solution of butanol is injected intramuscularly, the injection being repeated every six hours for the first day. After 24 to 48 hours, the butanol is administered orally in doses of one tablespoonful every four to six hours, and this is continued until after the eighth day following the operation. With this treatment, no severe bleeding has been seen. In several cases when the patient failed to follow instructions and did not continue taking butanol, hemorrhage resulted. In two cases, bleeding was relatively severe, the hemorrhage was brought under control by the intravenous injection of 10 to 20 cc. of the butanol solution. Administration of butanol afterward prevented subsequent bleeding. The value of butanol as a preventive of seventh day hemorrhage thus has been confirmed. (189)

Chapter 15, Note 1. Radio and Chemotherapeutical Essays

Though carried out only at the beginning of these studies, the application of this method, conducted by Leonard B. Goldman, M.D. in 1950 and 1951 appears interesting. As part of this investigation, lipids were used in association with radiotherapy. In the same research, a group of patients were also treated with lipids alone. A report on this series was presented by L. Goldman as part of a symposium on the therapy of advanced cancer patients, before the Radiological Section of the American Medical Association at its annual convention in Atlantic City on June 14, 1951 (327). His results with patients treated with X-ray and lipids and with lipid therapy alone are summarized in Table XVIA and XVIB.

TABLE XVIA
RESULTS OF LIPID THERAPY COMBINED WITH IRRADIATION
 (In excess of those expected with irradiation alone)

Type of Malignancy	Total Num- ber	Relief of Symptoms				Tem- porary Ar- rest	Re- gres- sion
		Num- ber	Slight	Mod- erate	Mark- ed		
Breast	8	7	2	1	4	5	2
Lymphoblastoma	7	4	2	1	1	1	0
Lung	6	5	2	0	3	1	1
Head and Neck	11	4	2	0	2	1	1
Gastro-intestinal	6	4	0	1	3	2	1
Gynecological	3	3	1	1	1	1	0
Genito-urinary	3	0	0	0	0	0	0
Sarcoma	2	1	0	0	1	0	0
Miscellaneous	4	2	1	0	1	1	1
Total	50	30 (60%)	10	4	16	12 (24%)	6 (12%)

TABLE XVIB
RESULTS OF LIPID THERAPY ALONE

Type of Malignancy	Total Num- ber	Relief of Symptoms				Tem- porary Ar- rest	Re- gres- sion
		Num- ber	Slight	Mod- erate	Mark- ed		
Breast	14	7	1	0	6	2	2
Lymphoblastoma	8	5	0	0	5	3	2
Lung	3	3	0	0	3	0	0
Head and Neck	3	2	1	0	1	0	0
Gastro-intestinal	6	3	1	1	1	0	0
Gynecological	3	1	0	0	1	1	1
Sarcoma	1	0	0	0	0	0	0
Miscellaneous	2	2	0	0	2	1	1
Total	40	23 (58%)	3	1	19	7 (18%)	6 (15%)

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