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THE CHEMISTRY AND BIOCHEMISTRY OF PANTOTHENIC ACID

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CONTENTS

I. Name

The name pantothenic acid (Gr. from everywhere) was given (1) because of the ubiquitous occurrence of the principle, before it was definitely known to be a vitamin. The retention of the name and the failure to use any letter-number designation for it marks an early departure from the traditional alphabetical vitamin nomenclature, and one which is almost certain to be followed in the future. It has been suggested that the name of the principle can, for certain purposes, be shortened conveniently to pantothen (2).

II. Isolation

Because of its hydrophylic and polyfunctional nature, making precipitation and crystallization difficult, and because methods have not been devised for isolating *acids* of this type, the isolation of pantothenic acid from natural sources in entirely pure form has never been accomplished. Five years after the original paper (1), Williams and co-workers (3) announced (1938) the isolation of about 3 gm. of 40% pure calcium salt and small quantities of over 90% pure salt, from 250 kg. of sheep liver.

The process involved (*a*) autolyzing liver to obtain a clear filtrate, (*b*) removal of bases by adsorption on fuller's earth, (*c*) adsorption of the principle on charcoal and subsequent elution, (*d*) evaporation to dryness on kieselguhr in the presence of brucine and brucine oxalate, (*e*) extraction of the residue with dry chloroform to obtain brucine pantothenate mixed with other brucine salts, (*f*) a long and laborious procedure, using fractional distribution of the brucine salts between chloroform and water, (*g*) conversion to the calcium salt, (*h*) fractionation of the calcium salts with various solvents and solvent mixtures.

The final material was about 11,000 times as potent as a rice bran preparation which originally had been a starting material, and was retained as a "standard."

Three other groups of workers independently reported progress in the isolation of what ultimately proved to be pantothenic acid.

Koehn and Elvehjem (4) in concentrating the "antipellagra factor" for chicks (1937) obtained a preparation which contained 40% of the vitamin present in the original liver extract, and only 0.64% of the solids. This represented a 62-fold concentration starting with the liver extract. When doses of 0.7 mg. per day were fed to chicks, pellagra was prevented and the chicks gained 67 gm. more than those on the control diet, in 6 weeks. Though exact evaluation is impossible, it appears that the preparation may have contained 5%, and possibly more, of pantothenic acid. In 1938 Woolley, Waisman, Mickelsen and Elvehjem (5) reported regarding this same material "no increase in potency has been obtained." The same preparation contained nicotinic acid because it cured black-tongue in dogs (4).

Snell, Strong and Peterson (6) studying an "accessory factor for lactic acid bacteria" obtained in the same year (1937) a preparation which subsequent work shows to have been approximately 10% pantothenic acid. In 1938 (7) they indicated "a long series of fractionation procedures [applied to the 10% material] resulted in only about twofold increase in activity." Their "best preparation contained approximately 26% pantothenic acid."

The collaborators at the Lister Institute and the University of Manchester (1939) obtained pantothenic acid (designated by them "liver filtrate factor") in approximately 25% purity (8, 9).

Subsequent to the publication of Williams and collaborators (3) two other groups of investigators reported serious attempts to purify pantothenic acid.

Subbarow and Hitchings (10) by modifying Williams' procedure obtained "510 mg. of white varnish-like calcium salt (corresponding to Williams' fraction C)" [*ca.* 76% pure] from 160 kg. of liver. This preparation stimulated rat growth when administered at the rate of 8 mg. per rat per week.

Kuhn and Wieland (11) used as raw material 4 tons of tunny fish liver. By using (*a*) several precipitation procedures to remove inert material at various stages, (*b*) adsorption and elution using charcoal, (*c*) precipitation of the active principle with barium hydroxide in methanol, (*d*) chromatographic adsorption on alumina, they obtained material about 6% pure. They concluded that the active material was the same as that obtained from mammalian livers. Pure pantoic lactone was obtained from their concentrate by hydrolysis and, in addition, β-alanine, leucine and what appeared to be a homolog of pantoic lactone. Only the pantoyl-β-alanine had physiological activity for *S. plantarum* which was used as a test organism.

III. Recognition of Physiological Importance

The earliest recognition of a physiological effect which may be ascribed predominantly to pantothenic acid may be ascribed to Ide (12) [Wildiers (13)] who in 1901 found "bios" to be indispensable for the growth of yeasts. Under the conditions of the experiments reported, involving very low seeding and prolonged incubation time (Hansen I yeast), pantothenic acid is effective and is the only substance which appears to have chemical and physiological characteristics similar to the active principle studied. Biotin, for example, while an exceedingly potent yeast growth stimulant when tested for under appropriate conditions, is too stable for "bios," and does not show its effect when small seedings and long incubation periods are used. During long incubation periods, biotin is synthesized by the yeast rapidly enough to produce extensive growth (14). The recognition of pantothenic acid as a single effective nutrilite did not come until 1931 (15) and 1933 (1).

The discovery of the effect of the principle now known as pantothenic acid on bacteria should be ascribed to Snell, Strong and Peterson (6, 7) who used lactic acid bacteria as test organisms in their concentration procedure, which resulted in highly potent material.

Possibly the first recognition of an effect on animal life due largely to pantothenic acid deficiency was that of R. R. Williams and R. E. Waterman (16) who discovered "vitamin B_3," necessary for weight maintenance in pigeons. The effect observed was doubtless complicated by other factors as would necessarily be the case so early in the history of "vitamin B." A syndrome in chicks which is now known to be due, in a considerable degree, to pantothenic acid deficiency was discovered by Norris and Ringrose in 1930 (17). A condition resembling uncomplicated pantothenic acid deficiency more closely was produced by Kline, Keenan, Elvehjem and Hart (18) who introduced the heated diet into studies of chick nutrition. The diet of Norris, *et al.*, was deficient in a number of the members of the "B complex" which are required by young chicks, whereas

that of Kline, *et al.*, was almost lacking pantothenic acid and was deficient in riboflavin (19), as well as an unknown factor or factors (20, 21). The identity of the "chick antidermatitis factor" and pantothenic acid was strongly indicated by Woolley, Waisman and Elvehjem (22) who showed the former was a β-alanine derivative (like pantothenic acid), and by Jukes (23) who tested highly potent concentrates furnished by Williams and co-workers.

The "liver filtrate factor" required by rats (8) which was concentrated by the English workers, was later found to be replaceable by pantothenic acid (9) and it is clear that these workers were concerned with pantothenic acid as a physiological principle required by the rat. Subbarow and Hitchings (10) first reported pantothenic acid, as prepared by Williams and co-workers, to be a growth substance for rats. Morgan and co-workers (24) found graying of the fur of rats accompanied by adrenal and other lesions (25) to be due to "filtrate factor" deficiency and suggested the possibility that pantothenic acid might be involved. Negative indications, however, were obtained. György, Poling and Subbarow (26) obtained positive evidence with regard to the efficacy of pantothenic acid in curing the gray hair condition in rats.

IV. Chemical Structure and Synthesis

1. Preliminary Findings

Long before pantothenic acid had been isolated or even concentrated, indirect experimental evidence was obtained indicating that its molecular weight was about 200; that it had in its structure no olefine double bond, aldehyde, ketone, sulfhydryl, basic nitrogen, aromatic or sugar group (1); that it was an acid with an ionization of about 3.9×10^{-5} (27) and possessed several hydroxyl groups (1) and a nitrogen atom with barely detectable basic properties (28). These extensive preliminary findings, which are unique in the history of the isolation of physiological principles, were made possible because a highly quantitative biological method for determining pantothenic acid could be used (1, 29, 30) and because of the extensive use of a relatively new tool, *i. e.*, fractional electrical transport (31).

2. β-Alanine as a Cleavage Product

The first definite step in the elucidation of the exact structure of pantothenic acid involved the discovery of β-alanine as a cleavage product (32, 33). Previous to this β-alanine had been found to be a yeast growth stimulant (34) when used in minute doses, and it later became clear that yeast used β-alanine as a "building stone" in the production of pantothenic acid. β-Alanine previously was known in the free condition and as a constituent of the peptides, carnosine and anserine, but its physiological significance was obscure. The combination of β-alanine in panto-

thenic acid was found to be different from that in carnosine or anserine, because in pantothenic acid no amino group was present. In the two peptides the carboxyl group of the β-alanine is involved in the linkage while in pantothenic acid the amino group is involved. β-Alanine was first identified as a cleavage product of pantothenic acid because of its physiological effects upon yeast. Later it was isolated and identified as α-naphthalene sulfo-β-alanine (33).

3. *Nitrogen-Free Portion of the Molecule*

Evidence was obtained by Williams and co-workers that the other cleavage product of the pantothenic acid molecule was an α-hydroxylactone in which the lactone was probably the γ-variety (35). Elementary analysis of the most potent pantothenic acid concentrates (36), however, had indicated that this lactone possessed five carbon atoms, instead of the six actually present. Later Williams and Major (37) announced that the lactone, as isolated in pure form in the Merck Laboratories from pantothenic acid concentrates, has the structure indicated by the name: α-hydroxy-β,β-dimethyl-γ-butyrolactone. Simultaneously Woolley (38) reported obtaining a small amount of crystalline material thought to be the non-nitrogenous portion of pantothenic acid, but no analysis or structure was given.

The lactone derived from pantothenic acid for which the appropriate name pantoic lactone has been suggested (39) was not a new compound as it had long before been synthesized and obtained in racemic form by Glaser (40). The natural lactone, m. p. 91–92°, $[\alpha]_D^{26}$ −49.8°, was isolated and its structure determined by degradation by Stiller, Keresztesy and Finkelstein (41) and was synthesized by Stiller, Harris, Finkelstein, Keresztesy and Folkers (42) following, with modifications, the work of Wessely (43) and Glaser (40) and Kohn and Neustädter (44). α,α-Dimethyl-β-hydroxypropionaldehyde was prepared by aldol condensation of isobutyraldehyde and formaldehyde (43). This aldol was converted into its bisulfite compound and this into the cyanohydrin which was saponified to produce the lactone in good yield. The racemic lactone was resolved by fractional crystallization of the quinine salts, and the (+) lactone racemized in order to increase the yield of the desired (−) lactone, by heating the sodium salt in water solution.

Reichstein and Grüssner (45) and Carter and Ney (46) have developed modified procedures for preparing the lactone, involving the direct reaction of calcium chloride and sodium or potassium cyanide on the aldol in aqueous

solution, and Major and Finkelstein (47) have successfully used optically active quaternary ammonium bases, such as quinine methohydroxide, to resolve the lactone. Grüssner, Gätzi-Fichter and Reichstein (48) and Parke and Lawson (123) have assigned to the (−) lactone the *d* configuration.

4. Methods of Condensation

Groundwork had already been laid for the synthesis of pantothenic acid as soon as the structure of the two constituent parts was known.

Williams and co-workers in June, 1938, before the presence of a β-alanine residue in the pantothenic acid molecule had been announced, had brought about a partial synthesis of pantothenic acid by condensing a β-alanine ester with the impure lactone obtained from a pantothenic acid concentrate; the ester so obtained was hydrolyzed to yield the physiologically active pantothenic acid (49). Similar partial syntheses were accomplished independently by Snell, Strong and Peterson (50), received February, 1939, and Woolley, Waisman and Elvehjem (51), received March, 1939, who were investigating an "accessory factor for lactic acid bacteria" and the "chick antidermatitis factor," respectively, from the standpoint of their relationship to pantothenic acid, which was now recognized as a β-alanine derivative of a dihydroxy acid (32).

There are two fundamental practical methods for the synthesis of pantothenic acid. One involves the reaction of pantoic lactone with a β-alanine ester. This was first used by Williams and co-workers (48, 49) using crude lactone from natural sources, and was developed on a practical scale using the synthetic lactone by Stiller and co-workers (42) and independently by Reichstein and Grüssner (45) and Kuhn and Wieland (52). In the early experiments of Snell, Strong and Peterson (50) and Woolley, Waisman and Elvehjem (22, 51) a modification of the ester-lactone condensation was used. Steps were taken to produce the acid chloride of the acetylated pantoic acid for use as a reactant, but since the acid chloride was not isolated it may well have been the acetylated lactone itself which condensed with the β-alanine ester. These experiments were carried out before the non-nitrogenous part of pantothenic acid was known to be a lactone which would itself react with β-alanine esters. Kuhn and Wieland (52) utilized the benzyl ester of β-alanine to react with pantoic lactone, and subjected the product to catalytic hydrogenation.

The use of β-alanine esters for the synthesis has two disadvantages. The simple alkyl esters, at least, are difficult to prepare or keep since they polymerize readily on standing (53). When an ester of β-alanine is used, the corresponding ester of pantothenic acid is produced, and a subsequent cleavage of the ester is necessary to obtain pantothenic acid, which is itself subject to hydrolytic cleavage under rather mild conditions.

The other general method of synthesis obviates both of the difficulties

mentioned above. It involves the reaction between the pantoic lactone and a salt of β-alanine to yield directly a salt of pantothenic acid. This was first carried out in alcoholic solution in Williams' laboratory, but a poor yield was obtained. Later when synthetic pantoic lactone became available, Williams, Mitchell, Weinstock and Snell (49) obtained a theoretical yield of sodium pantothenate by reacting dry pantoic lactone with sodium β-alanate. Independently Babcock and Jukes (54) carried out the reaction in concentrated water solution with good yields, and Woolley (55) proposed a synthesis along somewhat similar lines except that the unnecessary steps involving acetylation and the use of thionyl chloride to produce the acid chloride were included. Moore (56) has obtained pantothenic acid by condensation of pantoic lactone with free β-alanine.

5. *Resolution*

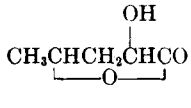
The resolution of pantothenic acid by use of its quinine salts was reported by Stiller, Harris, Finkelstein, Keresztesy and Folkers (42) simultaneously with their announcement of the total synthesis of pure pantothenic acid. As indicated above, the pantoic acid portion of the molecule bears the asymmetric carbon atom and the natural lactone is levorotatory. Free pantothenic acid has been obtained in the form of a pale yellow viscous oil with a rotatory power, $[\alpha]_D^{26}$ +37.5°. The calcium salt is likewise dextrorotatory, $[\alpha]_D^{26}$ +24.3°. Kuhn and Wieland (52, 57) also brought about the resolution of pantothenic acid by fractional crystallization of the quinine and cinchonidine salts, and Stiller and Wiley (58) used quinine methohydroxide for its resolution, as did Major and Finkelstein (47) for resolving pantoic lactone.

6. *Specificity*

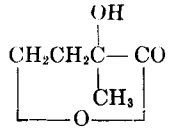
When β-alanine was discovered to be a nutrilite for yeasts (34) its action in this respect was found to be unique in that none of the common α-amino acids, or such closely related β-amino acids such as β-amino butyric acid or isoserine, $CH_2NH_2CHOH—COOH$, had a physiological effect which was in any way comparable with that of β-alanine (59). Weinstock and coworkers (60) have recently condensed various amino acids including α-alanine, β-aminobutyric acid, aspartic acid and lysine with pantoic lactone, and found the products to be biologically inactive. Kuhn and Wieland (11) found the leucine-pantoic lactone condensation product also to be inactive physiologically. These observations point to a definite biological

specificity so far as the β-alanine portion of the pantothenic acid molecule is concerned.

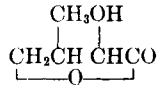
The non-nitrogenous portion of the pantothenic acid molecule can be altered in a number of ways without completely destroying the physiological potency. The optical antipode of natural pantothenic acid appears to have substantially no physiological activity either on microorganisms or on experimental animals (42); the configuration of the asymmetric carbon atom therefore cannot be inverted without destroying the physiological effectiveness. Among the lactones found in Williams' laboratories (35) to have some slight physiological activity when condensed with β-alanine, are the following:



α-Hydroxy-γ-valerolactone



α-Hydroxy-α-methyl-γ-butyrolactone

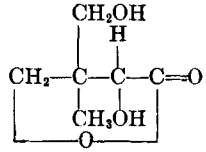


α-Hydroxy-β-methyl-γ-butyrolactone

Subbarow and Rane (61) and Woolley and Hutchings (62) have reported that α,δ-dihydroxyvaleryl-β-alanine is physiologically effective on certain strains of hemolytic streptococci but is required in much larger amounts than is pantothenic acid. Snell, Woolley and Strong (63) independently prepared and tested several of the β-alanine derivatives referred to above with similar findings. The activity for lactic acid bacteria (35) was in each case only a fraction of a per cent as great as that of pantothenic acid, but was nevertheless great enough to be readily detected. It may be noted that all of the compounds capable of yielding β-alanine derivatives with a trace of physiological activity, have an α-hydroxyl group.

Reichstein and Grüssner (45) found α,γ-dihydroxyvaleryl-β-alanine and α,δ-dihydroxyvaleryl-β-alanine to possess no more physiological activity for rats than free β-alanine.

Hydroxypantothenic acid, prepared by Mitchell and co-workers (64, 65) from hydroxypantoic lactone and sodium β-alanate, is definitely more active than any of the other known compounds closely related to panto-



Hydroxypantoic lactone

thenic acid. Its physiological activity varies from 1.5% to 25% of that of pantothenic acid depending upon the microorganism used and the conditions of testing. From this interesting fact it was deduced that probably hydroxypantothenic acid is not a naturally occurring substance, since preparations from natural sources do not vary in this manner when tested in comparison with synthetic pantothenic acid. Hydroxypantothenic acid was found to have physiological potency for rats but was an incomplete substitute for pantothenic acid (66).

In connection with the question of specificity, the finding of Kuhn and Wieland (11) of what was thought to be a homolog of pantoic lactone in the hydrolysis product of their pantothenic acid concentrate, is interesting, but the condensation product of this lactone and β-alanine did not possess physiological activity.

We must conclude that pantothenic acid, as it is found widespread in nature, is probably a single definite chemical substance. β-Alanine derivatives with closely related structures appear to have at most very slight physiological activity, or are incomplete substitutes for natural pantothenic acid.

It should be pointed out that substances which act as building stones for the production of pantothenic or which yield pantothenic acid on hydrolysis, may promote growth in a manner similar to pantothenic acid. Thus, for yeast, β-alanine serves as a precursor of pantothenic acid, but is in general much less effective even on an equal weight basis. β-Alanine is relatively effective in the presence of *low* concentrations of other amino acids (asparagine or aspartic acid), but is much less effective when the amino acid concentration in the medium is high (33). This is apparently due to a competitive action of other amino acids in preventing the absorption of β-alanine by yeast. β-Alanine also serves as a "growth substance" for the diphtheria bacillus (67). Pantothenic acid is effective at even lower concentrations and the evidence points clearly to the probability that β-alanine is a precursor of pantothenic acid.

Hoffer and Reichstein (68) in preliminary experiments found β-alanine to be physiologically effective for rats, but the importance of β-alanine in this connection has not been confirmed elsewhere (69, 70) or in Reichstein's laboratory (48).

Woolley (71) found that a strain of hemolytic streptococcus gave a growth response when pantoic acid without β-alanine was added to the culture medium. Again pantothenic acid itself was more effective and it was concluded that pantoic acid served as a precursor of pantothenic acid. Yeast is affected by relatively high concentrations of pantoic acid

in the presence of β-alanine, but too high concentrations were required to make it a useful test (72). Lactic acid bacteria, on the other hand, are, in general, not materially affected by moderate concentrations of either pantoic acid or β-alanine or hydrolyzed pantothenic acid which contains both (50).

Even animals are able to bring about some synthesis of pantothenic acid if the two fragments, β-alanine and pantoic lactone, are furnished in abundant quantities. Babcock and Jukes (54) reported that chicks responded definitely to such a mixture and calculated that roughly 0.06% coupling took place *in vivo*. Grüssner, Gätzi-Fichter and Reichstein (48) found that pantoic lactone (0.5 mg. per day) in the presence of β-alanine (0.5 mg. per day) promoted the growth of rats so that they gained 1.8 gm. per day as compared with 0.35 gm. per day for the controls. The question may be raised whether the synthesis of pantothenic acid in experiments such as these is being effected in the tissues of the animal or in the intestine by bacterial action.

The alkyl esters of pantothenic acid and their acetyl derivatives are ineffective as nutrilites for yeast or lactic acid bacteria. However, such derivatives are apparently hydrolyzed in the digestive tract of animals. Grüssner, *et al.* (48), found ethyl and methyl pantothenates to be effective in promoting the growth of rats and Unna and Mushett (73) found ethyl monoacetyl pantothenate and ethyl pantothenate to be as effective on rats as an equimolecular amount of calcium pantothenate. Ethyl monoacetyl pantothenate was also tested upon chicks and found to be fully effective. Woolley (74) found pantothenic acid diphosphate to be biologically inactive when tested on microorganisms.

Pantothenic acid as it occurs in natural foods is often in a combined form, and there are indications that it may be bound through an amide linkage to proteins (75). This combination certainly serves as an effective source of pantothenic acid, and digestive enzymes must cause its liberation. Bound pantothenic acid, like the esters and their acetyl derivatives, are, in general, ineffective for microorganisms and require preliminary liberation.

In connection with the problem of specificity, the "anti-vitamin" activity of pantoyl taurine discovered by Snell (76, 77) is interesting. This substance when introduced into the culture media for yeasts or lactic acid bacteria inhibits growth, but its effect is overcome by additional pantothenic acid. It appears that there is a competition between pantothenic acid and pantoyl taurine. When the ratio between the concentrations of pantoyl taurine and pantothenic acid is sufficiently high, pantothenic

acid is "blocked out" and growth ceases. This inhibition apparently is observed only in those organisms for which pantothenic acid is an essential nutrilite. Its effects on animals is being studied.

V. Origin-Distribution-Quantitative Determination

The fact that pantothenic acid appears to be everywhere in living matter, as the name suggests, has no bearing on the question of whether deficiencies exist. Recent studies (78, 79) indicate that pantothenic acid shares its universal presence in living matter with all the other B vitamins. Even though thiamin, for example, probably occurs universally, diets which are quantitatively deficient in thiamin are common.

Pantothenic acid has two types of origin both of which are probably important. Green plants under sterile conditions can, after the photosynthetic apparatus is functioning, produce pantothenic acid (80) and it must be assumed that this is an important source in the economy of nature. Various molds (1, 81), bacteria (79) and yeasts (82, 83) produce pantothenic acid when grown on a medium which is free from it. Some yeasts apparently require β-alanine as a building stone while others do not. Molds and bacteria may require a nutritional source of pantothenic acid or may be stimulated by it, but in any case it appears always to be present in the cells produced. The production of pantothenic acid in the soil by molds and bacteria may be important and its bacterial production in the rumen of herbiverous animals certainly is.

Rohrman, Burget and Williams (84) first made determination of the pantothenic acid content of various animal tissues, and noted the effect of autolysis. Jukes (86, 87), Jukes and Lepkovsky (85) and Bauernfeind (88) have given values for a number of foods and feed materials. Peterson and Elvehjem (82) have studied the content of yeasts; Waisman, Mickelsen and Elvehjem (89) meats and meat products. Strong and co-workers (90, 91) have assayed foods (and other materials) and found no great destruction in foods by cooking. Pearson (92) has determined the pantothenic acid content of blood of six species including human's. Waisman, Henderson, McIntire and Elvehjem (93) have found the pantothenic acid content of meats as determined microbiologically to be increased by enzymatic digestion. Wright and collaborators (94) have given assay values for various rat, mouse, beef and hog tissues (autolysates), and Williams and co-workers (95) have studied the content of tissues during embryonic development. These latter studies at the University of Texas have been extended, using enzymatic hydrolysis (96), to human tissues (97), diverse organisms (98), miscellaneous foods (99), bacteria (100), cell nuclei (101) and cancer tissues (102, 103). Miscellaneous additional information regarding the distribution of pantothenic acid will be found in articles referred to later in connection with assay methods. The very high content of "royal jelly" (104) is worthy of special notice.

The yeast growth method was used in the discovery (1) and isolation (3) of pantothenic acid but it was fully recognized, especially in the later stages of concentration, that the test was not entirely specific, when used on crude preparations. For testing potent concentrates it was highly specific and accurate (33) except for the β-alanine effect. When the method as applied to tissues was studied later it was abandoned in favor of a bacterial test, partly because of the possibility of β-alanine interference and partly because of the toxic effects of "old" extracts on yeast.

The methods involving the use of *Lactobacillus casei* are based upon the fundamental findings of Snell, Strong and Peterson (6, 7, 50) and have been developed by Pennington, Snell and Williams (105) and Strong, Feeney and Earle (91). The two methods give substantially the same results; in the latter case asparagin is added to the culture medium, but does not modify the assay values materially (91).

An ideal basal medium for these tests would be a completely synthetic one, but this is for the future, and it is necessary to introduce alkali-treated natural extracts (in which the pantothenic acid is destroyed). Various interfering substances may complicate the test (105, 106, 107, 108) but there are fairly satisfactory remedies for all the difficulties. The use of an alkali-treated extract of the material to be assayed, in the basal culture medium as suggested by Pennington, *et al.* (105), is an important expedient. Blood (or any material which is a very poor source of pantothenic acid) offers real difficulties, which, however, are not insurmountable (109).

Another bacterial method is based upon the observations of Pelczar and Porter (110) with regard to the pantothenic acid requirements of *Proteus morganii* and has been developed and applied by them to the assay of blood and urine (111, 112). Responses are obtained from 0.001 γ pantothenic acid per 10 ml. culture. Their values for blood are a little more than one-fourth of those obtained by Stanbery, *et al.* (109), and Pearson (92) using the *L. casei* method. The cause of this discrepancy is not known but may have been due to differences in the preparation of the samples for analysis or to differences in the ability of the organisms to utilize the pantothenic acid as it occurs in the blood. The values obtained by this method for urine (average 3.81 mg. per day) agree substantially with those of Gordon (113) (3.52 mg. per day) and Pearson (114) (3.19 mg. per day average for 3 subjects) and Wright and Wright (115) (*ca.* 3.3 mg. per day), as determined by using *L. casei*.

The number of bacteria which are potentially usable for pantothenic acid testing is large. Among those used rather extensively beside the two mentioned are *Streptococcus lactis* (35) and *Streptobacterium plantarum* (11). The existence of alternative microbiological methods by which pantothenic acid may be determined is an advantage, because they can be used to check one another, and if one fails for certain materials others may be applicable. All microbiological methods have the advantage of being applicable to small amounts of material. The method of Pen-

nington, *et al.*, has been adapted to determine 0.0002 γ to 0.001 γ (total) of pantothenic acid (116).

The chick growth method has been used most extensively by Jukes (86, 87) and since it involves only a two-week growth period on young chicks (which are readily available) it is relatively simple compared with other animal assay methods. The results are in general in good agreement with those obtained by bacterial assay (*L. casei*), but when certain materials such as liver extract, rice bran extract and, particularly, yeast are tested, the values by the chick method are much higher than the values obtained by bacterial assay (116). György (117) has indicated that the chick assay method is more reliable, because of difficulties of extraction involved in the bacterial methods. The writer's interpretation is that the chick assay method in some cases gives much too high values, simply because of the presence of growth-promoting substances for chicks, other than pantothenic acid, in the materials tested. It is and has been well recognized that a heated diet such as is used in chick assays is low in at least one other principle needed by chicks, besides pantothenic acid (20, 118, 119, 120).

A chemical method for determining pantothenic acid has been rather thoroughly investigated by Thompson (121) at the writer's suggestion. The method was based upon the selective oxidation of pantoic lactone by lead tetraacetate and the hope that other lactones obtained from natural sources would not be of the type to be affected. The oxidation was found to be erratic and not very selective when applied on a micro scale to natural extracts and there seemed no hope of devising a method which would be useful at the present time.

During earlier study of pantothenic acid various "units" were employed for designating quantities. One "gram unit" (= 80 γ Ca pantothenate) was employed by Williams and co-workers (3). A chick unit (= 14 γ Ca pantothenate) was utilized by Jukes (86) and a streptobacterium unit (0.02 γ) was used by Kuhn and Wieland (11). Since pure salts are now available these units are of no value, and a quantity of pantothenic acid can best be expressed in terms of weight.

Calcium pantothenate was the first salt prepared; it is available in pure crystalline form and since it has been used extensively as a standard substance there seems to be no good reason for changing, though because of its greater ease of crystallization, Gätzi-Fichter, Reich and Reichstein (122), and Parke and Lawson (123) have suggested the use of the sodium salt. The equivalent weights of sodium and calcium are similar, so that weights expressed in terms of the two standards would be about the same.

VI. Physiological Functioning

1. Fundamental Role or Roles

Among the first observations made with regard to the physiological functioning of pantothenic acid was that it stimulated the growth of alfalfa seedlings and caused an increase in carbohydrate production, without increasing the nitrogen assimilation (80). Later it was observed that glycogen storage in yeast was increased very definitely when pantothenic acid was supplied (124). These facts suggested that pantothenic acid probably plays some fundamental role in carbohydrate metabolism. As long as the effects are observed on living organisms, however, the question may always be asked: Is the effect direct or indirect; does pantothenic acid itself enter into the carbohydrate metabolism mechanism or does it affect the process by functioning in some other way necessary to the metabolic activity of the organism?

Pratt and Williams (125) reported that pantothenic acid had a slight definite effect on fermentation by dialyzed yeast maceration juice, but Teague and Williams (126) reported inability to confirm this observation. In the latter study the possibility of pantothenic acid entering into the fermentation process in the non-living system could not be ruled out, since no maceration juice capable of carrying on fermentation could be prepared, which did not contain combined pantothenic acid in appreciable amounts. It was concluded that pantothenic acid probably did not constitute a *dissociable* coenzyme, involved in any of the recognized steps. It may well be that there is some as yet unknown mechanism whereby pantothenic acid is *built into* an essential enzyme system, which is concerned with carbohydrate metabolism, and that failure to preserve this mechanism results in pantothenic acid becoming ineffective. In a living organism this hypothetical mechanism is always present and pantothenic acid becomes effective.

It must be admitted that up to the present time knowledge regarding the fundamental role or roles of pantothenic acid is very scanty. Many effects of deficiencies on animals will be mentioned in later paragraphs but these are mostly, without doubt, secondary.

Dorfman, Berkman and Koser (127) have recently studied the effects of pantothenic acid on the metabolism of *Proteus morganii* (110). Oxygen uptake by deficient cells with pyruvate as substrate was greatly stimulated by pantothenic acid, even though no increase in cell numbers occurred, but no experiments were carried out using non-living systems, and the position occupied by pantothenic acid in the metabolism mechanism could

not be ascertained. The authors concluded that probably pantothenic acid has some role in connection with the oxidation of pyruvic acid. If it is directly concerned in this, it must play a dual role (and this is not impossible) because pantothenic acid is absolutely essential to the metabolism of many organisms which yield lactic acid as an end product of metabolism. It seems not improbable that when bacteria utilize lactic or pyruvic acid as a fuel, complex preliminary syntheses must precede their utilization, and that pantothenic acid enters into some of these processes.

The presumption, in view of the known functions of thiamin, nicotinamide and riboflavin, is that pantothenic acid fits into some enzyme system (or systems) which is essential to metabolism. What this enzyme system is or what these enzyme systems are is not known. There are some facts which suggest that pantothenic acid may be concerned with carbohydrate metabolism, but this is not certain. Wright (128) has recently shown that glucose administration to rabbits causes a lowering of the pantothenic acid content of the blood. This again strongly suggests a function in carbohydrate metabolism, but the effect may be indirect.

It may well be emphasized that the role played by pantothenic acid is without doubt a fundamental one, since it appears to be present in every living cell. In a complex organism it is essential to all types of cells and to the functioning of all kinds of tissues. It is not surprising, in view of this fact, that diverse pathological changes may result from its deficiency.

2. Deficiencies and Requirements

General Discussion.—Among the symptoms which have been fairly well established as connected with pantothenic acid deficiency in animals or fowls are the following: dermatitis; keratitis; adrenal hemorrhage, atropy and necrosis; cortical fat depletion; "blood caked" whiskers; depigmentation of the hair (or feathers); failure to grow; loss of weight (adult); loss of appetite, emaciation; loss of coordination; loss of hair (alopecia); thymus involution; fatty livers; stomach and intestinal ulcers, diarrhea; heart damage; kidney damage; anemia; rapid respiratory rate; rapid heart rate; prostration or coma; sudden death; convulsions; gastrointestinal symptoms; loss of viability (eggs); paralysis; myelin degeneration, sciatic nerve and spinal cord damage; peripheral neuritis; sores about the mouth and nose; hemorrhages under the skin, severe oral lesions; abnormal cartilage (tibia); spinal curvature; increased appetite for salt.

It seems reasonable that only the lack of a substance, fundamental to cellular physiology in general, could cause such diverse symptoms. From

a study of the tissues of chicks deficient in pantothenic acid (129), it was concluded that every tissue became deficient, and presumably this would be true for any deficient animal. It seems that the particular part of the animal's mechanism, which appears to break down, depends upon the peculiarities of the species and the character of the examination made. If ways were available to carry out sufficiently delicate tests, every tissue in a deficient animal would presumably be found to be pathological. In individual species, particular tissues are susceptible or resistant, as the case may be, to pantothenic acid deficiency. The fact that in deficient mice the adrenal cortex may appear normal does not mean, of course, that pantothenic acid is non-essential for the adrenal cortex in the mouse, but rather that the adrenal cortex of the mouse has a more effective means of protecting itself against deficiency than the adrenal cortex of the rat.

A study of the data on the "B vitamins" in normal tissues (78) shows that in different species peculiarities in vitamin distribution in particular tissues exist, which may have a bearing on the question under consideration. Hog *muscle* (*not* hog tissues generally) is rich in thiamin and presumably might be resistant (or susceptible) to thiamin deficiency to a higher degree than other hog tissues. Beef *heart*, on the other hand, is considerably richer in thiamin than hog, rat or mouse heart, and this would be expected to have an effect on the resistance of this particular organ in this species to thiamin deficiency. Human muscle is richer in pantothenic acid than beef, hog, rat or mouse muscle (but not in other B vitamins), and one would expect this to have an effect on the ability of human muscle to resist pantothenic acid deficiency. It seems probable that the distribution of pantothenic acid (and other B vitamins) in the tissues of various species has an important bearing upon the question of how different animals react to deficiencies.

It will obviously be impossible in the available space to discuss all of the studies which have had to do with pantothenic acid deficiency. An attempt will be made to present the essential information regarding some of the more interesting and conclusive studies.

Experiments with Fowls.—The recognition of pantothenic acid as the "chick antidermatitis vitamin" has been discussed in an earlier section of this review. From the standpoint of the effects of pantothenic acid deficiencies on various animals discussed above, it becomes apparent how unsuitable the earlier name would have been for permanent retention, carrying with it the implication that the function of the vitamin is simply to prevent dermatitis, whereas in actuality all tissues are affected by its lack (129).

Phillips and Engel (130) have made a preliminary survey of the pathology of pantothenic acid deficiency in the chick. They found and described extensive spinal cord lesions, which were curable only by pantothenic acid. Sciatic nerve degeneration was observed to be associated with riboflavin deficiency. The conditions attributable to pantothenic acid deficiency included, aside from the spinal cord lesions, thymus involution, keratitis, dermatitis and fatty livers. The thymus involution seemed to occur when either pantothenic acid or riboflavin was deficient.

So far as the adult fowl is concerned Lepkovsky, Taylor, Jukes and Almquist (131) found the "filtrate factor" to have no apparent function in maintaining normal egg production or hatchability. The content of the eggs, however, was directly influenced by the diet. Bauernfeind and Norris (132, 20), however, found the antidermatitis vitamin to be essential for reproduction and hatchability, but not for egg production. Hens were on a diet supposedly free from the vitamins for 28 weeks, without developing any dermatitis and without any effect on egg production or mortality. In a later study from the same laboratory (119) after pure pantothenic acid became available, it has been shown conclusively that pantothenic acid is necessary for reproduction. On a basal diet even with added yeast filtrate and heated liver extract the hatchability of the eggs was zero. With suitable supplements, including pantothenic acid and unknown factors, the hatchability of the eggs could be brought up to 50–60%, but no diet lacking pantothenic acid could support reproduction. They concluded that pantothenic had some but not a great effect on egg production, and that its lack increased mortality. In seven weeks on the deficient diet, 80% of the hens developed a mild dermatitis on their lower shanks and feet.

The seemingly increasing importance of pantothenic acid for the mature fowl, as the result of more extended study, leads one to conclude that the diets used in the earlier studies contained appreciable amounts of undecomposed vitamin, and suggests the possibility that basal diets now in use may not be entirely free from it. Heating a diet in the dry condition may cause part of the vitamin to be "bound" in such a way that it is not extractable by ordinary procedures, and hence might not reveal its presence in a microbiological assay. Something of this sort happens to thiamin (133).

Snell, Aline, Couch and Pearson (134) studied quantitatively the effect of the diet of the hens on the pantothenic acid content of eggs. Eggs obtained from hens on a stock diet contained 17 γ pantothenic acid per gram; from hens on a deficient diet (75% whole corn) eggs contained

3.6 γ per gram. When extra pantothenic acid was fed the content became about double that on the stock diet. Snell and Quarles (135) found in connection with other studies that pantothenic acid, as might be expected, was not synthesized during incubation of eggs.

Taylor and co-workers (136) have found that increasing the pantothenic acid content of eggs by feeding the hens an extra amount or by injecting it directly into the incubating eggs modifies embryonic development. Hatchability was increased 15 to 30% over the controls, the hemoglobin content of the blood was increased 8 to 16%, and the heart size decreased as much as 17%.

The pantothenic acid requirement of chicks has been set by Jukes (137) at 1.4 mg. per 100 gm. of diet. According to a recent study by Bauernfeind (138) the requirement of single comb leghorn chicks is less than half of this, 600 γ per 100 gm. of diet, for maximum growth. This low figure is difficult to accept as an unqualified optimal requirement in view of the extensive work of Jukes and the observation that chicks may develop dermatitis on a diet containing 75% whole corn (134). Corn has been found to contain 700–1000 γ per 100 gm. (87, 88); accordingly 75% whole corn in the diet should furnish the optimal amount of pantothenic acid, and should by no means induce dermatitis.

Groody and Groody (139) recently have observed that pantothenic acid deficiency causes feather depigmentation in black chickens which is an interesting parallel with animal findings to be discussed later. They also observed that force-feeding chickens a diet low in pantothenic acid resulted in their death.

Lee and Hogan (140) have recently studied the requirements of the pigeon and conclude that "vitamin B_3" of Williams and Waterman (16) while multiple in nature is primarily pantothenic acid. Their conclusion is in line with the experimental findings of Carter and O'Brien (141).

Experiments with Rats.—Because rats are a favorite animal for nutritional studies, the reports dealing with pantothenic acid in rat nutrition are too numerous and extensive to be discussed adequately and individually in a short space. We shall consider various of the pathological features resulting from pantothenic acid deficiency, which appear most important at the present time.

Morgan and Simms (142) were the first to note adrenal damage, excessive vascularity and later atrophy of the cortex, due to "filtrate factor" deficiency. This deficiency in rats has been studied by Daft and Sebrell (143, 144) and from the standpoint of pathology by Ashburn (145), and more recently by Supplee and co-workers (146). The direct connection

between pantothenic acid deficiency and the adrenal condition has been established in a number of additional laboratories (147, 148, 149).

It appears that pantothenic acid deficiency in rats damages the adrenal cortex seriously and that a number of the symptoms associated with the deficiency are due to loss of function of the adrenal cortex. The adrenal cortex appears to have numerous functions among which regulation of salt balance and water balance is prominent. It is very interesting that a low salt content of the diet favors graying due to pantothenic acid deficiency (150) and that pantothenic acid deficiency increases the appetite of rats for salt (146). Also it is interesting that water deprivation results in a production of "blood-caked whiskers" in rats (151, 152), a condition commonly associated with pantothenic acid deficiency, and that no combination of vitamins will cure the condition as long as the water is withheld (152). Chronic poisoning with zinc chloride precipitates a syndrome similar to pantothenic acid deficiency and its effects can be neutralized by pantothenic acid (153).

The anti-gray hair effect of pantothenic acid has been studied extensively. The graying of hair due to lack of filtrate factor, observed by Morgan and co-workers (24), has already been mentioned. Following or accompanying the announcements of György and co-workers (154, 155) that pantothenic acid administration would cure the gray-hair condition, there was a considerable amount of contrary evidence. Nielsen, Oleson and Elvehjem in a note (156) indicated that they had obtained a small amount of crystalline material with low pantothenic acid content which was effective for rats at a level of 15 γ per day. Previous to this, workers in the same laboratory tested three pantothenic acid concentrates for anti-gray hair potency with negative results (157). Dimick and Lepp (158) gave evidence to indicate that the anti-gray hair factor is complex. R. R. Williams (159) found pantothenic acid to be of no value in protecting rats from gray hair, on the diets used, and Frost, Moore and Dann (160) found synthetic pantothenic acid ineffective whereas extracts containing comparatively little pantothenic acid were.

From these diverse results it is clear that the graying of hair is a complex process and that the condition may have more than one etiology, and be influenced by various factors. The effect of salt intake on graying (150) has already been mentioned. Cystine was found to decrease the time necessary for restoration of gray hair after pantothenic acid deficiency. Extracts of adrenals, pituitaries, thyroids or desoxycorticosterone were ineffective (161). Pyridoxin-deficient rats have a strong tendency to resist graying (162). Emerson and Evans (163) found pantothenic acid

to cure graying but not a stippling. Free (164) found graying in the rat to be due either to a vitamin lack or to a lack of minerals: iron, copper and manganese. Graying due to copper deficiency (165) was found by Elvehjem and co-workers (166) not to respond to pantothenic acid as did other graying.

As investigation has proceeded the importance of pantothenic acid as an anti-gray hair factor has increased. One of the most extensive investigations is that of Unna, Richards and Sampson (167) who not only found pantothenic acid effective, but found liver extract no better, on the basis of an equivalent amount of pantothenic acid. Their experiments indicated that inositol, *p*-aminobenzoic acid and biotin were ineffective either as substitutes for or supplements to pantothenic acid. On the basis of these experiments one would almost be tempted to justify designating pantothenic acid as *the* anti-gray hair vitamin. It is obvious, of course, that various vitamins of the B group, some of which may arise from bacterial action in the intestine, are important factors in maintaining healthy pigmented hair. The status of pantothenic acid as an anti-gray hair vitamin is strengthened by the finding of Wisconsin workers (166) that it prevents graying induced by a heated grain diet, and that *p*-aminobenzoic acid was ineffective. The work of Martin (185) with mice, to be discussed later, also stresses the importance of pantothenic acid as an anti-gray hair factor. The depigmentation of feathers of fowls due to pantothenic acid deficiency has been mentioned (139).

Other conditions which have repeatedly been observed in connection with pantothenic acid deficiencies in rats are dermatitis (168, 149, 169) sores about the mouth and nose (168, 149) so called "blood-caked whiskers" (170, 149), in which the deposit is porphyrin derived from the Harderian gland (171). The possible connection between this latter condition and the adrenal cortex has been mentioned. Dermatitis is, of course, an extremely indefinite condition and may have many causes. The fact that pyridoxin or pantothenic acid deficiencies may cause lesions which appear to be about the same (169) is not entirely surprising because both vitamins are necessary for a healthy skin, and there is no apparent reason why certain skin areas might not be susceptible to different deficiencies. Alopecia is another sign of pathological skin which has been observed in pantothenic acid deficiencies (168, 149).

Miscellaneous conditions which have been connected with pantothenic acid deficiencies in rats are: hemorrhages under the skin (149), kidney and heart damage (146), thinness of epiphyseal cartilage of the tibia (145) and sudden death (146).

It appears probable that with different levels of deficiencies, different lesions would appear. On a diet completely free from pantothenic acid probably few lesions would appear before death, as compared with those which might develop on a diet in which some pantothenic acid is supplied. The different types of syndromes which appear may be due largely to the degrees with which deficiency exists.

A few miscellaneous facts regarding the functioning of pantothenic acid in rat nutrition are worthy of note. Supplee and co-workers (172) have found that during the assimilation of food there is mobilization of riboflavin to the liver, and that pantothenic acid has a direct and specific function in connection with the process. Drill and Overman (173) have found that the pantothenic acid (also pyridoxin and thiamin) requirements of rats are increased during thyroid feeding. Elvehjem (174) has indicated that pantothenic acid may promote the intestinal production of other B vitamins in the rat. Taylor and co-workers (175) find increased litter size in rats, to result from pantothenic acid administration.

There is fairly good agreement among various workers who have studied the problem that the pantothenic acid requirement of the rat is about 80 γ to 100 γ per day. Unna (149) made a special study of the problem and concluded that about 80 γ per day yields optimal growth. About 100 γ per day is enough to support reproduction (176). Recently Unna and Richards (177) have studied the problem of the requirements of rats of different ages, and found that the requirement, unlike that of thiamin, decreases markedly with age. They suggest that the higher requirement during youth may be due to the association of pantothenic acid with the process of building new tissue. It may be that the decreased nutritional requirement with age may be associated with greater bacterial production in the cecum of the adult rat (178) rather than a decrease in the requirements of the adult tissues.

Experiments with Mice.—The study of the functions of pantothenic acid in mice has been complicated by the question of the exact status of inositol as a vitamin for mice, and by the existence of unknown vitamins necessary for mice and present in extracts.

Graying of hair in mice has not been studied as extensively as the same phenomenon in rats. György and Poling (155) found that graying could be induced in mice by pantothenic acid deficiency and cured by its administration. Like graying in rats it could not be cured completely for the maintenance of a normal pelt indefinitely by pantothenic acid alone; biotin also appeared to be necessary.

Alopecia has been observed a number of times in mice on diets deficient

in pantothenic acid (155, 179, 180, 181, 182). In the early experiments of Norris and Hauschildt (179) extensive alopecia was observed, for which the lack of some factor other than the ones then available was responsible. Later Martin (180) observed the same symptoms on the same diet and found that 150 γ of pantothenic acid per day caused immediate curative response. The effect of pantothenic acid on alopecia in mice is complicated by the question of the effectiveness of inositol, discovered to be a vitamin for mice by Woolley (183). His studies indicate that while pantothenic acid may have a curative effect on alopecia, its effect is indirect, by inducing intestinal synthesis of inositol (184). The situation from the dietary standpoint is complex, because he found that a deficiency of inositol could develop even when inositol is in the diet, provided pantothenic acid is absent.

Martin (185) finds that thiamin, riboflavin, pyridoxin, niacin, pantothenic acid and choline, added to a basal B complex-free diet, renders it adequate for mice and just as complete as one containing inositol and *p*-aminobenzoic acid in addition. Addition of either inositol or *p*-aminobenzoic acid precipitates a syndrome which can be overcome only by the addition of the other. He explains the finding of Ansbacher (186) with regard to the efficacy of *p*-aminobenzoic acid as an anti-gray hair factor, on the basis that inositol was introduced into the diet used, and only because of its presence was the striking effect of *p*-aminobenzoic acid observed. He also indicates that only when *p*-aminobenzoic acid is in the diet are the effects of added inositol readily and uniformly observed.

Some of the general symptoms and pathological changes resulting from pantothenic acid deficiency in mice have been described by two groups of workers (181, 182, 187). Sandza and Cerecedo (181) mention spinal curvature, serous exudate around the eyes, a kicking twitch of the hind legs in addition to alopecia. Lippincott and Morris (187) found myelin degeneration in sciatic nerves and spinal cord (accompanying paralysis of the hind quarters (182)), and hyperkeratotic, atrophic and desquamative dermatosis. The adrenal glands remained normal, in contrast to the observations on rats. Adult mice lost weight (182).

Morris and Lippincott (188) found that pantothenic acid deficiency in mice definitely lowers the rate of growth of mammary carcinomas, but simultaneously causes a severe interference with the host's nutrition. The average daily food intake of the mice was about the same before and after the administration of pantothenic acid. Lewisohn and co-workers (189) found the injections of yeast extracts to prevent tumor growth in 20% of implanted mice. This percentage was about doubled when panto-

thenic acid was administered with the yeast extract, and about tripled when riboflavin was used. Taylor and co-workers (175) found increased viability of eggs (increased litter size) could be induced in mice by pantothenic acid administration.

Sandza and Cerecedo (181) concluded that the pantothenic acid requirement of mice is about 30 γ per day. Morris and Lippincott (182) found 23–29 γ per day nearly as effective as over 200 γ per day, so there is substantial agreement on this point so far.

Experiments with Dogs.—A number of preliminary studies (189, 25, 190) had indicated the probable importance of pantothenic acid in the nutrition of dogs, before the more extensive report dealing directly with this subject was published by Schaefer, McKibbin and Elvehjem (192). This report gives in very satisfactory form about all that is known at the present time regarding pantothenic acid deficiency in dogs.

One of the characteristics of the deficiency is the suddenness with which the animals may fail. Often they eat normally up to the day of the onset, and must be observed frequently if treatment to save their lives is to be administered. There is sudden prostration or coma; usually, but not always, rapid respiration and heart rate; and gastro-intestinal symptoms. In some cases, treatment of a severe deficiency brought the animal back to health even though weeks were required. In other cases the dogs died in spite of treatment. Evidently pantothenic acid deficiency may result in severe damage which is difficult or impossible to repair.

The condition of coma may be related to the hypoglycemia which was observed in deficient dogs, and which disappeared when the deficiency was treated with pantothenic acid. The respiratory and gastro-intestinal symptoms and those involving the skeletal muscles (convulsions) may have had their origin in nervous lesions.

Necropsies were performed on all the dogs which died of deficiencies, but no histological examination of the tissues was recorded. All animals had light-colored mottled livers with very high fat content. The presence of fatty livers and hypoglycemia (and presumably low liver glycogen) on a diet containing 66% sucrose was pointed out as noteworthy, and suggestive of a fundamental impairment of carbohydrate metabolism. Mottled thymuses suggestive of hemorrhagic degeneration were generally observed. The kidneys were dark red in color and showed microscopic evidence of hemorrhage in cortex and medulla. Gastritis and severe enteritis were common and intussusception in the pyloric region and the lower ileum was observed.

The adrenal glands appeared macroscopically normal with the exception

of one out of six which was enlarged. The fact that the blood chlorides were about 20% low in the deficient dogs, and rose after treatment, suggested the possibility of impairment of the adrenals, which, however, did not in general appear macroscopically.

No observations were made in this report regarding the graying of the hair of the dogs except that graying had been observed in dogs which had abundance of pantothenic acid in the diet. Such graying did not necessarily have a nutritional origin.

The requirement of young puppies was estimated to be about 100 γ of calcium pantothenate per kilo of body weight per day. That of adult dogs is thought to be considerably less.

Three miscellaneous observations are worthy of note: Russell and Nasset (192a) found that carbohydrate digestion and absorption were increased 51% and 37%, respectively, when 2 mg. per day of calcium pantothenate was given to dogs on a Purina Chow diet. Other synthetic vitamins failed to have this effect. Morgan (193) in a preliminary report gave some evidence to indicate that dogs deficient in "filtrate factors"—nicotinic acid, pantothenic acid and unknown factors—were harmed rather than benefited by administration of nicotinic acid or pantothenic acid alone, and suggested the undesirability of an imbalance. Silber and Unna (194) studied urinary excretions of pantothenic acid by dogs and found that pantothenic administration had no effect on the riboflavin level of the blood. This is in contrast to the findings of Spies and co-workers (202) on humans to be mentioned later.

Experiments with Other Animals.—Most of the available published information regarding pantothenic acid in the nutrition of hogs is that from Hughes and co-workers (195, 196). Deficiency in this animal leads to poor growth (in pigs); rough dry coat; emaciation; loss of hair in some cases; congested, hemorrhagic and ulcerated areas in the stomach and large intestine particularly, and lack of co-ordination (goose stepping with the hind legs). As in the case of the dog the gastro-intestinal symptoms are prominent.

The requirement of growing pigs is thought to be 7.8–11.8 mg. daily per 100 lbs. of animal. Calculated on the same basis, this requirement is over twice that indicated for the growing pup (192).

It has been known for years that various B vitamins are produced in the rumen of cattle. Three studies have recently been made, dealing with the production of pantothenic acid in the rumen of cattle and sheep.

Wegner, Booth, Elvehjem and Hart (197) showed that pantothenic acid and other B vitamins are synthesized in a cow's rumen when the cow is

fed a diet deficient in B vitamins. When thiamin was added to the diet it seemed to stimulate the bacterial synthesis of other B vitamins.

Later the same authors (198) studied the synthesis of B vitamins including pantothenic acid in a heifer fed a natural diet of silage, hay and grain. The pantothenic acid content of the ration was about 10 γ per gram, whereas the rumen contents, obtained by fistula, were on the average nearly three times as rich.

McElroy and Goss (199) fed a diet to sheep and cows, which contained less than 2.8 γ of pantothenic acid per gram. The contents of the sheep rumen and reticulum contained 70 γ pantothenic acid per gram, and were therefore about 25 times as rich as the feed. In the cow the rumen contents were 20 to 30 times as rich in pantothenic acid as the feed. It is possibly significant that the pantothenic acid content of the rumen contents, according to these workers (using the chick assay method), was in general over twice as large as that given by the Wisconsin workers who used a microbiological assay. Phillips and co-workers (200) found that diarrhea in young calves could be prevented by administration of vitamins A and B. They concluded that nicotinic acid and pantothenic acid may be the effective members of the B family in this condition.

Chapman and Harris (201) found that monkeys maintained on diets deficient in pantothenic acid, and probably some other B vitamins, developed severe oral lesions, "marked general symptomatology" and showed short survival times. The oral lesions were accompanied by an increase in fusospirochetal flora.

Human Experiments and Deductions.—Spies and co-workers (202) were the first to present direct evidence to indicate that pantothenic acid has a function in human nutrition. They found that concentration of pantothenic acid in the blood of malnourished patients was substantially low compared with normal individuals. Injection of pantothenic acid produced a temporary rise in the pantothenic acid level of the blood, and also a 20–30% rise in the blood riboflavin level both in normal and malnourished individuals. These findings are of interest in connection with the more recent observations of Supplee and co-workers (172) on rats and Silber and Unna (194) on dogs. Pearson (114), Wright and Wright (115), Gordon (113) and Pelczar and Porter (112) have studied pantothenic acid excretion in man with substantial agreement as to normal excretion. "Normal" individuals excrete on the average something over 3 mg. per day. Gordon reports that the "test dose procedure" is not applicable as a diagnostic measure of human pantothenic acid deficiency.

Gordon (113) has recently reviewed the available evidence regarding

pantothenic acid deficiencies in man, and cites five cases of peripheral neuritis, one case of Korsakoff's syndrome in a male alcoholic with severe peripheral neuritis, two cases of delirium tremens (203), all of which responded promptly and markedly to pantothenic acid administration when other B vitamins had failed to elicit a response. In some cases there might be a question whether the beneficial response was not due to a delayed effect of vitamins previously administered.

He also indicated the possibility of non-tuberculous Addison's disease being due wholly or in part to pantothenic acid deficiency. In private correspondence he has cited one case from which autopsy sections of the adrenals showed marked hemorrhage and necrosis, and which he feels positive was a case of pantothenic acid deficiency.

The fact that the "anti-neuritic" properties of thiamin have been seriously questioned, is mentioned and the growing opinion that "nutritional neuropathy" is caused by multiple deficiency. Pantothenic acid may prove to be one of effective vitamins in this connection.

The amount of information regarding human pantothenic acid deficiencies and their treatment is meager, but the various lines of evidence with respect to animals suggest very interesting possibilities. The writer has personal knowledge of one case of a nurse, who gave evidence of having received benefit particularly on the mental side (especially memory) as a result of medication with pantothenic acid for a considerable period. This observation is suggestive because Gordon reported a "rapid clearing of the mental state" of one of his cases.

The writer is not aware of any significant report on the status of pantothenic acid as an anti-gray hair vitamin for humans, aside from one by Punnett and Bader published in a popular magazine (*Good Housekeeping*, September, 1941, and September, 1942) in which a fair proportion of the individuals of various ages are reported to be benefited. If these findings are real they suggest that mild pantothenic acid deficiencies may be common, and that gray hair in some cases may be an outward manifestation of a mild pathological state in various tissues.

The human requirement of pantothenic acid is not known with any certainty. Gordon, on the basis of a comparison of riboflavin requirement and excretion, with pantothenic excretion arrived at the "entirely speculative" figure of 9–11 mg. per day.

Independently and in ignorance of this figure, Williams (204) arrived at practically the same value by an entirely different means. Various natural foods and food mixtures were assayed for pantothenic acid (and other B vitamins) including human and cow's milk, and the results showed a striking uniformity in that about 10–12 mg. of pantothenic acid was invariably associated with 2500 calories of what we have reason to believe are suitable foods. From these findings it may be deduced that 10–12 mg.

of pantothenic acid per day (or per 2500 calories of food) is probably a perfectly safe level.

The subject of the human pantothenic acid requirements is, of course, in need of much study from several angles. Particularly since the needs of young animals seem to be greater than those of adult animals, the problem of the needs of children requires investigation. It may reasonably be supposed on the basis of present information that if human adult deficiencies exist (and there is some evidence that they do), deficiencies among children are more serious and widespread than those among adults.

3. *Pharmacology*

A noteworthy fact regarding pantothenic acid, which is shared to a greater or lesser degree by other vitamins, is that while it is essential to life, and detectable in extremely minute amounts, administration of it to a normal animal is practically without effect. Human beings also fail to give any response so far as blood pressure, pulse, temperature or respiration is concerned when about 10 times the normal daily intake (100 mg.) is injected (202). This suggests the probability that pantothenic acid in order to be effective must be built into tissue constituents; and that this is a relatively slow process and one which takes place only to an extent demanded by the needs of the organism.

Unna and Greslin (205, 206) have made a thorough study of its effects on animals. They found that a 10% solution could be instilled into the conjunctival sac, or injected (1 cc.) into rabbits without irritation or inflammation. The lethal dose is in terms of *grams* per kilo of body weight, in mice and rats.

Monkeys were fed one gram of calcium pantothenate daily for six months without any untoward effects or pathological changes. This is probably about 500 times their ordinary intake. Dogs and rats survived without damage daily administration for the same period of from 500 to 2000 times their daily requirement.

4. *Functioning in Miscellaneous Organisms*

It is well known that pantothenic acid is effective as a yeast growth substance and that its discovery and characterization came about for this reason (1). Unfortunately the term *yeast* is often used in a careless manner and sweeping and unwarranted statements are made on the basis of observations on one strain under a particular set of conditions. The term *Saccharomyces cerevisiae* while seeming to have a definite meaning is little better than *yeast* because it includes many strains of diverse behavior. This subject has been reviewed elsewhere (14). While pantothenic acid is stimulative of all strains tested, the differences in the responses were early noted (30).

The growth responses of yeast are complex because different strains do not have the same synthetic abilities for the various known nutrilites, thiamin, β-alanine, pantothenic acid, inositol, pyridoxin, biotin and folic

acid (207); and the amino acids (208). Furthermore, the synthetic powers of yeasts do not remain unchanged in all respects for an indefinite period (83). For these and other reasons the study of pantothenic acid in its relation to yeast physiology constitutes a difficult field of study.

The production of pantothenic acid by yeasts has been studied (82) and attempts to ascertain its fundamental role have been made (124, 125, 126), without coming to definite conclusions except that its "binding" by the yeast is preliminary to its utilization.

Green plants (and plant tissues, 125) may be stimulated by minute doses of pantothenic acid. This has been observed in the case of the liverwort, *Ricciocarpus natans* (209), alfalfa seedlings grown under sterile conditions (80) and pea embryos (210). The mobilization of pantothenic acid in a sprouting potato was early observed (209).

Elliott (211) observed the stimulating effect of a pantothenic acid concentrate on certain protozoa. The one species tested was found to be rich in pantothenic acid (98) as well as certain other B vitamins. Whether it is required by various protozoa is not known (212, 224).

The production of pantothenic acid by *Aspergillus niger* was early observed (1), and its stimulative effect on other molds (182, 213) has been studied. An extremely interesting finding is that of Beadle and Tatum (214) who were able to alter the genes in a mold, so that it became dependent upon an outside source of pantothenic acid. The observation that in one mold *any one* of several nutrilites had by itself the ability to promote growth, whereas growth was lacking in the absence of all (81), is worthy of note.

The relationship of pantothenic acid to various bacteria has been mentioned numerous times in this review. The requirement for pantothenic acid is shown by numerous lactic acid bacteria and some propionic acid bacteria as shown by Snell, Strong and Peterson (6), and others (215, 216). Among the other bacteria which require it as a nutrilite, or for which it is effective, are: diphtheria bacilli (67, 217), hemolytic streptococci (61, 218, 62), *Proteus morganii* (110), certain members of the *Pasteurella* group (219), pneumococci (220, 221), and certain non-sporulating anaerobes (222). Bacteria which do not require pantothenic acid produce it, retain it in the cells and release it into the culture medium (100). Microbiological methods for pantothenic acid assay using bacteria have been discussed (105, 91, 111, 35, 11). A study of the function of pantothenic acid in the metabolism of *Proteus morganii* has been discussed. Its production in the intestines of animals (174, 178, 223) and in the rumen of cattle and sheep (197, 198, 199) has been studied.

Comparatively little attention has been given to the study of insect nutrition using pure chemicals. Trager (224) has reviewed this field. Subbarow and Trager (225) have found mosquito larvae to require pantothenic acid for development. Insects are a comparatively rich source of pantothenic acid and certain other B vitamins (98). Their nutrition is complex and probably pantothenic acid is a general nutritional requirement for larvae. The high pantothenic acid content of "royal jelly" (104) is probably not accidental, and suggests important functions in the nutrition of bee larvae.

Bibliography

1. Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.*, **55**, 2912 (1933).
2. Williams, R. J., *Science*, **94**, 462 (1941).
3. Williams, R. J., Truesdail, J. H., Weinstock, H. H., Jr., Rohrman, E., Lyman, C. M., and McBurney, C. H., *J. Am. Chem. Soc.*, **60**, 2719 (1938).
4. Koehn, C. J., and Elvehjem, C. A., *J. Biol. Chem.*, **115**, 693 (1937).
5. Woolley, D. W., Waisman, H. A., Mickelsen, O., and Elvehjem, C. A., *ibid.*, **125**, 715 (1938).
6. Snell, E. E., Strong, F. M., and Peterson, W. H., *Biochem. J.*, **31**, 1789 (1937).
7. Snell, E. E., Strong, F. M., and Peterson, W. H., *J. Am. Chem. Soc.*, **60**, 2825 (1938).
8. Macrae, T. F., Todd, A. R., Lythgoe, B., Work, C. E., Hind, H. G., and El Sadr, M. M., *Biochem. J.*, **33**, 1681 (1939).
9. Lythgoe, B., Macrae, T. F., Stanley, R. H., Todd, A. R., and Work, C. Elizabeth, *ibid.*, **34**, 1335 (1940).
10. Subbarow, Y., and Hitchings, G. H., *J. Am. Chem. Soc.*, **61**, 1616 (1939).
11. Kuhn, R., and Wieland, T., *Ber.*, **73**, 962 (1940).
12. Williams, R. J., *Science*, **88**, 475 (1938).
13. Wildiers, E., *La cellule*, **18**, 313 (1901).
14. Williams, R. J., *Biol. Rev. Cambridge Phil. Soc.*, **16**, 49 (1941).
15. Williams, R. J., and Truesdail, J. H., *J. Am. Chem. Soc.*, **53**, 4171 (1931).
16. Williams, R. R., and Waterman, R. E., *J. Biol. Chem.*, **78**, 311 (1928).
17. Norris, L. C., and Ringrose, A. T., *Science*, **71**, 643 (1930).
18. Kline, O. L., Keenan, J. A., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, **99**, 295 (1932).
19. Lepkovsky, S., and Jukes, T. H., *ibid.*, **111**, 119 (1935).
20. Bauernfeind, J. C., and Norris, L. C., *J. Nutrition*, **18**, 579 (1939).
21. Dimick, M. K., and Lepp, A., *ibid.*, **20**, 413 (1940).
22. Woolley, D. W., Waisman, H. A., and Elvehjem, C. A., *J. Am. Chem. Soc.*, **61**, 977 (1939).
23. Jukes, T. H., *ibid.*, **61**, 975 (1939).
24. Morgan, A. F., Cook, B. B., and Davison, H. G., *J. Nutrition*, **15**, 27 (1938).
25. Morgan, A. F., and Simms, H. D., *ibid.*, **19**, 233 (1939).
26. György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, **132**, 789 (1940).

27. Williams, R. J., and Moser, R., *J. Am. Chem. Soc.*, **56**, 169 (1934).
28. Williams, R. J., Weinstock, H. H., Jr., Rohrmann, E., Truesdail, J. H., Mitchell, H. K., and Meyer, C. E., *ibid.*, **61**, 454 (1939).
29. Williams, R. J., McAlister, E. D., and Roehm, R. R., *J. Biol. Chem.*, **83**, 315 (1929).
30. Williams, R. J., and Saunders, D. H., *Biochem. J.*, **28**, 1887 (1934).
31. Williams, R. J., *J. Biol. Chem.*, **110**, 589 (1935).
32. Reported orally by R. J. Williams at the International Physiological Congress, Zurich, Switzerland, Aug., 1938, and at a meeting of the Am. Chem. Soc., Milwaukee, Wis., Sept., 1938.
33. Weinstock, H. H., Jr., Mitchell, H. K., Pratt, E. F., and Williams, R. J., *J. Am. Chem. Soc.*, **61**, 1421 (1939).
34. Williams, R. J., and Rohrman, E., *ibid.*, **58**, 695 (1936).
35. Mitchell, H. K., Weinstock, H. H., Jr., Snell, E. E., Stanbery, S. R., and Williams, R. J., *ibid.*, **62**, 1776 (1940).
36. Williams, R. J., Weinstock, H. H., Jr., Rohrmann, E., Truesdail, J. H., Mitchell, H. K., and Meyer, C. E., *ibid.*, **61**, 454 (1939).
37. Williams, R. J., and Major, R. T., *Science*, **91**, 246 (1940).
38. Woolley, D. W., *ibid.*, **91**, 245 (1940).
39. McIlwain, H., personal communication.
40. Glaser, E., *Monatsh.*, **25**, 46 (1904).
41. Stiller, E. T., Keresztesy, J. C., and Finkelstein, J., *J. Am. Chem. Soc.*, **62**, 1779 (1940).
42. Stiller, E. T., Harris, S. A., Finkelstein, J., Keresztesy, J. C., and Folkers, K., *ibid.*, **62**, 1785 (1940).
43. Wessely, L., *Monatsh.*, **21**, 231 (1900).
44. Kohn, M., and Neustädter, V., *ibid.*, **39**, 293 (1918).
45. Reichstein, T., and Grüssner, A., *Helv. Chim. Acta*, **23**, 650 (1940).
46. Carter, H. E., and Ney, L. F., *J. Am. Chem. Soc.*, **63**, 312 (1941).
47. Major, R. T., and Finkelstein, J., *ibid.*, **63**, 1368 (1941).
48. Grüssner, A., Gätzi-Fichter, M., and Reichstein, T., *Helv. Chim. Acta*, **23**, 1276 (1940).
49. Williams, R. J., Mitchell, H. K., Weinstock, H. H., Jr., and Snell, E. E., *J. Am. Chem. Soc.*, **62**, 1784 (1940).
50. Snell, E. E., Strong, F. M., and Peterson, W. H., *J. Bact.*, **38**, 293 (1939).
51. Woolley, D. W., Waisman, H. A., and Elvehjem, C. A., *J. Biol. Chem.*, **129**, 673 (1939).
52. Kuhn, R., and Wieland, T., *Ber.*, **73B**, 971 (1940).
53. Abderhalden, E., and Fodor, A., *Z. physiol. Chem.*, **85**, 112 (1913).
54. Babcock, S. H., and Jukes, T. H., *J. Am. Chem. Soc.*, **62**, 1628 (1940).
55. Woolley, D. W., *ibid.*, **62**, 2251 (1940).
56. Moore, M. B., U. S. Patent, 2,234,680 (1941).
57. Kuhn, R., and Wieland, T., *Ber.*, **74B**, 218 (1941).
58. Stiller, E. T., and Wiley, P. F., *J. Am. Chem. Soc.*, **63**, 1237 (1941).
59. Rohrman, E., and Williams, R. J., unpublished observations (1936).
60. Weinstock, H. H., Jr., May, E. L., Arnold, A., and Price, D., *J. Biol. Chem.*, **135**, 343 (1940).
61. Subbarow, Y., and Rane, L., *J. Am. Chem. Soc.*, **61**, 1616 (1939).

62. Woolley, D. W., and Hutchings, B. L., *J. Bact.*, **39**, 287 (1940).
63. Snell, E. E., Woolley, D. W., and Strong, F. M., unpublished data (1939).
64. Mitchell, H. K., Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, **62**, 1791 (1940).
65. Mitchell, H. K., U. S. Patent, 2,271,872 (1942).
66. Zschiesche, E., and Mitchell, H. K., *Proc. Soc. Exptl. Biol. Med.*, **45**, 565 (1940).
67. Mueller, J. H., and Klotz, A. W., *J. Am. Chem. Soc.*, **60**, 3086 (1938).
68. Hoffer, M., and Reichstein, T., *Nature*, **144**, 72 (1939).
69. El-Sadr, M. M., Hind, H. G., Macrae, T. F., Work, C. E., Lythgoe, B., and Todd, A. R., *ibid.*, **144**, 73 (1939).
70. Oleson, J. J., Woolley, D. W., and Elvehjem, C. A., *Proc. Soc. Exptl. Biol. Med.*, **42**, 151 (1939).
71. Woolley, D. W., *J. Biol. Chem.*, **130**, 417 (1939).
72. Mitchell, H. K., unpublished observations (1938).
73. Unna, K., and Mushett, C. W., *Am. J. Physiol.*, **135**, 267 (1942).
74. Woolley, D. W., *J. Biol. Chem.*, **134**, 461 (1940).
75. Williams, R. J., *Biol. Action of the Vitamins*, Univ. of Chicago Press, Chicago, 1942; p. 122.
76. Snell, E. E., *J. Biol. Chem.*, **139**, 975 (1941).
77. Snell, E. E., *ibid.*, **141**, 121 (1941).
78. *Studies on Vitamin Content of Tissue, I*, Univ. of Texas Publ., **4137** (1941).
79. *Studies on Vitamin Content of Tissue, II*, Univ. of Texas Publ., **4237** 1942.
80. McBurney, C. H., Bollen, W. B., and Williams, R. J., *Proc. Natl. Acad. Sci.*, **21**, 301 (1935).
81. Mosher, W. A., Saunders, D. H., Kingery, L. B., and Williams, R. J., *Plant Physiol.*, **11**, 795 (1936).
82. Peterson, W. H., and Elvehjem, C. A., *J. Nutrition*, **18**, 181 (1939).
83. Williams, R. J., Eakin, R. E., and Snell, E. E., *J. Am. Chem. Soc.*, **62**, 1204 (1940).
84. Rohrman, E., Burget, G. E., and Williams, R. J., *Proc. Soc. Exptl. Biol. Med.*, **32**, 473 (1934).
85. Jukes, T. H., and Lepkovsky S., *J. Biol. Chem.*, **114**, 117 (1936).
86. Jukes, T. H., *ibid.*, **117**, 11 (1937).
87. Jukes, T. H., *J. Nutrition*, **21**, 193 (1941).
88. Bauernfeind, J. C., *Poultry Sci.*, **21**, 136 (1942).
89. Waisman, H. A., Mickelsen, O., and Elvehjem, C. A., *J. Nutrition*, **18**, 247 (1939).
90. Strong, F. M., Earle, A., and Zeman, B., *J. Biol. Chem.*, **140**, CXXVIII, (1941).
91. Strong, F. M., Feeney, R. E., and Earle, A., *Ind. Eng. Chem., Anal. Ed.*, **13**, 566 (1941).
92. Pearson, P. B., *J. Biol. Chem.*, **140**, 423 (1941).
93. Waisman, H. A., Henderson, L. M., McIntire, J. M., and Elvehjem, C. A., *J. Nutrition*, **23**, 239 (1942).
94. Wright, L. D., McMahan, J. R., Cheldelin, V. H., Taylor, A., Snell, E. E., and Williams, R. J., *Univ. of Texas Publ.*, **4137**, 38 (1941).
95. Williams, R. J., Taylor, A., and Cheldelin, V. H., *ibid.*, **4137**, 61 (1941).
96. Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirard, B. M., *ibid.*, **4237** 15 (1942).

97. Taylor, A., Pollack, M. A., and Williams, R. J., *Univ. of Texas Publ.*, **4237**, 41 (1942).
98. Woods, A. M., Taylor, J., Hofer, M. J., Johnson, G. A., and Lane, R. L., *ibid.*, **4237**, 84 (1942).
99. Cheldelin, V. H., and Williams, R. J., *ibid.*, **4237**, 105 (1942).
100. Thompson, R. C., *ibid.*, **4237**, 87 (1942).
101. Isbell, E. R., Mitchell, H. K., Taylor, A., and Williams, R. J., *ibid.*, **4237**, 81 (1942).
102. Pollack, M. A., Taylor, A., and Williams, R. J., *ibid.*, **4237**, 56 (1942).
103. Taylor, A., Pollack, M. A., Hofer, M. J., and Williams, R. J., *Cancer Research*, **2**, 752 (1942).
104. Pearson, P. B., and Burgin, C. J., *Proc. Soc. Exptl. Biol. Med.*, **48**, 415 (1941).
105. Pennington, D., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, **135**, 213 (1940).
106. Scott, M. L., Randall, F. E., and Hessel, F. H., *ibid.*, **141**, 325 (1941).
107. Clarke, M. F., Lechycka, M., and Light, A. E., *ibid.*, **142**, 957 (1942).
108. Feeney, R. E., and Strong, F. M., *J. Am. Chem. Soc.*, **64**, 881 (1942).
109. Stanbery, S. R., Snell, E. E., and Spies, T. D., *J. Biol. Chem.*, **135**, 355 (1940).
110. Pelczar, M. J., Jr., and Porter, J. R., *Proc. Soc. Exptl. Biol. Med.*, **43**, 151 (1940).
111. Pelczar, M. J., Jr., and Porter, J. R., *J. Biol. Chem.*, **139**, 111 (1941).
112. Pelczar, M. J., Jr., and Porter, J. R., *Proc. Soc. Exptl. Biol. Med.*, **47**, 3 (1941).
113. Gordon, E. S., *Biol. Action of Vitamins*, E. A. Evans, Editor, Univ. of Chicago Press, Chicago, 1942; p. 136.
114. Pearson, P. B., *Am. J. Physiol.*, **135**, 69 (1941).
115. Wright, L. D., and Wright, E. Q., *Proc. Soc. Exptl. Biol. Med.*, **49**, 80 (1942).
116. Pennington, D., Snell, E. E., Mitchell, H. K., McMahan, J. R., and Williams, R. J., *Univ. of Texas Publ.*, **4137**, 14 (1941).
117. György, P., *Am. Rev. Biochem.*, XI, 329 (1942).
118. Johnson, E. L., Carrick, C. W., Roberts, R. E., and Hange, S. M., *Poultry Sci.*, **20**, 464 (1941).
119. Gillis, M. B., Heuser, G. F., and Norris, L. C., *J. Nutrition*, **23**, 153 (1942).
120. Mills, R. C., Briggs, G. M., Elvehjem, C. A., and Hart, E. B., *Proc. Soc. Exptl. Biol. Med.*, **49**, 186 (1942).
121. Thompson, R. C., Jr., unpublished.
122. Gätzi-Fichter, M., Reich, H., and Reichstein, T., *Helv. Chim. Acta*, **24**, 185 (1941).
123. Parke, H. C., and Lawson, E. J., *J. Am. Chem. Soc.*, **63**, 2869 (1941).
124. Williams, R. J., Mosher, W. A., and Rohrmann, E., *Biochem. J.*, **30**, 2036 (1936).
125. Pratt, E. F., and Williams, R. J., *J. Gen. Physiol.*, **22**, 637 (1939).
126. Teague, P. C., and Williams, R. J., *ibid.*, **25**, 777 (1942).
127. Dorfman, A., Berkman, S., and Koser, S. A., *J. Biol. Chem.*, **144**, 393 (1942).
128. Wright, L. D., *ibid.*, **142**, 445 (1942).
129. Snell, E. E., Pennington, D., and Williams, R. J., *ibid.*, **133**, 559 (1940).
130. Phillips, P. H., and Engel, R. W., *J. Nutrition*, **18**, 227 (1939).
131. Lepkovsky, S., Taylor, L. W., Jukes, T. H., and Almquist, H. J., *Hilgardia*, **11**, 559 (1938).

132. Bauernfeind, J. C., and Norris, L. C., *Science*, **89**, 416 (1939).
133. Lane, R. L., Johnson, E., and Williams, R. R., *J. Nutrition*, **23**, 613 (1942).
134. Snell, E. E., Aline, E., Couch, J. R., and Pearson, P. B., *ibid.*, **21**, 201 (1941).
135. Snell, E. E., and Quarles, E., *ibid.*, **22**, 483 (1941).
136. Taylor, A., Thacker, J., and Pennington, D., *Science*, **94**, 542 (1941).
137. Jukes, T. H., *J. Biol. Chem.*, **129**, 225 (1939).
138. Bauernfeind, J. C., Norris, L. C., and Heuser, G. F., *Poultry Sci.*, **21**, 142 (1942).
139. Groody, T. C., and Groody, M. E., *Science*, **95**, 655 (1942).
140. Lee, J. G., and Hogan, A. G., *Missouri Agric. Exptl. Station Research Bull.*, **342** (1942).
141. Carter, C. W., and O'Brien, J. R., *Biochem. J.*, **33**, 1810 (1939).
142. Morgan, A. F., and Simms, H. D., *Science*, **89**, 565 (1939).
143. Daft, F. S., and Sebrell, W. H., *U. S. Pub. Health Rep.*, **54**, 2247 (1939).
144. Daft, F. S., Sebrell, W. H., Babcock, S. H., and Jukes, T. H., *ibid.*, **55**, 1333 (1940).
145. Ashburn, L. L., *ibid.*, **55**, 1337 (1940).
146. Supplee, G. C., Bender, R. C., and Kahlenberg, O. J., *Endocrinology*, **30**, 355 (1942).
147. Mills, R. C., Shaw, J. H., Elvehjem, C. A., and Phillips, P. H., *Proc. Soc. Exptl. Biol. Med.*, **45**, 482 (1940).
148. Salmon, W. D., and Engel, R. W., *ibid.*, **45**, 621 (1940).
149. Unna, K., *J. Nutrition*, **20**, 565 (1940).
150. Ralli, E. P., Clarke, D. H., and Kennedy, E., *J. Biol. Chem.*, **141**, 105 (1941).
151. Figge, H. J., and Atkinson, W. B., *Proc. Soc. Exptl. Biol. Med.*, **48**, 112 (1941).
152. Smith, S. G., *ibid.*, **49**, 691 (1942).
153. Gross, P., Harvalek, Z., and Runne, E., *J. Invest. Dermat.*, **4**, 385 (1941).
154. György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, **132**, 789 (1940).
155. György, P., and Poling, C. E., *Proc. Soc. Exptl. Biol. Med.*, **45**, 773 (1940).
156. Nielsen, E., Oleson, J. J., and Elvehjem, C. A., *J. Biol. Chem.*, **133**, 637 (1940).
157. Oleson, J. J., Elvehjem, C. A., and Hart, E. B., *Proc. Soc. Exptl. Biol. Med.*, **42**, 283 (1939).
158. Dimick, M. K., and Lepp, A., *J. Nutrition*, **20**, 413 (1940).
159. Williams, R. R., *Science*, **92**, 561 (1940).
160. Frost, D. V., Moore, R. C., and Dann, F. P., *Proc. Soc. Exptl. Biol. Med.*, **46**, 507 (1941).
161. Mushett, C. W., and Unna, K., *J. Nutrition*, **22**, 565 (1941).
162. Lunde, G., and Kringstad, H., *ibid.*, **19**, 321 (1940).
163. Emerson, G., and Evans, H. M., *Proc. Soc. Exptl. Biol. Med.*, **46**, 603 (1941).
164. Free, H. H., *ibid.*, **44**, 371 (1940).
165. Gorter, F. I., *Z. Vitaminforsch.*, **4**, 277 (1935).
166. Henderson, L. M., McIntire, J. M., Waisman, H. A., and Elvehjem, C. A., *J. Nutrition*, **23**, 47–58 (1942).
167. Unna, K., Richards, G. V., and Sampson, W. L., *ibid.*, **22**, 553 (1941).
168. György, P., Poling, C. E., and Subbarow, Y., *Proc. Soc. Exptl. Biol. Med.*, **42**, 738 (1939).
169. Richardson, L. R., and Hogan, A. G., *ibid.*, **44**, 483 (1940).
170. Chick, H., Macrae, T. F., and Worden, A. N., *Biochem. J.*, **34**, 580 (1940).

171. McElroy, L. W., Salomon, K., Figge, F. H., and Cowgill, C. R., *Science*, **94**, 467 (1941).
172. Supplee, G. C., Jensen, C. G., Bender, R. C., and Kahlenberg, O. J., *J. Biol. Chem.*, **144**, 79 (1942).
173. Drill, V. A., and Overman, R., *Am. J. Physiol.*, **135**, 474 (1942).
174. Elvehjem, C. A., *Ind. and Eng. Chem.*, **33**, 707 (1941).
175. Taylor, A., Pennington, D., and Thacker, J., to be published.
176. Unna, K., *Am. J. Med. Sci.*, **200**, 848 (1940).
177. Unna, K., and Richards, G. V., *J. Nutrition*, **23**, 45055 (1942).
178. Taylor, A., Pennington, D., and Thacker, J., *Univ. of Texas Publ.*, **4237** (in press).
179. Norris, E. R., and Hauschildt, J., *Science*, **92**, 316 (1940).
180. Martin, G. J., *ibid.*, **93**, 422 (1941).
181. Sandza, J. G., and Cerecedo, L. R., *J. Nutrition*, **21**, 609 (1941).
182. Morris, H. P., and Lippincott, S. W., *J. Natl. Cancer Inst.*, **2**, 29 (1941).
183. Woolley, D. W., *Science*, **92**, 384 (1940).
184. Woolley, D. W., *J. Exptl. Med.*, **75**, 277 (1942).
185. Martin, G. J., *Am. J. Physiol.*, **136**, 124 (1942).
186. Ansbacher, S., *Science*, **93**, 164 (1941).
187. Lippincott, S. W., and Morris, H. P., *J. Natl. Cancer Inst.*, **2**, 39 (1941).
188. Morris, H. P., and Lippincott, S. W., *ibid.*, **2**, 47 (1941).
189. Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., Laszlo, D., and Block, K., *Science*, **94**, 70 (1941).
190. McKibbin, J. M., Madden, R. J., Black, S., and Elvehjem, C. A., *Am. J. Physiol.*, **128**, 102 (1939).
191. Fouts, P. J., Helmer, O. M., and Lepkovsky, S., *J. Nutrition*, **19**, 393 (1940).
192. Schaefer, A. E., McKibbin, J. M., and Elvehjem, C. A., *J. Biol. Chem.*, **134**, 321 (1942).
192a. Russell, R. A., and Nasset, E. S., *J. Nutrition*, **22**, 287 (1941).
193. Morgan, A. F., *Science*, **93**, 261 (1941).
194. Silber, R. H., and Unna, K., *J. Biol. Chem.*, **142**, 623 (1942).
195. Hughes, E. H., *J. Agr. Research*, **64**, 185 (1942).
196. Hughes, E. H., and Ittner, N. R., *J. Animal Sci.*, **1**, 116 (1942).
197. Wegner, M. I., Booth, A. N., Elvehjem, C. A., and Hart, E. B., *Proc. Soc. Exptl. Biol. Med.*, **45**, 769 (1940).
198. Wegner, M. I., Booth, A. N., Elvehjem, C. A., and Hart, E. B., *ibid.*, **47**, 90 (1941).
199. McElroy, L. W., and Goss, H., *J. Nutrition*, **21**, 405 (1941).
200. Phillips, P. H., Landquist, N. S., and Boyer, P. D., *J. Dairy Sci.*, **24**, 977 (1941).
201. Chapman, O. D., and Harris, A. E., *J. Infectious Diseases*, **69**, 7 (1941).
202. Spies, T. D., Stanbery, S. R., Williams, R. J., Jukes, T. H., and Babcock, S. H., *J. Am. Med. Assoc.*, **115**, 523 (1940).
203. Gordon, E. S., personal communication.
204. Williams, R. J., *J. Am. Med. Assoc.*, **119**, 1 (1942).
205. Unna, K., and Greslin, J., *Proc. Soc. Exptl. Biol. Med.*, **45**, 311 (1940).
206. Unna, K., and Greslin, J., *J. Pharmacol.*, **73**, 85 (1941).
207. Mitchell, H. K., Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, **63**, 2284 (1941).

208. Mitchell, H. K., and Williams, R. J., *Biochem. J.*, **34**, 1532 (1940).
209. Williams, R. J., and Rohrmann, E., *Plant Physiol.*, **10**, 559 (1935).
210. Bonner, J., and Axtman, G., *Proc. Natl. Acad. Sci. U. S.*, **23**, 453 (1937).
211. Elliott, A. M., *Biol. Bull.*, **68**, 82 (1935).
212. Johnson, W. H., *Quart. Rev. Biol.*, **16**, 336 (1941).
213. Shopfer, W. H., and Moser, W., *Protoplasma*, **26**, 538 (1936).
214. Beadle, G. W., and Tatum, E. L., *Proc. Nat. Acad. Sci.*, **27**, 499 (1941).
215. Krauskopf, E. J., Snell, E. E., and McCoy, E., *Enzymologia*, **7**, 327 (1939).
216. Möller, E. F., *Z. physiol. Chem.*, **260**, 246 (1939).
217. Happold, F. C., *Proc. Soc. Exptl. Biol. Med.*, **43**, 412 (1940).
218. McIlwain, H., *Brit. J. Exptl. Path.*, **20**, 330 (1939).
219. Berkman, S., Saunders, F., and Koser, S. A., *Proc. Soc. Exptl. Biol. Med.*, **44**, 68 (1940).
220. Rane, L., and Subbarow, Y., *J. Biol. Chem.*, **134**, 455 (1940).
221. Rane, L., and Subbarow, Y., *J. Nutrition*, **21**, Suppl. 17 (1941).
222. West, R. A., Lewis, K. H., and Militzer, W. E., *J. Bact.*, **43**, 155 (1942).
223. Mitchell, H. K., and Isbell, E. R., *Univ. Texas Publ.*, **4237** (in press).
224. Trager, W., *Physiol. Rev.*, **21**, 1 (1941).
225. Subbarow, Y., and Trager, W., *J. Gen. Physiol.*, **23**, 561 (1940).