XXX. A COMPARATIVE STUDY OF TUMOUR AND NORMAL TISSUE GROWTH.

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Introduction.

From early times the profound influence exerted upon the nutrition of an animal by the composition of its diet has been recognised, so that it is not surprising that a vast amount of research has been carried out upon the subject.

Whilst much of this research has furnished results of great value, it is as a result of the investigations of the last decade in particular that the theoretical basis of the science of nutrition has become so firmly established.

These results have permitted a far clearer definition of the many factors which control the nutritive value of a foodstuff than it was ever possible to deduce from calculations respecting the calorific value and nitrogen content.

Particular interest lies in the determination of the dietary factors which influence the growth and development of the young organism. Many of these factors are now recognised to such an extent that by an experimental application of them the growth of young animals may be controlled. This control may be so complete as to permit the total inhibition of the growth processes for a considerable period of time, without the subject suffering any apparent ill-health.

It is here that such results may be viewed in the light of cancer therapy, since the question arises as to whether dietary measures which inhibit the growth of the young cells of the animal organism may not be with justification applied as a means of arresting growth in tumour cells.

Tumours are frequently described as autonomous, in that their growth is self-regulated without regard for the laws governing the nutritive condition of the host. On the other hand there are facts, such as the relatively slow

growth of tumours in old or emaciated hosts, which lend support to the opposite view.

It is not easy to trace opinions, based upon clinical observations, which concern this subject, since they are scattered widely apart throughout the literature upon cancer.

Before proceeding to discuss the laws of the growth of tumours, it is necessary that the known facts concerning their metabolism be considered. Unfortunately, comparatively little is known with regard to the metabolic processes of the cancer cell. From what is known, it appears highly probable that there is a close similarity between the chemical processes occurring in the tumour cell and those which occur in the normal cell of similar type. It is unnecessary to give in detail an account of the experimental results which support this view, but Wells [1914] who has reviewed the whole subject very completely, expresses the opinion that little has been detected which indicates any important deviation of the chemical processes of tumours from those of normal cells of similar origin.

As this opinion has received the support of the majority of the experimental investigations since carried out, it will be seen that it is justifiable to apply factors which are known to influence the growth of normal cells in an attempt to influence in a similar manner the growth of tumour cells.

It is now necessary to review briefly what is known of these factors which may so profoundly influence the nutritional condition of the animal organism.

It is recognised as necessary that the diet of an animal shall satisfy certain requirements before it can be regarded as adequate to supply the demands of the organism throughout its life cycle.

As far as these requirements have been determined they are as follows:

1. The calorific value of the diet must be sufficient to supply the necessary potential energy.

2. Sufficient nitrogen must be supplied in a form suitable for the building up or repair of tissue.

3. This nitrogen must be supplied in a form which will ensure an adequate supply of certain amino-acids which the animal organism is unable to synthesise for its own use.

4. The diet must contain an adequate supply of inorganic salts capable of satisfying the mineral requirements of the animal.

5. Certain substances, probably two in number, the nature of which is at present unknown, but which have been provisionally termed "accessory growth promoting substances," must be present in a sufficient amount.

With regard to the first two of these requirements little need be said, for until recently they, together with the fourth stipulation, constituted the basis for the standardisation of the nutritive value of foodstuffs. The value and importance of the other two factors has, however, but recently been appreciated. Prior to 1911, repeated attempts had been made to rear and maintain animals upon dietaries composed entirely of chemically pure food units, but although every care was taken to ensure that the diets used contained satisfactory proportions of protein, fat, carbohydrates and mineral salts, yet such experiments met with repeated failure. It was Osborne and Mendel [1911] who first described an extensive research conducted along these lines which was attended with considerable success.

The results obtained by these two authors were of far-reaching importance and opened up a new field of research on protein metabolism.

Earlier studies than these had, however, determined the importance of the quality of the protein fraction of a diet, for Willcocks and Hopkins [1906] demonstrated the inferior nutritive value of the protein zein, which is totally deficient in the amino-acid tryptophan. Osborne and Mendel followed up their investigations along the lines they had previously adopted, and not only confirmed the indispensability of tryptophan [1912, 1] but also pointed out how the amino-acid deficiencies of such a protein as gliadin may influence the nutrition of an animal [1912, 2, 3; 1913]. Much of this earlier work has now received ample confirmation which, together with the results obtained by an extension of the study, have been the cause of very material alterations in the theory of protein metabolism.

That the nutritive value of a protein may be largely determined by its amino-acid content is now so fully appreciated as to have passed the stage when it was regarded as of academical interest alone, and to have become of great value when applied to certain problems of animal husbandry.

Not only may the nutritive value of protein be greatly influenced by the absence of certain amino-acids, but it may be limited by a low content of one such indispensable unit. The case of gliadin, which possesses a low nutritive efficiency as a result of a low lysine content has been referred to above, whilst a very striking example has been given more recently by Osborne and Mendel [1915, 1].

When the diet of a growing rat contains 18 % of protein in the form of caseinogen or lactalbumin, and is at the same time satisfactory with regard to the other requirements, a normal rate of growth is accomplished. Upon reduction of the plane of protein intake, it is found that a diet containing

9 % of caseinogen is of a lower food value to the growing rat than one containing the same percentage of lactalbumin. The explanation of this was found to lie in the fact that caseinogen has a low content of cystine, an amino-acid which the organism of the rat is unable to synthesise.

At the lower plane of intake, the caseinogen diet contained insufficient protein to supply the cystine requirements of the young rat, whereas no such limiting factor was in operation in the case of the lactalbumin diet. That this explanation was the correct one was proved by the addition of the pure amino-acid cystine to the deficient caseinogen diet, whereupon its nutritive value was at once raised to that possessed by the corresponding lactalbumin ration.

Such experiments as these have led to the establishment of an entirely new standard by which the food value of the proteins is judged [Osborne and Mendel, 1916, 1].

The necessity of ensuring an adequate supply of what are termed the "accessory growth promoting substances" in the diet, particularly that of a growing animal, is but now being fully recognised. That such substances existed and played important rôles in animal nutrition was indicated by the researches of Stepp [1909, 1912, 1913], Hopkins [1912] and Funk [1913, 1]. Their existence has been doubted by other investigators, amongst whom may be mentioned Abderhalden [1913, 1] and Röhmann [1916], but it is definitely established that in these cases the authors were not utilising food mixtures of sufficient purity. These substances have received many appellations and are frequently spoken of as "vitamines."

At first one of these substances was found in association with certain naturally occurring fats, such as butter fat and cod liver oil, by Osborne and Mendel [1912, 1] and almost simultaneously by McCollum and Davis [1913].

Later it was demonstrated by McCollum and Davis [1915, 1, 2, 3] that a second accessory exists in the aqueous extracts of certain foodstuffs, particularly seeds.

These authors have shown clearly the importance of these two unidentified substances which they have provisionally designated *the fat-soluble A* and *the water-soluble B.* Both substances are at present unidentified chemically, and indeed very little definite knowledge of their properties is possessed. It appears highly probable that the second substance is identical with the so-called "anti-beriberi vitamine," studied extensively by Funk, since they possess closely similar properties, Drummond [1917]. The need of the animal organism for an adequate supply of both these factors is now admitted by the majority of investigators in this field of research.

Having now very briefly considered the nature of the requirements which a dietary must satisfy before it can be considered adequate to supply the nutritive demands of the animal organism, the recorded experiments upon the influence of diet upon tumour growth will be considered in the light of this knowledge.

Haaland [1907] recorded that mice fed upon a diet of bread, oats, hempseed and milk were less resistant to inoculation with Ehrlich's mouse sarcoma than when fed upon bread and oats alone. This observation received support from Stahr [1908], who attributed a different degree of susceptibility to tumour inoculation exhibited by mice from Berlin and those from Düsseldorf to an influence exerted by differences in their diets. A somewhat similar conclusion was reached by Jensen [1909]. In all these cases, however, the explanation given by the authors was that which seemed best to account for the results, but no experimental proof was given which excluded the possible disturbing influence of other factors.

It was Moreschi [1909] who first attempted a study of the influence of diet upon tumour growth. He found that by underfeeding mice the growth of inoculated grafts of tumour tissue, as yet unvascularised, was retarded. Rous [1911, 1] made a similar study to this, but also examined the influence of such dietary measures applied after the tumour grafts had become established in the host. He obtained the interesting result that whereas a tumour may not take well when implanted into an underfed animal, yet the same tumour may show no retardation in growth as a result of dietary restriction applied after vascularisation of the graft.

Sweet, Corson-White and Saxon [1913] investigated the influence upon tumour growth exerted by a diet in which the protein was supplied in form of gluten. Such a diet had been found by Osborne and Mendel [1912, 1] to permit maintenance, but not growth, in rats owing to its low content of lysine.

Sweet, Corson-White and Saxon aimed at determining whether a cancer could grow in a body rendered incapable of normal cell-growth. They found that the number of successful inoculations of grafts of the Flexner-Jobling rat carcinoma was smaller in the animals which had been fed upon the gluten diet than in the normally fed controls. At the same time the grafts which succeeded in becoming established in the specially fed animals exhibited a slower rate of development and more frequently suffered retrogressive change than in the control series.

They were therefore led to suggest that tumour cells and somatic cells agree with respect to their laws of growth.

Van Alstyne and Beebe [1913] did not agree with the conclusion reached by Sweet, Corson-White and Saxon. They themselves examined the influence of carbohydrates upon tumour growth in rats, and found that a greater degree of success in tumour implantation was obtained in the animals which received a diet containing carbohydrate, as compared with those which received a carbohydrate-free diet.

It is unfortunate that these authors should have chosen lactose as the carbohydrate with which to work, for it has been pointed out that lactose is frequently contaminated with traces of nitrogenous impurities. The possible influence of these impurities upon the course of such experiments as were carried out by van Alstyne and Beebe has been pointed out by Funk [1914, 1] and by Sweet, Corson-White and Saxon [1915].

In spite of this, Woglom [1915] challenged the conclusion of van Alstyne and Beebe, since he found no effect upon tumour growth following the administration of lactose to the hosts. What was probably the correct explanation of these two diverse results was given by Drummond [1916, 1] who pointed out that lactose, unless carefully purified, usually carries traces of the water-soluble accessory factor, *B*. It was probably this factor which influenced tumour growth in the experiments of van Alstyne and Beebe, whereas no such influence would be exerted in Woglom's experiments since he added the lactose to a basal diet already rich in accessory substances.

Rous [1914] followed up his earlier studies and extended his investigations to the more practical determination of the influence of diet upon established tumours in mice. Rous appreciated the doubtful practical value of results such as those obtained by Sweet and his co-workers, and by van Alstyne and Beebe. Commenting upon the lower rate of tumour development obtained in the hosts which had been subjected to dietary measures prior to inoculation, he says, "Unfortunately it is not certain whether the results of these investigations are to be attributed to a specific lack in the foods employed or to the circumstance that the diet of the specially fed hosts differed from that of the animal furnishing the tumour transplanted to them." He therefore conducted a most important investigation in which he studied the effects of dietary restrictions upon spontaneous tumours in mice.

By underfeeding mice upon a gluten ration, similar in composition to that employed by Sweet, for several days prior to operation, the development of recurrences, following incomplete removal of the primary tumour, was in many cases considerably delayed. If, however, the special diet was employed after operation this delay was not brought about. Unoperated spontaneous

tumours seemed unaffected by rigorous dietary restriction and no cures were obtained in any dieted animal.

In the same paper he gives evidence which shows that the reactionary processes of the organism are considerably weakened by the malnutrition which results from the dieting. It therefore appears probable that this fact may furnish the explanation of the lower percentage of successful inoculations in poorly nourished hosts reported by Sweet, and by van Alstyne and Beebe.

Reviewing his work Rous [1915] has remarked, "Special experiments have shown that our results are to be attributed solely to the underfeeding and resultant loss of body weight, and not to the character of the food. The treatment was drastic. The best results were obtained with animals losing weight rapidly at the time of operation." In view of these results he does not feel justified in advocating the employment of underfeeding as a palliative treatment of cancer.

Following a somewhat different line of investigation Funk [1914, 1, 2] has made a study of the influence of what he terms "vitamines" upon the growth of a transplantable sarcoma of the fowl. Unfortunately his results must be regarded as inconclusive, chiefly as there is considerable doubt as to whether, under the conditions employed for the experiments, the chicken is a satisfactory subject for such studies [Drummond, 1916, 2].

Benedict and Rahe [1917] have recently investigated the influence of the "vitamines," or accessory growth factors, upon tumour growth. They employed dietaries similar in composition to those which have been used by Funk and Macallum [1915, 1916, 1, 2], and determined that tumour cells had no power to synthesise the accessory substances when these were absent from the diet of the host, but that at the same time a certain amount of tumour growth occurred under these circumstances, at the expense of the tissues of the animal.

From this brief summary of the more important work which has been carried out upon this subject, it will be realised how little is known regarding the influence of diet upon tumour growth. This is largely due to the disconnected character of the researches, and to the difficulty of co-ordinating the results they have yielded.

Few of these investigations can be said to have a close bearing upon the cancer problem, for the determination of the fact that underfeeding an animal will render it less susceptible to tumour inoculation scarcely applies to any phase of the disease in man. A low state of nutrition, whether induced by

poverty in the diet or by an intercurrent disease, is known to have an unfavourable influence upon tumour implantation.

Theoretically, all such studies as these should be carried out upon animals bearing spontaneous tumours, for they alone are to be regarded as the equivalent of the cancer patient. Rous has carried out a study upon such animals, which has already been referred to, but it is seldom that the opportunity of working with large numbers of these subjects presents itself.

The nearest approach to this ideal condition of cancer research is to study the implanted tumour after it has become established in the body of the host. It must be admitted that even this substitute for the cancerous animal falls regrettably short of what is desired, but it is at present the best available.

The present investigation was designed to study the influences exerted upon tumour growth by the following dietary factors.

(*a*) The plane of protein intake of the host.

(*b*) The character of the protein constituents of the diet of the host.

(*c*) The amino-acid content of the diet.

(*d*) The so-called accessory factors, the *fat-soluble A* and the *water-soluble B*.

Experimental.

Methods.

Rats were chosen as the subjects for this experimental research on account of their great suitability for such work. The great majority of the animals employed were of the albino variety of *Mus norvegicus*, but a few of the black hooded variety of the same animal were also used.

A large number of the experimental animals were those bred from the healthy laboratory stock, whilst others were purchased from reliable breeders.

All stock rats were kept under the closest observation during the period before they were used for experimental purposes. This provided the opportunity of weeding out unsuitable specimens. The general health of the stock was, however, so excellent that the number of rejected animals was very low indeed. As soon as batches of the selected animals had reached a suitable size and weight they were inoculated with the tumour under investigation and returned to the pens.

They were again closely observed during the initial stages of the development of the inoculated graft, until a number of them showed well established and actively growing tumours. These animals were then placed in special boxes and used for dietary experiments. The boxes used for these

experiments were shallow wooden ones with wire-covered tops and sawdust-sprinkled floors. At one end was placed an enclosed sleeping-box of a size proportionate to the number of occupants. All these boxes were cleaned out at least every three days, whilst occasionally the inmates were transferred to new and thoroughly cleansed quarters.

The experimental rats were fed every morning and evening with fresh supplies of the various food preparations. These latter were prepared by thoroughly incorporating the ingredients in a mechanical mixer and adding sufficient water to form the whole into a very stiff paste. This was then made up into small hard balls weighing approximately 5 grams apiece, in which form it was supplied to the animals. By this means it was possible to make a rough estimate of the twenty-four hours' food consumption. Small quantities of the diets were prepared at one time and stored at the temperature of the refrigerator room (1·1°), as the superior value of freshly prepared food mixtures was found to repay the trouble which the more frequent preparations entailed.

All experimental animals were subjected to the closest observation. Body weights were recorded every four days and tumours were charted once a week, whilst at the same time occasional observations on the body temperature were registered.

The tumours utilised for this work were two rat sarcomas which are designated for laboratory purposes, *A* and *S*.

The former was used in some of the earlier studies, but its employment was discontinued owing to certain characteristics it possessed which rendered it less suitable for the purpose than the *S* tumour.

The *A* tumour is a round celled sarcoma. Grafts inoculated into suitable rats usually develop satisfactorily in from 60—80 % of the cases. The tumour grafts generally show a rapid rate of growth, but the resulting tumours are particularly prone to early and extensive necrotic changes. It is frequently found that after a week or two's growth the whole central portion of the tumour is so extensively degenerated that there remains but a thin shell of growing tissue surrounding a mass of necrotic cell debris.

It was owing to this characteristic that the use of this particular tumour was discontinued and the *S* tumour adopted. The latter tumour is a spindle-celled sarcoma showing a high degree of virulence. Inoculated grafts in suitable hosts usually develop satisfactorily in 85—100 % of the cases. It exhibits a slightly slower rate of growth on the average than does the *A* tumour, but it is not nearly so prone to degenerative changes. The *S* tumour

remains firm until a relatively late stage in its development, and when necrotic changes in the centre of the growth do occur they do not extend rapidly.

Neither tumour is accompanied during its growth by microscopic evidence of the formation of metastases when the grafts are subcutaneous.

The inoculation of the rats was carried out by implanting small pieces of tumour tissue, of approximately equal size, subcutaneously in the neighbourhood of the right axilla by the trochar method. The question of the importance of what is termed the effective initial dose [Bashford, Murray, Haaland and Bowen, 1908] was considered before this method of inoculation was decided upon.

Several methods have been elaborated which aim at ensuring a closer approximation to uniform dosage at inoculation, but it is uncertain, having due regard to the more or less uncontrollable influences exerted by those factors which determine the susceptibility of the host, whether these methods are successful in attaining their object. In view of this uncertainty, the method of inoculating small pieces of tissue of an approximately equal size was considered sufficiently accurate for the purposes of this research. No animal which failed to show a satisfactory rate of tumour development during the period of observation following the inoculation was used for feeding experiments. All animals at the conclusion of the experiments were subjected to a post-mortem examination, at which pieces of the tumours and certain organs were removed and fixed in Zenker's fluid, for subsequent microscopical examination.

The mortality amongst the experimental animals from intercurrent disease was exceedingly low, only one or two cases occurring throughout the investigation.

The preparation of the individual components of the various dietaries was carried out by the processes described below.

Caseinogen was prepared from diluted skimmed milk by acidification as described by Osborne and Mendel [1911]. The crude protein was twice reprecipitated from solution in dilute alkali by the same method, and was then extracted by being shaken vigorously in repeated changes of distilled water, the first two changes containing a trace of acetic acid. The resulting product was dehydrated and repeatedly extracted with alcohol and ether at room temperature.

Lactalbumin was prepared by heat-coagulation of the proteins present in the faintly acidified filtrate from the precipitated caseinogen obtained in the last preparation. It was purified by several extractions with boiling

distilled water and subsequent treatment with changes of boiling alcohol and ether.

Edestin, zein and gliadin were prepared from hempseed, crushed maize, and wheat respectively by the processes described by Osborne [1910]. They were in each case subjected to careful purification, as directed by this author, to ensure preparations of a high degree of purity.

Ovalbumin was prepared from egg-white according to the process of Hopkins and Pinkus [1900]. It was a crystalline product of considerable purity.

Gelatin was that supplied by Coignet Père et Fils et Cie and bore the mark "Gold label, extra." It gave at the most only a faint coloration with Millon's reagent.

Starch was purchased as pure wheaten starch. It was submitted to two extractions with boiling alcohol before being included in the dietaries.

It is not out of place to discuss at this juncture the process of alcohol extraction which is somewhat commonly used to render foodstuffs free from traces of the accessory factors. Stepp [1909, 1912] found that alcohol extraction removed indispensable substances from natural foodstuffs, and it has been used extensively for this purpose by Funk [1914, 1] and others. From recent work, however, it appears that in the absence of water absolute alcohol will not dissolve the water-soluble accessory substance [Osborne and Mendel, 1917; Drummond, 1917]. It is therefore possible that alcohol extraction does not remove traces of this substance when the foodstuff is extracted in the dry state.

This point was, however, only determined after the present investigation was completed.

Agar was included in the majority of rations to provide an indigestible intestinal ballast or "roughage." It was purchased in the powder form and was subjected to the somewhat empirical "purification" by hot alcohol extraction.

Sucrose was not subjected to any further purification.

Lactose was added to some dietaries, not only to serve as a source of carbohydrate, but also as a means of adding a certain amount of the water-soluble growth accessory factor, which it usually contains [McCollum and Davis, 1915, 1; Drummond, 1916, 1]. In some instances the lactose used was a crude preparation, very rich in this unidentified dietary factor, which was very kindly supplied by the courtesy of "Casein, Ltd." It was a light sandy coloured crystalline powder representing the first crystalline fractions obtained in the commercial preparation of lactose.

The nitrogen content of specimens of lactose is to a large extent a measure of their impurity. A large number of samples of this sugar were analysed for nitrogen. Preparations sold as pure lactose were found to contain from 0 to 0·02 %. The nitrogen-free specimens were usually preparations consisting of large crystals, and were found to be uncontaminated by the presence of the water-soluble growth factor.

The crude lactose referred to above was found to contain 0·19 % nitrogen.

"*Protein-free milk*" was used as a source of lactose, inorganic salts and the water-soluble growth promoting factor in some rations. It was prepared as described by Osborne and Mendel [1911], and was a sandy coloured crystalline powder having a nitrogen content varying between 0·57—0·67 %.

The use of "protein-free milk" in artificial rations has not escaped criticism upon the grounds of its indefinite composition [Funk, 1914, 1; Funk and Macallum, 1914; McCollum and Davis, 1915, 4]. Whilst there is undoubtedly some justification for these criticisms, it must be recognised that the use of this product has greatly assisted the elucidation of many important points concerning the nutritive value of the proteins [Osborne and Mendel, 1916, 1, 2].

Lard was used as a source of fat when it was desired that the diet should be free from the fat-soluble *A* accessory substance, since it has been repeatedly shown that lard does not contain this substance.

Butter fat was used as a source both of fat and of the fat-soluble accessory factor. It was prepared by centrifugalisation of butter at a temperature just above its melting point, and separation of the clear fatty layer. Determinations of nitrogen by the Kjeldahl process indicated that the butter fat was nitrogen-free. There is, however, some uncertainty whether this fat does not usually carry traces of nitrogen and phosphorus [Funk and Macallum, 1914; McCollum and Davis, 1914; Osborne and Wakeman, 1915].

"*Yeast Preparation*" was used in a number of dietaries as a means of ensuring an adequate supply of the water-soluble growth accessory. The preparation was made by evaporating down to a thick syrup the aqueous dialysate of a commercial yeast extract (marmite), at low temperature and reduced pressure.

The resulting pale brown syrup is very rich in the water-soluble accessory substance. It also contains relatively large amounts of adenine, leucine, sodium chloride and potassium phosphate [Drummond, 1917].

Amino-acids were prepared by the usual methods from various protein hydrolysates. Tryptophan was obtained from the tryptic digest of caseinogen.

Histidine from hydrolysed ox-blood, lysine and arginine from hydrolysed gelatin, tyrosine from silk waste, and cystine from hair. In the case of the last two preparations the amino-acids tyrosine and cystine were freed from traces of each other by the phosphotungstic acid process described by Plimmer [1913].

Hydrolysed Meat-Protein. Fresh lean beef was minced and extracted three times with boiling water, which was slightly acidified with acetic acid at boiling point to complete the coagulation of proteins. The residue of tissue protein was well pressed out in a meat press and both dehydrated and extracted by treatment in a continuous extraction apparatus firstly with alcohol and subsequently with ether.

The resulting product, after being ground and passed through a fine mesh sieve, was submitted to hydrolysis with boiling 15 % sulphuric acid for 48 hours. From the resulting deep brown coloured fluid sulphuric acid was removed quantitatively by the careful addition of boiling saturated barium hydroxide solution. The bulky precipitate of barium sulphate, contaminated with much pigmented matter, was extracted by boiling with several changes of distilled water. The combined washings were united with the main filtrate and the whole was evaporated down until a thick syrup was formed. Extracted wheaten starch was then added until a stiff paste was obtained, which was spread out in a thin layer over heated glass plates and dried until it could be powdered and passed through a fine meshed sieve.

This powder possessed a pale brown colour and contained approximately 7 % of nitrogen. To render this product nutritively the equivalent of the original protein it was necessary to replace the destroyed tryptophan. This was done by adding 0·7 % of the pure amino-acid prior to the incorporation with starch.

Hydrolysed Meat-Protein, Monamino-acid fraction. Beef protein was submitted to acid hydrolysis as described in the preceding preparation. To the neutral filtrate after removal of the sulphuric acid was added sufficient of a 20 % solution of sulphuric acid to render the whole about 5 % in strength. A 30 % solution of phosphotungstic acid was then added until no further precipitation occurred. The heavy precipitate was allowed to stand for 24 hours at 2°, being then filtered off and well washed with an ice-cold 5 % solution of sulphuric acid. The filtrate and washings were quantitatively freed from the excess of sulphuric acid and the precipitant by the careful addition of baryta, evaporated down and incorporated with starch as described in the previous preparation.

The final product was a pale sandy coloured powder containing approximately 5 % of nitrogen.

"*Erepton.*" I was fortunate in being able to secure several hundred grams of a commercial preparation of hydrolysed meat-protein, placed on the market by Meister, Lucius and Brüning.

The product was labelled "Erepton. Vollständig abgebautes Fleischeiweiss, nach Prof. Dr Abderhalden." It consisted of a brown, granular, slightly hygroscopic mass and contained from 12·5—12·8 % of nitrogen. As far as could be ascertained the degradation of the protein had been completed. I have to thank Mr E. Scholl, of John George Haller and Co., for his great kindness in placing this preparation at my disposal.

Salt mixture. Inorganic salts were supplied in some of the rations in the form of a salt mixture possessing a composition identical with one of those used by McCollum in his extensive researches. It was made up as follows:

NaCl	1·73 g.	$CaH_4(PO_4)_2$, $2H_2O$	5·40 g.
$MgSO_4$ (anhydrous)	2·66 g.	Calcium lactate	13·00 g.
NaH_2PO_4, H_2O	3·47 g.	Ferric lactate	1·18 g.
K_2HPO_4	9·54 g.		

Traces of iodine were supplied in the drinking water once a week, as recommended by McCollum.

Alcoholic Extract of Dried Milk. This preparation has been extensively used by Hopkins and his co-workers [Ackroyd and Hopkins, 1916] as a means of adding to the dietary a sufficient amount of accessory substances.

The use of this preparation during this work has not been found as satisfactory for this purpose as the employment of the combination of butter-fat and yeast preparation. This is probably explainable by the fact that the water-soluble substance is insoluble in absolute alcohol, and unless the milk powder contains sufficient moisture, the extraction of this substance is liable to be incomplete. The results of the series of experiments carried out during this investigation will now be considered in detail.

The influence of the level of protein intake of the host upon normal and tumour growth.

Much important work has been carried out recently upon the influence which the protein content of the diet may exert upon the nutrition of the animal. The papers by Osborne and Mendel [1912, 2; 1915, 1, 2; 1916, 1], and Janney [1915] contain results of importance dealing with this subject.

From these results it is now known that a reduction in the level at which protein is furnished in the diet of a young animal will sooner or later induce a more or less complete inhibition of the growth processes of that animal. This will occur either when the nitrogen intake falls below the level required

	Days 14	21	28	35	42	Remarks	Weight of tumour g.	Weight of host − tumour g.	Change in weight of host g.	Change in weight of host per 1 g. tumour
♂						Tumour solid and rapidly growing	25	126	+30	
♂						Slight central necrosis of tumour	27	140	+37	
♂						—	15	132	+39	
♂						Tumour had ulcerated and undergone central necrosis	19	120	+24	
♀						—	22	130	+36	
♀						Tumour retrogressed	–	152	+44	
						Average	21·6	–	+35	+1·62

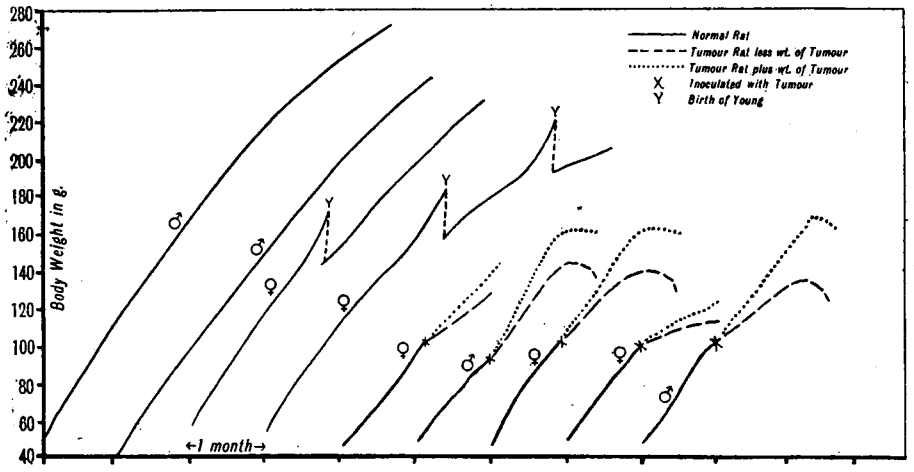

Chart 1. Tumour development, and growth curves of normal and tumour-bearing rats upon a normal diet of bread, oats, corn, greenstuffs and meat. Tumour *S*/113*a*.

to supply the demands of the organism, or when the lowered protein intake brings about a deficiency in the supply of one or more amino-acids which are indispensable to the animal. Examples of how this may occur have been given in the introduction to this paper. A large number of proteins, par-

ticularly those of vegetable origin, possess amino-acid contents which limit their nutritive value to no inconsiderable extent [Mendel, 1915].

Chart 1 serves as a control to a number of the experimental results of this work, for it illustrates the growth of young rats, the growth of tumour-bearing rats and the development of the tumours (*S*) they carry, upon a normal mixed diet of bread, seeds and greenstuffs, with occasional additions of meat or milk. The normal growth of the *A* sarcoma is given in Chart 2.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of host per 1 g. tumour
♀						Tumour ulcerated and very necrotic.	37·0	41·0	−9·0	
♀						ditto	18·0	62·0	+1·0	
♀						ditto	17·0	73·0	+6·0	
♂						ditto	15·0	87·0	+7·0	
						Average	21·7	—	−1·8	−0·08

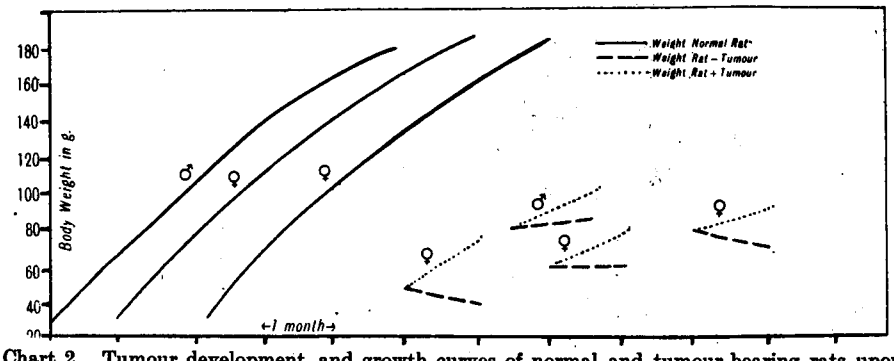

Chart 2. Tumour development, and growth curves of normal and tumour bearing rats upon complete artificial diet. Tumour *A*/30*d*. Diet: Caseinogen, 18 %, butter-fat, 20 %, "Protein-free milk," 30 %, Agar, 5 %, Starch, 27 %.

Chart 3 indicates the results which were obtained by feeding a "complete" artificial dietary composed as given below:

Caseinogen	18 %	Salt mixture	5 %
Starch	48 %	"Yeast preparation"	6 %
Agar	3 %	Butter-fat	20 %

It will be seen from Chart 3 that this diet is adequate to supply the nutritive requirements of the rat throughout its life cycle.

Tumour development proceeds at an equally normal rate in hosts fed upon this dietary, and is not accompanied by a loss in weight of the animal itself, until secondary disturbances, such as an ulceration of the tumour with resulting sepsis, set in.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀	•	●	●	●	●	Tumour firm and solid	10·7	11·3	+17·3	
♂	●	●	●	●	●	Slight central necrosis	25·5	140·5	+40·5	
♀	●	●	●	●		Animal died 17.4.17 Tumour firm	6·7·	141·3	+55·3	
♀	●	●	●	●	●	Central necrosis	24·0	146·0	+55·0	
♀	•	•	•	•	•	—	16·0	127·0	+42·0	
♂	•	•	•	•	•	Tumour retrogressed	—	—	—	
						Average	16·6	—	+42·0	+2·5

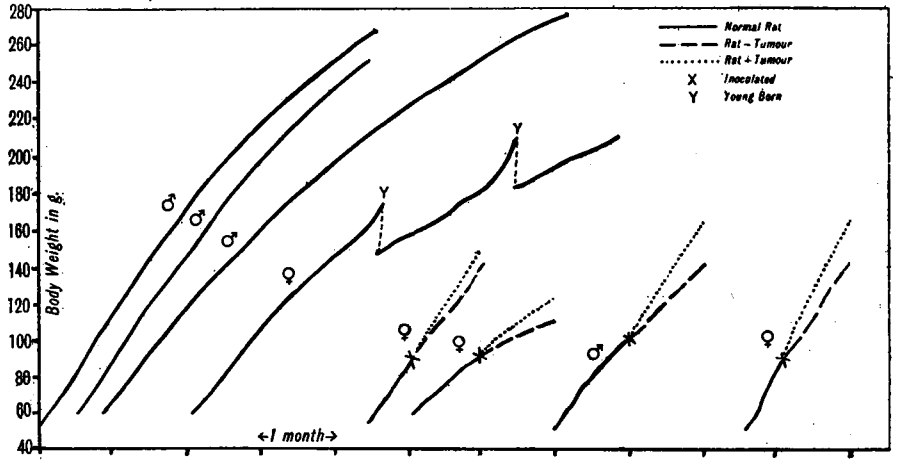

Chart 3. Tumour development, and growth curves of normal and tumour-bearing rats upon a complete artificial dietary. Tumour *S*/120*d*. Diet: Caseinogen, 18 %, Starch, 40 %. Agar, 3%, Salt Mixture, 5 %, "Yeast preparation" 6 %, butter-fat, 20 %.

As has already been stated, Osborne and Mendel [1915, 1] found that when the caseinogen content of such a diet as the above was lowered, a stage

was eventually reached where the low cystine content of the caseinogen became a factor limiting the growth processes of the animal. Accordingly the effect of a similar reduction in the protein content upon the growth of tumours was investigated. Two diets were compounded containing respectively 10 and 6 % of caseinogen, but in other respects possessing a composition similar to the diet described above. The results obtained in feeding these rations to the normal young rats support the work of Osborne and Mendel, although growth inhibition appears to have become marked at an earlier stage in the reduction of the protein level (Charts 4 and 5).

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♂						Much central necrosis of tumour	27·0	58·0	−23·0	
♀					–	Animal died 7. 1. 17. Tumour very necrotic and ulcerated.	14·0	63·0	−15·0	
♀						Firm tumour	8·5	60·5	−11·5	
♂						ditto	5·5	112·5	−17·5	
♀					–	Died 29. 12. 16. Tumour firm,	6·0	65·0	−15·0	
♂					–	Tumour retrogressed	–	–	–	
						Average	12·2	–	−16·4	−1·35

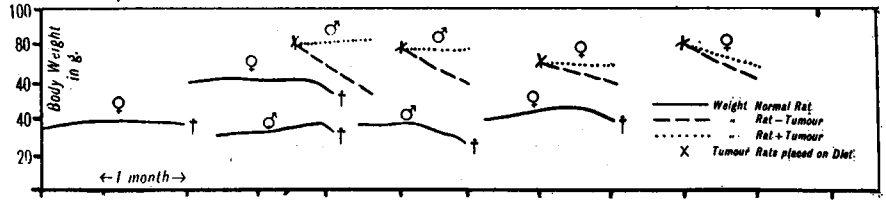

Chart 4. Tumour development, and growth curves of normal and tumour-bearing rats upon low protein diet. Tumour *S*/117*c*. Diet: Caseinogen, 6 %, Starch, 48 %, Agar, 3 %, Salt mixture, 5 %, "Yeast preparation" 6 %, Butter-fat, 20 %, Sucrose, 12 %.

The results yielded by the tumour-bearing animals may be conveniently summarised in tabular form.

TABLE 1.

% of caseinogen in diet	Average weight of tumours, g.	Average change in weight of host, g.	Average change in weight of host per 1 g. tumour
18	16·6	+42·0	+2·50
10	16·0	−19·4	−1·21
6	12·2	−16·4	−1·35

No evidence of tumour retardation is apparent in the case of the animals fed upon the 10 % caseinogen diet, but tumour growth is in these cases accompanied by a somewhat serious drop in the body-weight of the host. Where the diet contained 6 % of the protein there is a small but appreciable decrease in the size of tumour growth, also accompanied by a drop in the body-weight of the host. It will be noticed that the curves representing the weight of the

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat - tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀						Slight central necrosis	29·0	46·0	−26·0	
♀						Tumour firm	25·0	53·0	−17·0	
♀						ditto	14·0	86·0	−11·0	
♀					–	Animal died 20. 12. 16. Tumour firm	8·5	51·5	−24·5	
♀							3·5	96·5	−18·5	
♂						Tumour retrogressed	–	–	–	
						Average	16·0	–	−19·4	−1·21

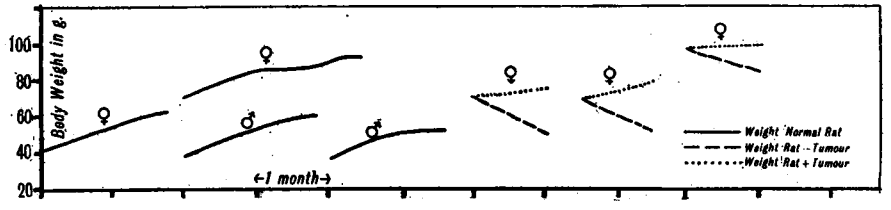

Chart 5. Tumour development, and growth curves of normal and tumour-bearing rats upon low protein diet. Tumour *S*/117*c*. Diet as in Chart 4, but containing 10 % of Caseinogen.

host plus tumour run roughly parallel to those which indicate the growth of the normal animal upon the same diet, whilst the weight of the host alone shows a gradual fall. This makes it probable not only that the food requirements of the tumour are satisfied before those of the host, but also, that if necessary the tissues of the host are drawn upon to supply deficiencies in the former.

Chart 6 illustrates the results of feeding a diet containing a low level of

protein to normal and tumour-bearing animals. The diet was composed as given below:

Dried whole-milk powder	15 %	Salt mixture	3 %
Starch	49 %	Butter-fat	10 %
Agar	3 %	Sucrose	20 %

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat–tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♂						Tumour ulcerated and very necrotic,	25·0	55·0	−24·0	
♂						Tumour very necrotic	20·0	82·0	−29·0	
♀						Ulcerated and necrotic	15·0	35·0	−29·0	
♂						ditto	16·0	74·0	−41·0	
♂						Tumour much degenerated	11·0	69·0	−22·0	
♀							3·0	59·0	−29·0	
						Average	15·0	—	−29·0	−1·94

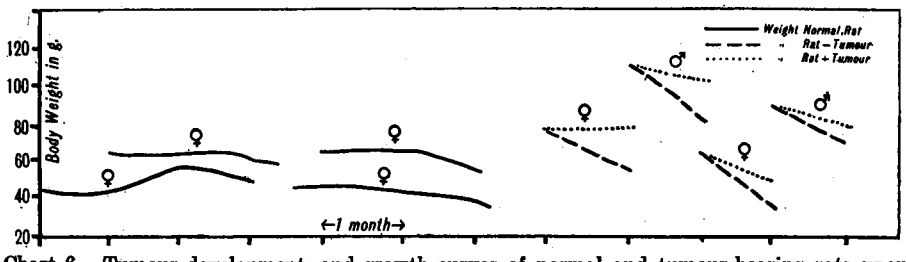

Chart 6. Tumour development, and growth curves of normal and tumour-bearing rats upon low protein level diet. Tumour *A*/24*a*. Diet: Dried milk, 15 %, Starch, 49 %, Agar, 3 %, Salt mixture, 4 %, Butter-fat, 10 %, Sucrose, 20 %.

Calculating from the known composition of the dried milk powder, this diet contained approximately 5 % of milk protein. Normal young rats fed upon this diet remain in good health, but show no change in body-weight for several months. The nitrogen balance and body temperature remain normal during this period of growth inhibition. Tumour growth in rats fed upon this same diet is however a little retarded, as is shown by the average weight of the tumours (15 g.) compared with the average weight (21 g.) grown on the normal dietary. A severe loss of body-weight upon the part of the host on the low protein diet may be observed in these cases also.

The influence of the character of the protein present in the diet upon normal and tumour growth.

Any consideration of the results of the preceding section of this paper cannot be dissociated from a study of the results given in this one, since they are so closely interrelated.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of rat, g.	Change in weight of rat per 1 g. tumour
♀						Tumour very necrotic and ulcerated.	31·0	59·0	−1·0	
♀						Tumour necrotic	12·0	68·0	+14·0	
♀						ditto	9·0	83·0	+13·0	
♀						ditto	10·0	90·0	−9·0	
						Average	15·5	—	+4·2	+0·27

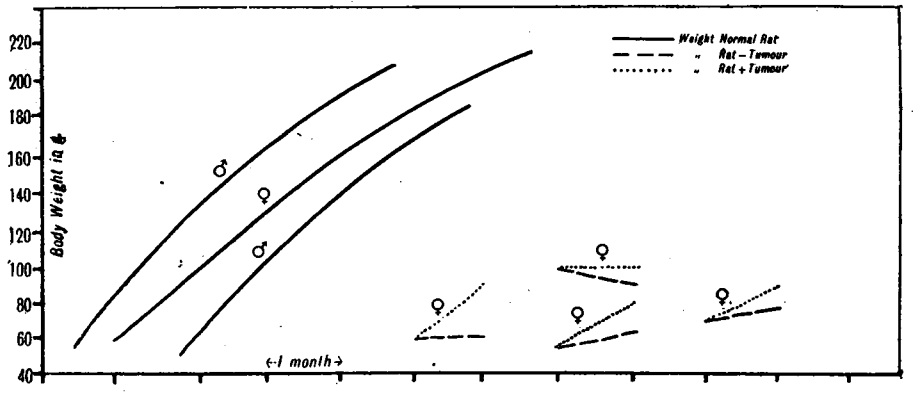

Chart 7. Tumour development, and growth curves of normal and tumour-bearing rats upon Edestin diet. Tumour *A*/30*d*. Diet as in Chart 2, but Edestin replacing Caseinogen.

The relative nutritive values of the proteins have already received attention in the introductory section, so that the results which are illustrated in Charts 2 and 7—11 may be considered forthwith.

The diets used in this series of experiments were similar in composition to those employed by Osborne and Mendel [1911].

Purified protein	18 %	Agar	5 %
Butter-fat	20 %	Starch	27 %
"Protein-free milk"	30 %		

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♂						Tumour very necrotic and ulcerated.	29·0	83·0	−9·0	
♀						ditto	19·0	77·0	+15·0	
♀					—	ditto	9·0	49·0	−11·0	
♀						Tumour necrotic	4·0	86·0	+26·0	
						Average	15·2	—	+5·2	+0·34

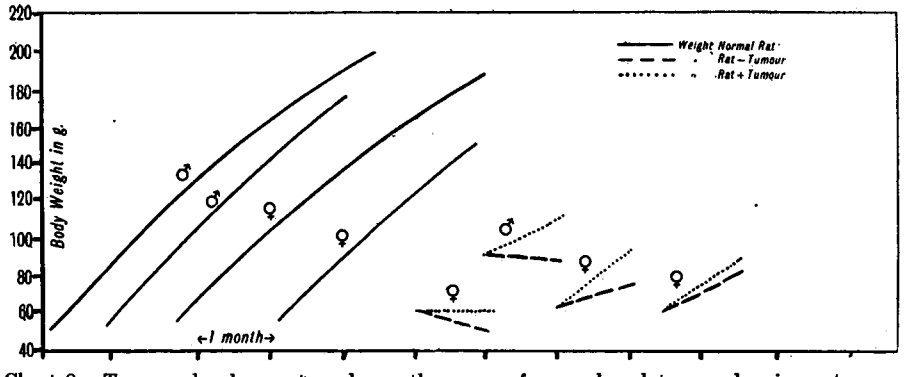

Chart 8. Tumour development, and growth curves of normal and tumour-bearing rats upon Lactalbumin diet. Tumour *A*/30*d*. Diet contains 18 % Lactalbumin.

The following proteins were investigated: caseinogen, lactalbumin, ovovitellin, edestin, gliadin, and zein.

The influence of these diets upon tumour growth may be summarised in the following table:

TABLE 2.

Protein in diet	Growth of normal animal on diet	Average weight of tumour, g.	Average change in weight of host, g.	Average change in weight of host per 1 g. tumour in four weeks
Caseinogen	normal	21·7	− 1·8	−0·08
Edestin	normal	15·5	+ 4·2	+0·27
Lactalbumin	normal	15·2	+ 5·2	+0·34
Ovovitellin	normal	17·0	+ 5·7	+0·34
Gliadin	cessation of growth and maintenance at constant weight	21·1	− 9·9	−0·47
Zein	rapid decline in body-weight ...	17·5	−21·2	−1·20

Rat	Days 12 19 26 33 40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of host. g.	Change in weight of rat per 1 g. tumour
♂		Tumour ulcerated and necrotic.	20·0	72·0	+11·0	
♀		Tumour extensively necrotic.	22·0	78·0	+9·0	
♀		Tumour very necrotic	16·0	85·0	−5·0	
♂		Slight central necrosis	10·0	90·0	+8·0	
		Average	17·0	—	+5·7	+0·34

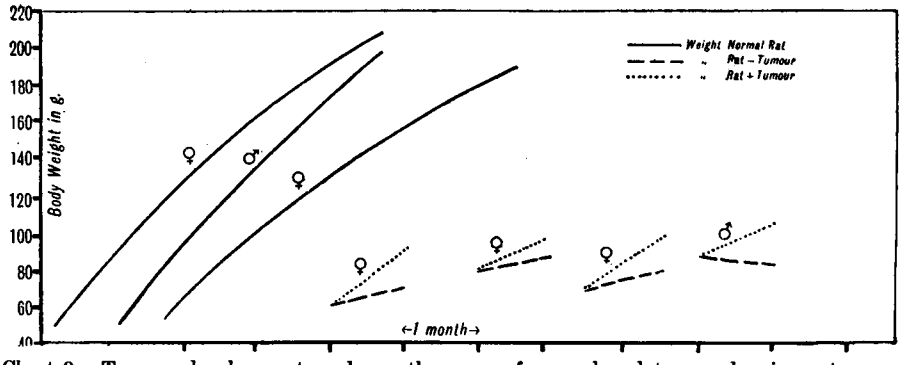

Chart 9. Tumour development, and growth curves of normal and tumour-bearing rats upon Ovo-vitellin diet. Tumour *A/30d*. Diet contains 18 % Ovo-vitellin.

For comparative purposes these results are valuable in that they indicate that tumour growth may be uninfluenced by dietary restrictions which

adversely affect the growth of the host. Individually, however, the results are unsatisfactory because of the irregularity in the changes in the body-weight of the rats on the first four dietaries. Thus there is no apparent explanation of the decrease of body-weight shown by the tumour-bearing rats fed upon the caseinogen diet. The only suggestion which can be advanced to explain this is that the *A* tumour was used throughout this experiment. As has already been mentioned this tumour tends to show an early

Rat	Days	Remarks	Weight of tumour g	Weight of rat – Tumour g	Change in weight of rat g	Change in weight of host per 1 g tumour
♀		Tumour extensively necrotic.	42·0	48·0	−10·0	
♀		Tumour very necrotic and ulcerated.	28·0	32·0	−19·0	
♀		Tumour firm, slight central necrosis	10·0	60·0	−12·0	
♀		Small fibrous growth	4·5	61·5	+1·5	
♀		Tumour retrogressed	–	–	–	
		Average	21·1	–	−9·9	−0·47

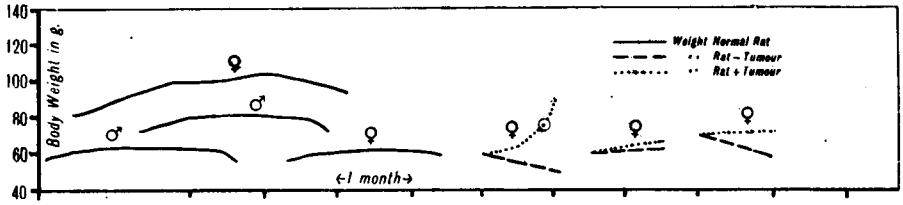

Chart 10. Tumour development and growth curves of normal and tumour-bearing rats upon Gliadin diet. Tumour *A*/30*d*. Diet contains 18 % Gliadin.

and extensive central necrosis, so that the possible deleterious influence upon the nutrition of the host caused by the absorption of the products arising from the degenerating tissue must be considered. Whatever may be the explanation of this point, it does not modify the importance of the observation that tumour growth may be practically normal in hosts whose growth is inhibited by the nature of their diet. The results obtained upon the gliadin

ration are of interest in view of the large amount of work which has been done upon the nutritive value of that protein.

Osborne and Mendel [1914, 1; 1916, 2] found that gliadin was adequate to supply the amino-acid requirements for maintenance in the rat, but that the addition of some 2 % of the missing unit, lysine, was necessary before the requirements for growth were satisfied.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g tumour
♂						Tumour very necrotic, animal extremely emaciated	31·0	29·0	−36·0	
♀						ditto	14·0	37·0	−11·0	
♂						ditto	15·0	38·0	−18·0	
♀						Animal emaciated Tumour shows slight central necrosis	10·0	52·0	−20·0	
♀						—	—	—	—	
♀						Tumour retrogressed	—	—	—	
						Average	17·5	—	−21·2	−1·20

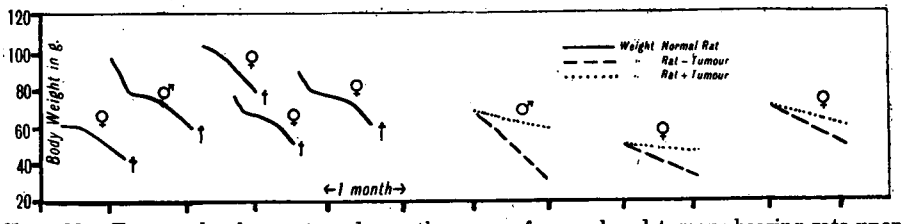

Chart 11. Tumour development, and growth curves of normal and tumour-bearing rats upon Zein diet. Tumour *A*/30*d*. Diet contains 18 % Zein.

It was the earlier results of Osborne and Mendel on this subject [1912, 1] that stimulated the investigations of Sweet, Corson-White and Saxon [1913], to which reference has already been made. The view which regards lysine as of great importance in growth receives support from the work of Buckner, Nollau and Kastle [1916] who worked upon the growth of chickens, and also from similar studies of Osborne and Mendel [1916, 4]. Other workers however do not attach as great an importance to the rôle of this amino-acid in nutrition. Thus McCollum, Simmonds and Pitz [1917] have shown that the

addition of gelatin, which possesses a high lysine content, does not improve the nutritive value of the lysine-poor proteins of the maize kernel, whilst Geiling [1917] does not regard lysine as necessary for the maintenance of the adult mouse. The true value of lysine in the nutrition of the organism is therefore at present uncertain.

Equal interest is to be attached to the results illustrated in Chart 11, where the influence of the well-known amino-acid deficiencies of the protein zein upon normal and tumour growth are indicated. The low nutritive value of this protein was shown by Willcocks and Hopkins [1906] to be largely due to the absence of tryptophan from its molecule. Their work has been repeatedly confirmed, and the serious nature of a tryptophan deficiency is now universally admitted. As is to be seen from Chart 11, young rats rapidly decline in body-weight and die upon a zein ration such as was used in this experiment.

It was, therefore, somewhat unexpected when no inconsiderable amount of tumour development was observed in rats fed upon the same ration. In the light of the results of some later experiments with zein dietaries, attention must be given to one of the criticisms which have been employed in deprecating the use of "protein-free milk," namely that the traces of milk protein which that product contains may possibly supply traces of the missing amino-acids, which may be of welcome assistance to the animal in its endeavour to overcome the serious deficiencies of its diet. Whether this will to some extent explain the result of this feeding experiment is uncertain, but it is apparent from a consideration of the experiment about to be described that a tryptophan deficiency, in the absence of any uncertain factor such as "protein-free milk," may cause retardation of tumour growth. Chart 12 illustrates the results which were obtained with a ration composed as given below:

Zein	18 %	Butter-fat	10 %
Lactose	30 %	Salt mixture	5 %
Starch	40 %		

Alcohol extract of 20 g. milk powder.

The summarised results of the experiments in this series are given in Table 3.

TABLE 3.

Diet	Average weight of tumour, g.	Average change in weight of host, g.	Average change in weight of host per 1 g. tumour in four weeks
18 % caseinogen	12·0	+19·9	+1·66
18 % zein	4·1	−21·1	−5·15
18 % zein + 0·5 % tryptophan ...	9·2	− 9·8	−1·06

As will be seen from the third series in this table, the influence of the addition of the missing amino-acid was investigated (Chart 13). In both the normal and tumour-bearing rats this addition markedly improved the nutritive value of the ration. Upon the zein diet there was a distinct retardation, but not a total inhibition, of tumour growth which was accompanied by a very rapid fall in body-weight of the host.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of rat g.	Change in weight of host per 1 g. tumour
♀						Tumour fibrous, but well vascularised	6·0	74·0	−13·0	
♂					—	Animal very thin.	5·0	53·0	−27·0	
♀						Tumour slow growing. Animal very thin.	2·5	58·5	−24·5	
♀					—	—	3·0	45·0	−20·0	
♂					—	Tumour retrogressed	—	—	—	
♂			—	—	—	ditto	—	—	—	
						Average	4·1	—	−21·1	−5·15

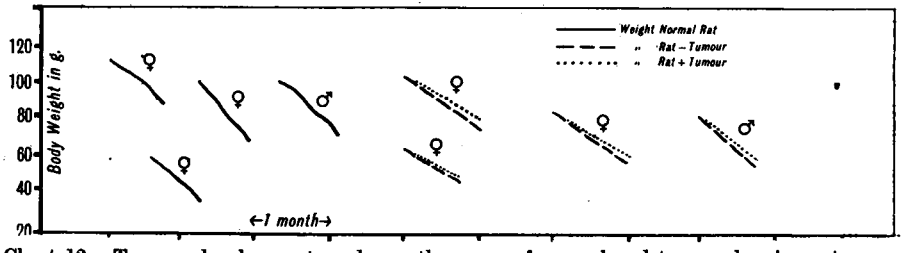

Chart 12. Tumour development, and growth curves of normal and tumour-bearing rats upon Zein diet. Tumour *S*/116*b*. Diet: Zein, 18 %, Crude lactose, 30 %, Starch, 40 %, Butter-fat, 10 %, Salt mixture, 5 % + Alcoholic extract 20 g. dried milk.

The addition of tryptophan to the extent of 0·5 % of the diet both assisted the growth of the tumour and lessened the sacrifice of the tissues of the host. It should be remarked that the consumption of the dietaries was satisfactory, except that of the zein ration during the fourth week, which declined considerably.

The low nutritive value possessed by gelatin has been recognised for many years. It has been shown that gelatin cannot serve to build up new tissue; whilst Kauffmann [1905] demonstrated that when the amino-acids

tyrosine, cystine and tryptophan are added to gelatin, and the deficiencies of the latter thereby to a large extent made good, nitrogen equilibrium can be established.

His work has received confirmation from that of Rona and Müller [1906] and Abderhalden [1912]. More recently the subject has been carefully investigated by Totani [1916], who found that the addition of tryptophan to gelatin greatly raised its nutritive value. The effect of adding the missing tyrosine was hardly appreciable; so that the author was led to suggest that the animal organism may have the power of synthesising the benzene nucleus.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♂						Tumour slightly necrotic. Animal thin.	14·0	78·0	−9·0	
♀						ditto	14·0	69·0	−12·0	
♀						ditto	7·0	92·0	−8·0	
♂						Tumour firm and fibrous. Animal thin.	2·0	90·0	−10·0	
♀						Tumour retrogressed	—	—	—	
						Average	9·2	—	−9·8	−1·06

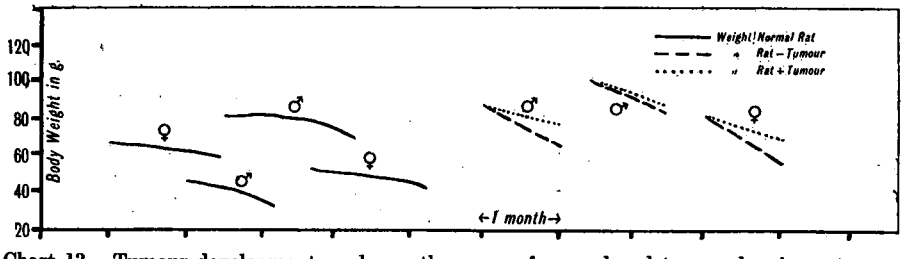

Chart 13. Tumour development, and growth curves of normal and tumour-bearing rats upon Zein diet (Chart 12) + 0·5 % tryptophan. Tumour *S*/116*b*.

The investigation of the influence of the amino-acid deficiencies of gelatin upon tumour growth, which are described in this paper, was carried out before the appearance of Totani's communication, so that his observation upon the more complete assimilation of gelatin administered in the hydrolysed

form was not applied. The gelatin was included in the diets in these experiments in the powder form.

The average results obtained in the four batches of experimental animals are tabulated in Table 4, which has been compiled from the data contained in Charts 14, 15 and 16.

Rat	Days 12	19	26	33	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♂					Rat very emaciated. Tumour firm, well vascularised and actively growing.	12·0	60·0	−40·0	
♂					ditto	10·5	74·5	−29·5	
♂					ditto	5·0	82·0	−28·0	
♀					ditto	4·0	73·0	−27·0	
♀					Tumour fibrous and slow growing. Rat very thin	2·5	65·5	−22·5	
					Average	6·8	—	−29·4	−4·3

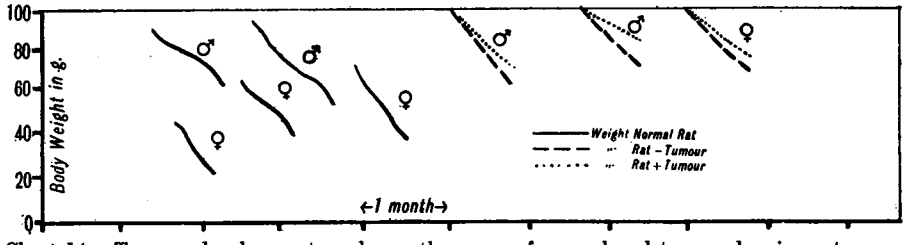

Chart 14. Tumour development, and growth curves of normal and tumour-bearing rats upon Gelatin diet. Tumour *S*/118*a*. Diet: Gelatin, 18 %, Starch, 52 %, Lard, 20 %, Agar, 5 %, Salt mixture, 5 % + Alcoholic extract 20 g. dried milk.

TABLE 4.

Diet	Average weight of tumour, g.	Average change in weight of host, g.	Average change in weight of host per 1 g. of tumour
18 % caseinogen	14·2	+19·0	+1·34
18 % gelatin	6·8	−29·4	−4·30
18 % gelatin + 0·5 % tryptophan	12·7	−19·3	−1·52
18 % gelatin + 0·5 % tryptophan + 1 % tyrosine	13·2	−16·5	−1·25

The influence of these dietaries upon the growth of the normal rats supports the work of Totani, in that the addition of tryptophan greatly raises the nutritive value of the gelatin diet, whereas the further addition of tyrosine has little if any such effect.

The retarding influence upon tumour growth which is exerted by the amino-acid deficiencies of the gelatin ration is well marked. As in other similar cases this is accompanied by a severe drop in the body-weight of the host.

Rat	Days 12	19	26	33	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of host per 1 g. tumour
♀					Rat thin Tumour shows slight central necrosis	18·5	71·5	−20·5	
♂					ditto	15·0	85·0	−28·0	
♂					ditto	14·0	94·0	−16·0	
♂					ditto	11·0	65·0	−25·0	
♀						5·0	83·0	−7·0	
					Average	12·7	—	−19·3	−1·52

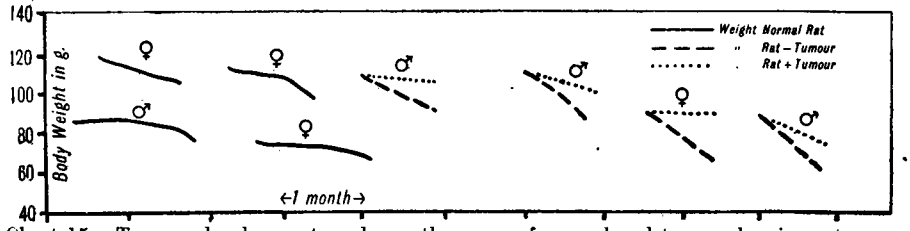

Chart 15. Tumour development, and growth curves of normal and tumour-bearing rats upon Gelatin diet, described in Chart 14, + 0·5 % tryptophan. Tumour *S*/118*a*.

When tryptophan has been added to the diet, tumour growth becomes normal, but there is a slight loss of weight upon the part of the host. The further addition of the missing tyrosine cannot be said appreciably to influence either tumour growth, or the sacrifice of the tissues of the animal.

The gelatin dietary to which the missing tryptophan and tyrosine have been added possesses a much lower nutritive value than the "normal"

caseinogen ration. This is probably to a large extent due to the defective assimilation of the solid gelatin, which has been observed by Totani. This imperfect utilisation does not apparently adversely affect tumour growth when tryptophan is added, but is reflected in the lowered nutrition of the host.

Rat	Days 12	19	26	33	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♂	•	●	●	●	Tumour shows slight central necrosis	17·0	79·0	−22·0	
♂	•	●	●	●	ditto	16·0	83·0	−17·0	
♀	•	●	●	●	ditto	11·0	75·0	−12·0	
♀	•	●	●	–	Animal died, and partially devoured 20.1.17	9·0	75·0	−15·0	
♀	•	•	•	•	Tumour fibrous, with small blood supply	–	–	–	
					Average	13·2	–	−16·5	−1·25

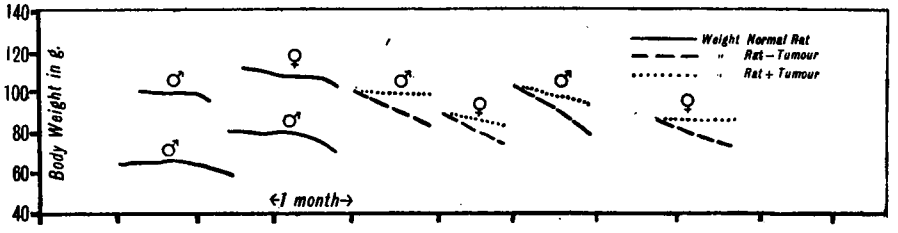

Chart 16. Tumour development, and growth curves of normal and tumour-bearing rats upon Gelatin diet + 0·5 % tryptophan + 2·0 % tyrosine. Tumour *S*/118*a*.

The effect upon normal and tumour growth of replacing the protein of a diet by amino-acid mixtures.

It is mainly due to the extensive researches of Abderhalden that it is now proved that an animal can be maintained, and even show an increase of body-weight, when it is fed upon a diet in which protein has been entirely replaced by an amino-acid mixture prepared from a completely hydrolysed protein. Of outstanding importance in this respect are the classical experi-

ments made by him, in which he accomplished the successful nutrition of dogs over long periods of time upon such diets [1913, 2; 1915].

He also found the same amino-acid preparation adequate to supply the nitrogen requirements of rats.

Abderhalden has extended his researches so as to ascertain the effect which the removal of certain amino-acids from his protein digest has upon the nutrition of the animal [1915].

By removing tryptophan and tyrosine the nutritive value of the hydrolysate was greatly lowered, becoming totally insufficient to maintain the body-weight of the animals. Restoration of the missing amino-acids was immediately effective in correcting this deficiency.

The removal of lysine was followed by a negative nitrogen balance, as was also the administration of a preparation of hydrolysed gliadin. The addition of the missing lysine did not in these two cases completely restore the food value of the rations. Other experiments dealing with the relative importance of histidine, arginine, and cystine gave somewhat indefinite results; although the evidence in the last case tended to show that cystine is an indispensable component of the protein molecule. Equally interesting results have been obtained by Hopkins and his co-workers in this country [Hopkins, 1916].

Ackroyd and Hopkins [1916] have again confirmed the absolute indispensability of tryptophan, and have also obtained most interesting and important results bearing upon the physiological value of the amino-acids arginine and histidine. They have presented evidence which indicates that these two substances are interconvertible in the animal organism and that an adequate supply of at least one of them in the diet is necessary for satisfactory nutrition. From their results it would also appear that these amino-acids can furnish the starting point of purine synthesis in the animal body.

Geiling [1917] has recently carried out an investigation the results of which have once again demonstrated the importance of the so-called diamino-acids in the processes of nutrition and growth.

This worker regards tryptophan and cystine as indispensable dietary units, and he adds confirmation of the relationship between arginine and histidine, described by Ackroyd and Hopkins. He does not regard lysine as of special importance.

Attempts have been made to effect the nutrition of animals upon diets in which protein has been replaced by artificial mixtures of amino-acids in

the pure state. Abderhalden [1912] encountered great experimental difficulties in attempting this, but was successful in maintaining a dog for eight days at constant body-weight upon a diet containing all the common amino-acids excepting oxyproline. The nutrition of the animal was far less satisfactory than when "erepton"—a completely hydrolysed meat-protein—was employed.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of rat g.	Change in weight of host per 1 g. tumour
♂						Tumour extensively necrosed and ulcerated. Animal thin.	32·0	134·0	+20·0	
♂						ditto.	27·0	99·0	−18·0	
♂						Tumour very necrotic	12·0	114·0	+14·0	
♀						Tumour small and fibrous	–	–	–	
						Average	23·6	–	+5·3	+0·22

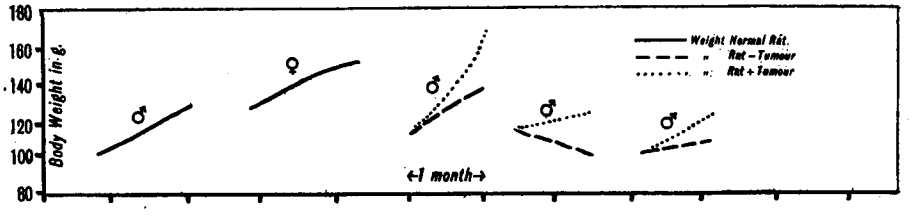

Chart 17. Tumour development, and growth curves of normal and tumour-bearing rats upon "Erepton" diet. Tumour *S*/121*d*.

Osborne and Mendel [1916, 2] record a failure to maintain rats upon a non-protein diet containing tryptophan, cystine, histidine, tyrosine, phenylalanine, proline and ammonium salts. Mitchell [1916] working with mice, has to some extent succeeded in surmounting the great difficulties which impede experimentation along these lines. His results support the indispensability of tryptophan, and indicate that the benzene nucleus may be omitted from the diet without adversely affecting nutrition.

It will be seen from a summary of these results that there is general agreement in regarding tryptophan, cystine and arginine or histidine as indispensable dietary components, but that opinion is divided regarding the value of lysine and the amino-acids containing the benzene nucleus.

The application of these results to the determination of the influence of individual amino-acids upon tumour growth was carried out in a similar manner to that employed in the study of the nutrition of the normal animal by Abderhalden and by Hopkins. Two amino-acid mixtures were prepared

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat–tumour g.	Change in weight of host g.	Change in weight of host per 1 g. tumour
♂	•	●	●	●	●	Tumour somewhat firm and fibrous. Animal very emaciated.	8·5	67·5	−35·5	
♀	•	•	●	●	●	ditto	8·0	56·0	−18·0	
♂	•	●	●	●	—	Tumour well vascularised, firm and slow growing. Animal very emaciated	8·0	67·0	−33·0	
♂	•	•	•	●	—	ditto	3·5	46·5	−15·5	
♀	•	●	●	—	—	Killed and devoured 28. 12. 16	—	—	—	
						Average	7·0	—	−25·5	−3·6

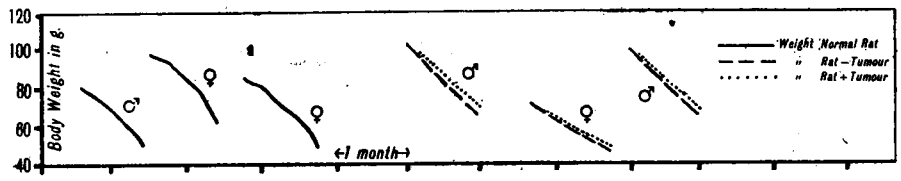

Chart 18. Tumour development, and growth curves of normal and tumour-bearing rats upon acid-hydrolysed meat-protein diet. Tumour *S*/117c.

from hydrolysed muscle protein, the one containing all the amino-acids excepting tryptophan, whilst from the other all the amino-acids which are precipitated by phosphotungstic acid had been removed. A commercial sample of completely hydrolysed meat-protein ("erepton") containing tryptophan, and prepared according to the method of Abderhalden, served as a control to these preparations.

The general composition of the rations employed is given below.

"Erepton" diet		Hydrolysed meat protein diet		"Monamino-acid fraction" diet	
"Erepton" (12·5 % N)	20 %	Acid-hydrolysed muscle preparation (7 % N)	40 %	"Monamino-acid fraction" preparation (5 % N)	50 %
Starch	40	Starch	20	Starch	25
Sucrose	15	Sucrose	15	Salt mixture	5
Salt mixture	5	Salt mixture	5	Agar	5
Agar	5	Agar	5	Butter-fat	15 +
Butter-fat	15 +	Butter-fat	15 +	Alcoholic extract of 20 g. dried milk	
Alcoholic extract of 20 g. dried milk		Alcoholic extract of 20 g. dried milk			

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀						Tumour necrotic and ulcerated. Animal thin.	19·0	55·0	−23·0	
♀						Tumour firm Animal thin.	16·0	62·0	−28·0	
♀						ditto	16·0	72·0	−25·0	
♀					–	ditto	10·0	57·0	−14·0	
♂					–	ditto	9·0	80·0	−10·0	
♀						ditto	3·0	77·0	−7·0	
						Average	12·0	–	−17·8	−1·5Z

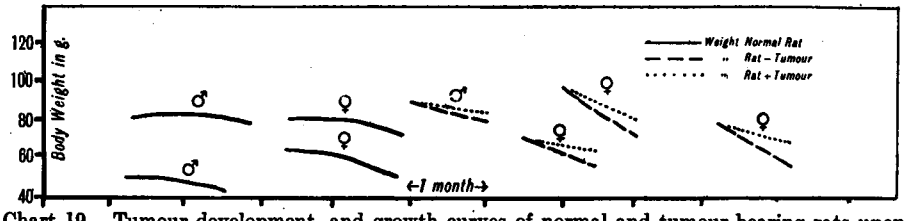

Chart 19. Tumour development, and growth curves of normal and tumour-bearing rats upon acid-hydrolysed meat-protein diet + 0·5 % tryptophan. Tumour *S*/117*c*.

Amino-acid additions were made, where indicated, in the following amounts:

Tryptophan	0·5 %	of total diet
Cystine	0·5	„ „
Lysine dihydrochloride (in neutral solution)	2·0	„ „
Arginine nitrate „ „	2·0	„ „
Histidine hydrochloride „ „	1·5	„ „
Tyrosine	2·0	„ „

Throughout the course of this series of experiments some difficulty was encountered in getting the rats to consume adequate amounts of the diets. This was particularly marked in the case of rations to which had been added the various pure amino-acids. Many attempts were made to render the diets more palatable without materially altering their composition but without much success.

The average results of this series of experiments are summarised in Table 5.

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀						Tumour firm. Animal very emaciated	15·0	53·0	−50·0	
♀						Slight central necrosis of tumour	13·0	48·0	−48·0	
♀						Tumour retrogressing	—	—	—	
						Average	14·0	—	−49·0	−3·5

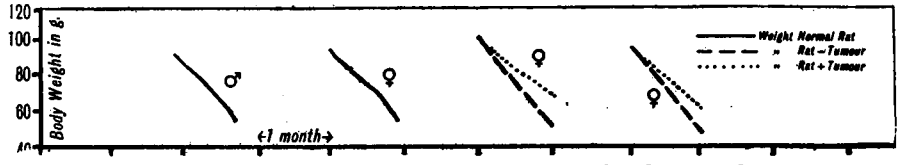

Chart 20. Tumour development, and growth curves of normal and tumour-bearing rats upon "monamino-acid fraction" diet. Tumour *S*/120*d*.

TABLE 5.

Diet	Average weight of tumour, g.	Average change in weight of host, g.	Average change in weight of host per 1 g. tumour
"Erepton"	23·6	+ 5·3	+0·22
Acid-hydrolysed protein	7·0	−25·5	−3·50
Acid-hydrolysed protein + 0·5 % tryptophan	12·0	−17·8	−1·52
"Monamino-acid fraction" + lysine, arginine, histidine, cystine and tryptophan	17·0	+1·50	+0·89
"Monamino-acid fraction" + lysine, arginine, histidine, and tryptophan	16·3	−10·3	−0·63
"Monamino-acid fraction" + lysine, arginine and histidine	15·0	−33·0	−2·20
"Monamino-acid fraction" + arginine and histidine ...	21·0	−39·0	−1·90
"Monamino-acid fraction" + tryptophan	19·5	−29·0	−1·49
"Monamino-acid fraction"	14·0	−49·0	−3·50

These results are illustrated more fully in Charts 17 to 25. It is unfortunate that the great labour involved in the preparation of the materials for this work should have necessitated a limitation of the number of experimental animals. This fact naturally greatly reduces the value of the average figures given in the preceding table, but it does not lessen the importance of observations that tumour growth was possible upon certain diets. The normal rats fed upon the "erepton" diet exhibited a sub-normal rate of growth, which may be attributable in part to the low food consumption of the animals. Tumour growth was quite normal in hosts fed upon the same ration, and was not accompanied by a loss of tissue upon the part of the animal (Chart 17).

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g	Change in weight of rat g	Change in weight of rat per 1 g. tumour
♀						Tumour somewhat necrotic and ulcerated	22	63	−39	
♂						Tumour firm	17	73	−19	
						Average	19·5	—	−29	−1·49

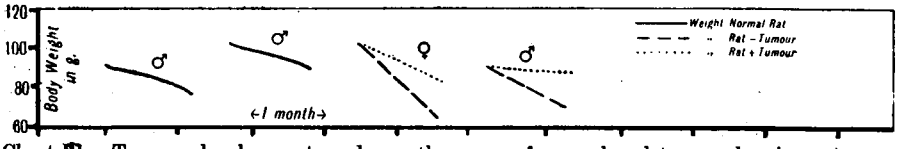

Chart 21. Tumour development, and growth curves of normal and tumour-bearing rats upon "monamino-acid fraction" diet + 0·5 % tryptophan. Tumour *S*/120*d*.

The diet prepared with the acid-hydrolysed meat-protein plus tryptophan should on theoretical grounds possess a nutritive value almost equal to that of "erepton." As a matter of fact it was found to possess a somewhat lower food value for normal rats, whilst tumour growth appeared somewhat retarded (Chart 19).

The adverse effects upon the nutrition of the animal which follow the withdrawal of tryptophan from the diet are illustrated in Chart 18. Normal animals quickly declined in weight and succumbed on this inadequate diet, whilst the tumour rats showed an appreciable inhibition of tumour growth, accompanied by a rapid fall in the body-weight of the host.

The low nutritive value possessed by an amino-acid mixture which is deficient in the so-called diamino-acids is illustrated in Chart 20. This ration was totally inadequate for the nutrition of normal rats, but the same restrictions imposed upon the tumour-bearing animals brought about comparatively little retardation of tumour growth.

As has already been remarked, it is not possible to place much reliance in the data given in Table 5, as in many cases they represent the average figures for a very small number of subjects.

28

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀						Tumour shows slight central necrosis Animal very thin	30	69	−49	
♀						ditto	12	75	−29	
						Average	21	—	−39	−1·9

29

♀						Tumour firm. Animal very thin.	15	75	−32	
♂						ditto	15	72	−34	
						Average	15		−33	−2·2

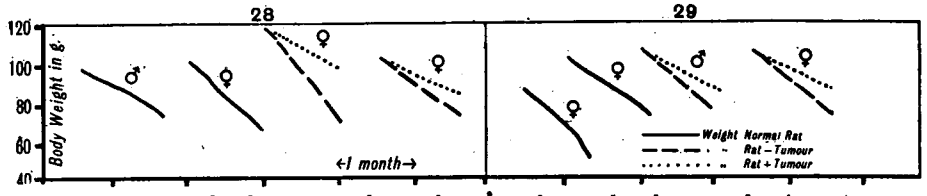

Chart 22. Tumour development, and growth curves of normal and tumour-bearing rats upon "monamino-acid fraction" diet, + 2 % arginine nitrate + 1·5 % histidine hydrochloride. Tumour *S*/120*d*.

Chart 23. Tumour development, and growth curves of normal and tumour-bearing rats upon "monamino-acid fraction" diet + 2 % arginine nitrate + 1·5 % histidine hydrochloride + 2 % lysine dihydrochloride. Tumour *S*/120*d*.

From the other Charts, 21—25, may be seen the influence exerted upon the nutrition of the experimental animals by additions of the various missing amino-acids.

The outstanding feature of this series of experiments is the demonstration

of the ability of the rat sarcoma to grow with comparatively little retardation in a host subjected to most drastic dietary restrictions.

Since it is apparent that the cells of these tumours can obtain considerable supplies of food units necessary for growth, when these are not present in the diet of the host, it becomes of importance to determine from what sources they are procured by the tumour.

30

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of host per 1 g. tumour
♂	•	•	●	●	●	Tumour much necrosed and ulcerated	24	96	−14	
♀	•	•	●	●	●	Tumour firm, shows slight central necrosis	14	96	+1	
♀	•	•	●	●	●	ditto	11	90	−18	
						Average	16·3	—	−10·3	−0·63

31

♀	•	•	●	●	●	Tumour firm, slight central necrosis	20	90	+1	
♀	•	•	●	●	●	ditto	14	99	+2	
						Average	17	—	+1·5	+0·89

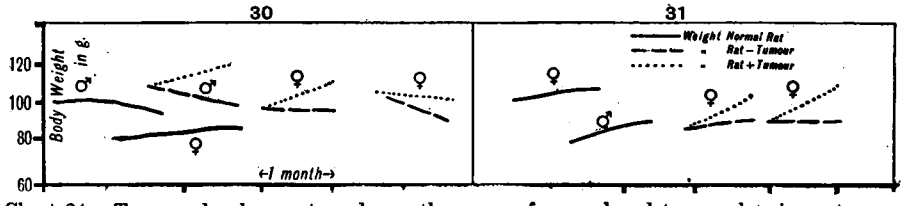

Chart 24. Tumour development, and growth curves of normal and tumour-bearing rats upon "monamino-acid fraction" diet, + 2 % arginine nitrate + 1·5 % histidine hydrochloride + 2 % lysine + 0·5 % tryptophan. Tumour *S*/120*d*.

Chart 25. Tumour development, and growth curves of normal and tumour-bearing rats upon "monamino-acid fraction" diet, + 2 % arginine nitrate + 1·5 % histidine hydrochloride + 2 % lysine dihydrochloride + 0·5 % tyrosine + 0·5 % cystine + 0·5 % tryptophan. Tumour *S*/120*d*.

Tumour cells require a comparatively large supply of the diamino-acids for their growth; since it has been shown by the analysis of a large number of tumour proteins that they contain a high percentage of these substances [Drummond, 1916, 3]. There is no evidence which indicates that tumour

cells differ from normal tissue cells in possessing the power to synthesise important cell units, such as the diamino-acids.

The question therefore arose as to whether the composition of the tumour proteins was in any way influenced by restrictions in the amino-acid intake of the host. Accordingly a number of the tumours grown in hosts fed upon certain of the diets employed in the last series of experiments were submitted to analysis in order to determine the amino-acid distribution of their proteins.

The preparation of the tumour material for analysis and the details of the latter were as described in a previous communication [Drummond, 1916, 3]. The partition of the various units constituting the diamino-acid fraction was not studied, in view of the fact that there appears to be some doubt as to the accuracy of the methods usually employed for that purpose.

A number of analyses were also carried out upon the proteins of the tissues of the host. Unfortunately the small amount of material furnished by individual organs prevented separate analyses being made of these. A representative sample of the tissues of the animal was therefore analysed. To ensure uniformity, the sample was composed, in each case, of the heart, lungs, liver, spleen, one kidney, equal sized strips of skeletal muscle, and one testicle or the ovary.

TABLE 6.

Partition of nitrogen in proteins of rat sarcoma S, and in the proteins of the tissues of hosts bearing these tumours.

Nature of diet of tumour-bearing rat	Protein analysed	No. of tissue	% N in dry tissue	Monamino-acid filtrate: % Amide N	% Humin N	% Monamino-acid N	% Nonamino-acid N	% Diamino-acid N	Total % N recovered
Normal diet	Organs	1	12·80	6·27	4·61	50·56	6·53	32·23	100·20
	Tumour	2	13·83	5·25	3·16	50·80	5·40	34·42	99·03
"Erepton" diet	Organs	3	12·62	5·51	5·66	55·93	4·77	30·97	100·64
	Tumour	4	13·55	4·42	3·59	51·77	6·73	32·42	98·93
Hydrolysed meat protein diet (tryptophan absent)	Organs	5	12·78	4·83	4·87	51·90	4·60	32·20	98·40
	Tumour	6	13·81	4·69	4·25	52·68	6·12	31·72	99·36
Monamino-acid diet	Organs	7	12·37	4·93	4·73	52·60	3·95	32·72	98·96
	Tumour	8	13·84	5·02	3·98	52·40	6·50	32·00	99·10

In the animals which had suffered a severe loss of body-weight upon certain of the diets, the post-mortem examination showed great wasting of the skeletal muscles. Blood examination of such animals carried out some days before death frequently revealed a state of anaemia. The number of animals under investigation was unfortunately too small to permit of a comparison being made of the weights of the various organs in these cases with the standard

data given by Donaldson [1915]. No apparent wasting was noticeable upon examination of the heart, spleen or kidneys, but the liver in some cases appeared smaller than normal.

Table 6 contains the results obtained from the examination of the tumour and tissue proteins referred to above. A study of this table indicates, as far as it goes, that the composition of both the tumour proteins and the proteins of the tissues of the host is unaffected as a result of deficiencies in the amino-acid content of the diets.

This chemical evidence is supported by the histological examination of the tumours and organs removed from the tumour-bearing rats. No appreciable differences could be detected between the microscopical appearance of sections of the tumours grown in animals fed upon the diets showing serious amino-acid deficiencies, and those removed from normally fed animals. Even the tumours removed from the rats fed upon the amino-acid diet deficient in histidine, arginine, cystine, lysine and tryptophan presented an apparently normal microscopical appearance.

The influence of the accessory growth factors "fat-soluble A" and "water-soluble B," upon tumour growth in rats.

The importance of these two unidentified dietary factors in the nutrition of the growing animal has received attention in the introduction to this paper. To investigate the possible influence which they might exert upon tumour growth the following four rations were prepared. Diet *A* contained both factors, and being in other respects adequate, may be considered a "complete" diet. Diet *B* lacked the "fat-soluble *A*" factor, but in other respects possessed a similar composition to *A*. Diet *C* was deficient only in the "water-soluble *B*" substance, whilst Diet *D* contained neither accessory factor.

The composition of the dietaries was as given below.

	A	*B*	*C*	*D*
Caseinogen	18	18	18	18
Starch	48	48	54	54
Agar	3	3	3	3
Salt mixture	5	5	5	5
"Yeast preparation"	6	6	—	—
Butter-fat	20	—	20	—
Lard	—	20	—	20

The results which were obtained are recorded in Charts 2, 26, 27 and 28 and are summarised in Table 7. Charts 29–31 refer to a similar experiment dealing with the fat-soluble *A*.

TABLE 7.

Diet	Deficiency in diet	Average weight of tumour, g.	Average change in weight of host, g.	Average change in weight of host per 1 g. tumour
A	Nil	16·6	+42·0	+2·5
B	Fat-soluble *A*	16·3	+12·9	+0·9
C	Water-soluble *B*	13·5	− 3·5	−0·3
D	Fat-soluble *A* and water-soluble *B*	12·7	− 5·0	−0·4

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀						Solid growth	10·5	87·5	−3·5	
♀						Tumour very necrotic, ulcerated 21.4.17.	22·5	71·5	−10·5	
♀						Tumour small and fibrous	2·0	90·0	−1·0	
♀						Tumour centrally necrotic	24·0	71·0	−4·0	
♂					–	Died 19.4.17	8·5	81·5	+1·5	
						Average	13·5	–	−3·5	·0·26

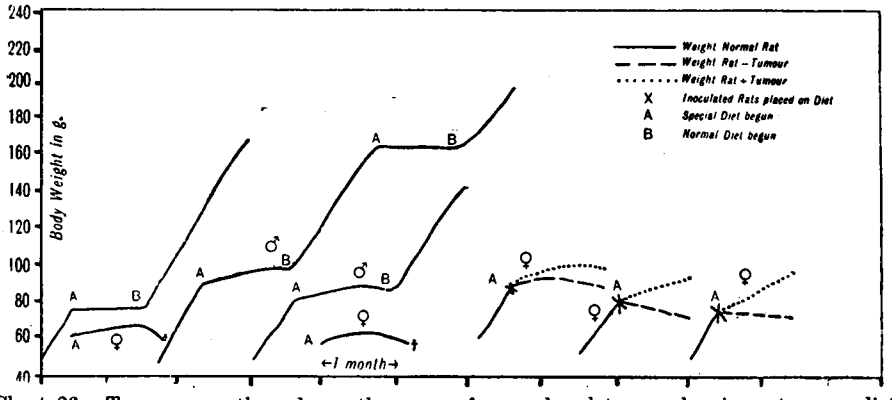

Chart 26. Tumour growth, and growth curves of normal and tumour-bearing rats upon diet deficient in "water-soluble *B*." Tumour *S*/120*d*. Diet similar to that employed for rats in Chart 2, except that "Yeast preparation" was omitted.

From these average figures, as well as from a study of the charts, it may be seen that no retardation of tumour growth followed a deficiency of the fat-soluble *A*, whereas the absence of the water-soluble *B* did cause a certain amount of inhibition. Even in the case of diet *D*, where both factors were

absent, the inhibition of tumour growth is only of the same order as that brought about by the deficiency of the water-soluble factor alone. The explanation of these results may be assisted by a study of the growth curves of the normal animals fed upon these rations. A deficiency in the supply of the fat-soluble factor in the diet of a growing rat is not always followed by

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g	Weight of rat − tumour g	Change in weight of rat g	Change in weight of rat per 1 g. tumour
♂						Tumour firm	7·0	114·0	+21·0	
♀						Large tumour extensively necrotic, small nodule solid	24·5	74·5	+2·5	
♂						Tumour firm	10·0	102·0	+2·0	
♂						Slightly necrotic at centre	38·0	128·2	+27·2	
♂						Hard fibrous growth	0·8	100·0	+12·0	
						Average	16·1	—	+12·9	+0·80

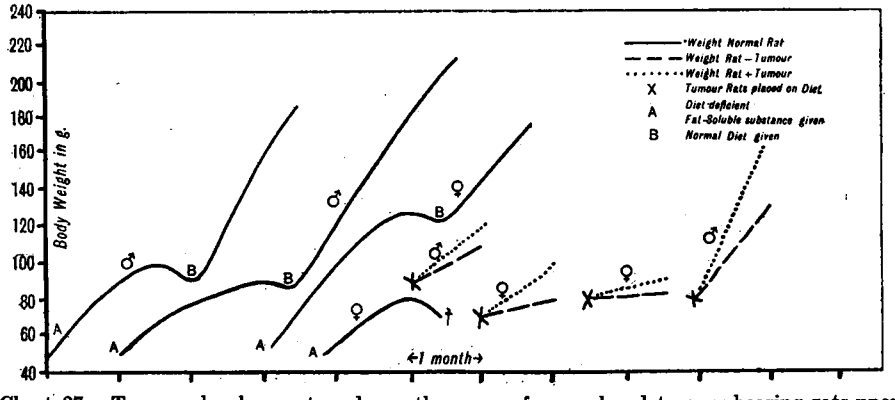

Chart 27. Tumour development, and growth curves of normal and tumour-bearing rats upon diet deficient in "fat-soluble *A*." Tumour *S*/120*d*. Diet similar to that employed in Chart 2, but butter-fat was replaced by lard.

an immediate cessation of growth. More usually, growth continues at a more or less subnormal rate for a period dependent upon the health and age of the animal. An animal which is well nourished at the outset of such an experiment may show such growth for several weeks; sooner or later, however, a cessation of growth will ensue which will be followed by a rapid

decline and death. Only a prompt addition of the missing factor to the diet will prevent this decline and restore health and growth.

These facts have led to the suggestion being advanced that the animal organism can mobilise existing stores of this dietary factor when a deficiency

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀	●	●	●	●	●	Tumour ulcerated and very necrotic. Rat very thin and anaemic	23·0	81·0	−13·0	
♂	●	●	●	●	●	Firm growth	6·5	84·5	+0·5	
♀	●	●	●	●	●	Firm growth	8·5	92·5	−9·5	
♀	●	●	●	●	●	Somewhat fibrous tumour	3·0	98·0	+2·0	
♂	●	●	●	●	—	Tumour retrogressed	—	—	—	
						Average	10·2	—	−5·0	−0·49

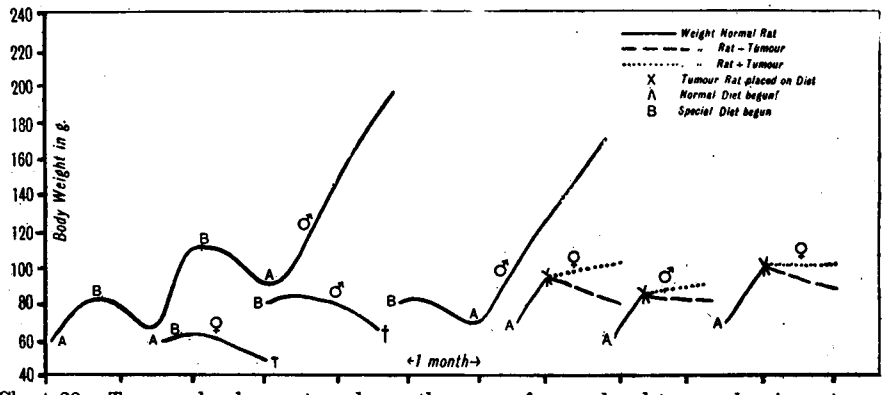

Chart 28. Tumour development, and growth curves of normal and tumour-bearing rats upon diet deficient in "water-soluble *B*," and "fat-soluble *A*." Tumour *S*/120*d*. Diet composed as described under Chart 2, but "Yeast preparation" omitted, and butter-fat replaced by lard.

occurs in its diet [Halliburton and Drummond, 1917]. Since there is evidence which indicates that the animal is able to grow for some weeks upon a diet deficient in the fat-soluble *A*, by making use of its own store of that substance, it is not surprising to find normal tumour growth in hosts placed under the same nutritive condition. Whether a deficiency in this factor

would exert a retarding influence upon tumour development after the available reserves had been exhausted is uncertain.

Animals are much less able to withstand the consequences following the withdrawal of the water-soluble *B* from the diet. As is seen from the curves of the normal rats, the withdrawal of this substance is practically immediate

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat – tumour g.	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀						Tumour extensively necrotic at centre	32·0	57·0	−13·0	
♂						Slight central necrosis	17·0	59·0	−2·0	
♀						—	7·0	103·0	+3·0	
♂						Ulcerated. considerable necrosis at centre of tumour	30·0	82·0	+6·0	
♀						ditto	40·0	57·0	−19·0	
						Average	25·2	—	−5·0	−0·20

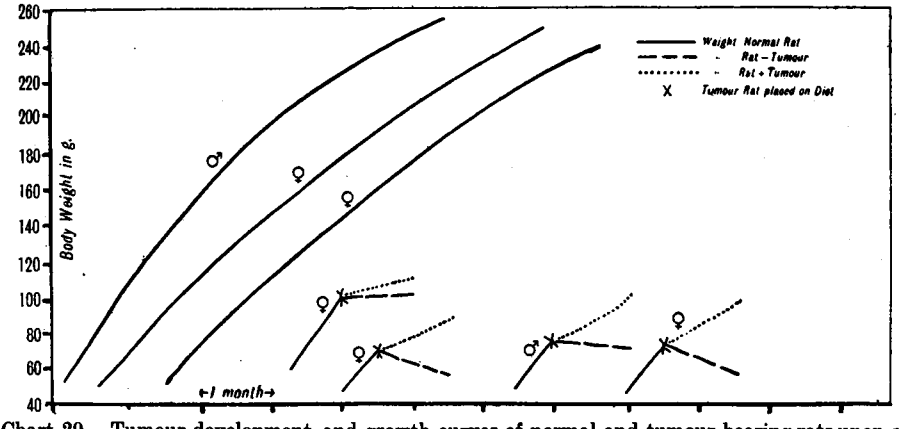

Chart 29. Tumour development, and growth curves of normal and tumour-bearing rats upon a complete artificial dietary. Tumour *S*/117*d*. Diet: Dried milk powder, 50 %, Starch, 40 %, Lard, 10 %.

in causing a cessation of growth. Then follows a period of maintenance or slow decrease in body-weight which eventually ends with a sharp decline and death. What is the state of the animal with regard to this dietary factor during this period is undefined. Drummond [1917] has recently shown that

the properties of the water-soluble growth factor and those of the so-called "beri-beri vitamine" are so closely similar as to render it very probable that the two substances are identical. If this is so certain facts known regarding the latter body may help to throw light on the question. Thus it is well known that the onset of the typical symptoms of the condition known as

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g.	Weight of rat − tumour g	Change in weight of rat g.	Change in weight of rat per 1 g. tumour
♀						Tumour shows slight central necrosis	21	43	−16	
♀						Firm tumour	20	74	−10	
♂						Firm, slow growing	5·5	78·5	−3·5	
♂						Firm tumour	10	70	−15	
♀						Centrally necrotic and ulcerated	40	39	−28	
						Average	19·3	—	−14·5	−0·75

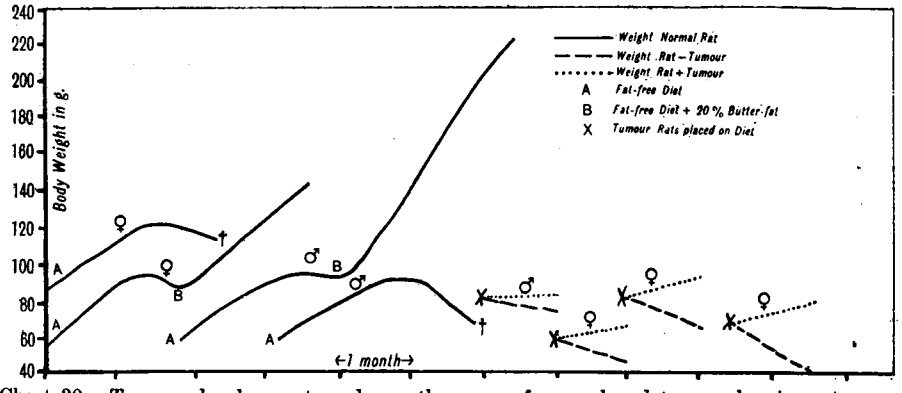

Chart 30. Tumour development, and growth curves of normal and tumour-bearing rats upon diet lacking both fats and the "fat-soluble *A*." Tumour *S*/117*d*. Diet: Ether extracted dried skimmed milk, 50 %, Starch, 50 %.

avian polyneuritis does not occur until the fowl has been fed upon a diet deficient in the so-called "vitamine" for a period of about three weeks. A gradual decrease of body-weight occurs during this period, which apparently corresponds with the period during which many rats show a more or less

successful maintenance of body-weight. The onset of the nerve symptoms in the fowl corresponds roughly with the rapid decline in weight and death of the rat.

Funk [1914, 3] has shown that the period preceding the onset of the nerve disturbances in the pigeon may be shortened by increasing the carbohydrate

Rat	Days 12	19	26	33	40	Remarks	Weight of tumour g	Weight of rat − tumour g	Change in weight of rat g	Change in weight of host per 1 g. tumour
♀						Tumour extensively necrotic and ulcerated.	27·0	105	+27	
♂						ditto	47·0	105	+3·0	
♀						ditto	26·0	55·0	−27·0	
♀						Tumour retrogressed	—	—	—	
♀					-	ditto	—	—	—	
						Average	33·3	—	+1·0	+0·03

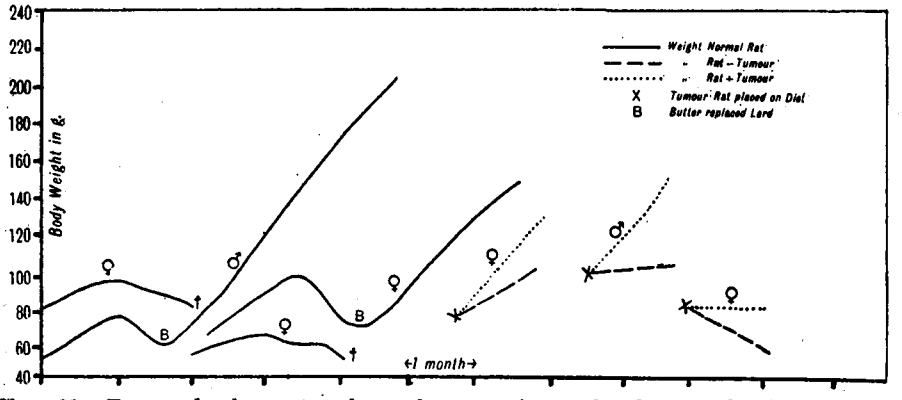

Chart 31. Tumour development, and growth curves of normal and tumour-bearing rats upon diet deficient in "fat-soluble *A*.' Tumour *S*/117*d*. Diet: Ether extracted dried skimmed milk, 50 %, Starch, 30 %, Lard, 20 %.

content of the diet, and he has suggested that a relationship may exist between carbohydrate metabolism and the utilisation of "vitamine." He is inclined to believe that the animal organism possesses a store of the latter substance which may be mobilised for use when a deficiency arises in the diet in this respect.

Whether it is the existence of such a reserve that enables rats to live for some time upon diets deficient in this accessory substance or not is hard to say. Certainly if such is the case the substance is utilised for the purpose of helping to maintain the life of the animal rather than in effecting growth. Tumour growth suffered a slight retardation under these dietary conditions, and was also accompanied by a loss of weight upon the part of the host (Chart 26). Post-mortem examination of these tumour-bearing rats revealed a considerable wastage of skeletal muscle, but the other organs were not visibly affected.

Benedict and Rahe [1917] whose work has already received attention, concluded that the tumour cells are as dependent upon exogenous supplies of this dietary factor, as are the normal cells of the growing animal.

Discussion.

Certain of the points which have arisen during this work have been discussed briefly in the preceding pages, so that the present consideration must be devoted to a more general survey of the results taken as a whole. The outstanding feature which is apparent from a glance at the charts presented with this paper is the comparative failure which has attended the attempts to inhibit the rate of tumour growth by dietary restrictions. In other words it is demonstrated that a considerable degree of tumour growth may occur in hosts fed upon diets which will not permit any normal tissue growth.

The established tumours which formed the subject of this investigation invariably appeared to possess the power of commandeering what part of the food supply of the host they required for their own use. Failing this source of nutrition it was usual to find the tissues of the host sacrificed to no inconsiderable extent in order to provide the requirements of the parasitic cells.

Not altogether unconnected with this subject is the question of tumour growth in pregnant females, a condition where two parasitic cell complexes, each possessing a high growth energy, may be said to be competing for possession of the available food supply.

Clinical observation both on human cases and on experimental animals, has repeatedly demonstrated a retardation of tumour growth during pregnancy, whilst in other instances an increased rate of development upon the part of the neoplasm has been noted under what were apparently identical conditions.

Haaland [1907], Cuénot and Mercier [1909], Uhlenhuth and Weidanz

[1909] are amongst those who have reported a slower rate of tumour growth during pregnancy.

It has been suggested by Jannovics [1912] that the pregnant female presents an unfavourable soil for tumour growth; but Rous [1911, 2] has pointed out that pregnancy under normal conditions is a stimulus to growth and nutrition. The inconsistences in the observations upon the relationship between the degree of tumour growth and pregnancy have been explained by Fichera [cited by Apolant, 1911] as being due to the influence of the number of embryos. Whether this explanation is the correct one, or whether it lies in the relative blood supplies of the tumour and embryos is uncertain, but there is much to support the latter view. It must be remembered that cancer cells perform no function, and that they are therefore free to expend the whole of their energies upon cell division and growth.

The development of the embryo may be for some reasons considered to possess certain parallel features to tumour proliferation, so that it is of interest to note the influence of inadequate dietaries, such as have been studied in this work, upon the former process.

It has been repeatedly observed in this laboratory that animals fed upon inadequate diets will not breed. If females are placed upon the diets soon after they are pregnant the results vary according to the nature of the dietary deficiency.

McCollum, Simmonds and Pitz [1916], found that when the accessory factors *A* and *B* are absent from the diet of the nursing mother, she is unable to rear her young, being only able to supply these substances so long as they are present in her own food supply.

Some results, recently obtained in this laboratory, indicate that in the absence of the water-soluble substance from the diet, growth of the young may continue for a short period after the imposition of the restriction upon the mother; but that sooner or later a rapid decline in the body-weight and health of the young will occur.

Nevertheless, there is unmistakable evidence of growth upon the part of the young for some time after the supply of this dietary factor is cut off from the mother. This result presents similar features to the tumour growth observed under similar conditions.

Returning to a consideration of the process by which tumour cells obtain supplies of food units such as certain amino-acids, when the latter are absent from the diet of the host, it is found that considerable light is thrown upon the subject by a study of certain other conditions.

It is well known that during inanition a transfer of protein "Bausteine"

may occur in the animal organism whereby vital organs, such as the heart, are enabled to exist and functionate as long as possible at the expense of the less important tissues, such as the skeletal muscle. Of particular interest are the classical investigations of Meischer, who described the development of the genital organs of the Rhine salmon at the expense of the musculature, during the period of starvation prior to spawning. In this instance he found that the muscle tissue of the fish might lose 55 % of its weight, without the apparent death of a single muscle cell.

Abderhalden, Bergell and Dörpinghaus [1904] showed that the protein of tissues in inanition possess the same amino-acid distribution as in their normal condition.

This result is confirmed by a few analyses made by the author who could trace no appreciable difference in the amino-acid partition of the tissue proteins of normal chickens, and others whose growth and development had been stunted by dietary inadequacies [Drummond, 1916, 3].

Other examples of a transfer of protein from comparatively unimportant tissues to organs where it is urgently needed are those furnished by the growth of the pregnant uterus and the maintenance of the protein content of the milk of a lactating animal. Both of these occur, as far as possible, at the cost of other tissues, when dietary restrictions necessitate the sacrifice.

The process by which this balance is attained is unknown. It appears however that it is tissues exhibiting at the time a high growth or functionating energy that are protected from the immediate effects of malnutrition by this means. Such for example are the cases which have been given, the uterus during pregnancy, the mammary gland during lactation and the genital organs of the salmon during the spawning period. At the same time it appears probable that all-important organs such as the heart are able to obtain the supplies necessary for their repair and function with equal ease.

Returning to the consideration of the growth of tumours under these circumstances, there seems no reason why the above explanation should not be equally applicable. Tumour cells, particularly those of actively growing sarcomas, possess a high growth potential, as compared with the tissues of the host. At the same time there is no waste of energy in the cancer cell upon functionating processes; cell-division being as far as is known the chief activity exhibited by these units. There is therefore no apparent reason why tumours in possession of an adequate blood supply should not be equally capable of satisfying their requirements by enforcing sacrifice of the tissue of the host, as is the developing embryo.

Any consideration of the results of this investigation must take into account

their bearing upon the treatment of cancer, but little if any hope of alleviating the course of the disease in man can be gathered from the preceding pages. All the results of this experimental research indicate that only the most drastic dietary restrictions, involving a very serious loss of weight upon the part of the host, would have any retarding influence upon tumour growth.

Summary.

1. By imposing certain restrictions upon the diet of a young animal, its growth may be completely inhibited. The present investigation has aimed at a determination of the influence which these dietary inadequacies exert upon the growth of tumours in tumour-bearing subjects.

2. The influence of the following dietary inadequacies upon normal and tumour growth has been studied:

(*a*) Low protein content of the diet.

(*b*) Nitrogen of the diet supplied in the form of a protein possessing a relatively low nutritive value.

(*c*) Absence of certain indispensable amino-acids from the diet.

(*d*) Absence of the equally indispensable accessory growth promoting factors, the "fat-soluble *A*" and the "water-soluble *B*."

3. In the event of a deficiency arising in the diet of the host, the tumour, provided it possesses a satisfactory blood supply, will continue to grow, although the host may be quite unable to do so. There is evidence that this proliferation will proceed at the expense of the tissues of the host, until these are no longer able to supply the missing units.

4. When the host is unable to make good the deficiency, by drawing upon its own reserves, the rate of tumour proliferation will decrease. This occurs at a comparatively early stage when the diet is deficient either in tryptophan or in the water-soluble accessory factor, *B*.

5. There is no evidence that the cells of tumours possess powers of synthetical action which the normal cell of similar type does not possess.

6. It does not appear possible to bring about an inhibition of tumour growth by an employment of dietary restrictions, such as have been used in this research, without the nutritive condition of the host being very seriously impaired.

7. There is, therefore, little hope of bringing about an alleviation of the disease in man by the imposition of dietary restrictions, such as are described in this communication.

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