THE IMPORTANCE OF INDIVIDUAL AMINO-ACIDS IN METABOLISM. Observations on the Effect of adding Tryptophane to a Dietary in which Zein is the sole Nitrogenous Constituent.

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THE behaviour of the animal body under the influence of deficiences in its supply of nitrogenous foodstuffs has hitherto been studied almost entirely from the aspect of fluctuations in nitrogenous equilibrium. It is, of course, clear that the existence of such equilibrium, if sufficiently prolonged, is the best proof that all the needs of the body are being satisfied. It is no less certain, however, that the full significance of nitrogenous equilibrium is as yet but imperfectly understood. Many metabolic factors are probably involved in its maintenance, and the condition is one which should be susceptible of some experimental analysis.

A deficiency in a nitrogenous dietary need not necessarily be one of quantity; the form in which the nitrogen is supplied may determine its efficiency. Thus, in the familiar case of gelatine it is, of course, a qualitative deficiency which makes that substance unable to maintain nitrogenous equilibrium. It is generally supposed that this qualitative deficiency is occasioned by the absence from gelatine of certain molecular groups which are present in true protein, but this hypothesis leaves unexplained the advantage possessed by gelatine over fats and carbohydrates as a protein sparer. It is assumed that gelatine, owing to its constitutional deficiences, cannot repair tissue waste, but can replace protein in so far as the latter functions as a source of energy; sharing with the proteid some unexplained advantage over fats and carbohydrates in this latter capacity.

Recent advances in physiology seem to justify a fresh attack upon this subject, upon somewhat different lines. It now seems necessary to differentiate between the minimal amount of protein necessary for actual tissue repair and that required for total maintenance; we have no reason for assuming that they are the same. We are no longer bound to Liebig's view, or to any modification of it which implies that the whole of the proteid consumed is utilized as intact proteid; nor are we even compelled to assume that the whole of what is broken down in the gut is resynthesised before utilization. Proteid products may function in other ways than in the repair of tissues or in supplying energy. It is highly probable that the organism uses them, in part, for more specific and more immediate needs. The discovery of substances absolutely essential to life, highly specific, and elaborated in special organs, suggests that some part, at least, of the protein products set free in the gut may be used directly by these organs as precursors of such specific substances. In adrenaline, for instance, we have an aromatic substance absolutely essential for the maintenance of life, and it is probable that the suprarenal gland requires a constant supply of some one of the aromatic groups of the proteid molecule to serve as an indispensable basis for the elaboration of adrenaline. If this be so it is certain that the gland itself could not, in starving animals, supply sufficient of such a precursor to outlast the observed survival periods[1]. Since adrenaline must be produced at all costs, the required precursor must, in starvation, be obtained by tissue breakdown outside the gland. We may be sure, moreover, that adrenaline is far from being the only substance elaborated to which such considerations apply. Similarly, in an animal upon a diet sufficient to supply energy, but lacking in some essential group, the minimal waste in the general tissues of the body will be determined by the special need of the organs for the missing group. On this basis we have a hypothesis to account for the special protein-sparing properties of gelatine. It shares with protein certain molecular groupings needed to satisfy specific needs, and is thus superior to fats and carbohydrates as a protein-sparer; it lacks, on the other hand, certain other necessary groupings, fails therefore to supply all such needs, and thus cannot replace true proteid.

Considerations such as these formed the basis for the experiments described in the present paper. The results obtained serve to show that even when tissue-equilibrium is not maintained the presence or absence of some one amino-acid in the diet may affect most materially the survival period and general well-being of an animal.

[1] See Hopkins. *Science Progress*, New Series, Vol. I. No. 1, July 1906.

Previous work on these lines has had the study of nitrogenous equilibrium as its basis. Voit and Munk showed in their classical work that gelatine could not maintain such equilibrium, while Escher showed that the addition of tyrosine at least improved its powers in this respect. During the progress of the present research a paper has been published by Kaufmann[1] giving the results of gelatine feeding with the addition of the missing amino-acids tyrosine and tryptophane. On replacing one-third of the proteid nitrogen of a diet by gelatine nitrogen equilibrium was no longer maintained. If now one-third to one-half of the gelatine nitrogen was replaced by nitrogen in tyrosine and tryptophane the animal could be—for a time at least—maintained in nitrogen equilibrium. The animals used were dogs, and the experimental period was of six days duration. Kaufmann performed a similar experiment upon himself in which he replaced all protein nitrogen by nitrogen in gelatine, tryptophane, and tyrosine. The experimental period was four days, and his nitrogen balance though not maintained was yet equal to that of a preliminary period when casein was taken.

It is necessary to point out that our own results could not have been obtained by observing the presence or absence of nitrogenous equilibrium; the significance of the observations would have been entirely missed if that alone had been considered.

Methods. The protein-like substance "zein" first described by Gorham[2] and later studied among others by Chittenden and Osborne[3], and especially by T. B. Osborne[4] himself, is, as is well known, obtained from maize (zea mais). It gives most of the proteid reactions—for instance Millon's reaction and the xanthoproteic and biuret reactions. Its decomposition products have not been fully studied, but it yields leucine, tyrosine, and abundant glutaminic acid. On the other hand Kossel and Kutscher have demonstrated that lysine cannot be obtained from it[5], and, as Osborne and Harris have shown, it fails to yield the Adamkiewicz reaction (glyoxylic reaction), thus indicating the absence of the tryptophane group[6].

1 Kaufmann. *Pflüger's Arch.* CIX. p. 440.

2 Gorham. *Berzelius Jahresb.* II. p. 124. 1822.

3 Chittenden and Osborne. *Amer. Chem. Journ.* XIII. Nos. 7 and 8, pp. 453, 529, and XIV. No. 1, p. 20. 1892.

4 T. B. Osborne. *Journ. Amer. Chem. Soc.* XIX. No. 7. 1897.

5 Kossel and Kutscher. *Zeitsch. f. physiol. Chem.* XXXI. p. 165. 1900.

6 Osborne and Harris. *Journ. Amer. Chem. Soc.* XXV. p. 853. 1905.

The whole significance of the observations to be described in the following section depends upon this absence of the indol group. In the paper by Szumowski referred to in a later paragraph, the statement is made that zein gives the glyoxylic reaction. The explanation for this discrepancy must be found, we think, in the fact, that preparations of zein are apt to give a purple colour with strong sulphuric acid atom. This colour change is chiefly, if not entirely, due to the yellow pigment which is always abundantly present in the raw product. After treatment with ether, which removes pigment, the purpling with sulphuric acid ceases, and it becomes easy to observe the complete absence of the glyoxylic reaction proper. We have hydrolysed pure zein by boiling with twenty per cent. sulphuric acid and no trace of tryptophane could be separated at any stage of the hydrolysis by the method of Hopkins and Cole. If finely powdered preparations are submitted to prolonged pancreatic digestion very complete hydrolysis occurs without the appearance of any colour reaction on the addition of bromine.

The behaviour of such a substance in metabolism could not fail to be of interest, and the immediate object of the experiments to be described was to discover whether an atypical protein of this kind can maintain growth in young animals, or whether in spite of its containing tyrosine the absence of other characteristic groups from its molecule causes it to take a position similar to that of gelatine as a nutritive agent. If the substance failed to maintain growth it would be clearly of further interest to observe the effect of adding an amino-acid absent from its breakdown products. If, again, on adding, say, tryptophane there should still be a failure to promote growth, there might yet be some favourable influence upon the animal, some effect independent of structural maintenance—a point which has received little attention in metabolism experiments.

Four years ago some feeding experiments relating to the fate of zein in metabolism were carried out under Kossel's direction, by W. Szumowski. The chief object of these was to discover whether this easily recognisable protein substance could be recognised in the tissue after it had been given by the mouth, and to compare in this respect the effects of intravascular injection. Geese and pigeons were fed for long periods with large amounts of maize, but showed when killed no trace of zein in their blood or tissues. Dogs fed upon pure zein showed very bad absorption of the substance. When injected into the blood stream zein was deposited in the liver, and a large proportion of what was injected could be recovered from that organ five hours afterwards. It is clear that Szumowski's interesting experiments, though differing somewhat in significance from the later ones of Abderhalden and Samuely on gliadine feeding, should be ranked with these as offering evidence for the deep seated processes which must be involved in proteid assimilation. They do not bear upon the topic of this paper.

Mice were employed for the feeding experiments described. Whatever objection may be attached to the use of small animals in metabolism experiments, they do not apply to observations of the simple kind necessary for the enquiry at its present stage. The observations are of a strictly comparative kind, in which one essential factor, and one only, is varied, and they are concerned only with the change of weight, the survival period, and the general well-being of the animals. It was desirable at the outset of the enquiry to work with a large number of individuals, and the difficulty and expense of preparing the special constituents of the dietary for the use of numerous large animals would be almost prohibitive. The general result being established, it will be possible to study the detailed effects of the dietary upon a smaller number of larger animals.

The origin and previous history of most of the mice employed were known, so that comparative series could be chosen with due regard to uniformity.

The zein was obtained by extracting maize-meal with 75 per cent. alcohol, as described by Osborne. The clear alcoholic solution obtained after filtering was concentrated and poured into a large quantity of water, in which the zein separated as tough fibrous masses. The product could be drawn out into long threads and in this condition could be washed with thoroughness. In most cases, after drying and removal of fat and pigment with ether, the protein could be shown to give no reaction with glyoxylic acid. In the case of one or two preparations a second solution in alcohol and reprecipitation by water were necessary to remove adherent traces of other proteid. Extraction by ether was quite necessary to make the dry product susceptible of being ground to powder; but no attempt was made to remove all traces of fat.

The zein was mixed with two and a half times its weight of carbohydrate, two parts of starch being used to one part of cane-sugar. The mixture was finely powdered and subsequently mixed with fat. The fat used was varied from time to time, such variation seeming to stimulate appetite; but in any one comparative series the changes were exactly uniform for all the mice concerned. Butter fat (filtered), etherial extract of cheese, clarified bacon fat, and olive oil were all used, with the occasional addition of a drop of cod liver oil. Zein does not contain phosphorus, and, in view of this, a little lecithin was added to the food. The salts were obtained by ashing a known weight of dog-biscuit and oats (these having formed the previous dietary of the animals). The ash was added to a corresponding weight of the prepared food. Macerated filter paper was added in the first experiments. Later this was replaced by charcoal. The complete mixture was eaten readily by the mice. In the tables of results which follow the above mixture is referred to under the term "Zein Diet."

In all the experiments the food was so given that the mice had as much as they could eat, and water was given freely.

As remarked above Szumowski found that pure zein given by the mouth to dogs was absorbed by them very badly. It does not of course follow that because a single large dose of such material forced upon a carnivorous animal was digested badly, that the results would be the same in such animals as mice, taking voluntarily a mixed dietary containing it. That zein is absorbed, both by man and animals, when taking ordinary maize or maize preparations, is proved by data quoted by Szumowski from the literature of the subject.

It is of course not easy to determine the degree of nitrogenous absorption in such small animals as mice, but in our experiments there was every indication that the food given was dealt with satisfactorily in this respect. In the great majority of cases the fæces were, throughout, perfectly normal, both in appearance and amount; while *post mortem* examinations of individuals which died while still taking their food freely, showed no accumulation of zein in the lower part of the intestines. It will be seen from what follows that a proof of complete utilization is of no importance to the significance of our results.

In the earlier experiments the mice which were upon the same diet shared a large cage; but in later experiments each animal had a separate compartment, in order to prevent any possibility of interference with one another. The cages were of wire or perforated zinc, and cotton wool was used for bedding.

Most of the mice used were young individuals not fully grown, as it was desired to take the maintenance of growth as a test of the efficiency of the diet. In each experiment exactly uniform conditions of feeding were maintained, the only variant being the presence or absence of added amino-acids as described in each case.

RESULTS OF FEEDING EXPERIMENTS.

Exp. I; Series A and B. This was a preliminary experiment made to test the power of zein to maintain growth in young mice. Series A had the zein diet as described above. Series B had the same diet but with the zein replaced by an equivalent quantity of casein.

A. Zein diet.		B. Casein diet.		
Mouse No.	Change in weight after 7 days feeding	Mouse No.	Change in weight after 7 days	Change in weight after 14 days
1	−11·8 %	6	+20·2 %	+68 %
2	−17·6	7	+21·8	+59
3	−13·1	8	+ 9·1	+27
4	−23·2	9	+21·0	+49
5	−27·1			

It is seen that the zein is quite unable to take the place of a normal proteid in maintaining growth. The loss of weight was observed from the first day onward. It is plain from the rapid gain in the case of series B that the non-nitrogenous constituents of the dietaries were in satisfactory proportions. The feeding of series B was continued for 14 days for comparison with the results of later experiments.

Exp. II. One of the mice from series A of Exp. I (No. 4) was now fed upon a dietary in which half the protein was zein and half casein. In 15 days it had gained in weight to the extent of 46 per cent., and was in fine condition. The gain commenced from the first day of taking the new diet. This experiment makes it clear that the prepared zein is in no sense actively deleterious.

Exp. III; Series C and D. This was a preliminary experiment to test the effect of adding tryptophane to the zein diet. It was not carried to completion. The food was precisely similar in each series, save that in D a little tryptophane was added to the zein diet described above.

C. Zein diet.			D. Zein diet + Tryptophane.			
Mouse No.	Survival period	Change in weight	Mouse No.	Survival period		Change in weight
10	12 days	−30 %	14	16 days	+	−18·2 %
11	12	−33·4	15	16	+	−26·8
12	12	−29·2	16	16	+	−25·6
13	12	−21	17	12		−29
			18	16	+	−23·7

In this particular experiment the mice on the zein diet all died together on the twelfth day of feeding. On the 16th day the experiment had to be stopped; but at this time four out of the five mice that received tryptophane were perfectly well and active. Those without tryptophane were torpid and apparently almost lifeless for four days before actual death. This symptom was always present in animals upon zein without tryptophane and will be discussed later. The addition of tryptophane is seen not to prevent loss of weight. The experiment was done in the summer, but the animals had to sustain the fluctuations of day and night temperature.

Exp. IV; Series E, F, and G. In this experiment three series were compared. The one had the zein diet alone; another had the same with tryptophane added to the extent of 2 per cent. of the zein present. The third series had the zein diet with tyrosine added to the extent of 2 % of the zein. Tyrosine is already present abundantly in zein, and on this account it was chosen as an addendum to a series forming a second control. That in this, as in subsequent experiments, its addition makes no difference to the effect of the zein diet, is sufficient to show that the modifications due to tryptophane are specific and not due merely to increasing the amount of aromatic amino-acids.

E. Zein diet.			F. Zein diet + Tyrosine.			G. Zein diet + Tryptophane.		
Mouse No.	Survival period	Change in weight	Mouse No.	Survival period	Change in weight	Mouse No.	Survival period	Change in weight
19	15 days	- 27·7 %	26	12 days	- 24·3 %	33	24 days +	40·4 % (alive)
20	15	- 32·2	27	15	- 31·5	34	24 +	39·0 (alive)
21	15	- 20·3	28	19	- 30·3	35	24 +	38·0 (alive)
22	17	- 32·6	29	19	- 27·7	36	24 +	27·6 (alive)
23	12	- 22·7	30	10	- 27·6	37	24 +	28·8 (alive)
24	17	- 36·5	31	10	- 17·3	38	16	33·7
25	17	- 32·9	32	12	- 32·8	39	10	34·8
Mean	15	- 29·2		14	- 27·3		21 + +	34·6

The experiment had, unfortunately, to be stopped on the 24th day. By this time all the mice without tryptophane had been dead for some days, while of the seven which received tryptophane five were still alive, and, though thin, were well and active. The temperature when the experiment was made was low, and all the mice without tryptophane were extremely torpid after exposure to cold in the night. They had to be warmed in front of a fire in the morning before becoming sufficiently active to seek their food. The tryptophane series were warmed at the same time to keep the conditions of experiment similar, but in their case there was no necessity for this; they were active throughout.

Exp. V; Series H and I. This experiment was on the same lines as the last, but the animals were exposed to the fluctuations of the temperature of the laboratory in the month of February, though provided with abundant cotton wool for bedding. Under these conditions the differentiation was rapid. Series H had the zein diet with tyrosine added to the extent of 2 per cent. of the zein; series I had the zein diet and a similar quantity of tryptophane. The experiment was continued till all the mice in both series had succumbed.

H. Zein diet + Tyrosine.			I. Zein diet + Tryptophane.		
Mouse No.	Survival period	Change in weight	Mouse No.	Survival period	Change in weight
40	8 days	− 25·8 %	45	37 days	− 34·9 %
41	10	− 27·4	46	13	− 20·7
42	7	− 17·3	47	33	− 40·1
43	5	− 24·1	48	27	− 35·0
44	14	− 28·9	49	17	− 28·8
Mean	9	− 24·7	Mean	25·5	− 31·9

A comparison of these results with those of Experiment IV, in which, as stated, all the mice were placed in a warm room each morning, shows the important part played by temperature in determining the survival period. The mice without tryptophane became rapidly torpid; those getting tryptophane were active throughout.

Exp. VI; Series J, K, and L. In this experiment the cages of all the mice were placed in a large well-ventilated thermostat, the temperature throughout being kept at 20° C. The amounts of tyrosine and tryptophane in series K and L respectively were 2 per cent. of the zein given.

J. Zein diet.		K. Zein + Tyrosine.		L. Zein + Tryptophane.	
Mouse No.	Survival period	Mouse No.	Survival period	Mouse No.	Survival period
50	19 days	58	25 days	66	18 days
51	21	59	24	67	45
52	21	60	23	68	45
53	16	61	16	69	Killed accidentally on 17th day by entanglement in wire of cage.
54	17	62	20		
55	15	63	15	70	34 days
56	21	64	17	71	34
57	22	65	18	72	29
				73	30
Mean	19	Mean	19·7	Mean	33·5

Exp. VII; Series M, N, and O. Exactly similar in all respects to Exp. VI. The cages were kept at 20° throughout. In this, as in the last experiment, the maintenance at uniform temperature appeared to delay the differentiation in the series. For some days the mice without tryptophane appeared as well as those supplied with it; but later on there was always the marked difference discussed elsewhere.

M. Zein diet.			N. Zein + Tyrosine.			O. Zein + Tryptophane.		
Mouse No.	Survival period	Change in weight	Mouse No.	Survival period	Change in weight	Mouse No.	Survival period	Change in weight
74	23 days	-41 %	81	21 days	-38 %	89	27 days	-34 %
75	19	-41	82	14	-21	90	43	-38
76	14	-37	83	8	-26	91	33	-43
77	14	...	84	26	-42	92	30	-22
78	14	...	85	8	-17	93	31	-38
79	16	...	86	34	-46	94	18	...
80	23	...	87	26	-43	95	33	-33
			88	21	-40			
Mean	18	...	Mean	19·7	-34	Mean	31	-30

Exp. VIII; Adult mice. In the foregoing experiments nearly all the mice were young growing animals, varying from about 3 weeks old to three-parts grown; only a very few were approximately full grown. In each experiment they were sorted, so that each series contained, so far as possible, mice of corresponding weights and ages. The behaviour of adult animals under similar conditions of feeding has not been fully studied. Only a short series can be given. Of six adult mice three were upon the zein diet with tyrosine, and three had the same diet with tryptophane. The amount of added amino-acid was in each case equal to 2 per cent. of the zein.

Zein + Tyrosine.			Zein + Tryptophane.		
Mouse No.	Survival period	Change in weight	Mouse No.	Survival period	Change in weight
96	19 days	-32·7 %	99	36 days	-42·7 %
97	21	-53	100	50	-50
98	40	-51	101	62	-57

The difference in the survival period in the two series is very great, but mouse No. 98 lived much longer than any other when upon a tryptophane-free diet. The condition of this animal was remarkable. On the 21st day it was torpid and seemed no better than its companion which died upon that day. The torpor lasted for most of the remaining period of its life, and though it mostly consumed food each day its movements were extremely sluggish; it made no response when handled, and if taken from the cage it remained quiet and motionless. The tryptophane mice were as usual lively and active till quite near their end.

Since a view of the death incidence is more instructive than averages of survival periods, a graphic representation of the survival periods of certain individual mice is given in the following diagram. It refers only to the young mice which were observed till death, and is compiled, therefore, from Exps. V, VI, and VII only. It shows the effect of adding tryptophane to the zein diet compared with that of adding tyrosine. The animals on zein only are not included.

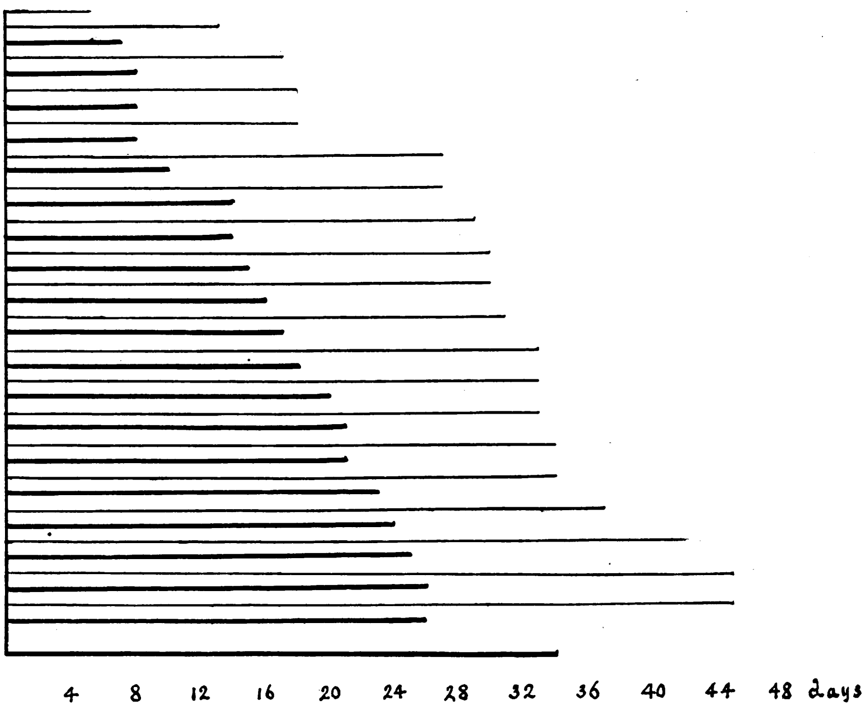

Diagram constructed from the results of Exps. V, VI, and VII. The thick lines show the survival periods (in days) of twenty-one individual mice upon the zein diet with tyrosine added. The thin lines show the same for nineteen mice upon the zein diet with tryptophane added.

General discussion of the results.

It may be again emphasized that in all the foregoing feeding experiments the conditions under which the mice were compared were exactly similar, except for the presence or absence of small quantities of tyrosine or tryptophane in the diet.

The food supplied was taken equally well by all the mice, at any rate till very near the end of life, when, in the case of those animals without tryptophane, the torpor which supervened introduced some slight variation in this respect. This circumstance, which certainly did not in the least affect the main bearing of the results, will be discussed immediately.

Considering first the *survival periods* on the several diets given, and taking together all those cases in which the animals were fed and observed till death supervened, we find that the average duration of life of young mice upon the zein diet alone was 16·0 days; upon zein and tyrosine 15·6 days; upon zein and tryptophane 30·0 days. Omitting Experiments III and V, in which all three diets were not compared simultaneously, we have for zein alone 17·3 days; for zein and tyrosine 17·5 days; and for zein and tryptophane 32·2 days. Again considering in each case the animals that were observed till they died, we find that of the mice on zein alone 20 out of 26, or 77 per cent., died within 20 days; of those on zein and tyrosine 21 out of 31, or nearly 70 per cent. died within the same period; while, of those receiving tryptophane, 7 out of 34, or 20 per cent. only, succumbed within this period. On the other hand, of those which lived for 30 days or more, there were none upon zein only; 2 only out of 26 upon zein and tyrosine (one of these being an adult mouse) and 15 out of 22 upon zein and tryptophane—nearly 70 per cent.

The effect of the presence or absence of small quantities of tryptophane upon the survival periods is therefore extremely well marked.

The figures last given (though not the averages) may appear to show a very slight advantage due to the addition of tyrosine. This is out of all proportion small when compared with the effects of adding tryptophane, and the result is probably accidental only. Consideration of the results as a whole will, we think, suggest that the addition of tyrosine was wholly without effect; and it is certain that the very marked superiority in the general condition of the mice during life, so evident in the tryptophane animals, was wholly absent from those with tyrosine. This does not, of course, show that the tyrosine group of protein plays no special part in metabolism; but only that this aromatic amino-acid being already present in the zein the addition of a further small quantity is—as might be expected—without effect. The interest however of adding it as a control in these experiments will be obvious.

It is desirable, we think, to insist upon the fact that the above numerical results do not adequately express the difference in the condition of the mice with and without tryptophane. The torpor already referred to was a very prominent feature in those animals which were without this amino-acid. To a certain degree it was observable as an early symptom, especially in animals which were not kept at a uniform temperature. Later on it always appeared, and for several days before death the mice were exceedingly inactive and made no movement when touched or handled. When in this condition the ears, feet and tail were cold to the touch, the eyes half closed and the coat glairy. If the external temperature rose there was in some cases a temporary improvement, and in those experiments in which the animals were kept uniformly warm the symptoms were delayed. Such symptoms are of course in part similar to those seen in animals when simply starving or wholly deprived of nitrogen; but there was a difference, more easy to recognize than to describe. The difference was possibly due to the longer duration of life, or to the fact that there was in the earlier stages more active metabolism than in starvation, carried on however in the absence of some essential factor. At any rate there was a most striking absence of such symptoms from the mice which received tryptophane. These, in spite of the daily loss of weight, were alert and active, and looked healthy, not only up to such a period as saw the other mice succumb, but also till very near the end of their own much prolonged lives. In very cold weather a slight degree of inactivity might be manifested; but it was never comparable with that seen in the animals without tryptophane. Notable as was the inactivity seen in the latter it is to be observed that nearly all showed appetite, and contrived to take food, till quite the last day or so of life; and, except in two or three special cases, the amount of food taken showed little or no difference from that consumed by the tryptophane mice during the same period. In the case of two long-lived mice in Exp. VII (Nos. 86 and 87) and especially in that of the adult mouse No. 98 (Exp. VIII) the matter was different. These were apparently moribund for several days before death and took then little or no food. Watching these mice, and comparing them with others, gave the impression that the absolute quiescence displayed by them was largely responsible for prolongation of their life.

It is of interest to consider how far actual tissue maintenance intrudes as a factor in producing the difference between the mice that received the missing amino-acid and those from which it was withheld.

Zein, it is clear, has no power whatever of maintaining growth in the young animal; loss of weight begins the moment it forms the sole nitrogenous supply. The addition of the missing tryptophane group to the zein has, it is also clear, no power to convert such loss into equilibrium or gain; a fact possibly due to other deficiences in the zein molecule, such as the absence of lysine, or the lack of some other amino-acid not yet observed. There was no close relationship in our experiments between the loss of weight and the length of survival period. In many individual cases the mice upon tryptophane lost a considerably larger percentage of their weight before death than, on the average, did those without it. Such differences may be largely due to differences in the nutritional condition of individuals at the outset, but the results appear to show that death was not determined by a critical percentage loss. On the other hand the figures show that, on the average, the loss of body weight was slower with tryptophane than without it. But this result might well be expected, even if the tryptophane administered undergoes utilization without directly contributing to tissue formation or structural maintenance. If it serves as a basis for the elaboration of a substance absolutely necessary for life—something, for instance, of an importance equal to that of adrenaline—then, in starvation, or when it is absent from the diet, a supply is likely to be maintained from the tissue-proteids; the demand for it would become one of the factors determining tissue breakdown. In the case of young animals which directly benefit from the addition of a protein constituent, otherwise absent from their diet, to the extent of a well nigh doubled life, and marked improvement in general condition, but at the same time steadily lose, instead of gaining, weight, the utilization of the constituent would seem to be of some direct and specific nature.

Histological examination of the various organs failed to show any essential differences between one set of mice and another, but such examination was very incomplete. It is noteworthy however that the suprarenal glands hardened in formaline and dichromate certainly exhibited no differences, and in the opinion of Mr T. R. Elliott none were deficient in adrenaline.

The Authors hope at some future time to make a more careful study of such points on larger animals and to observe the metabolic changes more completely.

Summary of results.

1. A dietary containing zein as its only nitrogenous constituent is unable to maintain growth in young mice.

2. The addition of tryptophane (an amino-acid absent from the decomposition products of zein) to such a dietary does not make it capable of maintaining growth.

3. On the other hand this addition greatly prolongs the survival period of animals fed upon zein, and materially adds to the well-being of such animals.

4. The addition of tyrosine (which is already present in zein), in equivalent amounts, has no such effect.

5. It is suggested that the tryptophane is directly utilized as the normal precursor of some specific "hormone" or other substance essential to the processes of the body.

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