XLV. FEEDING EXPERIMENTS WITH DEFICIENCIES IN THE AMINO-ACID SUPPLY: ARGININE AND HISTIDINE AS POSSIBLE PRECURSORS OF PURINES.

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We have abundant evidence to show that purine derivatives can be synthesised in the animal body when no preformed purine material is present in the diet, but the chemical events which are involved in the formation of tissue purines are yet unknown. It is of course possible that the synthesis of the purine ring may start at a low level from simple and remote precursors. Attention has been fixed for instance upon the interesting observation made several years ago by Knoop and Windaus [1905] who showed that 5-methyl-iminazole is formed when a solution of dextrose is exposed to sunlight in presence of the strongly dissociated compound $Zn(OH)_2$, $4NH_3$. We have here in effect the formation of the iminazole ring, an essential part of the purine structure, by the action of ammonia upon sugar. That this reaction is related to the chemical mechanism by which histidine and purines are formed in plants is highly probable. In the case of the animal, synthesis of such a type is perhaps less likely. Nevertheless the observations of Cathcart [1909], of Graham and Poulton [1913], and of Umeda [1915] have shown that when the diet is rich in carbohydrates the excretion of endogenous purines is greater than when fat predominates, yielding the suggestion that the former may be employed in synthesis.

It is by no means established however that such a synthesis from carbohydrate derivatives and unknown nitrogenous substances is the sole

[1] Several of the experiments described in this paper were made in 1914, the rest in 1915. My colleague has been long at the front, and in writing the paper I have been unable to consult him. He has had moreover no opportunity of reviewing the experimental results as a whole. If therefore it be held that the conclusions are not warranted by the facts I am alone responsible. F. G. H.

or chief origin for purines. We are at any rate justified in looking first to the food for a supply of more immediate precursors.

When some structural relation is evident between a food constituent and a tissue constituent it is justifiable to suspect that the former, and not some wholly unrelated substance, provides the immediate raw material for the manufacture of the latter. The recognition of such a chemical relation should, at any rate, lead to an experimental enquiry into the possibility of a metabolic relation.

The fact that histidine alone among the amino-acids from protein has a chemical connection with purines in that its molecular structure comprises the iminazole ring has led others to suppose that histidine might be the precursor of purines in metabolism. Abderhalden and Einbeck [1909] tested the point experimentally, but obtained negative results. In their experiments the administration of histidine produced no definite increase in the excretion of allantoin. The experiments were repeated later in conjunction with Julius Schmid [1910]. In the later work a starving dog was given on two occasions an isolated dose of 10 g. of histidine hydrochloride but no increase of allantoin followed.

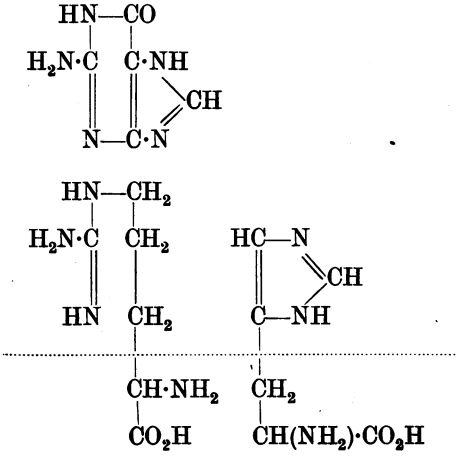
We venture to think however that these observers may not have secured the conditions necessary for properly testing the matter.

When an animal is in a state of full nutrition it does not follow that such a process as the synthesis of the purine ring would necessarily be much accelerated or increased by mere increase in the supply of its raw material. The accepted distinction between endogenous and exogenous metabolism and the recognised relative constancy of the former could scarcely hold were this the case. We know, it is true, that a large increase of pure protein in the diet does affect purine metabolism; but an individual amino-acid fed in excess of the immediate current needs of the tissues, as when it is added to an already efficient dietary, will almost certainly be rapidly broken down on more direct lines, even if it be a normal precursor of the purine (or other) synthesis in the body. We doubt, further, whether the administration of large isolated doses to a starving animal offers the best method for determining whether or not a substance suffers some special fate in metabolism. Doubtless synthesis of such essential tissue constituents as the purines continues during starvation, at the expense—as we are entitled to believe—of protein materials liberated by autolysis of the less essential organs. When however an excess of a single amino-acid enters the circulation of a starving animal in a single isolated dose it may well almost

completely escape such special utilisation. It appears suddenly in excess of current needs, and, because the processes of deamination and direct oxidation are always in action, it will almost certainly survive for but a short period as available material for synthesis. The increase in its concentration may momentarily accelerate the special reaction concerned, but the possible increase of reaction velocity in the tissue element is naturally limited, and the survival within the tissue cells of the abnormally increased amino-acid will probably be too short to affect appreciably the day's yield of a synthetic product. Much more satisfactory at any rate would seem to be experiments in which an animal, of which the normal metabolism is known, is first deprived entirely for considerable periods of the substance in question, and deprived of it alone. The effect of its absence being noted the substance can then be restored to the diet in normal amount and given continuously, when the results of its restoration can again be followed for considerable periods.

In the experiments to be described in this paper advantage was taken of the fact that from the complete mixture of amino-acids obtained upon hydrolysis of a typical protein, arginine and histidine can be efficiently removed by the well-known method of Kossel and Kutscher. Purine metabolism can thus be studied upon a dietary from which these two diamino-acids are absent and the effect of the deficiency as well as the result of restoring either or both can be observed.

The original incentive of the present research was, as a matter of fact, the idea that arginine and histidine might function together as precursors of the purine ring in metabolism, on some such lines as the following comparison of the structure of guanine with that of the two diamino-acids might suggest



Our original intention therefore was to test a possibility somewhat different from that which led to Abderhalden and Einbeck's experiments. During the progress of our observations however evidence was obtained which seems to show that in metabolism arginine and histidine are within certain limits equivalent to one another; equivalent probably because each can be converted into the other, just as phenylalanine can, up to a point at least, function in the place of tyrosine [Totani 1916] because in the body it can be converted into the latter (Embden). This circumstance removes some of the significance from the question as to whether both, rather than one only, of these two diamino-acids are concerned in the formation of the purine ring. The point however receives some further attention later on in this paper.

In most of our experiments, after a preliminary period during which a complete amino-acid mixture was administered, arginine and histidine were for some weeks removed from the diet, and then, in a third period, both were restored. The allantoin excretion was followed through each of these three periods.

In our opinion the results obtained offer good grounds for the belief that the molecules of arginine and histidine are as a matter of fact the most readily available raw material for the synthesis of purines in the body.

Methods.

The experiments were all made upon young growing rats. The rat when it has reached a weight of 80 to 100 g. continues to grow satisfactorily and for long periods on a diet which contains all its nitrogen in the form of a proper mixture of free amino-acids. It is certain however that successful feeding experiments when they involve the administration of abnormal diet can only be carried out on rats when care has been exercised in the choice of stock and when attention is given to the general care of the animals. Some stocks are more prone to disease, and especially to intestinal disorders, than are others, and, if such vulnerable animals are used, intercurrent disease may spoil a prolonged experiment just when it has reached its most interesting period. It is equally important that the experimental animals should be kept in healthy surroundings and at a uniform temperature. The cages should be kept clean and feeding should be carried out at regular intervals.

Admirably suited as is the rat for studies on growth it offers obvious difficulties when quantitative observations have to be made upon the urine.

The amount of urine is of course small and the proclivity of the rat when eating to hold food in its forepaws tends to scattering of the food and, in any ordinary collecting apparatus, to a contamination of the urine with the food.

The cages and apparatus used have been gradually modified by ourselves and by other workers in the laboratory and in their final form, as employed in the present research almost completely obviate any difficulty in collecting the urine quantitatively and with freedom from contamination. The

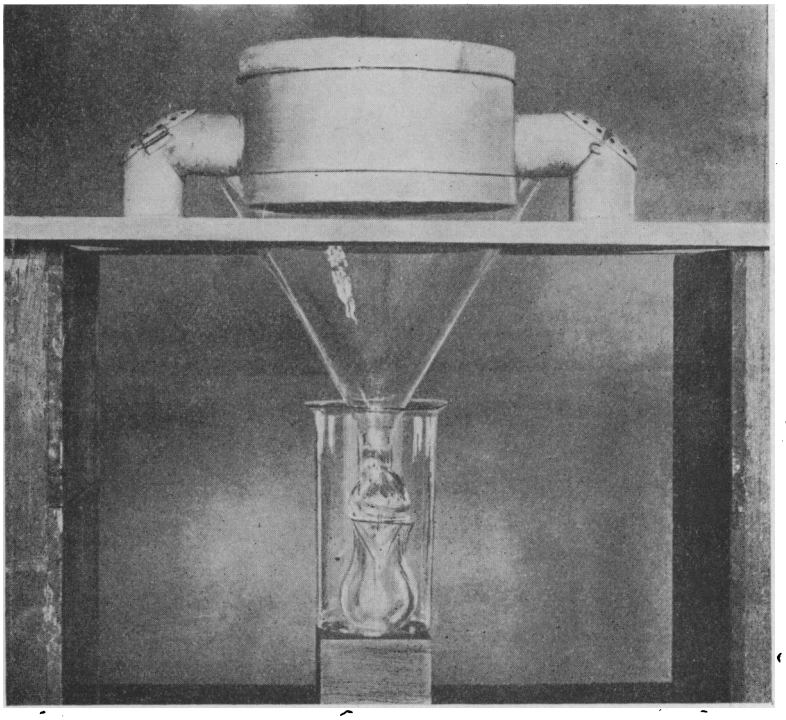


Fig. 1.

photographs illustrate the apparatus as used (Figs. 1 and 2). The cage itself is made of zinc. It is circular with a diameter of 10 inches, and a depth of $5\frac{1}{2}$ inches, and has a lid of perforated metal. The bottom consists of concentric circles of wire $\frac{1}{2}$ inch apart, held together by radial wires. The food and water are placed in glass vessels which fit into the vertical part of the metal side tubes. The tubes have a diameter of $2\frac{1}{4}$ inches. When the rat has to obtain its food under the conditions imposed by the tubes it seems unable to carry portions from the trough into the cage. This result

is more fully secured when the animal is wholly prevented from turning round while eating from the food-trough. For this reason inner tubes are provided, tapering somewhat towards their distal end. The diameter of these is adjusted to the size of the individual animals under experiment, and they are provided with radial flanges which slide tightly into the outer tube. Other details will be clear from the illustrations.

In our experiments no bedding was provided, as any material used is liable to soak up urine. The absence of bedding makes it important to

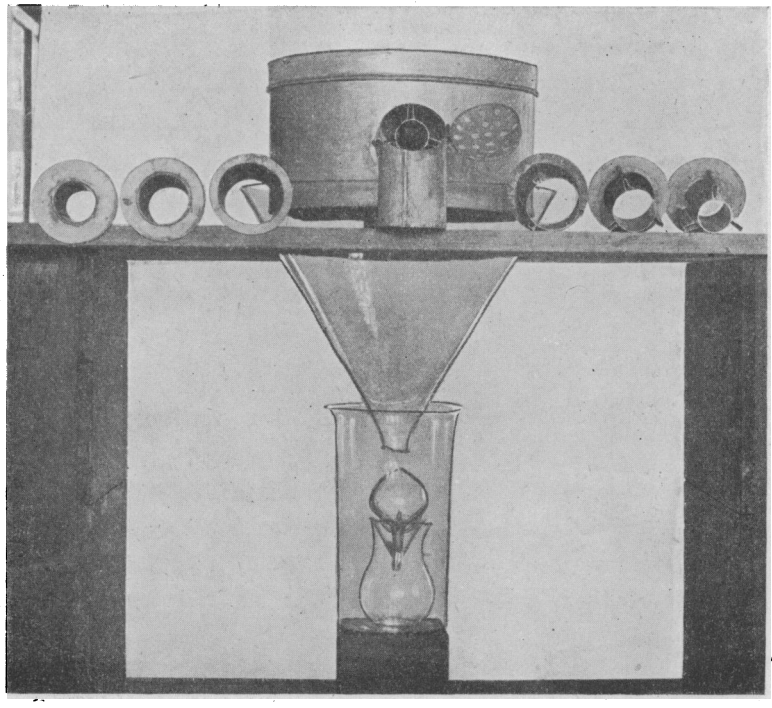


Fig. 2.

maintain the cages at a uniform temperature of about 65° F., and it is best that, as in our experiments, two animals should be fed in each cage. All our urine analyses represent the mean of the excretion of two rats.

For the collection of the urine we employed with slight modifications the apparatus first suggested by Paine and used by him at the Cancer Hospital, Fulham (Fig. 3). The metal cage stands within the upper part of a large glass funnel of somewhat larger diameter than its own. The short neck of the funnel reaches to within about $\frac{3}{4}$ inch of a glass globe.

This has a diameter of 2¼ inches and is provided with glass supports which allow it to stand in the neck of a small collecting vessel without touching the latter. An intermediary funnel as shown in the photographs may be used if necessary. The urine drops on to the globe without splashing and trickles over its surface into the collecting vessel whereas faeces and food particles are deflected and fall into an outer vessel. We have found it of advantage to give the glass globe a somewhat conical form as shown. The neck of the collecting vessel should have a diameter somewhat less than that of the globe.

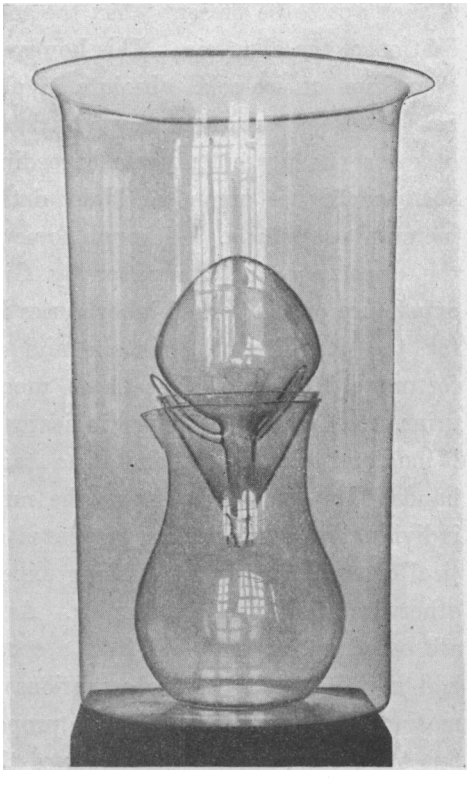


Fig. 3.

In each experiment the analyses were made upon the excretion of successive weeks. Shorter periods are unsatisfactory as their proper demarcation becomes impossible. The funnel in which the cage is placed was washed into the collecting vessel two or three times during the course of each day, small quantities of warm water being used. The urine and washings were transferred at frequent intervals to a stoppered bottle containing chloroform, the whole mixture being kept definitely acid throughout; acetic acid being added when necessary. During the week's collection the material was kept in an ice chest. At the close of the week it was made up to a standard volume for analysis.

For estimation of the allantoin Wiechowski's method was used. We first employed the method as originally described [1908] while in some of our later experiments the more recent modifications were used [Wiechowski, 1913]. In the case of rats' urine however we recommend the preliminary removal of ammonia by evaporation with magnesia and extraction with alcohol whatever modification of the process may be subsequently adopted.

As is well known the final stage of Wiechowski's process is reached either by determining the nitrogen in the mercury-allantoin precipitate, or by actually weighing the allantoin after its liberation from this compound. Wiechowski himself and especially Abderhalden and Einbeck look upon the latter procedure as the more trustworthy. This is not altogether in accord with our experience when working with diets containing free amino-acids. Sometimes a small amount of a syrupy non-nitrogenous substance is then apt to be present when the solutions are evaporated for the crystallisation of the allantoin. This however can be removed by the use of small quantities of ice-cold absolute alcohol. In several of our experiments both methods were used in every determination, and in the great majority of cases the agreement was exceedingly good. We finally came to rely with confidence upon Kjeldahl determinations of the nitrogen in the mercury precipitate. Although some of our experiments were made before the appearance of the paper by Givens [1914], in which he gives the experience of the Cornell laboratories bearing upon the conditions necessary for the stability of allantoin and other points of importance, we had fortunately observed just those precautions (in the preservation of the urine, etc.) which the paper indicates as necessary. Wiechowski's method is long and troublesome, and at no stage can it be entrusted to inexperienced hands. Its complexity limits the number of estimations possible for the individual worker and has prevented us from giving our results the statistical character for which feeding experiments upon small animals like rats otherwise afford the opportunity. As will be seen however our conclusions are based upon results got from a considerable number of individuals. We had hoped to carry out observations with the use of the method for estimating allantoin which has been proposed by Plimmer and Skelton [1914], but our leisure failed. The fault of that method lies in the fact that the allantoin nitrogen is determined by difference. Errors accumulate in estimating what is, after all, only a very small fraction of the total nitrogen. It is nevertheless very desirable that the method should receive full trial. In some experiments the total purine nitrogen of the urine was determined by the silver method of Camerer-Arnstein. Total nitrogen was estimated as usual by the Kjeldahl method.

The Diets Employed.

For experiments of this kind the standard control diet is prepared in the following manner (F. G. H.). Caseinogen, or better, a mixture of equal parts of caseinogen and lactalbumin is hydrolysed by boiling with 25 per cent. sulphuric acid for 20 to 30 hours. The sulphuric acid is quantitatively removed, and the fluid taken down to a syrup. At this stage a weighed quantity of potato starch, somewhat less than that meant to be present in the final diet is thoroughly incorporated with the syrup, and the resulting mixture spread out on a glass plate and allowed to dry in the air for two days. Tryptophane equal to 2 % of the protein originally taken for hydrolysis, and cystine equal to 0·5—1·0 %, are now added and thoroughly mixed with the whole. From this mixture, in which the nitrogen is determined, diets containing varying amounts of fat, additional starch, sugar, and mineral salts can be prepared with any desired nitrogenous content. Upon such diets well selected rats, if otherwise properly cared for, will show satisfactory, if not normal, growth, and will long remain in health. The effects of a tryptophane deficiency can be shown with great ease by refraining from adding that amino-acid to the hydrolysis mixture. Tryptophane certainly appears to be the only protein constituent of nutritive importance which is destroyed by acid hydrolysis, as the favourable results of its addition show. The addition of cystine is perhaps only of importance when the diet is given a low nitrogen content.

When in the present research the animals were to be fed in the absence of either arginine or histidine, or of both, the sulphuric acid of the original hydrolysis was reduced to about 1 %, and the solution of amino-acids well diluted (8 litres for 500 g. of original protein). It was kept at 40° and mixed with silver sulphate until sufficient was present to combine with all arginine and histidine (coloured precipitate on the addition of $Ba(OH)_2$). When working on a large scale this stage of Kossel and Kutscher's process occupies much time, but it is very essential that it should be properly carried out, and a full excess of silver added. The fluid was next saturated, while still at 40°, with powdered barium hydroxide. After cooling, the precipitate was filtered off at the pump and very thoroughly washed. From the bulky filtrate every trace of silver and barium was removed, and the whole evaporated to a syrup. In the preparation of the special diet this syrup was treated exactly as described above for the complete hydrolysis mixture. Tryptophane and crystine were added at the proper

stage, and if only one of the two diamino-acids was required to be absent a supply of the other was restored to the mixture. When the effect of restoring both to the food was under study the animals were not simply put upon a total hydrolysis mixture separately prepared, but arginine and histidine were restored to a mixture which had previously undergone treatment by the silver process, the results being thus more strictly comparable.

The silver method seems to remove both arginine and histidine with great completeness. If traces are left behind, and it is very difficult to disprove this possibility, the fact would not affect the bearing of our experiments, which were all comparative and controlled. The presence of traces in the diet of the experimental periods would tell against, rather than in favour of, our main conclusions.

The following example will illustrate the composition of the dietaries as fed:

Amino-acids from	200 g. of caseinogen
Potato Starch	250 ,,
Cane Sugar	125 ,,
Lard	45 ,,
Butter	45 ,,
[1]Ash from equal weights of Oats and Dog biscuit	21 ,,

This particular mixture contained 3·12 % of nitrogen; less than calculation from the original protein would indicate, but there is always some loss during the manipulation of the hydrolysis mixture. In some cases the diets have contained less nitrogen (down to 2 %) with proportionately more starch and sugar and fat. In each individual experiment the diet was identical throughout except for the qualitative variation in the amino-acid supply. Any change in purine metabolism was therefore independent of changes in the carbohydrates or fats.

The essential need of a vitamine supply comes to light with the greatest possible clearness when synthetic diets are prepared with amino-acids got by acid hydrolysis. Without a suitable addition there is no growth, and but short maintenance, when the assembly of amino-acids is complete. In our experiments this supply was given in the form of a protein-free alcoholic extract of fresh milk solids. Each rat received daily the equivalent

[1] There is no special reason for employing this particular mineral supply. It was originally used because oats and dog biscuits were the main food of stock rats in the laboratory. The ash has always proved satisfactory and large quantities were originally made so that its use has been continued.

of about 5 cc. of milk. This was true of every experiment described. To save printing we have not given the food consumption of our animals in the account of the experiments in the next section. We may state here that it was, throughout, sufficient to supply the energy not only for maintenance but for proper growth. This was true in the case of every experiment of which the protocol is given.

Experimental and Critical.

Effects upon Nutrition. Before giving the results of our allantoin estimations it will be convenient to deal separately with the effect upon body weight of the various dietetic deficiencies in connection with which we have studied the purine metabolism.

The pronounced effect upon nutrition produced by the withdrawal of tryptophane has, since the publications of Willcock and Hopkins, Osborne and Mendel, Abderhalden, Henriques and Hansen, and others, become well recognised. One of us (H.) has in various connections carried out a large number of experiments in which the nutritive condition of rats with and without tryptophane has been compared. Chart I summarises some of these results so far as the effect upon growth or body weight is concerned. The continuous lines show the effect of first withdrawing and of subsequently restoring tryptophane in the case of three separate rat pairs. The tryptophane was withheld on the 12th day (as shown in chart; the actual preliminary period was longer) of a diet containing a complete mixture of amino-acids. It was restored on the 35th day. The dotted lines show the average rate of growth upon the complete mixture (16 animals), and the average rate of loss in body weight when tryptophane is absent (8 animals). The average growth rate though satisfactory was not normal; but quite normal growth has been obtained in certain individuals when upon the complete mixture (hydrolysis products + tryptophane and a little cystine). On the other hand in a few cases not much more than maintenance without growth was attained [Asayama 1916] but this appears to have been due to deficiency in cystine as the result of prolonged hydrolysis of the protein, or to lack of adjustment in the vitamine supply. These cases were not included in the group yielding the average shown. After short hydrolysis of the protein (6 to 8 hours) maintenance, and some slight growth, has been observed in a few individuals, when no tryptophane was added to the hydrolysis products. Under these circumstances a certain amount of tryptophane escapes destruction and though certainly small it makes itself felt in nutrition. In all the

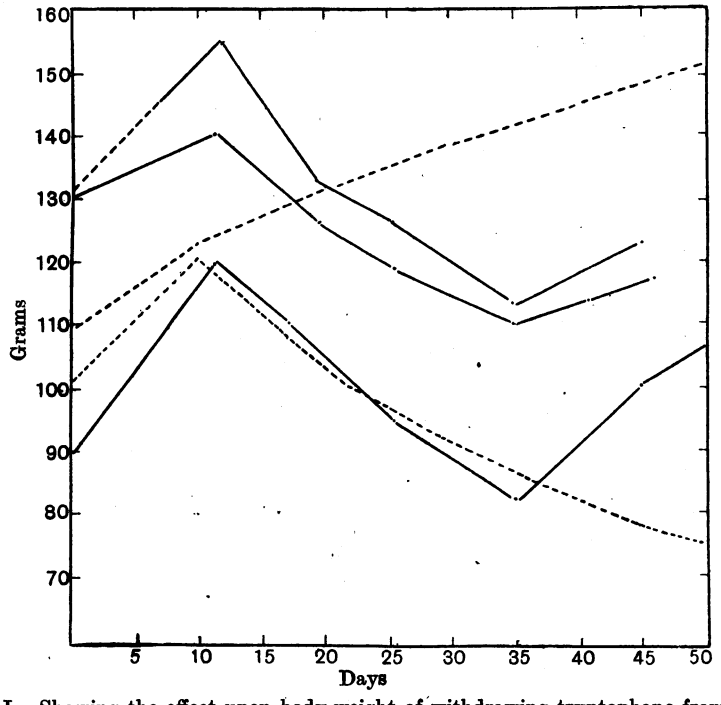


Chart I. Showing the effect upon body weight of withdrawing tryptophane from the diet. Continuous lines show mean weight of two rats in each case. Tryptophane removed on 12th day, restored on 35th day. The ascending dotted line shows average growth of 16 rats on complete amino-acid mixture. Lower dotted line average loss of 8 rats deprived of tryptophane.

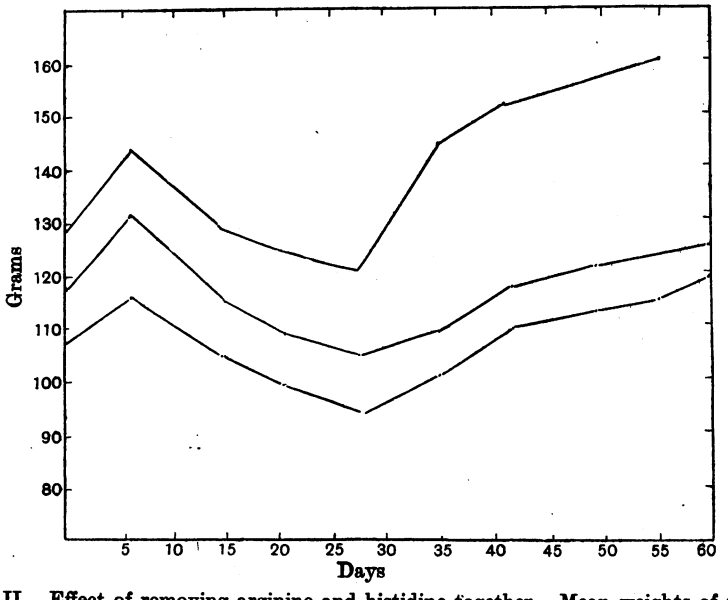


Chart II. Effect of removing arginine and histidine together. Mean weights of rat pairs. Diamino-acids removed on 7th day and restored on 28th day.

experiments yielding the results shown in Chart I the hydrolysis of the protein was continued for 24 hours or more.

When arginine and histidine are together removed from the products of protein hydrolysis the residual amino-acids are unable to support the animal. Chart II shows the effect upon body weight which the removal and restoration of these two diamino-acids respectively produce. When the removal has been as complete as possible the weight of animals taking the diet falls

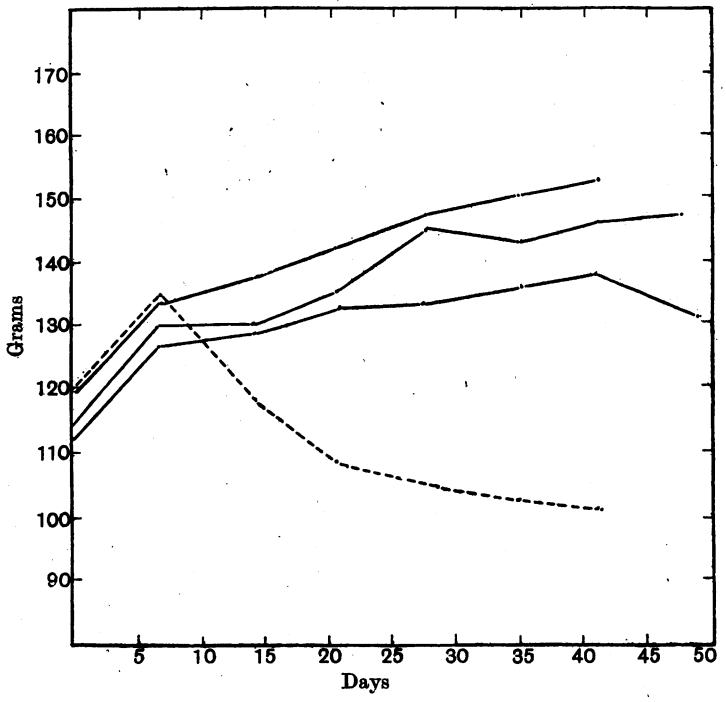


Chart III. Dotted line, body weight changes in the absence of both arginine and histidine. Lowest continuous line shows result when, of the two, only arginine was present. Two upper curves give weights when only histidine was present. Average weights of two rats in each case.

sharply. We have found however that this decline in weight becomes after a time less rapid than in the case of the tryptophane deficiency. There seems to be some adjustment to the lack of arginine and histidine. True, it must not be forgotten that, while prolonged hydrolysis destroys every trace of tryptophane, Kossel and Kutscher's method may leave behind minute amounts of the diamino-acids. The adjustment may be, not to the absence of arginine and histidine, but to the utilisation of a minute amount, sufficient for maintenance at a certain level, though not for growth. We believe

nevertheless that our preparations were free from these substances and that there are as a matter of fact different degrees of urgency in the need of an animal for a supply of this or that individual amino-acid in its food. On this assumption one of us has elsewhere briefly discussed the bearings of the present case [Hopkins, 1916].

The observations now to be mentioned illustrate a somewhat different aspect of the question as to how far the animal can dispense with individual protein units. They appear to us to yield an instance showing that the body can with some efficiency utilise one amino-acid vicariously for another.

The circumstance came to light when to the amino-acid mixture previously deprived of arginine and histidine, one, and only one of these two diamino-acids was restored.

The dotted line of Chart III shows once more the results when both are absent. It exhibits the average weights of two animals which were deprived of both substances for a somewhat longer period than in the earlier experiments (Chart II). In very marked contrast is the result of feeding when either one of the two is present in the diet. The two middle curves of Chart III show the behaviour of rat-pairs when receiving in two strictly comparable experiments either histidine without arginine (upper of these curves) or arginine without histidine (lower curve). There is no loss of weight such as follows when both are absent, but maintenance, and even slight growth. These results were obtained when the amount of arginine or histidine respectively amounted to only 3 % of the total amino-acid mixture. In another experiment (uppermost curve) histidine was added to the extent of rather under 5 % of the whole mixture and appreciable growth followed upon the diet, although arginine was absent. These results receive further attention in a later discussion.

Effects upon Purine Metabolism. We shall now give the protocols of experiments in which the allantoin excretion was determined week by week when arginine and histidine were first removed from, and afterwards restored to, the diet.

It will be seen from colums 4 and 7 of the protocol that in Experiment I the replacement of a bread and milk diet by one containing an amino-acid mixture deprived of the two diamino-acids, led, by the second week of the substitution, to a fall of over 40 % in the allantoin excreted. The excretion rose somewhat in the third week, but during the fourth it was only one half of the amount shown on the normal diet. When, in the sixth week of the experiment, arginine and histidine were restored to the amino-

acids of the diet the excretion of allantoin immediately rose, and after three weeks of feeding on the completed mixture it reached nearly 90 % of its original value.

In Experiment II, done under similar conditions, there was a fall of 47 % during the second week of deficiency feeding, and the results, in general, are very similar to those of Experiment I, save that there was a degree of

Arginine and Histidine absent from Food.

Experiment I.

Week 1914	Mean weight during week: Rat *A*	Rat *B*	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Purine N	Allantoin N	Allantoin N as percentage of total N.	Diet
1 (18. VI.–25. VI.)	124	132	0·458	0·147	0·00065	0·0116	7·8	Bread and Milk
2	116	126	0·296	0·095	0·00044	0·0080	8·4	Amino-acids without arginine or histidine
3	109	116	0·260	0·107	0·0003	0·0065	6·3	,, ,, ,,
4	105	109	0·319	0·116	0·0004	0·0080	6·7	,, ,, ,,
5	102	106	0·228	0·104	0·00023	0·0058	5·6	,, ,, ,,
6	113	114	0·347	0·134	0·00036	0·0090	6·7	Amino-acid + A. and H.
7	121	124	0·347	0·133	0·00034	0·0090	6·7	,, ,, ,,
8	131	131	0·400	0·157	0·00049	0·1010	7·0	,, ,, ,,
9	137	141	0·454	0·199	0·00069	0·1150	5·8	Bread and milk

Experiment II.

	Rat *C*	Rat *D*						
1	146	125	0·575	0·182	0·00063	0·0146	8·0	Bread and milk
2	139	119	0·406	0·105	0·00044	0·0103	9·8	Amino-acids – A. and H.
3	125	112	0·306	0·111	0·00046	0·0077	7·0	,, ,, ,,
4	119	106	0·390	0·142	0·00037	0·0099	7·0	,, ,, ,,
5	120	106	0·310	0·130	0·00039	0·0079	6·1	Amino-acids + A. and H.
6	125	115	0·413	0·119	0·00046	0·0105	8·8	,, ,, ,,
7	131	120	0·400	0·135	0·00053	0·0099	7·3	,, ,, ,,
8	133	127	0·486	0·136	0·00050	0·0123	9·0	,, ,, ,,
9	142	137	0·501	0·167	0·00051	0·0136	8·1	Bread and milk

lag in the effects of restoration. In the first week of giving the completed amino-acid mixture the excretion of allantoin remained nearly at its lowest, but it rose rapidly in the two succeeding weeks.

In the above experiments the food of the first control period was a purine-free, but otherwise normal, dietary (bread and milk). We have found that usually, though not always, a fall from 10 to 15 % in the allantoin seems to follow when the food is changed from bread and milk to a dietary containing even a complete amino-acid mixture. This result does not depend

upon any general failure of nutrition, as it may occur when the weight is going up and the total consumption of nitrogen greater than during the bread and milk period. It is not easy to explain. The bread and milk consumed by two rats during a week would—to judge from determinations made by one of us [Ackroyd 1911]—contain enough allantoin to add 10 to 12 mgs. to the week's excretion. It might possibly add more, but not enough to account for the result mentioned. If such views as those of Marěs [1888] are accepted it is possible that because liberated amino-acids instead of intact protein enter the alimentary canal there is less activity of the digestive glands and therefore some lessening of purine metabolism. Possibly the fact that the free acids are absorbed rapidly, rather than gradually as during normal digestion, may affect the efficiency of synthesis in the body. Growth upon the complete amino-acid mixture though satisfactory is seldom of normal velocity.

In any case the circumstance in no way affects the significance of the experimental results. Experiments I and II should be compared with Experiments VI and VII which were done under precisely similar conditions.

In the two experiments which follow a complete amino-acid mixture formed the nitrogen supply of the preliminary, as well as of the final, control period.

It may be further noted that in the first two experiments when arginine and histidine were restored to the diet they were not isolated but were added as recovered together from the silver-barium precipitate of the Kossel and Kutscher process. In the later cases they were added pure, in the form respectively of the nitrate and hydrochloride. Sodium carbonate was simultaneously added in quantity sufficient to correspond with the acid radical of the salt.

In Experiment III in which during the preliminary control period the complete amino-mixture and not bread and milk was given, the urine during the first week of feeding without arginine and histidine was unfortunately lost. That of the second week of the deficiency still contained nearly 85 % of the original output of allantoin. There was apparently therefore a delay in the effect of the deficiency not seen in the first experiments. During the third and fourth weeks however the allantoin fell to 60 % of its original value, and then rose immediately when the arginine and histidine were restored.

In Experiment IV the allantoin was sharply reduced when arginine and histidine were removed from the complete mixture of amino-acids first

given. In the second week of the deficiency it had fallen to one half. It rose somewhat in the two succeeding weeks, but in the fourth week it was only 64 % of its original value. On restoration of the missing amino-acids it quickly rose to the original amount.

Although the time incidence of the minimum varied in the above experiments the removal of arginine and histidine from the food never failed to produce a noteworthy decrease in the allantoin excreted.

Arginine and Histidine absent.

Experiment III.

Week 1915	Mean weight during week		Allantoin from two rats per week	Nitrogen per rat per day				Diet
	Rat *E*	Rat *F*		Total N	Purine N	Allantoin N	Allantoin N as percentage of total N	
1 (11. v.–18. v.)	136	125	0·366	0·121	—	0·0092	7·6	Complete amino-acid mixture
2	134	123	—	—	—	—	—	Arginine and histidine absent
3	127	115	0·312	0·120	—	0·0079	6·6	,, ,, ,,
4	126	114	0·237	0·110	—	0·0060	5·5	,, ,, ,,
5	123	110	0·221	0·112	—	0·0058	5·2	,, ,, ,,
6	137	113	0·356	0·150	—	0·0090	6·0	Arginine and histidine restored
7	149	121	0·356	0·147	—	0·0090	6·1	,, ,, ,,
8	155	128	0·343	0·130	—	0·0087	6·7	,, ,, ,,

Experiment IV.

	Rat *G*	Rat *H*						
1	124	115	0·400	0·118	—	0·0101	8·5	Complete amino-acid mixture
2	129	123	0·406	0·115	0·00051	0·0103	9·0	,, ,, ,,
3	120	118	0·301	0·107	0·00039	0·0076	7·1	Arginine and histidine absent
4	113	111	0·210	0·109	—	0·0053	4·8	,, ,, ,,
5	110	106	0·290	0·101	0·00029	0·0073	7·2	,, ,, ,,
6	106	102	0·256	0·098	0·00023	0·0065	6·6	,, ,, ,,
7	112	109	0·374	0·110	—	0·0095	8·6	Arginine and histidine restored
8	119	115	—	0·114	—	—	—	,, ,, ,,
9	126	119	0·405	0·121	0·00057	0·0102	8·4	,, ,, ,,

Experiments V and V*a* show the effects produced upon the allantoin when of the two diamino-acids only one is absent from the food. In the first arginine was present and histidine absent; in the second arginine was alone wanting. In the control periods the animals received the complete amino-acid mixture. In neither case was the fall at any period greater than 17 %. This is in marked contrast with the 40 to 50 % decrease induced by the absence of both substances. We hold that this interesting result supports the evidence from the body weight curves in suggesting that arginine and histidine are essentially equivalent in metabolism.

To control the experiments already described and to decide so far as possible whether the reduction of allantoin represents the failure of a specific synthesis in the body, and is not an indirect phenomenon depending upon the general failure in nutrition, we have studied the effect of other deficiencies.

The effect of removing tryptophane was first investigated. In Experiments VI and VII the diet of the first control period was bread and milk. This was immediately followed by food containing an amino-acid mixture

Histidine or Arginine removed.

Experiment V.

Week	Mean weight during week JA	Mean weight during week KA	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Nitrogen per rat per day: Allantoin N	Nitrogen per rat per day: Allantoin N as percentage of total N	Diet
1	120	122	0·410	0·170	0·0104	6·1	Total amino-acids
2	120	125	0·390	0·171	0·0099	5·8	Arginine present, histidine absent
3	118	127	0·351	0·152	0·0089	5·8	,, ,, ,,
4	115	127	0·337	0·140	0·0086	6·1	,, ,, ,,
5	114	128	0·343	0·134	0·0087	6·5	,, ,, ,,
6	114	125	0·343	0·130	0·0087	6·7	,, ,, ,,

Experiment V*a*.

Week	JH	KH					
1	135	131	0·448	0·189	0·0106	5·6	Total amino-acids
2	134	130	0·441	0·180	0·0105	5·8	Histidine present, arginine absent
3	137	131	0·400	0·144	0·0099	6·9	,, ,, ,,
4	138	139	0·374	0·137	0·0095	6·9	,, ,, ,,
5	137	138	0·374	0·142	0·0095	6·7	,, ,, ,,
6	134	131	0·356	0·140	0·0090	6·4	,, ,, ,,

deprived of tryptophane. These two experiments are therefore to be compared with Experiments I and II. In neither do we find the effect upon allantoin produced by the absence of arginine and histidine. In Experiment VI it is true a disturbance in the purine metabolism is shown which it is not easy to explain. After two weeks of the deficiency, showing no appreciable effect upon the allantoin, there was in the third week a sudden rise, and, in the following week what seems to be a compensating fall. The average of the two weeks represents an excretion which was nearly normal.

In Experiment VII there was no irregularity of this sort. The replacement of bread and milk by an amino-acid diet without tryptophane was associated with a maximal fall —in the second week—of 19 %. In the third week the excretion was only 9 % below that of the bread and milk period. Restoration of tryptophane to the diet produced no rise.

In Experiments VIII and IX the diet of the preliminary control period was one containing the complete amino-acid mixture. They are therefore comparable with Experiments III and IV. The removal of tryptophane produced no effect upon allantoin at all comparable with that seen when arginine and histidine are absent. In certain weeks when upon the deficiency diet the excretion of the animals was above that of the control period.

Tryptophane absent from food.

Experiment VI.

Week 1914	Mean weight during week		Allantoin from two rats per week	Nitrogen per rat per day			Diet
	Rat *M*	Rat *N*		Total N	Allantoin N	Allantoin N as percentage of total N	
1 (4. XI.–13. XI.)	106	98	0·410	0·170	0·0104	6·1	Bread and milk
2	118	105	—	—	—	—	,,
3	118	107	0·392	0·198	0·0098	4·9	Amino-acids, tryptophane absent
4	111	100	0·410	0·171	0·0104	6·1	,, ,, ,,
5	103	94	0·536	0·150	0·0136	9·0	,, ,, ,,
6	98	90	0·245	0·126	0·0062	4·9	,, ,, ,,
7	109	100	0·326	0·136	0·0083	6·1	Tryptophane restored
8	129	117	0·380	0·154	0·0096	6·2	,, ,,
9	135	122	0·406	0·167	0·0103	6·2	Bread and milk

Experiment VII.

	Rat *O*	Rat *P*					
1	107	107	0·393	0·141	0·0101	7·2	Bread and milk
2	107	107	0·351	0·157	0·0089	5·7	Amino-acids without tryptophane
3	102	102	0·335	0·160	0·0085	5·3	,, ,, ,,
4	91	92	0·357	0·153	0·0095	6·2	,, ,, ,,
5	92	95	—	0·130	—	—	Total amino-acids
6	104	98	0·319	0·123	0·0081	6·5	,, ,,

In Experiment X the periods observed were short. It is of interest however because in it the same rats were fed first without arginine and histidine and then, after an interval with normal feeding, without tryptophane. A marked fall in the allantoin (40 %) occurred under the former conditions, and a much smaller one (10 %) in the latter. In each case the food of the preliminary period was bread and milk.

The difference between the effect of removing tryptophane and that of removing arginine and histidine is throughout the experiments quite unmistakable.

Tryptophane absent.

Experiment VIII.

Week 1915	Mean weight during week: Rat *S*	Mean weight during week: Rat *T*	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Nitrogen per rat per day: Allantoin N	Nitrogen per rat per day: Allantoin N as percentage of total N	Diet
1 (10. II.–15. II.)	141	134	—	—	—	—	Total amino-acids
2	152	141	0·319	0·104	0·0081	7·8	,, ,,
3	146	135	0·319	0·179	0·0081	4·5	Tryptophane absent
4	130	122	0·347	0·160	0·0088	5·5	,, ,,
5	118	114	0·300	0·151	0·0076	5·1	,, ,,
6	109	108	0·304	0·130	0·0077	5·9	,, ,,
7	110	108	0·308	0·102	0·0078	7·6	Tryptophane restored
8	118	113	0·276	0·114	0·0070	6·1	,, ,,

Experiment IX.

	Rat *SS*	Rat *TT*					
1	220	225	—	—	—	—	Total amino-acids
2	215	222	0·347	0·276	0·0088	3·2	,, ,,
3	202	207	0·400	0·277	0·0099	3·6	Tryptophane absent
4	189	193	0·343	0·286	0·0087	3·1	,, ,,
5	176	178	0·300	0·196	0·0075	3·8	,, ,,
6	171	172	0·400	0·266	0·0099	3·7	,, ,,
7	181	179	0·356	0·202	0·0090	4·4	Tryptophane restored
8	199	197	0·420	0·322	0·0107	3·3	,, ,,

Experiment X.

Week 1914	Mean weight during week or period: Rat *AA*	Mean weight during week or period: Rat *BB*	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Nitrogen per rat per day: Purine N	Nitrogen per rat per day: Allantoin N	Diet, etc.
1 (6. III.–13. III.)	210	201	0·400	0·200	0·0010	0·0101	Bread and milk Three days on amino-acids minus arg. and hist.
2 (16. III.–23. III.)	182	188	0·239	0·1910	0·0006	0·0060	ib. (week)
3 (30. III.–5. IV.)	187	189	0·325	0·220	0·0008	0·0082	Total amino-acids Rats for three weeks on bread and milk
Ten day periods							
1	242	262	0·512	0·259	0·0010	0·0130	Bread and milk
2	228	251	0·458	0·229	0·0008	0·0116	Amino-acids minus tryptophane
3	213	242	0·458	0·2300	0·0008	0·0116	,, ,, ,,
4	215	244	0·441	0·1700	0·0005	0·0112	Tryptophane restored
5	259	278	0·473	0·225	0·0012	0·0120	Bread and milk

We have in one experiment studied the effects of quite another type of dietetic deficiency upon the allantoin excretion—the lack, namely, of exogenous growth hormones, or vitamines. As this involves nutritive failure of a marked kind the results offer further control of the arginine and histidine results.

Two rats were fed for eight weeks upon a synthetic diet containing purified caseinogen. During the first four weeks each animal received 2 cc. of fresh milk a day [Hopkins 1912]. In the last four weeks this was withheld, and, as the result of the absence of vitamines, the weight of the animals, which had risen during the first period, then fell rapidly, especially in the last two weeks. We give the results of the experiment in brief form.

	With Vitamine supply				Without Vitamine supply			
Week	1	2	3	4	5	6	7	8
Allantoin from two rats per week	0·263	0·319	0·265	0·285	0·294	0·350	0·340	0·296
Total N from two rats per week	2·47	2·83	2·91	—	3·25	3·38	4·08	3·50

The increase in food consumption after the withdrawal of the small dose of milk has been observed before. It was somewhat disconcerting in the present experiment. Nevertheless the first period was one of growth, and the second one of declining weight. We have further proof therefore that the decrease in allantoin due to the withdrawal of arginine and histidine is not due to nutritive failure alone, for in this experiment there was no fall.

It may be asked here how far the excretion of allantoin in the rat changes with increase or decrease of the total nitrogenous metabolism. All that we know concerning purine metabolism in other animals when the diet is purine-free would lead us to expect that there would be no direct proportionality.

In the protocols of our main experiments will be found a column giving the allantoin-nitrogen as a percentage of the total nitrogen. It will be seen that in general the relation is irregular because of disproportionate and independent variations in the two quantities. It may be noted however that the ratio of allantoin-N to total-N always tended to fall markedly as the result of arginine and histidine deficiency.

We carried out one brief experiment intended to bear specially on the relation of allantoin to total nitrogen. A pair of rats was for one week fed upon a diet of bread and water. Then for one week a large proportion of

caseinogen was supplied, while in a third week the animals returned to bread and water. The results were as follows:

	1st week	2nd week	3rd week
Allantoin	0·408	0·404	0·465
Total Nitrogen ...	2·26	4·27	2·35
Ratio of A.-N. to T.-N.	1 : 16	1 : 30	1 : 14

In this particular experiment the moderate rise in allantoin which occurred was not seen until the animals were again upon the diet containing the lower proportion of protein. Our main experiments were clearly not of a kind to afford evidence upon the point. The distinction between endogenous and exogenous purine excretion is, however, known to be vaguer than was once thought. A large increase of protein in the food has been shown to increase the output of purines, at least in some animals.

The behaviour of the tissues complicates the phenomena. We may perhaps picture the relations in the following way. Actual starvation increases the excretion of endogenous purines because of tissue breakdown. Once equilibrium is re-established on a proper food supply, the normal activities of the tissue determine and limit the amount of purine production through a somewhat wide range of protein intake; until ultimately, when the intake is large enough, the chemical reactions involved are inevitably accelerated by the continued high concentration of new material in the cell. It is clear that if a particular constituent of the protein molecule rather than any other is concerned in purine synthesis, experiments in which the tissues were at first wholly deprived of that constituent are those most likely to demonstrate the fact.

Discussion of Results.

Our observations show that the simultaneous removal of arginine and histidine from its food produces a marked decrease in the excretion of allantoin by the rat. The excretion observed when a normal purine-free diet was given, or that upon a diet containing a full assembly of amino-acids, was reduced by some 40 to 50 % during the period (1 to 4 weeks) when the animals were deprived of these two diamino-acids. The actual minimum of allantoin occurred sometimes early and sometimes later, but throughout the period of the deficiency it was always notably below the normal. On restoration of the missing diamino-acids it returned to or approached its original level. These effects seem to be definite and constant.

Since however deprivation of the two diamino-acids produces at the same time a marked effect upon the general nutrition of the animal, involving at first a rapid loss of weight, it is clear that the relation thus brought to light cannot be at once accepted as a proof that arginine and histidine are direct precursors of the purines of the body. The effect of their removal upon the allantoin excretion might be indirect, and a partial expression of the general nutritional failure. We know of no information in the present literature of the subject which could assist in measuring this possibility.

In actual starvation, to judge from Cathcart's observations upon a fasting man [Cathcart 1907], there is first a brief fall and, later, a rise in the purine excretion. The fall in the case studied by Cathcart lasted for three days only. A period of three or four days in the case of a man is out of all proportion small when compared with one of three or four weeks in the case of a rat. The brief fall at the beginning of starvation depends certainly upon factors different from those responsible for our results.

Moreover a qualitative deficiency in the amino-acid supply cannot be compared with actual starvation. The animals in our experiments were receiving an abundant energy supply throughout the periods observed, in spite of the failure in nutrition, and their nitrogenous metabolism was sometimes above the normal. There was no experimental information which could enable us to judge as to what might be the indirect effect if any of a general nutritive condition of this sort upon the special phenomena of purine metabolism.

By far the best method of controlling our results was—it seemed to us—to compare the effects of the deficiency in arginine and histidine with those of another deficiency in the diet, and, especially, of another amino-acid deficiency. We therefore fed rats upon a diet of which the nitrogenous supply was contained in an amino-acid mixture deprived of tryptophane. In the absence of this constituent the disturbance in the nutritional balance and the loss of weight are even more marked than when arginine and histidine are absent. In these control experiments all other factors in the diet, and all the other experimental conditions were made identical with those of the main experiments. The relative proportions of carbohydrate and fat, for instance, were the same. The only variation was in the nature of the amino-acid or acids removed from the food. Such a comparison should eliminate the influence of many unknown or uncertain factors.

Examination of the protocols given in the last section will show that the removal of tryptophane produced no fall in the excretion of allantoin

at all comparable with that seen when arginine and histidine are absent. The excretion was somewhat irregular; at times there was a small fall during part of the deficiency period, at other times there was some increase; on one occasion there was a considerable rise during the third week followed by a compensatory fall in the fourth. Taking the results as a whole however the difference between the effects of removing tryptophane and those which follow the removal of arginine and histidine is sharp and unmistakable. In the latter case the decrease in allantoin is constant and of marked degree.

It happens that in the case of the tryptophane deficiency the mean weekly excretion of the animals (17 weekly estimations) was identical with that of animals upon the complete amino-acid mixture (25 estimations) namely 0·135 g. allantoin per rat per week. This may be a coincidence but it is good evidence that there was no decrease of any importance.

In no case, it should be noticed, did we extend our observations concerning the allantoin excretion beyond the fourth week of feeding upon the deficient amino-acid mixture. At this stage and sometimes sooner we restored the missing constituent to the diet in order to control the results. We do not know what may happen to the allantoin at later periods. For the specific purpose of the research it seemed better to compare the effect of the different diets upon purine metabolism when the nutritional failure was as yet not too marked. Premortal changes might introduce complications and obscure the result.

A dietetic deficiency of quite another kind, the absence namely of growth hormones or vitamines, produced, in the one experiment carried out to test the matter, no fall in the excretion of allantoin, although the animals were losing weight as fast as those fed without the diamino-acids.

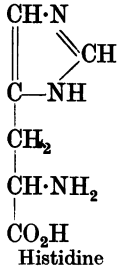
All our results therefore point to the probability that there is a specific metabolic relation between arginine and histidine and the purines of the body.

It was explained in the introductory paragraphs of this paper that we began our experiments in the belief that the molecules of arginine and histidine might prove to be used conjointly in metabolism as precursors of the purine ring. Our observations upon body weight, and, no less, others upon allantoin excretion seem to show clearly however that either one of these two diamino-acids can with a considerable degree of efficiency subserve the functions of the other. When both are removed from the diet the nutritive failure and the fall in allantoin excretion are unmistakable. When

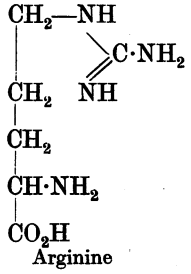
either alone is restored in sufficient amount there is maintenance and even growth, while no marked fall in allantoin is observed.

On our own view this is because each of these protein derivatives is, in metabolism, capable of being converted into the other, and is so converted when the supply of both is not harmoniously adjusted for the needs of the animal.

The chemical relations which justify this view are familiar, and become clear when the structural formulae are properly compared.



Histidine



Arginine

If arginine and histidine are, in the sense just discussed, to a large extent, equivalent in metabolism our original view that the molecules of both, rather than the molecule of histidine alone, are concerned in the synthesis of the purine ring loses some of its point. That the presence of both in the food yields the optimum conditions for purine synthesis seems nevertheless to be probable.

Summary.

(1) When arginine and histidine are together removed from the diet of rats which have been previously growing on a complete amino-acid mixture there is a rapid loss of body weight. This is promptly succeeded by renewed growth when the missing diamino-acids are restored to the diet.

(2) When arginine alone, or histidine alone, is restored to the food there is no loss of weight and there may even be growth. Nutritional equilibrium is possible in the absence of one of these related protein constituents though not in the absence of both. It is suggested that this is because each one of them can, in metabolism, be converted into the other.

(3) When arginine and histidine are both removed from the food the amount of allantoin in the urine is much decreased. When they are replaced the excretion returns to the normal. The decrease is very much less when either one of these diamino-acids is present alone.

(4) No decrease of allantoin excretion occurs when tryptophane is removed from the food, though nutritional failure is even greater than when arginine and histidine are withheld. No decrease was observed when animals were losing weight as the result of the absence of a vitamine supply.

(5) It is suggested therefore that arginine and histidine play a special part in purine metabolism, probably constituting the raw material (or the most readily available raw material) for the synthesis of the purine ring in the animal body.

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