LV. THE RELATION BETWEEN THE METABOLISM AND THE SPECIFIC DYNAMIC ACTION OF AMINO-ACIDS.

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IN a series of papers on "Animal Calorimetry," by Lusk and his collaborators [1912-1924], the view has been put forward that the specific dynamic action of proteins is due to a chemical stimulation of the body cells to a higher level of metabolism by some intermediary metabolites of the amino-acids other than the immediate products of their deamination. Accordingly one would expect, *a priori*, the amino-acids glycine and alanine—which exert a profound specific dynamic action—to be metabolised in the organism more rapidly and more completely than leucine, aspartic acid, etc., which are known to exert no appreciable specific dynamic action [Lusk, 1915; Atkinson and Lusk, 1918]. In other words, one would expect a smaller increase in the amino-N content of blood and a quicker return of the same to the normal level, following the ingestion of glycine or alanine, than after the ingestion of an equivalent amount of leucine or aspartic acid.

However, contrary to expectations based on Lusk's theory, the observations of Bang [1916, 2] show that whereas the amino-acid content of rabbit's blood increases very rapidly after an oral administration of glycine or alanine, and remains at a high level for about 6 hours, the ingestion of leucine causes no significant increase at all in the amino-acid content of blood.

In view of the importance and interest of the problem and the apparent inconsistencies existing between Lusk's theory and Bang's observations, it was decided to repeat Bang's experiments on a more extended scale. The results of this part of the investigation are described in Part I.

Further, having recently been impressed by the undesirability of applying facts based on observations on one species of animals to other species, we extended Bang's experiments to dogs in order to make sure that the vastly different points of view arising from the work of Lusk and Bang were not due to some intrinsic metabolic differences in the species of animals used by them. The modified procedure adopted in these experiments was designed firstly to eliminate any effect due to variable times of passage of the ingested material

through the pylorus, and secondly to enable us to follow the course of intestinal absorption more closely by analysing blood samples from the portal vein. This part of the investigation is dealt with in Part II.

A third series of experiments in which the amino-acids were administered to dogs by intravenous injection is described in Part III. These experiments were designed for the purpose of eliminating any effect due to variable rates of absorption from the intestine.

EXPERIMENTAL.

PART I

Rabbits of approximately uniform size were employed, each animal being used not more frequently than once a fortnight, during which interval it was kept on the ordinary routine diet. For 48 hours before the experiment the rabbit was kept in a clean metabolism cage and was given only water. On the experimental day, blood samples of about 1.5 cc. each were obtained from the ear at suitable intervals, and received into 0.15 cc. ammonia-free 2 % potassium oxalate solution. After drawing the first sample of blood a neutralised solution, at 38°, of the substance under investigation was administered by stomach tube. The total volume of the fluid ingested was 100 cc., and contained 560 mg. of amino-N (equivalent to 3 g. glycine).

Immediately after drawing each sample, the blood proteins were precipitated by the method of Folin and Wu [1919]. For the estimation of urea, 0.3 cc. molar phosphate solution of p_H 7.45, and 1 cc. of 0.5 % urease [Van Slyke and Cullen, 1914] filtered through glass-wool, were added to 4 cc. of the protein-free blood filtrate, and, after standing for 45 minutes at 40°, the ammonia was distilled (using 2 cc. saturated borax solution) into standard acid, and estimated by nesslerisation.

The amino-N was estimated in the blood filtrate by Folin's colorimetric method [Folin, 1923] which yielded very consistent and satisfactory results.

The water and other reagents used were ammonia-free, and all apparatus was washed with such water.

The amino-acids investigated were glycine, alanine, leucine, aspartic acid, glutamic acid, cystine, histidine and tryptophan, of which only the first three were used by Bang [1916, 2].

Results.

Table I shows the changes in the urea content of rabbit's blood during a period of about 7 hours following the ingestion of the various amino-acids, and Fig 1¹ represents the corresponding changes in the amino-acid content of the blood, under the same conditions.

¹ In the case of tryptophan the amino-N content of blood was reckoned as the difference between the total-non-protein N and urea-N, and the figures thus obtained being rather high, only two-thirds of their actual values were plotted in Fig. 1 for the sake of a clearer comparison.

Table I. *The Urea-values in this Table are expressed in mg. urea-N per 100 g. blood.*

Substance under investigation	Period after ingestion of amino-acid							Difference between initial and maximum urea values	Remarks
	0 min.	20-25 mins.	45-50 mins.	75-90 mins.	2½-3 hrs.	4-4½ hrs.	6-6½ hrs.		
Glutamic acid (6.0 g.)	9.6	10.3	10.9	12.6	14.7	14.8	18.6	9.0	
Glycine (3.0 g.)	18.0	—	19.6	17.6	19.1	22.2	26.7	8.7	
Aspartic acid (5.3 g.)	13.6	13.3	15.8	17.2	19.9	20.1	21.7	8.1	
<i>l</i> -Alanine (Kahlbaum) (3.6 g.)	15.8	—	—	18.8	23.4	23.4	16.4	7.6	
Cystine (4.8 g.)	12.4	12.4	—	17.9	18.7	14.0	15.1	6.3	Administered as the sodium salt
Tryptophan (4.5 g.)	14.3	16.1	16.6	18.3	20.0	19.4	18.2	5.7	Animal had acute diarrhoea about 1 hour after ingestion of tryptophan
<i>l</i> -Leucine (Kahlbaum) (5.2 g.)	14.5	14.4	15.7	18.3	17.5	17.5	18.8	4.3	Administered as the sodium salt
Histidine hydrochloride (8.3 g.)	21.6	22.9	22.3	22.0	19.3	21.2	21.7	1.3	
Histidine hydrochloride (repeated) (8.3 g.)	18.9	20.1	20.0	20.1	18.9	18.4	19.0	1.2	
Sodium chloride (1.2 g.)	10.7	8.5	8.8	9.6	10.1	—	6.1	-0.6	Control experiment

Fig. 1. Amino-N content of rabbit's blood.

These results (Table I) indicate that whereas there is no marked difference in the extent of urea formation in the case of glycine, alanine, aspartic acid and glutamic acid, there is a progressive decrease in the case of cystine, tryptophan, leucine and histidine, the last one producing practically no increase in the blood urea during the entire experimental period of 6 hours. On the other hand, the amino-acid content of blood (Fig. 1) after the ingestion of glycine, alanine or histidine not only attains a very high maximum, but persists at a high level all through the experimental period; whereas the amino-acid content of blood in the case of the other amino-acids examined, without ever rising very high, comes down to the normal or subnormal level within 3-6 hours, in confirmation of Bang's observations [1916, 2]. The significance of these results is discussed elsewhere.

PART II

Having in the main confirmed Bang's observations on rabbits regarding the amino-acid content of blood after the ingestion of glycine, alanine and leucine, and having further extended the investigation to other amino-acids, the experiments were repeated on dogs, in the following way:

Dogs weighing about 10 kg. each were used, and they were given water only for 48 hours before use to ensure freedom of the small intestine from food. The animals were anaesthetised with ether¹. A tracheal cannula was inserted. The femoral artery on one side and the femoral vein on the other were exposed. The abdomen was then opened and the splenic vein was tied off close to the portal vein. Ligatures were then applied to the small intestine, one just below the duodenum and the other above the caecum. The femoral artery was then ligatured distal to the profunda artery. Blood samples were taken by syringe at suitable intervals from the femoral artery and vein and from the portal vein, the latter being punctured about 2-3 inches from the porta hepatis. After drawing the first set of blood samples, the substance under investigation in a neutralised solution² at 38°, was injected into the small intestine by means of a hypodermic needle and pipette. The abdomen was promptly closed with artery clips. The total volume of the fluid injected was 100 cc. The animal was kept warm throughout the experiment.

The collection of blood samples (about 1.5 cc. each), the removal of blood proteins and the estimation of urea and amino-N in the blood filtrates, were done exactly as described before (p. 367).

The amino-acids investigated were glycine, alanine, leucine, aspartic acid, glutamic acid and cystine, the amounts used being the same as in the experiments on rabbits (see Table I).

¹ Ether anaesthesia has, according to Okada and Hayashi [1922] no significant effect on the amino-acid content of dog's blood, during the experimental period of about 2 hours.

² Cystine was administered as the sodium salt, whilst the leucine solution was neutralised just before injection.

Fig. 2. Amino-N content of dog's arterial blood.

Fig. 3. Amino-N content of dog's portal blood.

Results.

(i) In confirmation of the observations made on rabbits (see Part I), Figs. 2 and 3 show that the amino-acid content of blood after the ingestion of glycine or alanine not only attains a very high maximum but persists at a high level all through the experimental period, whereas in the case of the other amino-acids studied, the amino-N content remains at a comparatively low level throughout. The possible significance of this observation is discussed later.

(ii) The extent of urea formation in the case of glycine and alanine is higher than in the other cases (Fig. 4).

Fig. 4. Urea content of dog's arterial blood.

(iii) A comparison of Figs. 2 and 3 shows that at any one time the amino-acid value of portal blood is higher than the corresponding arterial value. This presumably results from the dilution of the portal blood with systemic blood. In the early stages of the experiment, the amino-N content of femoral-venous-blood¹ is also lower than that of the corresponding arterial sample. Later

¹ The figures for amino-N of femoral-venous blood were obtained in the case of glycine, alanine and cystine only, but they are not included in Fig. 2, in order to avoid unnecessary complication of the curves.

on, the two values tend to be almost identical. These observations, supported by the results of our injection experiments (Part III), point to a rapid absorption of the amino-acids from blood by the tissues. These conclusions are in agreement with similar findings of Folin and Denis [1912, 1] and of Van Slyke and Meyer [1912].

PART III

Dogs weighing about 10 kg. each were used. After being given only water for 48 hours, the animals were anaesthetised as described in Part II. The femoral artery on one side and the femoral vein on the other were exposed. The bladder was emptied by pressure. The femoral artery was then ligatured distal to the profunda artery. After drawing the first arterial blood sample by syringe, as described in Part II, the amino-acid under investigation, dissolved in 0.85 % NaCl solution, neutralised to litmus and warmed to 38°, was injected into the femoral vein as rapidly as possible (usually in less than a minute). Subsequent arterial samples were drawn at suitable intervals and analysed for amino-N and urea, as described in Part II. The total volume of the fluid injected was 25 cc., and it contained 186.6 mg. of amino-N. The amino-acids investigated were glycine, alanine, and aspartic acid.

At the end of the experimental period, the bladder was emptied and washed by catheterisation, the urine being subsequently analysed for amino-N.

Table II. 1.0 g. glycine injected intravenously.

Intervals before or after injection	mg. N per 100 cc. blood	
	Amino-N	Urea-N
Before injection	5.9	7.7
2 mins. after injection	10.0	7.9
8 " "	8.4	7.8
17 " "	6.7	8.7
28 " "	6.5	8.2
38 " "	6.3	8.7
57 " "	5.8	7.6
69 " "	5.8	7.7
94 " "	5.9	8.3
122 " "	5.4	8.5

Essentially similar results were obtained with alanine and aspartic acid. These results (Table II) show that:

(i) Immediately after injection, the amino-acid content of the blood falls very rapidly, reaching almost the normal level within 15 minutes.

(ii) During the experimental period of 2 hours, there is no significant increase in the blood urea.

No measurable amount of amino-N was eliminated in the urine during the course of the experiment.

From these observations it is concluded that, under the conditions of our experiments, the tissues absorb the amino-acids almost completely and with extreme rapidity from the blood, and that the formation of urea follows much later.

DISCUSSION.

We have shown (Parts I and II) that the extent of deamination of glycine and alanine is, in the case of rabbits of the same order, and in the case of dogs even greater than that of the other amino-acids studied. Again our injection experiments (Part III) have shown that under similar conditions the rate of absorption of the different amino-acids by the tissues is of a similar order, and also that the excretion of the free amino-acid is negligible under the conditions of our experiments. From these considerations it is clear that the earlier and greater increase in the blood amino-N, and its persistent circulation at a high level following the ingestion of glycine or alanine (both in rabbits and in dogs), is due neither to the lack of deamination nor to the non-absorption of these amino-acids by the tissues.

It is suggested that this phenomenon is due to the rapid absorption of glycine and alanine from the intestinal tract, as shown by the early and rapid rise in the amino-N of the portal blood, and its persistence at a high level for a long time (Fig. 3). Levene and Kober [1909] and Levene and Meyer [1909] have shown that glycine, alanine, glycyl-glycine and aspartic acid are rapidly metabolised, whilst leucine, phenylalanine, arginine and asparagine are metabolised more slowly. They ascribe these differences to the different rates of absorption of these amino-acids from the alimentary canal. Further, the work of Bang [1916, 1] and of Lewis [1918], on the relation between the amount of amino-acid ingested and the rise in the amino-N content of blood, lends additional support to the view expressed above.

Other contributory factors leading to this phenomenon of the rapid and continuous entrance into the systemic circulation of glycine and alanine (as distinct from other amino-acids), may be firstly, the permeability of the liver cells to glycine and the non-permeability to leucine, as observed by Bang [1916, 2], and secondly, the greater adsorbability of glycine and alanine by the blood corpuscles as suggested by the work of Sbarsky and Muchamedoff [1925] on the adsorption of glycine, alanine, leucine and tyrosine by an "erythrocyte-emulsion."

From the above-stated considerations it is obvious that Bang's observations [1916, 2] regarding the persistent circulation in blood of amino-N at a high level, following the ingestion of glycine or alanine, are not necessarily out of harmony with the views of Lusk on the cause of the specific dynamic action of these amino-acids. These two apparently inconsistent points of view are reconciled and explained by the difference in the rates at which the various amino-acids enter the circulation from the digestive tract.

It may be mentioned that the greater urea production in the case of glycine and alanine (Part II) is also probably a direct result of the circulation of larger amounts of these amino-acids and their consequent higher "partial pressure" in the sense of Folin and Denis [1912, 2].

The cause of the specific dynamic action of amino-acids.

Lusk [1915] considered that the increased heat production following the ingestion of glycine or alanine is not due to the oxidation of these amino-acids or of their metabolites, but that it is caused by the chemical stimulation of the body cells to a higher level of metabolism. In their attempts to discover the nature of this stimulant and the mechanism of its action, Lusk and his associates came to the following conclusions:

(i) That the specific dynamic action of amino-acids bears no relationship to the rate or extent of deamination, as shown by the facts that the period of maximum heat production [Lusk, 1912; Csonka, 1915] precedes the period of maximum nitrogen excretion [Janney, 1915] and that glutamic and aspartic acids and asparagine, though deaminised, exert no specific dynamic action [Atkinson and Lusk, 1918].

(ii) That acid radicals as such, or hydroxy- or keto-acids, which may possibly be the intermediary metabolites of some amino-acids, do not exert any specific dynamic action [Lusk, 1921; Atkinson and Lusk, 1918].

These results left the main problem still unsolved, namely, whether the specific dynamic action is due to a stimulus provided by the free amino-acid itself or by one or more of its yet unknown non-nitrogenous metabolites. Lusk [1915], however, subscribed to the latter view and the two chief arguments which he put forward in support of his theory need some attention.

Firstly, Lusk argued that if the free amino-acids exert any specific dynamic action, one should be able to observe an appreciable accumulation of amino-acids in the tissues following the ingestion of meat. Since Wishart [1915] failed to observe any such accumulation, Lusk concluded that the stimulus to increased heat production was not provided by the free amino-acids themselves. It appears to us, however, that this argument, besides being based on observations which are contradicted by Folin and Denis [1912, 2], Van Slyke [1914] and Mitchell [1918], is in itself not very conclusive. Our observations and those of numerous other workers have established the fact that following the ingestion of a protein or an amino-acid diet, the amino-N content of blood remains at a high level for a considerable period, in spite of the rapid absorption by the tissues. This fact indicates clearly that, under the conditions described, a fresh and continuous supply of amino-N reaches the tissues during a period of several hours. Therefore, even though there may not be a static accumulation of amino-acids in the tissues, there is no reason to assume that the abundant supply of constantly circulating amino-acids is not capable of stimulating the tissue cells to a higher level of metabolism.

The second argument put forward by Lusk in support of his theory is based mainly on the observations of Janney and Csonka. Janney [1915] showed that in phlorhizinised dogs the excretion of glucose, following the ingestion of a protein, was proportional to the amount of glucogenetic amino-acid radicals present in that protein, and that the glucose excretion attained

a maximum value during the second and third hours. Csonka [1915] observed that in phlorhizinised dogs the period of maximum heat production (second and third hours) after the ingestion of meat or glycine, corresponds to the period of maximum metabolism of the ingested amino-acids, as determined by the "extra-glucose" excretion method. From these observations Lusk concluded that the specific dynamic action of amino-acids was dependent upon and proportional to their metabolism. However, this conclusion is not justified in view of the facts that glutamic and aspartic acids, in spite of their glucogenetic properties [Janney, 1915], do not exert any specific dynamic action [Atkinson and Lusk, 1918], whilst leucine and tyrosine, though non-glucogenetic [Janney, 1915], cause a certain increase in heat production [Lusk, 1915]. Again, alanine produces only about half as much "extra heat" as an iso-glucogenetic quantity of glycine [Lusk and Richie, 1915], though, according to the "glucogenesis" theory, the two should produce the same effect.

And finally, the correspondence in the periods of maximum heat production and maximum glucose excretion cannot be brought forward as an argument in support of Lusk's theory, because it has been shown by us (Fig. 1) and by Bang [1916, 2] that, under similar conditions, the amino-N content of blood is also at its maximum level during the second and third hours¹. Hence the only conclusion that can be drawn from the above considerations is that the specific dynamic action may be due to some property of either the free amino-acid itself or its metabolites. The existing data do not preclude either of the two possibilities.

In this connection, however, the observations of Sandiford, Boothby and Giffin [1923], that "In all instances (of myelogenous leucaemia) in which amino-acids (of blood) were increased, there was some, but not necessarily a proportional increase in basal metabolism," are of interest. Similar observations on the association of pathological hyperamino-acidaemia with increased basal metabolism, have been recorded in cases of phosphorus poisoning [Atkinson and Lusk, 1918], and pernicious anaemia [Kraus, 1893; Magnus Levy, 1906; and Meyer and Du Bois, 1916]. But although an elevated basal metabolism, under physiological as well as pathological conditions, has been shown to be intimately associated with an increased concentration of amino-acids in the blood, it cannot be stated at this stage that the increased heat production is a direct consequence of hyperamino-acidaemia.

SUMMARY.

(1) Bang's observations on rabbits regarding the persistent circulation of amino-N in the blood at a high level, following the ingestion of glycine or alanine, and the absence of an appreciable increase in the amino-N content of the blood after the ingestion of leucine, are confirmed and extended to other amino-acids, using both rabbits and dogs.

¹ In the experiments on dogs, the maximum level of amino-N in the blood is reached a little earlier (Fig. 2) owing to more rapid absorption from the small intestine.

(2) In the case of histidine, although a high and sustained level of amino-N in the blood is maintained, as in the case of glycine and alanine, yet there is no increase in the urea content of blood even after 6 hours. All the other amino-acids studied caused appreciable urea production.

(3) The specific dynamic action of an amino-acid is proportional to its power of increasing the amino-N content of the blood, following the gastro-intestinal absorption of the amino-acid.

(4) An explanation is offered for the apparent inconsistencies in the views of Lusk regarding the cause of the specific dynamic action of amino-acids and the above observations of Bang and ourselves.

(5) The cause of the specific dynamic action of amino-acids is discussed.

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