PROTEIN METABOLISM FROM THE STANDPOINT OF BLOOD AND TISSUE ANALYSIS.

THIRD PAPER.

FURTHER ABSORPTION EXPERIMENTS WITH ESPECIAL REFERENCE TO THE BEHAVIOR OF CREATINE AND CREATININE AND TO THE FORMATION OF UREA.

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The hypothesis that the nitrogenous digestion products are resynthesized into albuminous materials while passing through the mucous membrane of the intestine and therefore cannot be detected in the blood was originally advanced to explain the absence of peptones in the blood during digestion. The subsequent discovery that protein digestion normally proceeds further than to the peptone stage and results in the formation of amino-acids suggested another explanation of the earlier negative results. As the attempts to find the amino-acids in the blood were no more successful than had been the earlier attempts to find the peptones, the hypothesis of the immediate resynthesis of the digestion products into albuminous substances was revived. Kutscher adopted it[1] and Abderhalden has become more and more positive that it represents the only adequate explanation of the negative results obtained by him and his coworkers in their search for peptones and amino-acids in the blood. Since the resynthesis hypothesis is based on negative findings alone it has no value after the presence in the blood of amino-acids absorbed from the intestine has been positively demonstrated. In our first paper[2] we showed that it is possible by means of our new analytical methods[3] to trace urea, glycocoll and pancreatic amino-acid mixtures not only into the blood, but

[1] *Zeitschr. f. physiol. Chem.*, xxxiv, p. 529, 1902.
[2] This *Journal*, xi, p. 87, 1912.
[3] *Ibid.*, xi, pp. 493–536, 1912.

also into the general tissues of the body. Moreover, the increases in the non-protein nitrogen of the blood and muscles which we have obtained in absorption experiments are large enough to account for practically all the nitrogenous material absorbed from the intestine. We do not claim to account exactly or quantitatively for all that has been absorbed. It is entirely possible that different tissues absorb different amounts from the blood, and we have therefore omitted all calculations tending to show that all the absorbed material is present in the animal in non-protein form. The results obtained indicate, however, that practically all the absorbed nitrogen can be accounted for, so that for the present at least the hypothesis of immediate protein synthesis in the walls of the intestine must be regarded as superfluous and untenable.

The protein regeneration idea served only as an explanation of the failure to find the absorbed amino-acids; it was anything but an explanation of the formation of those amino-acids in the intestine; it was inconsistent with the rapid urea elimination after protein feeding, and it necessarily presupposed that in chemical composition the serum proteins are very variable, whereas, on the contrary, they appear to remain decidedly uniform in composition.

As an explanation of the failure to find peptones or amino-acids in the blood when they should be there in unusual amounts, the immediate deaminization hypothesis therefore seemed more plausible than the protein regeneration doctrine, and was usually accepted as a working hypothesis by those who could not accept the older theory of protein synthesis.

The supposedly high ammonia content of the portal blood, and the results reported by Cohnheim[4] on the production of ammonia and volatile bases accompanying the disappearance of peptone and amino-acids from the digestive tract of fishes (when isolated and suspended in blood or salt solutions) pointed certainly in the direction of deaminization. The experiments of Jacobi[5] and of Lang[6] on the deaminization of amino-acids by tissue extracts

[4] *Zeitschr. f. physiol. Chem.*, lix, p. 239, 1909; lxi, p. 181, 1909; lxxvi, p. 293, 1912. In his last paper Cohnheim reports only the finding of ammonia and no amino-acids.

[5] *Zeitschr. f. physiol. Chem.*, xxx, p. 149, 1900.

[6] *Hofmeister's Beiträge*, v, p. 321, 1904.

seemed to furnish direct evidence of the presence of deaminizing ferments in the intestine and liver, and helped therefore materially to strengthen the theory of immediate deaminization as the characteristic feature of intermediary protein catabolism. Folin's theory of protein metabolism is based on the same conception. Folin did not commit himself to the localization of the deaminization process in the intestine and liver, though he believed that those organs were chiefly responsible for the ammonia and urea formations.

The immediate deaminization theory failed of course to explain why the non-protein nitrogen did not increase in the blood during active absorption. The adherents of that theory had to content themselves with the "calculations" of Bergmann and Langstein[7] and others that the speed of the blood was so great as to remove the urea practically as fast as it was formed. In such an application of these calculations it is tacitly assumed that the excretory capacity of the kidneys is quite as efficient as the absorptive capacity of the intestine, for if such were not the case, the volume and speed of the blood stream in the mesenteric and portal circulation could not prevent a temporary accumulation of the absorbed digestion products in the blood.

The results reported in our first paper indicated that the deaminizing power ascribed to the intestine and liver is by no means adequate to prevent the accumulation of amino-acids in the blood; and in our second paper[8] we showed that no specialized deaminization process is located in the intestine, and that the ammonia in the portal blood is very small in amount and represents the absorption of ammonia produced in the intestinal lumen, chiefly by putrefactive bacteria.

The results reported in our first two papers are surprising in so far as they failed to reveal any urea formation at all except when ammonia was present in the material used for absorption. This result might be regarded as inconsistent with the fact shown by ordinary feeding experiments that the urea elimination is rapidly increased when protein or amino-acids are fed to men or to animals. The inconsistency is, however, more apparent than real. The "rapid urea elimination" after the intake of nitrogenous food is

[7] *Hofmeister's Beiträge*, vi, p. 27, 1904.
[8] This *Journal*, xi, p. 161, 1912.

measured by hours, whereas in our absorption experiments we were dealing with minutes, and did not carry the experiments beyond one hour. The negative results obtained for the urea during the first stages of absorption prove that the urea formation is not localized in the intestine and liver for the purpose of holding back all amino-acids which are not needed for the rebuilding of body tissues, because if such were the case the increase in the urea contents of the blood should begin practically at once, and should be much greater than the accumulation of amino-acid nitrogen. On the other hand, unless we could show that the urea formation does take place later there would remain a discrepancy between our findings and the facts established by ordinary feeding experiments. In this paper we wish to report results which seem to clear up in a measure the formation of urea from amino-acids.

Before taking up the consideration of experiments specifically planned to throw light on the urea formation we wish to record a few additional absorption experiments with substances which are not adapted for the study of the urea production.

For the sake of brevity we wish to state here certain details which represent the procedure followed in all the experiments recorded in this paper unless otherwise described. (1) For anesthetics we have used ether alone, or ether together with a subcutaneous injection of morphine sulphate, or ether with chloretone (the latter injected, together with the substance investigated, into the intestine). After the first few minutes the ether was administered by means of a tracheal cannula. (2) As soon as the animals were unconscious we laid bare the common carotids to have them ready when wanted and inserted a tracheal cannula in the usual manner. (3) The first sample of blood was then taken from one of the femoral arteries by means of a 2 or 5 cc. pipette, as described in this journal,[9] the gracilis muscle of the opened leg was dissected out, and 5 grams of it was immediately cut fine with scissors and immersed in pure methyl alcohol. (4) The abdomen of the animal was then opened and ligatures applied to the small intestine, one just below the stomach, the other just above the caecum. (5) Unless otherwise stated the kidneys were not ligatured or disturbed. (6) The substance under investigation, dissolved in from 50 to 100 grams of water, was then injected into the small intestine by means of a large syringe and a hypodermic needle and the abdomen promptly closed by means of artery clips. (7) The animal was kept warm by means of an electric stove below the holding frame. (8) The alcoholic blood and tissue extracts were prepared as described.[10] (9) The analytical results are calculated in terms of

[9] This *Journal*, xi, p. 527, 1912.

[10] *Ibid.*, xi, p. 528, 1912.

milligrams of nitrogen per 100 grams of blood or tissue. (10) The difference between the amount of nitrogen obtained by washing out the intestine at the end of the experiment and the amount of nitrogen contained in the injected product is given as the amount of substance absorbed. A small error may be involved in this procedure, but in order to keep the animals as normal as possible we did not want to wash out the intestine before beginning the experiment. We have washed out the intestine at the beginning and have found only a few (30 to 40) milligrams of nitrogen, so the error is certainly of very little significance. (11) Unless otherwise stated, no account has been taken of the absorption of water from the intestine or of urine elimination. In nearly all of our experiments there was practically no elimination of urine as the bladder remained collapsed, also there was very little water absorption, for the intestine remained full.

ABSORPTION OF ASPARAGINE.

EXPERIMENT 1. Cat 41 (weight, 3163 grams). Last feeding, twenty-four hours before the operation. Anesthetic, ether and morphine. After ligaturing the blood supply of both kidneys 10 grams of Kahlbaum's asparagine dissolved in about 100 cc. of water were injected into the ligatured intestine. Asparagine is not very soluble and the absorption was allowed to continue for two hours. The following analytical results were obtained.

		Milligrams.
	Asparagine nitrogen injected	2154
	Asparagine nitrogen absorbed	1054
I.	Non-protein nitrogen, control blood	43
II.	Non-protein nitrogen, jugular vein, twenty-two minutes after injection	49
III.	Non-protein nitrogen, portal vein, twenty-four minutes after the injection	69
IV.	Non-protein nitrogen, carotid artery, forty-seven minutes after the injection	52
V.	Non-protein nitrogen, carotid artery, one hundred and twenty minutes after the injection	62
VI.	Non-protein nitrogen, portal vein, one hundred and twenty-three minutes after the injection	70
VII.	Non-protein nitrogen, mesenteric vein, one hundred and twenty-nine minutes after the injection	83
VIII.	Non-protein nitrogen, inferior vena cava, one hundred and thirty-two minutes after the injection	62
	Non-protein nitrogen in muscle before the injection	200
	Non-protein nitrogen in muscle one hundred and thirty-seven minutes after the injection	235

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