XL. STUDIES ON THE METABOLISM OF ARGININE AND HISTIDINE.

PART I. ARGININE AND HISTIDINE AS PRECURSORS OF PURINES.

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THE experiments described in this paper were undertaken in the hope of confirming the suggestion, advanced by Ackroyd and Hopkins [1916], that arginine and histidine are utilised in the animal body as precursors of purines. Not only did Abderhalden and his collaborators [1909, 1910], in testing this very idea, fail to obtain evidence in support of it, but since 1916 the validity of Ackroyd and Hopkins' conclusions has been called in question by several workers. Thus Lewis and Doisy [1918], maintaining normal men on purine-free high-protein diets of high and low content of arginine and histidine, found that no differences in the excretion of purines followed the ingestion of the two types of diet. More recently, Christman and Lewis [1923] have observed a marked diminution in the daily elimination of allantoin following the feeding of amino acids (glycine, alanine and glutaminic acid) to rabbits—results in agreement with those of Abderhalden and Einbeck [1909], who found the excretion of allantoin decreased when histidine was fed to dogs. On the other hand, Harding and Young [1919], feeding placenta (which is rich in arginine and histidine) to young dogs, with a control diet containing an equivalent amount of muscle protein (which is poor in these amino acids) found that the uric acid and allantoin excretions were parallel, and were markedly increased during the placenta feeding.

Not only does there remain some doubt as to whether arginine and histidine actually do function as purine precursors, but the whole of the evidence, both for and against, seems to have been derived from feeding experiments. In these circumstances, the author considered that some useful results might be obtained by employing the method of liver perfusion, in which the use of the single organ eliminates certain of the complications inherent in the use of the intact animal.

It is not necessary, of course, that a substance should be metabolised along one line only, and indeed, we have abundant examples to the contrary. Hence other suggestions as to the fate of arginine and histidine need not be considered contradictory to the hypothesis that they are or may be utilised in the syn-

thesis of purines. In the case of arginine, Thompson [1917] has shown that hydrolysis to urea and ornithine takes place, and Felix [1923] claims that when arginine is perfused through the surviving liver, this hydrolysis is nearly quantitative. Dakin and Wakeman [1911] have demonstrated the production of acetoacetic acid; and Inouya [1912], Thompson [1917], and Gross and Steenbock [1921] have adduced evidence in support of the view that arginine is a precursor of creatinine. As to the changes undergone by histidine, there seems to be no suggestion other than that of Ackroyd and Hopkins [1916]. Its capability of conversion to acetoacetic acid [Dakin and Wakeman, 1911] and to glucose in the diabetic organism [Dakin, 1913] have been examined, but in both cases with negative results.

EXPERIMENTAL.

The perfusions were carried out with excised cat livers in the apparatus designed by Dixon [1922], Ringer's solution being used as the perfusion fluid.

The Control. One great objection to the perfusion method is the difficulty of obtaining an adequate control. If, as is usual, separate livers are used for the positive and control experiments, the comparison can only be valid when the number of perfusions is so large that the statistical method may fairly be applied to the results. The labour involved in so large a number of perfusions is necessarily very great, and an attempt has been made in the experiments here described to secure an efficient control and yet obtain a definite result with a relatively small number of perfusions.

Each liver was perfused for a definite time with a known volume of Ringer's solution only. This fluid was then removed and replaced by an equal volume of Ringer's solution to which had been added the substance under examination, the p_H and osmotic pressure being adjusted to the normal value. This was perfused for the same length of time with, of course, the same rate of flow and the same means of aeration—a slow stream of oxygen. Estimations were carried out on both perfusates. It remained to be shown that this procedure provided an *efficient* control—*i.e.* that any increase of the substance estimated was really due to the substance perfused. A series of experiments was therefore carried out in which the liver was perfused as before with two successive equal volumes of Ringer's solution to neither of which, however, was added any other substance. Estimations were carried out as before, and it was found that there was no increase in the second perfusate—in fact the second usually gave a slightly lower result than the first. This was true not only of allantoin, but also of urea, acetone bodies and sugar. It may fairly be considered, therefore, that this method of double perfusion affords an adequate control, each positive perfusion being compared with the corresponding blank. In this way it is possible to obtain a definite and reliable result with only some half-dozen perfusions.

The Criterion. The allantoin production was taken as the criterion of purine formation since allantoin has been amply shown to be the normal

end-product of purine metabolism in the lower animals [*e.g.* Ackroyd, 1910, 1911, 1914]. It would seem that any increase in the end-product would be accompanied by an increase in the amount of each intermediate, so that in the case of purines, the more easily estimated uric acid might be used as the test-substance. Unfortunately, however, the colorimetric methods of estimating uric acid, which are best adapted for dealing with the small amounts here present, are not applicable in the presence of certain iminazole compounds which themselves give a blue colour with the various uric acid reagents.

Method of Estimation. The method of estimation was that of Wiechowski [1913] modified at the end to remove small quantities of iminazole compounds which escape precipitation in the earlier stages and are then thrown down by the allantoin reagent. Allantoin condenses with xanthydrol [Fosse and Heuille, 1923], while histidine does not, at any rate under the same conditions. The amount of condensation product isolated never corresponded to the whole of the allantoin, but, under strictly-defined conditions, it was always possible to isolate a fixed proportion.

A weighed amount of allantoin, mixed with histidine, was dissolved in the minimum amount of water and an equal volume of glacial acetic acid was added to the cold solution. Xanthydrol, in amount equal to twice the weight of the mixture (sufficient if the "mixture" was really pure allantoin), was dissolved in a volume of glacial acetic acid equal to that added to the allantoin solution; the two cold solutions were mixed, and allowed to stand for half an hour. Less time gave a smaller yield, more gave an impure product. The condensation product was then filtered off, washed four times with 5 cc. of cold water, dried at 100°, and weighed. As shown in the table below, this product was pure and corresponded to 90 % of the allantoin.

Weight of allantoin	·100 g.	·100 g.	·107 g.	·361 g.
Weight of histidine	—	·220 "	·250 "	·342 "
Weight of condensation product	·214 "	·214 "	·231 "	·777 "
Melting point of product (pure product melts at 214°) ...	214°	215°	213°	214°
% of allantoin recovered ...	89·9	89·9	90·0	89·8

In applying this method, the liquid, after perfusion, was made faintly acid with acetic acid, boiled and filtered from the precipitated protein. A measured volume of the filtrate was treated exactly as described by Wiechowski [1913]. After the addition of the allantoin reagent the liquid was allowed to stand overnight. The precipitate was then filtered off, suspended in hot water, and decomposed with hydrogen sulphide. The liquid was evaporated to dryness on the water-bath and the residue was thoroughly extracted with hot water. The combined extracts were evaporated to dryness and the residue was weighed. It was then dissolved in water and the solution evaporated to about 0·5 cc. An equal volume of glacial acetic acid was added and, after cooling, an equal volume of glacial acetic acid containing xanthydrol in amount equal to twice the weight of the residue. The condensation product of allantoin and xanthydrol was prepared as already described, its purity being

tested by determination of the melting point. Throughout the estimation equal volumes of control and positive fluids were used and the liquids received exactly similar treatment. Thus the final weighings, without any calculation, gave a strict comparison of the amounts of allantoin in the two fluids, though, of course, not the absolute amounts.

RESULTS AND DISCUSSION.

The results of one typical perfusion are given.

	Control	Positive
Time of perfusion	2 hours	2 hours
Volume of liquid	500 cc.	500 cc.
Volume taken for estimation ...	450 "	450 "
Weight of condensation product	37.0 mg.	37.6 mg.
Melting point	214°	214°

The perfusion of arginine or histidine through the excised liver caused no real increase in the production of allantoin. The slight increase shown in the experiment quoted is within the limits of experimental error. The results are therefore disappointing in that they fail to confirm the hypothesis that these amino acids are precursors of purines. They are, however, not sufficient of themselves to invalidate the hypothesis.

SUMMARY.

The method of liver perfusion has failed to afford evidence in support of the hypothesis that arginine and histidine function in the animal body as purine precursors.

A method of controlling liver perfusion experiments is described.

A method is given for the estimation of allantoin in presence of iminazole compounds.

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