XXXIV. THE SUPPOSED SYNTHESIS OF URIC ACID FROM ITS DECOMPOSITION PRODUCTS BY TISSUE EXTRACTS.

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It has long been known that extracts of the organs of the lower animals possess the power of destroying uric acid [Stockvis, 1860; Brunton, 1905; Wiener, 1899, 1902, 1903]. Of the various decomposition products which have been reported at one time or another, allantoin has been shown by Wiechowski [1907] to be the real one, and this observer has demonstrated the quantitative conversion of the acid into this substance by the agency of an active ferment preparation from dog's liver. Ackroyd [1911] obtained the same result on perfusing surviving rabbit's liver with Ringer's solution containing dissolved sodium urate. Bezzola, Izar, and Preti [1909] found that uric acid dissolved in defibrinated ox blood was destroyed by perfusion through surviving dog's liver, and maintained that the uric acid was regenerated on saturating with carbon dioxide the solution containing the decomposition products and again passing it through the organ. Ascoli and Izar [1908] also demonstrated such a re-synthesis of uric acid from its decomposition products in the case of extracts of ox liver. When ox liver extract to which was added sodium urate was aërated and incubated, the uric acid was destroyed, but on saturating the resulting mixture with an inert gas such as carbon dioxide, hydrogen, or nitrogen, or by simply incubating the product in a closed vessel, the uric acid was quantitatively regenerated. Later, the same observers [1909] set out to determine what

substances could give rise to uric acid under these conditions. Having unsuccessfully tried several substances, allantoin among them, they found that dialuric acid and urea combined to form uric acid in presence of ox liver extract under anaërobic conditions.

The present paper is a repetition of their work. Their technique was employed in the preparation of the extract and during the incubation, but the uric acid was estimated by Hopkins' method, and not by the Ludwig-Salkowski silver method. The liver was obtained fresh from the slaughter-house, and was finely minced and digested for one hour with Ringer's solution or with 0.85 % sodium chloride solution in an incubator, followed by shaking for one hour. It was then strained through muslin. A solution containing a known weight of uric acid was added, together with 1 per cent. chloroform and 1 per cent. toluene, and the flask containing the mixture was placed in a thermostat at 37° and a current of air drawn through. At the end of the incubation, two equal volumes of the liquid were taken, and the uric acid determined in one of them at once. The second portion was then placed in a flask with more chloroform and toluene, stoppered, and again incubated, after which the uric acid was estimated. The proteins were coagulated in presence of dilute acetic acid in the heat, and the coagulum was filtered off, transferred back to the basin, boiled out with water and again filtered, the process being again repeated. To the filtrate and washings, ammonium chloride was added to the extent of 20–22 per cent. and 2 cc. per cent. of strong ammonia. The ammonium urate was decomposed with hydrochloric acid and titrated as in Hopkins' process.

The author was unable to show any difference in the uric acid content of the fluid before and after the anaërobic conditions, but found that the amount of uric acid in the latter case was equal to that which had not been oxidised by the uricolytic ferment in the extract. Therefore the results of Ascoli and Izar were not confirmed.

Experiment I. 500 g. of finely minced ox liver in 2000 cc. of Ringer's solution was maintained for one hour at 37°, and was then shaken for one hour. It was strained through muslin and 1 per cent. chloroform and 1 per cent. toluene added. A solution of 1.5608 g. of uric acid in 87 cc. of 0.1914 N lithium carbonate and water to 250 cc. were added and air was drawn through for 67 hours at 37°. Total volume was 2225 cc. after this period. Two portions *A* and *B* were then taken. *A* equalled 556 cc., *B* was 1124 cc. *A* was coagulated at once; *B* after anaërobic incubation at 37° for 114 hours with further addition of 1 per cent. chloroform and 1 per cent. toluene

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was divided into two equal portions and the uric acid determined in one of them.

	Found	Calculated
<i>A</i>	Uric acid = 0.1957 g.	
<i>B</i>	Uric acid = 0.1824 g.	$\frac{1}{4} \times 1.5608 = 0.3902$ g.

Experiment II. 250 g. of dog's liver in 1000 cc. Ringer's solution was treated as before. 1.1882 g. of uric acid dissolved in 74 cc. of 0.1914 N lithium carbonate solution made up to 250 cc. were added. Air current for 45 hours at 40°. Final volume was 1230 cc. Two portions *A* and *B* were taken, each of 500 cc. *A* was coagulated at once, *B* after 72 hours of anaërobic incubation at 37° with a further portion of chloroform and toluene.

	Found	Calculated
<i>A</i>	Uric acid = 0.1282 g.	
<i>B</i>	Uric acid = 0.1050 g.	$\frac{500}{1230} \times 1.1882 = 0.4833$ g.

Experiment III. 347 g. of ox liver in 1500 cc. of Ringer's solution was treated as above. A solution of 1.0118 g. of uric acid in 60 cc. of 0.1914 N lithium carbonate solution and water to 250 cc. were added. Air current 42 hours at 37°. Final volume was 1800 cc. Two portions *A* and *B* were taken, each of 450 cc. *A* was coagulated at once, *B* after 72 hours of anaërobic incubation at 37° with a further addition of chloroform and toluene.

	Found	Calculated
<i>A</i>	Uric acid = 0.0791 g.	
<i>B</i>	Uric acid = 0.0435 g.	$\frac{1}{4} \times 1.0118$ g. = 0.2529 g.

Experiment IV. 350 g. of ox liver in 1750 cc. of 0.85 per cent. sodium chloride solution was treated in the same way. A solution of 1.8155 g. of Merck's monobasic sodium urate (= 1.4120 g. of uric acid) in 240 cc. water and 10 cc. N NaOH solution were added, and air drawn through for 41 hours at 37°; a further 15 cc. of N NaOH was added after 19 hours as the reaction was acid. Two portions *A* and *B* were taken, each equal to a fourth of the mixture. *A* was coagulated at once, *B* after 46 hours of anaërobic incubation at 37° with a further addition of chloroform and toluene.

	Found	Calculated
<i>A</i>	Uric acid = 0.1326 g.	
<i>B</i>	Uric acid = 0.1426 g.	$\frac{1}{4} \times 1.4120 = 0.3530$ g.

Experiment V. 330 g. of ox liver in 1500 cc. of 0.85 per cent. sodium chloride solution was treated as usual. 25 cc. of N NaOH and a solution of 1.6013 g. of sodium urate (= 1.2454 g. of uric acid) in 500 cc. of water were added. Air stream for 42 hours at 37°. Two portions *A* and *B* were

taken, each equal to one-fourth of the total. *A* was coagulated at once, *B* after 27·5 hours of anaërobic incubation at 37° with further addition of chloroform and toluene.

	Found	Calculated
<i>A</i>	Uric acid = 0·0498 g.	
<i>B</i>	Uric acid = 0·0530 g.	$\frac{1}{4} \times 1·2454 = 0·3114$ g.

In the attempted synthesis of uric acid from dialuric acid and urea, two Erlenmeyer flasks with ground-glass stoppers were partially filled with equal volumes of liver extract. In one of them was placed a solution containing 2·0 g. of dialuric acid and 0·5 g. of urea dissolved in 0·85 per cent. sodium chloride solution. In the other, an equal volume of 0·85 per cent. sodium chloride solution was placed. To the contents of each flask 1 per cent. chloroform and 1 per cent. toluene were added. The flasks were thus filled to within 25 cc. of their total volume. They were stoppered and incubated for some days at 37°. The flasks were vigorously shaken twice a day. After the incubation, the contents were coagulated, and an endeavour was made to estimate the uric acid. This, however, was not found, as the following experiments show.

Experiment VI. 250 g. of ox liver and 1000 cc. of 0·85 per cent. sodium chloride solution as before. In flask *A* were placed 500 cc. of the extract together with 75 cc. of 0·85 per cent. sodium chloride solution and 1 per cent. of chloroform and toluene. In flask *B* were placed 500 cc. of the extract and a solution of 2·0 g. of dialuric acid and 0·5 g. of urea in 75 cc. of 0·85 per cent. sodium chloride solution, and 1 per cent. of chloroform and toluene. Incubation period was 220 hours at 37°. The contents were then coagulated.

A No precipitate with ammonium chloride and ammonia.

B No precipitate with ammonium chloride and ammonia.

Experiment VII. Similar to last, but incubation period was 92 hours.

A Very slight precipitate of ammonium urate.

B No precipitate of ammonium urate.

Experiment VIII. Similar to the above, but the mixture was made with 180 g. of ox liver and 1000 cc. of 0·85 per cent. sodium chloride solution, and the incubation was for 70 hours. Again no precipitate was obtained in either case.

I am unable to explain my failure to repeat the work of the Italian investigators. Their technique, so far as their published statements allow, was adopted in all essential details except in the method of estimating uric acid.

That the ammonium chloride method is reliable for the estimation of uric acid in tissue extracts is shown by the following observations upon human liver, which, as Walter Jones and his co-workers have shown, contains no uricolytic ferment.

Experiment IX. To each of two mixtures, *A* and *B*, containing 250 g. human liver in 250 cc. Ringer's solution, a solution of 0.6050 g. of uric acid in sodium carbonate and 250 cc. Ringer's solution were added.

A Coagulated at once gave 0.6055 g. of uric acid by the ammonium chloride process.

B Coagulated after six hours' aëration gave 0.6075 g.

Experiment X. A similar experiment, but in each case a solution of 0.7050 g. of uric acid in sodium carbonate and Ringer's solution to 250 cc. were added.

A Coagulated at once gave 0.6995 g. of uric acid.

B Coagulated after six hours' aëration gave 0.6980 g.

SUMMARY.

The experiments of Ascoli and Izar were repeated, but no uric acid could be shown to have been formed:

(1) From the decomposition products of uric acid under the influence of ox liver extracts under anaërobic conditions;

(2) From dialuric acid and urea under the influence of ox liver extracts with exclusion of air.

I wish to express my sincere thanks to Professor Hopkins for suggesting this research and for his kind interest and advice.

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