URIC ACID METABOLISM IN DOGS *

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Wiechowski¹ in 1907 showed that preparations of the livers of dogs and oxen when incubated with sodium urate for about eight hours converted the uric acid into allantoin. In each of his three experiments the uric acid disappeared completely, urea could not be isolated, and allantoin was obtained in the pure crystalline state. Only the second of these was quantitative, and in this 75 per cent. of the uric acid which had been destroyed was converted into allantoin. From this he concluded that uric acid is converted quantitatively into allantoin under the conditions of his experiments while admitting that more prolonged incubation, or incubation under anaerobic conditions, might possibly lead to different results (p. 305). It may also be added that the mode of preparation of his ferment solutions might favour the production of allantoin and not that of other substances; moreover, 25 per cent. of the uric acid which had been destroyed was not accounted for in his experiment. He made an exhaustive criticism of the work of previous observers, and showed clearly that no satisfactory proof had been brought forward that urea, whether by way of glycoll or allantoin, was the nitrogenous end point of purin metabolism in mammals.

In a later paper² he describes a new method of estimating allantoin, and by the use of this showed that it was a constant constituent of normal urine of dogs, rabbits, cats and a monkey. Although allantoin had been found in the normal urine of calves,³ dogs, cats, rabbits⁴ and a cow,⁵ it had not been regarded as constantly present in the urine of the lower animals, but rather as an occasional occurrence or produced by exceptional circumstances, such as poisoning by hydrazin,⁶⁻⁸ limitation of air supply,⁹ or administration of excessive quantities of purin substances.¹⁰⁻¹⁹ Wiechowski put forward the view that in the lower animals allantoin is the normal end product of purin metabolism, and

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that none of the urea excreted is derived from uric acid. He supported this opinion by metabolism experiments on dogs and rabbits. In one experiment uric acid injected subcutaneously into dogs was excreted to the extent of 59 per cent. as allantoin (the uric acid excreted was not estimated in this experiment); while in another (p. 116) 21·4 per cent. of the uric acid injected was excreted unchanged and the whole of the remainder appeared as allantoin. In this last experiment the excretion of urea was much increased after the injection of uric acid, and it cannot be regarded as clearly shown that some of the allantoin excreted might not have been due to the effect of a large dose of uric acid on the endogenous purin metabolism, while a part of that actually injected might have been excreted in some other form; at any rate, the increased excretion of urea was out of all proportion to the nitrogen of the uric acid administered. Uric acid given to a rabbit by the mouth was excreted to the extent of 92 per cent. as allantoin, while when injected subcutaneously into another rabbit 5·6 per cent. was excreted unchanged and 53·2 per cent. as allantoin. In this case the whole nitrogenous metabolism was altered by the injection, and Wiechowski considered that the subcutaneous method should not be employed in rabbits owing to this effect on metabolism. The same objection to subcutaneous or intravenous injection for metabolism experiments applies most probably to other animals (Schittenhelm and Seisser²⁰); the method of administration is abnormal, the substance given not reaching the liver at once, but acting first, if it have any action, on other tissues.

In the case of dogs, Schittenhelm²¹ has brought forward strong support in favour of Wiechowski's view; he administered thymo-nucleic acid by the mouth, and recovered in three experiments 102 per cent., 99 per cent. and 88 per cent. of the purin nitrogen given as purin bases, uric acid, or allantoin; of this, 95 per cent., 94 per cent., and 93 per cent., respectively, was excreted as allantoin. In another experiment, in which the animal was starving, 98 per cent. of the purin substance given was recovered, and of this 95 per cent. was allantoin. Schittenhelm, however, gives an observation (p. 83) concerning Wiechowski's method of estimating allantoin. According to this, the allantoin appears to be greater in quantity when calculated from the nitrogen of the mercury-acetate precipitate than when isolated from that precipitate in the pure state. He found that this difference might amount to as much as 30 per cent., which seems to indicate that nitrogen containing substances other than allantoin may be carried down by mercury acetate. Since

Schittenhelm seems to have calculated his results from the nitrogen, his values for the allantoin excretion may be too high; they certainly show that allantoin is the principal form in which purin substances are excreted by dogs, but there remains the possibility that other substances, perhaps urea, might also be formed, though in small amounts. This would, perhaps, have little practical interest were it not that Wiechowski²² has recently revived the older views of Loewi,²³ and of Soetbeer and Ibrahim,²⁴ that man has not the power to destroy uric acid but excretes it all unchanged. At first he was unable to isolate allantoin from human urine, but later²⁵ he showed that man regularly excretes about ten milligrams of allantoin daily: this observation was confirmed by Schittenhelm and Wiener.²⁶ Wiechowski takes this quantity (0.01 g. per diem) as the measure of man's power to destroy uric acid, but Schittenhelm and Franz²⁷ consider that man readily destroys uric acid and converts it into urea, or more correctly into some substance, not allantoin, which is estimated as urea by the Pflüger-Bleibtreu method.

It seems quite possible that uric acid metabolism in the lower animals may proceed in two directions, the one leading to allantoin and perhaps ending there, the other to urea or intermediary bodies. Nor is it improbable that these different mechanisms may be used to a varying extent by the same animal under different metabolic conditions, and still more is this likely to be the case in different classes of animals. The lower animals may possess both mechanisms but use chiefly that producing allantoin, while in man little allantoin may be formed, uric acid metabolism proceeding mainly in another direction. It was from this standpoint that the experiments on dogs here recorded were undertaken with the view of ascertaining whether these animals had the power to convert uric acid into any other substance than allantoin.

The method of perfusion of the surviving liver was chosen for the investigation, since thereby the normal balance of ferment action is less disturbed than by the use of organ extracts, whether partially purified or not. The perfusions were carried out in a modified form of the apparatus described by Brodie and Dixon.²⁸ No food was given to the dogs for twenty hours before the experiment, they were killed by pithing, cannulae were tied into the portal vein and superior vena cava, the liver was washed free from blood with normal saline solution* followed by fluid of the same

* Normal saline solution as used in these experiments was of the following composition :— Sodium chloride, 0.9 g. per cent. ; potassium chloride, 0.025 g. per cent. ; sodium bicarbonate, 0.015 g. per cent. ; calcium chloride, 0.02 g. per cent. and distilled water *Ad* 100 per cent.

composition as that subsequently used for perfusion, thus preventing any dilution of the perfusion fluid.

The liver was then excised and placed in the perfusion chamber. Nine hundred cubic centimetres of the perfusion fluid, kept well oxygenated, were passed through the liver in a continuous stream, the whole quantity passing through about ten times per hour by force of gravity alone (the fluid ran down from a container placed 50 cms. above the portal vein). The temperature both of the fluid and of the chamber containing the liver was kept at 38° to 40° C.

At the end of the experiment, proteid was removed from the perfusion fluid by acidifying with acetic acid and boiling, the fluid when cool was made up to its original bulk with distilled water and the coagulated proteid filtered off. Uric acid was estimated by the Ludwig-Salkowski method. Attempts were made to isolate urea by the method given in Hoppe-Seyler's Hdb., 8th edit., p. 651. Allantoin was estimated by Wiechowski's method, the very dilute solutions being previously concentrated on the water bath to a known volume so as to avoid great dilution in the final precipitation; the quantity present was afterwards calculated in terms of the original solution, of which never less than 600 c.c. were used. The allantoin was always isolated and weighed. Allantoin was identified by its crystalline form, its melting point with decomposition at 231° C., and when sufficient material was obtained, by its percentage nitrogen composition determined by Kjeldahl's method (theoretical = 35.44 per cent.). Its solutions tested with silver nitrate alone gave no precipitate, but a voluminous one on further adding a little ammonium hydrate; this was soluble in excess, but came down again on adding more silver nitrate. A trace of the solid substance boiled with strong sodium hydrate gave the glyoxylic reaction, while addition of acetic acid and calcium chloride brought down microscopic crystals of calcium oxalate.

Experiment I.—Normal saline solution was perfused for one hour. No urea could be isolated from the perfusion fluid at the end of the experiment.

Experiment II.—Normal saline solution containing 0.076 g. per cent. of uric acid as sodium urate was perfused for two and a half hours. At the end of the experiment the percentage of uric acid was 0.0504 g. No urea could be isolated from 140 c.c. of the solution.

Experiment III.—Normal saline solution containing 0.1554 g. per cent. of uric acid dissolved by the addition of a minimal quantity of sodium hydrate was perfused for two hours. At the end of the experiment the percentage of uric acid had fallen to 0.0947 g. No urea could be isolated by Hoppe-Seyler's method from 272 c.c. of the solution, but 0.0625 g. of a nearly white substance crystallising in fine prisms like allantoin was obtained. When heated this

began to brown at 220° C., and melted with decomposition at 225° C. It gave the reactions described for allantoin, and was precipitated from solutions by Wiechowski's mercury acetate reagent, not by phospho-tungstic acid or basic lead acetate.

Experiment IV.—Normal saline solution containing 0.0645 g. per cent. uric acid as sodium urate was perfused for two hours. The uric acid at the end of the experiment had fallen to 0.045 g. per cent. No urea could be isolated from 280 c.c. of the fluid, but a few crystals microscopically resembling allantoin were obtained; the quantity was not sufficient for further investigation.

Experiment V.—Normal saline solution containing 0.1048 g. per cent. of uric acid brought into solution by a minimum quantity of sodium carbonate was perfused for one and a half hours. At the end of the experiment the percentage of uric acid had fallen to 0.092 g. per cent. No urea could be isolated from 350 c.c. of the fluid.

These experiments seem to indicate that the liver of dogs, while able to destroy uric acid, cannot convert it into urea, but on the other hand allantoin is formed. Further experiments were carried out to ascertain if the allantoin produced represents quantitatively the uric acid destroyed.

Experiment VI.—A dog's liver was perfused for five hours with normal saline solution. At the end of the experiment no uric acid was present in the solution, but by Wiechowski's method allantoin was found to be present to the extent of 0.011 g. per cent. This allantoin, when heated, began to brown at 225° C., and melted with decomposition at 229° C. It burnt completely on platinum foil, leaving no residue; it crystallised in fine prisms, and its chemical reactions were those already given for allantoin.

Experiment VII.—A dog's liver was perfused for five hours with normal saline solution. At the end of the experiment no uric acid was present, but the fluid contained 0.0079 g. per cent. of allantoin, melting with decomposition at 228° C., having the typical crystalline form, and giving the chemical reactions of allantoin.

Experiment VIII.—A dog's liver was perfused for five hours with normal saline solution containing 0.0542 g. per cent. of uric acid as sodium urate. At the end of the experiment the percentage of uric acid was 0.024 g. From 820 c.c. of the fluid 0.1585 g. of allantoin were isolated. This melted at 230° C., contained 36 per cent. of nitrogen (theoretical for allantoin is 35.44 per cent.), and in crystalline form and chemical reactions agreed with allantoin.

Experiment IX.—Normal saline solution containing 0.0758 g. per cent. of uric acid as sodium urate was perfused for five hours. At the end of the experiment the fluid contained 0.0422 g. per cent. of uric acid and 0.019 g. per cent. of allantoin, which melted at 228° C., burnt, leaving no residue, crystallised in prisms, and gave the usual chemical reactions.

Experiment X.—Normal saline containing 0.0447 g. per cent. of uric acid as sodium urate was perfused for five hours. At the end of the experiment the percentage of uric acid was 0.0181 g. and the fluid contained 0.0243 g. per cent. of allantoin, which melted at 226° C., contained 35.44 per cent. of nitrogen, crystallised in typical prisms, and gave the usual chemical reactions.

The control experiments VI and VII show that in five hours' perfusion of a dog's liver with normal saline solution a quantity of

allantoin, approximately 10 mgs. per cent., is produced from the liver itself, while any uric acid formed is at once decomposed. Whether this allantoin is to be regarded as part of the normal endogenous metabolism of the liver, or as the result of early death of the tissues, cannot be certainly decided, but the absence of uric acid and the close agreement of the two experiments, quantitatively, seems to be in favour of the former view. In none of the other experiments VIII, IX and X was the uric acid which had been destroyed replaced even approximately quantitatively by allantoin. Allowing 10 mgs. per cent. as the amount of allantoin produced from the liver in a five hours' perfusion, the percentage of uric acid destroyed which was recovered as allantoin in these three experiments was 32.7, 28.5 and 57.2. It is possible that more allantoin had been formed but had been converted into some other substance. The following experiment shows that this was not the case.

Experiment XI.—Normal saline solution containing 0.2576 g. per cent. of allantoin (Merck) was perfused for five hours. At the end of the experiment the solution contained 0.2571 g. per cent. of allantoin having a melting point of 231° C., and containing 35.52 per cent. of nitrogen.

Allowing for 10 mgs. per cent. produced during the perfusion, this gives 96 per cent. of the allantoin recovered in the pure state, which agrees with the observation of Poduschka,⁷ who found that of allantoin administered to dogs 90 per cent. could be recovered unchanged in the urine, while Minkowski,¹³ using the same animals, recovered 70 per cent.

In all the last six experiments an attempt was made to isolate urea from the filtrate after precipitation with mercury acetate by addition of mercuric nitrate; none, however, was found.

These experiments show that the liver of dogs can destroy uric acid and can partially convert it into allantoin, but the amount of this substance formed falls far short of corresponding in quantity to the uric acid destroyed, when allowance is made for the amount of allantoin produced from the liver itself during perfusion. The deficit could not be accounted for as urea, nor could Ascoli,²⁹ in his perfusion experiments (experiment XIII, p. 344), isolate urea in the pure crystalline state, though he believed that it had been produced. This opinion rested on estimations by the Pflüger-Schöndorf method, which would, however, include allantoin as urea. The same objection applies to Subkow's³⁰ perfusion experiments, the urea being estimated by a nitro-metric method in the filtrate from precipitation by phospho-tungstic acid. Although perhaps too much weight should not be put on the quantitative side of

perfusion experiments, I think it is extremely unlikely that the uric acid unaccounted for as allantoin had been retained in the liver either mechanically or physiologically, and the amount of the deficit is far too great to be accounted for by experimental error. It seems, therefore, more likely that allantoin is not the sole end product of purin metabolism in dogs, though it is undoubtedly the main one. Since dog's liver cannot destroy allantoin, any other substances formed from uric acid must originate in a primary cleavage of the molecule other than that by which allantoin is formed. Glycocoll was believed by Wiener to represent an intermediate stage in the conversion of uric acid to urea in rabbits³¹ and oxen,³² while such a very simple chemical reagent as hydrogen peroxide can convert uric acid through tetra-carbonimide³³ and carbonyl di-urea to urea³⁴ itself. Failure in these experiments to isolate urea does not prove that it is not formed; for the amount of it or any one intermediary substance might be so small as to escape detection.

Experiments on rabbits on similar lines to these on dogs are now being carried out so as to ascertain if in those animals any decomposition products of uric acid other than allantoin can be isolated.

SUMMARY

The liver of dogs when perfused with normal saline solution produces a small quantity of allantoin.

When sodium urate is added to the perfusion fluid, a part of the sodium urate is destroyed: part of this can be recovered as allantoin, but not the whole. No urea could be isolated from such a perfusion fluid.

The liver of a dog has not the power to destroy allantoin.

REFERENCES

1. Wiechowski, *Hofmeister's Beitr.*, Vol. IX, p. 295, 1907.
2. Wiechowski, *Hofmeister's Beitr.*, Vol. XI, p. 109, 1907.
3. Wöhler, *Liebig's Ann.*, Vol. LXX, p. 229, 1849.
4. Meissner, *Ztsch. f. rat. Med.*, Vol. XXXI, p. 144, 1868; quoted from *Jahresb. u. d. Leistungen i. d. Med.*, Vol. I, p. 69, 1868.
5. Salkowski, *Ztsch. physiol. Chem.*, Vol. XLII, p. 218, 1904.
6. Borrisow, *Ztsch. physiol. Chem.*, Vol. XIX, p. 501, 1894.
7. Poduschka, *Arch. f. exp. Path. u. Pharmak.*, Vol. XLIV, p. 59, 1900.
8. Pohl, *Arch. f. exp. Path. u. Pharmak.*, XLVIII, p. 367, 1902.
9. Frerichs and Staedler, *Müller's Arch.*, p. 393, 1854.
10. Salkowski, *Ber. d. Deutsch. chem. Gesell.*, Vol. IX, p. 719, 1876.
11. Salkowski, *Ber. d. Deutsch. chem. Gesell.*, Vol. XI, p. 500, 1878.
12. Cohn, *Ztsch. physiol. Chem.*, Vol. XXV, p. 507, 1898.
13. Minkowski, *Arch. f. exp. Path. u. Pharmak.*, Vol. XLI, p. 389, 1898.
14. Salkowski, *Centralbl. f. med. Wissen.*, Vol. XXXVI, p. 929, 1898.
15. Mendel and Brown, *Amer. Journ. Physiol.*, Vol. III, p. 261, 1900.
16. Salkowski, *Ztsch. physiol. Chem.*, Vol. XXXV, p. 495, 1902.
17. Swain, *Amer. Journ. Physiol.*, Vol. VI, p. 38, 1902.
18. Mendel, Underhill and White, *Amer. Journ. Physiol.*, Vol. VIII, p. 376, 1903.
19. Mendel and White, *Amer. Journ. Physiol.*, Vol. XII, p. 85, 1905.
20. Schittenhelm and Seisser, *Ztsch. f. exp. Path. u. Ther.*, Vol. VII, p. 116, 1909.
21. Schittenhelm, *Ztsch. physiol. Chem.*, Vol. LXII, p. 80, 1909.
22. Wiechowski, *Arch. f. exp. Path. u. Pharmak.*, Vol. LX, p. 185, 1909.
23. Loewi, *Arch. f. exp. Path. u. Pharmak.*, Vol. XLIV, p. 22, 1900.
24. Soetbeer and Ibrahim, *Ztsch. physiol. Chem.*, Vol. XXXV, p. 1, 1902.
25. Wiechowski, *Biochem. Ztsch.*, Vol. XIX, p. 368, 1909.
26. Schittenhelm and Wiener, *Ztsch. physiol. Chem.*, Vol. LXIII, p. 283, 1909.
27. Schittenhelm and Franz, *Ztsch. physiol. Chem.*, Vol. LXIII, p. 269, 1909.
28. Brodie and Dixon, *Journ. Physiol.*, Vol. XXX, p. 476, 1904.
29. Ascoli, *Pflüger's Arch.*, Vol. LXXII, p. 340, 1898.
30. Subkow, *Inaug. Diss. Pharmak. Lab. Univers. Moskau*, quoted from *Jahresb. Thier-Chem.*, Vol. XXXIII, p. 873.
31. Wiener, *Arch. f. exp. Path. u. Pharmak.*, Vol. XL, p. 313, 1897.
32. Wiener, *Arch. f. exp. Path. u. Pharmak.*, Vol. XLII, p. 375, 1899.
33. Scholtz, *Ber. d. Deutsch. chem. Gesell.*, Vol. XXXIV, p. 4130.
34. Schittenhelm and Wiener, *Ztsch. physiol. Chem.*, Vol. LXII, p. 100, 1909.