

EXPERIMENTAL LIVER NECROSIS; IV. NUCLEIN METABOLISM.¹

BY HOLMES C. JACKSON, PH.D.,
Adjunct Professor of Physiological Chemistry,

AND

RICHARD M. PEARCE, M.D.,
*Professor of Pathology and Bacteriology,
Albany Medical College.*

(From the Bender Laboratory, Albany, N. Y.)

The experiments here detailed were undertaken for the purpose of determining the process or processes intimately concerned in the increased elimination of uric acid which, as has been shown elsewhere,² accompanies the augmented output of total nitrogen when hæmotoxic serum is injected. The fact that the principal lesion produced by the toxic serum is in the liver lends peculiar interest to the problem, in view of the important part which this organ is supposed to play in the formation of endogenous uric acid. The direct object of the experiments was to determine whether the increased elimination of uric acid in dogs under these conditions was the result of the breaking down of nuclear material during the necrosis which follows the injection, or whether it signified simply a diminished oxidative power on the part of the hepatic cell by which the uric acid normally oxidized to simple complexes is eliminated unchanged. Or, more concisely, does an actual increase in the production of uric acid from the nucleic acids of the decomposed nuclei of the necrotic cell occur or is there a simple rearrangement of enzymotic equilibrium by which less uric acid is decomposed than normally.

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²See third paper of this series "Nitrogenous Metabolism" in this number of the *Journal*.

Schittenhelm³ has isolated two enzymes from the spleen and liver, one of which causes a hydrolytic splitting off of the amino group of guanin and adenin transforming these bodies thereby into xanthin³ and hypoxanthin respectively; the other an oxydase, xantho-oxidase (Jones), oxidizes the latter compounds into uric acid. Since adenin, guanin, xanthin and hypoxanthin are found in varying amounts in the different nucleic acids which give character to the nucleoproteid of the nucleus we have a fairly definite process by which uric acid results, it may be assumed, from the decomposition of the nucleus by means of autolytic enzymes aided perhaps by a special oxydase.

On the other hand, however, the fact has long been known that various tissues are capable of decomposing uric acid. Wiener,⁴ Schittenhelm⁵ and Almagia⁶ have recently studied the question in detail and describe the presence of uricolytic enzymes in various tissues of several animals. Schittenhelm claims that the calf's kidney is most active in this regard with the liver of the same animal second in power. Pfeiffer⁷ was able to prove that ninety to ninety-five per cent. of the uric acid added to human kidneys suffered decomposition. In the horse Almagia found the most active enzyme to be in the liver. These results indicate that the liver among other organs possesses the power of forming uric acid by oxidization and perhaps also by synthesis,⁸ while at the same time it is

³ Schittenhelm, A., Ueber die Harnsäurebildung in Gewebsauszügen, *Zeit. f. physiol. Chem.*, 1904, xlii, 251; Ueber die Harnsäurebildung und die Harnsäurezersetzung in dem Auszügen der Rinderorgane, *ibid.*, 1905, xlv, 121.

⁴ Wiener, H., Ueber Zersetzung und Bildung der Harnsäure im Thierkörper, *Arch. f. exper. Path. u. Pharm.*, 1899, xlii, 375.

⁵ Schittenhelm, A., Ueber das uricolytische Ferment, *Zeit. f. physiol. Chem.*, 1905, xlv, 161.

⁶ Almagia, M., Zur Lehre vom Harnsäurestoffwechsel, Ueber die Zersetzung der Harnsäure durch die Organe des Säugetheirs, *Beit. z. chem. Phys. u. Path.*, 1905, vii, 459.

⁷ Pfeiffer, W., Zur Lehre vom Harnsäurestoffwechsel, Ueber die Zersetzung der Harnsäure durch menschliche Nierengewebe, *Beiträge z. chem. Phys. u. Path.*, 1905, vii, 463.

⁸ Burian, R., Ueber die oxydative und die vermeintliche synthetische Bildung von Harnsäure im Rinderleber Auszug, *Zeit. f. physiol. Chem.*, 1905, xliii, 497; Die Herkunft der endogenen Harnpurine bei Mensch und Säugethier, *ibid.*, 1905, xliii, 532.

capable of decomposing it through the stages of allantoin and glyoxylic acid.

Austin⁹ has raised the point, however, that the alkali in which the uric acid is dissolved is capable of splitting up the latter and that all preparations of uricolytic enzymes contain purin bases which readily become transformed into uric acid. Notwithstanding the latter criticism it seems fairly well founded that the reaction under discussion is a reversible one in which synthetic and analytic processes come into a state of equilibrium in the cell and that alterations in the activity of one or the other set of enzymes increase or diminish the elimination of uric acid without the occurrence, necessarily, of an increase or decrease in the amount of purin material formed from the nucleic acid of the nucleus.

If the increased elimination of uric acid in dogs injected with toxic sera is the result of altered uric acid equilibrium of the cell, as just described, one would expect to find that, as more xanthin the hypoxanthin are oxidized to uric acid, less of these purins should be eliminated in the urine. On the other hand, if the uric acid is the result of a new formation from the nucleic acids then an increase in purins also would be expected and at the same time an augmentation in elimination of phosphoric acid in some form¹⁰ as the result of the splitting of the nucleic acid molecule. In the formation of uric acid by the first process no change in phosphoric metabolism should take place.

Methods.—The experiments presented in Tables I, II and III were carried out in the following manner: The animals were placed upon nitrogenous equilibrium after which estimations were made during a three day fore period. An injection was then given, and in two instances a second injection after a lapse of one and three days respectively. The observations were continued until after the maximum effect was reached. The purin-free diet was a casein,

⁹ Austin, A. E., The Uricolytic Enzyme in Animal Organs, *Jour. of Med. Research*, 1906, xv, 309. The Uricolytic Enzyme, *ibid.*, 1907, xvi, 7.

¹⁰ Jackson, H. C. and Blackfan, K. D., Action of Certain Drugs on the Elimination of Uric Acid During a Nitrogen-free Diet, *Albany Medical Annals*, 1907, xviii, 24.

cracker dust and lard mixture. The urine was examined for total-nitrogen by the Kjeldahl-Gunning method, the uric acid and purin bases were determined by the Salkowski procedure, and the inorganic phosphates by titration with uranium nitrate, using potassium ferrocyanide as an indicator. The method used for total phosphorus was fusion of the evaporated residue of the urine with sodium hydroxide and potassium nitrate, precipitation with ammonium molybdate in the presence of ammonium nitrate, solution of the ammonio-phosphomolybdate in ammonia and re-precipitation with magnesium mixture. This precipitate was filtered off, incinerated and weighed as magnesium pyrophosphate.

The question of the presence of phosphorus in the urine in organic form has recently received attention from various investigators among whom Bergmann¹¹ and LeClerc and Cook¹² incline to the opinion that all of the phosphorus of the urine exists in the inorganic form. The latter observers studied the phosphorus content of the urine by means of determinations made by Neumann's¹³ method, and by that outlined above, and compared the results with those obtained by the uranium-titration procedure. They show that the results by the dry fusion method are uniformly higher by three to four per cent. than those given either by uranium acetate or by Neumann's method; hence they incline to the view that this difference is within the limits of error and that no organic phosphorus is present in normal urine.

We cannot agree with these conclusions in view of the results obtained by one of us in a study of the elimination of organic phosphorus after the administration of sodium salicylate.¹⁴ Nor do they receive support from the results which are presented in this

¹¹ Bergmann, W., Ueber die Ausscheidung der Phosphorsäure beim Fleisch- und Pflanzenfresser, *Arch. f. exper. Path. u. Pharm.*, 1901, xlvii, 77.

¹² LeClerc, J. A., and Cook, F. C., Metabolism Experiments with Organic and Inorganic Phosphorus, *Jour. of Biol. Chem.*, 1906, ii, 203.

¹³ Neumann, A., Einfache Veraschungsmethode (Säuregemisch-Veraschung), *Zeit. f. physiol. Chem.*, 1902, xxxvii, 115.

¹⁴ Jackson, H. C. and Blackfan, K. D., Action of Certain Drugs on the Elimination of Uric Acid During a Nitrogen-free Diet, *Albany Medical Annals*, 1907, xviii, 24.

communication. The constant uniformity of difference shown by the fusion method over that of titration seems to indicate that the difference is not due to the factor of error in the method. We have performed in this connection some preliminary experiments with a view to explaining the differences reported and expect to continue them more in detail. At present we can say that Neumann's method apparently yields results which agree closely with those obtained by titration with uranium nitrate, using potassium ferrocyanide as indicator. When the fusion method is employed the figures obtained are uniformly higher than by both of the other methods. At present, therefore, we incline to the opinion that phosphorus in other than inorganic form is present in normal urine.

TABLE I.
Nuclein Metabolism. Dog 59.

Date.	Grams Nitrogen as			Grams P ₂ O ₅ .		Notes.
	Total.	Uric Acid.	Purin Bases.	Inorganic.	Organic.	
Apr. 10	7.86	0.0015	0.001	0.899	0.147	
11	5.51	0.0159	Lost	0.652	0.093	
12	4.70	0.0141	0.0082	0.690	0.087	
13	4.49	0.0121	0.0097	0.576	0.050	Injection 10 A. M. toxic serum; dose 1:785.
14	6.97	0.0126	0.0231	1.009	0.085	
15	12.50	0.0246	0.0169	0.690	0.121	Injection 11 A. M. toxic serum; dose 1:1745.
16	11.08	0.0127	Lost	0.848	0.072	Hburia.
17	13.81	0.0319	0.0287	0.629	0.083	
18	11.93	0.0203	0.0265	0.866	0.023	Killed.

TABLE II.
Nuclein Metabolism. Dog 61.

Date.	Grams Nitrogen as			Grams P ₂ O ₅ .		Notes.
	Total.	Uric Acid.	Purin Bases.	Inorganic.	Organic.	
Apr. 22	3.33	0.0042	0.0086	0.478	0.078	
23	3.83	0.0063	0.0072	0.554	0.052	
24	3.12	0.0050	0.0079	0.584	0.097	Injected 12 M. toxic serum; dose 1:937; vomited.
25	4.57	0.0167	0.0103	0.549	0.085	Hburia.
26	5.74	0.0336	0.0107	0.387	0.074	Hburia.
27	6.83	0.0241	0.0124	0.387	0.084	
28	6.54	0.0200	0.0235	0.326	0.046	
29	5.69	0.0112	0.0086	0.356	0.118	Killed.

TABLE III.
Nuclein Metabolism. Dog. 63.

Date.	Grams Nitrogen as			Grams P ₂ O ₅ .		Notes.
	Total.	Uric Acid.	Purin Bases.	Inorganic.	Organic.	
May 14	4.74	0.0057	0.0061	0.520	0.062	
15	4.41	0.0054	0.0068	0.557	0.109	
16	4.26	0.0068	0.0076	0.550	0.072	Injected 11 A. M. toxic serum; dose 1 : 835; vomited.
17	5.29	0.0249	0.0110	0.641	0.062	
18	4.24	0.0143	0.0088	0.508	0.078	
19	4.34	0.0109	Lost.	0.535	0.066	Injected 11 A. M. toxic serum; dose 1 : 557; vomited.
20	4.75	0.0133	0.0181	0.576	0.066	Hburia.
21	7.64	0.0141	0.0116	0.435	0.090	Hburia.
22	8.38	0.0095	0.0187	0.440	0.075	Hburia.
23	6.21	Lost.	0.0133	0.550	0.111	Killed.

Results.—From the figures in the above tables it is evident that the increase of uric acid after injection, as noted in the study of the nitrogenous metabolism,¹⁵ is constant and that there also occurs an increase of purin bases and with one exception of phosphorus pentoxide. This general increase occurs not only after the first but also after subsequent injections. It reaches its maximum on the second day and then falls away. The uric acid increase is greater proportionately than that of the total nitrogen, hence an augmentation of the percentage uric acid in terms of total-nitrogen takes place. The uric acid nitrogen, however, returns to the normal sooner than the latter. The same facts hold true for the purin bases the elimination of which runs parallel, in a general way, to the uric acid output. In the one instance (Table II, Dog 61) in which the phosphorus pentoxide elimination remained constant there still occurred the markedly increased uric acid and purin base output. That the phosphorus pentoxide elimination did not follow that of the uric acid and purin bases is to be explained by the fact that after the injection the animal ate only a quarter of the daily food allowance. Although on the day following the injection the

¹⁵ See third paper of this series "Nitrogenous Metabolism" in this number of the *Journal*.

amount ingested of phosphorus pentoxide was therefore diminished, the elimination of inorganic phosphates remained unchanged. This would indicate that an actual increase in endogenous phosphorus pentoxide formation took place, thus bringing the apparent exception in agreement with our other observations. The organic phosphorus elimination was unaltered.

The question as to the cause of the increased output of uric acid during a purin-free diet, observed after various experimental procedures, has been variously explained. Beebe¹⁶ concludes that the increase found after alcohol administration is to be attributed to a diminished oxidative power on the part of the hepatic cells although the purin bases were likewise increased. In commenting upon this view Jackson and Blackfan, from results which they obtained with alcohol, inclined to the opinion that the facts would warrant rather the assumption that the alcohol causes an increased new formation of uric acid and purin bases, and they adduced as evidence, among other factors, the augmented excretion of organic phosphorus.

According to the view of Schittenhelm, concerning the probable way in which the uric acid is formed from the nucleo-proteid, we would expect, if for any cause oxidization in the hepatic cell is diminished, the xantho-oxydase would be affected, and as a result less uric acid would be formed from the purin bases than under normal circumstances. An increase in purins would also occur if the hydrolytic splitting enzymes were unaffected. An augmentation of uric acid would take place either as the result of a diminished power of the uricolytic enzymes or as the result of an increased new production, other things being equal. In the latter case the purin would also be increased.

The results reported in the paper on nitrogenous metabolism do not indicate that the injection of a hæmotoxic serum causes a prolonged or decided decrease in the oxidative power of the hepatic cell. As a whole the organ continues to perform its functions in the normal way. We must therefore explain the increase in purins and uric acid as a decomposition of nuclear material in the autolysis occurring in the areas of necrosis. This is brought about by

¹⁶ Beebe, S. P., The Effect of Alcohol and Alcoholic Fluids upon the Excretion of Uric Acid in Man, *Amer. Jour. of Physiol.*, 1904, xii, 13.

the hydrolysis of the nucleic acids. Such an explanation agrees with the feeding experiments performed by Sweet and Levene¹⁷ upon a dog with an Eck fistula. They found the ingestion of nucleic acids to be followed by a rise in uric acid and total phosphorus elimination. The fact that the increased excretion of these nuclear compounds usually reaches its maximum in our experiments on the second day, when the autolysis is at its height, would also strengthen this explanation.

The increased elimination of inorganic phosphates which accompanies the output of uric acid would likewise point to the new formation of the latter from the nucleic acids formed as a step in the autolysis of nuclear material. The phosphoric acid radical of these acids is evidently excreted in inorganic form and thus the mechanism differs from that observed in connection with the administration of alcohol and salicylic acid under which circumstances the phosphoric acid is apparently eliminated in organic combination, since no increase in inorganic phosphates occurs.

Allantoïn.—In the dog, the largest part of the quantity of uric acid ingested, as shown by Swain,¹⁸ disappears and is not excreted. In the experiments outlined above it is not improbable, therefore, that a much larger amount of uric acid was produced in the necrosis of the cell nucleus than was eliminated. Of the total amount formed only a small proportion escapes hydrolysis and appears in the urine. Swain also has shown that the ingestion of large amounts of uric acid is followed by the elimination of small quantities of allantoïn.

In order to obtain evidence upon this point the urine of the six animals under observation in the experiments reported in the paper on nitrogenous metabolism, was examined for allantoïn according to the method of Loewi.¹⁹ The results are not uniform but indicate that usually the increase of uric acid is accompanied by an increase in allantoïn; for example, in one the amount of uric acid the day

¹⁷ Sweet, J. E., and Levene, P. A., Nuclein Metabolism in a Dog with Eck's Fistula, *Jour. of Exper. Med.*, 1907, ix, 229.

¹⁸ Swain, R. E., Formation of Allantoïn from Uric Acid, *Amer. Jour. of Physiol.*, 1901, vi, 38.

¹⁹ Loewi, O., Beiträge zur Kenntnis des Nucleinstoffwechsels, *Arch. f. exp. Path. u. Pharm.*, 1900, xlv, 20.

before injection was 0.018 gram and of allantoin 0.319; in the urine of the twenty-four hour period following the former rose to 0.337 and the latter to 0.669.

CONCLUSIONS.

In necrosis of the liver of the dog produced by hæmotoxic immune sera, the increased excretion of uric acid, purin bases and inorganic phosphorus pentoxide is the result of the hydrolysis of nuclear material occurring during the autolysis of the necrotic tissue.