CCLXXX. NITROGEN METABOLISM OF CERTAIN INVERTEBRATES.

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NEEDHAM [1935] made the statement that "in the insects excretion of uric acid as the main end product of nitrogen catabolism is widespread, if not universal". Przyłęcki's paper [1926] on the nitrogen metabolism of invertebrates indicates that this statement is perhaps more comprehensive than the facts would warrant. For this, and other reasons, the problem in question was reinvestigated, not only for a number of insects but also for members of other phyla.

The present research, whilst concerned chiefly with purine catabolism, also includes data for other nitrogenous catabolites. In most cases, the presence or absence of uricase, xanthine oxidase and urease was established and in addition the uric acid content of the animals was determined wherever possible.

GENERAL EXPERIMENTAL METHODS.

The animal material was ground up in a mortar with 10 times its weight of water (in the cases of Mollusca and earthworms clean sand was added). The suspension was strained through gauze to retain the larger fragments (chiefly the chitin integument in the case of insects and smooth muscle in that of other animals) and the filtrate was centrifuged (2000 r.p.m.; 5 min.). Portions of the centrifugates were examined for xanthine oxidase by adding an equal volume of 0.1 % suspension of xanthine in water and heating one portion at 100° for 5 min. Toluene was then added, and the mixtures were placed in a thermostat at 37°; portions of the solutions were examined after 24 and 48 hours for uric acid.

Urease was detected by adding 4 ml. of 0.5 % urea to 4 ml. of centrifugate and determining NH_3 by aeration after 2 hours at room temperature. The controls consisted of similar systems in which the centrifugates had been heated at 100° before addition of urea, or in which water was taken in place of the centrifugates.

Uricase was detected in the residues obtained by centrifuging the suspensions or in suspensions of whole animals by suspending the precipitates in 10 ml. of 0.1 % uric acid solution (prepared by dissolving 1 g. of uric acid in 500 ml. of boiling saturated aqueous Li_2CO_3 , cooling and diluting to 1000 ml.), heating part of the suspension at 100° for 5 min., cooling and determining the uric acid content of the heated and unheated systems after 24 and 48 hours at 37°.

Uric acid, when present in the aqueous extracts of the animals, was determined colorimetrically, by the original method of Folin and Denis, using a reagent not containing molybdc acid.¹ The reagent did not give any coloration with phenols, but gave positive reactions with sulphides and substances containing the SH group. The interfering effect of sulphides and thiol compounds is however only apparent and may lead to misleading results only when the fresh

¹ Merck's sodium tungstate is suitable, without purification, for preparation of the reagent.

extracts are studied; thus, a solution of Na_2S which gave a coloration corresponding with 1.2 mg. of uric acid per ml. immediately after preparation gave no coloration whatsoever after incubation at 37° for 18 hours. Analogous results were obtained with alloxan and with thiolacetic acid. In practice therefore the coloration given by the extracts after 24 hours at 37° may be assumed to be due chiefly, if not exclusively, to uric acid.

The presence of uric acid was also established in certain cases by positive murexide reactions given by the dry residues of deproteinised extracts.

Determinations of uric acid in the extracts were also performed as follows: the solutions were made feebly acid with acetic acid and boiled, the filtrates were made alkaline with Na_2CO_3 (roughly to p_{H} 9), and 0.2 g. of dry ox-kidney uricase (prepared by a method of Truszkowski and Gubermanówna [unpublished]) was added to a portion of the solution. The solutions, with and without uricase, were incubated at 37° for 24 hours, and uric acid was determined; in most cases the systems containing uricase gave no coloration, but where a positive reaction persisted the uric acid content was taken as being proportional to the differences in intensity of coloration of the two systems.

In view of the specificity of the action of uricase, it must be considered that the method described is one of the most trustworthy, both for the detection and for the determination of uric acid and has the further merits of simplicity and economy of time.

RESULTS.

1. *Crayfish* (*Potamobius astacus*). Only the liver was examined; the results (Table I) are in accordance with the findings of Truszkowski and Goldmanówna [1933] that uricase is present. Other enzymes were not investigated in this case.

Table I.

Animal	mg. uric acid per ml.			Uric acid content in mg. per g. of animals	mg. of NH ₃			
	In control	Oxidised after			In 4 ml. of extract of 0.5% urea	In 4 ml. of extract of 0.5% urea	Produced	
		24 hours	48 hours					
Crayfish (liver)	0.35	0.15	0.25	—	—	—	—	—
Black-beetle (<i>Blatta orientalis</i>)	0.35	0	0	2.4	0.9	1.2	0.2	0.1
Cockroach (<i>Blattella germanica</i>)	0.32	0	0	15	0.4	0.8	0.2	0.2
May-beetle (<i>Melolontha</i>)	0.41	0	0	6.85	—	—	—	—
<i>Aphis</i>	0.32	0	0	0	—	—	—	—
House-fly (<i>M. domestica</i>)	0.28	0.08	0.23	0	1.3	2.9	1.0	0.6
Blow-fly (<i>M. carnaria</i>)	{ 0.2 0.28	{ 0.12 0.11	{ 0.2 0.28	0	—	—	—	—
Honey-bee (<i>Apis mellifica</i>)	0.31	0	0	0	—	—	—	—
Earthworm (<i>Lumbricus</i>)	0.4	0	0	0	—	—	—	—
<i>Anodonta</i>								
foot }	0.41	0.18	0.21	0	0.63	1.56	0.82	0.11
mantle }	0.41	0	0		—	—	—	—
<i>Helix pomatia</i>	0.39	0	0	+	0.85	3.52	1.2	1.47
<i>Limnaea stagnalis</i>	0.22	0	0	—	—	—	—	—

2. *Black-beetle* (*Blatta orientalis*). Uricase and urease were not present. In this, as in all the remaining cases, xanthine oxidase was not found. The aqueous extract contained uric acid, the content of which was roughly determined as follows: 8 individuals, weighing 2 g., were ground up with 25 ml. of water, and the uric acid content of the filtrate was determined in this manner: 5 ml. of filtrate gave a coloration corresponding with 0.19 mg. of uric acid per ml. after 24 hours at 37°, whilst after incubation for the same length of time with uricase no coloration was given, whence the content of uric acid per g. of insect was calculated to be 2.4 mg. The actual value is probably considerably higher, as it is doubtful whether all the uric acid was extracted under the above conditions.

An extract (in 10 ml. of boiling saturated aqueous Li_2CO_3) of 5 mg. of faeces collected from five beetles fed for 4 weeks on potatoes and beetroots gave only a very faint reaction for uric acid (<0.015 mg. per ml.); assuming the faeces to have the same uric acid content as the entire insects, 0.012 mg. might be expected. A larger quantity of faeces, accumulated over a period of several months, gave a uric acid content of 2.1 mg. per g.

3. *Cockroach* (*Blattella germanica*). Uricase and xanthine oxidase were not found. The uric acid content of these insects is higher than that of black-beetles. Ten insects, weighing 0.6 g., were ground up with 15 ml. of water; the uric acid content of the deproteinised filtrate was 0.6 mg./ml. corresponding with 15 mg. of uric acid per g. of live wt. Owing to the difficulty of accumulating the faeces of this insect in sufficient quantity, the presence of uric acid in the excreta was established only qualitatively.

4. *May-beetle* (*Melolontha*). The specimens were collected in Polesie (East Poland) in June, and were sent by post to Warsaw, where they arrived in a moribund condition. They were examined only for uricase and uric acid; uricase was absent, and the aqueous extract contained an amount of uric acid corresponding with 6.85 mg. per g. of insect.

5. *Aphides*. The specimens were taken from nasturtium plants growing in window-boxes in Warsaw. Uricase, xanthine oxidase and uric acid were not found.

6. *House-fly* (*M. domestica*). Various species of *Musca* were taken, without any attempts at closer identification. The uricolytic activity of the residue after centrifuging the extract was quite considerable and traces of urease were present. Uric acid was not found in the extracts, indicating either that it is not a metabolite of these insects, or that, if formed, it is completely decomposed by uricase. Owing to the difficulty in obtaining centrifugates completely free from uricase, it was not attempted to detect xanthine oxidase in this and in the succeeding cases.

7. *Blow-fly* (*M. carnaria*). As in the preceding case, closer identification was not attempted. The uricolytic activity of these insects is even greater than is that of house-flies; it is probable that the activity of the preparation of uricase from this source surpasses that of any other studied up to the present. Uric acid was absent as in the case of house-flies.

8. *Honey-bee* (*Apis mellifica*). Worker-bees in good condition were taken. Uricase was not found, but the extracts gave only a very faint reaction for uric acid.

9. *Earthworm* (*Lumbricus*). Specimens from garden soil were examined by grinding with sand and water. Uricase, xanthine oxidase and uric acid were not found.

10. *Anodonta*. These were collected from semi-stagnant water near Warsaw. Uricase was present in the foot, but not in the mantle (the same results were obtained by Truszkowski and Goldmanówna [1933]), xanthine oxidase was

not found and urease is probably absent. Uric acid was not present in the extracts.

11. *Helix pomatia*. Uricase and xanthine oxidase were not found, urease and uric acid were present. These results are in agreement with those of Przylęcki [1922; 1926] and of Baldwin and Needham [1934].

12. *Limnaea stagnalis*. The specimens were taken from pools in the vicinity of Warsaw. Uricase and xanthine oxidase were not found.

DISCUSSION.

The findings for the somewhat heterogeneous group of invertebrates studied do not support the view that uric acid is invariably an end-product of the nitrogenous metabolism of insects; the notable exceptions were the house-fly and the blow-fly, which possess high degrees of uricolytic activity and which probably surpass any other known source of uricase in this respect. The same insects possess appreciable quantities of urease, which is absent from the other animals studied. The type of metabolism corresponding with these findings is one which would enable the flies to derive energy from the waste products of other animals, and this is in accordance with the mode of life of these insects and with their capacity of dealing with the most varied nutritive substances.

Amongst the Insecta uric acid was found in appreciable amount in the black-beetle, cockroach and May-beetle, the highest value (15 mg. per g.) being found for the cockroach. In view of the absence of xanthine oxidase and of uricase, the uricotelic type of metabolism is the most probable for these insects.

Certain of the animals examined possessed neither uricase nor uric acid (*Aphides*, earthworm, *Limnaea stagnalis*); it must be supposed that the nitrogenous metabolism of these animals is one in which uric acid is not an intermediate or final product. The position of the honey-bee is somewhat uncertain, since uricase is absent but only traces of uric acid are present; this animal requires closer investigation.

The type of nitrogenous metabolism corresponding with the enzymic equipment of *Anodonta* would be one in which uric acid is not a normal metabolite, although uricase is present. Truszkowski [1928] expressed the view, based on determinations of purine nitrogen before and after autolysis of the tissues of this animal, that the end-product of their purine metabolism is xanthine; the present findings support this opinion.

Finally, none of the animals examined gave evidence of the presence of xanthine oxidase, from which it might be concluded that uric acid in the invertebrates has a different origin from that of mammals.

SUMMARY.

1. Xanthine oxidase was not found in any of the phyla examined.
2. House- and blow-flies were found to be strongly, and crayfish and fresh-water mussels feebly, uricolytic; uricase was absent from the black-beetle, cockroach, May-beetle, honey-bee, *Aphis*, earthworm and Roman and pond snails.
3. A simple and trustworthy colorimetric procedure for the detection and determination of uric acid in presence of substances also reducing Folin's reagent has been described.
4. Uric acid was found in the extracts of the black-beetle, cockroach, May-beetle, honey-bee and Roman snail and in the excreta of the first two animals.
5. Urease was present in the house-fly and the Roman snail.

6. A uricotelic nitrogenous metabolism is probable, on the basis of the above findings, for the black-beetle, cockroach and May-beetle and for the Roman snail.

7. Przyłęcki's rule [1925] that uricotelic organisms do not possess uricase is confirmed.

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REFERENCES.

- Baldwin and Needham (1934). *Biochem. J.* **28**, 1372.
Needham (1935). *Biochem. J.* **29**, 238.
Przyłęcki (1922). *Arch. int. Physiol.* **20**, 103.
—— (1925). *Arch. int. Physiol.* **24**, 317.
—— (1926). *Arch. int. Physiol.* **27**, 159.
Truszkowski (1928). *Biochem. J.* **22**, 1299.
—— and Goldmanówna (1933). *Biochem. J.* **27**, 612.