CLXI. THE PURINOLYTIC ENZYMES OF THE LEECH (*HIRUDO MEDICINALIS*) AND THE FRESH-WATER MUSSEL (*ANODONTA*).

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INTRODUCTION.

URIC acid is in almost every case a stage or else the final product of purine metabolism within the living organism. Certain cases have, however, been reported where this stage appears not to enter. Thus Ulpiani and Cingaloni [1906] found that certain bacteria derived from poultry excreta degrade aminopurines in a different manner, involving the formation of guanidine; in this case, therefore, neither uric acid nor allantoin can be amongst the products of purinolysis.

Certain invertebrate forms are known [Przylecki, 1926], *e.g.* leeches and fresh-water mussels, amongst the final products of whose purine metabolism uric acid or allantoin cannot be found. In the case of leeches uric acid added to their tissues is not oxidised, whilst *Anodonta* is able quantitatively to destroy added uric acid. Two explanations of these phenomena might be given. One would be that the purine catabolism of these animals runs a course similar to that of Ulpiani and Cingaloni's bacteria, whilst the other would be that purine catabolism stops short at some stage prior to the appearance of uric acid. The first supposition would involve a fall in the purine-N content of the tissues after autolysis of over 20 %; in the second case the loss of purine-N due to autolysis should not exceed 20 % if both guanase and adenase are present, or some smaller figure if only one of these enzymes exists, and would be nil if neither of these ferments is possessed by these animals.

EXPERIMENTAL.

A. *Leeches*. These animals were first placed in concentrated sodium chloride solution, with the object of forcing them to disgorge any blood present in the alimentary canal, and were then washed, roughly dried with filter paper, and comminuted using either scissors or a mincing machine. Four portions of 50 g. each of the well-mixed mince were weighed out and placed in litre flasks, to which 500 cc. of water were added. Toluene was added to two flasks, which were left in a thermostat at 37° for about two weeks. Sulphuric acid to 5 %

was then added and the contents of the flasks were boiled under reflux for 4 hours, after which total- and purine-N were determined by the method of Krüger and Schittenhelm, as described in a previous paper [Truszkowski, 1926]. A portion of mince was also weighed out for the determination of dry content, being dried at 110° to constant weight. The contents of the remaining two flasks were hydrolysed immediately. The results are given in Table I, each value being the mean of two determinations. The dry content varies from 19.25 to 27.2 %, average 22.5 %, and the total-N content from 2.718 to

Table I. *Total- and purine-N contents of leeches and fresh-water mussels before and after autolysis.*

Material No.	% solid substance	% N live wt	% N dry wt	Before autolysis		Days autolysis	After autolysis		% diminution in purine N
				Purine-N mg. per 100 g. live wt	Purine-N mg. per 100 g. dry wt		Purine-N mg. per 100 g. live wt	Purine-N mg. per 100 g. dry wt	
Leeches 1	21.15	2.718	12.85	68.4	323.6	17	63.0	280.2	7.9
2	19.25	2.344	12.18	48.0	249.1	15	36.3	189.2	24.4
3	27.2	3.675	13.53	53.8	197.7	16	44.2	161.8	17.8
Mean	22.5	2.912	12.85	56.7	256.8	—	47.8	210.4	16.7
Mussels 1	12.7	1.179	9.27	36.2	284.8	—	—	—	—
2	13.0	1.093	8.41	34.0	261.5	15	28.1	216.5	17.4
3	12.75	0.963	7.55	36.0	282.6	16	31.8	249.5	11.7
4	11.1	1.001	9.10	34.6	314.3	13	29.8	270.8	13.9
Mean	12.39	1.059	8.58	35.2	285.8	—	29.9	245.6	14.3

3.675 %, average 2.912 %. Considerable variations are found in purine-N, from 48.0 to 68.4 mg. per 100 g., mean 56.7 mg. per 100 g. After autolysis (15–17 days) the mean purine-N content falls to 47.8 mg. per 100 g. giving a mean loss of 16.7 %. This indicates deamination of both aminopurines present. The absence of uniformity of the results obtained is probably due to the incomplete elimination of blood from the alimentary tract.

B. *Fresh-water mussels.* These were taken from clay-pits in the vicinity of Warsaw at various seasons of the year, from spring to autumn; all belonged to various sub-types of the genus *Anodonta*. After separation from the shell, they were allowed to drain for a few minutes, and passed through a mincing machine. The well-mixed mince was then treated as above for the determination of dry content, total- and purine-N. In the determination of purine-N, however, it was found that Krüger and Schittenhelm's method was not, without certain modifications, applicable. This was due to the extraordinarily high calcium content of these animals; thus in No. 2, the filtrate after acid hydrolysis contained 1.4 g. of calcium oxide, amounting to over 21 % of the dry weight of tissue taken and the residue from hydrolysis contained a further quantity of insoluble calcium sulphate. This calcium, whilst soluble in strongly acid solution, begins to separate as a flocculent precipitate at about p_H 4, precipitation being complete at p_H 8, at which p_H sodium hydrogen sulphite

and copper sulphate are added for the precipitation of purines, and accompanies the purines through all the subsequent stages of the determination. Its harmful influence is particularly noticeable in the dissolution of the purine-copper double salt by means of sodium hydrosulphide, when, even after adding comparatively very large quantities of the sulphide, copper is continually found in the filtrate. This effect appears to be due to preferential formation of calcium sulphide or hydrosulphide.

This source of error was eliminated by the addition of excess ammonium oxalate to the hydrolysate, after its neutralisation and feeble acidification with acetic acid.

The dry content of *Anodonta* (Table I) varies from 11.1 to 13.0 %, mean value 12.4 %. The nitrogen content varies from 0.963 to 1.179 %, mean value 1.059 %, and the purine-N content from 34 to 36 mg. per 100 g., mean 35.2 mg. per 100 g. After autolysis (13–16) days the mean purine-N content fell to 29.9 mg. per 100 g., a decrease of 14.3 %. It appears therefore that in this case, too, both guanase and adenase are present.

DISCUSSION.

Since both leeches and fresh-water mussels are capable of deaminising guanine and adenine, but do not produce uric acid, it follows that in these animals, purine catabolism stops at some stage short of uric acid. That these animals do not degrade purines in some other way not involving deamination and the production of uric acid is indicated by the limited loss of purine-N during autolysis.

It hence follows that the end-product of purine catabolism of leeches and *Anodonta* must be xanthine and/or hypoxanthine. Przylecki's classification [1926] of leeches as uricostatic-uriconegative forms and of *Anodonta* as uricolytic-uriconegative forms is thus confirmed.

SUMMARY.

1. Purine-N has been determined before and after autolysis of the tissues of leeches and of fresh-water mussels.

2. The results obtained indicate that the end-product of their purine catabolism is xanthine and/or hypoxanthine.

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