XANTHINE OXIDASE AND URICASE IN LIVER AND KIDNEY OF SOME AMPHIBIANS

by

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Since the distribution of xanthine oxidase in the organs of the frog *Rana hexadactyla* is somewhat unusual[1] it was decided to extent observations on purine catabolism to the tissues of some other amphibians. Liver and kidney homogenates (1:10) were tested for the presence of xanthine oxidase and of uricase. Xanthine oxidase activity was followed as before[1] in the THUNBERG experiment in the presence of PALITZSCH's borate buffer (pH 7.6) so that 40 mg of tissue acted on 0.5 mg of alkali-dissolved xanthine in a total volume of 2.4 ml, containing 1 ml buffer and 0.5 ml methylene blue (1:5000). Uricase activity was detected by assaying the uric acid[2] remaining after a test of 2.5 ml containing 0.5 mg uric acid (phosphate-dissolved[2]) was incubated at room temperature (29–31° C) with homogenate of 40 mg fresh tissue in the presence of 1.5 ml borate buffer (pH 8.69) and chloroform, the mixture being kept well shaken.

As in the case of *R. hexadactyla*, xanthine oxidase was found absent from one day old refrigerated liver homogenates of the burrowing frog *Cacopus systoma* and of the bull frog *R. tigrina* (specimens of the latter available only after chloroform poisoning). The enzyme was, however, present in the liver of the toad, *Bufo melanostictus*, even after the animal had been starved for five days. It was present in the kidneys of all these animals. Enzymes for purine breakdown are evidently not identically distributed in all amphibians.

This conclusion was confirmed by observations on the distribution of uricase, for no uricase action could be detected in fresh liver homogenates of eight different specimens of *R. hexadactyla*, while liver preparations of *R. tigrina* and of *B. melanostictus* in corresponding tests had completely oxidised the uric acid after one hour. *Cacopus* liver strongly interfered with uric acid assay and so could not be tested for uricase activity without a manometer. Kidney homogenates of all the four amphibians completely oxidised the uric acid within an hour. Liver of *R. hexadactyla* contains no uricase inhibitor, since addition of liver homogenate to kidney homogenate, either immediately or half an hour before the substrate, did not affect the extent of uric acid oxidation (36%) after six minutes of incubation. It is of interest to note that in the case of *R. esculenta*, the liver has actually been reported[3] to be slightly more urico-oxidising than the kidney. *R. hexadactyla* constitutes an exception to BALDWIN's statement[4] that uricase "occurs in the livers of animals which are not uricotelic".

The example of *R. tigrina*, as also of dog (where liver lacks xanthine oxidase[5] but contains uricase[3]) shows that inability of liver to oxidise xanthine does not necessarily mean an analogous incapacity for the subsequent oxidation of uric acid. Such cases indicate that different organs, and not merely different enzymes and different parts of one cell, may cooperate in the step by step degradation of one metabolite.

Borate has been shown[6] to be slightly inhibitory to xanthine oxidase. Since the present experiments were performed with borate buffer (appr. 0.07 *M* final concentration, pH 7.6), its action on xanthine oxidase was compared to that of SØRENSEN's phosphate buffer (0.028 *M* final concentration, pH 7.4), using frog kidney homogenate as enzyme. In one experiment the decolourisation time in presence of borate was 19 min, of phosphate 30 min, of water 16 min; in another experiment with weaker enzyme the times were 1 h, 2 h, and 1 h respectively. Hence any significant inhibition to xanthine oxidase under the conditions of these experiments could be excluded, while a weaker molar concentration of phosphate was much more inhibitory.

REFERENCES

1 H. C. FRIEDMANN, *Biochim. Biophys. Acta*, 11 (1953) 585.
2 S. R. BENEDICT AND E. FRANKE, *J. Biol. Chem.*, 52 (1922) 387.
3 R. TRUSZKOWSKI AND C. GOLDANÓWNA, *Biochem. J.*, 27 (1933) 612.
4 E. BALDWIN, *Dynamic Aspects of Biochemistry*, Cambridge University Press, 1949, p. 101.
5 E. J. MORGAN, *Biochem. J.*, 20 (1926) 1282.
6 A. ROUSH AND E. R. NORRIS, *Arch. Biochem. Biophys.*, 29 (1951) 344.

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