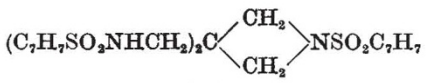
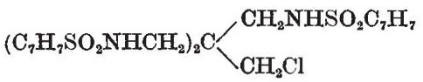
Methylation of the tetramine readily yields the octa-methyl compound, $C(CH_2N(CH_3)_2)_4$, which even with an excess of methyl iodide gives the bi-quaternary iodide $C(CH_2N(CH_3)_2)_2(CH_2N(CH_3)_3I)_2$: our attempts to prepare the quadri-quaternary iodide, $C(CH_2N(CH_3)_3I)_4$ have not at present succeeded.

The preparation of the tetrasulphonamide (I) gives a small quantity of a trisulphonamide, $C_{26}H_{31}N_3O_6S_3$, of m.p. 214°, as a by-product. This compound has apparently the 4-membered ring structure (IV) : hydrolysis with sulphuric acid opens the ring and gives the monohydroxy-triamine (V). Hydrochloric acid also

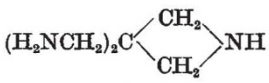


(IV)

(V)



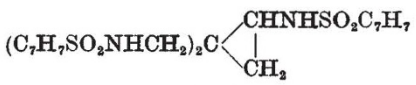
(VI)



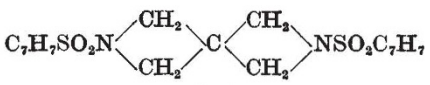
(VII)

opens the ring giving the chloro-compound (VI), which on complete hydrolysis followed by steam distillation undergoes ring closure again to give the monocyclic triamine (VII).

Monoacetyl-tribromo-pentaerythritol, $C(CH_2Br)_3(CH_2OCOCH_3)$, when heated with sodium *p*-toluene sulphonamide, gives two derivatives. The first is a trisulphonamide, of m.p. 171°, isomeric with (IV), and having apparently the *cyclo*-propane structure (VIII) : the second is the spirocyclic disulphonamide (IX), of m.p. 185°. Both (VIII) and (IX) on hydrolysis



(VIII)



(IX)

with hydrochloric acid followed by steam distillation give the spirocyclic diamine (III). The constitution which we have provisionally allotted to the isomeric compounds (IV) and (VIII) is supported by the fact that their sodium derivatives on treatment with benzyl bromide give a dibenzyl and a tribenzyl derivative respectively.

The tetramine $C(CH_2NH_2)_4$ is now being used to investigate the stereochemistry of its spirocyclic organic and complex metallic derivatives.

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[1] *Proc. Roy. Acad. Sci. Amsterdam*, **37**, 156 (1934).
[2] *Rec. Trav. Chim.*, **57**, 265 (1938).

Purified Uricase

IN the attempt to isolate the enzyme uricase from pig liver, I have obtained a preparation 550 times purer than the enzyme powder used as starting material. The purified preparation contains 0·15–0·18 per cent Fe, 14·4 per cent N, traces of copper, and no cobalt or manganese. It is almost colourless, and therefore is not a hæmin derivative. It is insoluble in water, almost insoluble in phosphate buffer *p*H 7·4, and soluble in borate buffer *p*H 10. Truszkowski[1] has already shown that uricase is soluble in alkaline solution.

The highest specific activity obtained, as measured by the oxygen uptake during the oxidation of uric acid in manometers, is 85 c. mm. per mgm. enzyme per minute as compared with a value of 0·15 c. mm. per mgm. per minute for the powder used as starting material. The test is arranged as follows : The main chamber of the manometer vessel contains 1·0 c.c. *M*/5 borate buffer *p*H 9 and 1·5 c.c. enzyme preparation plus water. The potash tube contains 0·2 c.c. 10 per cent caustic potash, and the side bulb 2·24 mgm. uric acid (as *M*/30 lithium urate). The gas space contains pure oxygen and the oxygen uptake is measured at 38° over a period of 30 minutes.

If it is assumed that the catalytic activity of the enzyme is due to the iron (although this has not yet been proved), then 1 mgm. iron brings about the reaction of 57,000 c. mm. oxygen per minute.

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March 24.

[1] Truszkowski, R., *Biochem. J.*, **28**, 62 (1934).

Lactoflavin in the Eyes of Fish

SOME years ago we found[1,2,3] that the retinal pigment epithelium of the eyes of a number of fish contained relatively large amounts of flavin. Liver and kidney, which are known to be the materials richest in flavin, contain about 20 γ flavin per gm. tissue, while an approximate calculation of our results indicated in the pigment epithelium of some fish eyes 500 γ flavin per gm. tissue.

Flavin occurs in tissues almost entirely combined with protein as an enzyme which does not show the characteristic fluorescence of flavin and is not dissociated at neutral reaction. It was, therefore, a surprising observation that flavin of the pigment epithelium showed green fluorescence in water extract and was almost completely dialysable[2,3]. When the eye of a freshly killed fish was frozen in acetone - carbon dioxide mixture and cut by a meridional section the flavin contained by the pigment layer could be directly observed in the fluorescence microscope[4]. The spectrum of the green fluorescence light was identical with that of the flavin. The flavin from haddock eyes was also isolated and identified by Karrer[5] as lactoflavin. From these observations we concluded that flavin in the eye occurred in the free state or in the form of a highly dissociated protein compound but not as flavin enzyme. Thus the eye is the only tissue known which contains flavin mostly or entirely in the free form. Otherwise free flavin occurs only in milk and urine[6].

There was one detail of the problem not clear yet, namely, the question whether flavin in the fish eye originally occurs in the form of lactoflavin or as its