

hormone preparation. Addition of ρ -aminobenzoic acid in concentrations of 2.5 to 10 γ per c.c. did not increase the yeast crop and, in some concentrations, gave a suggestion of slight inhibition, which may not be significant.

The work may be summarized as follows: Cell-free wound hormone preparations from ultra-violet injured yeast cells maintain a considerable degree of their proliferation-promoting potency for yeast in media containing not only the required inorganic constituents and sugar but also containing amino acids, inositol, thiamin, pantothenic acid, biotin, vitamin B₆, riboflavin, uracil, choline, acetyl choline, ethanolamine, nicotinic acid and ρ -aminobenzoic acid. The wound hormone preparation therefore contains active substances in addition to these.

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¹ Loofbourow, Dwyer and Cronin, *Biochem. J.*, **35**, 603 (1941).

² Loofbourow, Cook and Stimson, *NATURE*, **142**, 573 (1938). Cook, Loofbourow and Stimson, *Atti X^o Cong. Intern. Chim.*, **5**, 26 (1939).

³ Reader, *Biochem. J.*, **21**, 901 (1927).

⁴ Cook and Cronin, *Studies Inst. Divi Thomæ*, **3** (1941) in the press.

⁵ Williams and Saunders, *Biochem. J.*, **28**, 1887 (1934).

Bacterial Reduction of Tetrathionate

THE power of reducing nitrate is widespread among micro-organisms, while the reduction of sulphate is restricted to a few species¹. It has long been recognized, however, that many bacteria are able to reduce less highly oxidized inorganic sulphur compounds (tetrathionate, thiosulphate, sulphite, etc.) to hydrogen sulphide². In the course of attempts to improve selective media for isolating *Salmonellas* and *Bact. typhosum* from excreta, we have found that, so far as some organisms are concerned, the tetrathionate is first reduced to thiosulphate. This reaction takes place rapidly and quantitatively, and estimations at different stages show that the molecular concentration of the thiosulphate formed is twice that of the tetrathionate which has disappeared, in agreement with the equation:



The subsequent further reduction of thiosulphate to hydrogen sulphide is, in comparison, so slow that the reduction of tetrathionate can be followed accurately by direct iodometric titration of the thiosulphate, provided that the high acidity developing from accumulation of thiosulphuric acid is controlled by a phosphate buffer.

The ability to reduce tetrathionate is possessed by most of the common *Salmonellas*, such as *Bact. typhosum*, *paratyphosum B* and *C*, *typhi-murium*, *thompson*, *reading* and *dublin*, and by members of the *Proteus* group, but not by most other faecal organisms tested including *Bact. coli*, *Bact. aerogenes* and dysentery bacilli.

Our experiments have been done almost entirely with *Bact. paratyphosum B*. The reaction can be followed in growing broth cultures, or by the use of thick washed suspensions. Unless, however, some

suitable hydrogen donor such as mannitol or glucose is added to the washed suspensions, the reaction proceeds comparatively slowly. When prepared from organisms grown on plain nutrient agar, washed suspensions show an apparent lag phase before the rapid reduction of tetrathionate begins. This lag phase is not present when the organisms have been grown on agar containing tetrathionate. Suspensions grown on plain agar can be 'adapted' to reduce tetrathionate without the initial lag phase by incubation for only three or four hours with tetrathionate and mannitol, without additional source of nitrogen.

The reaction appears to be similar to other known types of anaerobic oxidation processes³ and we have evidence that it involves a hydrogen donor (for example, glucose or mannitol), a specific dehydrogenase, a heat-stable co-enzyme-like factor present in tryptic digest broth and acting as a hydrogen carrier, a reducing enzyme mechanism, and a hydrogen acceptor (tetrathionate). The 'adaptive' effect mentioned above, taken in conjunction with the fact that tetrathionate reduction is restricted to certain organisms, strongly suggests a true acceptor specificity.

The discovery of this system is of interest from several points of view:

(1) It has brought to light certain facts of obvious importance in any attempt to explain the selective action of tetrathionate in media for isolating *Salmonellas*, etc.

It is significant that, in general, organisms which actively reduce tetrathionate are able to grow well in media containing it in relatively high concentration, and that, in competitive growth such as occurs in cultures from faeces, tetrathionate selects those organisms which can reduce it. The reason may be partly that the ability to reduce tetrathionate provides these with a mechanism of energy release not available to other organisms. Further, it is of interest that the substance produced in the bacterial reduction of tetrathionate is thiosulphate, which can be shown to be much less toxic than the tetrathionate from which it is formed. It is not clear, however, whether in mixed cultures this would operate entirely in favour of the tetrathionate reducers.

(2) It provides an interesting example of an oxidation mechanism of a kind known to be present in facultative anaerobes⁴, and especially likely to be of value to tetrathionate reducers under conditions of low oxygen tension.

(3) It affords an opportunity of investigating the important questions of acceptor specificity and enzyme adaptivity.

(4) The simple titration technique, though limited in application, is an accurate and convenient method for the study of enzyme kinetics.

It is intended to publish shortly a fuller account of this work.

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² Beijerinck, M. W., *Z. Bakt.*, Abt. 2, **7**, 93.

³ Green, D. E., Stickland, L. H., and Tarr, H. L. A., *Biochem. J.*, **28**, 1812 (1934).

⁴ Quastel, J. H., Stephenson, M., and Whetham, M. D., *Biochem. J.*, **19**, 305 (1925).