Note on the Manometric Determination of Succinic and α -Ketoglutaric Acids

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Small quantities of succinic and α -ketoglutaric acids, from 0.2 mg. upwards, are now commonly determined manometrically with the help of succinic dehydrogenase (Szent-Györgyi & Gözsy, 1935; Krebs, 1937, 1938). The object of this paper is to draw attention to two sources of errors which we have recently encountered.

Inhibition of succinic dehydrogenase by phosphotungstates

In some determinations of succinate it was noted that the ethereal extract contained a powerful inhibitor of succinic dehydrogenase, resulting in delayed or incomplete oxidation of the succinate. This was found to be phosphotungstate which arises from the tungstate added as a deproteinizing agent and from phosphate present in the tissue preparations. Phosphotungstic acids are known to be readily extractable with ether.

Whilst Na tungstate ($2 \times 10^{-2}M$) has no effect on succinic dehydrogenase, Na phospho-18-tungstate ('A-acid' of Wu, 1920) is a strong inhibitor in relatively low concentrations. For example $0.5 \times 10^{-4}M$ caused an inhibition of 64% (pH 7.4; succinate concentration 0.01M; 40°). Na phospho-24-tungstate, also prepared according to Wu (1920), is likewise an inhibitor, but higher concentrations, about five times that of the 18-acid, are required to produce the same effect.

These inhibitions might be expected as it is known that succinic dehydrogenase possesses SH groups (Hopkins & Morgan, 1938), and that phosphotungstates readily oxidize SH compounds to the disulphides (Folin & Marenzi, 1929; Schöberl & Ludwig, 1937; Schöberl & Rambacher, 1938). The inhibition of succinic dehydrogenase depends on the concentration of succinate. This again is not unexpected since Hopkins, Morgan & Lutwak-Mann (1938) have shown that succinate protects the SH groups of the enzyme against other reagents.

When solutions of tungstate which do not contain phosphate are acidified, practically all the tungstic acid is precipitated, but in the presence of phosphate, tungstic acid in excess of that reacting with protein and similar substances remains in the solution as complex phosphate. The formation of inhibitory

quantities of phosphotungstate can easily be prevented by avoiding an excess of tungstate. A minimum amount, ascertained on a sample of the material, is recommended. We find that 4 ml. of a neutralized tissue suspension containing 0.4 g. of fresh tissue and diluted to 15 ml. with water or washings is satisfactorily deproteinized by the addition of 0.25 ml. of 10% (w/v) Na tungstate and subsequent acidification with H_2SO_4 (blue to congo red). We retain tungstate as a deproteinizing agent because, unlike other deproteinizing agents, it yields filtrates which do not froth when ether is bubbled through the solution at a rapid rate.

Interference of glutamic acid in the determination of α -ketoglutaric acid

$KMnO_4$ in acid solution is used for the oxidation of α -ketoglutaric to succinic acid, and it has been pointed out previously (Krebs, 1938) that several substances which may occur in biological material, such as α -hydroxyglutarate and arginine, also yield succinate when treated with the reagent. When these substances are present α -ketoglutarate must be separated prior to the oxidations. This can be effected by the conversion into the 2:4-dinitrophenyl-hydrazone and ethereal extraction of the latter. In the absence of interfering material a simplified procedure may be adopted, i.e. direct oxidation with $KMnO_4$ of the deproteinized solution (Krebs, 1938; Krebs & Eggleston, 1940).

In recent experiments we found that under some conditions glutamic acid, which is frequently present in biological material, yields succinate on treatment with $KMnO_4$. It is noteworthy that in pure solution glutamic acid does not react significantly. Previous findings on this point (Krebs, 1938) were confirmed by recent tests; it was further found that no oxidation of glutamic acid occurred in mixtures of glutamic acid with α -ketoglutaric acid, or glucose, or pyruvate, or lactate and several amino-acids. But in deproteinized homogenates of liver and other tissues added glutamic acid was found to yield varying amounts of succinate, the maximum being 70% of the theoretically possible value. Deproteinized suspensions of minced liver had little or no effect on the oxidation of glutamic acid.

We have not been successful in identifying the substance in liver and other tissue homogenates responsible for the oxidizability of glutamic acid by acid KMnO_4 . The following substances added to a pure solution of glutamic acid had no effect on the oxidizability: CuSO_4 , FeSO_4 , MnSO_4 , haemin, the substances of rabbit bile, the amino-acids contained in casein hydrolysate.

In view of the widespread occurrence of glutamic acid in biological material the direct treatment of deproteinized solutions with permanganate must be deprecated. We separate α -ketoglutaric from glutamic acid by extraction of the strongly acidified solution with ethyl ether, using a continuous extractor of the Kutscher-Steudel type with the dimensions described by Krebs, Smyth & Evans (1940). The deproteinized filtrate is acidified with 0.1 vol. of 50% (v/v) H_2SO_4 . The rate of extraction is illustrated by the following data. When 5.84 mg. of α -ketoglutaric acid, dissolved in 15 ml. of 2N- H_2SO_4 were extracted, 71% appeared in the extract within 15 min., 90% within 30 min., 95% within 60 min. These figures depend of course on many factors, especially the design of the apparatus and the rate at which ether passes through the solution; it is therefore necessary to check the rate for each

set up. In order to have a safety margin we extract for 2 hr. The ether is then evaporated and the residue is dissolved in a few ml. of water. It is usually divided into 2 parts; in one succinate is determined directly, whilst the other is acidified and treated with an excess of KMnO_4 as described by Krebs & Eggleston (1945).

This procedure eliminates interference by glutamic acid, glutamine and arginine, but if α -hydroxyglutarate is present the dinitrophenylhydrazone method (Krebs, 1938) remains the method of choice.

SUMMARY

1. Succinic dehydrogenase is inhibited by phospho-18-tungstate and phospho-24-tungstate, presumably because these reagents oxidize the SH groups of the enzyme.

2. Glutamic acid, whilst stable in the presence of potassium permanganate in pure solution, is oxidized by this reagent to succinic acid in the presence of certain deproteinized tissue extracts.

3. The above reactions may cause errors in the manometric determination of succinate and α -ketoglutarate. Procedures are described which avoid such errors.

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New Zealand Fish Oils

5. COMPOSITION OF THE FATS OF THE SCHOOL SHARK (*GALEORHINUS AUSTRALIS*, MACLEAY)

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The school or snapper shark (*Galeorhinus australis*) occurs in both New Zealand and Australian waters and, in common with related species found elsewhere, such as the North West Pacific soupfin shark (*G. zyopterus*) (Byers, 1940), the South American tollu (*G. mento*) (Pister, 1936a, b) and the South African soupfin shark (*G. canis* Rond) (Molteno, Rapson, Roux, Schwartz & van Rensburg, 1945) is an im-

portant source of vitamin A-rich liver oil (cf. Davies & Field, 1937; Jowett & Davies, 1938; Cunningham & Slater, 1939). Little, however, is known concerning the composition of oils from these species.

In most work on the fatty acid composition of fish oils from a given species, one sample only has been used so that there is no information as to the extent of the variation between individuals. This may be