reverse swelling[9,14]), thus pointing to a role of oxidative phosphorylation[7,8] in the maintenance of mitochondrial structure. As regards the nature of the substrate, it appeared that succinate was (much) more effective than glutamate, α-ketoglutarate, pyruvate or β-hydroxybutyrate in counteracting the spontaneous and thyroxine-induced swelling.

Glutamate may, in fact, cause an immediate swelling of the fresh particles, especially when diphosphopyridine nucleotide was also present; diphosphopyridine nucleotide ($3 \times 10^{-4} M$) alone was sometimes similarly active[4].

The difference in effect of the various substrates might have been due to the fact that the phosphorylation accompanying succinate oxidation, or an intermediate reaction thereof, is especially involved in the contraction of the mitochondrial membranes[7] and/or to the fact that the corresponding processes of the diphosphopyridine nucleotide-linked oxidations are less stable[15], so that in the latter case the postulated 'change' may occur more readily under the conditions of the present experiments, which are certainly unfavourable to the maintenance of these integrated systems. It appears that the 'change' sets the trigger for swelling, while the actual swelling is dependent upon the presence of substrate, that is, on electron transport in (part of) the respiratory chain[2,3].

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Presence of Protein in Some Serum Filtrates

IN the determination of creatinine in plasma or serum the method of preparation of protein-free filtrates is known[1] to influence the total chromogen content of such fluids. Moreover, varying amounts of non-creatinine chromogen in such filtrates are known[2] to be partly responsible for differences in the total chromogen content.

It is generally assumed that a 1 : 5 'neutral' tungstic acid filtrate (pH $>$ 2·5), prepared as follows: 6 ml. distilled water, 2 ml. serum, 1 ml. 10 per cent sodium tungstate, 1 ml. 0·66 N sulphuric acid, and filtered through a Whatman No. 1 filter paper, is totally free of protein. Yet, it is well known that the precipitation of proteins by tungstic acid and other 'protein precipitants' consists of a series of changes in the protein molecule, which may, under certain conditions, be reversible. For it often happens that more than one denatured protein is formed by the action of these protein precipitants on a solution containing different amounts of albumins and globulins. Moreover, the isoelectric points of these proteins are different, so that a certain proportion of the metaprotein so formed may remain in solution at any given reaction. Further, these changes often consist in the opening up and extension of the highly organized coiled polypeptide chains of the native proteins which effect their viscosity, particle-size and solubility, etc.

Thus, a 'neutral' tungstate filtrate contains a small amount of protein (3·08 mgm./100 ml. serum) which can be precipitated by 5 per cent phosphotungstic acid in 2 N hydrochloric acid (1 ml. to 5 ml. filtrate). A 1 : 10 tungstate filtrate, prepared as follows: 7 ml. distilled water, 1 ml. serum, 1 ml. 10 per cent sodium tungstate and 1 ml. 0·66 N sulphuric acid, contains no such detectable protein. It is evident, therefore, that a 1 : 5 'neutral' filtrate is not entirely 'protein-free' but contains some material which has escaped total precipitation or has been held in solution under the conditions of precipitation. Further, it is possible that other low molecular weight carbohydrate-containing proteins, particularly the mucopolysaccharides, may also be present in plasma or serum filtrates, as some of the acid mucopolysaccharides have a very acid isoelectric point.

The protein precipitated by phosphotungstic acid has a concentration of 0·08 mgm./100 ml. serum (in terms of creatinine) in normal pooled sera. Moreover, since protein is 'chromogenic' to alkaline picrate solution, it is possible that some filtrates may contain small amounts of protein which may be related to the so-called 'interfering material' (non-creatinine chromogen) in the determination of creatinine by the Jaffé reaction in biological fluids. For this figure is in fairly good agreement with that found by Owen, Iggo, Scandrett and Stewart[3] using the NC-creatinine destroying bacteria of Miller and Dubos[4] for a 'neutral' tungstate filtrate (1 : 5). Further, Lloyd's reagent, which is used to remove the 'interfering material' from a serum filtrate[3], is also widely used for removing proteins at a pH 2·0–3·0 from protein-containing solutions.

Thus, it should be remembered that the various protein precipitants differ in their ability to produce a plasma or serum filtrate which is completely free of protein, and of low molecular weight protein fragments (proteoses and peptones, etc.) which can react and interfere with the Jaffé reaction.

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