

A final concentration of 0.01–0.02 *M* in the substrate seems to give the best result. When, after fourteen days of cultivation, sodium citrate was added to a medium containing casein digest with a concentration of 120 mgm. nitrogen per litre, an inhibition zone of 17 mm. was reached after seven days. Stimulation by citrate was also shown on media with ammonium sulphate and asparagine as nitrogen sources. In this case also the sodium citrate decreased the lag phase in production of antibiotic and increased the final concentration of antibiotics.

I have also observed a stimulating effect of sodium citrate upon the production of antibiotic by *Marasmius ran. ealis*. The antibiotic agent produced by this fungus seems not to be identical with that obtained from *M. urens*. It has earlier been reported by Koffler *et al.* that sodium citrate exerts a stimulatory effect upon penicillin production, the degree of stimulation varying with the strain used³.

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¹ Melin, E., Wikén, T., and Öblom, K., *Nature*, **159**, 840 (1947).

² Bendz, G., Wallmark, G., and Öblom, K., *Nature*, **162**, 61 (1948).

³ Koffler, H., Knight, S. G., Emerson, R. L., and Burris, R. H., *J. Bact.*, **60**, 549 (1945).

Effects of Oxygen Carriers and Oxygen Tensions on Fluoroacetate Inhibition of Citrate Utilization

AFTER comparing the action of fluoroacetate on the respiration and citrate utilization in pigeon breast muscle and nematode parasites, Massey and Rogers¹ reported that the poison had little action on the pigeon breast muscle. In these experiments, methylene blue, 0.0003–0.0005 *M*, was present in the brei. Later experiments carried out to discover why the fluoroacetate affected the parasite tissue but not the pigeon breast muscle showed that the inhibition obtained was associated with the nature of the oxygen carriers present, and with the oxygen tension at which the experiments were carried out.

Fluoroacetate (0.01 *M*) inhibition of citrate utilization in pigeon breast muscle in air was greatest (100 per cent) when cytochrome *c* (5×10^{-6} *M*) was added. With methylene blue (0.0005 *M*) inhibitions of 20–30 per cent were obtained; without added carrier, the inhibition was about 50 per cent.

The oxygen tension had a profound effect on the inhibition in pigeon breast muscle mince to which no carrier had been added. Under anaerobic conditions, inhibition has not been noted; in air, inhibition was about 50 per cent, and in oxygen about 100 per cent.

These results suggested that there might be a relationship between the oxidation-reduction potential and the efficiency of the fluoroacetate as an inhibitor. Accordingly, the inhibition of citrate utilization in the presence of fluoroacetate (0.01 *M*) was examined at different oxidation-reduction potentials, maintained by electrolysis by the method of Hanke and Katz². At E_h levels below + 50 mV., fluoroacetate caused inhibitions of 0–25 per cent. At levels above + 120 mV., the inhibition varied from 65 to 100 per cent.

It has been suggested by Liébecq and Peters that fluoroacetate itself is not an inhibitor, but is activated and brought into the tricarboxylic acid cycle to form

Gas phase, pre-incubation period	Nitrogen	Oxygen	Oxygen	Oxygen
Gas phase, experimental period	Air	Air	Oxygen	Nitrogen
Inhibition of citrate utilization	26 per cent	86 per cent	100 per cent	Nil

a fluoro-compound, probably fluorocitrate, which causes a 'jamming' of the cycle. Further experiments were carried out to discover whether the oxygen tension affected the formation of the fluoro-compound, or the actual inhibiting reaction, or both. The pigeon breast muscle mince, which contained 0.0025 *M* oxalacetate, with and without 0.0025 *M* fluoroacetate, was pre-incubated for 15 min. under oxygen, or under oxygen-free nitrogen. Thereafter, the citrate utilization, occurring under air, oxygen or nitrogen, during a period of 30 min. was determined. The results, which are shown in the accompanying table, showed that high oxygen tensions favoured both the formation of the fluoro-compound and its activity as an inhibitor.

A full account of this investigation, which was part of the research programme of the Division of Animal Health and Production, Commonwealth Scientific and Industrial Research Organization, will be published elsewhere.

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¹ Massey, V., and Rogers, W. P., *Nature*, **165**, 681 (1950).

² Hanke, M. E., and Katz, Y. J., *Arch. Biochem.*, **2**, 183 (1943).

³ Liébecq, C., and Peters, R. A., *Biochem. Biophys. Acta*, **3**, 215 (1949).

Fractionation of Serum Proteins with a Quaternary Ammonium Detergent

THE fractionation of serum proteins with the aid of quaternary ammonium compounds has met with difficulties, because the detergent/protein ratio must be maintained at a very constant level¹. We have shown that this is no longer necessary if 'Desogen' (methylphenyl dodecyltrimethyl-ammonium-methosulphate, Geigy S.A., Basle) is used. Separation into at least four components can be performed by the following method.

To 2 ml. diluted human serum ((*a*) 1 : 100 in 1.00 per cent sodium chloride for pH 4–8; or (*b*) 1 : 500 in 0.03 per cent sodium chloride for pH 8–11) is added 1 ml. Michaelis buffer² of constant ionic strength 0.1. After mixing, 1 ml. of 0.15 per cent 'Desogen' solution in distilled water is added. A very faint, stable turbidity has completely developed after 15 min., and the transmission is read in a photo-electric colorimeter at 620 m μ .

For the pH range 8–11 the sodium acetate in the Michaelis buffer is replaced by glycine. The minimum admissible detergent/protein ratio is 1.0, the maximum varying from 4.0 at pH 8.5 to about 30 for pH 5.0.

The results are illustrated by the accompanying graph. The first step in the transmission–pH curve