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The Oxidation of Glutathione by a Preparation of Glucose Oxidase from *Aspergillus niger*

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A commercial preparation of glucose oxidase from *Aspergillus niger* (Fermcozyme 653 AM, lot no. 1917; Hughes and Hughes Ltd., Romford, Essex, U.K.) catalyses the reaction:

The catalytic activity is unaffected by dialysis or the presence of 50 mM-EDTA, but is abolished by heating at 90°C for 10 min.

Measurements with an oxygen electrode show that in phosphate-citrate buffer, pH 5.6 (20 mM- Na_2HPO_4), containing 1.6% (v/v) of Fermcozyme, 50 mM-EDTA and 0.005% (w/v) of catalase, 0.25 mol of O_2 is consumed/mol of GSH added. The concomitant disappearance of free thiol can be demonstrated by using 5,5'-dithiobis-(2-nitrobenzoate) (Roberts & Agar, 1971). All the added GSH is recoverable as GSSG, as assayed by a modification of the method of Srivastava & Beutler (1968). The evidence for H_2O_2 as another reaction product is that in the presence of Fermcozyme and peroxidase GSH oxidizes gum guaiacum to a blue compound, the maximum extinction at 610 nm being 40% of that given by an equimolar amount of glucose. These findings are consistent with the equation given above.

The apparent Michaelis-Menten parameters of Fermcozyme for GSH, estimated by the procedure of Wilkinson (1961) from initial rates of O_2 uptake in the presence of excess of catalase and 50 mM-EDTA at pH 5.6, 37°C and about 230 μM - O_2 , are: $K_m = 0.97 \pm 0.04$ (10 mM); $V = 16.3 \pm 0.3$ (10) μmol of O_2 /min per ml of Fermcozyme. In these conditions, the rate of O_2 uptake with 5 mM-cysteine is less than 10% of that with an equimolar concentration of GSH.

When Fermcozyme is saturated with glucose (110 mM) or GSH (5 mM), the ratio v (glucose)/ v (GSH) is 11. The corresponding ratio for a purer preparation of glucose oxidase from *Aspergillus niger* (Glucose Oxidase 250, lot no. 6X; Hughes and

Hughes Ltd.) is 440. Therefore Fermcozyme may contain an enzyme that oxidizes GSH and is not glucose oxidase.

One implication of our finding that may be of practical importance is that, when glucose in the diffusate from a typical haemolysate is measured by using Fermcozyme-peroxidase-gum guaiacum, the GSH also present raises the estimate by about 5%.

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Differences in Triacylglycerols from Acoustic Tissues and Posterior Cranial Blubber of the Narwhal (*Monodon monoceros*)

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Acoustic tissues of the porpoises *Phocoena phocoena* (Varanasi & Malins, 1970b) and *Tursiops gilli* (Varanasi & Malins, 1970a), as well as the Beluga whale (*Delphinapterus leucas*) (Litchfield *et al.*, 1971), contain large amounts of isovalerate (3-methylbutyrate) and long-chain saturated iso acids. However, cranial blubber of *T. gilli*, having no acoustic function, comprises primarily acids characteristic of normal mammalian adipose tissues (Varanasi & Malins, 1971). Moreover, in *T. gilli* large amounts of di-isovaleroyl glycerides in acoustic tissues appear to play a vital role in sound transmission (Varanasi & Malins, 1972).

We analysed in the narwhal (*Monodon monoceros*), an unusual Arctic whale, the triacylglycerols from acoustic tissues at the attachment point of the mandibular waveguide to the bulla. Comparisons were made with triacylglycerols from non-acoustic posterior cranial blubber.

The acoustic tissues and blubber each contained 94.5% of lipid comprised of 82.2% and 85.5% of triacylglycerols respectively. The triacylglycerols were separated and purified, and component acids were analysed by g.l.c. (Varanasi & Malins, 1970b). Triacylglycerols from the acoustic tissues comprised 47.8 mol of isovalerate/100 mol and 18.7 mol of long-chain iso acids (isopentadecanoate, 11.4 mol/100 mol, was the major component)/100 mol. Moreover, straight-chain monounsaturated acids ($\text{C}_{16:1}$ acid, 10.4 mol/100 mol; $\text{C}_{18:1}$ acid, 2.2 mol/100 mol) were

found in significant amounts, and some polyunsaturated acids ($C_{16:2}$ acid, 2.2 mol/100 mol; $C_{16:3}$ acid, 2.8 mol/100 mol) were detected. Triacylglycerols from the blubber contained 4.2 mol of isovalerate/100 mol and 0.9 mol of long-chain iso acids/100 mol, together with large amounts of straight-chain mono-unsaturated acids ($C_{14:1}$ acid, 2.5 mol/100 mol; $C_{16:1}$ acid, 22.9 mol/100 mol; $C_{18:1}$ acid, 23.9 mol/100 mol; $C_{20:1}$ acid, 12.5 mol/100 mol; $C_{22:1}$ acid, 4.6 mol/100 mol) and polyunsaturated acids ($C_{18:2}$ acid, 1.1 mol/100 mol; $C_{20:5}$ acid, 4.8 mol/100 mol; $C_{22:5}$ acid, 2.2 mol/100 mol; $C_{22:6}$ acid, 4.1 mol/100 mol.).

The triacylglycerols from the acoustic tissues contained 66.0% of structures comprising 2 mol of isovalerate and 1 mol of long-chain acid/mol. The diisovaleroyl glycerides were not found in the blubber. Thus the findings strengthen the hypothesis (Varanasi & Malins, 1972) that di-isovaleroyl glycerides are intimately involved in the biosonar of marine cetaceans. Further, the lipid structural requirements for acoustic tissues now appear to be similar for marine cetaceans from both warm and very cold waters.

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The Effect of Local Anaesthetics on Lipolysis in Fat-Cells

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Local anaesthetics are inhibitors of hormone-stimulated lipolysis in rat fat-cells, the most potent being dibucaine (Hales, 1970). The amount of inhibition does not depend on the lipolytic stimulus. With a cell concentration of 55 mg dry wt./ml 1 mM-dibucaine gave 88% inhibition of lipolysis stimulated by adrenaline (10 μ g/ml), theophylline (1 mM) or both together. The effect is reversed by washing the cells. Similar concentrations of dibucaine have little effect on insulin- or adrenaline-stimulated glucose uptake.

Since the action of lipolytic hormones is thought to be mediated by adenylate cyclase, the effect of dibucaine on the concentration of cyclic AMP (adenosine 3':5'-cyclic monophosphate) was in-

vestigated. When the cell content of cyclic AMP was raised by adrenaline, theophylline or the two combined, the addition of 1 mM-dibucaine caused still greater accumulation of cyclic AMP in both cells and incubation medium.

To test the possibility that dibucaine inhibited the lipase, it was added to cells sonicated after lipase activation by adrenaline. At 4 mM dibucaine completely inhibited the adrenaline effect in whole cells, but only partially in the sonicated cells. It was concluded that the main effect of dibucaine is not on adenylate cyclase or the lipase.

Local anaesthetics may interfere with activation of lipolysis by preventing production of an activating factor other than cyclic AMP. The lipase cannot be activated in cell-free systems to a degree comparable with that in whole cells (Hales *et al.*, 1968), even with added protein kinase (Huttunen & Steinberg, 1971). Ionic changes may be necessary for maximum lipase activation. The potencies of various local anaesthetics as lipolytic inhibitors parallel their anaesthetic potencies, and therefore their ability to interfere with ion movements. Ca^{2+} activates the analogous phosphorylase system in muscle (Ozawa & Ebashi, 1967), and the general relationship of Ca^{2+} to the action of cyclic AMP has been discussed (Rasmussen, 1969). Local anaesthetics inhibit Ca^{2+} release from sarcoplasmic reticulum (Johnson & Inesi, 1969). We therefore suggest that hormonal stimulation of lipolysis in adipose tissue requires increased availability of Ca^{2+} for activation of the lipase system, and that local anaesthetics inhibit the stimulation by preventing the necessary movement of Ca^{2+} .

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