The Incorporation of ^{14}C-Acetate into the Lipids of Vacuolated Rat Liver Cells

In earlier studies on the metabolism of liver undergoing cell vacuolation by hypoxia it has been observed that the incorporation of labeled amino-acids into the proteins is impaired in vacuolated rat liver slices. The peptide bond formation is also inhibited, as we have demonstrated in studies on the synthesis of *p*-aminohippurate from *p*-aminobenzoate and glycine *in vitro*[1,2]. On the other hand, glycogen synthesis in vacuolated rat liver slices occurs at a normal rate, with either glucose or fructose as substrate[3]. In order to get a better understanding of the various synthetic reactions in rat liver cells which have undergone vacuolation by hypoxia, we have carried out the present investigation on lipid synthesis and we have measured the incorporation of sodium acetate-1-^{14}C into the phospholipids, the cholesterol and the fatty acids of normal and vacuolated rat liver slices.

Overnight-fasted albino rats, weighing 150–180 g were used throughout. Liver cell vacuolation was induced as described in a previous paper[1], by keeping the animals in a continuously renewed atmosphere of N_2 + 3% O_2 for 2 h. The rats were killed by decapitation: the livers were quickly removed and sliced by hand with a razor. The slices were then incubated in KREBS-HENSELEIT saline[4] with 10 μc of sodium acetate-1-^{14}C and a certain amount of unlabelled sodium acetate to give a final concentration of 0.005 *M*. The incubation was carried out in O_2 + 5% CO_2 at 37°C, in a conventional Warburg apparatus. At the end of the experiment the slices were quickly transferred to a homogenizer tube, and extracted with ethanol-ethyl ether (3:1) as described by COOK[5]. The filtrate was dessicated under N_2 stream, the residue was dissolved in petroleum ether, washed with an aqueous solution of sodium acetate and water, and finally dried over anhydrous Na_2SO_4. The phospholipids were isolated and purified according to JOHNSON and ALBERT[6]. Samples of the final phospholipid solution in chloroform were taken for the measurement of radioactivity and for the determination of phospholipid phosphorus[7,8]. The supernatant solution remaining after precipitation of phospholipids was evaporated to dryness, the residue was redissolved in ethanol and saponified[6] for 5 h in the presence of KOH. Cholesterol was removed, precipitated as the digitonide, washed, and finally dissolved in methanol: part of this solution was plated for the measurement of radioactivity, while separate aliquots were used for the determination of cholesterol by the modified SPERRY-SCHOENHEIMER method[9]. The mixture remaining after the extraction of cholesterol, was acidified with HCl, and the fatty acids were extracted with petroleum ether. The ethereal phase was washed with water, dehydrated over anhydrous Na_2SO_4, and evaporated to dryness under N_2. The fatty acids were dissolved in acetone, and samples were plated for the measurement of radioactivity. Finally, acetone was evaporated, and the fatty acids were determined quantitatively by titration in an ethanolic solution with KOH, and calculated as stearate.

Radioactivity was determined in all the samples after plating aliquots of essentially zero mass on cigarette paper in stainless steel planchets: they were counted with a thin-mica-window G.M. counter, and a conventional scaler. The radioactivities reported are subject to a standard error in counting of no greater than 2%.

An appropriate analysis of variance was carried out on the results of these experiments, which are shown in the Table.

In all the lipid fractions which have been considered, the specific activities increase significantly ($P < 0.05$: phospholipids and fatty acids), or very significantly ($P < 0.01$: cholesterol), at least up to the second hour of incubation. On the contrary, there is no difference between the specific activities of the phospholipids, cholesterol, and fatty acids from normal and vacuolated rat liver slices ($P > 0.05$).

The results reported in the present paper indicate that the incorporation of acetate-1-^{14}C into the lipids occurs at a normal rate in vacuolated rat liver cells. Obviously, the statement applies within the limits of our experimental conditions. It cannot be excluded that lipid synthesis is in some way impaired *in vivo*, as a consequence of a general and transitory reaction to hypoxia, when the rat is kept at the reduced O_2 tension needed to induce vacuolation of liver cells. But an inhibition of lipid synthesis is not a biochemical feature of rat liver cells which have undergone cell vacuolation.

Similarly, glycogen synthesis *in vitro* is normal in vacuolated liver slices. Therefore the impairment in protein synthesis—as revealed by the reduced incorporation of labeled amino acids into the proteins of vacuolated liver cells incubated *in vitro*—cannot be interpreted as a consequence of a general metabolic failure of vacuolated liver cells, but should be considered as a more specific damage whose mechanism is still largely unknown to-day.

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Incorporation of sodium acetate-1-^{14}C into different lipid fractions of normal and vacuolated rat liver slices incubated *in vitro*. Incubation at 37°C in 5.2 ml of Krebs-Henseleit saline, with 10 μc (26 μ*M*) of sodium acetate-1-^{14}C. Phospholipids: c.p.m./10 μg phospholipid phosphorus. Cholesterol: c.p.m./100 μg of cholesterol. Fatty acids: c.p.m./mg of fatty acids, calculated as stearate. Means of 10 experiments ± s.e.m.

Time of incubation	Phospholipids		Cholesterol		Fatty acids	
	normal	vacuolated	normal	vacuolated	normal	vacuolated
1 h	140 ± 15	145 ± 15	237 ± 46	256 ± 65	119 ± 25	157 ± 50
2 h	171 ± 11	180 ± 15	458 ± 72	539 ± 118	215 ± 38	267 ± 74

Riassunto. È stata studiata l'incorporazione dell'acetato-1-^{14}C nei fosfolipidi, nel colesterolo e negli acidi grassi di sezioni di fegato normale e vacuolizzato di ratto. Essa non è variata, in nessuna delle frazioni lipidiche considerate, nelle sezioni di fegato colpito da degenerazione vacuolare. Si discutono i risultati in rapporto alle precedenti osservazioni sulla inibizione dell'incorporazione degli amino acidi marcati nelle proteine delle sezioni di fegato vacuolizzato.

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Inhibitory Effect *in vitro* of some Polysaccharides and of Horse Serum Albumin on Mitochondrial Swelling

It is well known that certain substances, such as ATP, Mg^{++}, EDTA, K^{+1-3}, protect mitochondria, which are suspended in an isotonic saccharose solution, from spontaneous swelling. This property has been demonstrated recently in this Institute by Casu [1] in the case of serotonin (5-hydroxytryptamine).

In this study, the possible inhibitory effect *in vitro* on mitochondrial swelling by certain polysaccharides is examined, such as rabbit liver glycogen, apple pectin, gum Arabic, and by horse serum albumin, the protective effect of which on old or damaged mitochondria has already been shown by other authors [2,3].

These substances were used at various concentrations, and solutions 70, 35, and 17.5 mg of glycogen, pectin, and gum Arabic in 100 ml of 0.25 *M* sucrose were prepared.

The concentrations of albumin in 0.25 *M* sucrose were $1 \cdot 10^{-5}$ *M*, $0.5 \cdot 10^{-5}$ *M*, $0.25 \cdot 10^{-5}$ *M*.

Substances were supplied by: British Drug Houses, Liverpool (England): glycogen; Nutritional Biochemicals Corporation, Cleveland, U.S.A.: pectin and gum Arabic; Istituto Sieroterapico Toscano, Siena (Italy): albumin.

Assays were carried out by reading, on a DU model Beckmann spectrophotometer, the extinction values of the mitochondrial suspensions, judging the degree of swelling by the gradual decrease in optical density, according to Cleland [4].

Mitochondria were obtained by fractioned centrifugation of albino rat liver homogenates, according to Schneider [5]. They were then suspended in 4 ml of 0.25 *M* sucrose. 10% homogenates in 0.25 *M* sucrose were prepared from 1 g of the tissue.

Two containers were used for each experiment, one of which contained 3 ml 0.25 *M* sucrose solution, the other an equal quantity of sucrose solution with substance dissolved in it. All 0.25 *M* sucrose solutions were buffered

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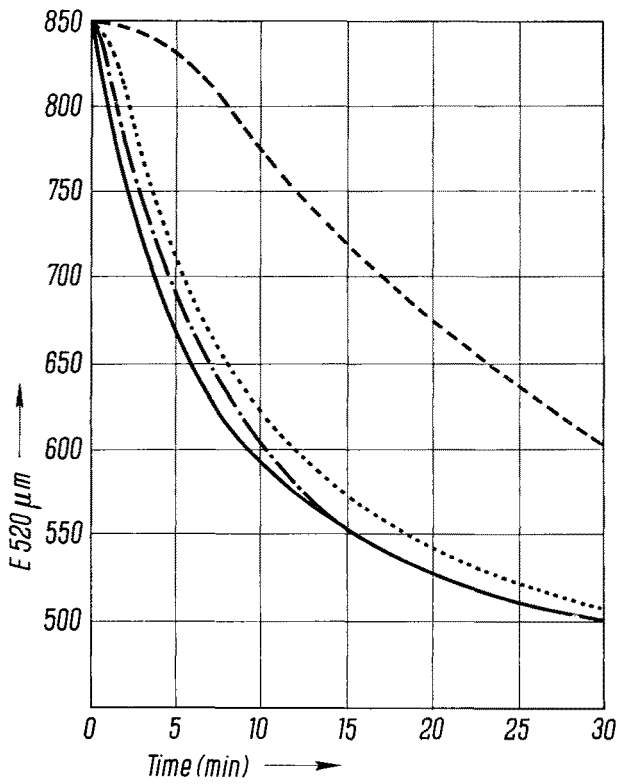

Fig. 1.
Inhibiting effect on mitochondrial swelling produced by glycogen.

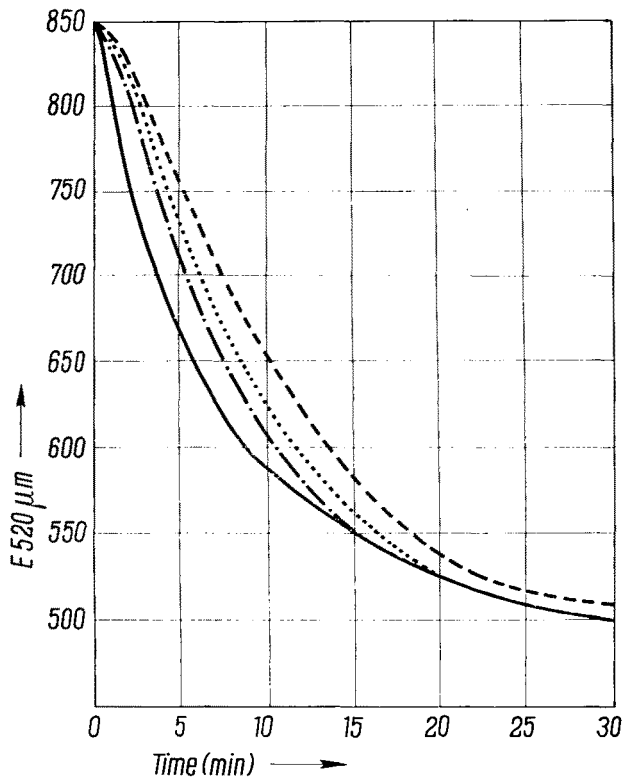

Fig. 2.
Inhibiting effect on mitochondrial swelling produced by pectin.