CYTOPLASMIC LOCATION OF CERTAIN SMALL MOLECULES AFTER THEIR METABOLISM BY THE ENDOPLASMIC RETICULUM OF RAT LIVER

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1. Introduction

In addition to endogenous metabolites, a large number of drugs and other exogenous substances are also metabolized by enzymes bound to the membranes of the liver endoplasmic reticulum (ER) [cf. 1]. Many of these, especially the drugs, eventually appear in the blood and/or the bile. The exact route of migration of exportable proteins is established, and includes the channels of the ER and the Golgi apparatus [2–4]. The intracellular pathway taken by small molecules, on the other hand, is not clarified, but they are generally considered to follow the same route as exocrine proteins. For this reason, *in vitro* and *in vivo* experiments were performed to study the possible routes taken by certain substances metabolized by various associated with the ER.

2. Material and methods

Starved rats weighing 180–200 g were used. Microsomes were prepared as described by Ernster et al. [5]. The medium for ATPase and G6Pase (glucose-6-phosphatase) was adjusted to 0.25 M sucrose and the incubations were performed as before [6]. For G6Pase, deoxycholate was omitted because of its detergent effect on microsomal membranes. After incubation, the medium was supplemented with cold 0.25 M sucrose and centrifuged at 105,000 *g* for 60 min. The supernate was decanted and the pellets suspended in 0.25 M sucrose, which was followed by determination of orthophosphate and ADP. Naphthalene-^{14}C from the Radiochemical Centre, Amersham, was diluted with Ringers solution and injected into the femoral vein (5 μCi/100g). Microsomes and supernate were separated as above, and appropriate aliquots were shaken in plastic tubes in order to remove non-hydroxylated naphthalene [7]. Radioactivity was measured in a toluol scintillator; all vials contained 0.1 ml formic acid. Protein was determined according to Lowry et al. [8].

3. Results and discussion

The distribution of P_i and ADP after incubation in the presence of G6P and ATP, respectively, are shown in fig. 1. In both cases, the products were detected almost exclusively in the medium, indicating the absence of the products in the microsomal lumen. No glucose determination was performed in the G6Pase experiment since it penetrates microsomal membranes [9].

Theoretically, microsomes can be damaged after high-speed centrifugation, resulting in a release of the intravesicular content. Such a rupture would lead to a relocation of intramicrosomal substances to the medium. In order to find out whether any damage occurred, Millipore filtration – instead of centrifugation – was used for the separation of microsomes from the incubation medium (table 1). The filtrate was analyzed for the presence of both P_i and albumin. Similarly to the results in fig. 1, the bulk of P_i released was recovered from the filtrate. On the other hand, the filtrate only contained trace amounts of albumin. Since the albumin of isolated microsomes is present intraluminally, its release requires breakage of the

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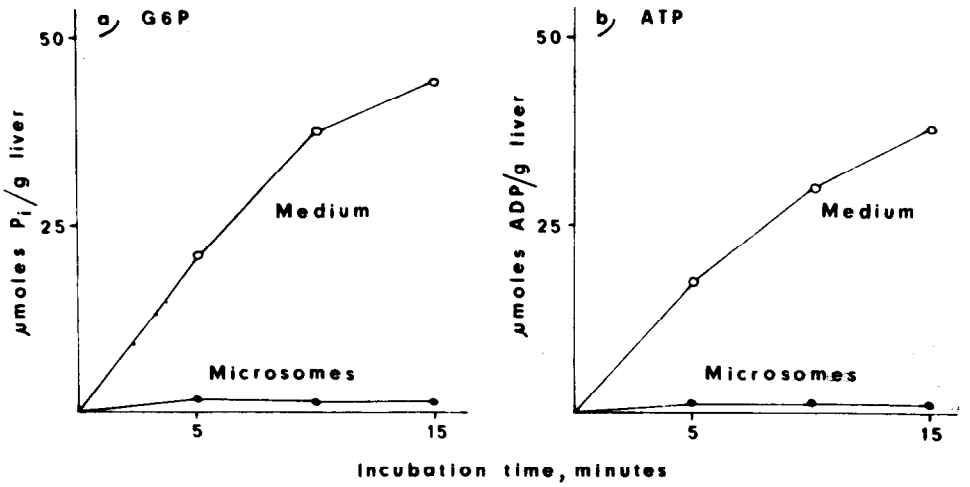

Fig. 1. Appearance of P_i and ADP after G6P and ATP hydrolysis *in vitro*.

membranes [10]. The finding suggests, therefore, that neither incubation nor centrifugation caused rupture in our experiments.

Table 1
Appearance of P_i and albumin in Millipore filtrate after incubation of microsomes in the presence of G6P.

Incubation time (min)	P_i (μmoles/g liver)		Albumin (mg/g liver)	
	Total	Filtrate	Total	Filtrate
10	16.5	14.9	–	–
20	36.2	34.1	0.8	0.15

The incubation medium was filtered by suction pump through a filter of 0.22 μm (Millipore Filter Co., Bedford, Mass.). Albumin was isolated as described earlier [3].

The liver ER is the site of hydroxylation of many drugs and other exogenous substances. After leaving the liver, the metabolized and often conjugated drug is excreted via the bile or urine. In this way drugs can be important in studying the intrahepatic transport of small molecules in an *in vivo* system. The agent employed in such an investigation must fulfil several conditions: (a) the product should not penetrate the microsomal membranes; (b) it should be at the endpoint of a metabolic sequence; (c) it must be identifiable; and (d) it must be separable from the substrate itself. Naphthalene appears to be ideal for this purpose: it is metabolized *in vivo* mainly into 1-naphthol [11]; being a charged molecule, it is not able to penetrate the membrane; it is easily separable from its substrate

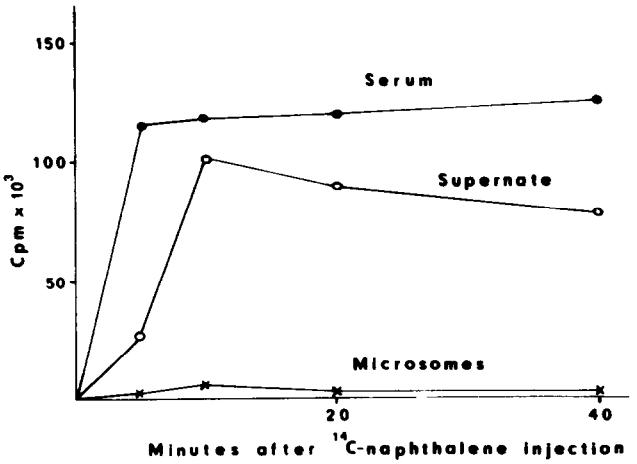

Fig. 2. Transport of hydroxylated naphthalene *in vivo*. The radioactivity in the microsomes and the liver supernate is given for the total amount isolated from one rat. The values for serum were calculated on the assumption of 3.5 ml serum/100 g rat [2].

by using a polyethylene procedure [7], and by making use of its radioactive form it can be identified.

In *in vivo* experiments, the appearance of the hydroxylated product was followed in the microsomes and supernate of the homogenate as well as in serum after injection of naphthalene-^{14}C (fig. 2). A maximal level of radioactivity was reached in both serum and supernate as soon as 10 min after the administration. Only trace amounts of label could be detected in the microsomal pellets.

The experiments described above strongly indicate that small molecule metabolites produced by enzymic systems of the ER do not appear within the channels but are released to the cytoplasmic side. This pathway of transport does not necessarily have generalized validity, but in our case it proved to be the route when studying a drug, a nucleotide and an intermediary component of the carbohydrate metabolism. Therefore, the intracellular transport of a number of metabolites does not follow the pathway described for secretory proteins, e.g. rough ER, smooth ER and Golgi system. Instead, they reach the blood or bile via the cytoplasm.

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