THE METABOLISM OF DRUGS BY SUBFRACTIONS OF HEPATIC MICROSOMES[1]

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A variety of drug metabolizing enzymes are localized in hepatic microsomes (Brodie et al., 1958). The microsome fraction is composed of fragments of the endoplasmic reticulum of which there are at least two types: rough-surfaced (caused by granules rich in RNA) and smooth-surfaced. Certain areas in the hepatic cell contain only one form of reticulum; e.g. Porter and Bruni (1959) state that areas rich in glycogen are associated only with smooth-surfaced reticulum.

We have noted marked changes in glycogen levels associated with changes in microsomal drug-metabolizing enzyme activities (Dixon et al., 1961). It seemed likely that drug-metabolizing enzymes could be localized mainly in the smooth-surfaced reticulum and in the microsomes derived therefrom, rather than be distributed randomly throughout the endoplasmic reticulum.

Smooth-surfaced microsomes can be separated from the rough-surfaced variety in a two-phase system described by Rothschild (1961), which takes advantage of their lower specific gravity; both the rough-surfaced microsomes and the small RNA-rich granules are more dense.

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This difference in specific gravity can even lead to a partial separation of the two kinds of microsomes in a one phase system. Fouts and Brodie (1957) reported hepatic nitro reductase to be localized in both microsomes and soluble fraction. In later research (Fouts, unpublished) it was shown that the "soluble" nitro reductase was in reality associated with light microsomes which sedimented very slowly--the definition of soluble enzyme as one not sedimented at 72,000 x g for one hour being incorrect. Remmer (1960) has also observed certain oxidative enzymes responsible for drug metabolism associated with a microsome fraction of low specific gravity.

The present report gives evidence of the presence of several drug-metabolizing enzymes almost exclusively in smooth-surfaced vesicles derived in all probability from only one kind of endoplasmic reticulum.

Livers (from Dutch, male rabbits) were homogenized in 0.88 M sucrose (1 g. liver ground with 3 ml sucrose in a Potter homogenizer with a plastic pestle), and mitochondria and nuclei removed by centrifugation at 9000 x g for 25 minutes. This supernatant was then diluted with 5 volumes of cold water and layered over 1.31 M sucrose. Two centrifuge rotors have been used at 4° in the model L Spinco ultracentrifuge for separations. Cytologically the following times of centrifugation were equivalent: 10-12 hrs. in type SW39L Spinco rotor or 8 hrs. in type 40 Spinco rotor. The best results were obtained with the following volumes of 1.31 M sucrose as the bottom layer in these rotors: 1 ml in the type SW39L or 2 ml in the type 40.

Electron micrographs of the microsome fractions (total, rough-surfaced, and smooth-surfaced) revealed essentially complete separation of rough from smooth surfaced microsomes. With the type 40 Spinco rotor, the time of centrifugation in the two phase system is critical; electron micrographs showed the smooth-surfaced vesicles were contaminated with the rough-surfaced ones when less than 7 hrs. centrifugation was used, whereas more than 10 hrs. centrifugation caused contamination of rough-surfaced vesicles with smooth. Also the enzyme distribution was altered; too short a centrifugation gave a "smooth-surfaced" microsome fraction with all the drug-metabolizing and TPNH oxidase activity, while too long a time smeared these activities between both "rough- and smooth-membraned" fractions. Centrifugation times were less critical in the swinging bucket rotor.

After centrifugation, the rough-surfaced microsomes were packed in the bottom of the tube, and the smooth-surfaced ones in a layer at the boundary of the two phases; the smooth-surfaced microsomes were aspirated off and sedimented at 140,000 x g for 1 hr. To assay drug enzyme activity, both types of microsomes were resuspended in normal rabbit liver soluble fraction, i.e., 140,000 x g supernatant--at such volume that each ml contained that type of microsomes from 1 g. liver.

Drug metabolisms studied were the side chain oxidation of hexobarbital, the O-dealkylation of codeine, the ring-sulfur oxidation of chlorpromazine, and the N-demethylation of aminopyrine. Disappearance of substrate was measured to follow the metabolisms of hexobarbital (method of Brodie and Axelrod, 1950) and

chlorpromazine (method of Salzman and Brodie, 1956). The appearance of a metabolite was measured to follow the metabolisms of aminopyrine (the metabolite, 4-aminoantipyrine, determined by the method of Cooper and Brodie, 1955) and codeine (the metabolite, morphine, determined by use of a phenol reagent (Snell and Snell, 1937) after extraction from tissue with chloroform). The incubation conditions used were described by McLuen and Fouts (1961). TPNH oxidase was assayed at 25° by the optical density change at 340 mμ of 3 ml of solution containing 2.5 ml tris buffer (pH 7.5, 0.05 M), 0.2 ml microsomes suspended in the same tris buffer, and 0.4 μmoles TPNH. Nitrogen determinations (by Kjeldahl) were made on subfractions resuspended in water.

The drug enzyme and TPNH oxidase activity of smooth-surfaced microsomes was greater than the values for the rough-surfaced ones, i.e., 3 to 5 times with drugs as substrates and 5 times with TPNH as substrate (table 1). The nitrogen content of the 2 types of microsome was roughly equivalent (table 1).

The high concentrations of sucrose used in the separation procedures were shown not to affect the enzyme activity. Fouts (unpublished) had observed that sucrose added <u>in vitro</u> to incubation mixtures at or above a final concentration of 0.4 M inhibited drug metabolism by microsomes plus soluble fraction. Therefore, the total microsome fraction, isolated from a 9000 x g supernatant, was divided and one part resuspended in 1.15% KCl, the other in 1.31 M sucrose. The suspensions were allowed to stand over night at 4°, diluted with an equal volume of water, resedimented, and resuspended in soluble fraction. Comparison of

Table 1

ENZYME ACTIVITIES AND NITROGEN CONTENT OF ROUGH-VS SMOOTH-SURFACED MICROSOMES

Substrate	Enzyme Activity[1,2] in Rough	Smooth	Ratio-Smooth/Rough
	μmoles/g.N/2 hrs	μmoles/g.N/2 hrs	
Chlorpromazine	248 ± 29 (6)	680 ± 160 (6)	2.7
Codeine	112 ± 31 (7)	395 ± 91 (7)	3.5
Hexobarbital	155 ± 32 (6)	633 ± 53 (8)	4.1
Aminopyrine	51 ± 15 (5)	278 ± 82 (5)	5.4
	μmoles/g.N/min.	μmoles/g.N/min.	
TPNH	9.5 ± 4 (6)	47 ± 7 (5)	4.9
	mg/g. liver	mg/g. liver	
Nitrogen	2.3 ± 0.5 (9)	1.9 ± 0.2 (7)	0.83

(1) Drug enzyme activity in μmoles drug disappeared (chlorpromazine and hexobarbital) or metabolite produced (codeine and aminopyrine). The type of metabolic change is given in text.
(2) TPNH oxidase in μmoles TPNH disappeared (decreased absorption at 340mμ). Figures in the table are averages ± standard deviation. Number of assays with each type of microsome is within parenthesis. A different animal was used for each assay.

drug enzyme activity showed no difference in specific activity between these two treatments. Thus, sucrose was not significantly retained by microsomes sedimented from sucrose solutions and was not inhibitory under procedures described in this paper.

Separation of the smooth-surfaced microsomes represents a type of purification of the drug enzyme systems since a significant amount of the total microsome fraction (the rough-surfaced vesicles) can be discarded as relatively inactive. Of more interest will be whether these smooth-surfaced particles offer any advantage over the total microsome fraction in the solubilization

and further purification of the drug enzyme systems. Finally it will be interesting to study this type of microsome both biochemically and morphologically in livers from animals metabolizing drugs at abnormal rates (e.g., after stimulation by phenobarbital, and in conditions where activity is very low or absent as in the newborn animal).

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