Fractionation of microsomal proteins by a non-ionic detergent

A considerable amount of evidence[1,2,3] has been accumulated to show that the combined proteins of the microsome fraction incorporate labelled amino acids more readily than those of any other cell fraction. It would be of great interest to know the nature of the proteins into which this incorporation occurs. Attempts have been made to obtain protein constituents of microsomes by extraction with sodium deoxycholate[4] or sodium chloride followed by sodium hydroxide[5]. The protein fractions which remained associated with ribonucleic acid (RNA) after such treatments were usually found, after short periods of incorporation, to have higher specific radioactivities than those devoid of RNA.

In an attempt to find other significant methods of fractionation of the proteins of the microsome fraction a non-ionic detergent (Lubrol W) was used here as a first treatment. Such an agent was chosen in preference to ionic detergents since it might be expected to be less likely to denature the proteins[6], so that specific protein complexes may be extracted in their original state.

Male Wistar albino rats were killed by perfusion with ice-cold 0.25 *M* sucrose under anaesthesia induced by pentobarbitone sodium. In experiments designed to examine the incorporation of radioactive phenylalanine, the rats were killed by breaking their necks. The livers were quickly removed and immersed in ice-cold 0.25 *M* sucrose solution (time taken: 45 sec). Microsome pellets were prepared according to the technique of LITTLEFIELD *et al.*[4], except that the microsomes were centrifuged from the mitochondrial supernatant liquid which had been diluted with 0.7 volumes of 0.25 *M* sucrose. The microsome pellets were resuspended with the aid of a polythene pestle in Lubrol W dissolved in 0.25 *M* sucrose. They were then centrifuged at 105,000 × *g* for 2 h to give the "detergent pellet", which had the same appearance as the microsome pellet. The supernatant liquid was sometimes freeze-dried and excess detergent was extracted with acetone. All samples were then analysed for RNA and total proteins according to LITTLEFIELD *et al.*[4]. Lipid phosphorus was determined on the chloroform–ethanol–ether extract. The final protein residue was assayed for radioactivity on polythene planchets (1 cm²) in an end-window counter connected to a scaling unit (Type 100C, Panax Equipment Ltd.).

Fig. 1 shows the effect of different concentrations of Lubrol W on the composition of the detergent pellet. It can be seen that, at all concentrations used, virtually all the RNA is recovered in the detergent pellet. The proportion of protein remaining in this pellet reached a plateau at a value of approximately 50% in the presence of detergent concentrations between 0.175% to 2.0%. In contrast the lipid phosphorus fell progressively until, at a concentration of Lubrol W of 1.5%, it reached a value of approximately 20% of that found in the microsome pellet. In the detergent pellet the amount of protein found was approximately 4½ times the amount of RNA. It may be noted that this amount of protein is considerably greater than that which LITTLEFIELD *et al.*[4] observed after treatment of the microsome pellet with sodium deoxycholate.

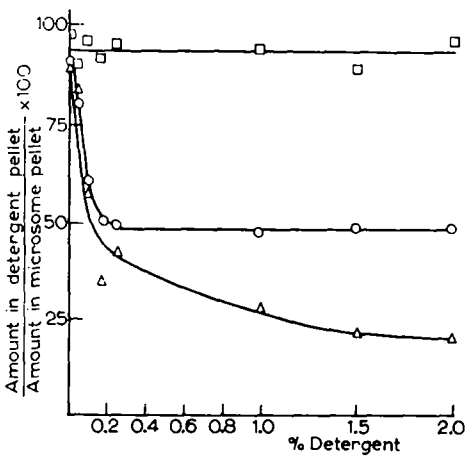

Fig. 1. Fractionation of microsomal constituents by Lubrol W. □—□ RNA; ○—○ protein; △—△ lipid phosphorus.

Table I shows the effect of the length of the period of incorporation on the specific radioactivities of the proteins of the two microsome fractions in comparison with those of the microsomes as a whole and the final supernatant liquid. After periods of 6 min or longer the proteins of the fraction solubilized by Lubrol W had a specific radioactivity approximately twice that of those proteins which remained in association with virtually all the RNA of the microsome pellet. It can also be seen that variations from 0.25% to 1.0% in the concentrations of detergent used did not affect the specific radioactivities of the protein of the detergent pellet or its supernatant liquid. It therefore appeared that, in these experiments, the microsome fraction in which incorporation occurred to the greater extent is less firmly bound to the RNA than the remainder.

When the period of incorporation was reduced to 2 min, the specific radioactivity of the proteins of the detergent pellet was observed to be twice as high as that of the proteins of the supernatant liquid. If the detergent pellet was solubilized by means of sodium deoxycholate and the liquid centrifuged[4], the resulting sediment contained approximately equal amounts of protein and RNA (85–90% of that found in the detergent pellet). The lipid phosphorus present was

TABLE I

SPECIFIC RADIOACTIVITIES OF PROTEINS OF MICROSOMAL FRACTIONS AND SUPERNATANT LIQUID

Body weight (g)	Period (min)	Concn. of detergent (%)	Specific radioactivities (μc/g) of proteins					
			Microsomes	Detergent treatment				Final supernatant liquid
				Pellet	Supernatant liquid	Pellet resuspended in Na deoxycholate		
						Sediment	Supernatant liquid	
350	2*	0.25	0.71	0.90	0.46	1.95	0.39	0.11
335	6	0.25	0.94	0.78	1.33			0.25
		1.0		0.70	1.16			
230	10½**	0.25	2.22	1.34	2.91	1.43	1.38	0.68
333	10½	1.0	2.06	1.07	2.56			0.43
221	31½	0.25	4.59	2.47	5.40			1.53
		1.0		2.45	5.12			
		1.0		2.40	5.85			

Each rat received 0.2 mc/kg of DL-phenylalanine(3-^{14}C) (2 mc/mmole) intraperitoneally, except:
* Injection into tail vein under light ether anaesthesia.
** Injection into femoral vein under light ether anaesthesia.

negligible. It can be seen that these ribonucleoproteins, not solubilized by sodium deoxycholate, were responsible for much of the high initial specific radioactivity of the detergent pellet. In contrast, the lipoproteins of the detergent pellet had a lower specific radioactivity than the proteins of the supernatant liquid obtained by the treatment of the microsomes with Lubrol W. When the time of incorporation was extended to 10.5 min, the two protein fractions produced by the treatment of the detergent pellet with sodium deoxycholate had the same specific radioactivities.

An effect of the non-ionic detergent Lubrol W on microsomes is thus to produce a fraction whose proteins become readily labelled within a few minutes of the administration of phenylalanine-^{14}C.

Acknowledgements. The authors thank Mrs. J. TAPLEY for technical assistance, and Imperial Chemical Industries Ltd. for providing a sample of Lubrol W. The DL-phenylalanine-^{14}C used in this study was prepared by Dr. V. C. E. BURNOP.

This investigation has been supported by grants to the Royal Cancer Hospital and the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

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Received April 15th, 1957