

been isolated from among the products of bacterial action on either glycerol or lactate, although the formation of glucose in cultures of *Poria vaillantii* on a glycerol medium has been reported by Sison and Schubert⁴, while Horecker⁵ has suggested a cyclic mechanism by which this conversion may be effected.

It is proposed to report the work in further detail elsewhere.

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C. P. JACKSON
K. RAMAMURTI

College of Science and Technology,
University of Manchester.

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Distribution of Protein-bound Aminoazo Dyes in the Rat Liver Microsome

EVIDENCE has been obtained that rat liver microsomes are the site where the formation of protein-bound aminoazo dyes occurs¹⁻³. On the other hand, one of the most important parts played by the microsome is known to be protein synthesis. Littlefield *et al.*⁴ further fractionated the microsome by means of sodium deoxycholate and pointed out that the protein in the deoxycholate-insoluble fraction, ribonucleoprotein particles, showed a particularly high turn-over rate, suggesting that protein is in fact synthesized in this fraction. This suggestion was supported by other authors⁵⁻⁷.

In investigating the carcinogenesis due to aminoazo dyes, it is an inevitable question whether the protein-bound dye is detectable even in the fraction with such a high turn-over rate as the microsome ribonucleoprotein fraction. Despite the fact that Arcos and Arcos⁸ are inclined to the view that no protein-bound dye was found in this fraction, we repeated a similar experiment but on a somewhat larger scale, since their evidence seemed to be inconclusive.

Rats which were fed 3'-methyl-4-dimethylaminoazobenzene coated on rice (0.06 per cent) were killed after 2 weeks when the protein-bound dye is considered to be a maximum. The liver microsomes were prepared according to Schneider's method⁹ and washed once with 0.25 M sucrose solution. The undimented fluid was designated as 'supernatant fraction'. The microsomes were further fractionated by means of sodium deoxycholate as proposed by Littlefield *et al.*⁴. Thus we obtained three fractions, namely, deoxycholate-insoluble fraction (ribonucleoprotein fraction) and deoxycholate-soluble fraction from microsomes and the supernatant fraction. A small portion of each fraction was withheld for the protein⁹ and ribonucleic acid⁴ assays and the rest was subjected to protein-bound dye (polar dye) determination^{10,11}.

The results are summarized in Table 1. This clearly shows that a definite amount of protein-bound dye is detected in the ribonucleoprotein fraction. The total amount of this protein-bound dye in the ribonucleoprotein fraction is obviously lower than that in the other fractions. If, however, the protein-bound dye concentration is expressed on a per unit

Table 1. DISTRIBUTION OF POLAR DYE AMONG CELL FRACTION

Exp.	Fraction	Microsome deoxycholate		Supernatant
		-insoluble	-soluble	
Exp. 1	Liver used (gm.)	87		
	Protein (mgm.)	105	750	
	Ribonucleic acid (mgm.)	63	3.0	
	Polar dye (γ)*	2.6	27	
Exp. 2	Polar dye (γ)/100 mgm. protein	2.5	3.6	
	Liver used (gm.)	60		
	Protein (mgm.)	65	530	2,700
	Ribonucleic acid (mgm.)	56	18	
	Polar dye (γ)*	2.0	15	93
	Polar dye (γ)/100 mgm. protein	3.1	2.8	3.4

* The amount of polar dye is expressed in terms of DAB tentatively.

mgm. protein basis, the figures for the three fractions stand very closely to each other.

The significance of the finding must await further experiments, which are now being carried out here.

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T. YAMADA
M. MATSUMOTO
S. KANDA
H. TERAYAMA

Department of Biophysics and Biochemistry,
Faculty of Science,
University of Tokyo,
Tokyo.

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Water-soluble, Non-fibrous Deoxyribonucleoprotein from Calf Thymus Nuclei

A WATER-SOLUBLE, non-viscous form of deoxyribonucleoprotein has been isolated from purified calf thymus nuclei by a process that minimizes enzyme degradation. Previously the only preparation of deoxyribonucleoprotein generally accepted as undegraded has been the fibrous one, forming gels in water and only slightly soluble, with a molecular weight of 17 million¹.

Fresh calf thymus glands are frozen immediately on removal from the animal and ground to a powder in the frozen state in the presence of 'dry ice'. Only this procedure has been found to produce intact, round nuclei, as confirmed by microscopic examination, without excessive clumping, when the ground material is suspended in the purification medium at 0-4° C.

The purification medium is modified from those of Douce *et al.*² and Philpot and Stanier³, and is composed of 0.44 M sucrose, 40 per cent glycerol, 0.039 M sodium glycerophosphate, 0.019 M citrate buffer (pH 6.1, ionic strength 0.15).