vitro of corticosterone as compared to adrenals with the "normal" high lipid content. Since the cholesterol concentrations of DBA mice resemble those of the AC (Fig. 1), it remains to be seen whether the phenomena of low adrenal cholesterol and adrenal lipid depletion have a common genetic cause. Vicari reported (*13*) lower adrenocortical sudanophilia in young DBA mice than in young C57 mice.

The biochemical mechanism controlling ester concentration in the adrenal is still obscure. There is good evidence that blood supplies the bulk of metabolically active cholesterol and that any synthesis in the gland itself contributes relatively little (*14*). Shima and Pincus (*15*) have noted a rapid synthesis and secretion of radioactive corticosterone by the adrenals of rats infused with [7α-^{3}H]cholesterol. Nevertheless, a consideration of adrenal ester concentrations in relation to the plasma concentrations in Table 2 makes it clear that some specific sterol ester-concentrating mechanism must control the concentrations in the adrenal independently of the prevailing concentrations of cholesterol in the plasma. Dexter *et al.* (*16*) have shown that ACTH stimulates the accumulation of cholesterol by the rat adrenal when the utilization of cholesterol was blocked at the same time.

The chief interest of the findings in Fig. 1 derives from the opportunity to employ genetic analysis in the problem of correlating adrenal hormone production with cholesterol concentrations. There is a large difference between DBA/2J and C57BL/10J mice in the capacity of the liver to generate reduced nicotinamide adenine dinucleotide phosphate with implications for a number of major metabolic pathways (*17*). Bartke (*18*) has observed a much lower ratio of ester to free cholesterol in the testes of DBA than in those of C57 mice. These findings can be exploited in the study of the relation of biochemical and endocrine characters by determining their intercorrelation in segregating generations. Genetic analysis could be used as a tool for the study of the interrelationship of a number of biochemical and physiological systems which would be difficult to approach by other means.

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Similarity and Limited Multiplicity of Membrane Proteins from Rough and Smooth Endoplasmic Reticulum

Abstract. *During electrophoresis on acrylamide gel 30 to 45 percent of the protein of hepatic microsomal membranes migrates as a single band corresponding to a molecular weight of 52,000. Rough and smooth microsomal membranes exhibit essentially identical electrophoretic patterns. Different findings by previous workers may be the result of contamination of the membranes by adsorbed and entrapped nonmembrane protein.*

Recent studies (*1, 2*) suggest that most animal cell membranes contain many different proteins instead of a single predominant protein, as had been proposed earlier (*3*). Some of these studies also indicate that there is considerable variation in the protein composition of different membranes, even when closely related membranes from the same tissue are compared. However, an alternative possibility that these findings represent contamination of the membranes by extraneous proteins has not been excluded.

Indeed, evidence from our laboratory and others (*4, 5*) suggests that hepatic microsomal membranes, as normally isolated, contain relatively large quantities of protein that do not appear to be required for their structural or functional integrity. A procedure for selectively removing these proteins with salt and detergent has been briefly described (*4*). We have examined the protein composition of membranes both before and after removal of these proteins.

Rough and smooth microsomes, isolated from rat liver by the method of Dallner *et al.* (*6*), were washed sequentially with 0.14*M* sodium chloride, 1.0*M* sodium chloride, and 0.1*M* sodium carbonate with 0.1*M* sodium bicarbonate (8 ml of each solution per fraction from 1 g of liver). This procedure removes membrane-attached ribosomes and large amounts of adsorbed protein without extracting significant amounts of phospholipid or several microsomal enzymes that have been examined (*4*). Finally, in order to remove protein that appears to be trapped within the closed vesicles, we washed the membranes once with half the above volume of 0.075 percent sodium deoxycholate, *p*H 7.6.

Unwashed and washed microsomal fractions were analyzed by electrophoresis on polyacrylamide gel in a sodium dodecyl sulfate (SDS) system by means of a modification of a meth-

od developed by Maizel *et al.* (*7*) as modified by Kiehn *et al.* (*2*). The buffer for electrophoresis consisted of 0.1*M* tris(hydroxymethyl)aminomethane (tris) acetate buffer (*p*H 9.0), 1.0 percent SDS, 0.1 percent β-mercaptoethanol, and 0.01 percent ethylenediaminetetraacetate (EDTA). The polyacrylamide gels were polymerized in 0.1*M* tris acetate buffer (*p*H 9.0), 1.0 percent SDS, 2.0*M* urea, and 0.001 percent EDTA. The polymerizing agents were 5.0 percent acrylamide, 0.133 percent bis acrylamide, 0.05 percent ammonium persulfate, and 0.05 percent *N,N,N′,N′*-tetramethylethylenediamine. The membranes were solubilized in a solution consisting of 0.01*M* tris acetate buffer (*p*H 9.0), 1.0 percent SDS, 0.001 percent EDTA, 2.0*M* urea, and 0.1 percent β-mercaptoethanol; the solution was adjusted to 10 percent sucrose before being applied directly to gels 100 mm by 5 mm. After electrophoresis at 1 ma per gel for 4 hours, the gels were stained for 3 hours with a solution of 1.0 percent amido black, 7.0 percent acetic acid, and 40.0 percent ethanol, and destained overnight in a solution of 7.0 percent acetic acid and 40.0 percent ethanol.

Samples were run in duplicate with cytochrome c added to one sample to provide a reference point for obtaining an estimate of molecular weights by the method of Shapiro *et al.* (*8*).

The relative amount of protein in each electrophoretic band was estimated from scans of absorbancy of the gel at 570 nm. As backgrounds, the absorbancy of the gel in the region ahead of the fastest migrating material or of several of the lowest troughs in the absorbancy scan were used.

The electrophoretic patterns of rough and smooth microsomes before and after the washing procedure are shown in Fig. 1. The very complex pattern of the unwashed rough microsomes, which is probably due in part to the presence of heterogeneous ribosomal protein and adsorbed cytoplasmic protein, serves to illustrate the high resolving power of the electrophoretic method as adapted for this study. The clear difference between the patterns of the unwashed rough and smooth microsomes confirms that separation of the two different cell fractions has been achieved. In contrast to the unwashed preparations, the patterns of the washed membranes from rough and smooth microsomes are essentially identical (Fig. 1b). This result provides strong additional evidence in support of earlier proposals (*9*) that the two membranes are closely related.

Recent studies of membranes prepared from rough microsomes by other methods (*1, 2*) found more complex patterns that were clearly different from the pattern of smooth microsomal membranes. We have confirmed these findings despite the fact that we obtained low yields and considerable variability in the patterns of the membranes prepared by one of these methods (*2*). The reason for the difference between these and the present results therefore seems to reside in the method for preparing the membranes. We feel that the present method is more efficient and selective in its ability to remove extraneous proteins from the membranes.

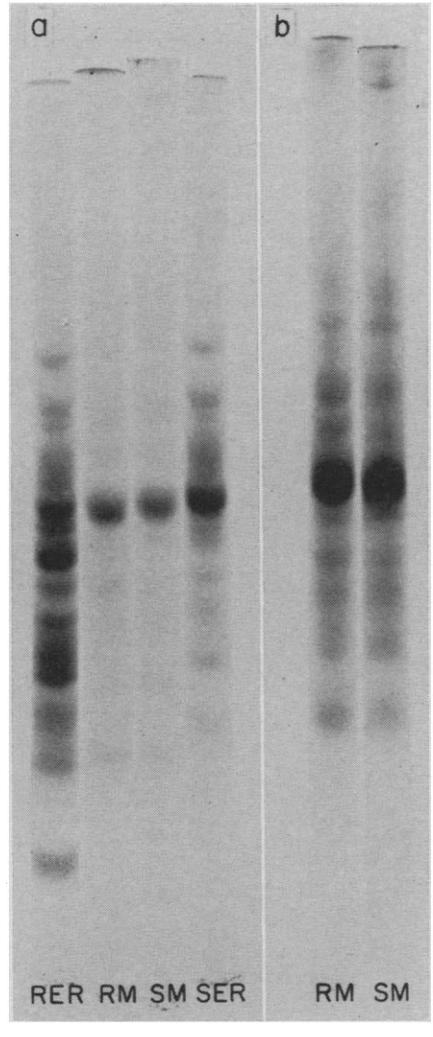

Fig. 1. Acrylamide gel electrophoresis of rough endoplasmic reticulum (*RER*), smooth endoplasmic reticulum (*SER*), and the washed membrane preparations from these two fractions (*RM* and *SM*). The gels of the membrane preparations in (a) and (b) contained 25 and 80 μg of protein, respectively.

The similarity in the patterns of the unwashed and washed preparations of smooth microsomal membrane (Fig. 1) provides further evidence that the membrane itself is not significantly altered by the washing procedure. The major change occurring during the washing steps is the disappearance of protein migrating just behind the major band, which corresponds in molecular weight to rat serum albumin. This protein is known to be present in significant amounts in smooth microsomes (*10*).

Finally, both washed membrane preparations exhibit a single predominant band whose molecular weight has been estimated at 52,000. Integration of scans of the gels, against the two different backgrounds, indicates that this band contains 30 percent of the total membrane protein or 45 percent of the protein migrating in the gel as distinct bands. This distribution, as well as the shape of the overall patterns, was observed to remain essentially unchanged when the amount of protein applied to the gels was varied between 25 and 100 μg.

We still have not rigorously proven that this band represents a single protein component of the membranes nor have we determined to what extent the results of this study apply to other cell membranes. Despite these limitations, our results tend to support the earlier (*3*) but now controversial conclusion that a single protein constitutes a major structural element of at least certain membranes.

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