slowly, indicating that degradation of DDT was proceeding beyond these two metabolites. Others have suggested a number of other degradative products, most of which do not have chlorine atoms on the ethane or ethene portion of the molecule and which are considerably more polar (*13*, *14*). The loss of chlorine, if accompanied by an increased polarity, may account for a loss in electron sensitivity. The analysis by gas chromatography of a sample of pure DDA synthesized in our laboratory failed to detect a peak even at high concentrations, indicating that this carboxylic acid is electron-insensitive.

The possible role of reduced porphyrins in the degradation of DDT (*6*, *8*) is of interest because, with repeated freezing and thawing, the erythrocytes would be hemolyzed, exposing the insecticide to a high concentration of free hemoglobin. Experiments in which we added purified hemoglobin to chicken serum showed that degradation proceeded at approximately the same rate, the results being quite similar to those shown in Fig. 1. We have also mixed DDT with serum samples showing a small amount of hemolysis and have observed an initial decline in the concentrations of *o,p'*-DDT and *p,p'*-DDT for approximately 2 weeks, after which the plotted line was parallel to the base line for the remainder of the experiment.

Considering the above results and those reported for degradation by microorganisms (*2–6*), we question the concept of enzymatic dechlorination of DDT. Possibly, tissues and microorganisms which contain large quantities of reduced coenzymes, porphyrins, and other metalloproteins could carry out these degradative steps by a simple chemical redox reaction.

D. J. ECOBICHON
P. W. SASCHENBRECKER
Department of Physiology and Pharmacology, University of Guelph, Ontario, Canada

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1. Abbreviations: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; *o,p'*-DDT, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane.
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Plasma Membranes of Rat Liver: Isolation of Lipoprotein Macromolecules

Abstract. *Three high-density lipoprotein classes and one protein were separated from rat-liver plasma membranes that had been treated with mild sonic oscillation. The lipoproteins were separated and identified by techniques in which ultracentrifugation was used. Enzyme markers and electron-microscopic examination revealed membrane preparations essentially free of contaminating cellular particulates.*

Electron micrographs of the unit membrane structure of various cells led several investigators to postulate that these membranes were constructed of layers of lipids and proteins (*1*). Chemical analyses of membranes from atypical cells, such as erythrocytes (*2*) and Schwann cells (*3*), suggested that they consist of lipids and proteins, which exist as bimolecular leaflets. A new structure for membranes in general, consisting of base pieces with lipoproteins attached, was proposed by Green and Perdue (*4*).

Lipoprotein macromolecules of plasma membranes from rat liver can be isolated and separated by the classical procedures for flotation of lipoproteins by increasing density gradients. Their presence can be verified in the optical ultracentrifuge (*5*), as well as by the chemical analytical procedures that give their lipid (*6*, *7*) and protein components (*8*).

Plasma membranes were isolated from rat liver by a modification of the procedure of Neville (*9*), in which an additional sucrose density (*d*) gradient [$d = 1.18$ g/ml (*7*)] that efficiently separates contaminating mitochondria from the plasma membranes was used.

The bulk of each sample consisted of membranes of various lengths and configurations (Fig. 1). A considerable portion of this membranous material consisted of pairs of elongated membranes that possessed, at intervals, small, dense regions resembling the desmosomes of liver parenchymal cells. The fractions also contained an occasional swollen mitochondrion with fragmented cristae, and a small amount of amorphous material and small vesicles of unknown origin. Microsomes and free ribosomes were virtually absent.

Several enzyme markers were determined on fresh membrane preparations. To show enrichment of the membrane preparations, we determined the activity of the adenosine triphosphatase dependent upon Na^+, K^+, and Mg^{2+} (*10*). We measured the activities of three mitochondrial enzymes [cytochrome *c* oxidase (*11*), monoamine oxidase (*12*), and malate dehydrogenase (*13*)] to determine the possible presence of mitochondria and the different mitochondrial membranes. We measured the activity of glucose-6-phosphatase (*14*) to determine the presence of rough endoplasmic reticulum.

Isolated membranes from ten preparations (200 g of fresh rat liver) were washed with 0.001*M* sodium bicarbonate buffer (*p*H 7.5). Membranes were resuspended in 0.02*M* phosphate buffer (*p*H 7.5, ionic strength 0.05) and treated with sonic oscillation for 90 seconds (M.S.E. ultrasonic disintegrator, 60 watt, 20 kc/sec). The cloudy suspension of membranes became a clear opalescent solution from which only small amounts of debris could be removed by centrifugation at 10,000*g* (12,500 rev/min) in the Spinco No. 40 rotor for 30 minutes. The resulting supernatant fluid will be referred to as the "sonicated" supernatant (SS).

The lipoproteins in the SS were separated into three classes based upon their flotation in KBr solutions of different densities. Suitable samples were reserved for extraction of total lipid with a mixture of one part methanol and two parts chloroform. The densities of all solutions were measured at 25°C with a micropycnometer. Preparative separations were done in the Spinco Model L ultracentrifuge, No. 50 rotor at 50,000 rev/min for 19 hours, 45 minutes. The top 2.5 to 3.0 ml of floating material was removed and analyzed

in the Spinco Model E at 31,410 rev/min in a double-sectored cell. The typical inverted boundaries produced by lipoproteins (since their refractive-index gradients are negative) were observed in all cases.

The SS containing the lipoproteins (in 0.02*M* phosphate buffer) released by sonic oscillation was adjusted to a solution density of 1.125 g/ml first with crystalline KBr and, in additional experiments on new membrane preparations, with crystalline sucrose. The density of the infranatant fluid from the preparative centrifugation was adjusted to 1.170 g/ml, and that of the infranatant from this separation was adjusted to 1.210 g/ml. Samples to be analyzed for total protein were removed at each step before analytical ultracentrifugation.

The isolated and concentrated membranes had greater activities of Na^+-K^+-Mg^{2+} adenosine triphosphatase (typical mean from ten experiments equals 30 μmole of P_i released per milligram of protein per hour at 37°C) than were obtained for the whole homogenate. For example, 4.6 μmole of P_i were released from 1 mg of homogenate protein as compared to 58 μmole of P_i released from 1 mg of membrane protein, under the conditions of assay for this enzyme. The 12-fold increase in specific activity of the Na^+-K^+-Mg^{2+} adenosine triphosphatase indicates a purification of the plasma membrane preparations. That no cytochrome *c* oxidase, no monoamine oxidase nor malate dehydrogenase activity could be measured in isolated membranes, indicates that there were few, if any, mitochondria, or fragments from ruptured mitochondria. When an additional sucrose gradient was used, those mitochondria which remained suspended were layered at the interface between the solution containing the original mixture of sucrose ($d = 1.34$ g of sucrose per milliliter) mixed with the membrane suspension (final $d = 1.22$ g of mixture per milliliter), and the sucrose gradient above ($d = 1.18$ g of sucrose per milliliter). The membranes, which have a pale tan color, were concentrated at the interface between the sucrose gradient $d = 1.18$ and the overlayer of sucrose ($d = 1.16$ g/ml). The zone between the two interfaces was clear and free of visible particles. (These discontinuous gradients were obtained with SW 39 rotor tubes in the swinging-bucket rotor at 30,000 rev/min for 75 minutes in the Spinco Model L preparative ultracentrifuge.)

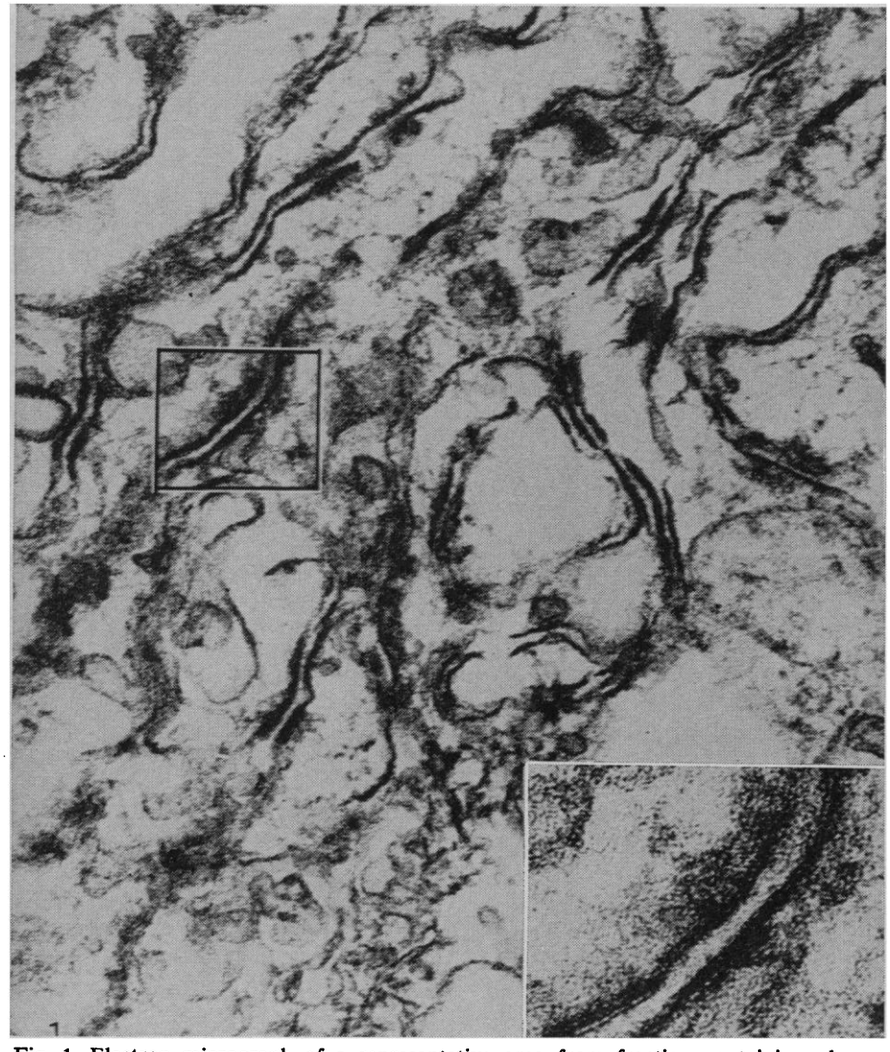

Fig. 1. Electron micrograph of a representative area from fraction containing plasma membranes isolated from rat liver, showing long lengths of plasma membrane interrupted at intervals by dense regions interpreted as desmosomes. Material was fixed 1 hour in the cold, buffered with 1 percent osmium tetroxide, left overnight in 1.6 percent formalin in cacodylate buffer, and treated with 0.5 percent uranyl acetate (*15*) to reveal unit membranes; it was then dehydrated in alcohols and embedded in Epon 812. The section was stained with lead citrate. Magnification, × 36,510; inset, enlargement of a desmosome showing unit membranes (× 98,010).

Whereas no activity of cytochrome *c* oxidase was measurable in the layer containing membranes, substantial activity was measured in the layer below (that is, between $d = 1.22$ g/ml and $d = 1.18$ g of sucrose per milliliter). No glucose-6-phosphatase activity could be measured in either layer.

Figure 2 shows the three classes of lipoproteins at the densities employed with KBr. Figure 2A illustrates the type of inverted schlieren boundary obtained at a solution density of 1.107 g/ml after the usual redistribution of the salt during the preparative ultracentrifugation at a starting solution density of 1.125 g/ml. This boundary began rather sharply but tended to move upward (from the bottom of the cell at the right toward the top of the cell at the left) quite rapidly with some diffusion. Six to eight minutes after a speed of 31,410 rev/min was reached the entire boundary appeared. This class had a negative sedimentation, or flotation, rate (S_f) of $14.2_{(1.107)}$.

The lipoproteins floating in a preparative solution density of 1.170 g/ml and removed subsequently for analytical ultracentrifugation are shown in Fig. 2B. The solution density analyzed in the Model E was 1.155 g/ml ($<$ 1.170 g/ml). This class produced a much sharper boundary that diffused

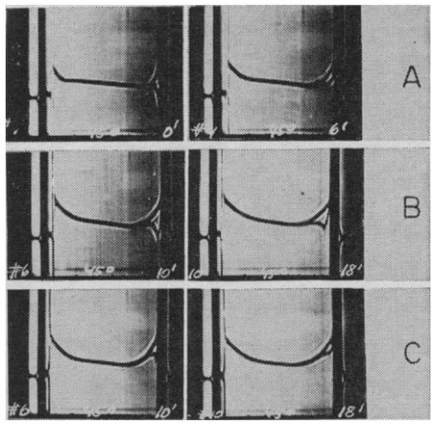

Fig. 2. Analytical ultracentrifuge photographs of the three lipoprotein components separated from plasma membranes treated with sonic oscillation followed by fractionation in KBr gradients. Movement of boundaries is from right to left, in the centripetal direction. Speed was 31,410 rev/min in a double-sectored cell. Schlieren diaphragm angle is 45° for all pictures taken at different times (0, 6, 10, and 18 minutes) after a speed of 31,410 rev/min was attained.

less rapidly and that floated more slowly than did the component shown in Fig. 2A. The total boundary was seen after 16 to 18 minutes of centrifugation at 31,410 rev/min. This class had an S_f of $9.1_{(1.155)}$.

Figure 2C shows the class of lipoproteins obtained after preparative centrifugation of the infranatant from above, at a solution density of 1.210 g/ml. The final solution density was 1.193 g/ml, and the lipoprotein had an S_f of $5.1_{(1.193)}$. Recentrifugation of solutions containing the lipoproteins caused no visible alterations in the boundaries at any of the densities.

We dialyzed the final infranatant solution ($d = 1.210$ g of KBr per milliliter) against 0.15*M* NaCl to remove the large amount of KBr. Only one sedimenting component was observed in the analytical ultracentrifuge; its sedimentation coefficient was 2.6. When the infranatant was dialyzed against phosphate-NaCl buffer (*p*H 7.5, ionic strength 0.20), only one sharp boundary was obtained in moving-boundary electrophoresis. It had a mobility of -4.87×10^{-5} cm^2 volt^{-1} sec^{-1}.

Isolated plasma membranes, not treated with sonic oscillation, routinely contained 25 percent protein that was soluble in 0.15*M* NaCl and that had the same sedimentation coefficient (2.6) as the single protein remaining in the ultracentrifugal residue ($d = > 1.21$ g/ml) from membranes treated with sonic oscillation (SS). The remaining 75 percent of protein from untreated membranes was best solubilized by 1 percent sodium dodecylsulfate. When this protein was subjected to ultracentrifugation, three sedimenting components were resolved during the sedimentation procedure.

Lipoprotein macromolecules can be isolated from plasma membranes of liver from the rat, when suitable, meticulous procedures are employed. No low-density lipoproteins (those that would float in solution density of < 1.0635 g/ml) were ever observed. All the lipoproteins isolated were high-density and had different ratios of lipid to protein. Current experiments suggest that those lipoproteins floating in solution density of 1.125 g/ml have a higher ratio of lipid to protein than the other two classes.

M. Barclay, R. K. Barclay
E. S. Essner, V. P. Skipski
O. Terebus-Kekish

Divisions of Experimental Chemotherapy and Cytology, Sloan-Kettering Institute for Cancer Research, New York, and *Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York 10021*

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Steroid-Sensitive Single Neurons in Rat Hypothalamus and Midbrain: Identification by Microelectrophoresis

Abstract. *Minute amounts of Na-dexamethasone-21-phosphate administered by microelectrophoresis to the immediate extracellular environment promptly suppressed electrical activity of 15 out of 115 hypothalamic and mesencephalic neurons, the effect being readily reversible. Such neurons marked with fast green were found to lie in circumscribed areas of the periventricular gray of the third ventricle and aqueduct, and may represent a site of action of adrenocortical steroids in the regulation of corticotrophin releasing factor and/or adrenocorticotropin secretion by negative feedback.*

Studies using electrical stimulation (*1*), placement of electrolytic lesions (*2*), pituitary transplants (*3*), and especially implantation of steroid cristals (*4*) have implicated several hypothalamic and midbrain structures in the negative feedback exerted by adrenocortical steroids upon the synthesis and/or release of adrenocorticotropin (*5*). In particular, the basomedial hypothalamus (nuclei arcuatus and ventromedialis) and the mesencephalic reticular formation seem to be involved. While the results so obtained are remarkably consistent if species variations and limitations inherent in these experimental approaches are taken into account, there is still no agreement on the *exact* location of the presumed cerebral steroid receptors. Furthermore, little information is available on the kinetics of these feedback reactions, most experiments being of necessity carried out in chronic preparations which do not necessarily reflect short-term physiological interactions. The use of more refined techniques operating on the level of the single neuron (*6*, *7*) therefore appears to be in order.

The technique of microelectrophore-