# Increased Cholesterol Biosynthesis Following Phenobarbital Induced Hypertrophy of Agranular Endoplasmic Reticulum in Liver.* (30396)

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It has been suggested by Christensen and Fawcett(1) that the enzymes necessary for cholesterol biosynthesis are associated with the smooth endoplasmic reticulum. This suggestion was based largely upon their observation of the prominence of agranular reticulum in interstitial cells of the opposum testis, and on the biochemical study of Morris and Chaikoff(2), who demonstrated that in the rat a considerable percentage of the cholesterol utilized for production of testicular steroids is synthesized in the testis. Other endocrine organs known to produce substantial amounts of cholesterol such as the adrenal(3) and corpus luteum(4), have been shown to have correspondingly large quantities of smooth reticulum(5,6,7), whereas the hamster adrenal gland which is exceptional in that it does not appear to produce cholesterol(8,9) contains a relatively small amount of agranular reticulum(5).

Of particular significance in relation to the present study is the report of Bucher and McGarrahan(10) that the enzymes necessary for cholesterol biosynthesis from acetate by liver homogenates are localized in the microsomal fraction. Subfractionation of the microsomes into smooth and rough surfaced categories was not feasible at that time, but from what is now known of the fine structure of the liver it can be presumed that their microsome fraction was derived in part from the agranular reticulum. More recently, the smooth surface microsomal subfraction of liver has also been found to contain enzymes involved in the metabolism of certain drugs. The activity of these enzymes is markedly increased in the livers of animals that have received repeated doses of phenobarbital or certain other lipid-soluble drugs(11,12,13, 14). Remmer and Merker(15) observed with the electron microscope an increase in smooth endoplasmic reticulum in livers of rats, rabbits and dogs, concomitant with the rise in drug-metabolizing enzyme activity. This drug-induced cytological alteration in the liver, now confirmed in the hamster(16), offered an attractive method of selectively increasing the quantity of this category of cytoplasmic membranes, and of evaluating its possible biochemical activity with respect to functions other than drug metabolism. In the present study our interest was centered in the enzyme systems responsible for cholesterol synthesis.

*Materials and methods.* Nine male golden hamsters weighing 100 to 130 g, were given 80 mg/kg of phenobarbital intraperitoneally for 4 days and then fasted for 24 hours. Nine other 24-hour-fasted male hamsters of similar weight were used as controls. The animals were killed by decapitation, and a small portion of the right lobe of the liver was removed and processed for electron microscopy.

Approximately one gram of the liver remaining was sliced with a Stadie-Riggs microtome, the slices rinsed in 0.9% NaCl, blotted, weighed and incubated for 1 hour at 37-38°C in 8 ml of Krebs-Ringer bicarbonate buffer containing 1 mg glucose per ml, 1 microcurie acetate-1-$C^{14}$ per ml, and gassed with 95% $O_2$ - 5% $CO_2$. Total tissue lipids were extracted by the method of Folch *et al* (17). After addition of tracer cholesterol-7-$H^3$, the cholesterol was isolated by 2-dimensional thin layer chromatography in the following solvent systems: First dimension: Hexane:ether:acetic acid (90:10:1) followed by Hexane:ether:acetic acid (80:20:2); second dimension: Methylene chloride:ether (5:2). After spraying each chromatogram with rhodamine-6-G the sterol spot was located under ultraviolet light and eluted with chloroform:methanol (2:1). The specific ac-

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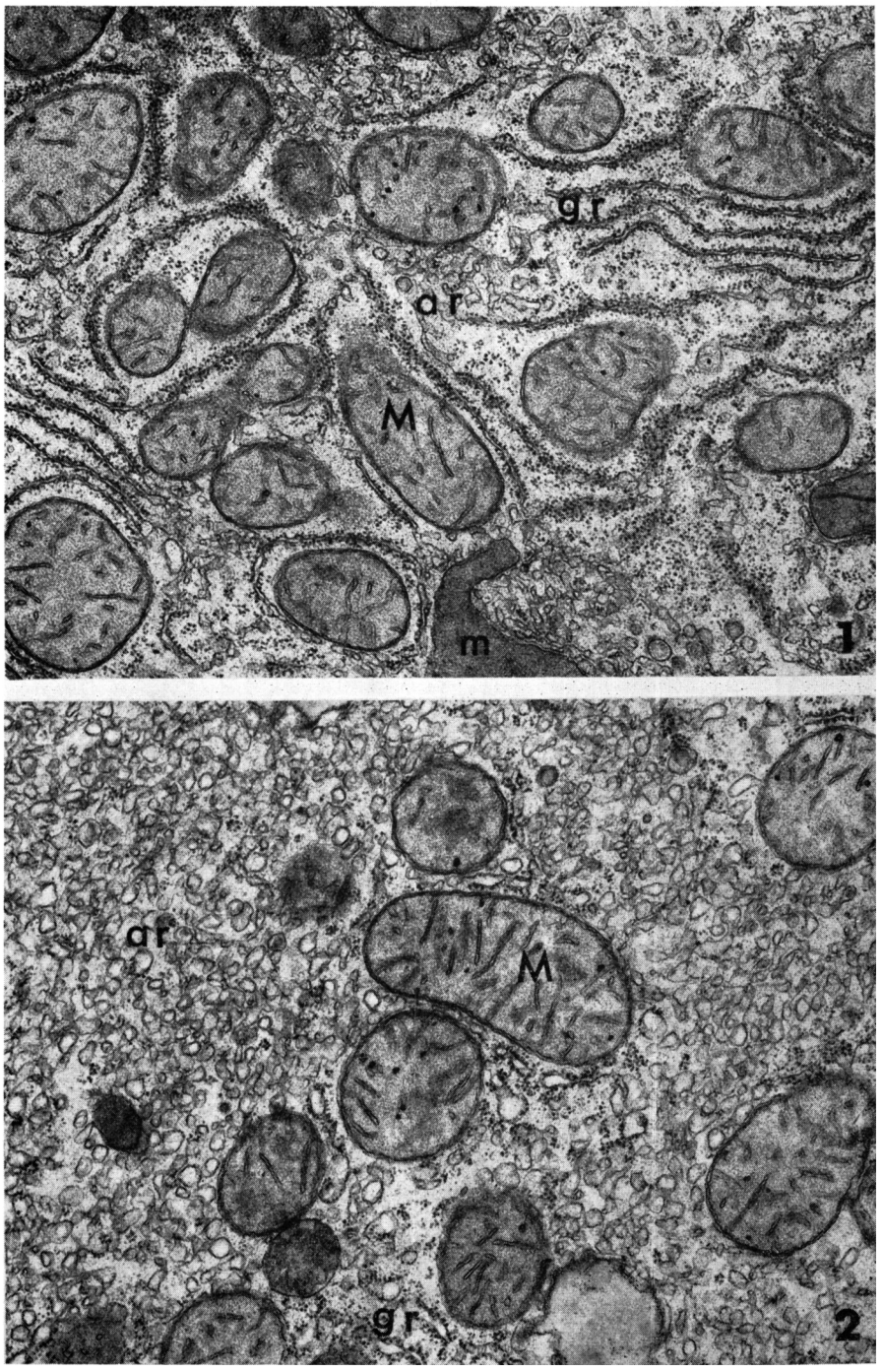


FIG. 1. This electron micrograph is from a hepatocyte of a control animal fasted for 24 hr. Small areas of agranular reticulum (ar) are dispersed among the lamellar profiles of granular reticulum (gr), the mitochondria (M) and microbodies (m). 18,500 ×.

FIG. 2. This portion of a liver cell is from a phenobarbital-injected animal. Note the remarkable increase of smooth membranes interspersed among the other cell organelles. Although the majority of the granular reticulum is crowded into localized areas of the cell not included in this field, a few small profiles can be observed here. 18,500 ×.

Livers were fixed in phosphate buffered osmium tetroxide and the sections stained with lead.

TABLE I. Effect of Phenobarbital Treatment upon Cholesterol Synthesis by Hamster Liver Slices *in vitro*.

| Treatment | No. of animals | DPM acetate-1-$C^{14}$ incorporation into cholesterol per g of liver | Liver cholesterol specific activity, DPM/$\mu$g |
|---|---|---|---|
| Control | 9 | 24.7 ± 5.7 | 11.2 ± 2.7 |
| Phenobarbital | 9 | 102.9 ± 30.9* | 49.1 ± 13.0* |

* Using Wilcoxon's 2-sample rank test $P < .01$.

tivity of the sterol was determined by analysis of one aliquot of eluate by a modified Lieberman Burchard reaction(4), and an assay of the $C^{14}$ and $H^3$ content of another aliquot in a Packard-liquid scintillation spectrometer. To the remainder of this eluate was added 20 mg of carrier cholesterol, and bromination was carried out by the procedure of Fieser(18). The insoluble dibromide derivative was washed 5 times with glacial acetic acid, redissolved in chloroform, with an aliquot taken for counting and the specific activity of the cholesterol calculated on the basis of the $C^{14}/H^3$ ratio obtained.

*Results and discussion.* Animals given 80 mg/kg of phenobarbital each day intraperitoneally were found to develop a striking morphological modification in their hepatocytes manifested by hypertrophy of the smooth endoplasmic reticulum. The granular reticulum retained its lamellar profile but tended to be crowded into more localized areas rather than distributed throughout the cytoplasm (Fig. 1, 2). In general, the remainder of the cell organelles appeared unaltered. The pronounced increase of smooth surfaced membranes observed cannot be explained by a mere loss of ribosomes from the granular reticulum and is considered to represent an absolute increase resulting from formation of new membrane. A more detailed treatment of the morphological changes induced by phenobarbital will be published later.

As seen in Table I, phenobarbital treatment resulted in highly significant (approximately 4-fold) increases in cholesterol biosynthesis from acetate-1-$C^{14}$, whether expressed as total incorporation of radioactivity per gram of liver, or as cholesterol specific activity (dpm/$\mu$g cholesterol). This increased rate of cholesterol synthesis was not accompanied by an increased accumulation of cholesterol in either the liver or the plasma.

The present study confirms in the hamster the hypertrophy of the smooth reticulum observed by Remmer and Merker(15) in the liver cells of rat, rabbit and dog following phenobarbital administration. In addition a concomitant increase in the capacity of the liver to synthesize cholesterol from acetate has been reported for the first time. This finding provides biochemical evidence indirectly supporting the earlier interpretations of the abundant smooth membranes in steroid producing cells as sites of biosynthesis of cholesterol(1,5,20) and lends credence to the suggestion by Fawcett(19) that the agranular reticulum in liver probably plays a major role in cholesterol formation in that organ.

*Summary.* Previous workers have correlated hypertrophy of the agranular reticulum in the liver with increase in drug-metabolizing enzymes following repeated injections of phenobarbital. Our investigation confirms their morphological observation and adds additional biochemical data which suggest that the enzyme systems responsible for cholesterol biosynthesis are associated with the smooth reticulum and are also increased following phenobarbital stimulation.

1. Christensen, A. K., Fawcett, D. W., J. Biophys. and Biochem. Cytol., 1961, v9, 653.

2. Morris, M. D., Chaikoff, I. L., J. Biol. Chem., 1959, v234, 1095.

3. Srere, P. A., Chaikoff, I. L., Dauben, W. G., ibid., 1948, v176, 829.

4. Armstrong, D. T., O'Brien, J., Greep, R. O., Endocrinology, 1964, v75, 488.

5. Fawcett, D. W., Intracellular Membraneous Structure, eds., S. Seno, E. V. Cowdry, Japan Society for Cell Biology, 1965.

6. Ross, M. H., Pappas, G. D., Lanman, J. T., Lind, J., J. Biophys. and Biochem. Cytol., 1958, v4, 659.

7. Enders, A. C., Lyon, W. R., J. Cell. Biol., 1964, v22, 127.

8. Marks, B. H., Alpert, M., Kruger, F. A., Endocrinology, 1958, v63, 75.

9. Schindler, W. J., Knigge, K. M., ibid., 1959, v65, 748.

10. Bucher, N. L. R., McGarrahan, K., J. Biol. Chem., 1956, v222, 1.

11. Fouts, J. R., Ann. N. Y. Acad. Sci., 1963, v104, 875.

12. Fouts, J. R., Biochem. and Biophysical Res. Commun., 1961, v6, 373.

13. Fouts, J. R., Fed. Proc., 1962, v21, 1107.

14. Remmer, H., Merker, H. J., Science, 1963, v142, 1657.

15. Remmer, H., Merker, H. J., Klin. Wochschr., 1963, v41, 276.

16. Fawcett, D. W., J. Cell. Biol., 1964, v23, 30A.

17. Folch, J., Lees, M., Sloane-Stanley, G. H., J. Biol. Chem., 1957, v226, 497.

18. Fieser, L. F., J. Am. Chem. Soc., 1953, v75, 5421.

19. Fawcett, D. W., Proc. Inter. Symposium on Lipid Transport (Charles C Thomas, 1964).

20. Christensen, A. K., J. Cell. Biol., in press.

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## Particle Retention by the Renal Glomerular Capillaries in the Generalized Shwartzman Reaction.* (30397)

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During late pregnancy, the Syrian hamster (*Mesocricetus auratus*) regularly develops the generalized Shwartzman reaction (GSR) in response to a single intraperitoneal injection of colchicine(1,2). At 24 hours, the reaction is characterized by extensive fibrin thrombosis of the renal glomerular capillaries and renal cortical necrosis. When a suspension of carbon particles is administered intravenously from 4 to 6 hours after colchicine, at a time when renal histologic changes are minimal or absent, there is striking accumulation of carbon particles in the glomerular tuft (Fig. 1). Three possible mechanisms of particle retention by the glomerular capillaries may be considered: i) Stasis resulting from vasodilatation. This has been postulated to account for the similar phenomenon of particle retention observed during the development of the GSR in the rabbit(3); ii) Increased endothelial permeability. Endothelial "leaks" are responsible for particle retention by postcapillary venules during inflammation(4); or iii) Adherence, either to an altered endothelium or to a previously-deposited sticky material. The early appearance of fibrinoid in the glomerular capillaries during the development of the GSR in the rabbit has been demonstrated by electron microscopy (5).

The present study was undertaken to determine which of these 3 mechanisms, vasodynamic, endothelial permeability or adherence, is responsible for entrapment of carbon particles in the glomerular capillaries during the development of the GSR.

*Materials and methods.* On the 14th day of pregnancy (16 day gestation period), 6 Syrian hamsters were given a single injection of colchicine (300 mg/kg body weight, i.p.). After 2, 4 or 6 hours, 2 hamsters at each time were anesthetized with ether and 2 ml of a 1% carbon particle suspension (Pelikan special biological ink, C11/1431a, particle size 200-300 Å) was slowly injected into the lingual vein. The hamsters rapidly recovered from the anesthesia. Between 1 and 1½ hours later, they were anesthetized with pentobarbital sodium, and small portions of the kidney were fixed in 1% osmium tetroxide and embedded in Vestopal-W(6). Sections cut at 0.5 $\mu$-1 $\mu$ were mounted on glass slides, stained with toluidine blue and examined by phase contrast microscopy. Photographs were taken at a magnification of 400 on 35 mm Kodak High Contrast Copy Film. In addition, portions of the kidney were fixed in

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