above the normal limit, was obtained. The other patient, a 54-year-old male with Hgb SC disease and infected leg ulcer, showed 9 total porphyrin values from 4 to 149 μg/24 hr and only one value, 208 μg/24 hr above the normal limit of 180 μg/24 hr.

Discussion. Our findings reveal a rather consistent moderate, occasionally marked increase of urinary copro-, total, and uroporphyrins—in this order—and to a slight extent of porphyrin precursors ALA, PBG, and AA during painful and febrile sickle cell crises. Although the number of cases is small, it is our impression that total urinary porphyrin excretion generally parallels the severity of clinical symptomatology and is normal during recovery and the steady state.

Of considerable interest is the close resemblance of prominent clinical features in sickle cell crisis and acute porphyria, particularly evident in cases with episodes of excruciating abdominal pain which in both conditions has led to unnecessary laparotomies (9,10,11). Besides increase of urinary porphyrins there is evidence of vasoconstriction in both diseases(9,10,12,13).

Summary. Porphyrin excretion in urine was studied in 18 patients with sickle cell disease, including multiple observations in 6 patients during 7 typical painful and febrile crises. With few exceptions there was moderate to marked elevation of copro-, total, and uroporphyrins at time of pain and fever. Aminolevulinic acid, porphobilinogen, and aminoacetone during crises were within range of normal variation or only slightly increased. Following subsidence of pain and fever, total porphyrin values returned toward normal. Similarities between clinical features of painful sickle cell crisis and acute porphyria are pointed out.

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An Electron Microscopic Study of Lipoprotein Production and Release by the Isolated Perfused Rat Liver.* (31388)

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(Introduced by D. W. Fawcett)
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It has been well established that liver plays an active role in lipoprotein synthesis (1-5). If the theoretical dimensions of the high and low density lipoproteins calculated by Oncley(6) are correct, they should both be visible with the electron microscope. Nevertheless, this tool has been very little used

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to study their ultrastructure, site of production and mechanism of release in the liver cell. Hayes and Hewitt(7) using a shadow casting technique have found that lipoprotein molecules of d $<$ 1.007 are 300-800 Å in diameter while those of d 1.007-1.063 and d $>$ 1.063 have approximate diameters of 350 and 150 Å respectively. More recently Casley-Smith(8) has observed that lipoproteins isolated from rat chyle appeared as irregular spheres which are similar in size to the bodies described by Hayes and Hewitt.

For a number of years electron microscopists have drawn attention to 300-1000 Å electron dense granules of similar appearance in the cisternae of the liver cell Golgi apparatus, in the smooth surfaced endoplasmic reticulum and occasionally in the space of Disse of normal rats(9,10) and hamsters(11,12). These granules have been reported to become more numerous after partial hepatectomy(13) and after ethanol administration(14). Although considered by some investigators to be albumin granules(10), Trotter has postulated that they are lipid in nature(13) while Stein and Stein have suggested that they are lipoproteins(14).

Nearly all of the above studies utilized livers of intact animals. The complex homeostatic mechanisms of the intact organism, however, increase the difficulties of interpretation, because of the interplay of numerous hormones and other uncontrollable factors in the *milieu interieur*. To obviate these difficulties and yet have morphologically adequate tissue the use of the isolated perfused liver offers unique advantages and is an experimental system virtually unexplored by the morphologist. The present study describes ultrastructural observations before and after perfusion of livers from fasted rats with high concentrations of linoleic acid.

Materials and methods. Two hundred fifty to 300 g male Sprague-Dawley rats were anesthetized with intraperitoneal nembutal (3 mg/100 g body wt) after an overnight fast. The bile duct and portal vein were cannulated *in situ* and a preperfusion with oxygenated Krebs-Ringer bicarbonate buffer (KRB) was immediately started. After removal from the animal the liver was placed in a modified Miller apparatus(15) in which it was perfused with 90-100 ml of medium composed of KRB, 4 g/100 ml albumin (fat free Cohn fraction V),† 5 mg/100 ml streptomycin, 5 mg/100 ml penicillin and 20 mg/100 ml heparin. The perfusate for livers which were to receive fatty acid contained 2 μM/ml sodium linoleate in their initial medium and received an additional 120 μM/hr from a constant infusion pump. Control livers received no fatty acid. Liver viability was judged by flow rate, bile production, oxygen extraction and gross appearance.

Biopsies were taken from livers immediately after being placed on the perfusion apparatus (0 time) and then at 2, 5 and 30 minutes or at 60 minutes. From these biopsies, the most centrally located portion of initial liver slices was dissected out and cut into small cubes, fixed in 0.05 M phosphate buffered 1% OsO_4, dehydrated and embedded in Epon 812(16).

Aliquots of initial and final media were fixed in equal volumes of 2% OsO_4 as were samples of d $<$ 1.006 fraction of human and rat serum. The latter were obtained by ultracentrifugation following removal of the $S_f >$ 400 fraction(17). Following a 2-hour "fixation" the mixture was centrifuged at 20,000 $\times$ *g* for 60 minutes. In those cases where a pellet was obtained, it was treated in the same manner as the fixed cubes of liver tissue. All thin sections were stained with lead(18) and examined with a RCA EMU 3G electron microscope.

Results and discussion. At 0 time the livers appeared normal. Dense 300-800 Å spherical granules were observed in moderate numbers in the hepatocyte Golgi apparatus while an occasional granule was found in the tubules of the smooth surfaced endoplasmic reticulum or in isolated vesicles. Upon careful examination of the contents of the space of Disse, an occasional cluster of 300-800 Å granules could be found (Fig. 1).

At the end of only 2 minutes of perfusion with medium enriched with fatty acid there was an increased number of osmiophilic granules (300-800 Å in diameter) in the

† Mann Biochemical, New York.

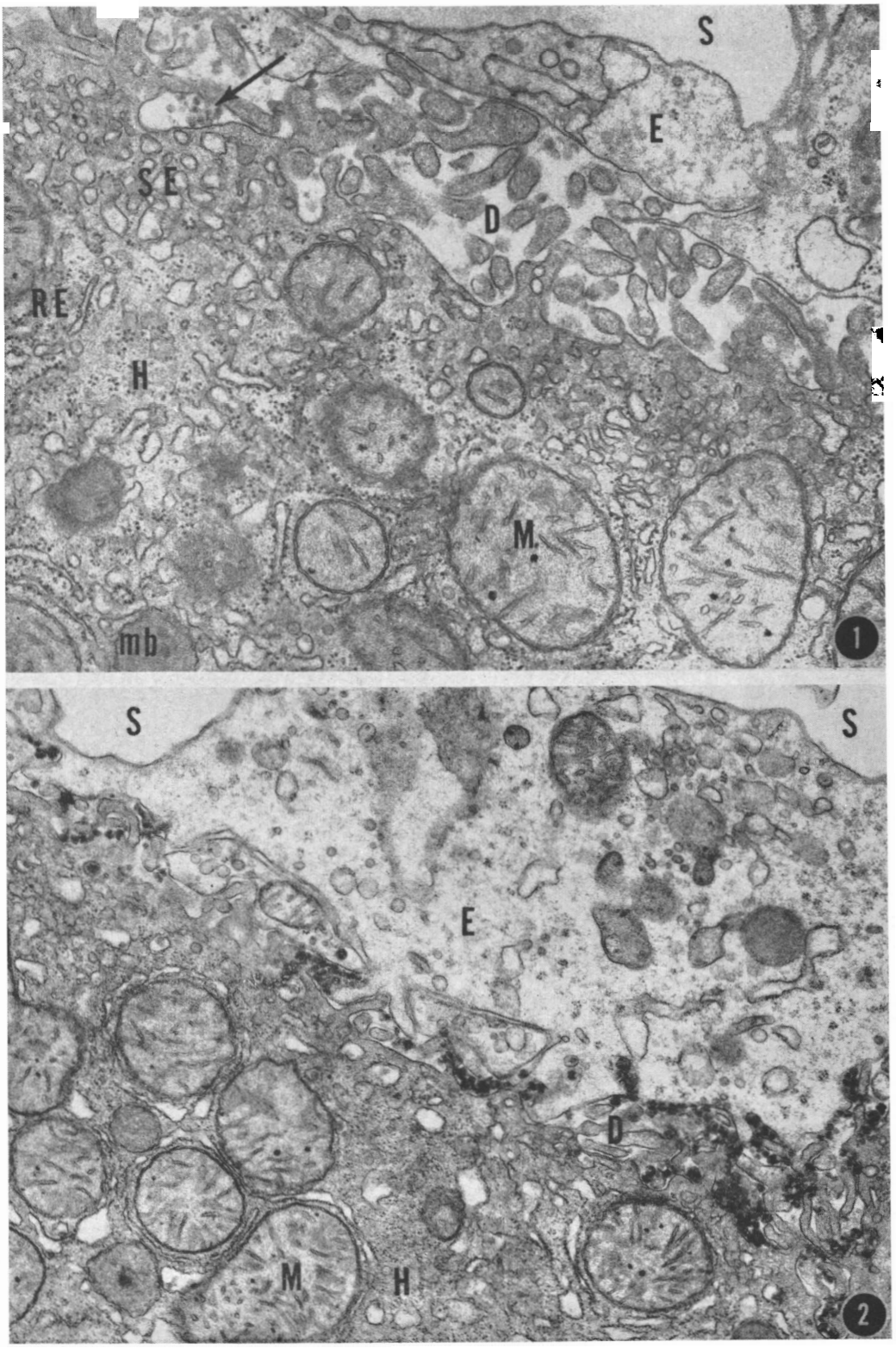
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FIG. 1. An election micrograph illustrating a portion of a hepatocyte (H), space of Disse (D), endothelial cell (E) and the blood vascular space of the hepatic sinusoid (S) from an experimental animal at zero time. A few 300 - 800 Å granules (at arrow) are observed within the space of Disse. Mitochondria (M); microbodies (mb); rough surfaced endoplasmic recticulum (RE) and smooth surfaced endoplasmic reticulum (SE) are also identifiable. 18,000 ×.

FIG. 2. After 1 hr perfusion with free fatty acid there is a striking accumulation of the 300 - 800 Å dense bodies within the space of Disse. The increase in electron density of these bodies over those found in the control (Fig. 1) is likely due to the fact that the predominant fatty acid contained in these lipoproteins is highly unsaturated linoleic acid which is quite osmiophilic. 18,000 ×.

Golgi and within the agranular reticulum. These findings were quite striking at the end of 1 hour of perfusion (Fig. 3). Such granules were seen in the space of Disse rather infrequently in the 2-minute biopsies but in those taken after 5 minutes or more of fatty acid perfusion, there was a remarkable increase in the abundance of osmiophilic granules seen in this region (Fig. 2)

No particulate material was found upon centrifugation and fixation of the initial medium or in the final medium of livers perfused without addition of fatty acid. On the other hand final media of livers perfused with fatty acid yielded a small pellet which contained large numbers of closely packed granules whose size and shape corresponded to that of the dense bodies observed in thin sections of liver, both in the cells and the space of Disse (Fig. 4). Similar dense particles were found in the $d < 1.006$ fraction of rat and human sera. They were not observed in media containing linoleate which circulated through the perfusion apparatus for 60 minutes in the absence of a liver.

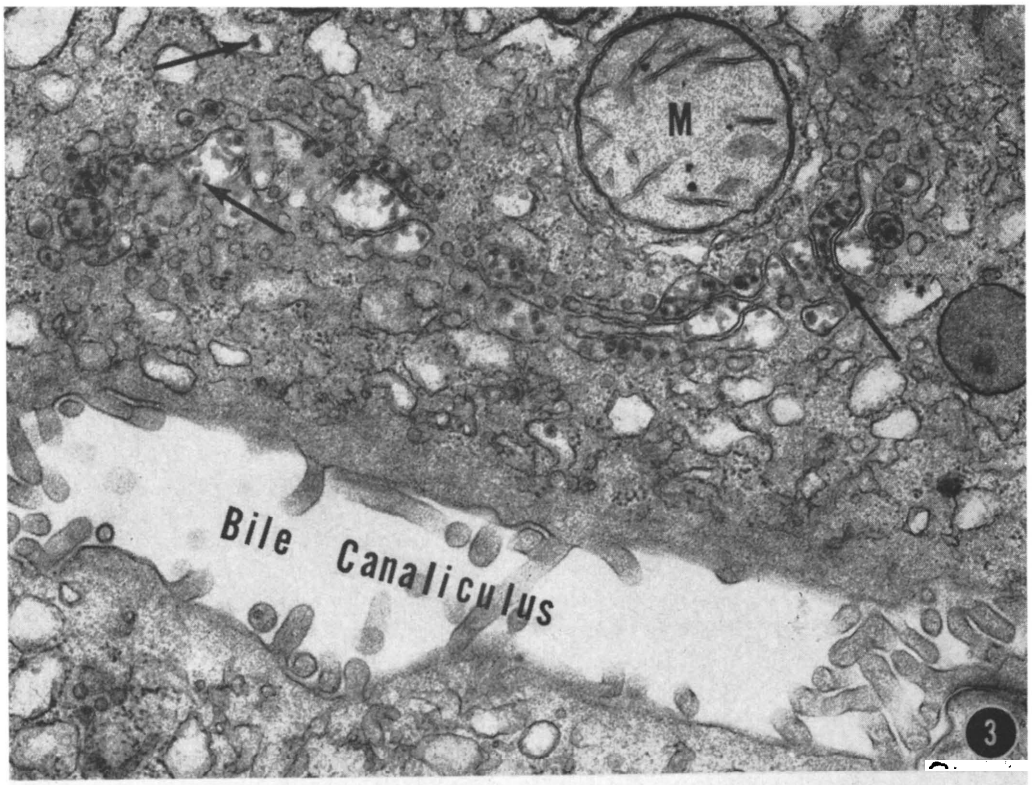
From the preceding studies, the following conclusions seem justifiable. The granules observed in the final medium were produced by the liver. The fact that they could only be detected following perfusion with free fatty acid, that they were morphologically similar to the osmiophilic bodies found in the $d < 1.006$ fraction of human and rat sera strongly suggest that they are lipoproteins.

The finding of the newly synthesized "lipoprotein" within the cisternae of the smooth reticulum, near their sites of confluence with the rough surfaced reticulum, suggests that both categories of cytoplasmic membranes may play a role in their synthesis. It is tempting to think that the ribosome-studded granular reticulum may provide the protein component while the smooth surfaced cytoplasmic membranes, which have previously been implicated in cholesterol(19) and triglyceride synthesis(29) may provide the lipid. The apparent early accumulation of these particles in the Golgi region has yet to be explained, but it may play a role in stabilizing the granules for transport. Finally, the appearance of the granules in the space of Disse only after their appearance in the endoplasmic reticulum suggests that this is a later event which must await transfer of the particles out of the liver cell. The relation of these granules, if any, to the apolipoprotein described by Roheim *et al*(21) is not clear.

During the course of these studies Hamilton *et al*(22) reported finding similar osmiophilic bodies upon perfusing fed rat liver for 10, 90 or 120 minutes with a medium containing palmitate or oleate. In addition, they observed these bodies in the $d < 1.019$ fraction of their post-perfusion medium using negative phase microscopy. Although their experimental system differed from ours with respect to composition of the basic perfusion medium and the fatty acids used, the findings of both studies appear to be mutually supporting.

The present study does not establish with certainty the sequence of cytological events in the formation and transport of lipoprotein within the liver. Nevertheless, it does demonstrate that the perfused rat liver provides an excellent experimental system which will enable the morphologist to contribute to the study of lipoprotein metabolism. Investigations utilizing this system in combination with electron microscopy and autoradiography may be expected to yield valuable new information on the sites of synthesis and pathways of intracellular transport of lipoprotein.

Summary. Dense osmiophilic bodies, 300-800 Å in diameter, appear in isolated rat livers perfused with high concentrations of linoleic acid. After 2 minutes of perfusion, these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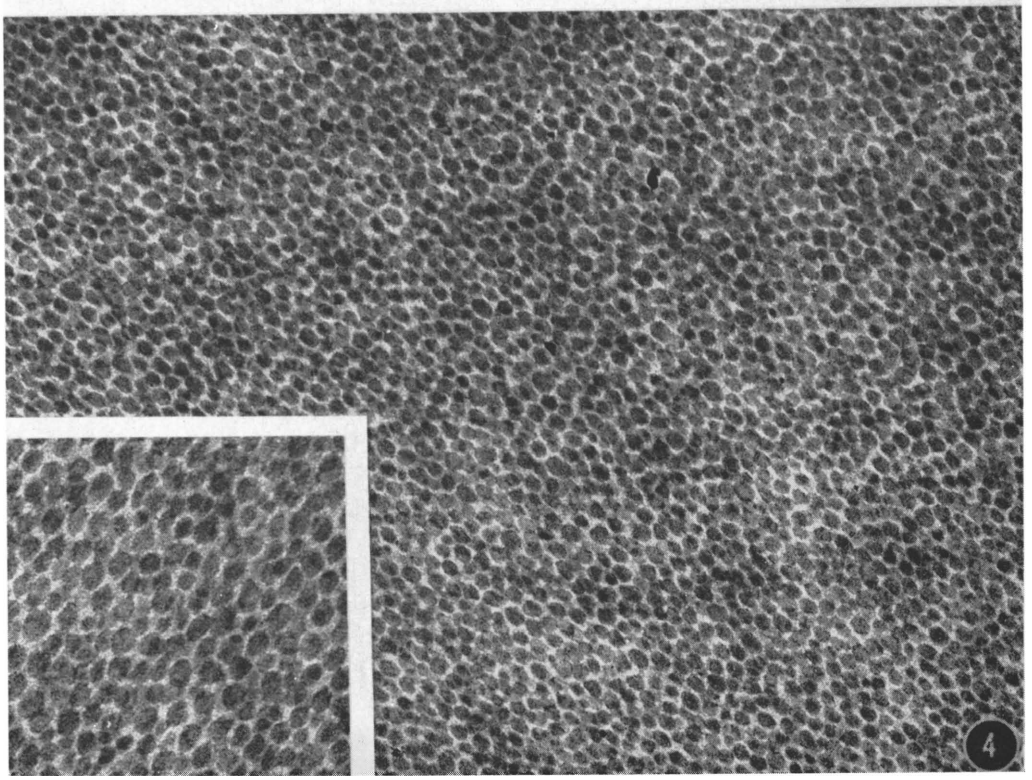
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FIG. 3. Beginning at 2 min of fatty acid perfusion and continuing for 60 min (as shown here) there appears to be an increase in granules, morphologically similar to those in the space of Disse, within the cisternae of the Golgi system (G) and the endoplasmic reticulum (at the arrows). 29,000 ×.

FIG. 4. An electron micrograph of a portion of the pellet obtained after fixation and centrifugation of the free fatty acid perfusate after 1 hr of liver perfusion. The electron dense granules shown are of the same dimension as those in the liver and space of Disse. At slightly higher magnification (see insert) they appear to have an irregular outline and a stippled substructure. 44,500 × ; Insert, 62,500 ×.

bodies are very numerous in the endoplasmic reticulum and Golgi apparatus. They are first seen in significant numbers in the space of Disse at 5 minutes. Similar granules are present in the final media of livers perfused with fatty acid and the d $<$ 1.006 fraction of human and rat sera. The data suggest that these bodies are very low density lipoprotein and indicate that the isolated perfused liver is an excellent experimental system for morphological study of lipoprotein metabolism.

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Comparison of Biological Activity of Orally Administered and Injected L-Thyroxine, L-Triiodothyronine and Thyroprotein* in Rats. (31389)

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Oral administration of thyroid hormones as salts, desiccated thyroid, or thyroactive iodinated casein (thyroprotein) is the most practical method of administration of these hormones. The reduced effectiveness of thyroxine, or thyroxine-containing preparations by oral administration has been recognized for some time.

Sturnick and Lesses(1), using PBI levels

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