$PGF_{2\alpha}$ (26.5±10.5 pg/ml) in the arterial blood samples, the levels were not statistically different from those of venous samples.

The figure depicts the effect of anaesthesia (pentobarbital 30 mg/kg) on prostaglandin levels measured in venous blood, results obtained by direct puncture (n=5) and from venous catheter (n=8) have been combined. Both PGE_2 and $PGF_{2\alpha}$ decreased during anaesthesia, though this change was significant only for $PGF_{2\alpha}$. 3 h after injection of pentobarbital, both prostaglandins rose to their initial levels.

The results obtained in the present studies indicate that chronic catheterization of aorta and inferior vena-cava in dogs does not significantly affect circulating levels of prostaglandins. Furthermore, the levels of prostaglandins measured in the plasma collected from chronically implanted catheters are not different from those obtained after direct puncture. These studies further suggest that circulating levels of $PGF_{2\alpha}$ and possibly PGE_2 decrease during anaesthesia. The values of PGE_2 and $PGF_{2\alpha}$ observed in the present studies are similar to those reported earlier by Dunn et al.[7] from the same laboratory. Terragno et al.[8] (1977) reported that anaesthesia as such did not change levels of renal venous prostaglandins but that laparotomy markedly increased prostaglandin levels. The values of PGE_2 and $PGF_{2\alpha}$ observed in conscious dogs show more variability than those observed during anaesthesia. This may be due to variable physical activity in conscious dogs before blood sampling, as suggested by one experiment, where the effect of 10 min running on circulating levels of PGE_2 and $PGF_{2\alpha}$ was studied. The blood was collected from a venous catheter before and at the end of exercise. The respective values of PGE_2 and $PGF_{2\alpha}$ before exercise were 3.5 and 24.1 pg/ml plasma and after exercise 7.3 and 53.7 pg/ml.

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Lipid accumulation in mouse hepatocytes after morphine exposure[1]

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Summary. After morphine injection lipid accumulation in mouse hepatocytes begins within 2 h and continues for 24 h when most hepatocytes are filled with lipid droplets. In spite of morphine maintenance the liver recovers as the accumulated lipids are coupled with protein and subsequently transported and released into the perisinusoidal space of Disse.

The effects of morphine on liver function is poorly understood but bile secretion can be inhibited, catalase activity diminished[2] and morphological lesions produced[3]. In respect to lipid metabolism there may be species differences. Thus rat liver lipids are not significantly changed after acute or chronic morphine exposure[4]. Non-tolerant guinea-pigs show an increased phospholipid metabolism[5] but tolerant animals do not[6]. During preparation of mice treated with morphine for ultrastructural studies of the cerebellum[7] we noted that most animals exhibited a very pale, apparently fatty, liver after 24 h of morphine exposure (figure 1). This dramatic change in apparent lipid metabolism prompted the present ultrastructural investigation.

Material and methods. Male ICR mice weighing 25-30 g were implanted s.c. with a pellet containing 75 mg of morphine base which becomes continuously released[8] or with placebo pellets. Other mice were injected with 40 mg of morphine/kg or with saline every 8 h to exclude that the apparent steatosis of the liver was produced by pellet components other than morphine or by surgical manipulation. Some morphine and placebo treated mice were fasted for 15 h to determine if the development of a fatty liver was due to reduced food intake only[9] rather than to morphine treatment per se. The livers were perfused with 4% formaldehyde and 1% glutaraldehyde freshly prepared in Millonig's phosphate buffer at pH 7.2. Portions from the caudal lobe of livers that blanched immediately during perfusion were postfixed in 2% osmium tetroxide at pH 7.2, dehydrated and embedded. The fixation took place at 1, 2, 5, 8, 24 h, 3 and 9 days after morphine treatment was initiated. Mice treated for 9 days received a new pellet every 3rd day to circumvent the effects of encapsulation. At least 3 mice were used in each group. A preliminary study of lipids in liver extracts from groups of 3 morphine-treated and control mice sacrificed after 24 h or 3 days was performed by the clinical laboratory.

Results and discussion. The livers and hepatocytes from control mice were normal. Lipid droplets in the hepatocytes varied in size and number and were generally restricted to the cell periphery. They were increased slightly in fasted mice but never filled the cells. Particles, 30-100 nm in diameter, occasionally were present within the cisternae of the endoplasmic reticulum and Golgi apparatus. Similar particles were infrequently observed in the space of Disse. They were interpreted to represent very low density lipoproteins[10] formed in the endoplasmic reticulum, subsequently coupled with carbohydrate in the Golgi apparatus[11] and finally transported and secreted into the space of Disse[12].

The most dramatic changes in hepatocyte ultrastructure were observed 8 and 24 h after morphine injection or pellet implantation. At these times the hepatocytes contained numerous lipid droplets which varied in diameter between 50 nm and several μm and were commonly fused into aggregates (figure 2). Many mitochondria were condensed and closely associated with the lipid droplets. Only a few of the membrane stacks of the granular endoplasmic reticulum remained and many ribosomes were detached. Golgi cisternae and saccules were abundant and filled with the presumptive very low density lipoprotein particles. These particles were also numerous among the microvilli in the space of Disse (figure 3). Liver extracts showed an increase of 106.7% lipids over controls.

None of the ultrastructural changes involving lipoprotein particles and lipid droplets were evident after 1 h of morphine exposure. However, 2 h after injection (figure 4)

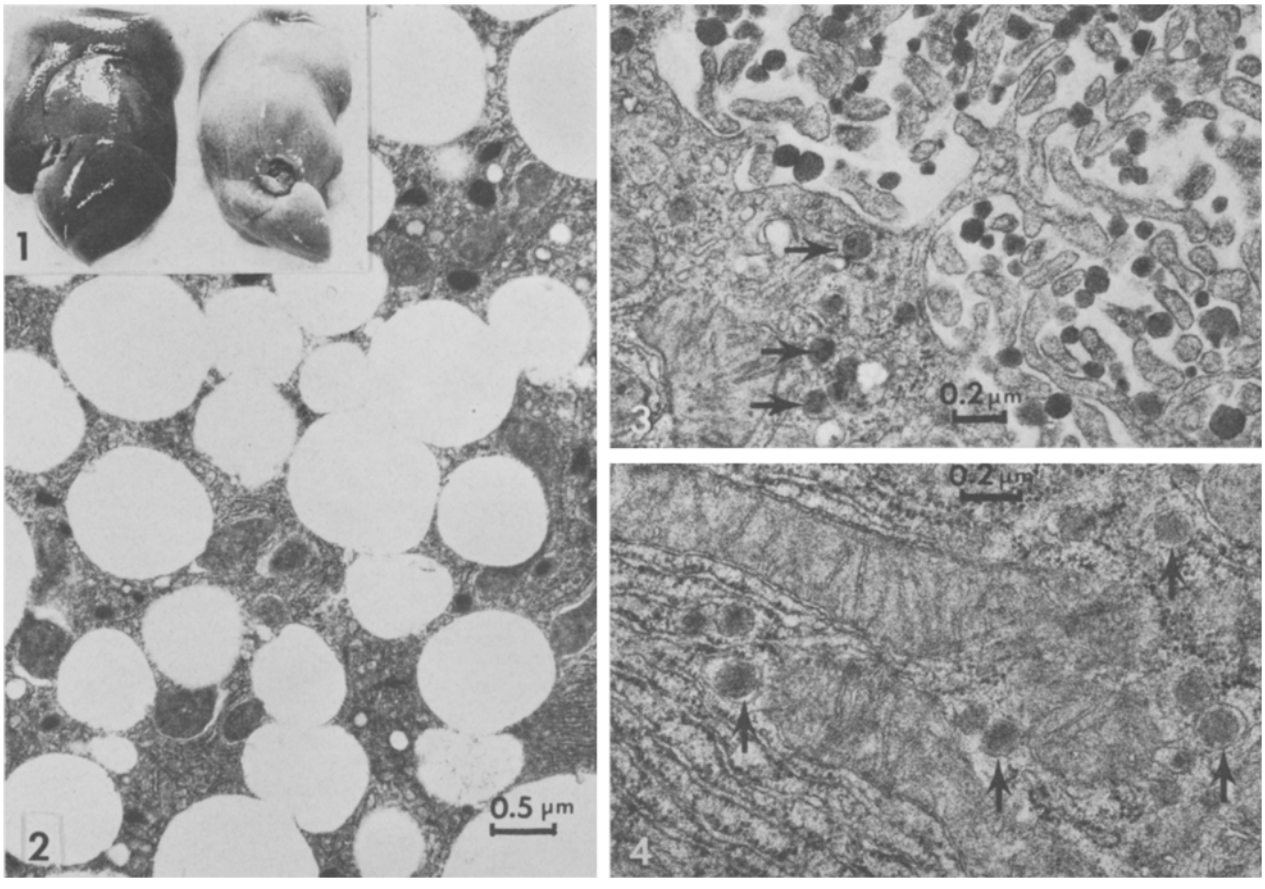

Fig. 1. Liver from mouse exposed continuously to morphine for 24 h (right) is pale and has a fatty appearance in comparison to control liver (left). Fig. 2 and 3. Portions from hepatocytes after 24 h of morphine exposure. Fig. 2. Lipid droplets, often in aggregates, are present. Condensed mitochondria are often closely associated with the lipid droplets. Fig. 3. Electron-opaque particles believed to represent very low density lipoprotein are present in the cytoplasm (arrows) and numerous among the microvilli in the space of Disse. Fig. 4. Portion from hepatocyte 2 h after morphine injection. Many lipoprotein particles (arrows) are present among the mitochondria and granular endoplasmic reticulum membranes.

or 5 h after morphine pellet implantation the number of lipid droplets and lipoprotein particles in the endoplasmic reticulum and Golgi apparatus were increased significantly. The lipid droplets continued to accumulate through 24 h and remained approximately the same by the 3rd day of treatment. At this time the liver lipids were still increased 86.7% over the control value. After 9 days of treatment the lipoprotein particles remained abundant but the number and size of the lipid droplets were reduced. The granular endoplasmic reticulum appeared normal and was again arranged in stacks.

Fatty livers occur under many conditions including deficiences in diet, inhibition of protein synthesis and after a marked release of fatty acids from adipose tissue. The effects of morphine may directly or indirectly involve some of these conditions. For example, morphine can temporarily suppress protein synthesis[13] which may be indicated by the changes in granular endoplasmic reticulum during morphine treatment. Morphine also released about 50% of the adrenaline content from the adrenal medullae of mice treated for 24 h while leaving noradrenaline unchanged[14]. Adrenaline augments fatty acid release from adipose tissue[15] which causes increased liver uptake[16] and lipid formation[17]. The lipids can not be removed until coupled with protein and carbohydrate. The steatosis may be exacerbated by a somewhat decreased food intake after morphine. It is noteworthy that the mice apparently can adapt to the morphine and their livers return towards normal in spite of drug maintenance. This contrasts to fatty livers produced by many other agents, e.g. alcohol, carbon tetrachloride and tetracycline. The steatosis observed after these drugs can only be reversed by stopping their administration or by adding protective compounds to the regimen.

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