naloxone. The excellent technical assistance of Mr Gianni Montorsi is gratefully acknowledged.

2 R.M. Quock and A. Horita, Proc. West. pharmac. Soc. *16*, 68 (1973).
3 R. Harris, D. Snell, H.H. Loh and L.E. Way, Proc. West. pharmac. Soc. *19*, 448 (1976).
4 R.M. Quock, Life Sci. *20*, 2005 (1977).
5 L. Terenius, W.H. Gispen and D. De Wied, Eur. J. Pharmac. *33*, 395 (1975).
6 W.H. Gispen, J. Buitelaar, W.M. Wiegant, L. Terenius and D. De Wied, Eur. J. Pharmac. *39*, 393 (1976).
7 J. Chang, B. Fong, A. Pert and C.B. Pert, Life Sci. *18*, 1473 (1976).
8 M.G. Baxter, D. Goff, A.A. Miller and I.A. Saunders, Br. J. Pharmac. *59*, 445P (1977).
9 W. Ferrari, G.L. Gessa and L. Vargiu, Ann. N.Y. Acad. Sci. *104*, 330 (1963).
10 A. Bertolini, W. Vergoni, G.L. Gessa and W. Ferrari, Nature *221*, 667 (1969).
11 A. Bertolini, G.L. Gessa and W. Ferrari, in: Sexual Behavior: Pharmacology and Biochemistry, p.247. Ed. M. Sandler and G.L. Gessa, Raven Press, New York 1975.
12 C.H. Sawyer, D.M. Baldwin and C.K. Haun, in: Sexual Behavior: Pharmacology and Biochemistry, p.259. Ed. M. Sandler and G.L. Gessa, Raven Press, New York 1975.
13 S. Levine and L.E. Jones, J. comp. Physiol. Psychol. *59*, 357 (1965).
14 D. De Wied, Proc. Soc. exp. Biol. Med. *122*, 28 (1966).
15 B. Bohus, C. Nyakas and E. Endröczi, Int. J. Neuropharmac. *7*, 307 (1968).
16 J.A. Gray, A.R. Mayes and M. Wilson, Neuropharmacology *10*, 223 (1971).
17 S. Guth, S. Levine and J.P. Seward, Physiol. Behav. *7*, 195 (1971).
18 S. Bjorkman and H. Sievertsson, Naunyn-Schmiedebergs Arch. Pharmac. *298*, 79 (1977).
19 A. Cowan, Adv. Biochem. Psychopharmac. *8*, 427 (1973).

Effects of anaesthesia and chronic catheterization on circulating levels of prostaglandins (PGE_2 and $PGF_{2\alpha}$ in dogs[1,2]

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Summary. Chronic catheterization of aorta and inferior vena cava in dogs did not significantly affect circulating levels of prostaglandins (PGE_2 and $PGF_{2\alpha}$). Pentobarbital (30 mg/kg i.v.) anaesthesia produced a significant decrease in $PGF_{2\alpha}$.

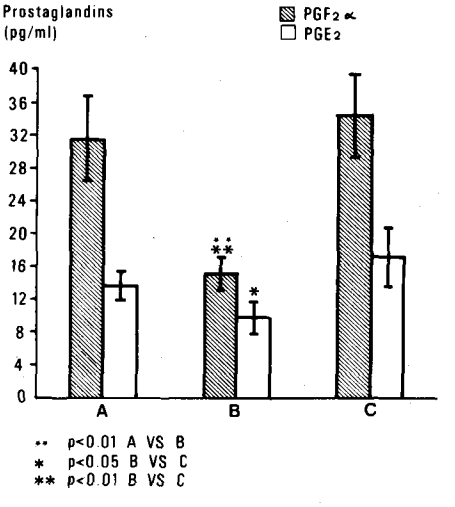
Normal circulating levels of prostaglandins have been difficult to determine due to limited sensitivity of many available assay procedures. Furthermore, values obtained in anaesthetized and conscious animals might differ, thus complicating comparison of the results reported by various authors. It was therefore felt necessary to evaluate the effect of anaesthesia on circulating levels of prostaglandins. The influence of chronic catheterization of blood vessels, a method often used when studying conscious dogs, was also determined.

Materials and methods. Nine mongrel dogs of average b.wt 23.8±1.3 kg (SEM) were used. Aortic and vena-caval catheters were inserted in aseptic conditions under pentobarbital anaesthesia (30 mg/kg) through iliac vessels, brought s.c. to the back of the animal and exteriorized at the level of intrascapular region. The catheters were filled with heparin (1000 U/ml) and flushed twice a week; in addition they were rinsed after each blood collection with 5 ml of normal saline followed by 1 ml (dead space of catheter) of diluted heparin solution.

A period of at least 1 week, and usually 2 weeks, preceded the first experiment (7-40 days). Intervals between experiments were no less than 3 days (3-26 days).

Collection of blood. About 10 ml of blood were drawn in a vacutainer containing EDTA (14 mg) either from direct puncture of a leg vein and/or from aortic and venous catheters. The first 3-5 ml of blood collected from the catheters were discarded. Hematocrit, determined in each dog, ranged from 37 to 45%. Immediately after collection, the blood was transferred in plastic tubes kept in ice and containing 0.1 ml of meclofenamic acid (500 µg/ml) in Tris hydrochloric acid 0.1 M, pH 8.4. The blood was centrifuged at 4500 rpm for 15 min at 4 °C. Supernatant plasma was collected without contamination by white blood cells and platelets. The plasma was stored at −20 °C till subsequent radioimmunoassay (2-10 days after blood collection). Radioimmunoassay of prostaglandins (PGE_2 and $PGF_{2\alpha}$) was conducted following the technique of Dray et al.[6]. The values are expressed as pg/ml of plasma±SEM in the text and figure. Blood was collected before anaesthesia (A), during anaesthesia (15-30 min after injection of pentobarbital 30 mg/kg) (B) and after anaesthesia (3 h after injection of pentobarbital 30 mg/kg) (C).

Results and discussion. The mean values of PGE_2 and $PGF_{2\alpha}$ in 5 conscious uncatheterized dogs were 9±2.4 and 20.4±9.1 pg/ml, respectively. The levels of both prostaglandins were slightly but not significantly higher (12.2±1.9, 36.9±9.4 pg/ml) in 5 conscious catheterized dogs. The levels of PGE_2 and $PGF_{2\alpha}$ in the plasma obtained from these 5 catheterized dogs were similar, whether it was collected by direct venous puncture (12.2±1.9, 36.9±9.4 pg/ml) or from the chronically implanted venous catheter (14.35±2.9, 28.2±6.7 pg/ml). Although there were wide variations in values of PGE_2 (27.4±12.95 pg/ml) and



Peripheral venous levels of prostaglandins before (A), during (B) and after anaesthesia (C) with pentobarbital 30 mg/kg i.v. (n = 13).

$PGF_{2\alpha}$ (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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6 F. Dray, B. Charbonnel and J. Maclouf, Eur. J. clin. Invest. *5*, 311 (1975).
7 M.J. Dunn, J.F. Liard and F. Dray, Kidney Int., in press (1977).
8 N.A. Terragno, D.A. Terragno and J.C. Mcgiff, Circulation Res. *40* (6), 590 (1977).

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)