to 18.00 h). All animals received tap water and food ad libitum. Groups II and IV, however, had MEL dissolved in the drinking-water. Based on preliminary observations of the quantity of water drunk by the animals we calculated that in order to obtain a MEL ingestion of approximately 50 µg/day/hamster, we had to use a concentration of 2.5 mg/500 ml water. The 2.5 mg MEL were dissolved in 200 µl ethanol which were then mixed with 500 ml water. MEL-containing drinking-water was refreshed every week. Ethanol (200 µl/500 ml tap water) was added to the drinking-water of the control groups (I, III). After 8 weeks the hamsters were sacrificed and the testes and accessory sex organs (seminal vesicles and coagulating glands) were dissected and weighed. The results were statistically analyzed using Student's t-test and are given as means ± SEM.

Results and discussion. As shown in figures 1 and 2, MEL orally administered via the drinking water caused a marked loss of testicle and accesory sex organ weight in hamsters maintained in long photoperiod, suggesting a strong antigonadotropic effect. Short photoperiod by itself is known to induce gonadal atrophy in hamsters[2,3] and this is visible when we compare the control groups submitted to short days (figs 3 and 4) with control groups submitted to long days (figs 1 and 2). MEL does not seem to have any effect in hamsters maintained in short photoperiod, at least when the testes are considered (fig. 3). Looking at the sex organs, however, it appears that orally administered MEL could also have an inhibitory effect. The weight of the accessory organs of the MEL-treated hamsters (fig. 4) is, indeed, significantly lower than in the control group. This result seems to suggest that MEL could also have an antigonadal effect in hamsters kept in short photoperiod, an effect which would be masked by the gonadal atrophy induced by the short photoperiod. If this interpretation is correct, such a result could be of great importance for the understanding of pineal physiology.

Kennaway and Seamark[7] have observed that s.c. injections of MEL resulted in an extremely rapid rise in plasma hormone titres to peak within 15 min, with an apparent half life for MEL of about 30 min. It appears thus that after injection, MEL disappears quickly, and indeed these authors have observed that after 4 h no MEL was detectable in the blood. Oral administration, on the contrary, resulted in blood MEL levels which rose within 30 min to a plateau which was sustained for at least 7 h. It is difficult at present to extrapolate these results obtained in ruminants to the hamster, but in our opinion it seems sound to assume that orally administered MEL induces an increase in blood MEL concentration for a longer time than does s.c. injection of MEL. How, therefore, can we integrate our observation that orally administered MEL induced gonadal atrophy in the hamster with the observations of other authors that in this species MEL is progonadotropic (or counterantigonadotropic) when constantly available, and antigonadotropic when injected late in the afternoon[2,3]? S.c. injections of MEL, however, induced gonadal atrophy not only when injected in the late afternoon but also when injected at the beginning or at the end of the dark period[8]. The hamster is a nocturnal animal. This means that it is active during the dark period, especially at the beginning and end of it, and that consequently it eats and drinks at these times. This probably explains why orally administered MEL in the hamster has an antigonadotropic effect. On the other hand, as it has been demonstrated that multiple daily injections of MEL induced gonadal atrophy in hamster[9,10], and as the animals probably drink more than once a day, it could be possible that it is this multiple daily ingestion of MEL which is responsible for the observed antigonadotropic effect.

In conclusion, it appears that the inhibition of gonadal function normally observed after multiple daily injections, or late afternoon injections, can also be obtained by simply adding MEL to the drinking-water. For future studies, MEL orally administered via the drinking-water seems to be a very effective and especially a very practical way of administration.

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The effect of o,p'-DDD on the adrenal cortex in sheep[1]

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Summary. The effect of o,p'-DDD (200 mg/kg/day given p.o. for 100 consecutive days) on the sheep adrenal gland was studied. The results suggest that this ruminant species is highly resistant to the adrenocorticolytic activity of o,p'-DDD when compared with dogs.

The compound 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane, known by its trivial name o,p'-DDD, was shown first in the dog to be selectively toxic to the adrenal cortex[3,4]. Histologic and ultrastructural studies in dogs[5,6] suggest a direct toxic effect on mitochondria, resulting in cellular necrosis and atrophy of the adrenal gland. Cytotoxicity is most pronounced in the zona fasciculata and zona reticularis whereas the zona glomerulosa is only mildly affected. In addition, o,p'-DDD has been shown to block ACTH-induced steroidogenesis[7] and to modify the peripheral metabolism of steroids[8]. Today, o,p'-DDD is widely used for the treatment of adrenal cortical neoplasia and hyperadrenocorticism in man[9,10] and in animals[11]. In order to extend our studies about the endocrine relationship

between adrenal and gonadal steroids in domestic animals[12], the main goal of the present study was to develop a method for suppressing adrenocortical activity in the ovine (ruminant) species with o,p'-DDD, thus avoiding the risk of bilateral adrenalectomy.

Materials and methods. Six ewes of the White Mountain breed weighing between 45 and 60 kg were used for this study. 3 animals were given o,p'-DDD (Lysodren®, Bristol Laboratories) p.o. at a dose of 200 mg/kg b.wt once daily for 100 consecutive days. The drug was presented as tablets containing 0.5 g of o,p'-DDD. 3 animals served as controls. Blood samples (5 ml) were withdrawn from the jugular vein into heparinized tubes every 5 days between 09.00 and 10.00 h just prior to treatment. The blood was immediately centrifuged and plasma fractions stored at −20 °C until analyzed for cortisol[13] and alkaline phosphatase[14]. Upon termination of the experiment, all animals were killed and tissues from the liver and adrenal glands were fixed for histologic and ultrastructural examination. Comparison of means between the 2 experimental groups was made using Student's t-test.

Results and discussion. Although cytotoxic effects of o,p'-DDD have been studied in various laboratory animals, no reports exist in the literature using sheep as experimental animals. Results from this study indicate that the ovine species seems to be highly resistant to the adrenocorticolytic activity of o,p'-DDD, as is true for other species which have been tested such as rats, rabbits, mice and monkeys[3,15]. Oral administration of 200 mg/kg/day of Lysodren® during a period of 100 days did not alter peripheral plasma cortisol levels (fig. 1) but significantly increased alkaline phosphatase (fig. 2) from 20 days after initiation of treatment. Histologic and ultrastructural examination of the adrenal cortex and the liver revealed no pathological lesions. Furthermore, no toxic effects such as gastrointestinal disturbances, neuromuscular signs or dermal changes were observed clinically during the trial.

Our data are in contrast to results in dogs[16–18], where severe damage to the innermost zones with decreased cortisol secretion is reported to occur within 2–14 days of treatment with a daily dose of 50–100 mg/kg. Also, in dogs and rats[19] a reversible degeneration of liver cells with a concomitant

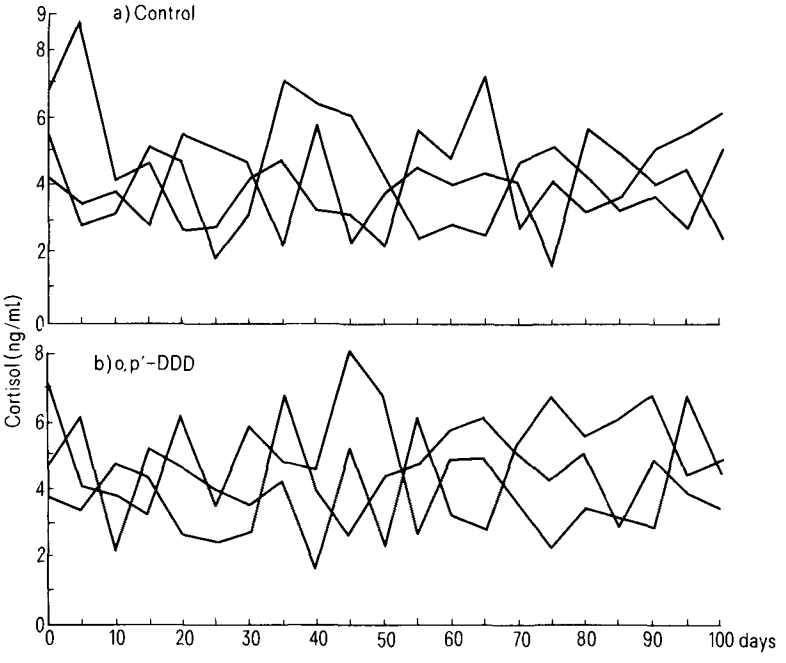

Figure 1. Plasma cortisol values in 3 control sheep (a) and 3 sheep treated with o,p'-DDD (b) for 100 consecutive days. Mean (± SEM) concentrations in ng/ml for the 2 groups are 4.25 ± 0.18 vs 4.44 ± 0.18.

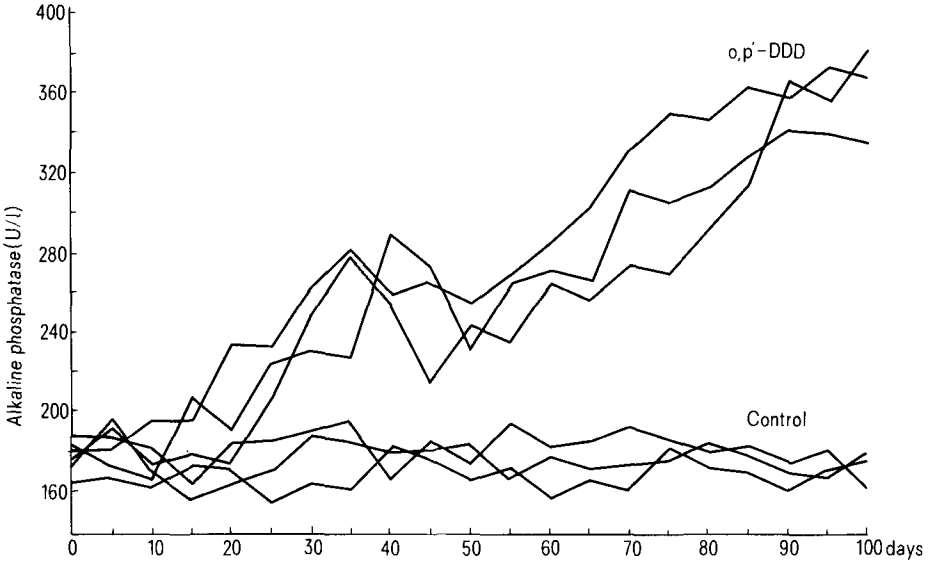

Figure 2. Alkaline phosphatase activity in serum of 3 control sheep and 3 sheep treated with o,p'-DDD for 100 consecutive days.

rise in serum alkaline phosphatase has been observed after oral o,p'-DDD treatment. In guinea-pigs[20], an oral dose of 300 mg/kg/day given for 12 days reduced circulating cortisol levels and killed one of the animals. In the dog[21] and in man[22] absorption of o,p'-DDD from the gastrointestinal tract has been shown to be poor. Although no absorption studies were performed in our preliminary experiment, the dramatic rise in alkaline phosphatase (over 100% increase) noticed at the end of the treatment period points towards a blood level of o,p'-DDD sufficient to have affected the adrenal cortex. However, as hepatic lesions were absent, the exact source of increased alkaline phosphatase activity must await further investigation. To summarize, the sheep (and perhaps ruminants in general) seems to be quite resistant to the cytotoxic effects of oral o,p'-DDD and, therefore, is not recommended for use for research purposes such as those outlined in the introduction. A definite judgement, however, is not possible until more data have been presented, especially about dosage, route of administration and bioavailability of the drug.

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Uptake of fat by fluorescent granular perithelial cells in cerebral cortex after administration of fat rich chow[1]

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Summary. As reported previously, fluorescent granular perithelial cells (F.G.P.) are distributed along small blood vessels, possibly postcapillary venous vessels, in the cerebral cortex; these cells take up intraventricularly administered horseradish peroxidase efficiently. In this study it is shown that lipid substances of the blood are easily incorporated into F.G.P. and stored in their cytoplasm. The quantity of fat deposits in F.G.P. varies with the age of the animal and is very marked in old rats. The administration of elastase suppresses the fat uptake and/or facilitates the fat metabolism in F.G.P.

In a previous paper[2] the authors have pointed out that the blood-brain barrier is not always as absolute as Westergaard and Brightman[3] suggested, and shows diurnal variations. The diffusion of substances through the walls of cerebral blood vessels is dependent on their molecular weight and their fat solubility. However, it is also possible that the transport of fat through the endothelium involves a vesicular component. Fluorescent granular perithelial cells (F.G.P.), discovered by the authors, are a specific type of histiocytes which are localized adjacent to small cerebral vessels, possibly postcapillary venous vessels, measuring 7-30 µm in diameter; they play an important role for the uptake, segregation and digestion of foreign material and of waste products in the central nervous system. They may thus be designated as cerebral scavenger cells. This paper is mainly concerned with the passage of nutritinal fat from the blood into the F.G.P. In addition, the effect of elastase on fat metabolism in F.G.P. was studied.

Material and methods. 16 Wistar rats, 8 months old, and 16 rats, 2.5 years old, were used; they were divided into the 2 groups A and B. Both groups were fed with a fat rich chow (Oriental Co., Tokyo, Japan), containing 2% cholesterol, soybean oil, 10% lard and 0.2% methylthiouracil for 1 day (group A) and 15 days (group B). Half of each group (4 young rats and 4 old rats) was s.c. injected with 5 mg/kg of elestase (Eisai Co., Tokyo, Japan) dissolved in 0.5 ml of physiological saline once a day; the other half received a control injection of the same volume of physiological saline. No irritations were seen at the injection sites.

The rats were sacrificed by decapitation, and the parietal region of the cerebral cortex was sliced using a blade. For light microscopy, specimens were stretched on glass slides, fixed with formaldehyde gas and stained with hematoxylin eosin (Mayer), PAS (periodic acid Schiff reaction) or sudan black B. For electron microscopy, the specimens were placed in 2.5% glutaraldehyde and 2% paraformaldehyde buffered with 0.1 M phosphate solution (pH 7.4), and then transferred into osmic tetroxide buffered with 0.1 M phosphate solution (pH 7.4). Then they were embedded in Epon 812, and cut with a Porter-Blum MT-2B ultramicrotome. The investigation was restricted to intracortical small vessels with a diameter of 10-15 µm.

Results and discussion. One day after feeding the fat rich chow (group A) the stainability of the intracellular granules in F.G.P. with eosin and PAS was somewhat diminished in both young and old rats, as compared with the controls. In the specimens stained with sudan black B, intracellular