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The effect of two synthetic steroids on the ultrastructure of the liver of *Rattus norvegicus* L.

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Flumedroxone acetate and an analogue [17-acetoxy-3β(β-carboxypropionyloxy)-6-trifluoromethylpregn-5-ene-20-one] each produce a liver weight increase and a change in hepatic cell ultrastructure, following chronic administration in mice and rats. In all liver cells there is much proliferation of the smooth endoplasmic reticulum, which arises from the ergastoplasm, or rough membranes. An effect on esterase enzyme specificity and the evidence for the induction of an esterase isoenzyme after treatment with these steroids, is referred to. The distribution of the new smooth endoplasmic reticulum is of interest as it varies with each analogue.

Flumedroxone acetate (17-acetoxy-6α-trifluoromethylpregn-4-ene-3,20-dione; Demigran) and its analogue, 17-acetoxy-3β(β-carboxypropionyloxy)-6-trifluoromethylpregn-5-ene-20-one, have been reported to increase the liver weight of rats, *Rattus norvegicus*, L. Porton strain. These increases followed the daily intraperitoneal or oral administration of either steroid at various dose levels for a period of 3 to 14 days (Hines, 1967). Experiments on various strains of mice also produced liver weight increases; here the subcutaneous route was used and the steroids were suspended in arachis oil (Hines, unpublished observations). Pantelouris & Hines (1968) reported that these two steroid compounds cause a widening of the substrate specificity of a particular "fast running" esterolytic enzyme from the liver and serum of the adult laboratory rat, as detected by starch-gel electrophoresis, and that this wider specificity is normal in the young animal. This paper describes changes in ultrastructure of rat liver (Wistar strain) after chronic treatment with flumedroxone acetate and its analogue.

EXPERIMENTAL

Virgin female albino Wistar rats (*Rattus norvegicus*, L.) were used as they consistently produced a greater liver weight increase than male rats at similar dose levels (Hines, unpublished observations). Each rat weighed approximately 250 g, and was maintained on Lever Brothers Spittal No. 4 rat cubes with water *ad libitum*. Each steroid was suspended in water (1 ml) using compound tragacanth powder and was given by gastric intubation. Some animals were given tragacanth only by the same route, as a vehicle control; other controls received no treatment. Control and treated rats were killed by cervical fracture 24 h after the final treatment, samples were immediately taken for ultrastructural studies and the liver and the body weights were recorded.

Tissue samples for electron microscopic investigation were removed from each of the liver lobes of six rats randomly chosen from each experimental group (see Table 1).

These were fixed immediately in 1% osmic acid in veronal acetate buffer, pH 7·4, for 1 h (Palade, 1952). The samples were then rinsed in veronal acetate buffer, dehydrated through graded acetones and propylene oxide, then embedded in Araldite (Luft, 1961). Preliminary treatment for staining was by uranyl acetate incorporated in the acetone dehydrating fluids and then the sections were stained in Reynolds lead citrate (Reynolds, 1963). The sections were cut on an LKB ultra-microtome with glass knives and examined in an AEI-EM-6B electron microscope at an accelerating voltage of 60 kV.

RESULTS AND DISCUSSION

The daily administration of flumedroxone acetate or of its unsaturated analogue (17-acetoxy-3β(β-carboxypropionyloxy)-6-trifluoromethylpregn-5-ene-20-one) for 9 days produced an increase in the liver weight of Wistar rats in each case (Table 1). Flumedroxone acetate evokes a liver enlargement of 65–70% with Wistar rats compared to 85–90% with the Porton strain (see Table 1 and Hines, 1967). The stage of development and sample size, rather than strain difference probably account for the variation in overall liver weight response. Durand, Fauconneau & Penot (1965) considered that rat liver attains its "functional equilibrium" at 10 weeks but continues to grow for some weeks more; the Wistar rats that I used were at least 12 weeks old.

Table 1. *Liver weight of rats after treatment with steroid compound for 9 days*

Drug	Dose mg/kg	Mean body weight g (No. of rats)		Liver weight g/100 g body weight (range, g)	
		Porton rats†	Wistar rats	Porton rats†	Wistar rats
Flumedroxone acetate	50	138·2 (4)	225·4 (10)	7·2 (8·7–10·6)	6·2 (12·6–15·1)
17-acetoxy-3β(β-carboxypropionyl-oxy)-6-trifluoro-methylpregn-5-ene-20-one	50	136·7 (4)	248·2 (8)	7·8 (9·4–12·1)	7·0 (15·5–18·9)
Compound tragacanth powder ..	700*	137·2 (6)	—	3·9 (4·3–6·0)	—
	200	—	245·0 (14)	—	3·8 (8·3–10·6)
None	—	138·1 (8)	249·2 (10)	3·8 (4·4–7·5)	3·7 (8·1–10·7)

* Treatment continued for 14 days.
† After Hines (1967).

The fundamental ultrastructural modification after the treatment of Wistar rats with each of these synthetic steroids is classical. Comparison of liver cells from treated rats (Fig. 1B and C) with liver cells from untreated rats (Fig. 1A), shows hypertrophy of the smooth endoplasmic reticulum (ser) of the treated animals. This hypertrophy differed with each steroid. With flumedroxone acetate (Fig. 1C) a lattice work of smooth tubules (ser) grow from the nearby parallel ergastoplasm (rough membranes; rer), and occupy the whole hyaloplasm of the liver cell, a picture comparable to that following phenobarbitone treatment, 80 mg/kg i.p. daily for 5 days (Remmer, 1966). This formation of smooth (ser) from rough (rer) membranes is

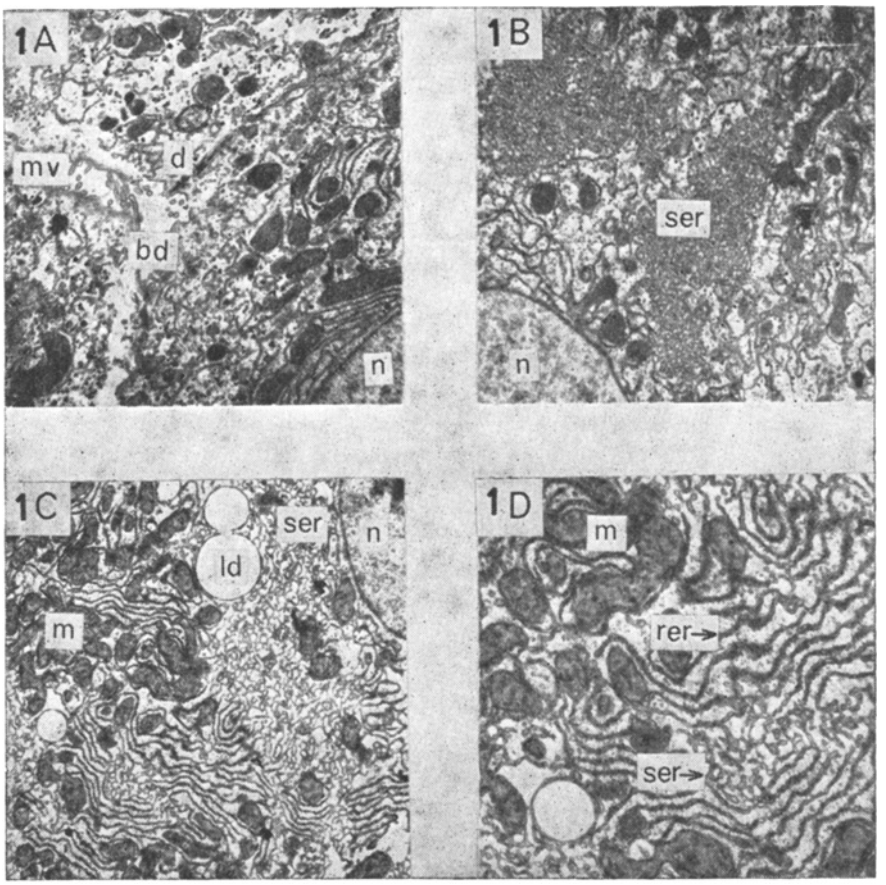

FIG. 1. Electron micrographs of rat liver cells. A. Untreated control, 1:7,400. B. 17-acetoxy-3β(β-carboxypropionyloxy)-6-trifluoromethylpregn-5-ene-20-one, 1:4,200. C. Flumedroxone acetate, 1:6,000. D. Flumedroxone acetate, 1:14,000. Abbreviations: m = mitochondrion; n = nucleus; mv = microvillus; bd = bile duct; d = desmosome; ld = lipid; ser = smooth endoplasmic reticulum; rer = rough endoplasmic reticulum.

shown more clearly in Fig. 1D. Such electron microscopic evidence indicates that smooth endoplasmic reticulum membranes are formed not through *de novo* protein synthesis, but through a process of cytoplasmic rearrangement. It has been observed that the rough endoplasmic reticulum membranes increase in liver cells even *in vitro* when respiration or oxidative phosphorylation are depressed (Loewe & Jung, 1965). For the unsaturated analogue of flumedroxone the distribution of the newly formed smooth membranes is seen to be zonal (Fig. 1B). This phenomenon was a constant feature with this steroid and has not previously been described. The origin of lipid is thought to be the hyaloplasm or the endoplasmic reticulum (du Boistesselin, 1966). In cells (e.g. sebaceous glands) engaged in the synthesis of lipids for export, it is common to find the cytoplasm filled with smooth surfaced tubules of ser (Porter, 1966). The proximity between smooth membranes and lipid (Fig. 2A), and an association between golgi, ser and lipid (Fig. 2B) was often seen following treatment with flumedroxone acetate. This suggests that the exogenous flumedroxone acetate has stimulated lipid formation through ser proliferation. On no occasion were any mitochondrial lesions observed with either steroid.

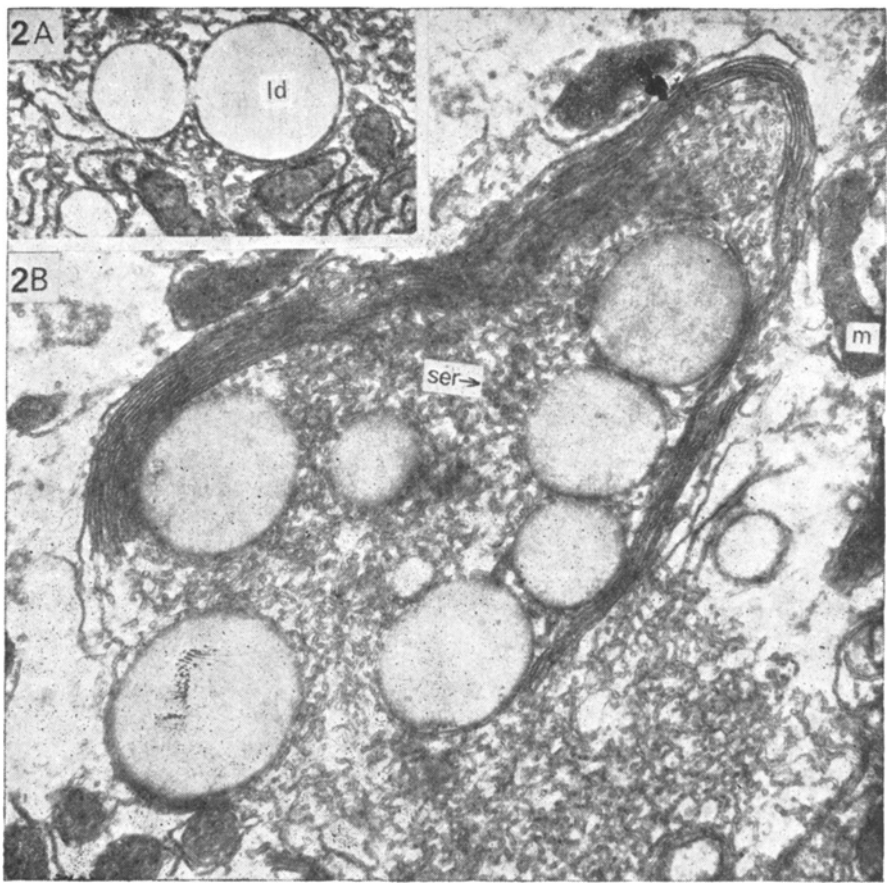

FIG. 2. Electron micrographs of rat liver cells treated with flumedroxone acetate. A. Smooth endoplasmic reticulum (ser) boundary to lipid, 1:15,500. B. Close association between ser, golgi and lipid, 1:14,000.

The chronic dosage of experimental animals with these progesterone-like synthetic steroids resulted in the following: an increase in the liver weight of various animals independent of the route of administration; a change in liver cell ultrastructure; and the appearance of two esterase isoenzymes one of which, like glucokinase (Golberg, 1966), appears in its broader reactive form as it does in the neonatal rat's liver and the second, a fast-running isoenzyme may be seen as a product of enzyme induction (Greengard, 1967; Pantelouris & Hines, 1968). Both isoenzymes were located in the microsomal fraction, a mixture of rough and smooth membranes. Chlorpromazine, phenylbutazone, SKF525-A and benzydamine can each produce in mice, after weekly treatment, a liver weight increase, a reduction in hexabarbitone sleeping time and a decrease in the retention of blood serum phosphatase (Silvestrini, Catanese & Del Basso, 1966). These drugs have been implicated in the induction of microsomal enzymes, which can activate the breakdown of the inducer itself, or their entirely different compounds (Remmer, 1964): the latter seemingly operating in this instance.

Meldolesi (1967) reviews those drugs known to bring about ser hypertrophy and suggests that 'ser hypertrophy in hepatic cells is always produced by one and the same mechanism", and further that the exogenous material which acts as the "inducer"

must itself be metabolized by enzymes which lie in the microsomal compartment of the cell. The latter fits this case, as both drugs are steroids and, as yet, are unproven inhibitors of protein synthesis. Although the overall mechanism might be the same for all ser hypertrophy, some difference must be operating in this case between the two steroid analogues. In one the ser is distributed through the cell, in the other it is zonal.

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