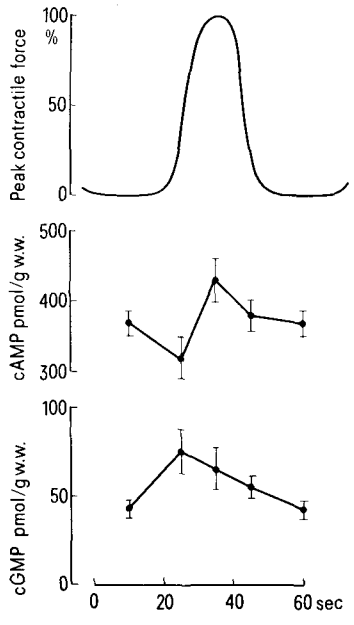
of estradiol valerianate, the cyclic GMP content of uterus horns was 149.2 ± 12.2 pmoles/g wet weight but, 68 h after injection, a value of 64.2 ± 10.5 pmoles/g wet weight (n = 8) was found. The difference was significant $p < 0.01$).

The results of the cyclic nucleotide determinations during the contraction cycle of estrogen dominated uterus are summarized in the Figure. The highest determined cyclic GMP level was found 5 sec after the start of the spontaneous contraction. At this time, the level was 30.6 ± 11.5 pmoles/g higher ($n = 8$, $p < 0.05$) than in the resting muscle. During the subsequent stages of contraction, the level fell towards the resting value. The cyclic AMP level reached its minimum level 321 pmoles/g 5 sec after the start of the contraction and its maximum 429 pmoles/g at the peak of contraction. The difference between the highest and the lowest cyclic AMP concentration (108 ± 35, $p < 0.05$) was probably significant.

In rats treated with progesterone (4 mg/kg) for 3 days before sacrifice, the spontaneous contractions of their uteri were small, 0.3–0.4 g, and the frequency was irregular. In these preparations it was not possible to follow the fluctuation of the cyclic nucleotides. The mean value of cyclic AMP for 8 animals was 370 ± 50 pmoles/g. The cyclic GMP level was 26 ± 2.5 pmoles/g, and significantly lower than in estrogen-dominated uteri.



Levels of cyclic AMP and cyclic GMP in rat uterus during different stages of a spontaneous contraction. Mean ± SEM of the results of 8–12 animals.

Our results demonstrate that during rhythmic contraction of rat uterus, oscillations in uterine concentrations of cyclic nucleotides occurred. This is in contrast to the report of DIAMOND and HARTLE[8]; these authors could not demonstrate significant fluctuations of cyclic nucleotides in rat uterus. However, when looking at their data the cyclic nucleotides tended to fluctuate in the same way as have been found in the present work, but the variations of their values were much larger than ours.

An interesting question is the causal relationship between the contraction-relaxation cycle and the observed changes of the cyclic nucleotide levels. In estrogen-dominated uterine muscle cells, the membrane potential is close to threshold for spontaneous discharge[9]. The rhythmic contractions have been suggested to be elicited by a synchronized discharge of actions potentials from the muscle cells[9]. The time-relationship between the electrical activity of the cell membrane and the changes of the cyclic nucleotides ought to be studied. If the electrical phenomena preceeds the nucleotide changes, the latter may be a consequence of the process of excitation.

It has been suggested that the spontaneous activity of uterus is associated with intramural generation of prostaglandins. Both these properties could be abolished by indomethacin[10]. Prostaglandins are also potent agents to increase the cyclic nucleotide levels in uteri[7]. The variations of the nucleotide levels as well as the electrical events in uterine muscle may therefore reflect prostaglandin generation.

Summary. Oscillations on the concentrations of cyclic nucleotides occurred during spontaneous rhythmic contractions in rat uterus. The levels of cyclic GMP and cyclic AMP were highest at the beginning of the contraction respectively the relaxation.

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Pathological Changes in the Liver of Mice Given 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (dioxin) is a potential contaminant of technical 2,4,5-trichlorophenol with a high toxicity for several species, including the rabbit[1], chicken[2,3], guinea-pig[3,4] and rat[3,4]. In all these species death occurs some time after administration of a single oral dose of dioxin. In the course of investigations[5] of biochemical changes in the liver of rodents, it was noticed that 7–10 days after dosing C57BL/6 mice the liver was pale and fatty. This communication describes studies arizing from this observation.

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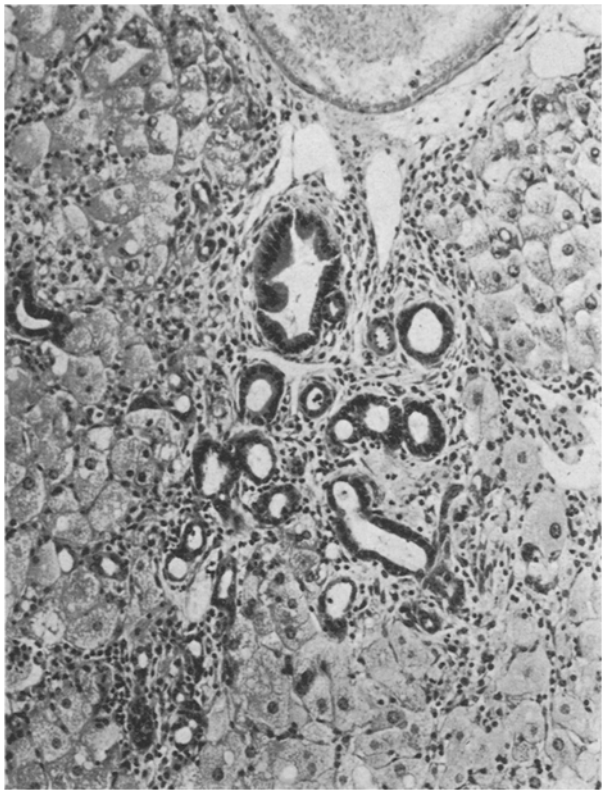

Fig. 1. 16 days. Bile duct proliferation and fibrosis in a portal tract. Note also the presence of dilated lymphatics. Formal Alcohol fixed; H & E, ×150.

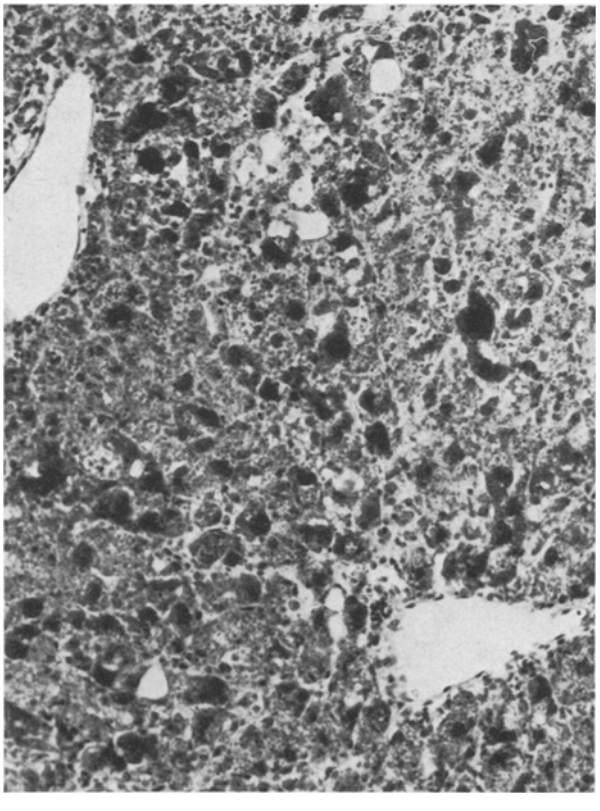

Fig. 2. 16 days. Parenchymal cells in all areas contain multiple vacuoles of lipid. Formal Calcium fixed, Oil Red O. ×150.

Male mice (7–15 weeks old, 14–30 g) of the C57BL/6 strain bred in this laboratory were maintained on Sterolit litter with access to MRC diet 41B and water. The 35 day LD_{50} of dioxin[3] following oesophageal intubation of solutions in arachis oil (5 ml/kg) was estimated[6] to be 126 μg/kg (95% confidence limits 86–183 μg/kg). In this experiment the mean time to death (± SE) was found to be 21.0 ± 1.6 days ($N = 8$).

Groups of 4 C57BL/6 male mice, given 250 μg/kg of dioxin p.o. in arachis oil, were killed and the organs taken for histological examination at 24 h, 3, 5, 8, 12, 16 or 35 days. At 8 days ,and thereafter, the livers of the dosed group were larger and paler than controls. A few moribund animals had subcutaneous oedema, and haemorrhage into the gastrointestinal tract. A progressive necrotic centrilobular lesion developed over the first 16 days after dosing.

The first change, increased eosinophilia of the cells in the centrilobular zone, occurred by 24 h. By 5 days, an infiltrate of mononuclear cells and a few polymorphonuclear leucocytes accompanied the lesion. Adjacent centrilobular areas were connected by bands of inflammatory cells and connective tissue. This extensive lesion gave the surface of the liver a granular appearance. In addition, at 12–16 days, a diffuse infiltration of the portal tracts and sinusoids by mononuclear cells was present. Bile duct proliferation and an increase in connective tissue occurred in the larger portal tracts. These bile ducts were lined by tall columnar cells with basal oval nuclei (Figure 1). Vacuolation of the parenchymal cells in all zones was present by 16 days (Figure 1). Their cytoplasm contained multiple fat vacuoles (Figure 2) and this increase in lipid was associated with dilatation of the lymphatics in the portal tracts (Figure 1). By 35 days the diffuse inflammatory infiltration of the liver had resolved, leaving a few foci in the centrilobular zones and adjacent to large portal tracts. Fibrosis remained a prominent feature of the larger portal tracts. A regenerative response of the viable parenchymal cells was observed at 5 and 8 days and mitotic figures were plentiful in the mice surviving 35 days.

The liver composition was examined in groups of C57BL/6 mice receiving either dioxin or oil p.o. Body weights were measured after 5, 8, 12 and 16 days and random groups of treated and control animals were killed at these times. Weighed portions of the livers were dried to constant weight for determination of their water content. Samples of 20% aq. liver homogenates were analyzed for proteins[7] and DNA[8]. Ethanol-diethyl ether extracts (3:1, v/v; 20 volumes) of the homogenates were assayed for total esterified fatty acids[9] and, by the Liebermann-Burchard reaction, for cholesterol.

The data in the Table demonstrates that the treated mice showed an increasing loss of weight as compared with the controls. This was possibly due to a reduction in food intake. However, in all but the day 8 group, the wet weight of the liver of the dosed animals, as a percentage of body weight, was significantly higher than that of the controls.

The analyses indicate that an increased lipid content was responsible, at least partially, for the greater liver weights. The lipids were significantly elevated in the treated animals at day 5, and thereafter increased until on day 16 the esterified fatty acids accounted for 21%

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Effect of a single oral dose of 250 μg dioxin/kg on body weight, liver weight and liver constituents of male C57 BL/6 mice

Days after dosing		Change in BW since dosing (%)	Liver wet weight (g/100 g BW)	Liver water content (% w/w)	Esterified fatty acids (μequiv/g wet liver)	Cholesterol (mg/g wet liver)	Protein (mg/g wet liver)	DNA (mg/g wet liver)
5	Controls	+ 5.5 ± 0.59 (16)	5.18 ± 0.20	67.2 ± 0.2	112 ± 2.9	2.95 ± 0.24	161 ± 2.3	2.15 ± 0.11
	Treated	+ 2.4 ± 0.54[c] (20)	6.45 ± 0.18[b]	68.9 ± 0.4[b]	156 ± 12.4[a]	5.02 ± 0.30[b]	154 ± 2.6	2.19 ± 0.03
8	Controls	+ 5.8 ± 1.98 (12)	4.24 ± 0.23	68.4 ± 0.2	115 ± 5.0	3.40 ± 0.04	174 ± 1.8	2.52 ± 0.21
	Treated	− 1.5 ± 1.16[b] (16)	4.56 ± 0.23	69.1 ± 0.4	263 ± 22.7[c]	7.33 ± 0.57[c]	156 ± 2.2[c]	2.77 ± 0.18
12	Controls	+ 3.8 ± 1.40 (8)	4.45 ± 0.25	67.6 ± 0.3	89 ± 6.8	3.47 ± 0.37	163 ± 2.8	2.71 ± 0.11
	Treated	− 8.3 ± 2.52[c] (12)	5.33 ± 0.13[a]	61.9 ± 2.9	540 ± 93[b]	10.55 ± 1.35[b]	113 ± 11.1[b]	2.58 ± 0.23
16	Controls	+ 7.2 ± 1.51 (4)	5.01 ± 0.14	68.3 ± 0.7	99 ± 4.7	3.46 ± 0.14	167 ± 5.0	2.45 ± 0.10
	Treated	−16.9 ± 3.82[c] (6)	8.07 ± 0.87[b] (6)	55.5 ± 4.3[a] (7)	742 ± 14.6[b] (7)	11.19 ± 0.72[c] (7)	107 ± 7.4[c] (7)	2.29 ± 0.24 (7)
2[d]	Controls	−18.9 ± 0.71 (7)	4.69 ± 0.08 (7)	—	348 ± 40.8 (7)	4.73 ± 0.19 (7)	—	—
	Treated	−17.7 ± 0.51 (7)	5.57 ± 0.11[c] (7)	—	402 ± 60.7 (7)	5.64 ± 0.11[b] (7)	—	—

Dioxin was dissolved in arachis oil (50 μg/ml), controls received an equivalent volume of arachis oil. Values are means ± SEM for groups of 4 mice (unless number of mice is otherwise indicated in brackets). Those marked differ significantly from the controls: [a] $p < 0.025$; [b] $p < 0.01$; [c] $p < 0.001$. [d] Food was withheld from both groups immediately following dosing.

of the liver wet weight as compared with 3% in the control mice. At later periods these increased lipid levels of the treated mice were associated with decreased liver protein and water contents. The fact that the DNA content of the liver was unaltered despite the increase in liver size may have been due to the inflammatory infiltration of the liver. Starvation of mice following dosing with either dioxin or oil resulted in elevated liver esterified fatty acid levels in both groups. However the dioxin treatment had caused significant increases in mean liver weight and cholesterol content (Table).

The pathological findings in the liver are similar to those reported by GOLDSTEIN et al.[10], and differ in many respects from the liver lesion induced in rats by dioxin[3, 11]. Extensive centrilobular necrosis, bile duct proliferation and lipid accumulation are not features of the lesion in rats and multinucleate hepatocytes were not seen in mice. In surviving animals of both species, the liver lesion regressed with little change in the basic liver architecture.

Although these results clearly indicate that dioxin induced a fatty liver in C57BL/6 mice the mechanism of this induction is possibly indirect. Mice are known to develop fatty livers following starvation and the loss of body weight following dioxin treatment would suggest that the lipid accumulation was, at least partially, a consequence of a depression of food intake. The increased cholesterol in the livers of dioxin treated mice may be associated with the synthesis of new membrane-bound enzymes[5, 12].

Summary. In C57BL/6 mice a single oral dose of 2, 3, 7, 8-tetrachlorodibenzodioxin (LD_{50} 126 μg/kg) results in loss of body weight and death with an enlarged fatty liver after ca. 21 days. A progressive necrotic centrilobular liver lesion is also seen.

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Quaternary Isoarecoline: a Potent Muscarine-Like and Nicotine-Like Acting Agonist

In our studies of cyclic and semi-rigid analogs of acetylcholine, we have synthesized and tested derivatives of isoarecaidine (1-methyl-1, 2, 5, 6-tetrahydroisonicotinic acid)[1, 2].

In several types of preparation (rat blood pressure, smoth muscle, heart muscle) the quaternary methylester (isoarecoline) shows a high muscarinic activity. On the guinea-pig ileum, it has the same intrinsic activity and affinity ($pD_2 = 8.0$) as acetylcholine (Figure 3).

It has been reported that arecoline (methyl 1-methyl-1, 2, 5, 6-tetrahydronicotinate) acts as a potent muscarinic agonist through the release of endogenous acetyl-

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