beeinflussen sich die kompensatorischen und reparativen Vorgänge nicht, sondern laufen nacheinander ab.

Summary. Following 2/3 hepatectomy + CCl_4, the labelling- (^{3}H-thymidine) and mitosis-index of liver epithelia increases 8 h later than after an unique 2/3 hepatectomy, and the peak of compensatory regeneration is reached 16 h later. Then the curve declines and 48 h post operationem reparative regeneration due to CCl_4 necrosises starts.

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Reversible Inhibition by CCl_4 of Bile Secretion During Isolated Liver Perfusion

A number of recent publications[1-3] deal with early hepatotoxic effects of carbon tetrachloride. The first detectable events are biochemical changes in the endoplasmic reticulum, occurring about 5 min after application of CCl_4. Little attention, however, was paid to alterations in bile secretion as response to the hepatotoxin. Such an alteration is the decrease in bile secretion cumulatively measured over periods of some hours after intragastric administration of CCl_4 in rats[4].

To avoid extrahepatic influences, we used isolated liver perfusion for studying early effects of CCl_4 on bile secretion. The haloalkane was introduced in gaseous form. Furthermore we measured bile production by visual counting of drop frequency to achieve the detection of quickly occurring bile flow changes.

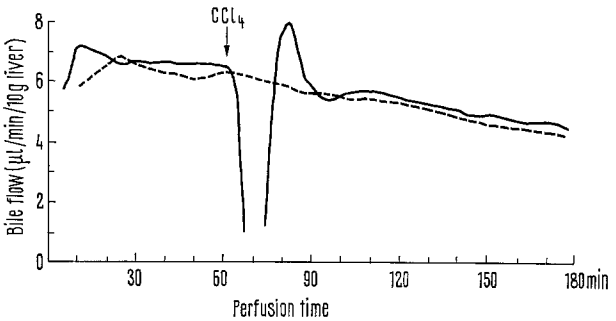
Material and method. 15 livers of male Wistar rats (200–250 g) were perfused according to Schimassek[5] with a suspension of bovine erythrocytes in albumin containing Tyrode solution. CCl_4 was introduced by switching the oxygen flow for 5–10 min from the humidifier to a gassing tower filled with CCl_4. Thereby oxygen supply was maintained during CCl_4 application. In 5 experiments CCl_4 (20 µl) was injected directly into the portal vein cannula[6]. Hexobarbital metabolism was measured by estimation of the disappearance of substrate[7,8].

Results and discussion. The most striking observation was a sharp decrease in bile flow occurring at about 3 min after introduction of gaseous CCl_4 (Figure); 2–3 min later the bile flow stopped entirely. At this point the CCl_4 gassing was finished. After a lag period of 2–10 min the bile flow accelerated to a maximum and returned to control values at 30–50 min after CCl_4 treatment. In control perfusions, a steady slow decrease of bile flow rate was measured. During CCl_4 induced stop of bile secretion, a slight increase in blood flow (10–20%) could be observed. The pH of the perfusion medium was not affected by the CCl_4 treatment. Hemolysis in perfusion experiments with CCl_4 did not exceed control values (1.2–1.7% after 3 h).

We also investigated the effect of CCl_4 when injected into the portal vein. The changes in bile flow (reversible decrease by about 30%) were less than in the case of gaseous CCl_4, but blood flow simultaneously decreased as response to the hepatotoxin. In addition multiple anemic regions located subcapsularly and irregular granulation of the liver surface appeared within 30 min after CCl_4 injection. Similar liver alterations could be observed with gaseous CCl_4 only at 120–180 min after application or with gassing times longer than 10 min. Because of these disadvantages the intraportal injection was abandoned.

The course of bile flow inhibition by gaseous CCl_4 leads to following considerations:

1. The response of bile flow to application of gaseous CCl_4 during liver perfusion occurs with at least the same rapidity as the changes in the endoplasmic reticulum. Therefore the described decrease in bile flow is, to our knowledge, the earliest change in liver function as response to CCl_4.
2. The occurrence of a maximum after restoration of bile flow suggests a retention of bile constituents inside the hepatocyte due to secretion inhibition during CCl_4 influence. A congestion in bile ducts due to smooth muscle contraction and following release of bile seems rather unlikely, since blood flow is increased at the same time. Changes in blood flow within the range of our experiments did not influence bile flow.
3. The reversibility of inhibition may be explained by the hypothesis of a physicochemical action of CCl_4[7]: mediated by its lipid solubility, CCl_4 can enter the lipid layer of membranes and thereby impair membrane properties. The restoration of normal membrane function in respect to bile secretion could be caused by rediffusion of CCl_4 out of the membranes after the end of application.



Bile flow during isolated perfusion of rat liver. Solid line: flow changes caused by gaseous CCl_4. Dotted line: control perfusion. Each line represents one single perfusion experiment.

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4. CCl_4 is known to reduce the activity of drug metabolizing enzymes as soon as 60 min after intragastric application[7]. Hence we measured the oxidation of hexobarbital at various intervals after introduction of the haloalkane. We did not find, however, any decrease in hexobarbital metabolism when compared with control experiments from 0–150 min after CCl_4 treatment. Perhaps this lack of drug enzyme damage is due to the short time of CCl_4 influence during liver perfusion. Therefore the method of introducing gaseous CCl_4 into a liver perfusion system can be regarded as a 'pulse' treatment, allowing the detection of early and reversible functional changes.

Zusammenfassung. Durch kurzzeitige Applikation von gasförmigem Tetrachlorkohlenstoff während Leberperfusion tritt eine rasche und reversibel verlaufende Gallesekretionshemmung ein. Diese Hemmung könnte auf Veränderungen von Transport- oder Permeabilitätseigenschaften der Leberzellmembran durch CCl_4 zurückgeführt werden.

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An Histochemical Study of the Nerve Supply to the Developing Alimentary Tract

Histochemical techniques for the demonstration of substances involved in nerve transmission processes have been used by several workers in the study of alimentary canal nerve structures in adult mammals and birds. Fibres and nerve cells rich in acetylcholinesterase[1–6] and adrenergic fibres[7–9] have been demonstrated in Auerbach's and Meissner's plexuses and in the muscle layers of the intestinal wall. Very little work, however, has been done in this field as far as embryonic and postnatal development is concerned[10–12].

The aim of the present study was to investigate the differentiation of intrinsic nerve cells as well as the ingrowth of extrinsic nerve fibres, in the developing alimentary tract of the rat, rabbit and chicken using KOELLE's[1] method for the demonstration of acetylcholinesterase activity. Histochemical findings were compared with morphological differences in silver-stained embryos from the early stages of development to birth.

Rat and rabbit embryos from the 11th day[13] and chick embryos from stage 23 H.H.[14] were treated with the method of Koelle-Friedenwald as modified by GEREBTZOFF[15]. The reaction was carried out on in toto dissected alimentary tract and on 20–40 μ thick sections; the samples were pre-incubated with $1 \times 10^{-6} M$ Mipafox to inhibit non-specific cholinesterases. Silver impregnation of embryos at the same stages of development was carried out by means of Cajal-De Castro, Bielschowsky-Gros and Bielschowsky-Boeke techniques.

Nerve fibres from the vagus nerve are found in the oesophagus and stomach wall, from the 13th day on, in rat and rabbit embryos; in the chick, they appear at stage 24–25. In the stomach these fibres are gathered into 2 flattened bundles on the anterior and posterior face (of both proventriculus and gizzard in the chick), but are absent from the greater and lesser curvatures. The fibres show a clear acetylcholinesterase-positive reaction, as do the rare unipolar neuroblasts observed in these organs (Figure 1).

From the 14th day on in the rat, and from the 16th day on in the rabbit, the vagal fibres spread randomly over all the stomach and beyond the pylorus into the duodenal loop. A negative reaction is observed in the remaining parts of the anterior and posterior intestine. Furthermore, Koelle-positive nerve fibres proceed from the coeliac

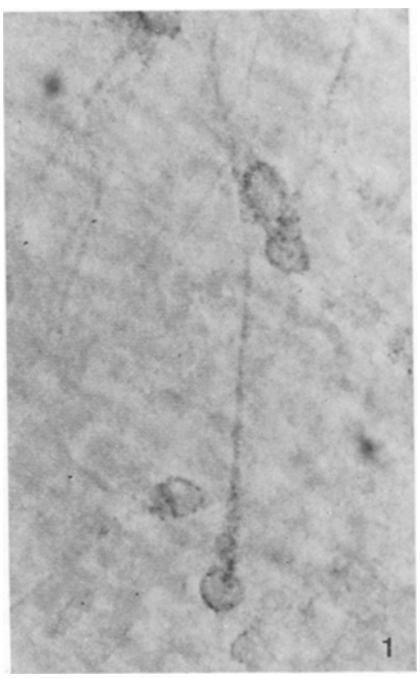

Fig. 1. Monopolar neuroblasts in the wall of the proventriculus show a clear acetylcholinesterasic activity in a small perinuclear area as well as in their processes. Chick embryo at stage 25 H.H. Koelle method. × 600.

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