EFFECT OF LONG-TERM CARBON TETRACHLORIDE ADMINISTRATION ON BILE COMPOSITION IN RATS

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The action of carbon tetrachloride (CCl_4) on the body leads to the appearance of metabolic and structural changes in the liver [1-4, 12, 13] which may be both damaging and compensatory in character [3]. The trend of these changes during long-term administration of the hepatotoxin determines the rate of subsequent development of the pathological process. Changes in the bile-secreting function of the liver and in the composition of the organic constituents of the bile under these conditions have received little study [8, 9]. Meanwhile specific interaction of CCl_4 with the microsomal oxidative system of the hepatocyte, leading to binding of the iron of hemocytochrome P-450 and a decrease in its content in the cell [1, 10, 13], and to an indirect effect on the activity of hydroxylases involved in the conversion of cholesterol into bile acids [11], suggests changes in the synthesis and secretion of individual bile acids by the liver.

The object of this investigation was to study the effect of long-term CCl_4 administration on the time course of bile secretion and composition.

EXPERIMENTAL METHOD

An experimental model of toxic liver damage was formed in rats by subcutaneous injection of 50% CCl_4 solution in olive oil in a dose of 1 ml/kg body weight twice a week. The longest period of injection was 6 months. Experiments were carried out on 175 male albino rats weighing 150-250 g. The secretory function of the liver was studied repeatedly after the 1st, 2nd, 4th, and 7th injections of CCl_4 and 1, 2, 4, and 6 months after the beginning of injection of the poison. The bile duct was cannulated. Bile was collected from fasting animals in the course of 3 h during an acute experiment under ether-hexobarbital anesthesia. The volume of bile was measured and its content of conjugated bile acids [5], cholesterol (the Liebermann–Burchard reaction), bilirubin (Van-den-Bergh's method), and phospholipids (as lipid phosphorus) determined. The results were expressed as the concentration (in mg/liter) of each component per gram weight of liver. In some experiments gamma-glutamyl transpeptidase (GGTP) activity was determined in the blood serum and bile (using standard kits from Czechoslovakia). A parallel electron-microscopic investigation of the liver was made with the EMV-100L microscope after perfusion of the organ through the portal vein. Animals receiving olive oil only and rats receiving no injection whatever served as the control group.

EXPERIMENTAL RESULTS

A particular feature of this experimental model is the use of olive oil as the solvent for CCl_4, for administration of dietary fats could affect the composition of the bile [7]. Long-term injections of olive oil in the present investigation also gave rise to definite changes in bile secretion in the rats, and to take these into account, in each series of the investigation results obtained in rats receiving CCl_4 and rats receiving only olive oil were compared.

The acute period of liver poisoning was characterized by qualitative and quantitative changes in the secretory process. For instance, a single injection of CCl_4 caused a small

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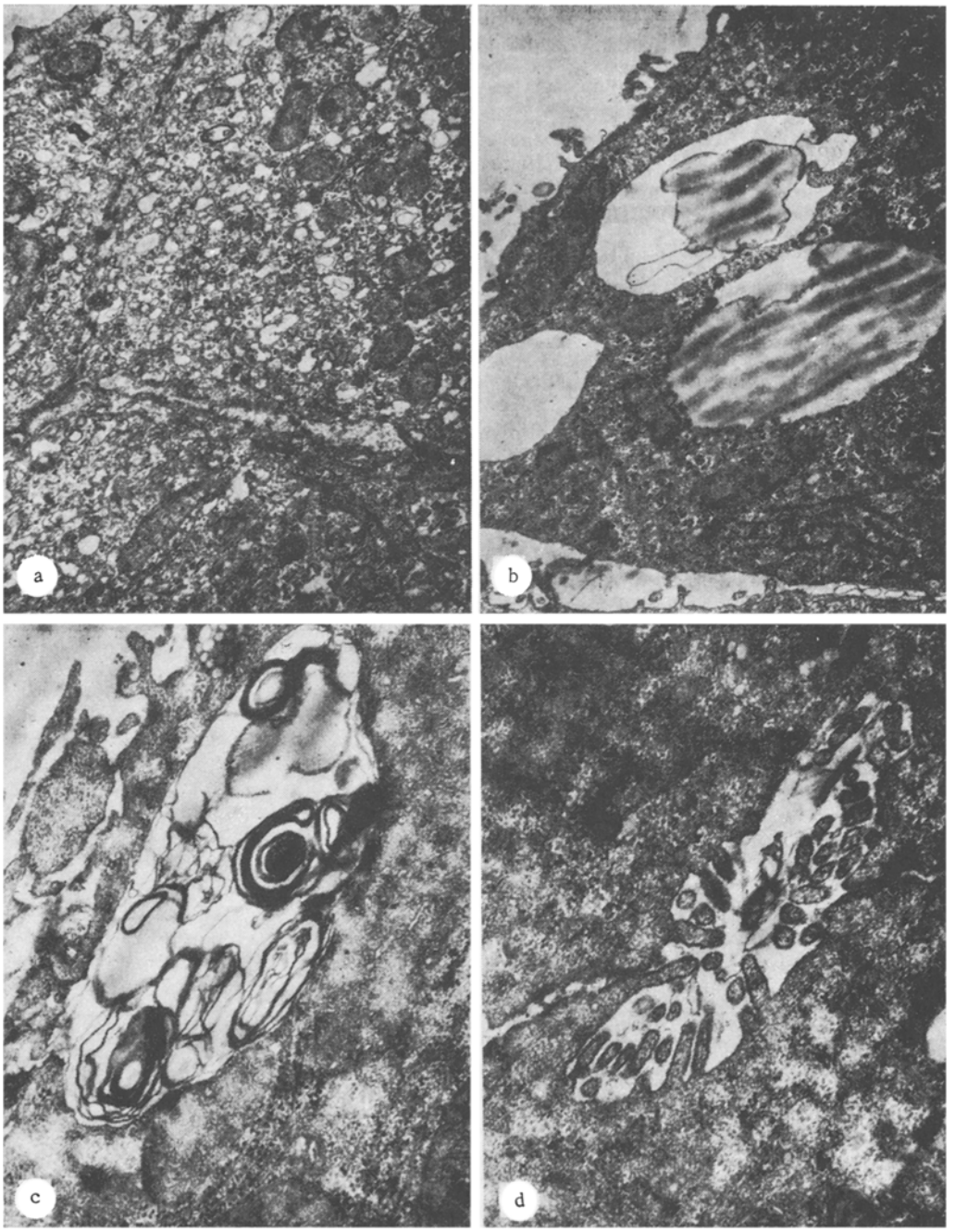

Fig. 1. Ultrastructural changes in hepatocytes at various times of action of CCl_4: a) vacuolation and fragmentation of rough and smooth endoplasmic reticulum after a single dose of CCl_4; b-d) after CCl_4 poisoning for 6 months: fatty degeneration of hepatocytes and widening of intercellular space (b), myelin-like structures in hepatocytes (c), biliary canaliculus containing myelin-like structures (d). Magnification: a) 15,000, b) 20,000, c, d) 30,000×.

(not significant) decrease in the volume of bile secreted in the course of 3 h from 0.16 ± 0.01 to 0.14 ± 0.01 ml/g weight of liver ($P = 0.2$). The total concentration of conjugated bile acids in the bile fell under these circumstances from 0.54 ± 0.04 to 0.40 ± 0.02 g/liter/g weight of liver ($0.91 > P > 0.001$). Modification of bile acid metabolism led to a reduction by one-third in the ratio between trihydroxy- and dihydroxycholanic acids and a reduction in the glycine–taurine ratio by 1.1 times. A small decrease (not significant) in the bilirubin concentration in the bile by 22.2% ($P = 0.2$) was observed, whereas the cholesterol concentration was 40.6% higher than in the control ($0.05 > P > 0.02$).

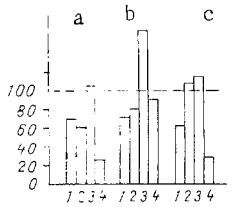

Fig. 2. Changes in concentration of bile components at different periods of toxic liver damage. Time after beginning of CCl_4 injection: a) 1 month, b) 4 months, c) 6 months. 1) Bile acids, 2) bilirubin, 3) cholesterol, 4) phospholipids. Abscissa, changes in secretion of above-mentioned components; ordinate, concentration of components of bile (in % of control, taken as 100).

A similar trend of change in these components was still present after conversion to absolute amounts in milligrams secreted with the bile during a 3-h period, per 100 g liver. All these facts are evidence of a definite disturbance of bile formation in rats under the influence of each sessional dose of CCl_4 injected. This conclusion is confirmed by the increase in GGTP activity observed at this time in the blood serum (by 2.5 times), and the very considerable (by 30 times) increase in its activity in the bile, most probably connected with a compensatory intensification of the synthesis of this enzyme in the liver in response to damage, for this enzyme plays an important role in the transport and assimilation of amino acids and also in regulating the tissue glutathione level, and it provides a sensitive test for use in liver diseases [12]. Investigation of the ultrastructure of the liver tissue showed that hepatocytes are most exposed to the action of CCl_4. Among the characteristic injuries of the liver cells in the acute period vacuolation and fragmentation of the rough and smooth endoplasmic reticulum were observed (Fig. 1a), for the principal biochemical processes connected with synthesis of bile acids take place on its membranes. Mitochondria with a translucent matrix could be seen. Lipid drops of different sizes appeared in the hepatocytes.

Further introduction of CCl_4 induced an acceleration of pathological processes in the liver, accompanied by certain undulatory changes of its secretion activity (Fig. 2). The volume of bile secreted one month from the beginning of CCl_4 injection decreased to 0.8 ± 0.1 ml/g weight of liver ($P < 0.5$); this comprised 66.7% of the control level. Formerly the lowest volume of bile in the secretions was 0.45 ± 0.08 g/liter/g weight of liver, $P < 0.05$. There was also a lessened secretion of bilirubin and particularly of phospholipids (down to 70.3%). The concentrations of cholesterol in the bile had similar activity; GGTP levels definitely did not distinguish themselves from the control values ($P > 0.5$). At the same time a nine-fold increase in serum GGTP activity indicated further disturbance in the liver cells.

Some increase in the intensity of bile secretion by the liver was observed 2 months after the beginning of CCl_4 injection, as shown by an increase in the volume of bile secreted up to the control values ($P > 0.2$). However, both the concentration of the principal organic components of the bile and their absolute amounts during a 3-h period of secretion were, as before, lower than in the control. The decrease in the content of bile acids in the bile in this case was due mainly to a decrease in the synthesis and secretion of taurine conjugates. This reorganization of synthesis of individual bile acids led to an increase of 2.8 times in the glycine—taurine ratio and to an increase of 1.7 times in the ratio between trihydroxy- and dihydroxycholanic acids.

Somewhat higher activity of bile secretion was observed 4 months after the beginning of CCl_4 administration (Fig. 2). During this period bile secretion again exceeded the control level ($P = 0.01$). Although the concentration of bile acids as before remained low, their absolute content reached 90.2% of the control level. The concentration of phospholipids in the bile increased appreciably, as also did that of bilirubin, although to a rather lesser degree. There was a particularly sharp rise (up to 162.5%) in the concentration of cholesterol in the bile ($P < 0.001$), and its absolute secretion rose from 1.91 ± 0.18 to 3.68 ± 0.46 mg/

100 g weight of liver (P = 0.001). Further observation, however, indicated a more intensive development of the pathological changes in the liver. Whereas after CCl_4 poisoning for 6 months the volume of bile secreted was indistinguishable from the control, the concentration and absolute secretion of bile acids were considerably reduced during this period (0.01 > F > 0.001). Secretion of phospholipids, another very important component of bile, was very low. The concentration of cholesterol and bilirubin exceeded the control level but not significantly The fall in serum GGTP activity below normal during this period indicates, in turn, a disturbance of synthetic processes in the liver. Further changes were observed in hepatocyte ultrastructure: vacuolation, degranulation, and fragmentation of the rough and smooth endoplasmic reticulum, the development of fatty degeneration (Fig. 1b), accumulation of myelin-like structures (Fig. 1c), the appearance of which in the cell is linked with the action of the poison and may be one sign of hepatocyte pathology [6]. Widening of the intercellular spaces was observed. Individual biliary canaliculi were dilated and some of them also contained myelin-like structures (Fig. 1d).

Prolonged administration of CCl_4 thus has a marked effect on the character of bile secretion in rats. The most profound changes in this condition affect biochemical processes connected with the synthesis of bile acids, the principal secretory component of bile. This is confirmed both by a decrease in their content in the bile throughout the period of investigation and by changes observed in the relative proportions of individual fractions. The most likely cause of this disturbance is a decrease in the content of cytochrome P-450 in the hepatocytes under the influence of CCl_4 [10] and a consequent reduction in the rats of hydroxylation of cholesterol in the 7α-position, which may lead to an increase in cholesterol concentration in the liver and, to some extent also, in the bile. Meanwhile the fluctuating nature of the changes in bile secretion with time may indicate that compensatory processes play a definite role in this state.

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