and aspartic acid are important. This is in keeping with the suggestion of Perlmann (1955) that phosphoproteins and peptides are rich in glutamic acid and aspartic acid in addition to serine. About 20 % of the amino acids of hen's-egg phosvitin are basic; none could be detected in herring-egg phosvitin. This would explain the low N:P molar ratio of the herring-egg phosvitin (1.38; that of hen's-egg phosvitin is 2.72). Another fish-egg protein, obtained by Ito *et al.* (1963), contains only six amino acids; of these four are common to herring-egg phosvitin, namely serine, aspartic acid, glutamic acid and valine.

The lack of aromatic amino acids in the phosvitins results in their showing unusual ultraviolet-absorption spectra when compared to that of a mixture of 'typical' proteins, namely human γ -globulins (Fig. 1). The low plateaux in the 250–290 $m\mu$ region are probably due to light-scattering and the increase in extinction at lower wavelengths is probably due to the peptide bond (Beaven & Holiday, 1952).

It is probable that herring-egg phosvitin is an important reserve of both phosphorus and energy for the growing embryo, a reserve which would be particularly important in the herring egg with its long period of incubation (10 months) and its impermeability to orthophosphate (Needham, 1931).

SUMMARY

1. A phosphoprotein (herring-egg phosvitin) has been isolated from the eggs of the herring by a procedure involving extraction with butanol-1-ol of a water-soluble lipoprotein complex.

2. The calcium salt of the phosvitin contained 7.6 % of nitrogen and 12.0 % of phosphorus.

3. The protein contained only five amino acids, namely serine, aspartic acid, glutamic acid, valine

and threonine. Only serine was phosphorylated in the intact material.

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Lipid Metabolism in Liver Injury Caused by Carbon Tetrachloride in the Rat

By A. S. AIYAR, PREMA FATTERPAKER AND A. SREENIVASAN
Central Food Technological Research Institute, Mysore 2, India

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The fundamental metabolic deviation that results in fatty degeneration of liver in rats given carbon tetrachloride is not yet fully understood, though the biochemical and histological alterations in liver caused by this hepatotoxin have been investigated intensively in recent years.

Extensive studies have indicated that the administration of carbon tetrachloride causes damage to the integrity and function of mitochondria, resulting in an impaired ability to retain cofactors (Christie & Judah, 1954; Judah & Rees, 1959) and a marked uncoupling of oxidative phosphorylation

(Dianzani, 1954, 1955, 1957). Investigations in our Laboratory have revealed loss of mitochondrial nucleotides (Kasbekar & Sreenivasan, 1956; Kasbekar, Rege & Sreenivasan, 1959b), phospholipids (Kasbekar, Lavate, Rege & Sreenivasan, 1959a) and coenzyme Q (Aiyar & Sreenivasan, 1962) in livers of animals treated with carbon tetrachloride.

That breakdown of mitochondrial function is the fundamental lesion in the liver of animals poisoned with carbon tetrachloride has been doubted by several investigators. In particular, it has been pointed out that the time of arrival of carbon tetrachloride in the liver coincides with the increase in liver fat and precedes by several hours the time of onset of mitochondrial alterations (Recknagel & Anthony, 1959; Recknagel & Litteria, 1960). On the other hand, marked dilatations of the cisternae of the endoplasmic reticulum are discernible as early as 1 hr. after administration of the steatogen (Bassi, 1960; Oberling & Rouiller, 1956; Smuckler, Iseri & Benditt, 1961) and are followed by changes in microsomal enzyme systems (Neubert & Maibauer, 1959). Recknagel & Lombardi (1961) have suggested that, in liver injury caused by carbon tetrachloride, the hepatic triglyceride secretory mechanism (Byers & Friedman, 1960), probably localized in the membranous component of the endoplasmic reticulum, is inhibited or destroyed and is the key focus involved. Maling, Frank & Horning (1962) reported that, when [^{14}C]-palmitate is injected intravenously, the ^{14}C -labelled triglycerides appearing in the plasma of the rats treated with carbon tetrachloride are much less than those of the control. Similarly, the formation of plasma lipoprotein (Robinson & Seakins, 1962) and the phosphorylcholine-glyceride-transferase activity (Wilgram, Smith, Ingli & Kennedy, 1961) are also decreased in fatty livers. These observations, together with those on the impaired synthesis of plasma protein (Smuckler *et al.* 1961), are of importance in view of the involvement of protein moieties in the mobilization of lipids from liver. An impairment of lipoprotein synthesis in the liver (Seakins & Robinson, 1963) and consequently of outward hepatic triglyceride transport (Heimberg, Weinstein, Dishmon & Dunkerley, 1962) may be the aetiology of the fatty livers caused by poisoning with carbon tetrachloride.

In view of the above observations, several phases of lipid metabolism in rats given carbon tetrachloride have been investigated for a better understanding of the mode of action of this hepatotoxin.

EXPERIMENTAL

Management of animals. Adult male albino rats (Wistar strain), weighing 155–170 g. and reared on the laboratory

stock diet [made up of (percentages by weight): wheat flour, 75; casein, 12; whole milk powder, 2; dried yeast, 2; arachis oil, 4; shark-liver oil, 1; sodium chloride, 2; and calcium carbonate, 2], were used in all the studies. The experimental animals were injected intraperitoneally with 2 ml. of carbon tetrachloride/kg. body wt., and the control rats received corresponding amounts of distilled water. At intervals of 3 and 24 hr. after the injection, the animals were killed by cervical section except when blood was required.

Preparation of tissues. Blood was withdrawn from the inferior vena cava of animals kept under light ether anaesthesia, clotted at room temperature for 30 min. and centrifuged for serum. Livers were excised quickly, chilled in 0.25M-sucrose solution for 5 min., dried with filter paper, weighed accurately and portions made into 10% suspensions with a Potter-Elvehjem-type glass homogenizer fitted with a Teflon pestle (A. H. Thomas and Co., Philadelphia, Pa., U.S.A.). Representative samples of liver lobes from each animal were cut into thin uniform slices of less than 0.5 mm. thickness with a Stadie-Riggs tissue slicer (A. H. Thomas and Co., Philadelphia, Pa., U.S.A.). For electrophoretic separation of the lipoproteins of the liver supernatant, the liver was perfused with 0.9% sodium chloride solution through the dorsal aorta while the animal was kept under light ether anaesthesia. Portions (4 g.) of the perfused liver, each homogenized in 2 ml. of 0.25M-sucrose, were centrifuged at 105000g for 1 hr. in rotor no. 40 of the Spinco model L preparative centrifuge (Beckman Instruments Inc., Belmont, Calif., U.S.A.), after prior centrifugation in the International model PR-2 refrigerated centrifuge (International Equipment Co., Boston, Mass., U.S.A.) at 5000g for 10 min. to remove nuclei and mitochondria. The clear supernatants were used for electrophoresis.

Lipid composition of liver. Total lipids of liver were extracted by the procedure of Sperry (1954) and determined gravimetrically.

Portions of liver homogenate (2 ml. of the 10% suspension) were treated thrice with cold 10% (w/v) trichloroacetic acid to extract acid-soluble phosphorus. The residue was resuspended in water (1 ml.) mixed with cold 95% (v/v) ethanol (4 ml.), centrifuged and then repeatedly extracted by boiling with ether-ethanol (1:3, v/v) (Schneider, 1945). The combined ethanol and ethanol-ether extract containing phospholipids was digested with perchloric acid, and phosphorus was determined by the method of Tausky & Shorr (1953). The weight of phospholipids was calculated by multiplying the weight of phosphorus by 22.7. The total cholesterol in liver was determined by the method of Sperry & Webb (1950).

The fatty acids of acetone-soluble lipids were determined by extraction of total lipids of liver with acetone, hydrolysis with potassium hydroxide and liberation of free fatty acids with sulphuric acid, as described by James, Lovelock, Webb & Trotter (1957). The free fatty acids obtained were weighed.

Incorporation of [$^{14}\text{C}_2$]acetate into lipid fractions by liver slices. Liver slices (approx. 500 mg.) were incubated in Krebs-Ringer bicarbonate solution, pH 7.4 (Umbreit, Burris & Stauffer, 1949), containing 1 μmole of uniformly labelled [$^{14}\text{C}_2$]acetate with 1 μC of radioactivity (The Radiochemical Centre, Amersham, Bucks.), in Warburg flasks with air as the gas phase for 3 hr. at 37°. After incubation, the suspending solution was decanted off and

the slices after washing twice with Krebs-Ringer bicarbonate solution were homogenized in 3 ml. of cold 95% ethanol. The total lipids, phospholipids, cholesterol and fatty acids of acetone-soluble lipids were fractionated and purified essentially as described by Kline & De Luca (1956). Suitable samples of the lipid fractions were plated on tared stainless-steel planchets, dried under an infrared lamp and counted at infinite thinness by using a Tracerlab SC-16 windowless gas-flow counter in conjunction with an SC-51 autoscaler (Tracerlab Inc., Boston 10, Mass., U.S.A.). Specific activities were calculated as net counts/min./mg. of lipid in the sample.

Oxidation of octanoate and [1-¹⁴C]palmitate by liver homogenates. Liver homogenates corresponding to 100 mg. fresh wt. of tissue were incubated in the system described by Kennedy & Lehninger (1950) with either unlabelled octanoate (caprylate) or [1-¹⁴C]palmitate (The Radiochemical Centre, Amersham, Bucks.) as substrate in Warburg flasks at 37° for 30 min. The palmitate solution was prepared according to the method of Stumpf (1955). Oxygen consumption was recorded every 10 min., and, after the incubation with [1-¹⁴C]palmitate, the contents of the central well were transferred with three washings to 15 ml. centrifuge tubes and excess of 5% (w/v) barium chloride solution was added to each. The precipitated barium carbonate was centrifuged, washed twice with water, transferred as a slurry to stainless-steel planchets, dried under an infrared lamp, counted as detailed above and the radioactivity corrected for infinite thinness.

Coenzyme A in the liver homogenate was determined, because of its important role in fatty acid activation and oxidation reactions, by the method of Kaplan & Lipmann (1948).

Electrophoretically separated serum proteins and liver lipoproteins. Fractionation of serum proteins and of liver lipoproteins was carried out by electrophoresis on Whatman 3MM paper in a horizontal open-strip-type cell (Evans Electroselenium Ltd., Harlow, Essex).

For the separation of proteins, 10 μ l. samples of serum were spotted on strips (5 cm. $\times$ 34 cm.) and separation was effected by using barbital buffer, pH 8.6 and *I* 0.075, with a constant current of 2.5 ma per strip for 16 hr. at room temperature. At the end of the run the strips were dried, stained as described by Jencks, Jetton & Durrum (1955) and scanned after rendering them translucent by treatment with liquid paraffin.

For the extraction of lipids from lipoprotein fractions, 200 μ l. samples of serum were spotted and electrophoresis

was carried out by the 'zone-sharpening' procedure of Hjertin (1959). The strips were cut into two portions between the α - and β -globulins, and the total lipids from α - and β -lipoproteins extracted separately and determined as detailed by Brown, Michaels & Kinsell (1956).

The lipoproteins from 100 μ l. of liver cell sap (supernatant after centrifuging at 105000g) were separated by electrophoresis for 5 hr. with borate buffer, pH 8.6 and *I* 0.06, at a constant current of 4 ma per strip (M. M. Bhargava & A. Sreenivasan, unpublished work). The paper strips, after being dried, were stained with a 0.1% solution of Sudan black B in 60% (v/v) ethanol. The bands were arbitrarily named α_1 , α_2 and α_3 respectively from the point of application. The extinctions of the bands were estimated at 590 m μ , after elution in 20% (v/v) acetic acid in ethanol (Swahn, 1952), in a Beckman model DU spectrophotometer.

RESULTS AND DISCUSSION

Results on the accumulation of lipid in liver after the administration of carbon tetrachloride are presented in Table 1. The content of total lipids shows a considerable increase after 3 hr. and is almost doubled after 24 hr. Total cholesterol shows a slight though insignificant increase during the same period. The fatty acids of acetone-soluble lipid show a rapid increase in the early period of poisoning, being doubled after 3 hr. and tripled after 24 hr. In marked contrast with other lipid fractions, phospholipids are considerably decreased in the animals treated with carbon tetrachloride. The rapid increase in total lipids and in particular of fatty acids of acetone-soluble lipid during the early period of poisoning are in accordance with the observations of Schotz & Recknagel (1960).

The pattern of incorporation of uniformly labelled [¹⁴C]₂acetate into the different lipid fractions by liver slices is considerably altered in the rats poisoned with carbon tetrachloride (Table 2). Though the incorporation into fatty acids of acetone-soluble lipid is but little affected, the incorporation into cholesterol showed a slight increase which is not significant. However, the labelling of phospholipids is considerably decreased

Table 1. *Lipid composition of rat liver*

Adult male rats (155–170 g.) were given 0.2 ml. of carbon tetrachloride/100 g. body wt. and killed at intervals as indicated. The results are means $\pm$ S.E.M. of at least four independent observations. The moisture contents of liver did not vary much after the administration of carbon tetrachloride: average values obtained were in the range 70–72%.

Group	Lipid content (mg./g. fresh wt. of liver)			
	Total lipids	Fatty acids of acetone-soluble lipids	Phospholipids	Total cholesterol
Normal	56.8 $\pm$ 3.4	21.6 $\pm$ 2.9	23.1 $\pm$ 2.3	3.6 $\pm$ 0.4
Carbon tetrachloride-treated (after 3 hr.)	79.0 $\pm$ 7.4	44.7 $\pm$ 6.9	21.8 $\pm$ 1.6	3.8 $\pm$ 0.4
Carbon tetrachloride-treated (after 24 hr.)	101.4 $\pm$ 14.7	69.6 $\pm$ 9.3	16.6 $\pm$ 2.1	4.2 $\pm$ 0.3

Table 2. *Incorporation of [$^{14}\text{C}_2$]acetate into lipid fractions by liver slices*

Liver slices (500 mg.) were incubated in Krebs-Ringer bicarbonate solution, pH 7.4, containing 1 μmole of sodium [$^{14}\text{C}_2$]acetate (1 μC) in a total volume of 5 ml. in Warburg flasks with air as gas phase at 37° for 3 hr. The lipids were fractionated and the radioactivities determined as detailed in the text. Other details were as given in Table 1. The results are means $\pm$ S.E.M. of four independent observations.

Group	Specific radioactivity (counts/min./mg. of lipid)		
	Fatty acids of acetone-soluble lipids	Phospholipids	Total cholesterol
Normal	3250 $\pm$ 240	1740 $\pm$ 80	2140 $\pm$ 190
Carbon tetrachloride-treated (after 3 hr.)	3080 $\pm$ 110	1280 $\pm$ 140	2460 $\pm$ 140
Carbon tetrachloride-treated (after 24 hr.)	1940 $\pm$ 170	1130 $\pm$ 40	1390 $\pm$ 130

Table 3. *Oxidation of octanoate and of [$1\text{-}^{14}\text{C}$]palmitate by liver homogenate*

Homogenates corresponding to 100 mg. of liver were incubated in 0.02M-potassium phosphate buffer, pH 7.4, containing 0.07 μmole of cytochrome *c*, 4.5 μmoles of adenosine triphosphate (potassium salt), 150 μmoles of potassium chloride, 20 μmoles of magnesium chloride, 250 μmoles of sucrose and either 30 μmoles of potassium octanoate or 10 μmoles (1 μC) of [$1\text{-}^{14}\text{C}$]palmitate as substrate (total vol. 3 ml.) in a Warburg vessel containing 0.2 ml. of 20% (w/v) potassium hydroxide in the centre well. Incubations were carried out at 37° and manometric readings taken every 10 min. for 30 min. The $^{14}\text{CO}_2$ absorbed by potassium hydroxide was counted as detailed in the text. Other details were as given in Table 1. The results are means $\pm$ S.E.M. of four independent observations.

Group	Oxidation of octanoate ($\mu\text{l. of O}_2$ uptake/hr./g. fresh wt. of liver)	Oxidation of [$1\text{-}^{14}\text{C}$]palmitate ($^{14}\text{CO}_2$ counts/min./flask)	Coenzyme A (units/g. fresh wt. of liver)
Normal	2430 $\pm$ 140	1264 $\pm$ 40	136 $\pm$ 22
Carbon tetrachloride-treated (after 3 hr.)	2120 $\pm$ 80	866 $\pm$ 110	124 $\pm$ 13
Carbon tetrachloride-treated (after 24 hr.)	1360 $\pm$ 70	442 $\pm$ 60	98 $\pm$ 11

3 hr. after the administration of carbon tetrachloride. This decrease in phospholipids is in accordance with the observations of Rossi & Zatti (1963). Incorporation into the first two fractions is greatly diminished without much change in the phospholipids after 24 hr. It would thus appear that, early during poisoning, the effects are specific, but that, with progress of time, cell function in general is impaired. These observations exclude increased lipogenesis as a possible cause for the accumulation of fat in liver injury caused by carbon tetrachloride.

Liver homogenates from rats given carbon tetrachloride exhibit some decrease in the oxidation of octanoate after 3 hr., whereas after 24 hr. the activity is markedly depressed (Table 3). With [$1\text{-}^{14}\text{C}$]palmitate as substrate the radioactivity of respired carbon dioxide is considerably decreased even after 3 hr. and is greatly diminished after 24 hr. The decrease in the capacity to oxidize palmitate as compared with octanoate by liver homogenates from rats treated with carbon tetrachloride may be due either to the detergent

action of the longer-chain fatty acid salt on the already partially damaged mitochondria, or to a dilution of the label in the substrate by the increase in endogenous fatty acids in the injured livers. It is possible that a selective destruction of the mitochondrial enzymes more directly concerned with the oxidation of fat may take place in poisoning with carbon tetrachloride. The decrease in fatty acid oxidation is paralleled by a decrease in the coenzyme A content of liver. It is likely that the decrease in coenzyme A and in adenosine triphosphate (Dianzani, 1957) may lead to impairment in oxidation since both these are necessary for the activation of fatty acids before their oxidation. Recknagel & Lombardi (1961) have reported no difference in the oxidation of octanoate by livers 2 hr. after poisoning with carbon tetrachloride, whereas in some rats the oxidation of octanoate was depressed to 50% of control rates 4 hr. after the poisoning. However, it appears that an impairment in fatty acid oxidation is perhaps not the major cause of fat accumulation during the early period of injury. This has been confirmed by

observations by Neubert & Maibauer (1959) and by Recknagel & Lombardi (1961) on the destruction of microsomal enzymes at a time when mitochondrial oxidative phosphorylation is intact.

The electrophoretic pattern of plasma proteins of the poisoned animals shows a decrease in total proteins with a significant decrease in albumin after 3 hr. that is subsequently less marked after 24 hr. The changes in the other fractions are comparatively small (Table 4). This decrease in plasma albumin is similar to that reported in a deficiency of vitamin B₁₂ (Mulgaonkar & Sreenivasan, 1958); Kasbekar *et al.* (1959*a*) observed that loss of vitamin B₁₂ from liver is one of the primary effects of poisoning with carbon tetrachloride. Further, decreased synthesis of plasma albumin by liver has been observed both in liver injury caused by carbon tetrachloride (Smuckler *et al.* 1961) and in simple vitamin B₁₂ deficiency (S. Venkataraman & A. Sreenivasan, unpublished work). The decrease in plasma albumin is of considerable significance because of the involvement in the transport of free fatty acids from adipose tissue to liver (Frederickson & Gordon, 1958), and probably rules out the possibility of increased mobilization of lipids into liver (Calvert & Brody, 1961). It is suggested that, since vitamin B₁₂ has been implicated in protein biosynthesis (Wagle, Mehta & Johnson, 1958), a deficiency of this vitamin in liver caused by the

administration of carbon tetrachloride may result in an impaired synthesis of specific proteins such as plasma albumin.

Results on lipids of plasma lipoprotein, particularly of the β -fraction, also show some decrease observable 3 hr. after the administration of carbon tetrachloride. Seakins & Robinson (1963) also provide evidence for a decreased formation of plasma lipoprotein at an early stage after the liver injury. These results are in conformity with the decrease in the specific activity of the serum lipoproteins when liver slices from rats given carbon tetrachloride were incubated in a serum medium containing ¹⁴C-labelled amino acid (Robinson & Seakins, 1962). It is possible that an adequate synthesis of the lipoproteins by the liver is a prerequisite to the transport of triglycerides from the liver.

The electrophoretic pattern of the lipoproteins of the liver cell sap (supernatant after centrifuging at 105 000*g*) resolves into two sharp bands, α_1 and α_2 , and a diffuse band, α_3 , from the point of application. There is a marked decrease in the lipid content of the α_2 - and α_3 -fractions even after 3 hr. and of the α_1 -fraction after 24 hr. (Table 5). The total decrease in the three fractions amounts to 49%, which further increases to 61% by 24 hr. It is believed that the synthesis of lipoproteins occurs at the endoplasmic reticulum from triglycerides syn-

Table 4. *Plasma proteins and lipids of plasma lipoproteins*

Plasma samples (10 μ l. or 200 μ l.) were electrophoretically separated as indicated in the text. Other details were as given in Table 1. The results are means $\pm$ S.E.M. of four independent observations.

Group	Plasma proteins (g./100 ml.)						Lipids of plasma lipoproteins (mg./100 ml.)	
	Total	Albumin	α_1 -Globulin	α_2 -Globulin	β -Globulin	γ -Globulin	α -Fraction	β -Fraction
Normal	5.76 $\pm$ 0.19	2.34 $\pm$ 0.09	0.99 $\pm$ 0.03	0.46 $\pm$ 0.08	0.89 $\pm$ 0.02	1.01 $\pm$ 0.08	116 $\pm$ 19	153 $\pm$ 22
Carbon tetrachloride-treated (after 3 hr.)	5.43 $\pm$ 0.23	2.03 $\pm$ 0.07	0.97 $\pm$ 0.06	0.47 $\pm$ 0.03	0.83 $\pm$ 0.03	0.96 $\pm$ 0.03	101 $\pm$ 13	113 $\pm$ 7
Carbon tetrachloride-treated (after 24 hr.)	4.96 $\pm$ 0.17	1.81 $\pm$ 0.11	0.91 $\pm$ 0.07	0.43 $\pm$ 0.02	0.87 $\pm$ 0.09	0.96 $\pm$ 0.05	99 $\pm$ 11	83 $\pm$ 9

Table 5. *Lipoproteins of cell sap*

A portion (100 μ l.) of liver cell sap (supernatant after centrifuging at 105 000*g*) was electrophoretically separated, the bands were stained and the extinctions in the eluted bands were determined as described in the text. Other details were as given in Table 1. The results are means $\pm$ S.E.M. of four independent observations.

Group	Extinction (unit/100 μ l.)			Total decrease in extinction over normal (%)
	α_1 -Band	α_2 -Band	α_3 -Band	
Normal	0.117 $\pm$ 0.020	0.146 $\pm$ 0.010	0.135 $\pm$ 0.010	—
Carbon tetrachloride-treated (after 3 hr.)	0.104 $\pm$ 0.030	0.032 $\pm$ 0.006	0.070 $\pm$ 0.006	49
Carbon tetrachloride-treated (after 24 hr.)	0.056 $\pm$ 0.010	0.027 $\pm$ 0.005	0.072 $\pm$ 0.003	61

thesized elsewhere (Heimberg *et al.* 1962). These lipoproteins are probably in dynamic equilibrium with the supernatant lipoproteins, and a decrease in the latter may indicate a block in the lipoprotein-synthesizing mechanism of the liver microsomes. This can be in part attributed to a decreased synthesis of phospholipids by the liver (Table 2); phospholipids, apart from being the major component of lipoproteins, also play an active part in the transport mechanism across the cell membrane.

These observations are consistent with the view that the increase in the concentration of triglycerides in the livers of rats given carbon tetrachloride is due primarily to an impairment in the synthesis of plasma lipoproteins resulting in a decreased mobilization of lipids from the liver and that decreased oxidation of fatty acids occurs in addition in the later stages of the development of fatty liver.

SUMMARY

1. The increase in liver lipids in rats given carbon tetrachloride is restricted to fatty acids of the acetone-soluble lipids. The phospholipid concentration is significantly decreased.

2. The incorporation of uniformly labelled [$^{14}\text{C}_2$]acetate into the fatty acids and cholesterol of liver slices is little affected in the early period (after 3 hr.) of poisoning with carbon tetrachloride, when there is a significant decrease in the specific activity of the phospholipids. The labelling of all these fractions is greatly diminished after 24 hr.

3. A significant decrease in rate of oxidation of octanoate and of [$1\text{-}^{14}\text{C}$]palmitate by liver homogenates from rats given carbon tetrachloride is observable 3 hr. after the administration; the activity is considerably impaired after 24 hr.

4. Poisoning with carbon tetrachloride results in decreases in plasma albumin and in lipids of plasma lipoproteins even 3 hr. after the administration, followed by a considerable decrease in the lipids of the lipoproteins of the cell sap.

5. An impairment of lipoprotein synthesis and consequently of lipid transport may be the primary cause of the accumulation of lipid in liver injury caused by carbon tetrachloride.

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