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EDITORIAL*

BIOCHEMICAL ASPECTS OF CARBON TETRACHLORIDE POISONING

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INTRODUCTION

CARBON TETRACHLORIDE has been employed in therapeutics against hookworm infestation and, in industry, this compound is used for degreasing metal, for extracting fats, as a solvent in the rubber industry and in the lacquer manufacture. Fatal poisonings are constantly being reported in humans subjected to its vapours (Forbes,[27] Phelps and Hu,[66] Gardner *et al.*[31]). Its toxicology is therefore an important object of investigation.

This review, while not intended to be complete, proposes to focus on the research relating biochemistry to the problems of fat infiltration and liver necrosis. Biochemical investigations, while limited, have permitted better insight as to the mechanism of CCL_4 poisoning.

In intoxication with repeated doses of this drug, some early authors described hepatic changes resembling portal cirrhosis (Bollman[6], Mayer and Pessoa[57]). In 1936 Cameron and Karunaratne[16] published their classical paper on the histological development of cirrhosis in CCl_4-poisoned rats. The ease with which it produces liver lesions and the precise histological control rendered this drug a useful tool for liver studies. The significance of the pathological changes and the site of the primary lesion in the liver cell have elicited much controversy. The pioneering researchs of Christie and Judah[18] on the enzymes of liver mitochondria and of Gallagher and Rees[30] on the liberation of pyridine nucleotides from mitochondria in CCl_4 poisoning deepened insight as to the biochemical processes operating in the liver cell.

In animals pathological studies have demonstrated that the liver is the primary site of attack of the drug. The minimum toxic dose producing histologic changes in the liver of the rat by the subcutaneous route was 0·033–0·006 ml/kg body weight (Cameron and Karunaratne[16]).

The onset of the pathological changes depends on dose, tissue, and route of administration. Small doses produce liver lesions and generally cause some kidney damage

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(Sherlock[88]). Large doses lead to acute symptoms and kidney injury and cause death in few hours. Repeated small doses have an additive effect.

An hour after a single injection the liver appears normal but some degree of sinusoid congestion is seen after 4½ to 5 hr when typical hydrotopic degeneration appears. Six hours after administration of repeated doses of CCl_4 given at short intervals, the liver shows microscopically an early cirrhosis (Lacquet[49]). When 0·25 ml CCl_4 is given intragastrically to the normal rat centrilobular necrosis and fat infiltration are produced. The intensity of the lesions is more pronounced after 24 hr and regeneration starts later, usually after 2 weeks.

The conditions necessary for inducing CCl_4 cirrhosis in the rat were postulated by Cameron and Karunaratne[16] as follows:

(a) the dose must exceed the minimal toxic dose for the liver;

(b) the CCl_4 must be administered continously over prolonged period and at short intervals;

(c) the intervals must be short to stop regeneration of the parenchyma.

When the acute intoxication is sublethal, the liver starts to regenerate near the affected region. The time for complete repair depends on the dose and the time elapsed after feeding the drug. The diet during and before the administration of the drug influences regeneration: the liver of rats on a good protein diet is more resistant to the onset of liver lesions; high-fat diets speed fat infiltration. Ethanol when given with CCl_4 increases fat infiltration.

During regeneration Tsuboi *et al.*[95] found that increase in nucleic acids paralleled the degenerative and necrotic changes.

By autoradiograph technic with tritriated thymidine Leevy *et al.*[50] studied liver regeneration in rats treated with a single oral dose of CCl_4. They demonstrated that synthesis of deoxyribonucleic acid was chiefly in non-necrotic areas. The regenerative process was diffuse and involves the acinar, intermediate, centrilobular, and periportal regions of the liver. Maximal rate of regeneration was observed 36–72 hr after administration of the drug. After 120 hr the regenerative process was completed and the histological and autoradiographic pattern returned to normal.

The liver, the organ most damaged by the drug, has fatty infiltration as the most striking cellular change. Fatty infiltration is followed by necrosis; necrosis is a sequel of fatty infiltration. The effectiveness of CCl_4 in producing liver necrosis in the rat is seen mainly 48 hr after administration of a single dose (0·2 ml/100 g body weight) (Wrenn and Feichmeier[108]).

Regeneration then accelerates and is practically complete in 6–7 days. The kidney is also affected when large doses of CCl_4 are inhaled. In acute poisoning by intraperitoneal injection of doses above 0·5 ml/100 g body weight, which causes the death of the rat in 5 or 6 hr, a typical necrosis of the proximal and outer tubules have been observed (Jennings[38]). Subcutaneous injections of the same dose of CCl_4 are not fatal and the kidney is not significantly affected. Several human cases of poisoning have been caused by inhalation or ingestion of the drug: the patients usually showed centrilobular necrosis of the liver, 'lower nephron nephrosis' of the kidney, and pulmonary edema. Some acutely fatal cases showed extensive renal damage besides liver infiltration and necrosis. In man, repeated exposure to low concentrations may cause serious

poisoning. As noted ethanol and dietary factors may predispose the liver and the kidney to damage. Gardner *et al.*[31] obtained extensive fatty infiltration and necrosis of the proximal tubules of the kidney in dogs poisoned with 0·5 ml/kg CCl_4. In human cases the most striking feature is observed in the lower nephron generally diagnosed as 'lower nephron nephrosis'. Jennings and Rearns[39] think that the earlier kidney lesion in man is in the proximal tubules and that nephron nephrosis a later manifestation. In human fatal cases the kidney is always severely attacked. Many cases due to inhalation or ingestion of CCl_4 have been reported in the literature of the last 13 years.[38, 39, 92, 94]

Rees, Sinha and Spector[75] used the antihistamine Phenergan to show the dissociation in CCl_4 poisoning between liver necrosis, fatty infiltration and mitochondrial damage. Phenergan 10-(2-dimethylamino-isopropyl) phenothiazine HCl) prevents only necrosis and mitochondrial damage; it is ineffective against fat infiltration. Necrosis and mitochondrial damage both reflect permeability changes. Fatty infiltration is a separate manifestation: one recalls that Recknagel and Anthony[69] demonstrated that fatty changes preceded by many hours the mitochondrial damage. Calvert and Brody[14] showed the intraperitoneal injection of ethylenediamine tetraacetate (EDTA) in rats poisoned with CCl_4 prevented mitochondrial damage without preventing fatty infiltration (Recknagel and Lombardi[70]).

All these experiments suggest that mitochondrial damage is a late occurrence, detectable through the overflow of liver dehydrogeneses into the blood, and that fatty infiltration and cytoplasmic disturbances are early events in liver damage.

Activation of fatty acids in CCl_4 *poisoning*

That liver can secrete large amounts of triglycerides into the blood plasma was shown through the experiments of Byers and Friedman[13] who used the detergent Triton to prevent the release of triglycerides from the liver. CCl_4-poisoned rats appear to have deranged hepatic triglyceride secretory mechanism. Two hours after administration of the drug triglycerides accumulated in the liver and decreased in the blood plasma. Triton injection prevented this increase. The triglycerides are therefore stored in the liver, accumulation of fat being a later process. A statistically significant increase was observed in rats 3 hr after CCl_4 feeding (Recknagel and Anthonyı[69] Recknagel *et al.*[71]).

Beside the triglycerides which increase markedly in liver, some lipids (phosphatides, cholesterol) including triglycerides, showed decreased values in the blood (Robinson and Seakins,[81] Maximchunk and Rubinstein[55]). Low figures for the lipoproteins of plasma are generally observed soon after administration of the drug. In rabbits Pierce and Gofman[67] reported an increase in all 3 classes of serum lipoproteins which later returned to normal, but these animals behaved differently from rats in respect to lipids. The release of liver triglycerides into plasma was inhibited by CCl_4 as demonstrated by Heimberg and Weinstein[35] using perfused rat liver. The liver from animals fasted 48 hr in contrast to those from fed rats did not release triglycerides into the perfusate. The mechanism of this inhibition by CCl_4 is unknown: perhaps it is mediated through adrenergic mechanisms as suggested by Calvert and Brody.[15] Accumulation of triglycerides in liver during CCl_4 poisoning results not from a defect in release of triglycerides into plasma but is perhaps due to an increase in hepatic synthesis of triglycerides.

Experiments with fed (1-^{14}C) palmitate have shown that the total radioactivity incorporated into liver triglycerides and phospholipids in poisoned rats was greater than in control animals (Maling, Frank and Horning[53]).

Serum free fatty acids decrease markedly in CCl_4 poisoning; phenoxybenzamine administered together with CCl_4 has an additive effect. However, phenoxybenzamine is ineffective in the protection of liver against exposure to CCl_4 vapour (Fox, Dinman and Frajola[28]). Rees *et al.*[75] showed that Phenergan decreased hepatic necrosis and the activity of liver mitochondrial enzymes; liver non-particulate enzymes were not affected consistently. This fact has been interpreted to mean that Phenergan affects both the mitochondria directly and the permeability of the mitochondrial membrane.

The enzyme responsible for binding of phophorylcholine to tri-diglyceride is very sensitive to CCl_4 *in vitro* (Kennedy[47]). It was suggested that fatty infiltration of liver caused by CCl_4 could be due to binding of a molecule of diglyceride with acetyl-CoA to form a molecule of fat (Kennedy and Weiss[38]). Serum protein do not change appreciably in CCl_4 poisoning (Wrenn and Feichmeier[108]).

Increased total lipids and phospholipids were found in rats injected with CCl_4; the amount of fat seemed to parallel the fat infiltration shown microscopically. In this condition the elevated serum lipids and β-lipoproteins may explain increase in cholesterol. CCl_4 when injected to normal rabbits induced an increase of the three classes of lipoproteins S_1 3–12, 12–20, and 20–40 (Pierce and Gofman[67]). After the cessation of the effect of the drug the lipoproteins having the highest sedimentation rate (S_1) returned to normal; when cholesterol was also fed the lipoproteins increased unless no more CCl_4 was injected.

Nakatsuka[60] mentions a slight increase in total fat, cholesterol and phospholipids in blood of rabbits intoxicated with CCl_4 and Ribeiro[79] found a significant increase in the percentage values for serum β-lipoproteins, measured by paper electrophoresis, 24 hr after administration of the drug.

Recently, Seakins and Robinson[86] showed that rats poisoned with 1 : 1 CCl_4 in olive oil had a lowered incorporation of DL-(1-^{14}C) leucine into plasma proteins and also of labeled acetate into liver and plasma cholesterol. They suggest that fatty livers could be due to inhibition of formation of plasma lipoproteins.

Conjugation and oxidation of phenobarbital is retarded (Glasson and Benakis[32]) and conjugated morphine is less excreted in the urine of CCl_4-poisoned dogs (Gross[34]).

The rate of oxidation of tyrosine and of *p*-hydroxyphenylpyruvic acids is decreased in the liver of CCl_4-treated rats. Addition of α-ketoglutaric acid to the liver did not restore the normal rate of oxidation, while 2,6-dichlorophenol-indophenol completely restored the normal rate of tyrosine oxidation (Wang-Chung-yen[107]). Vitamin B_{12} injected 3 hr before the administration of CCl_4 reversed the decreased rate of tyrosine oxidation. This protection of vitamin B_{12} has been also observed in relation to the production of fatty infiltration and necrosis of the liver.[45]

Liver and blood enzymes

When the liver is damaged by CCl_4 several enzymes appear in the blood in elevated concentration. A similar process has been revealed for other tissues, e.g. heart and muscle, where the size of the tissue injured and amount of enzymes liberated into the blood stream correlate.

Blood levels of isocitric dehydrogenase and malic dehydrogenase increase very markedly. Six hours after administration of the drug, isocitric dehydrogenase increased more than malic dehydrogenase. After 24 hr all 3 enzymes reached a maximum level, declined at 36 hr, and then returned to normal (Rees and Sinha[74]).

The initial lesion produced by CCl_4 seems to be cytoplasmic. Mitochondrial damage comes later. In early stages of intoxication, cytological lesions were more pronounced in the ergastoplasm, as confirmed by electron micrographs (Bassi[3]), leakage of cytoplasmic enzymes resulting from this injury. A confirmation came from study of xanthine oxidase and quinine oxidase in CCl_4 poisoning in rats and rabbits. The blood plasma of the rabbit lacks quinine oxidase activity but became rich in this enzyme as little as 2 hr after CCl_4 was injected. In the rat the increase in blood xanthine oxidase was also rapid. Since none of these enzymes exist in mitochondria, they therefore appear in the blood only when the cytoplasm is damaged (Villela and Mitidieri[105], Villela,[101, 102] Affonso *et al.*[1]).

Aldolase and phosphohexose isomerase of serum and liver of mice were analysed by Bruns and Neuhaus[10, 11] in intoxicated animals. The serum enzymes increase with a corresponding decrease in the liver. Since neither hexokinase nor fructokinase were detected in serum it was concluded that glycolysis must be disturbed (Bruns and Neuhaus[11]).

A single intraperitoneal injection of 0·04 ml CCl_4/100 g body weight do not change the succinoxidase of the rat kidney, while larger doses significantly decreased this enzyme as soon as 1 hr after the administration of the drug (König *et al.*[46]). Serum tributyrinase, serum amylase, and alkaline phosphatase increase slightly according to the same authors. Alkaline phosphatase as well as 5-nucleotidase also increase 24–48 hr after administration of high doses of CCl_4 when hepatobiliary injury is produced (Villela and Assis,[103] Villela and Mello[99]). Increased alkaline phosphatase was also reported by Koch-Weser[45] in blood serum and liver tissue of CCl_4-poisoned rats.

The rapid increase in serum glutamic-oxalacetic transaminase (S GO-T) may reach a maximum 8–12 days after administration of CCl_4 and may serve as an index of hepatocellular integrity (Molander, Wroblewski and La Due[59]). Extent of zonal necrosis and S GO-T levels in blood serum seemed correlate. Restoration of hepatocellular integrity after acute injury is easily followed by measuring the S GO-T in serum; this seems a better index than serum cholinesterase or alkaline phosphatase. Quinine oxidase appears 2–5 hr after acute CCl_4 poisoning in rabbits.[101]

As noted quinine oxidase is only found in the liver and its appearance in blood plasma denotes injury of liver-cell cytoplasm. A decrease of this enzyme appears concommitantly in liver.[100]

Another enzyme localized exclusively in liver is ornithine carbamyl transferase. In dogs acutely intoxicated with CCl_4 the enzyme was detected the second day after injection (Reichard[77]). This test is not as sensitive for ascertaining the degree of liver damage as quinine oxidase in the rabbit or S GO-T in the rat.

Xanthine oxidase increased in blood serum 2–5 hr after the injection of CCl_4; higher values for liver were also reported (Villela and Mitidieri,[105] Block and Cornish[5]). It was suggested that xanthine oxidase is found in liver and in blood plasma as a lipoprotein complex ruptured by CCl_4 (Villela and Mitidieri[104]).

The events in the rat liver after CCl_4 administration according to Rees, Sinha and Spector[76] may be summarized as follows: after 6 hr liver fat increases sharply and

contributes to the later induction of necrosis. Isocitric and malic dehydrogenases of liver appear in the blood. After 12 hr centrilobular necrosis and gross fatty infiltration are seen in liver cells glutamic dehydrogenase start to move into the blood serum. Phenergan protected rats from the liver necrosis produced by CCl_4, and can alter the sequence of events. At this stage blood enzymes are slightly increased and necrosis is absent. Fatty infiltration remains unaltered.

Rees and Sinha[74] concluded from their experiments on enzymes released in blood serum after CCl_4 poisoning that mitochondrial damage is a late manifestation.

One concludes that mitochondrial injury is not the primary lesion of the liver cell, that other changes in the cytoplasm are much earlier. Liver dehydrogenase dependent on pyridine nucleotides became inactivated, but their activity could be restored by addition of nicotinamide adenine nucleotide (NAD) (Gallagher[29]).

The loss of pyridine nucleotides from liver cells following CCl_4 poisoning is not an early event and is not a result of necrosis (Gallagher and Rees[30]). Nicotinic acid and tryptophan, precursors of pyridine nucleotides, protect the liver from the necrosis and restore the fall in NAD of liver homogenates (Gallagher[29]). The extinction coefficient of liver and blood extracts measured at 260 $m\mu$, was considered an indication of the pyridine nucleotides present in the oxidized form. Gallagher applied this test to show that CCl_4 produces a decrease in the concentration in these coenzymes in liver and their increase in blood serum.[28]

Intracellular damages

CCl_4 diffuses rapidly and is found in the liver to a maximum level $1\frac{1}{2}$ hr after its administration and falls thereafter continously (Recknagel and Litteria[72]). These results indicate that the poison attacks the cells very early. Christie and Judah[18] suggested that the changes in the liver indicate a direct physical attack of the drug on liver mitochondria, degeneration and death of the cells resulting from the disorganization of mitochondrial structure.[43]

Christie and Judah[18] found that the enzymes of the tricarboxylic cycle were disorganized 10–15 hr after administration of the drug. Oxidation of malate, citrate, octanoate, pyruvate and glutamate were inhibited but not of succinate. Pyridine nucleotides reversed these inhibitions and are quickly liberated from mitochondria as demonstrated with isolated mitochondria.

Clearly, then, CCl_4 increases the permeability of the mitochondrial membrane and allows the pyridine nucleotides to leak from mitochondria, inactivating all enzymes needing these cofactors.

Accumulation of fat in the liver according to Dianzani[21] results from decreased mitochondrial fat oxidation. As shown by Recknagel and Anthony[69] mitochondrial damage appeared only many hours after the fatty changes in the liver. The accumulation of fat was not dependent on mitochondrial injury, as shown by Calvert and Brody,[14] since the administration of Versene (EDTA) to rats poisoned with CCl_4 led to fatty livers without visible mitochondrial damage.

Since some enzymes which are confined to the liver mitochondria appear in blood of poisoned rats after the first cell damages, Rees and Sinha[74] concluded that mitochondrial damage also happens late. Recknagel, Lombardi and Schotz[71] verified that mitochondrial oxidation of octanoate and mitochondrial respiration generally are unaffected 2 hr after CCl_4 administration, while liver triglycerides are elevated.

These observations confirmed that the mitochondrial damage is secondary and possibly not related to fatty infiltration and liver degeneration.

Gross loss of mitochondrial function is not evident until about 20 hr after CCl_4 feeding in rats. The ability of potassium-depleted mitochondria to accumulate K also indicates that mitochondrial degeneration does not set in for many hours after the peak level of CCl_4 in the liver has been reached (Share and Recknagel[87]). Since the hepatic lesions appear very early before the damage of the mitochondria, the loss of mitochondrial function cannot by caused in turn by the initial biochemical lesion.

Oberling and Rouiller[63] by electron microscopy observed few alterations in the liver parenchyma after CCl_4 intoxication. More marked changes were present in the ergastoplasm. The electron microscopy study of Bassi[3] revealed also that 2 hr after injection of 0·25 ml CCl_4/100 g body weight the mitochondria were normal although the endoplasmic recticulum showed some morphological modifications.

According to Rosin and Doljanski[82] there is evidence that the centrilobular parenchymal cells were free of pyrinophilic granules, probably due to loss of cytoplasmic ribonucleic acid (RNA) as early as one hour after administration of CCl_4. Through chemical determinations, Richter[80] demonstrated a decrease of RNA of the microsomal fraction and increase in the supernatant of the mouse liver 2 hr after poisoning by the drug. These results suggested to Richter that CCl_4 poisoning may change the molecular weight of RNA from 360,000 to 120,000.

There is loss of basophilia of liver cells of the central and intermediate zones of the hepatic lobules 24 hr after CCl_4 feeding. Accordingly, the RNA and DNA contents of liver tissue drops (Farber, Koch-Weser, Szanto and Popper[23]).

Prevention of liver fat infiltration and liver necrosis

Several papers dealing with the protection of liver damage by CCl_4 appeared since 1933. Sato[85] and Yoshida[106] claimed that a liver preparation, 'Yakriton' was effective against several hepatotoxic drugs including CCl_4 (Tria[96]). In 1936, Forbes and Neale[25] prepared a liver extract which was later purified and appeared very active in protecting against liver nectosis produced by CCl_4. Forbes and McConnell[26] crystallized this antitoxic factor; Neale and Winter identified it as sodium xanthine (Forbes,[27] Lima and Koch-Weser,[52] Nasio[61]). Other purines were less active but still had some protective effect. Ricinolate was also shown to protect the rat against the drug and Villela[97] prepared an active purified liver extract which was effective against arsenobenzol, $CHCl_3$ and CCl_4. This extract restored the decreased liver glutathione when injected in the rat before administration of a toxic drug.[98] Drill and Ivy[22] suggested that CC_4 damages the liver cell by combining with sulfhydryl groups, but Eden and Harrison found no evidence of combination *in vitro* with glutathione and CCl_4 (Stoner and Magee[92]). Gallagher[29] protected rats against acute poisoning by means of tryptophane and nicotinic acid injected intraperitoneally and so reduced considerably the death rate. Normal values for the extinction coefficient at 260 mμ (pyridine nucleotides) of acid extracts were obtained in rats protected with DL-tryptophane or with nicotinic acid.

In acute CCl_4 poisoning methionine favors recuperation of liver parenchyma (Miller, Ross and Whipple,[56] Beattie, Herbert, Wechtel and Steel[4]). In treated human cases Shiels[90] obtained improvement of their condition. Coenzyme A concentration also decreases in CCl_4 poisoning (Chenoweth and Hake[17]) possibly due to loss of SH groups.

An interesting finding was reported in relation to the prevention of liver damage. Adrenalectomy and blocking of spinal tract protected against liver necrosis. Therefore it has been suggested that CCl_4 may work through hormonal and nervous mechanism (Brody and Calvert,[8] Maximchunk and Rubinstein[55]).

Vitamin B_{12} is a protective factor but is non-specific.[2] Its effect is better obtained when associated with methionine or liver extracts rich in sulfhydryl compounds. It must be recalled that recently protein-free liver extracts were used with success in preventing fat infiltration produced by CCl_4 administration (Tanyol *et al.*[93]).

Theories of CCl_4 *poisoning*

The theories to explain the characteristic liver changes in CCl_4 poisoning are of two kinds: one centers on the vascular and the other on the damage to mitochondria. The vascular theory attributes the liver lesions to a reduction in hepatic blood flow (Himsworth[36]).

Based on the histologic changes and on some experiments in which India ink injected into the portal system of the anesthetized rats caused a restriction of the intralobular circulation as early as 4 hr after subcutaneous injection of CCl_4, Himsworth emphasized vascular changes as the primary cause of CCl_4 damage. According to him, necrosis is centrilobular because the sinusoids are narrowed by the swelling of the parenchymal cells.

Liver rapidly absorbs CCl_4 from the blood and the cells exposed to the highest concentrations—the periportal cells—survived while those less exposed to its action—the centrilobular cells—became necrotic. Nevertheless, when larger doses are given the necrosis expand to the rest of the lobule. Necrosis is only seen in cells which are distant from the portal tracts, a fact which suggested that necrosis is intimately related to blood supply.

The cells most in contact with the poison are those most severely damaged. This situation happens with the vascular changes produced by the drug. The restriction of intralobular circulation was not confirmed by the use of different methods. The radiographic studies of Daniel, Richard and Reynall[19] showed that there is no alteration in the liver. The rate of portal blood flow seemed not very altered.

In the early stages there is some dilation of the sinusoids and vasoconstriction when necrosis set in. By thermocouple technique necrosis was produced in the rat without reduction in hepatic blood flow.[91]

The decrease of vascular circulation was not confirmed by Stoner;[92] there is a dissociation between appearance of fat infiltration and necrosis.

Rats exposed to low O_2 tensions show far more severe lesions than the control animals in air (Glynn and Himsworth[33]) notwithstanding that O_2 can decrease the necrosis produced by CCl_4. Evidently therefore, O_2 supply must be important in producing and localizing the lesions. Himsworth concluded from his experiments that centrilobular necrosis is due to ischemia of these cells resulting from O_2 lack. The severity of the lesions produced by CCl_4 can be modified if the O_2 supply from the spleen is altered. This fact led to the finding that the liver lesions can be reduced by processes interfering with the sympathetic nerve supply to the liver: cordotomy at level T_7, also sympatholytic agents like Dibenamine reduce effectively the histological and biochemical changes in liver damage. While some authors believe in a primary

vascular damage driven by a sympathico-adrenal discharge which produce the liver lesion, the general opinion is not to attribute to this the primary causation.

The primary lesion once established, any inadequate blood supply extends the damaged area; a better O_2 supply likewise improves the changes of survival of the damaged tissue. The benefits of O_2 inhalation in poisoned subjects exemplifies this situation.

The attractive biochemical theory of Christie and Judah,[18] supported by Dianzani,[21] is based on the changes in the mitochondrium due to increased permeability of the membrane. Fat infiltration was considered to result from decreased fat oxidation (Dianzani[21]).

A test system for mitochondrial integrity based on the ability of isolated mitochondria to concentrate potassium was developed by Share and Recknagel.[87] They showed that CCl^4 feeding reduced the K content of rat liver mitochondria and the ability of depleted mitochondria to reaccumulate K. The changes occurred about 10 hr later. Since fatty infiltration in liver is demonstrable as early as 3 hr following the poisoning, the most important increase in fat is around 9 hr and mitochondria are normal at this time, the primary locus of CCl_4 poisoning is not in the mitochondria.

There is some relationship between increase in calcium concentration in the liver and CCl_4 poisoning. Mitochondrial Ca increases more than 10-fold 16 hr after CCl_4 feeding. Ca harms mitochondria in that it produces swelling, loss of bound NAD, uncoupling of oxidative phosphorylation. EDTA protected mitochondria against the loss of NAD but not of fat infiltration which is a different process (Judah[40, 42]). Share and Recknagel[87] do not support this view since mitochondria are disturbed during fatty infiltration.

Calvert and Brody[15] formulated the hypothesis that CCl_4 acts indirectly through release of catechol amines from the adrenal medula. The decrease of epinephrine and norepinephrine content of the adrenal is detected 20 hr after CCl_4 treatment. Other agents such as ethanol and ethionine cause a similar effect and large doses of epinephrine lead to hepatic lesions comparable to those of CCl_4. This hypothesis merits further investigation.

SUMMARY AND CONCLUDING REMARKS

CCl_4 in single or repeated doses produces characteristic hepatic lesions, inducing in turn in the liver further biochemical and pathological disturbances. The sequence of events after CCl_4 administration may be summarized as follows as seen in rats:

1. *Diffusion of CCl_4.* CCl_4 is found $1\frac{1}{2}$ hr after administration of the drug (Recknagel and Litteria[72]).

2. *Morphological changes in the cytoplasm* (recticulum). Observed in electron micrographs (Bassi[3]; Oberling and Rouiller[63]). Increase in water content of liver (Seneviratne[89]; Stoner and Magee;[92] D. Monte and Fonnesu[58]).

3. *Fat infiltration.* Accumulation of triglycerides in liver and decrease in blood serum (Recknagel and Anthony;[69] Maximchunk and Rubinstein[55]). Drop in blood serum esterase (Koch-Weser[44, 45]). Vascular changes and ischemia of sinusoids (Himsworth[36]).

4. *Decrease of cytoplasmic enzymes of liver.* Xanthine oxidase and quinine oxidase, L-glutamic dehydrogenase, and passage to blood serum (Villela;[102] Villela and Mitidieri;[105] Rees and Sinha[74]). Increase in serum aldolase and phosphohexose

isomerase (Bruns and Nehaus[10, 11, 12]). Changes in intracellular distribution of RNA (Richter[80]).

5. *Modification in electrolyte distribution* (K *and* Ca). Mitochondrial injury with permeability changes. Loss of pyridine nucleotides from mitochondria (Gallagher[29]) and concommitant decrease in the activity of NAD-dependent dehydrogenases (isocitric, malic) Rees and Sinha;[74] Christie and Judah[18]). Impaired fat oxidation (Dianzani[21]). Increased liver and serum alkaline phosphatase (Koch-Weser;[15] Villela and Mello[99]).

6. *Centrilobular necrosis.* Drop in nucleic acid content of liver (Villela[100]). Increase in serum glutamic oxaloacetic transaminase (Molander, Wroblewski and La Due[59]) and of ornithine carbamyl transferase (Reichard[77]).

7. *Liver regeneration.* Increase in nucleic acid content in non-necrotic areas of the liver (Tsuboi *et al.*[95]).

The diffusion of CCl_4 is very rapid and its distribution in the lipid fractions must be considered in interpreting the sequelae in acute and chronic administration. CCl_4 is immiscible with water, hence its diffusion must obey the distribution coefficient water: lipid. Therefore, CCl_4 is carried possibly by the lipids of lymph and plasma and retained in the fat of liver. Very soon after administration of CCl_4 one can detect it in liver tissue (Recknagel and Litteria[72]). CCl_4 may disappear from tissues before the appearance of necrosis—a fact bespeaking a chain of events after direct damage. The liver cell membrane then the endoplasm are attacked, as demonstrated by the early release of cytoplasmic enzymes into blood plasma.

At this stage enzymes like xanthine oxidase, not present in the mitochondria, are activated by CCl_4, possibly by breakage of the lipoprotein bonds between the enzyme and the lipid support, which suggests that CCl_4 alters cell lipoproteins.

Mitochondrial disorganization may then be set off by the entrance of poison through the cell membrane and to the uncontrolled action of the cytoplasmic enzymes present in the ruptured lysosomes (De Duve[20]) which in turn may provoke changes in the mitochondrial membrane.

The diverse studies outlined in this reveiw open new avenues of research on how CCl_4 attack cells. The lipids in the cell and mitochondrial membranes may well be the most vulnerable sites.

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