Biochemical Mechanisms of Liver Injury

J. D. JUDAH, M.D.
A. E. M. McLEAN, M.D.
E. K. McLEAN, M.D.

London, England

Mechanisms of liver injury are considered from the points of view of fat accumulation, necrosis and the role of toxic metabolites. The actions of microsomal enzymes in producing such toxic metabolites, or else detoxicating other chemicals, are related to the nutritional control of microsomal enzyme activity and the effects of enzyme inducers.

The question whether the biochemical changes observed have any relevance to the development of pathologic states is discussed. It is concluded that useful conclusions can be derived only by comparative studies using either different poisons or animals in differing physiologic states.

A great deal of work has been done on the mechanisms of liver injury using experimental models based on a variety of toxic agents of greater or lesser specificity. In this review we consider three major topics which illustrate the advances and the difficulties of such an approach to disease. The accumulation of triglyceride fat in liver cells may be considered the result of imbalances imposed upon the system responsible for the transport of these molecules. Since the normal mechanism for this is reasonably well known, the study has been rewarded with a fair degree of success. The question of necrosis is hedged about with formidable difficulties, and correspondingly little success has been experienced in the attempts to solve it. Finally, we discuss the metabolism of some of the toxic agents used in these studies and show how the liver cells may be induced to act as mediators of their own fate.

Several reviews have recently appeared on this and related topics [1–4].

ACCUMULATION OF TRIGLYCERIDES IN LIVER CELLS

The mechanism of the production of fatty livers was first investigated by using toxic substances which induce the accumulation of triglyceride fat within liver cells (e.g., carbon tetrachloride (CCl_4), used by Cameron and Karunaratne [5]. It was not at first appreciated that the accumulation of fat could be functionally separated from the cell necrosis, which is also seen in many instances of experimentally produced liver injury, and it was not until these processes were separated in the 1960's that progress

From the Department of Experimental Pathology, University College Hospital Medical School, London, WCIE 6JJ, England. Requests for reprints should be addressed to Dr. J. D. Judah, Department of Experimental Pathology, University College Hospital Medical School, University Street, London W.C. 1, England.

was made. Christie and Judah [6] suggested a biochemical mechanism for CCl_4 toxicity based on mitochondrial damage, which was thought to lead both to accumulation of fat and to death of the liver cell. This theory was rapidly shown to be untenable when electron microscopy [7] revealed that the endoplasmic reticulum (ER) was damaged within thirty minutes of the administration of CCl_4 whereas the mitochondria survived apparently unaltered for several hours. Biochemical evidence that endoplasmic reticulum was damaged was not hard to find. Smuckler, Iseri and Benditt [8] showed that protein synthesis was reduced within two hours of CCl_4 poisoning and thought that this event could be responsible for the whole pathologic sequence caused by CCl_4. Decisive evidence that cell necrosis and the accumulation of neutral fat were separate came from several observations: (1) drugs could reduce cell necrosis without affecting the fat accumulation; (2) fat accumulation began within sixty minutes of the onset of intoxication by CCl_4, whereas necrosis was not seen until ten to twelve hours had elapsed; (3) poisons which brought about fat accumulation without causing necrosis were used to separate many of the changes caused by CCl_4 and to make a logical hypothesis which accounted for these changes. To understand this we must return to the mechanisms proposed for the secretion of triglyceride by the hepatocytes [9–12]. Fatty acids move from the peripheral fat depots to the liver. There they are either oxidized or formed into triglyceride. The triglycerides are then conjugated with a globulin to yield lipoproteins, which are then secreted. This mechanism is thought to give rise to the plasma lipoproteins. Figure 1 illustrates the cycle of events. If the synthesis of the carrier globulin is blocked, lipoprotein will not be formed, and fat will accumulate in the liver cell. This is the significance of the inhibition of protein synthesis early in CCl_4 poisoning. Triglyceride secretion is much reduced in isolated perfused livers examined as early as three hours after CCl_4 poisoning in rats [13]. Investigations of ethionine, the ethyl analogue of methionine [14], seemed to support the view that inhibition of protein synthesis caused a large accumulation of triglyceride fat. Ethionine does not cause any detectable cellular necrosis, and the work done upon it has yielded the final evidence that cell necrosis and the accumulation of fat in the liver are separate phenomena. These studies also suggest that diminution of protein synthesis has no major role in the pathogenesis of cell death. Figure 1 indicates the general mechanism of the production of fatty liver, but the study of ethionine, whilst it increased our knowledge, also brought to light new problems.

Role of Adenosine Triphosphate (ATP). Farber et al. [14] found that ethionine causes a profound fall in the level of adenosine triphosphate (ATP) in the livers of poisoned female rats. Male rats were not so susceptible to the poison and did not develop any pathologic changes. The decrease in ATP is due to the reaction ATP + ethionine → S-adenosylethionine + inorganic orthophosphate.

This is analogous to the reaction undergone by methionine but, in contrast, S-adenosylethionine is inert and acts as an adenosyl trap, thus reducing the level of ATP. The administration of adenine to poisoned rats allowed a new synthesis of ATP and reversed the toxic effects of ethionine. It was thought that the chain of events following upon ethionine poisoning was low ATP → reduced protein synthesis → reduced lipoprotein synthesis → fat accumulation.

Role of the Ribosomes. The investigations of the effects of CCl_4 and of ethionine here drew close together. The electron microscope has shown extensive damage to the endoplasmic reticulum, upon the membranes of which are attached the spirals composed of ribosomes (polysomes) that are the sites of protein synthesis. Shortly after administration either of CCl_4 or of ethionine the ribosomes are seen to be detached from the membranes. When cell-free preparations of poisoned livers are examined by ultracentrifugal analysis it is found that the polysomes are disaggregated into smaller assemblies and even into single particles [15]. All these findings were in accord with the diminished incorporation of [^{14}C] amino acids

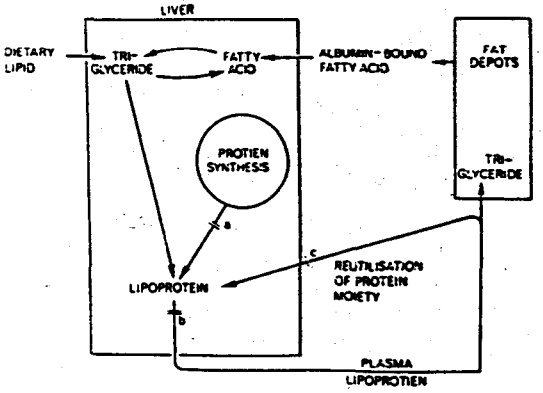

Figure 1. The fatty acid cycle. The scheme shows movements of fatty acids from depots to liver and the return of surplus as triglyceride.

into liver proteins in vivo. When the cell-free preparations were tested, it was found that their ability to incorporate amino acids into proteins was also reduced. Plainly, a ribosomal lesion had been found. Since messenger RNA (m-RNA) is thought to hold the polysomes in the spiral form [16], the nature of the lesion seemed to be clear. In ethionine poisoning the low level of ATP was thought to cause a block in m-RNA synthesis, which in turn would allow the polyribosomes to break up. Protein synthesis would thus be reduced. The nature of the initiating damage in CCl_4 poisoning was not clear, but there was the possibility of direct attack on the endoplasmic reticulum membranes: this possibility was supported by the work of Recknagel and his group [3].

Other Inhibitors. If this hypothesis is correct, it may be assumed that other inhibitors of protein synthesis would also give rise to fat accumulation in liver cells. Puromycin [17] when given to rats does indeed cause a fatty liver, but effective doses of cycloheximide [18] do not. The administration of actinomycin D, which blocks m-RNA synthesis, causes no fat accumulation. To confuse matters even more, Stewart and Farber [19] have shown that an injection of adenine given after a dose of actinomycin D still reverses the ill effects of ethionine and allows realignment of the ribosomes. This can only mean that the low level of ATP has its effect in some other way than through block of synthesis by actinomycin D.

The Transport Problem. In a review [1] we drew attention to two aspects of the problem of fat accumulation in the liver. The first concerned synthesis of the carrier protein (apoprotein) to which triglyceride is attached before it can be moved out of the cell. If it had a relatively long "life" and could be re-used for formation of lipoprotein, then hypotheses that tried to account for the rapid development of fatty liver (within a few hours of poisoning CCl_4 or by ethionine) could not be based on failure of synthesis of protein alone.

Secondly, we pointed out that failure of cation transport also took place in ethionine poisoning [20,21] and potassium ion is lost from the liver cells, an effect shown to be due to low levels of ATP. Could it be that the accumulation of triglyceride fat in the liver is part of a more general failure to transport more than one material?

Evidence suggesting that a re-usable apoprotein exists for triglyceride transport is provided by the work of Roheim et al. [22]. Administration of orotic acid causes a failure in lipoprotein secretion, which is not associated with failure to synthesize protein but apparently with failure to form lipoprotein from the component triglyceride and apoprotein. New evidence has also been obtained to support the idea of a failure of "transport" of protein and lipoprotein in ethionine poisoning. Low potassium ion levels are sufficient by themselves to bring about considerable (50 to 70 per cent) reductions in the rate of transfer of serum albumin and of lipoproteins across the membrane of the liver cell [23]. When potassium ion levels are restored, the rate of appearance of both groups of protein in the extracellular fluid is restored after a delay of about thirty to forty minutes. This effect of potassium ion can be demonstrated in the presence of blocking levels of actinomycin D and is therefore independent of the synthesis of m-RNA. The low potassium ion levels found in ethionine poisoning are sufficient by themselves to cause a failure of lipoprotein secretion. The fatty liver of ethionine can thus be seen to arise in the sequence low ATP → low cell K^+ → reduced lipoprotein secretion → triglyceride accumulation.

We can explain the experiments in which administration of adenine in the presence of actinomycin D reversed the effects of ethionine, since adenine elevates cell potassium ion within thirty minutes of administration.

Present Status of the Problem. Previous work on triglyceride accumulation following CCl_4 or ethionine intoxication suggested the following sequence of events: (1) disaggregation of polyribosomes (?due to failure of m-RNA synthesis); (2) failure of protein synthesis; (3) failure to form apoprotein and hence lipoprotein, leading to (4) accumulation of triglycerides (fatty liver). However, other observations indicate that this explanation is not completely adequate. For example: (1) actinomycin D (which blocks m-RNA synthesis) causes no fat accumulation; it also fails to stop reversal of the manifestations of ethionine intoxication by adenine; (2) not all inhibitors of protein synthesis cause fatty livers when administered to animals; (3) apoprotein may circulate for some time and can be re-utilized by the liver to form lipoprotein.

New evidence suggests interference at different points in the process of accumulation of fat in liver cells at (1) the point of attachment of triglyceride to apoprotein and (2) the sites of transport of lipoprotein (and other proteins) across the cell membrane.

The original cautionary remarks are therefore justified. When we study the mechanism of an abnormal process, we have to be aware of the spreading of secondary disturbances that follow

the primary assault on the cell, and have to decide which are crucial for the next step and which will lead nowhere.

LIVER CELL NECROSIS

If the present knowledge of the development of the fatty liver is still doubtful in many details, that of the mechanisms of cellular necrosis is in a worse state. This is not surprising in view of the many ways in which cells may be killed. In the process of fat accumulation, the fate of a single group of molecules, the triglycerides, together with their associated protein carriers, is being studied by excellent methods. When, however, the chemistry of cell death is considered, it is hard to know where to begin. Different lines of inquiry have to be pursued, until a common pattern may emerge.

A few of the main suggestions that have been made will be discussed, but it must be admitted that progress so far is relatively slight. The first difficulty is one of definition. Necrosis is a histologist's conception. The dead cell is recognized by changes that are the consequences of cell death, or autolysis, which are seen when the degradative enzymes of the dead cell attack its structures and destroy them. These signs take some time to develop, hence one is ignorant of the precise moment of cell death. Whole chains of secondary changes have to be distinguished. CCl_4 poisoning leads to death of the liver cell and has been much investigated. Its history proves instructive.

Role of the Mitochondria. Mitochondrial damage was early put forward as the initial cause of liver damage in CCl_4 poisoning. This theory was inadequate to explain all changes, but it is still just possible that cell death might follow from a primary attack on these organelles. Two possible mechanisms have been suggested:

(1) Attack on the mitochondrial membrane by CCl_4 or a metabolite of CCl_4: This possibility has never been ruled out. The earlier work of Christie and Judah [6] could explain cell necrosis, especially if newer methods of analysis were applied. The crucial factor is time. More sensitive methods for determining changes in the mitochondrial membrane might show that mitochondrial damage occurs quite early.

(2) Mitochondrial damage resulting from calcium accumulation: Mitochondria in vitro take up an enormous amount of calcium (Ca^{2+}) from the surrounding medium, and this Ca^{2+} eventually destroys the mitochondria, causing irreversible changes in their membranes, disrupting the energy-conserving mechanisms of oxidative phosphorylation and allowing the loss of intramitochondrial components such as nucleotides, potassium ion and Mg^{2+} [24,25]. The possibility that Ca^{2+} is a killing agent had been suggested by Thiers, Reynolds and Vallee [26], who showed that a very early rise in liver Ca^{2+} was a feature of CCl_4 poisoning. This is an attractive idea, and the nature of the Ca^{2+} accumulation has been studied extensively. But little firm information has resulted. It has been found by use of ^{45}Ca in vivo that the rate of Ca^{2+} uptake early in CCl_4 poisoning (up to five hours) is normal; this can only mean that the rate of exit is impaired.

Further investigation [27] shows that Ca^{2+} taken up by the poisoned liver cell exchanges very slowly with external Ca^{2+} and that this unexchangeable fraction increases rapidly with time. Thus about 20 per cent of the normal cell Ca^{2+} is slowly exchanging, but within sixty minutes of CCl_4 poisoning this figure is 40 per cent, and it rises steadily thereafter. Some intracellular Ca^{2+} may migrate to an abnormal site and Ca^{2+} may flow in from without to compensate for this. Unfortunately, there is no evidence that shifts of Ca^{2+} play any part in cell death from other toxic agents. There is no early shift of Ca^{2+} after poisoning with thioacetamide, dimethylnitrosamine or by pyrrolizidine alkaloids, all of which kill liver cells. These changes in Ca^{2+} levels may, therefore, represent a curiosity of CCl_4 poisoning.

Role of Peroxidative Changes. Recknagel [3] and Dianzani and associates [28] showed that lipid peroxidation accompanies CCl_4 intoxication. Since the first signs of peroxidation appear within a few minutes of CCl_4 administration, and since the endoplasmic reticulum appears to be the first site of the change, it might well be of crucial importance in initiating cell necrosis.

The importance of lipid peroxidation is twofold: (1) The phospholipids, with a high level of unsaturated fatty acids, are an obvious target for such reactions. This in turn might damage membranous structures, which of course contain much phospholipids. (2) Peroxidation involves the formation of free radicals. This means that a small initiating reaction can rapidly spread, as the free radicals by their attack form others to initiate a chain reaction. The damage is amplified as more and more molecules are attacked and in turn form reactive substances which attack others. The chemistry of free radical formation as it affects cellular injury is discussed by Slater [29].

The significance of the suggested mechanism is

increased by experiments which suggest that CCl_4 itself has to be metabolized to a toxic intermediate. The most likely toxic substance formed from CCl_4 is itself a free radical, CCl_3, the CCl_4 having undergone a homolytic split, losing a Cl. and leaving the residue with a highly reactive unpaired electron. The implication of this is clear. The CCl_3, radical would attack unsaturated fatty acids, which would undergo peroxidative degradation, themselves forming further free radicals that amplify the damage, which would then spread through the cell.

This hypothesis is unproved, for it has yet to be shown that the peroxidation reactions are the cause and not the result of the cell damage. Some of the normal pathways of cellular oxidation involve free radical formation. Normally these are controlled by the intrinsic organization of the electron-transport chain, which is geared to promote a free flow of electrons in a given direction. Alternatively, the liver cell contains large amounts of antioxidants, such as reduced glutathione, which will mop up free radicals. In damaged cells these protective mechanisms might be lost and a random chain reaction might start.

Evidence against this hypothesis is derived from experiments in which antioxidants are administered to rats. Phenothiazines [30] and alpha-tocopherol [31] have both been used to prevent necrosis due to CCl_4. However, McLean [32] finds but a small effect, and if the spread of liver cell necrosis in CCl_4 intoxication depended upon the autocatalytic effect of lipid peroxidation, then antioxidants should be protective, which has not been demonstrated in CCl_4 poisoning. Promethazine, a potent antioxidant, is not much more effective than such nonantioxidant stabilizers as diphenhydramine [30].

Although the liver injury caused by choloroform is similar to that produced by CCl_4, there is no evidence of lipid peroxidation [33,34].

ROLE OF DRUG-METABOLIZING ENZYMES IN CHEMICAL TOXICITY

The drug-metabolizing enzymes in the endoplasmic reticulum of liver cells are usually thought of as detoxicating enzymes. In some cases toxins may be inactivated but recent work has shown that these enzymes may in fact activate otherwise inert substances into toxic molecules [35,60].

When an animal is exposed to phenobarbital or DDT a massive synthesis of the whole system, including cytochrome P-450, takes place. This induction requires DNA-dependent RNA synthesis and also protein synthesis [36,37]. When an animal is fed a purified low protein (3 per cent casein) diet, the activity of the enzyme system and the cytochrome P-450 content of the liver fall to a third, or less, of the normal level in four days. Induction of enzyme synthesis by phenobarbital or DDT is also markedly inhibited [38,39]. However, synthesis of the enzyme system can be induced in protein-deficient animals to levels above those found in animals fed stock diets (Table I).

TABLE I Effect of Nutritional State and Inducing Agents on Microsomal Hydroxylating Enzymes Activity and on Lethal Action of Carbon Tetrachloride

Diet	Treatment	Pyramidon Demethylation (μM gm liver hr)	Mean LD_{50} (ml CCl_4 kg body weight)
Protein-free	—	0.1	14.7
Protein-free	DDT	0.6	4.3
Standard cube diet	—	0.7	6.4
Standard cube diet	Phenobarbital	2.6	0.5

NOTE: CCl_4 was given orally in liquid paraffin oil to male rats, allowed food throughout the experiment. DDT was given as a single subcutaneous dose of 100 mg/kg one week before CCl_4. Phenobarbital was given in the drinking water as a 1 mg/ml solution for one week, and plain drinking water was substituted at the time of CCl_4 dosage.

The induction of microsomal enzymes by exposure to DDT and phenobarbital is an extreme example of a common phenomenon. Many of the non-nutrient components of food are inducers of the enzyme system. Ethanol, oxidized fats and several anti-oxidants are also inducers.

Carbon Tetrachloride and Chloroform. The feeding of a low protein diet almost abolishes the lethal and hepatotoxic effects of CCl_4 in rats (Table I). This effect is reversed by the administration of DDT or phenobarbital. The toxicity of CCl_4 to any animal goes hand in hand with the activity of the microsomal hydroxylating enzymes, which metabolize CCl_4 [38,40,41]. In each situation the lethal and hepatoxic effects are proportional to the amount of CCl_4 metabolized (as measured by conversion of CCl_4 to carbon dioxide both in vivo and in vitro).

Thus the same amount of liver damage is produced by a dose of 2.5 ml CCl_4/kg body weight in a rat fed stock diet as is found after a dose of 0.25 ml/kg body weight to a phenobarbital-treated rat. The concentrations of CCl_4 in the liver differ by a factor of 9, but the amount of CCl_4 metabolized is 12 mg/kg body weight/six hours in each case.

TABLE II Effect of Diet on Chloroform Poisoning

Diet	LD_{50} (ml kg body weight)	Log Plasma ICD
Standard cube diet	1.3	1.9
Standard cube diet + phenobarbitone	0.5	>2.9
Protein-free	1.1	1.4
Protein-free and DDT	0.4	2.4

NOTE: Plasma isocitric dehydrogenase (ICD) activity is expressed as log mμM/ml/min. In unpoisoned rats these values are about 0.15. The rats were given oral doses of chloroform 0.75 ml/kg body weight, and killed twenty-four hours later for determination of ICD activity.

It seems likely that CCl_4 combines with microsomes at the P-450 site [42] and is there converted, as already mentioned, to a highly reactive CCl_3 free radical by the hydroxylation enzyme system [43.44]; this radical in turn attacks numerous cell sites. This mechanism would explain the observations that only cells containing hydroxylation enzymes are sensitive to CCl_4, that low protein diets protect, and that inducers of microsomal hydroxylation potentiate CCl_4 toxicity.

The failure of vitamin E to protect against the hepatotoxic effects of CCl_4 [32] suggests that the autocatalytic lipid peroxidation observed by Recknagel [3] may not be relevant to cell injury.

Chloroform. Protein depletion does not materially alter the toxicity of chloroform given as a single oral dose (Table II). However, enzyme induction renders rats more sensitive to the lethal and hepatotoxic actions of chloroform [33].

Retrorsine. Pyrrolizidine alkaloids such as retrorsine cause diseases of animals and man [35.45]. Neither low protein diet or enzyme induction alters the acute toxicity of retrorsine in vivo [33]. However, the in vitro conversion of retrorsine to a toxic metabolite is dependent on microsomal hydroxylation and is altered by diet and enzyme induction in the same way as pyramidon demethylation or CCl_4 metabolism [46]. It is possible that retrorsine is metabolized to a toxic product which in turn is further metabolized to an inactive substance. Altering the total amount of hydroxylating enzyme may alter the rate of both reactions and need not change the amount of toxic substances reaching the sensitive site [47].

Dimethylnitrosamine. There is a well marked protection against acute dimethylnitrosamine (DMN) poisoning in rats fed protein-deficient diets. The LD_{50} is almost doubled, and the liver damage is greatly reduced [48].

DMN requires demethylation in a microsomal enzyme system before it exerts its toxic and carcinogenic effects. In protein-depleted animals DMN metabolism is greatly reduced in the liver but unaffected in the kidney. The decreased liver clearance of DMN in the protein-deficient animals allows more DMN to come into contact with the kidneys [49].

If DMN is given as a single injection of 60 mg/kg body weight, normal rats almost all die in a few days, with severe liver necrosis. In protein-deficient animals the severity of the liver lesions is much reduced, and most of the animals survive. All the survivors die with kidney tumors in twelve months. Here the protein-deficient diet has changed the action spectrum of DMN. Instead of a predominant hepatotoxic effect, the single dose produces malignant renal tumors in the majority of animals.

Aflatoxin. Madhavan and Gopalan [50] showed that the acute toxicity of aflatoxin was greatly enhanced in protein-deficient rats. This effect can be reversed by dosing with DDT [51]. It appears likely that aflatoxin B_1 and perhaps other aflatoxins are themselves the toxic and carcinogenic agents, and that they are converted to nontoxic metabolites in the liver by the hydroxylation system. In current experiments on the carcinogenic action of aflatoxin, rats were fed peanut meal containing aflatoxin for nine weeks, with simultaneous dosing with phenobarbital to some groups. At the time of writing this paper, eighteen months after cessation of administration of aflatoxin one of twenty of the aflatoxin group is alive, the remainder having succumbed with massive liver tumors. Of the group that received phenobarbital as well as aflatoxin, eleven of twenty survive.

It is a sad irony that two toxins, aflatoxin and DMN [52], likely to occur in the diet of man in areas in which protein deficiency is common, should both have their carcinogenic action enhanced by the suppression of hepatic microsomal hydroxylation attendant upon protein deficiency.

COMMON MISCONCEPTION RELATING TO LIVER DAMAGE

There is a widespread belief that protein deficiency and malnutrition increase the toxic effects of all poisons, especially in their actions on the liver. This belief is incorrect but it is interesting how it came about. It rests upon three assumptions about which our views have changed in the past thirty years, and upon a fourth which was purely

mythical but which has achieved the status of fact by transcription from one textbook to another.

(1) In the late 1940's it was thought that protein deficiency, and especially deficiency of methionine, caused liver necrosis. Schwarz [53] showed that dietary liver necrosis was caused not by protein deficiency but by a simultaneous deficiency of vitamin E and selenium.

(2) About the same time it became clear that there was widespread and serious protein deficiency among children of many developing countries [54]. In kwashiorkor, excessive fat accumulates in the liver, and it was thought that the high incidence of cirrhosis and cancer of the liver in many tropical countries [55] was a sequel to kwashiorkor. Since then it has become clear that kwashiorkor alone does not lead to cirrhosis and hepatoma. Other factors, such as fungal toxins in foodstuffs, may account for these diseases.

(3) Experiments have shown that dogs depleted of protein by plasmapheresis, and rats made protein-deficient with various diets, had more liver necrosis, histologically assessed, after chloroform anesthesia [56]. These results conflict directly with our own (Table II), and it is not easy to resolve this conflict. Factors in our work which might account for the difference include larger numbers of animals, quantitative assessment of liver damage, oral administration of chloroform instead of by inhalation, and highly purified diets. It is possible that when chloroform is inhaled the limiting factor is the amount of chloroform that remains in the liver, and this will depend on the amount of fat present [57].

(4) It has been assumed that protein deficiency sensitizes animals to acute CCl_4 poisoning. There has never been any evidence for this view. Opie and Alford [58] and Campbell and Kosterlitz [59] showed that low protein-high carbohydrate diets protected against CCl_4 and chloroform poisoning, when compared with high protein or high fat diets. For unknown reasons this work never achieved adequate recognition.

REFERENCES

1. McLean AEM, McLean E, Judah JD: Cellular necrosis in the liver induced and modified by drugs. Int Rev Exp Path 4: 127, 1965.
2. Magee PN: Toxic liver necrosis. Lab Invest 15: 111, 1966.
3. Recknagel RO: Carbon tetrachloride hepatotoxicity. Pharmacol Rev 19: 145, 1967.
4. Magee PN, Swann PF: Nitroso compounds. Brit Med Bull 25: 240, 1969.
5. Cameron GR, Karunaratne WAE: Carbon tetrachloride cirrhosis in relation to liver regeneration. J Path Bact 42: 1, 1936.
6. Christie GS, Judah JD: Mechanism of action of carbon tetrachloride on liver cells. Proc Roy Soc B 142: 241, 1954.
7. Oberling C, Rouiller C: Les effets de l'intoxication aiguë au tétrachlorure de carbone sur le foi du rat. Étude au microscope electronique. Ann Anat Path (Paris) 1: 401, 1956.
8. Smuckler EA, Iseri OA, Benditt EP: An intracellular defect in protein synthesis induced by carbon tetrachloride. J Exp Med 116: 55, 1962.
9. Rees KR, Shotlander VL: Fat accumulation in acute liver injury. Ann NY Acad Sci 104: 905, 1963.
10. Eder HA, Roheim PS, Gidez LI, Switzer S: Regulation of lipid transport in the liver. Proceedings of an International Symposium on Lipid Transport, Nashville, Tenn (Meng HC, ed), Springfield, Ill, 1963, Charles C Thomas, p 202.
11. Robinson DS: The uptake and release of lipids by the liver. Proceedings of an International Symposium on Lipid Transport, Nashville, Tenn (Meng HC, ed). Springfield, Ill, 1964, Charles C Thomas, p 194.
12. Lombardi B: Considerations on the pathogenesis of fatty liver. Lab Invest 15: 1, 1966.
13. Heimberg M, Weinstein I, Dishmon G, Dunkerley A: The action of carbon tetrachloride on transport and metabolism of triglycerides and fatty acids by isolated perfused rat liver and its relationship to etiology of fatty liver. J Biol Chem 237: 3623, 1962.
14. Farber E, Shull KH, Villa-Trevino S, Lombardi B, Thomas M: Biochemical pathology of acute hepatic adenosine triphosphate deficiency. Nature (London) 203: 34, 1964.
15. Smellie RMS: Biochemistry of deoxyribonucleic acid and ribonucleic acid replication. Brit Med Bull 21: 195, 1965.
16. Arnstein HRV: Mechanism of protein biosynthesis. Brit Med Bull 21: 217, 1965.
17. Collins JF, Antibiotics, proteins and nucleic acids. Brit Med Bull 21: 223, 1965.
18. Farber E: Personal communication
19. Stewart GA, Farber E: Reformation of functional liver polyribosomes from ribosome monomers in the absence of RNA synthesis. Science 157: 67, 1967.
20. Judah JD, Ahmed K, McLean AEM, Christie GS: Ion transport in ethionine intoxication. Lab Invest 15: 167, 1966.
21. Christie GS, Judah JD: Cations of mitochondria in ethionine intoxication. Lab Invest 18: 108, 1968.
22. Roheim PS, Switzer S, Girard A, Eder HA: The

mechanism of inhibition of lipoprotein synthesis by orotic acid. Biochem Biophys Res Commun 20: 416, 1965.
23. Judah JD, Nicholls MR: Role of liver cell K in secretion of serum albumin and lipoproteins. Biochem J 116: 663, 1970.
24. Harris EJ, Judah JD, Ahmed K: Ion transport in mitochondria, Current Topics in Bioenergetics, vol I (Sanadi DR, ed), New York and London, 1966, Academic Press, p 255.
25. Lehninger AL, Carafoli E, Rossi CS: Energy-linked ion movements in mitochondrial systems. Advances Enzym 29: 259, 1967.
26. Thiers RE, Reynolds ES, Vallee BL: The effect of carbon tetrachloride poisoning on subcellular metal distribution in rat liver. J Biol Chem 235: 2130, 1960.
27. Judah JD, McLean AEM: Unpublished results 1966.
28. Dianzani MU, Baccino FM, Comporti M: The direct effect of carbon tetrachloride on subcellular particles. Lab Invest 15: 149, 1966.
29. Slater TF: Free radical mechanisms in tissue injury, Methods and Achievements in Experimental Pathology (Bajusz E, Jasmin G, eds), Basle, 1969, Karger, p 30.
30. Rees KR: Cellular injury by drugs. Ciba Foundation Symposium, jointly with the Co-ordinating Committee for Symposia on Drug Action on "Enzymes and Drug Action," (Mongar JL, Reuck AVS de, eds) London, 1962, Churchill, p 344.
31. Gallagher CH: The effect of antioxidants on poisoning by carbon tetrachloride. Aust J Exp Biol Med Sci 40: 241, 1962.
32. McLean AEM: The effect of diet and vitamin E on liver injury due to carbon tetrachloride. Brit J Exp Path 48: 632, 1967.
33. McLean AEM: The effect of protein deficiency and enzyme induction on the toxicity of chloroform and retrorsine. Brit J Exp Path 51: 317, 1970.
34. Klaassen CD, Plaa GL: Comparison of the biochemical alterations elicited in livers from rats treated with carbon tetrachloride chloroform 112 trichloroethane and 111 trichloroethane. Biochem Pharmacol 18: 2019, 1969.
35. McLean EK: Toxic actions of pyrrolizidine alkaloids. Pharmacol Rev (in press).
36. Conney AH, Miller EC, Miller JA: The metabolism of methylated aminoago dyes. V. Evidence for induction of enzyme synthesis in the rat by 3 methylcholanthrene. Cancer Res 16: 450, 1956.
37. Conney AH: Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 19: 317, 1967.
38. McLean AEM, McLean EK: The effect of diet and DDT on microsomal hydroxylation and on sensitivity of rats to CCl_4 poisoning. Biochem J 100: 564, 1966.
39. Marshall WJ, McLean AEM: The effect of oral phenobarbitone on hepatic microsomal cytochrome P450 and demethylation activity in rats fed normal and low protein diets. Biochem Pharmacol 18: 153, 1969.
40. Seawright AA, McLean AEM: Effect of diet on carbon tetrachloride metabolism. Biochem J 105: 1055, 1967.
41. Garner RC, McLean AEM: Increased susceptibility to carbon tetrachloride poisoning in the rat after pretreatment with oral phenobarbitone. Biochem Pharmacol 18: 645, 1969.
42. McLean AEM: Effect of hexane and carbon tetrachloride on microsomal cytochrome P450. Biochem Pharmacol 16: 2030, 1967a.
43. Slater TF: Necrogenic action of carbon tetrachloride in the rat. A speculative mechanism based on activation. Nature (London) 209: 36, 1966.
44. Reynolds ES, Yee AG: Liver parenchymal injury. V. Relationships between patterns of chloromethane C^{14} incorporation with constituents of liver in vivo and cellular injury. Lab Invest 16: 591, 1967.
45. McLean EK, Bras G, György P: Veno-occlusive lesions in livers of rats fed Crotalaira fulva. Brit J Exp Path 45: 242, 1964.
46. Mattocks AR: Toxicity of pyrrolizidine alkaloids. Nature (London) 217: 723, 1968.
47. Butler WH, Mattocks AR, Barnes JM: Lesions in the liver and lungs of rats given pyrrole derivatives of pyrrolizidine alkaloids. J Path 100: 169, 1970.
48. McLean AEM, Verschuuren HG: Effect of diet and microsomal enzyme induction on the toxicity of dimethyl-nitrosamine. Brit J Exp Path 50: 22, 1969.
49. Swann PF, McLean AEM: The effect of diet on the toxic and carcinogenic actions of dimethylnitrosamine. Biochem J 107: 14, 1968.
50. Medhavan TV, Gopalan C: Effect of dietary protein on aflatoxin liver injury in weanling rats. Arch Path 80: 123, 1965.
51. McLean AEM, McLean EK: Protein depletion and toxic liver injury due to aflatoxin (abstract). Proc Nutr Soc 26: xiii, 1967.
52. McGlashan ND, Walters CI, McLean AEM: Nitrosamines in African alcoholic spirits and oesophageal cancer. Lancet 2: 1017, 1968.
53. Schwarz K: Vitamin E trace elements and sulfhydryl groups in respiratory decline. Vitamins Hormones 20: 463, 1962.
54. Waterlow JC, Cravioto J, Stephen JML: Protein malnutrition in man. Advances Protein Chem 15: 131, 1960.
55. Doll R: Prevention of Cancer: Pointers from Epidemiology. London, 1967, The Nuffield Provincial Hospitals Trust, p 40
56. Miller LL: Nutritional factors affecting the toxicity of hydrocarbons. Occup Med 5: 194, 1948.
57. Kutob SD, Plaa GL: The effect of acute ethanol intoxication on chloroform induced liver damage. J Pharmacol Exp Ther 135: 245, 1962.
58. Opie EL, Alford LB: The influence of diet upon necrosis caused by hepatic and renal poisons. I. Diet and the hepatic lesions of chloroform phosphorus or alcohol. J Exp Med 21: 1, 1915.
59. Campbell RM, Kosterlitz HW: The effect of short term changes in dietary protein on the response of the liver to carbon tetrachloride injury. Brit J Exp Path 29: 149, 1948.
60. McLean AEM: Conversion of inactive to toxic molecules by the liver, Mechanisms of Toxicity, Biological Council Symposium (Aldridge WN, ed) London, MacMillan & Co, 1970.

n Store REG US1C Ord 1316667 B Slot 042W

Ime 1-DEC-94 @ 12:17 Needs By: To Location: 3CA00Staffer: N

mber: 10023936
itle: DEUT MED WOCHSCHR

lume: 97 Issue: ISSN/ISBN:
DATE: 1972
AGES: 1922-
itle: SKLERODERMICARTIGE HAUTVERAUNDERUNGEN....

thor: JOHE, S; LANGE, CE

Page Count	2
Reason Not Filled	

Note: CAON

73

Num.: 5393
ce 1: 5393
ce 2: BAN
: US1C Ord 1316667 B Slot 042W

BFG36132