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TOXIC EFFECT OF CARBON TETRACHLORIDE ON THE LIVER CELL

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Rats and mice were given injections of carbon tetrachloride into the portal vein or into the spleen, and the localization of the resulting hepatocellular lesions was studied. In contrast to some statements in the literature, and confirming some older reports, damage was found to be limited to the periportal areas. A range of changes leading to cell necrosis was found, which was frequently associated with pronounced vasodilatation. These features were considered to be evidence in favour of a direct hepatotoxic effect of carbon tetrachloride, and are difficult to reconcile with the view, formulated again recently by some workers, that carbon tetrachloride acts indirectly on the liver cell by producing vasoconstriction.

Although carbon tetrachloride is one of the most widely used experimental hepatotoxic agents, its mode of action is not well understood and has given rise to considerable discussion in the literature. In the main there are two interpretations of the morphological and biochemical changes to be found after treatment of experimental animals with carbon tetrachloride. One school of thought, represented mainly by earlier workers such as Cameron, Karunaratne & Thomas (1937), considers that the effect is a direct toxic action on the liver cells. Other workers, however, hold that the effect is essentially an indirect one, mediated by changes in the blood supply to the liver cells. To some extent this view appears to be supported by the well-known observation that liver cell damage following the oral or subcutaneous administration of carbon tetrachloride is localized predominantly in the central zone of the liver lobule (for references see Aterman, 1954). It has even been stated that following the intraportal or intrasplenic injection of carbon tetrachloride (Myren, 1956) the early liver lesions are to be found not in the periportal regions, where they reasonably could be expected and where, indeed, pronounced changes are found following the injection of other fluids (Aterman, 1958a), but in areas removed from that zone. The problem of the mode of action of carbon tetrachloride has in the last few years repeatedly been reviewed, for instance, by Drill (1952), Myren (1956), Stoner & Magee (1957) and, more recently, by Brody, Calvert & Schneider (1961). Brody *et al.* (1961) have reported experiments, on the basis of which they proposed that " . . . carbon tetrachloride does not act directly upon the liver parenchymal cell but rather (that) the effects usually observed are promulgated via an action upon the central nervous system, or more precisely the sympathetic outflow of the autonomic nervous system. This sympathetic discharge may cause a constriction

of blood vessels supplying the liver with a resultant decrease in blood flow and a consequent anoxia. This produces the centrilobular necrosis characteristic of carbon tetrachloride poisoning." This particular hypothesis has prompted me to present here the results of some experiments which suggest that, under certain circumstances at least, carbon tetrachloride acts directly on the liver cells. They were undertaken in order to study the discrepancy between the results reported by Cameron *et al.* (1937) and by Myren (1956) following the injection of carbon tetrachloride directly into the portal circulation. Some aspects of this work have been briefly referred to in a different context (Aterman, 1958b).

METHODS

Fourteen albino rats, weighing 150 to 200 g, and eight mice, of both sexes, were used in these experiments. Carbon tetrachloride, in doses ranging from 0.01 ml. to 0.1 ml., was injected either directly into the portal vein or into the spleen of the experimental animals which had been lightly anaesthetized with ether. Representative sections of the various lobes of the liver were taken immediately, or at intervals up to 4 hr after injection. In view of the evidence presented by Myren (1956), his experimental technique was largely adhered to in the experiments in which mice were used. The mice, therefore, were killed very shortly after injection, irrespective of whether they had been treated with carbon tetrachloride intraportally or into the splenic substance. The specimens of the liver were fixed in 10% buffered formalin, cut at 7 μ, and stained with haematoxylin-eosin.

RESULTS

The effects of injections of carbon tetrachloride into the portal vein or into the splenic substance have been described in detail by Cameron *et al.* (1937) and will, therefore, not be reported extensively here. Of interest for the purpose of the present paper is mainly the localization of the changes to be seen in the liver. In contrast to the experiments described by Myren (1956), all the animals examined in the present series showed the changes to be essentially localized in the periportal region of the liver lobules (Figs. 1 to 5). Where, however, the changes had become extensive, similar to the " toxic infarcts " of Cameron *et al.* (1937), this periportal localization was no longer so clearly seen. The extent of the detectable damage varied considerably, even in the same section. The zone of histologically altered cells, for instance, did not always surround the entire circumference of the portal vessels, particularly of the larger ones; it was, on occasion, possible to find a distinctly segmental arrangement (Fig. 5), in that an irregularly shaped but well-demarcated zone of damaged cells extended into the liver lobule from about one-half of the vessel only, as if the wave of injected carbon tetrachloride had hit only that part of the vessel wall and had leaked out through this portion into the adjacent parenchyma. The contrast between the damaged cells surrounding the injured part and the apparently intact cells surrounding the unaffected part of the vessel was striking, and is difficult to reconcile with an indirect effect of carbon tetrachloride, but is highly suggestive of direct contact of the cells with this noxious agent. It is possible that asymmetrical extensions of damaged cell zones of this type, combined with the occasionally almost complete escape of a branch of a portal vessel from immediately detectable impairment of the surrounding cells, give rise to the

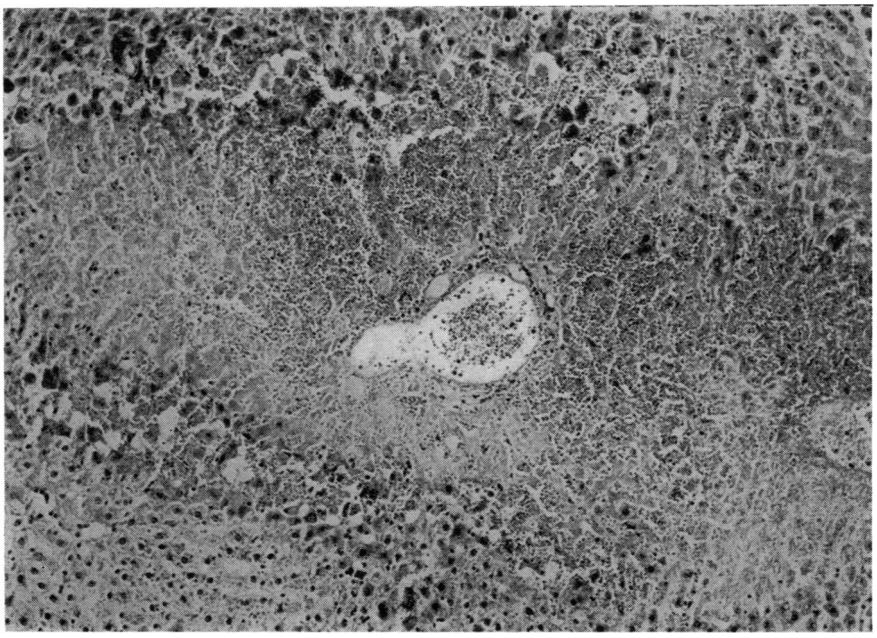

Fig. 1. Periportal necrosis in the liver of a rat given 0.05 ml. of carbon tetrachloride into the spleen and killed after 3.5 hr. Note the portal tract in the centre of the lesion, the sharp demarcation of the area of damage, and the presence of engorged and dilated sinusoids. Formol-saline, 7 μ, H.E., $\times$180.

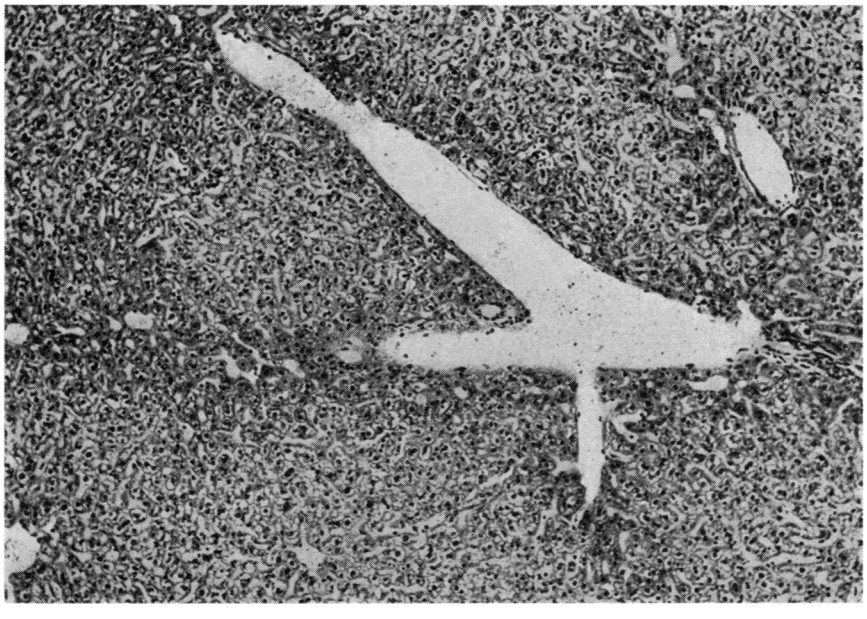

Fig. 2. Liver of a mouse, injected with 0.04 ml. of carbon tetrachloride into the spleen and killed very shortly afterwards. Note the periportal localization of the early hepatocellular changes. Formol-saline, 7 μ, H.E., $\times$180.

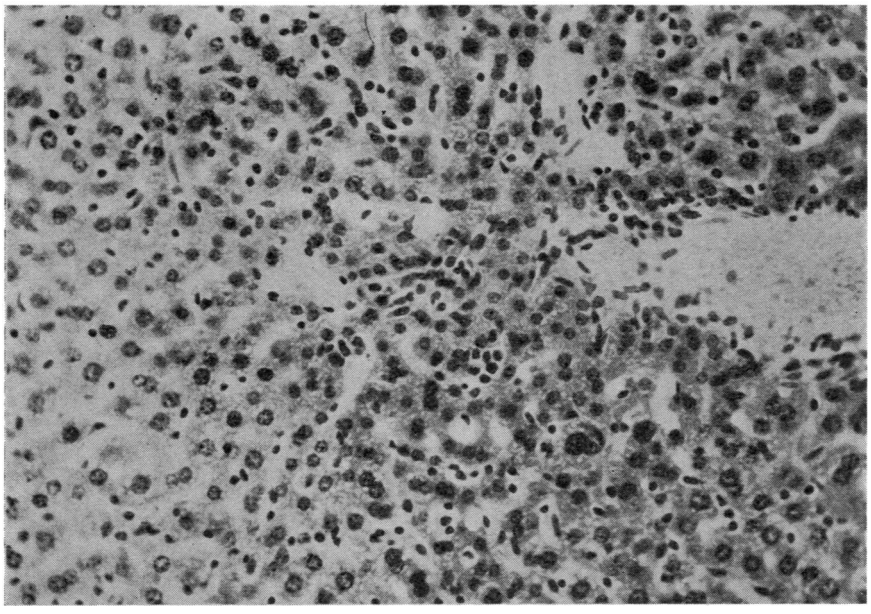

Fig. 3. Same section as Fig. 2. Note the blurred outline of the cells and trabeculae and the fine cytoplasmic vacuolation in the zone of early damage in the periportal region. Formol-saline, 7 μ, H.E., $\times$450.

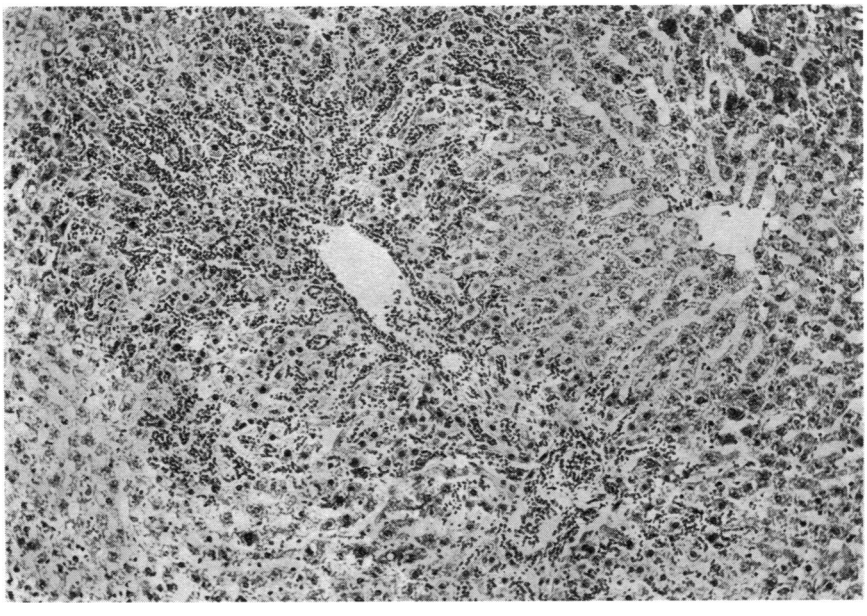

Fig. 4. Liver of a rat given 0.1 ml. of carbon tetrachloride into the spleen. The animal died after 20 min. Note the engorgement of vessels in the periportal region, and the wide but relatively empty sinusoids in the central zone of the liver lobule. Formol-saline, H.E., 7 μ, $\times$180.

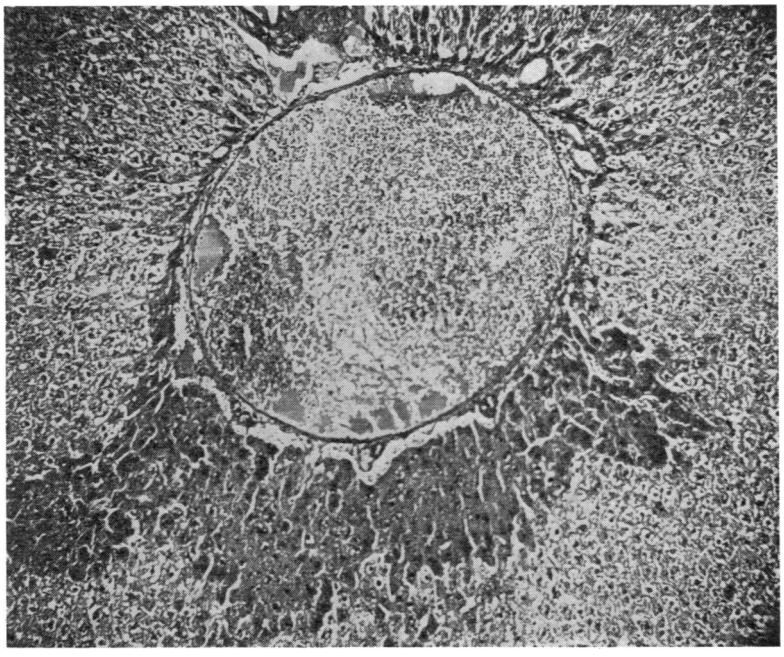

Fig. 5. Periportal necrosis in the liver of a rat given 0.05 ml. carbon tetrachloride into the spleen and killed shortly afterwards. Contrast the area of distinct damage surrounding one half of the circumference of a large branch of the portal vein with the area of apparently intact cells surrounding the other half of the vessel. In the same section there could be seen another branch of the portal vein with only scanty signs of surrounding cell damage. Formol-saline, H.E., 7 μ, $\times$120.

occasional impression of areas of cell damage not obviously related to the portal tracts in an individual section. It must, however, be stated quite clearly that central damage as a primary localization has not been seen in any of these sections, in contrast to the findings reported by Myren (1956). Histologically the lesions, depending on their age, were characterized by a spectrum of changes varying from a blurring of trabecular and cellular outlines (Fig. 3) with cytoplasmic eosinophilia, the formation of fine cytoplasmic vacuoles, and a denser appearance of the nuclear chromatin with loss of nuclear detail, to unmistakable liver necrosis (Figs. 1, 5). Histochemical studies presented elsewhere (Aterman, 1960) suggest that at least some of these changes can be interpreted as a loss of glycogen and a redistribution of the basophile substance of the liver cell. The changes seen took place with great rapidity. Morphological evidence of narrowing or obliteration of the sinusoidal bed in the areas of cell damage, either by compression or by contraction of the sinusoids, was not noted in these sections. On the contrary, there was present a distinct dilatation of the sinusoids which were packed with red blood corpuscles (Figs. 1, 4).

DISCUSSION

The findings in rats and mice, presented here, agree with the results reported by earlier workers following the intraportal injection of carbon tetrachloride in dogs

(Gardner, Grove, Gustafson, Maire, Thompson, Wells & Lamson, 1925) or in rabbits (Cameron *et al.*, 1937). It is surprising that so little attention has been paid to the findings and the conclusions of these workers. Cameron *et al.* (1937), for instance, state that " . . . there seems little doubt, therefore, that the changes in the liver are due to a direct action of these poisons on the liver cells." They specifically draw attention to the fact that central or midzonal necrosis was not seen in their experiments. It is important to emphasize this, since Myren (1956) quotes Cameron *et al.* (1937) as having found " . . . the early changes centrilobularly irrespective of the manner of administration." Moreover, Gardner *et al.* (1925) and Lamson, Gardner, Gustafson, Maire, McLean & Wells (1924) have quite clearly pointed out that the effects of carbon tetrachloride depended on the mode of administration, a point also made by Himsworth (1950), who is frequently quoted in support of their view by those workers who postulate an indirect effect of carbon tetrachloride on the liver cells. In the light of these early observations it is difficult to understand how the categorical statement, found repeatedly in the literature, that carbon tetrachloride produces centrilobular necrosis, has arisen. Drill (1952), for instance, states that " substances such as chloroform, carbon tetrachloride, tannic acid or mushroom toxin cause injury in the central part of the lobule, etc." Stoner & Magee (1957) also list carbon tetrachloride as a toxic agent producing centrilobular necrosis, whereas Brody *et al.* (1961) speak of a decrease in the blood flow and a consequent anoxia which " . . . produces the centrilobular necrosis characteristic of carbon tetrachloride poisoning." Generalizations of this type have, presumably, led to the continued resurrection of the hypothesis of the indirect effect of carbon tetrachloride, mediated through changes in the circulation of the liver. It is, therefore, particularly pertinent to point out that the evidence for this indirect effect is not clear-cut. Dunn, Ellis & Freedman (1961), for instance, were unable to confirm the findings of Brody *et al.* (1961), who stated that " . . . adrenergic blocking agents protected against the centrilobular necrosis seen with carbon tetrachloride," with all the morphological and biochemical changes which follow the subcutaneous injection of this agent. There is little concrete proof that the changes postulated to occur in the hepatic circulation actually do take place. There are, on the contrary, observations and suggestions present in the literature which appear at variance with this postulate. Daniel, Prichard & Reynell (1952), for instance, studied the passage of contrast media through the liver and concluded that " . . . it does not seem likely that ischaemia due to retardation of blood flow is responsible . . . for the centrilobular necrosis seen in acute CCl_4 poisoning. . . ." Stoner (1956) even found, by means of internal calorimetry, an increase in the blood flow of the liver during the time of development of the hepatic lesions, and concluded that circulatory disturbances could not be invoked to explain the production of centrilobular necrosis. Similarly, Seneviratne (1949), who observed the vascular changes in the liver by means of transillumination, noted shortly after the subcutaneous injection of carbon tetrachloride dilatation of the sinusoids and an active flow of blood at a time when hepatocellular changes began to appear in the central zone of the liver lobule. He specifically states that " the portal vessels, hepatic arterioles and hepatic venous radicles showed no appreciable change." He did, however, notice an almost instantaneous contraction of these vessels when carbon tetrachloride was injected

directly into the portal vein. Since a similar blanching effect was seen following the injection of hypertonic saline or glucose—an observation which I can confirm—he attributed this change to a " non-specific response to a strong irritant." It is of interest, for the purpose of this paper, to draw attention to the discrepancy inherent in the fact that on the one occasion when the contraction of vessels, which is postulated to cause the centrilobular changes, is known to occur, the pathological changes, as described here, take place in the periportal and not in the central region of the liver lobule. Since the nature and the localization of these changes are highly suggestive of a direct hepatotoxic effect of carbon tetrachloride, at least if given intraportally, it is questionable whether an indirect effect of such an agent can be invoked.

REFERENCES

ATERMAN, K. (1954). Studies in fibrosis of the liver induced by carbon tetrachloride (relation between hepatocellular injury and new formation of fibrous tissue). *A.M.A. Arch. Path.*, **57**, 1–11.

ATERMAN, K. (1958a). Observations on the nature of " watery vacuolation." The response of the liver cell to the intravenous injection of hypertonic saline, Evans blue, dextran and heparin. *Lab. Invest.*, **7**, 577–605.

ATERMAN, K. (1958b). Some observations on the sinusoidal cells of the liver. *Acta Anat.*, **32**, 193–213.

ATERMAN, K. (1960). The dark and the light cells of the liver. Seventh International Anatomical Congress, New York.

BRODY, T. M., CALVERT, D. N. & SCHNEIDER, A. F. (1961). Alteration of carbon tetrachloride-induced pathologic changes in the rat by spinal transection, adrenalectomy and adrenergic blocking agents. *J. Pharmacol. exp. Ther.*, **131**, 341–345.

CAMERON, G. R., KARUNARATNE, W. A. E. & THOMAS, J. C. (1937). Massive necrosis (" toxic infarction ") of the liver following intra-portal administration of poisons. *J. Path. Bact.*, **44**, 297–303.

DANIEL, P. M., PRICHARD, M. M. L. & REYNELL, P. C. (1952). The portal circulation in rats with liver-cell damage. *J. Path. Bact.*, **64**, 61–64.

DRILL, V. A. (1952). Hepatotoxic agents: Mechanism of action and dietary relationships. *Pharmacol. Rev.*, **4**, 1–42.

DUNN, M. R., ELLIS, S. & FREEDMAN, H. (1961). The failure of an adrenergic blocking agent (dibenzyline) to prevent hepatic necrosis caused by carbon tetrachloride. (Abstract of 58th Annual Meeting of Amer. Ass. Pathologists and Bacteriologists, Chicago, April, 1961.)

GARDNER, G. H., GROVE, R. C., GUSTAFSON, R. K., MAIRE, E. D., THOMPSON, M. J., WELLS, H. S. & LAMSON, P. D. (1925). Studies on the pathological histology of experimental carbon tetrachloride poisoning. *Bull. Johns Hopkins Hosp.*, **36**, 107–133.

HIMSWORTH, H. P. (1950). *The Liver and Its Diseases.* Oxford: Blackwell Scientific Publications.

LAMSON, P. D., GARDNER, G. H., GUSTAFSON, R. K., MAIRE, E. D., MCLEAN, A. J. & WELLS, H. S. (1924). The pharmacology and toxicology of carbon tetrachloride. *J. Pharmacol. exp. Ther.*, **22**, 215–288.

MYREN, J. (1956). Injury of liver tissue in mice after single injections of carbon tetrachloride. *Acta. Path. Microbiol. scand., Suppl.*, **116**, 1–64.

SENEVIRATNE, R. D. (1949). Physiological and pathological responses in the blood-vessels of the liver. *Quart. J. exp. Physiol.*, **35**, 77–110.

STONER, H. B. (1956). The mechanism of toxic hepatic necrosis. *Brit. J. exp. Path.*, **37**, 176–198.

STONER, H. B. & MAGEE, P. N. (1957). Experimental studies on toxic liver injury. *Brit. med. Bull.*, **13**, 102–106.