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THE DIFFERENT SUSCEPTIBILITY OF RAT LIVER LOBES TO CARBON TETRACHLORIDE AND DIMETHYLNITROSAMINE

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Summary.—The extent of liver damage in rats dosed with carbon tetrachloride (CCl_4) or dimethylnitrosamine (DMN), by intragastric or intraperitoneal injection, has been compared in the different lobes. The level of activity of DMN-demethylase in the lobes has also been measured as an index of the activity of the microsomal enzymes. DMN-demethylase activity was greater in the left and left median lobes than in the right and right median lobes. The extent of liver damage (disruption of the basophilic bodies and necrosis) was greater in the right than in the left lobes of animals dosed with CCl_4 but was greater in the left lobes of animals given DMN. The route of injection made no difference. The distribution of liver damage may be explained by the distribution of microsomal enzymes.

IT IS OFTEN assumed that the lobes of the liver all react to the same degree to hepatotoxic agents. Biochemical changes in the liver of experimental animals, accordingly, are often assayed in preparations of the whole liver. However, occasional reports suggest that the histological changes in different lobes differ in extent. Of course, the histological changes caused by hepatotoxic agents and drugs are not constant in the hepatic lobules from the portal tract to the hepatic veins (Rouiller, 1964). The distributions of cytological structures and enzymes within the lobules also vary with the distance from the portal tracts (Novikoff and Essner, 1960; Bruni and Porter, 1965; Loud, 1968).

This work was prompted by observed differences in the extent of carbon tetrachloride (CCl_4) and dimethylnitrosamine (DMN) induced lesions between the lobes of the liver in rats. These agents themselves are not considered hepatotoxic but are metabolized by microsomal enzymes to active intermediates (Brouwers and Emmelot, 1960; McLean and McLean, 1969; Slater, 1966, 1972; Pound and Lawson, 1974). It seemed possible that the observed differences might be associated with differences in the drug metabolizing enzymes.

MATERIALS AND METHODS

Animals.—Random bred male Sprague–Dawley rats from the University animal house were used. They weighed 200–300 g and were maintained on the high protein diet used previously (Pound, Lawson and Horn, 1973). The diet and water were freely available.

Chemicals.—Carbon tetrachloride A.R. (CCl_4) was obtained from Ajax Chemical Company Pty Ltd, Auburn, N.S.W. Dimethylnitrosamine (DMN) was purchased from Schuchardt, West Germany. CCl_4 was administered as a solution in olive oil and DMN as a solution in saline. The drugs were given by stomach tube (i.g.), or by intraperitoneal injection (i.p.).

Histological methods.—Tissue for histological examination was fixed in 4% phosphate buffered formol saline, pH 7·2, dehydrated in alcohols and embedded in paraffin. Sections cut at 5 μm were stained by routine methods with haematoxylin and eosin.

Groups of 4 rats were dosed with CCl_4 or DMN, and killed 24 or 48 h later for histological examination of the different lobes of the liver. Control rats had no treatment.

Determination of DMN-demethylase.—DMN-demethylase activity was determined by measuring the amount of formaldehyde produced from DMN by a microsomal preparation (Pound and Lawson, 1974), obtained by the calcium salt precipitation technique (Baker, Coons and Hodgson, 1973). Groups of 2 rats were given CCl_4 and DMN-demethylase determined in the different lobes of the liver 3, 24 and 144 h later. Control rats had no CCl_4.

RESULTS

The liver lobes are referred to as the right, right median, left median and left lobes following the usual nomenclature. The small caudate lobes were left out of consideration.

Livers from untreated rats.—The broad histological structure of the liver does not vary between the lobes but there are cytological differences between the right and left lobes. The basophilic bodies (ergastoplasm) of the hepatocytes (Jones and Mills, 1973) are more numerous, and the cytoplasmic basophilia generally is more evident, in the centrilobular zones than in the periportal zones of the lobules. The cells in the periportal areas appear more vacuolated due to the presence of glycogen. The centrilobular zone with the more closely packed ergastoplasm is larger in the left lobes (Fig. 1) than in the right lobes (Fig. 2). The 2 right lobes and the 2 left lobes do not differ significantly.

Histology of lesions after CCl_4.—The histological effect of CCl_4 on the liver of these rats under the prevailing laboratory conditions has been described briefly (Pound and Lawson, 1974). The centrilobular zone of necrosis after 24 and 48 h is larger in the right lobes than in the left lobes (Fig. 3, 4). Similarly, after non-necrogenic doses, the zone of dispersal of the basophilic bodies is larger in the right lobes, and there is more vacuolation due to fat (Fig. 5, 6).

The extent of the lesions has been measured as the fraction of the distance involved from the central vein to the portal tracts (Pound and Lawson, 1974) and the differences between the lobes set out in Table I. With the largest dose, 2·5 ml/kg, the area of necrosis is 15–30% more extensive in the right lobes than in the left lobes. With a dose of 0·6 ml/kg, which produces necrosis only in occasional

EXPLANATION OF PLATES

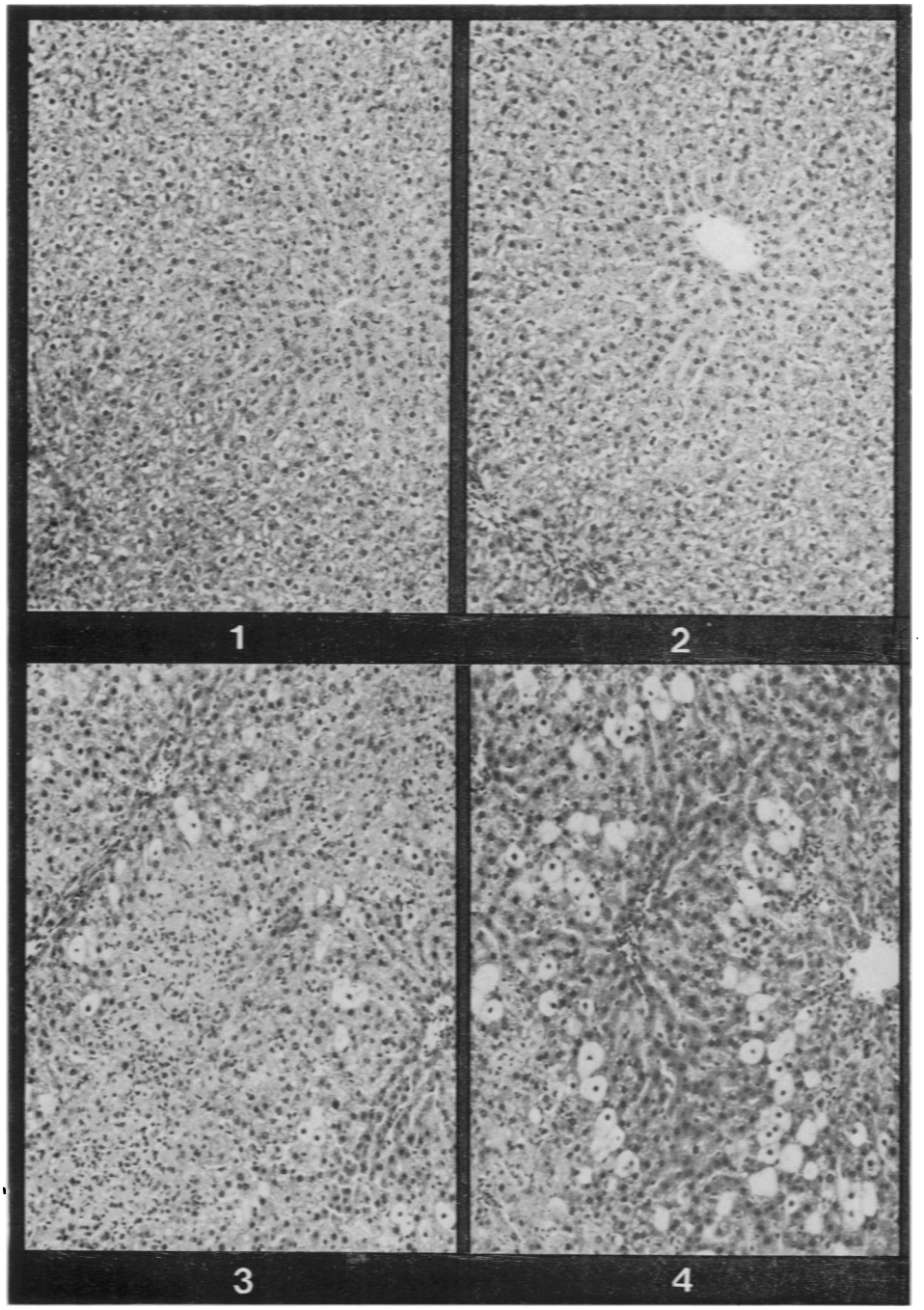
FIG. 1, 2.—Liver from normal untreated rat. The basophilic bodies are more numerous and the cytoplasmic basophilia is more evident in the zone around the central vein. The peripheral zone of cells is vacuolated due to the presence of glycogen. The zone of basophilic bodies occupies a larger proportion of the lobule in the left lobe, Fig. 1, than in the right lobe, Fig. 2. H. and E. × 100.

FIG. 3, 4.—Section of liver of rat 24 h after dosing with 2·5 ml/kg CCl_4 i.g. showing large area of centrilobular necrosis. The area of necrosis is larger in the right lobe, Fig. 3, than in the the left lobe, Fig. 4. H. and E. × 100.

FIG. 5, 6.—Liver from rat given 0·15 ml/kg CCl_4 i.g. 24 h previously. This dose does not produce necrosis but there is dispersal of the cytoplasmic granulation over a large part of the lobule. The change is more extensive in the right lobe, Fig. 5, than in the left lobe, Fig. 6. H. and E. × 100.

FIG. 7, 8.—Liver from rat given 20 mg/kg DMN i.g. 48 h previously, showing typical haemorrhagic necrosis with rapid removal of tissue. The area of necrosis is more extensive and the lesion is more advanced in the left lobe, Fig. 7, than in the right lobe, Fig. 8. H. and E. × 100.

FIG. 9, 10.—Liver from rat given 10 mg/kg DMN i.p. 24 h previously, showing the centrilobular zone of dispersal of the basophilic bodies and the eosinophilic change in the cytoplasm. This dose does not produce necrosis. The zone of cytological change is more extensive in the left lobe, Fig. 9, than in the right lobe, Fig. 10. H. and E. × 100.



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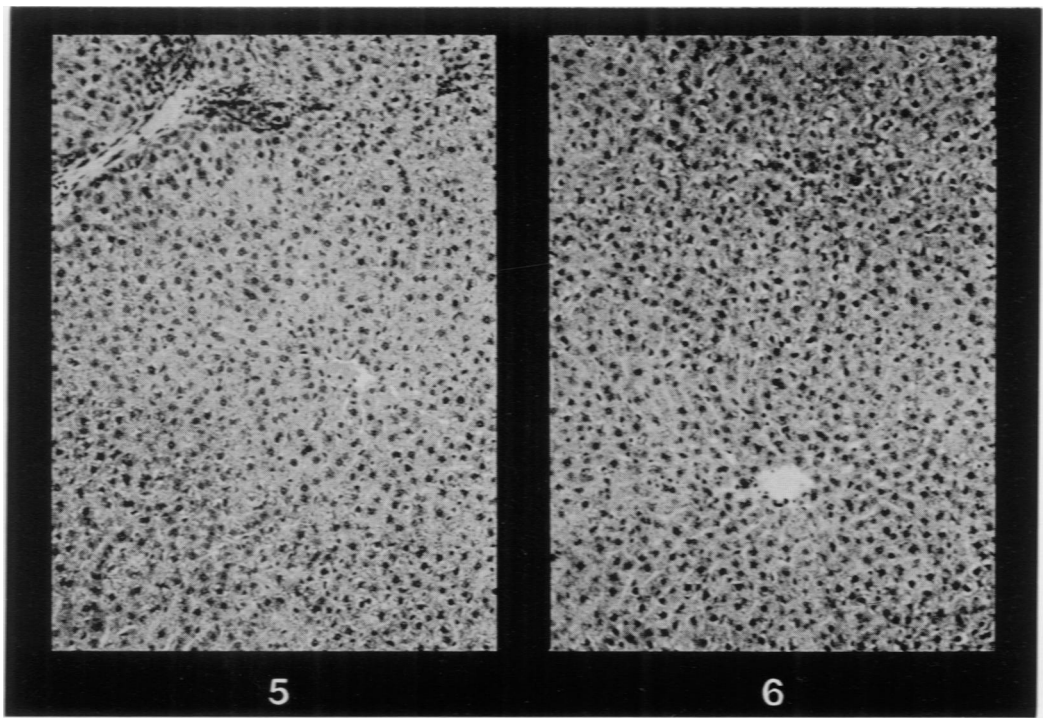
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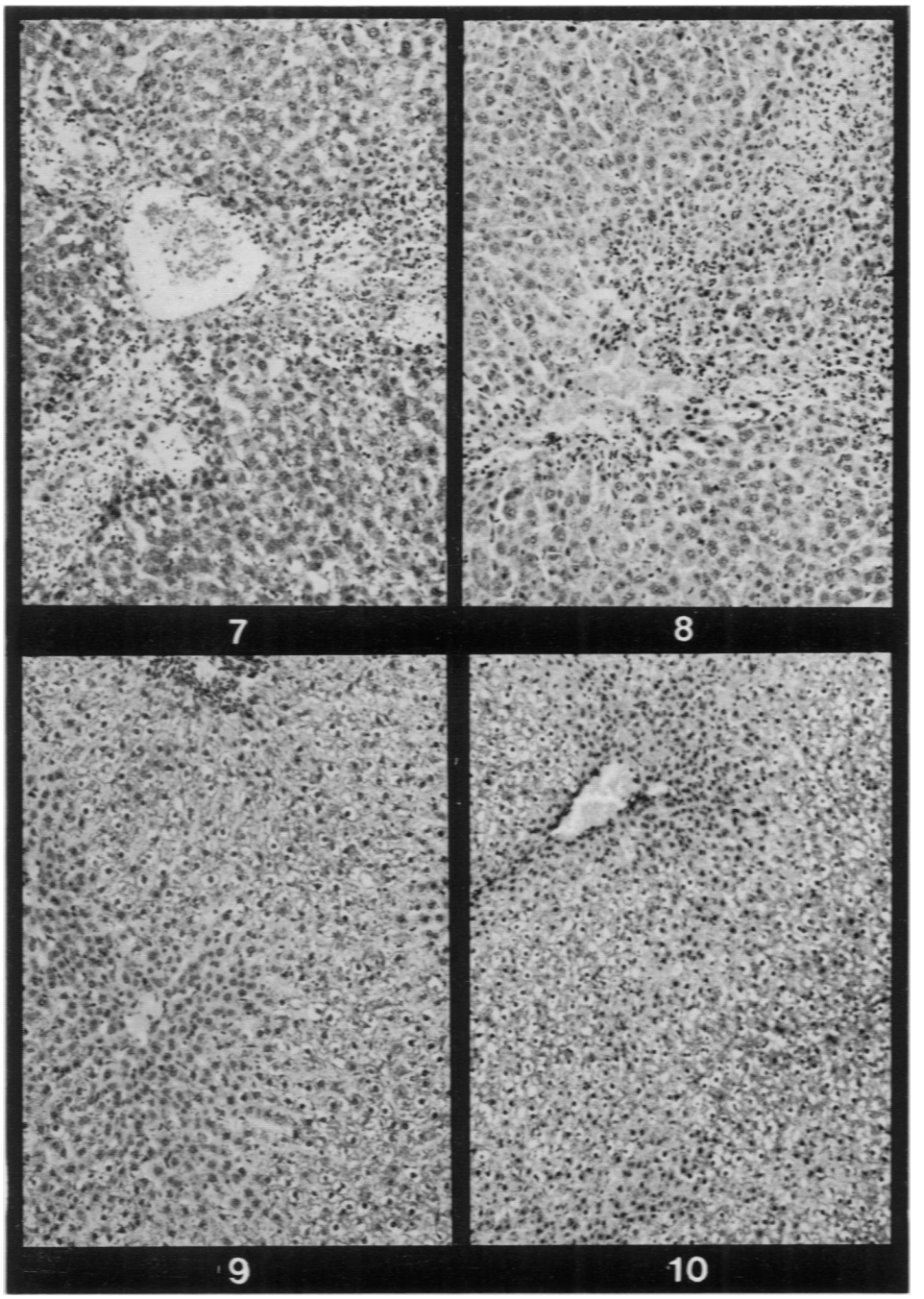
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TABLE I.—*Extent of Necrosis and Cytological Change in Different Lobes of Rat Liver after a Single Dose of Carbon Tetrachloride*

Dose of CCl_4 ml/kg	Route*		Right lobe	Right median lobe	Left median lobe	Left lobe
2·5	i.g.	Cytological change	0·9	0·9	0·9	0·9
		Necrosis	0·7 ±0·04 (4)†	0·8 ±0·13 (4)	0·58±0·05 (4)	0·6 ±0·11 (4)
0·6	i.p.	Cytological change	0·85±0·11	0·75±0·07	0·6 ±0·13	0·61±0·07
		Necrosis	0·15 (2)†	0·1 (2)	Nil	0·°5 (1)
0·15	i.g.	Cytological change	0·7 ±0·12	0·6 ±0·12	0·4 ±0·03	0·5 ±0·08
		Necrosis	Nil	Nil	Nil	Nil
0·04	i.p.	Cytological change	0·5 ±0·09	0·5 ±0·07	0·4 ±0·10	0·3 ±0·07
		Necrosis	Nil	Nil	Nil	Nil

* Four rats in each group.
† Number in parentheses is the number of animals with necrosis.
The figures represent the mean radial involvement of the liver lobules.

animals, this occurs more frequently in the right lobes. The zone of dispersal of the basophilic bodies is also greater in the right lobes, even with a dose as small as 0·04 ml/kg. Similar differences were found after intraperitoneal or intragastric administration. No recognizable difference was noted between the 2 right lobes or between the 2 left lobes.

Histology of lesions after DMN

The liver lesions produced in these rats by DMN (Pound and Lawson, 1974) conform to those described elsewhere (Barnes and Magee, 1954). With a dose of

TABLE II.—*Demethylation of Dimethylnitrosamine by Different Lobes of Rat Liver after a Single I.G. Dose of Carbon Tetrachloride*

nmol HCHO/30 min/mg microsomal protein

Time after dosing (h)	Nil	Dose CCl_4 0·01 ml/kg			0·6 ml/kg		
	Control†	3*	24†	144†	3*	24†	144†
Right lateral lobe	9·1±0·3	6·4	6·6±0·5	11·3±1·7	5·7	5·7±0·9	
Right median lobe	6·5±0·3	6·8	7·4±2·1	6·9±0·4	4·0	3·9±0·5	6·7±0·2
Left median lobe	11·0±0	7·7	7·3±0·8	12·3±0·2	6·3	4·8±0·9	12·2±0·1
Left lateral lobe	13·6±0·2	7·4	5·0±1·9	12·2±0·2	7·4	5·0±2·1	12·2±1·7

* Two rats in each group.
† Four rats in each group.

20 mg/kg, the centrilobular zone of necrosis is greater in the 2 left lobes than in the 2 right lobes (Fig. 7, 8).

With doses of 10 mg/kg or less, which do not produce significant necrosis, the zone involved in the cytological changes of dispersal of the basophilic bodies and the curious eosinophilic staining of the cytoplasm that occurs after DMN poisoning is also greater in the left lobes (Fig. 9, 10). The route of administration was not important.

DMN-demethylase

The DMN-demethylase levels for the different lobes of untreated rats and for animals 3, 24 and 144 h after dosing with CCl_4, Table II, show that the activity of this enzyme is greater in the left and left median lobes than in the right and right median lobes of untreated animals. The levels of activity are reduced after dosing with CCl_4 but return to normal after 144 h. The reduction is greater with the dose 0·6 ml/kg than with 0·04 ml/kg and is probably proportionately greater in the case of the left lobes.

DISCUSSION

There are significant differences in the effects of CCl_4 and DMN between the right and left lobes of rat liver. It is unlikely that this is a consequence of absorption from a particular part of the intestine and preferential carriage to the liver lobes of one side due to the differences in distribution of venous drainage from the gut (Brauer, 1963) as similar differences were found after intragastric and intraperitoneal administration. Since the toxic effects of DMN are mediated through metabolic demethylation to an active intermediate by microsomal enzymes (Brouwers and Emmelot, 1960; McLean and McLean, 1969; Emmelot and Benedetti, 1960; Pound and Lawson, 1974), a reasonable explanation of the greater effect of this toxin in the left lobes may be found in the greater levels of DMN-demethylase in microsomal preparations from these lobes of the liver. This might be valid even if DMN were preferentially carried to the right lobes because of the vascular drainage of the gut.

However, whereas the left lobes were more susceptible to DMN, the right lobes showed the more extensive lesions due to CCl_4. The hepatotoxic activity of CCl_4 also appears to be mediated by metabolic change to an active intermediate by processes involving microsomal enzymes (Slater, 1966, 1972; Judah, McLean and McLean, 1970). CCl_4 reacts with cytochrome P450 extremely rapidly, with consequent rapid inactivation of the cytochrome *in vivo* (Recknagel and Glende, 1973) and *in vitro* (Uehleke, Hellmer and Tabarelli, 1973). Inactivation of many drug metabolizing enzymes follows. Both such inactivations may influence the extent of the lesion differently in the different lobes. It is also possible that the active site within the microsomal system involved in CCl_4 activation does not have the same distribution as the DMN-demethylase even though both involve the cytochrome P450.

The levels of microsomal enzyme activity (as measured by DMN-demethylase) in the lobes appear to follow the extent of the basophilic granulation and cytoplasmic basophilia which, as described earlier (Deane, 1946), appears to be more prominent in the central zones of the liver lobules. Electron microscopy shows that this is due to variations in the distribution of ergastoplasmic membranes

(Loud, 1968) with which the microsomal enzymes are associated. The mixed function oxidase systems incorporating the P450 cytochromes, however, appear to be associated, not with the rough, but rather with the smooth membranes which appear to be more evenly distributed (Loud, 1968). Evidence of cytological damage in the form of dispersal of the basophilic bodies occurs very rapidly after both CCl_4 and DMN (Oberling and Rouiller, 1956; Reynolds, 1963; Calafat, den Engelse and Emmelot, 1970). Interpretation of the changes in terms of the intracellular distribution of cytological structures and associated enzymes therefore would need adequate histochemical study.

The existence of such differences must impress caution in the interpretation of a variety of experimental results. It must not be assumed that the differences are in the same direction at all times, or that they are part of the differentiation pattern of the animal. Indeed, they are likely to be conditioned by the environment. The activity of liver toxins and microsomal enzymes is greatly affected by diet (McLean and McLean, 1969) and a wide variety of drugs that either enhance or depress activity or are known to cause changes in distribution of the ergoplasmic membranes. Such drugs may be absorbed from different parts of the gut and carried to different lobes of the liver (Brauer, 1963) to condition the behaviour of the liver, or the animal, to another and possibly hepatotoxic drug.

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