The screening test and part of the metronidazole work was carried out on the fresh homogenate (i.e. within 4 hr of its removal from the body) and the rest of the work was carried out on the homogenate after it had been stored at −20° for 11 days, by which time it had lost 63 per cent of its original activity.

The results obtained with metronidazole are as follows:

pH	Concentration of metronidazole	Percentage inhibition	
		Fresh homogenate	Stored homogenate
8·8	$4{\cdot}8 \times 10^{-3}$ M	20·5	39·1
	$5{\cdot}4 \times 10^{-3}$ M	—	48·8
	$6{\cdot}4 \times 10^{-3}$ M	—	64·3
11·0	$4{\cdot}8 \times 10^{-3}$ M	49·7	—

From this data it is computed that the concentration of metronidazole producing 50 per cent inhibition of the stored, crude preparation of atypical human alcohol dehydrogenase at pH 8·8 is $5{\cdot}5 \times 10^{-3}$ M and the concentration of metronidazole producing 50 per cent inhibition of the fresh crude preparation at pH 11·0 is very nearly $4{\cdot}8 \times 10^{-3}$ M.

The figure we reported[2] for the concentration of metronidazole producing 50 per cent inhibition of a fresh, crude preparation of typical human liver alcohol dehydrogenase at pH 11·0 was $6{\cdot}6 \times 10^{-3}$ M.

We conclude that the inhibitory effect of metronidazole on crude preparations of atypical and typical human liver alcohol dehydrogenase is of the same order.

Acknowledgements—We wish to thank the Wellcome Trust for the gift of the spectrophotometer, and May and Baker Ltd. for the metronidazole.

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Biochemical Pharmacology, Vol. 16, pp. 2027–2030. Pergamon Press Ltd. 1967. Printed in Great Britain

The effect of *N*-hydroxyurethane upon the rapidly labelled RNA of the mouse

(*Received* 1 *May* 1967; *accepted* 14 *June* 1967)

COUNTER-current distribution (CCD) analysis has been used to detect possible precancerous changes in the rapidly labelled RNA isolated in association with DNA from the liver of rats given hepatocarcinogens in their diet[1, 2] and it was considered of interest to compare the early responses of two tissues with different susceptibility to a carcinogen. Urethane was considered a suitable carcinogen

for investigation since although it readily induced lung adenomas when given to adult mice hepatomas were rarely caused unless infant mice were injected.[3] It was decided to use *N*-hydroxyurethane in these experiments since Boyland and Nery[4] had suggested that this metabolite was a more proximal carcinogen than urethane. This view has been challenged[5] but the carcinogenic action of *N*-hydroxyurethane was attributed to its rapid conversion back into urethane when injected into mice.

The pooled livers and pooled lungs of forty 6–8 week old Chester Beatty A strain (CBA) mice (weight 20–25 g) given 20 per cent protein rat cake and water *ad libitum* were used for each experiment. Male mice were normally used but the occasional use of females showed no sex difference in the CCD patterns. CBA mice have a low spontaneous incidence of pulmonary adenomas when under one year old but numerous adenomas were found in the lungs seven months after the first administration of urethane to 8–12 week old mice.[6] No hepatomas were observed.

The labelling and isolation of the rapidly labelled RNA-DNA from the liver and lungs of the mice and its subsequent CCD analysis was as described by Kidson and Kirby.[1] The mice were injected i.p. with 0·1 ml of uridine-^{3}H (1 mc/ml, 500–1,000 mc/m-mole) and killed 25–30 min later; the optimal time for labelling the interfacial rapidly labelled RNA of mouse liver and lungs was found to be similar to that reported for rat liver.[1] An Ultra–Turrax blender was used to break up the fibrous lungs before extraction of the RNA–DNA.

Most of the test mice were given a single injection of *N*-hydroxyurethane (0·25 g/kg dissolved in 0·1 ml pH 7·4 0·1 M phosphate buffer) 6 or 24 hr before they were killed, the carcinogenic action of urethane on lung tissue having been found to be largely confined to the first 24 hr.[7] Some mice, however, were given an injection once a week for five weeks and killed after six weeks.

Reproducible CCD patterns were obtained from the rapidly labelled RNA–DNA (Fig. 1a) except for variation in the amount of a component running with the front of the organic phase. The small peak at the left of the pattern was due to some RNA remaining in association with the DNA. Dedgradation with crystalline ribonuclease caused the changes shown in Fig. 1b.

The effect of *N*-hydroxyurethane upon the CCD patterns of the RNA–DNA isolated from liver and lungs are compared in Figs. 1c and 1d. Six hours after injection of the carcinogen there was some suppression of the amount of labelled RNA in the RNA–DNA mixture from liver and a slight shift of the main peak in the CCD pattern towards the aqueous phase of the solvent system. Lung RNA–DNA showed a similar shift but here labelling had been enhanced. There was an improvement in the labelling of the liver RNA–DNA after 24 hr but the main peak continued its movement towards the left of the CCD pattern. In contrast CCD patterns obtained from lung RNA–DNA were now almost normal although labelling was still higher than in the controls.

The changes were not permanent. Mice given 5 weekly injections of *N*-hydroxyurethane and left a week before being killed gave RNA–DNA mixtures with CCD patterns similar to the controls although there may still be a new peak (transfer no. 10–15) remaining in the liver CCD pattern.

Histological examination showed no evidence of neoplasia or gross changes in the cell population of the liver and lungs over the duration of these experiments.

The presence of labelled material in the 18-4 *S* region of pH 5 sucrose gradients showed that the mouse rapidly labelled RNA–DNA preparations were probably more degraded than those which could be obtained from normal Chester Beatty rats. This may be due to ribonuclease activity; the 'free' and 'total' acid ribonuclease activity of normal CBA mouse liver (assayed by the method of De Duve, Pressman, Wattiaux and Appelmans[8]) was found to be twice that of these rats.

Some of the alterations induced in CCD patterns by carcinogens may be a result of changes in the degradation rather than changes in the base composition of the rapidly labelled RNA. The injection of *N*-hydroxyurethane into mice 6 or 24 hr previously gave a 3 to 5-fold increase in the 'free' acid ribonuclease activity of the liver although the 'total' activity was unchanged. Nodes and Reid[9] have found that 4′-fluoro-4-dimethylaminoazobenzene caused a marked increase in the 'free' ribonuclease activity of rat liver and this carcinogen also caused changes in the rapidly labelled RNA–DNA CCD pattern.[1]

The identification of induced changes in the composition of messenger RNA may be of great assistance in understanding the mode of action of carcinogens. Recent work[10] however has shown that present isolation and analytical techniques need refinement before they can be used for this purpose; alterations in experimental conditions which induced changes in the degradation of the mRNA during isolation and in the aggregation which occurred during analysis also gave reproducible but different results.

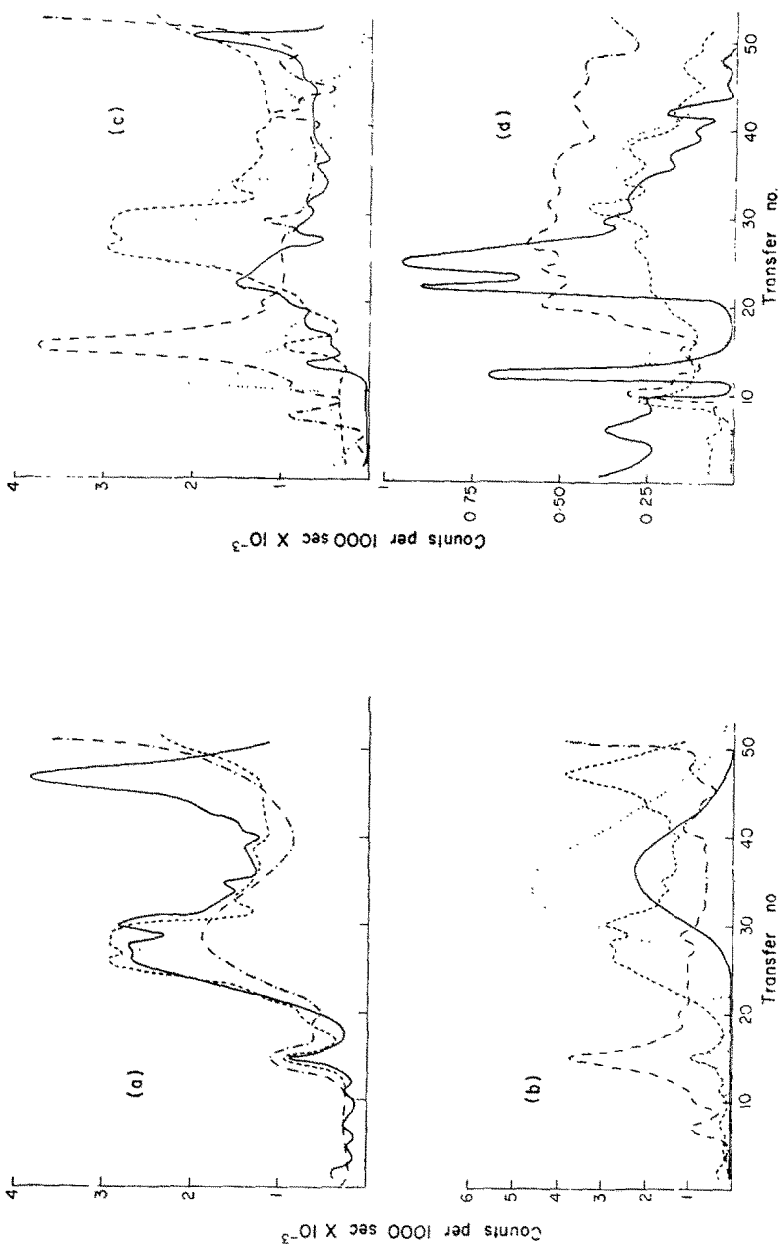

FIG. 1. Counter-current distribution patterns of the rapidly labelled RNA from CBA mice: (a) liver RNA; female, no injections ——; male, no injections, -·-·-·-; males injected with pH 7·4 buffer 24 hr previously — — —; (b) liver RNA; control mice — — —, after treatment with ribonuclease · · · ·; mice injected with *N*-hydroxy urethane 24 hr before death -·-·-, after treatment with ribonuclease ——: (c) and (d) liver and lung RNA respectively; control pattern - - - - -, mice injected with *N*-hydroxyurethane 6 hr previously ——, 24 hr previously -·-·-·-, injected once a week for five weeks and killed the sixth week · · · · ·.

Acknowledgements—I wish to thank Professor E. Boyland for his interest and encouragement, Dr. C. E. Dukes for the histological examinations, Professor K. S. Kirby and Dr. J. H. Parish for valuable advice and Mr. W. Down for his excellent technical assistance. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by the Public Health Service Research Grant No. CA-03188-09 from the National Cancer Institute, U.S. Public Health Service.

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Biochemical Pharmacology, Vol. 16, pp. 2030–2033. Pergamon Press Ltd. 1967. Printed in Great Britain

Effect of hexane and carbon tetrachloride on microsomal cytochrome (P450)

(*Received* 1 *May* 1967; *accepted* 14 *June* 1967)

THIS communication reports that carbon tetrachloride, (CCl_4) chloroform, halothane, and hexane all produce a spectral change in microsomes, similar to that produced by hexobarbital. Remmer *et al.*[1] and Imai and Sato[2] have shown that when substrates are added to microsomal preparations, difference spectra can arise. Two types of difference spectrum are found, with hexobarbital there is a decrease of optical density around 420 mμ and an increase around 390 mμ, with aniline there is an increase around 430 mμ and decrease around 390 mμ. These changes are thought to be due to interaction between the haem of cytochrome P450 and the enzyme substrate complex.

MATERIALS AND METHODS

Male Wistar rats were fed a cube diet, and given a single injection of dicophane (DDT) 100 mg/kg s.c. 1–4 weeks before use, to stimulate synthesis of microsomal hydroxylating enzymes.[3, 4] The rats were starved overnight, killed by exsanguination, and the microsome fraction prepared by the method of Kato and Gillette.[5] The microsome fraction was diluted to 4 mg protein per ml in 0·1 M KCl with 50 mM Na–PO_4 buffer, pH 7·4.

Volatile solvents were added in Warburg flasks. Three ml of the dilute microsomal suspension was placed in the main compartment of the flask which was kept cold in a tray of ice. The solvent was added to the side arm, which was kept at room temperature, the flask was closed and the tray rocked gently for 20 min. Control preparations of microsomal suspensions differed only in the absence of solvent or substrate. If the control was not diluted, stored and handled in exactly the same way as the suspension containing solvent, a spurious difference spectrum was produced.