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SPECIES AND SEX DIFFERENCES IN THE METABOLISM OF A CHLORINATED EPOXIDE BY HEPATIC MICROSOMAL ENZYMES

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Abstract—A dimethylated chlorocyclodiene epoxide (DME), proposed as a suitable substrate for the study of microsomal monooxygenases in rats, yields two major metabolites, M1 and M2, when incubated with NADPH-supplemented hepatic microsomes from adult male rats but only one when incubated with those from adult female rats. Similar microsomes from male and from female mice, pigeons and chickens produce both metabolites. The ratio of the amounts of the two metabolites formed varies with the species but no sex difference within any one of these species has been demonstrated. The rate of metabolism of DME varies both with sex and with species: hepatic microsomes from female mice metabolise it faster than do those from males whereas hepatic microsomes from male rats metabolise it faster than do those from females. Hepatic microsomes from both sexes of the domestic chicken metabolise DME slowly, converting it predominantly to metabolite M2. The use of [^{14}C]DME has established that the apparent absence of metabolite M2 from the incubates containing liver preparations from female rats is not attributable to low metabolism nor is it caused by metabolite M2 being converted to other metabolites as soon as it is formed. Little radioactivity remains behind in the aqueous phase after hexane extraction of incubation media containing [^{14}C]DME and female rat liver microsomes. Florisil column separation of the components of the hexane extracts indicates that little radioactivity is present in eluate fractions other than those showing a g.l.c. peak for DME or for metabolite M1 or for metabolite M2. Hepatic microsomes from young rats of both sexes metabolise DME at similar rates until they approach 28 days of age. Thereafter, the rate in those from male rats increases whereas that from those from female rats falls. The hepatic microsomes from male rats castrated when seven days of age metabolise DME in adulthood at the same rate as those from intact female rats. The relevance of these observations to the proposed use of DME is discussed.

It is well known that species differences exist in the rates at which many foreign compounds are metabolised both by intact animals and by liver preparations derived from them [1–3]. However, rats differ from other common laboratory animals in that NADPH-supplemented liver microsomes from adult males metabolise many xenobiotics much more rapidly than do those from livers of adult females [4,5]. Qualitative differences in primary metabolism are less common although several steroids (studied as xenobiotics rather than at physiological concentrations) are metabolised differently by liver microsomes from male and from female rats [6–8]. A cyclodiene epoxide, DME‡, related to the insecticide dieldrin, provides an example of a non-steroid xenobiotic which is apparently metabolised in qualitatively different ways by hepatic microsomes from male and from female rats [9].

NADPH-supplemented hepatic microsomes from adult female rats convert DME (Fig. 1) into only one metabolite, M1, which is present in sufficient quantity to be recognisable by g.l.c., whereas those from male rats make two metabolites, M1 and M2 [9, 10]. Young male and young female rats metabolise it in a qualitatively similar manner, much metabolite M1 and a small amount of metabolite M2 being made by animals of both sexes. At puberty, the amount of metabolite M2 made by liver microsomes from male rats rapidly increases whereas in the female rat liver preparations the amount of metabolite M2 apparently decreases to zero [11]. It has also been shown that hypophysectomised adult female rats make considerable quantities of metabolite M2 [12] and that intact females pre-treated with phenobarbital make it in large quantities [10]. Such differences as these could reflect the existence of several forms of cytochrome P-450, the types and proportions of which vary with the physiological circumstances of the animal from the neonatal period up to the time of killing.

The work described in the present communication had two objectives. The first of these was to establish how DME was metabolised by species other than the rat, both to determine the rate at which it was metabolised by different species and to find out whether metabolites M1 and M2 are produced in the same ratio as they are by male rat liver microsomes. The second objective was to establish whether metabolites M1 and M2 are themselves rapidly metabolised and thus removed from the incubation

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‡ Abbreviations used: DME, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-dimethyl-6,7-epoxy-1,4-methanonaphthalene; M1, M2, two metabolites, probably possessing the partial structures shown in Fig. 1; g.l.c., gas–liquid chromatography; and HCC, hexachlorocyclopentadiene.

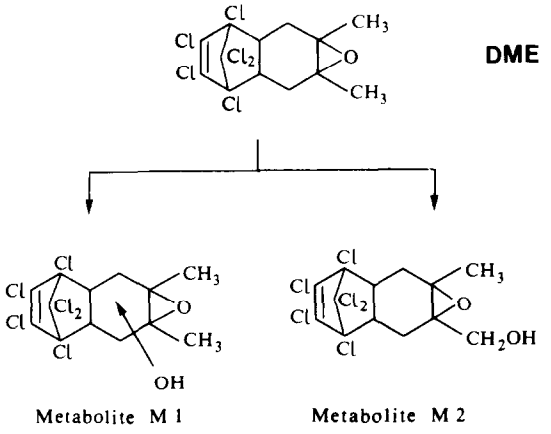

Fig. 1. Formula of 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-dimethyl-6,7-epoxy-1,4-methanonaphthalene (DME), and the tentative structures of metabolites M1 and M2 which are based on mass spectrographic evidence (see Ref. [9]).

medium. Such data are necessary to validate the earlier suggestion that DME might prove to be a useful substrate for the investigation of multiple forms of the monooxygenase system in the rat.

MATERIALS AND METHODS

Materials. Sprague–Dawley CD strain rats and Hauschka and Mirand CD strain mice were purchased from Charles River Ltd., Margate, U.K. Feral pigeons were obtained from Bioserve Ltd., Worthing, U.K. Domestic meat chickens (White Leghorn breed) were provided by Reading University farm, U.K. Animals were housed in a temperature-controlled building lit for 12 hr a day.

Technical grade *n*-hexane and acetone were purchased from BDH Ltd., Poole, U.K., and redistilled. $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were Boehringer products (Lewis, U.K.), florisil was obtained from Sigma Ltd.(London, U.K.), and the scintillation fluid was Fisons' dioxan-based Cocktail 'D'.

The route by which non-radioactive DME was synthesised was described earlier [9]. It involves an inefficient first step in which vinyl chloride combines with hexachlorocyclopentadiene (HCC). For the micro synthesis of [^{14}C]DME this step was modified as follows. A solution in carbon tetrachloride of 800 μmole of uniformly ^{14}C-labelled HCC, sp. act. 2 μCi/μmole, was transferred to a 500 cm^3 Quickfit flask. After removing the solvent by means of a stream of nitrogen, 300 mg (1.1 mmole) of unlabelled HCC was added. The total HCC present requires 120 mg (1.9 mmoles) of vinyl chloride to convert it to heptachloronorbornene. The flask was fitted with a glass tap, wired into position, and the nitrogen was displaced by vinyl chloride. With the tap open, the flask was heated in an oven until the temperature reached 130°. The tap was then closed and the flask rotated to spread the oily HCC around the sides. The oven was maintained at 175° for 30 hr, the flask being rotated every 30 min. Without purification, the product was added to unlabelled heptachloronorbornene, and thereafter the synthesis was carried out as previously described. The overall efficiency of the four-step synthesis of [^{14}C]DME was 17%. After recrystallisation from methanol an *n*-hexane solution was put on a florisil column and DME eluted with 5% diethyl ether in *n*-hexane. The specific activity of the final product was 0.15 μCi/μmole.

Methods. Livers were sliced, washed with ice-cold 0.15 M KCl and homogenised using 2 cm^3 of 0.15 M KCl/g of liver. The supernatant obtained by centrifuging at 12,000 *g* for 30 min was centrifuged at 105,000 *g* for 60 min. The pellet was re-suspended in 0.15 M KCl, re-centrifuged at 105,000 *g* for 30 min and finally re-suspended in 0.15 M KCl. All operations were carried out at 4°. A quantity of this suspension, containing for different purposes between 2 and 13 mg protein, was added to a mixture containing 2.0 cm^3 of 0.14 M NaH_2PO_4/0.1 M NaOH buffer, pH 7.2, and 0.8 cm^3 of 0.15 M KCl in glass-stoppered 25 cm^3 flasks. Where applicable, microsomal suspensions were supplemented by adding an NADPH-generating mixture comprising 6.3×10^{-4}M $NADP^+$, 3×10^{-3}M glucose-6-phosphate and 1.6 units of glucose-6-phosphate dehydrogenase (EC 1.1.1.49). The DME was added as 10 μl of an ethanolic solution containing approximately 3 μg/μl.

Incubations varied in length from 5 to 30 min, the flasks being placed in a metabolic shaker at 37° and shaken at 100 strokes/min. After stopping the reaction by adding 2 cm^3 of acetone, unchanged reactant and reaction products soluble in *n*-hexane were twice partitioned into *n*-hexane and the combined volumes of the extracts adjusted to 10 cm^3. Five microlitre portions were examined by injection into a g.l.c. fitted with a 1 metre SE52 column heated at 185° and with a ^{63}Ni source electron capture detector [9].

Radioactivity was measured using 0.5 cm^3 portions of *n*-hexane extract or of residual aqueous phase. These portions were added to 7.5 cm^3 of dioxan-based scintillation fluid, the counting being done in a Nuclear Enterprises 8310 scintillation counter. Efficiency of counting was determined using an external standard of [^{14}C]hexadecane.

Residual substrate and *n*-hexane-extractable metabolites were separated by evaporating the extract to 0.5 cm^3 and then adding it to a florisil column already containing *n*-hexane. Elution was effected by using increasing amounts of ether in *n*-hexane. Elution characteristics of the three main components have been provided elsewhere [9].

The protein present in microsomal suspensions was determined by the method of Lowry *et al.* [13].

RESULTS

A major objective of this work was to establish the extent to which rat liver preparations metabolise DME to products other than the primary metabolites M1 and M2. Using concentrated 12,000 *g* × 30 min supernatant preparations incubated for 30 min, the percentage of substrate metabolised was determined by g.l.c., assuming the amount extractable from the zero time controls to be the amount of DME put into all the flasks (Table 1). Also shown in the table is the distribution of radioactivity, originally present in the DME, between each *n*-hexane extract and the

Table 1. Metabolism of [^{14}C]DME by concentrated 12,000 g ×30 min supernatant fluid of livers from male and from female adult rats

Sex	Incubation time (min)	Exogenous NADPH-generating mixture	Per cent of total in n-hexane	^{14}C recovered in the residual aqueous phase	Per cent of DME metabolised in 30 min
Male	30	−	93	5	8
	30	+	79	18	58
Female	30	−	94	3	6
	30	+	92	5	33
Male	0	−	94	4	−
Female	0	−	94	3	−

Results are averages of triplicates in four experiments, two with the 12,000 g × 30 min supernatant fluid of livers from each sex of rat. The percentage of DME metabolised was determined by g.l.c., the amount present after incubation being subtracted from the average amount (approx. 29 μg) recovered from the unincubated samples. Protein concentration per incubation flask, 10–13 mg. Details of the incubation medium and extraction procedure appear in the Methods section.

residual aqueous phase. Approximately 97% of the radioactivity originally present in the DME was recovered in the two phases, indicating that losses caused by volatilisation or adsorption seldom exceeded 3%.

It is also evident from Table 1 that, in the presence of NADPH-generating mixture, three times as much radioactivity remained in the aqueous layers derived from male rat liver preparations after extraction with two 5 cm^3 portions of n-hexane than remained in those derived from female rat liver preparations. In the absence of NADPH-generating mixture the residual radioactivity in the aqueous phase was similar to that remaining in the aqueous phases of non-incubated samples that had been inactivated with acetone before DME was added. (Additional experiments have shown that some of this residual activity persists even after six n-hexane extractions. This is presumably a consequence of sorption of substrate on to liver constituents, for DME added directly to pH 7.2 buffer is extracted with more than 99.5% efficiency after three extractions.)

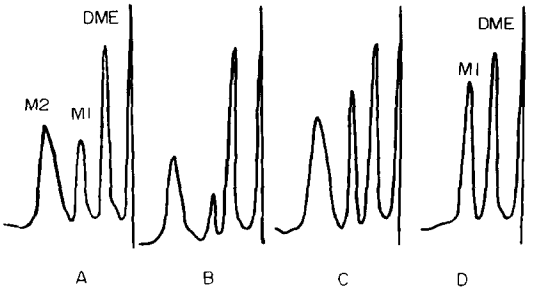

Fig. 2. Typical gas chromatograms showing the relative amounts of metabolites M1 and M2 produced by hepatic microsomes from adult male and female animals of four species. Details of the incubation medium and extraction procedure are given in the Methods section; incubation time, 10 min. A: Male and female pigeons (4 independent experiments for each sex); B: male and female chickens (3 independent experiments for each sex); C: male mice, female mice and male rats (6 mice provided livers bulked for each of two independent experiments for each sex); D: female rats (more than 200 experiments have been carried out with rats of both sexes). The relative peak areas should be compared to the metabolite ratios shown in Table 3.

In one of the experiments which provided data for DME metabolism by male rat liver for Table 1, additional flasks containing NADPH-supplemented supernates were sequentially removed to monitor for radioactivity at 5, 10, 15 and 20 min from the commencement of incubation. The percentage of radioactivity not readily extractable with n-hexane increased progressively from 4% at zero incubation time to 6% after 10 min, 10% after 15 min and 15% after 20 min. As already stated it reached an average of 18% after 30 min.

The metabolism of DME was investigated using hepatic microsomes from adult pigeons, chickens, mice and rats. In each case supplemented microsomes from male and from female animals of one species were studied simultaneously. The g.l.c. chromatograms in Fig. 2 are typical of those obtained for each species, the level of replication appearing in the legend to the figure. It is noteworthy that the difference in metabolite peak area ratios (i.e. the area of the peak representing metabolite M2 divided by that for metabolite M1) for each of the four species, as well as the lack of discernible difference in such ratios between the sexes of three of those species, are in agreement with observations based on an independent scintillation counting technique (see below and Table 3).

Both species and sex differences have been observed in the *rate* of metabolism of [^{14}C]DME by hepatic microsomes. The same four species were used, two independent experiments being done on each sex of each species. In each case male and female animals were studied simultaneously. Within experiments each treatment was done in quadruplicate. The results summarised in Table 2 show that NADPH-supplemented hepatic microsomes from male rats metabolised DME three times as fast as did those from females but that hepatic microsomes from female pigeons and female mice metabolised it significantly faster than did those from males. No sex difference in the rate of metabolism of DME by hepatic microsomes was observed for chickens.

Distribution of radioactivity amongst components of n-hexane extracts of incubation media was studied as follows. Similar n-hexane extracts (derived from quadruplicate treatments in two experiments) from the preceding experiments were bulked for each of

Table 2. Rate of metabolism of DME by NADPH-supplemented hepatic microsomes from adult pigeons, chickens, mice and rats

Species and sex	Metabolic rate ± S.E. (μg/min/mg protein)	Ratio of metabolic rates (male/female)	Level of significance of sex difference in rates	Protein present (mg)
Pigeons				
Male	0.162 ± 0.008	0.79	$P < 0.05$	4.8
Female	0.206 ±0.009			4.9
Chickens				
Male	0.096 ± 0.002	1.05	NSD*	4.7
Female	0.091 ± 0.003			5.4
Mice				
Male	0.225 ± 0.007	0.72	$P < 0.01$	3.6
Female	0.313 ± 0.005			3.9
Rats				
Male	0.328 ± 0.006	2.83	$P < 0.001$	2.0
Female	0.116 ± 0.003			3.5

Microsomal suspensions in pH 7.2 phosphate buffer and supplemented with NADPH-generating mixture were incubated for 7 min at 37°. Additional flasks were run to confirm that metabolism was linear with time for 10 min (male rats, female mice) or longer. Details of replication are given in the Results section.

* NSD, no significant difference.

Table 3. Percentages of the total *n*-hexane extractable radioactivity associated with DME and its metabolites in eluates from florisil columns

	Pigeons		Chickens		Mice		Rats	
	Male	Female	Male	Female	Male	Female	Male	Female
DME	70	61	75	68	74	66	60	80
M1	6	9	3	4	7	8	12	10
M2	12	18	13	18	11	13	18	(2)
In other fractions, or left on the column, or not accounted for	12	12	9	10	8	13	10	8
Metabolite Ratio, M2/M1	2.0	2.0	4.3	4.5	1.6	1.6	1.5	(0.2)
100 M1/M1 + M2	33	33	19	18	39	38	40	—

Microsomes were incubated for 7 min with [^{14}C]DME and components soluble in *n*-hexane were extracted as described in the Methods section. Concentrated extracts were put on to florisil columns after sub-sampling for total radioactivity. Losses of radioactivity (glass adsorption, volatility and error) did not exceed 2%. Using g.l.c. to monitor the separation, DME was eluted by *n*-hexane containing 4–10% diethyl ether, M1 by *n*-hexane containing 10–20% diethyl ether, and M2 by *n*-hexane containing 20–40% diethyl ether (compare [9]). Approximately 35–50% of the uncategorised radioactivity remained attached to the florisil after elution with 100% diethyl ether.

Table 4. The effect of castration, when 7 days of age, on the metabolism of DME by hepatic microsomes from adult male rats

Experiment	Age at sacrifice (months)	Metabolic rate μg/min/mg microsomal protein		
		Intact female	Castrated male	Intact male (apparent rates only)
1	3	0.116	0.108	0.196
2	5	0.125	0.125	0.196

Microsomal suspensions containing 6–7 mg protein per flask were supplemented with NADPH-generating mixture and incubated for 10 min under the conditions described in the Methods section. Each experiment involved only 3 animals but these were littermates. Results shown in the table are averages of closely agreeing quadruplicates. Additional flasks run for 5 and 15 min showed that metabolism proceeded linearly with time except for microsomes from intact males. Apparent metabolic rates for intact males are therefore lower than anticipated values, for which see Table 2.

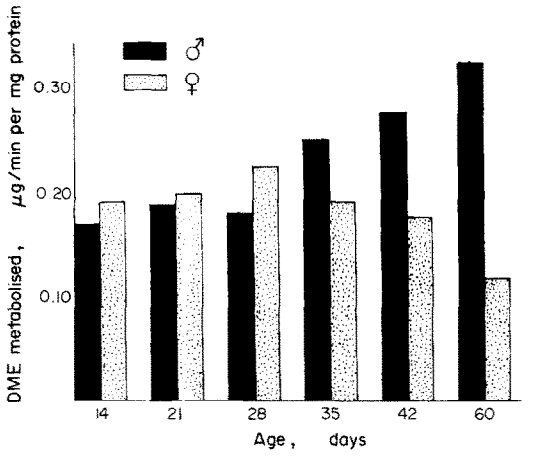

Fig. 3. The rates of metabolism of DME by hepatic microsomes from rats of different ages. Incubation was for 10 min and rate is expressed as μg DME metabolised/min/mg protein. Livers from 5 animals (14 days), 4 animals (21 days) or 3 animals (other ages) provided microsomes for each experiment, treatments within experiments being done in triplicate. Two independent experiments were done on each sex at each age. Histograms represent mean figures. The results of statistical analysis of these data are described in the Discussion section. Figures for 60 day old animals are not included in the statistics and are derived from Table 2.

the eight variables (two sexes, four species). After drying with anhydrous sodium sulphate each was evaporated to 2 cm^3. After determining the total radioactivity by removal from each of a 0.2 cm^3 sample, the remainder was evaporated to 0.5 cm^3 and each of the eight samples was added to a separate florisil column. Elution was carried out using eluting fluid containing progressively more diethyl ether in *n*-hexane, ending with 100% diethyl ether [10]. Appropriate amounts of eluting fluid to achieve maximal separation of the three known components (DME, metabolite M1 and metabolite M2) were selected by concurrent g.l.c. monitoring of small eluate fractions. Samples that were apparently almost pure with respect to each of the three components were bulked, adjusted to a suitable volume and sampled for scintillation counting. Table 3 shows the percentage of the total ^{14}C added to each of the eight columns which was recovered in each of these three elution fractions, as well as that which appeared in other fractions from which the three main metabolites were absent.

The percentage figures given in Table 3 are based on the assumption that the radioactivity in each of the three main fractions is exclusively associated with the component giving the g.l.c. peak, and not with undetected compounds. This is regarded as a valid assumption because ^{14}C labelling is confined to the chlorinated ring and this ring (which is the moiety influencing the response of the electron capture detector) almost certainly remains intact in DME metabolites of relatively low polarity. No g.l.c. peak for metabolite M2 was observed in florisil eluates of *n*-hexane extracts from adult female rat preparations and the small amount of radioactivity in the fraction eluted with 15–30% diethyl ether in *n*-hexane is probably associated with traces of metabolite M1 held back on the column. The figures in brackets in Table 3 must therefore be regarded as maximal values.

Figure 3 summarises the results for 20 experiments in which the rate of metabolism of DME was measured using hepatic microsomes from male or from female rats 14, 21, 28, 35 or 42 days *post partum*. For each sex two independent experiments were done at each age but the effects of individual variation were minimised by the fact that, because of their small sizes, livers from 3, 4 or 5 individuals were bulked to provide the homogenate from which microsomes were prepared. In these experiments the amount of protein varied from 3.5 to 4.5 mg per incubation flask and the incubation time was 10 min. To complete the series the rates of metabolism found for hepatic microsomes from adults (Table 2) are also shown in Fig. 3.

In two exploratory experiments measurements were made of the rate of metabolism of DME by liver microsomes from adult male rats which had been castrated when they were seven days of age. Similar microsomes from intact male and from intact female littermates acted as controls and the results are shown in Table 4. The rate of metabolism by hepatic microsomes from the two castrated males is almost identical to that of hepatic microsomes from female rats used as controls. On the other hand, the observed rate of metabolism by hepatic microsomes from intact male rats was on both occasions higher than either. These two experiments were primarily designed to discover whether traces of metabolite M2 were made by liver microsomes from castrated animals and concentrated microsomal suspensions were used. This has resulted in an underestimation of the true rate of metabolism in the intact male (for which see Table 2) and the recorded 40% reduction of metabolic rate in the male caused by early castration (Table 4) is in consequence a minimal figure.

DISCUSSION

A qualitative (or a very large quantitative) difference exists in the rate of metabolism of DME by NADPH-supplemented hepatic microsomes from adult male and from adult female rats [9]. It has been suggested that such easily recognised differences render this substrate a useful probe to investigate apparent variations in the monooxygenase system(s) present in livers of maturing rats [11], of adult animals [10], of male rats that have been castrated [11], of female rats that have been hypophysectomised [12], and of female rats after enzyme induction with phenobarbital [10].

Figure 2 shows that the relative amounts of the metabolites M1 and M2 produced from DME by NADPH-supplemented hepatic microsomes vary considerably from species to species, but that the qualitative sex difference observed when rat liver microsomes are used is not evident when those from three other species are employed. Marked interspecies differences do, however, exist in the relative amounts of the two metabolites produced, although no difference in the metabolite peak area ratio has been detected between males and females of any

species other than the rat. Both metabolite M1 and metabolite M2 are produced by hepatic microsomes from both sexes of rabbits and of Japanese quail (unpublished results).

Such differences as those above may reflect the existence of varying poportions of different monooxygenases in the microsomes from livers of various species of animals. Another possibility, however, is that they might be caused by the distorting effects of reactions leading to the further or secondary metabolism of metabolites M1 or M2, with the consequent establishment of different steady states. We believe for the following three reasons that neither the different proportions of metabolites M1 and M2 produced by mice, pigeons and chickens, nor the absence of metabolite M2 in the adult female rat, can be explained in such terms.

Firstly, Table 3 shows that in both sexes of the four species of animals that were studied, approximately 90% of the total radioactivity extractable with *n*-hexane is recovered in florisil eluates showing g.l.c. peaks for the three main known constituents, namely, DME, or metabolite M1 or metabolite M2. Almost all of the remainder was accounted for either in the fraction eluted with 50% diethyl ether in *n*-hexane or remained tightly bound to the florisil column. Although metabolites of DME with longer retention times than that of metabolite M2 do exist, they are most evident in rapidly metabolising systems such as those containing hepatic microsomes from the male rat and those containing hepatic microsomes from male or female rats pre-treated with phenobarbital [10]. It is therefore improbable that the absence of metabolite M2 from incubates of hepatic microsomes from the uninduced intact adult female rat is a consequence of it being rapidly converted to significant quantities of other *n*-hexane-soluble metabolites.

Secondly, the use of concentrated $12{,}000\,g \times 30$ min supernatant preparations from rat livers would be expected to exacerbate problems associated with further or secondary metabolism, yet the data in Table 1 indicate that little radioactivity from [^{14}C]DME is unextractable, using *n*-hexane, from female rat liver incubation mixtures. Considerably more remains unextracted from male rat liver preparations yet even for these the unextracted radio activity is low at short incubation times. When dilute suspensions of washed microsomes are used the contribution of further or secondary metabolism to the overall metabolic pattern is likely to be minimal.

Thirdly, it is possible that a low level of metabolism *per se* could in some way favour a steady state situation which obscured the recognition of metabolite M2 in female rats (the limits of detection for DME, metabolite M1 and metabolite M2 by g.l.c. are 10, 80 and 120 pg, respectively [11]). Comparison of the metabolic pattern for the four species (Fig. 2) with the rates at which DME disappears (Table 2) shows that this is improbable. Chicken liver microsomes from animals of both sexes metabolise DME less rapidly than do microsomes from the livers of female rats, yet the former produce readily detectable quantities of metabolite M2.

We conclude from the observations in the three preceding paragraphs that, while further metabolism of metabolites M1 and M2 probably occurs slowly in both sexes of all species studied, there is no evidence to suggest that such metabolism explains the absence of metabolite M2 in the adult female rat. It is considered more likely that the difference reflects some difference in the hepatic monooxygenase systems in male and in female rats. Partial separation of functionally distinct forms of cytochrome P-450 has been achieved by several investigators (e.g. [14, 15]) and immunochemical differences have also been reported [16]. On the other hand the possibility cannot be excluded that sex and age dependent variations in foreign compound metabolism could result from coupling and decoupling of the components of the total cytochrome P-450 system consequent upon changes in the physical state of lipids within microsomal membranes [17]. It has also been suggested that some sex differences in metabolism are associated with a lack of phosphatidylcholine.

The g.l.c. chromatograms in Fig. 2 and the data in Table 3 demonstrate that the *relative amount* of metabolites M1 and M2 produced by NADPH-supplemented hepatic microsomes varies with the species but that, except for the rat, it does not vary with sex. In contrast, the data in Table 2 show that, of the species studied, only in the case of the domestic chickens is the *rate* of metabolism of DME by hepatic microsomes from the two sexes of comparable magnitude.

Figure 3 shows the rate of metabolism of DME by liver microsomes from rats of different ages. The rates at which hepatic microsomes from males and from females metabolise this substrate are not significantly different for rats that are 14 days of age. The same is true at 21 and 28 days of age. However, those from the male metabolise it significantly faster than do those from the female at 35 days of age ($P < 0.05$) and at 42 days of age ($P < 0.001$). Statistical analysis for combined data for hepatic microsomes from juvenile males (14, 21 and 28 days of age) and similar combined data for adolescent males (35 and 42 days of age) show that those from juvenile males metabolise DME less rapidly than do those from adolescent males ($P < 0.001$). The ontogenesis of metabolism in the female is less clear but hepatic microsomes from juvenile females possibly metabolise it faster than those from adolescent females ($0.10 > P < 0.05$).

It is noteworthy that the quantitative changes in the rate of DME disappearance which occur during the period between 28 and 42 days of age (Fig. 3) parallel the qualitative changes in the pattern of DME metabolism by maturing rats of both sexes. In particular, it has been shown [11] that, during the period from about 28 to 42 days *post partum* the amount of metabolite M2 produced by hepatic microsomes from the intact male rapidly increases whereas the amount produced by those from the female decreases to vanishing point.

This parallelism between qualitative and quantitative metabolism probably reflects some aspect of the physiological changes which occur during this period. One likelihood is that hormonal influences play an important role in determining the activity, and perhaps the nature, of the monooxygenases pres-

ent in liver endoplasmic reticulum. Correlation of monooxygenase activity with past or present hormonal status is probably also illustrated by the data of Table 4. Similar quantitative effects consequent upon neonatal castration have been observed with other substrates [19 20].

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