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STUDIES OF THE MECHANISMS OF METABOLISM OF DIETHYL *p*-NITROPHENYL PHOSPHOROTHIONATE (PARATHION) BY RABBIT LIVER MICROSOMES*

BRENDA J. NORMAN, WILLIAM K. VAUGHN and ROBERT A. NEAL

Center of Environmental Toxicology, Department of Biochemistry, Vanderbilt University, Nashville, Tenn. 37232, U.S.A.

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Abstract—Based on the protein content of microsomes, the administration of 3-methylcholanthrene (3-MC) and phenobarbital (PB) to adult rabbits leads to an increased rate of metabolism of parathion (diethyl 4-nitrophenyl phosphorothionate) by rough-surfaced and whole microsomes but not by smooth-surfaced microsomes. Although prior administration of both PB and 3-MC increased the cytochrome P-450 content of the microsomes, when the rate of metabolism of parathion was calculated on the basis of the concentration of cytochrome P-450 in these microsomes, there is no difference in the rate of metabolism of parathion by rough-surfaced and smooth-surfaced microsomes from the untreated, 3-MC-treated and PB-treated animals. However, based on the cytochrome P-450 concentration, the rate of metabolism of parathion by whole microsomes from 3-MC and PB-treated animals is less than the rate with whole microsomes from untreated animals. Further studies have shown there is no correlation between the concentration of high spin or low spin cytochrome P-450 in any of the microsomal fractions or subfractions and the rate of metabolism of parathion to paraoxon or diethyl phosphorothionate.

THE CHOLINERGIC insecticide, parathion (diethyl 4-nitrophenyl phosphorothionate), is metabolized by the mixed function oxidase enzyme systems of mammalian liver to paraoxon (diethyl 4-nitrophenyl phosphate), a toxic metabolite,[1] and to diethyl phosphorothionate (DEPT), a nontoxic metabolite.[2,3] Paraoxon is metabolized further to diethyl phosphate by a number of esterases present in animal tissues.

It was shown by Neal[2] that pretreatment of rats with 3,4-benzpyrene increased the rate of formation of paraoxon from parathion to a greater extent than the rate of formation of DEPT. This differential stimulation of paraoxon formation was confirmed by Chapman and Leibman[4] using 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT), chlordane and 3-methylcholanthrene (3-MC) as inducers. These and other data[5] suggested that two mixed function oxidase enzymes are involved in the metabolism of parathion in the rat. Subsequent work in rabbits[6–8] also indicated there were two mixed function oxidase enzymes involved in the metabolism of parathion in this species.

Cytochrome P-450, the terminal enzyme of the mixed function oxidase enzyme system, is a hemoprotein. This enzyme exists in rabbit liver microsomes in two or more forms. Two of these are forms in which the iron in the heme moiety exists in a high spin and a low spin state.[9–11] A method for determining the absolute amount of

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the high spin and low spin forms of cytochrome P-450 in rabbit liver microsomes has recently been reported.[12]

The ratio of the concentrations of the high spin and low spin forms of cytochrome P-450 in microsomes isolated from rabbit liver can be altered by pretreatment of the animals with 3-MC.[10] Thus, the ratios of the concentrations of the high spin to low spin forms of cytochrome P-450 are in the order: phenobarbital (PB)-treated $\simeq$ untreated $<$ 3-MC-treated animals. It has been suggested that the high spin and low spin forms of cytochrome P-450 may account in some degree for the multiplicity of mixed function oxidase activity seen with liver microsomes.[10]

In the studies reported here, we have examined the effect of pretreatment of rabbits with PB and 3-MC on the rates of formation of paraoxon and DEPT using hepatic microsomes from the various treatment groups. Because the mixed function oxidase enzyme system catalyzing the formation of either paraoxon or DEPT may be selectively concentrated in the smooth-surfaced or rough-surfaced microsomes, the rates of formation of these two metabolites in these microsomal subfractions were also examined. In addition, we have compared the ratios of the rates of formation of paraoxon and DEPT using microsomes and subfractions of microsomes isolated from the livers of rabbits in the various treatment groups with the ratios of the concentrations of the high spin and low spin forms of cytochrome P-450 in these same microsomes and subfractions of microsomes. The rationale for this latter comparison was that perhaps the high spin or low spin cytochrome P-450 selectively catalyzed the metabolism of parathion to paraoxon or DEPT.

METHODS

New Zealand male rabbits weighing approx. 3 kg were used throughout these studies. The animals were divided into three groups of three animals. The first group received a dosage of 50 mg/kg/day of sodium phenobarbital, i.p., for 5 days. The last injection was made approx. 18 hr before sacrifice. 3-Methylcholanthrene was administered to the second group as a single dose of 20 mg/kg in corn oil 3 days before sacrifice. The last group of rabbits received no treatment and served as controls.

Preparation of microsomes. All animals were sacrificed by cervical dislocation. The livers were removed immediately, perfused with cold 1·15% KCl and weighed. Equal portions of the central lobe of the liver of each of the three rabbits in a group were pooled and homogenized in either 3 vol. of 0·88 M sucrose or in 3 vol. of 0·05 M phosphate buffer (pH 7·6). The homogenization involved first chopping the tissue in a Waring blender (10 sec at 19,500 rev/min) and further homogenizing in a motor-driven Teflon–glass homogenizer (eight strokes).

Rough-surfaced and smooth-surfaced microsomes were then separated from the tissue homogenized in 0·88 M sucrose by the method of Rothschild.[13] Each pellet of rough-surfaced and smooth-surfaced microsomes, equivalent to 1 g of liver, was resuspended in 1 ml of 0·05 M phosphate buffer (pH 7·6).

Whole microsomes were obtained from the homogenate of the livers in 0·05 M phosphate buffer by centrifuging the 23,000 *g* supernatant of this homogenate at 110,000 *g* for 1 hr. Whole microsomes equivalent to 1 g were also resuspended in 1 ml of 0·05 M phosphate buffer (pH 7·6).

Assays. Cytochrome P-450 was measured by a modification of the method of Omura and Sato.[14] The microsomes were suspended in 0·1 M phosphate buffer,

pH 7·0, at a concentration of 2·0 mg/ml. A few milligrams of dithionite was added to both the reference and sample cuvettes and the base line recorded. Carbon monoxide was then bubbled through the sample cuvette for 2 min and the CO–cytochrome P-450 spectra recorded. All operations were carried out at room temperature. The concentration of high spin and low spin cytochrome P-450 in the various microsomal fractions was measured by the method of Jefcoate *et al.*[12] using a Cary 15 spectrophotometer equipped with a high intensity light source. Protein was measured by the biuret method,[15] modified to include 0·1 ml of 1% sodium deoxycholate in each sample.

Incubation procedures. The incubations were done in triplicate for each microsomal preparation as follows: phosphate buffer (pH 8·0), 100 μmoles; NADP, 1·3 μmoles; glucose 6-phosphate, 5·6 μmoles; glucose 6-phosphate dehydrogenase, 0·5 unit; ^{32}P-parathion, 0·68 μmole (added as a 20 mM solution in ethanol); and microsomes equivalent to 500 mg of liver. In the case of smooth-surfaced microsomes isolated from phenobarbital-treated animals and whole microsomes from all treatment groups, the equivalent of 300 mg of liver was used. Distilled water was added to all incubations to a final volume of 2·0 ml. The mixture was incubated at 37° at a shaking rate of 200 rev/min in a gyroaction shaker. The incubations were carried out in 30-ml beakers containing a glass marble. Incubation time was 20 min, in an atmosphere enriched with oxygen. The rates of formation of paraoxon and diethyl phosphorothionate were determined as described previously.[2]

Materials. ^{32}P-parathion was synthesized as described by Neal.[2] ^{32}P-disodium phosphate was purchased from the New England Nuclear Corp.

Glucose 6-phosphate, NADP and glucose 6-phosphate dehydrogenase were purchased from the Boehringer, Mannheim Corp. The *n*-octylamine was obtained from Aldrich Chemicals.

Statistics. All metabolism experiments were done in triplicate and the mean and the standard deviation of the mean were calculated. The levels of significance between treatment groups and between microsomal fractions of the same treatment group were determined using the Duncan multiple range test.[16]

RESULTS AND DISCUSSION

Rough-surfaced, smooth-surfaced and unfractionated (whole) microsomes isolated from the livers of untreated, PB-treated and 3-MC-treated animals were incubated with ^{32}P-parathion, and the rates of formation of paraoxon and DEPT were determined. The results (Table 1) indicate a greater concentration of the mixed function oxidase activity toward parathion in the smooth-surfaced as compared to the rough-surfaced microsomal fraction of untreated animals. However, microsomes from PB- and 3-MC-treated animals are about equal in activity toward parathion in these two microsomal subfractions. Induction by PB or 3-MC significantly increased the rate of metabolism of parathion to both paraoxon and DEPT by the rough-surfaced microsomal subfraction. However, no significant induction by either PB or 3-MC of the metabolism of parathion to either metabolite was seen using the smooth-surfaced microsomal subfraction. Using whole microsomes, both PB and 3-MC significantly induced the metabolism of parathion to paraoxon but not to DEPT as compared to untreated animals.

Some caution needs to be exercised in comparing the enzyme activity in the rough-surfaced microsomal subfraction from 3-MC-treated animals with the same subfraction

Table 1. Mixed function oxidase activity toward parathion in rough-surfaced, smooth-surfaced and whole microsomes from livers of untreated, phenobarbital-treated and 3-methylcholanthrene-treated adult male New Zealand rabbits*

Treatment	Enzyme source					
	Rough microsomes		Smooth microsomes		Whole microsomes	
	Paraoxon (nmoles formed/20 min/mg protein)	DEPT (nmoles formed/20 min/mg protein)	Paraoxon (nmoles formed/20 min/mg protein)	DEPT (nmoles formed/20 min/mg protein)	Paraoxon (nmoles formed/20 min/mg protein)	DEPT (nmoles formed/20 min/mg protein)
Untreated	3·57 ± 1·34	1·72 ± 0·25	12·33 ± 2·89	8·07 ± 1·76	15·63 ± 1·67	9·45 ± 1·81
Phenobarbital	12·33 ± 2·70†	7·29 ± 1·78†	16·60 ± 5·63	9·97 ± 4·21	28·92 ± 2·40†	12·02 ± 1·82
3-Methylcholanthrene	17·09 ± 5·20†	6·93 ± 3·63†	13·36 ± 6·55	7·39 ± 5·34	27·25 ± 2·47†	11·45 ± 3·57

* These values are the mean ± S.E.M. of three separate experiments in the case of the untreated and 3-MC-treated animals and two experiments using the PB-treated animals. In each experiment the microsomes were isolated from the pooled livers of three rabbits. The incubations were done in triplicate in each experiment, and the mean values ± S.E.M. for the rates of formation of each metabolite shown in Table 1 were calculated from the nine estimates of the rates in the case of untreated and 3-MC-treated animals and six estimates in the case of PB-treated animals.

† These rates are significantly different ($P < 0·05$) from the rate of formation of the same metabolite using the equivalent microsomal fraction or subfraction from the untreated group. The statistical significance was examined using the Duncan multiple range test.[16]

from untreated and PB-treated rabbits. Electron microscope studies by Gram *et al.*[17] have shown that the rough-surfaced microsomal subfraction from 3-MC-treated rabbits isolated by the method of Dallner[18] is contaminated with smooth-surfaced microsomes to a greater extent than the same subfraction isolated from untreated and PB-treated animals. Whether the same degree of contamination is seen in the rough-surfaced microsomal subfraction isolated by the method of Rothschild[13] has not been determined.

The specific activity of the whole microsomes is considerably higher than the specific activity of the smooth-surfaced or rough-surfaced subfractions. The incubation of parathion with whole microsomes usually commenced about 4 hr after sacrificing the animals, whereas the incubations with the smooth-surfaced and rough-surfaced microsomal subfractions usually began about 15 hr after sacrifice. To examine if a time-dependent loss of activity during the extended period of time required to isolate the microsomal subfractions were responsible for the decreased activity, whole microsomes were isolated from the pooled livers of three 3-MC-treated rabbits using conditions similar to those used to isolate the microsomal subfractions, i.e. a 10-hr centrifugation at 110,000 *g* in 0·14 M sucrose. The incubations using the whole, rough-surfaced and smooth-surfaced microsomes were done at the same time, and the rates of metabolism of parathion to paraoxon and DEPT by the whole microsomes were compared with the rates of formation of these same metabolites using the rough-surfaced and smooth-surfaced microsomal subfractions isolated from the same pooled sample of livers. The rate of metabolism of parathion by a combined sample of rough-surfaced and smooth-surfaced microsomal subfractions was also examined. These data are shown in Table 2. The activities in the rough-surfaced and smooth-surfaced microsomal subfractions fell in the lower range of the values shown in Table

TABLE 2. COMPARISON OF THE MIXED FUNCTION OXIDASE ACTIVITY TOWARD PARATHION IN ROUGH-SURFACED, SMOOTH-SURFACED, RECOMBINED ROUGH-SURFACED AND SMOOTH-SURFACED AND WHOLE MICROSOMES FROM POOLED LIVERS OF THREE 3-METHYLCHOLANTHRENE-TREATED ADULT MALE NEW ZEALAND RABBITS

	(nmoles/20 min)			
	Rough	Smooth	Rough + smooth	Whole
	A. Paraoxon formation			
Per mg microsomal protein*	10·26 ± 0·77	9·20 ± 1·21	9·94 ± 0·30	18·68 ± 1·36
Per nmole P-450†	5·82	5·94	5·93	11·73
	B. Diethyl phosphorothionate formation			
Per mg microsomal protein*	2·29 ± 0·21	3·47 ± 0·79	2·94 ± 0·40	7·16 ± 1·39
Per nmole P-450†	1·30	2·24	1·75	4·49

* These values are the means ± S.E.M. of three determinations using microsomes and microsomal subfractions isolated from the same pooled samples of three rabbit livers. The rough-surfaced and smooth-surfaced microsomes were isolated by the method of Rothschild.[13] The whole microsomes were isolated by centrifuging the 23,000 *g* supernatant from a homogenate of the livers in 0·14 M sucrose for 10 hr at 110,000 *g*.

† These values are the nanomoles of metabolite formed per milligram of protein in a given sample of microsomes divided by the nanomoles of cytochrome P-450 per milligram of protein in the same sample.

1 for 3-MC-treated animals. However, the specific activity of the whole microsomes especially the rate of formation of paraoxon, was considerably less than the mean value for whole microsomes shown in Table 1. Whether this decreased rate was due to inactivation of the mixed function oxidase enzyme system during isolation or to animal variation cannot be determined by this experiment. However, it is clear there was a considerable loss of enzyme activity on the basis of both protein and cytochrome P-450 during the process of subfractionation. This loss in activity is not the result of the loss of some factor in the smooth-surfaced or rough-surfaced microsomal subfractions which stimulates the activity in the other, because the combined rough-surfaced and smooth-surfaced microsomes have about the average of the activity seen in the rough-surfaced and smooth-surfaced subfractions incubated separately.

The concentrations of cytochrome P-450 in the various microsomal fractions are shown in Table 3. Gram *et al.*[19] have reported the ratio of cytochrome P-450 in

TABLE 3. CYTOCHROME P-450 CONCENTRATIONS IN ROUGH-SURFACED, SMOOTH-SURFACED AND WHOLE MICROSOMES FROM POOLED LIVERS OF UNTREATED, PHENOBARBITAL-TREATED AND 3-METHYLCHOLANTHRENE-TREATED ADULT MALE NEW ZEALAND RABBITS*

Treatment	Enzyme source		
	Rough (nmoles/mg protein)	Smooth (nmoles/mg protein)	Whole (nmoles/mg protein)
Untreated	0·51 ± 0·08	1·49 ± 0·22	1·16 ± 0·09
Phenobarbital	1·29 ± 0·22†	2·56 ± 0·68†	2·67 ± 0·03†
3-Methylcholanthrene	1·72 ± 0·68†	1·72 ± 0·34	1·98 ± 0·28†

* These values are the means ± S.E.M. of the cytochrome P-450 concentrations in the microsomes isolated from three separate pooled samples of three rabbit livers in the case of untreated and 3-MC-treated animals and two separate pooled samples of three rabbit livers in the case of PB-treated animals.

† These concentrations are statistically different ($P < 0.05$) from the concentrations in equivalent microsomal fractions or subfractions isolated from the untreated animals. The cytochrome P-450 concentration in whole microsomes from 3-MC-treated animals is also significantly different than the concentration in whole microsomes from PB-treated animals.

smooth-surfaced and rough-surfaced microsomes isolated from the livers of untreated Dutch rabbits by the method of Rothschild[13] to be 24·8 : 1 and by the method of Dallner[18] to be 2·3 : 1. In our hands, the method of Rothschild[13] gave a smooth to rough ratio of 2·9 : 1. The reason for this marked difference in the results of the two laboratories concerning the ratios of cytochrome P-450 in these two subfractions using the method of Rothschild[13] is not known.

Comparison of the cytochrome P-450 content in each microsomal fraction and subfraction among the various treatment groups indicates significant differences in the cytochrome P-450 content of rough-surfaced microsomes from 3-MC and PB-treated as compared to untreated animals. For the smooth-surfaced microsomes, the PB-treated animals had a significantly higher mean concentration of cytochrome P-450 than did the untreated or 3-MC-treated groups, and for the whole microsomes every pairwise comparison was statistically significant (i.e. 3-MC-treated animals had a higher mean concentration than untreated, and PB-treated animals had a higher mean concentration than 3-MC-treated animals).

TABLE 4. COMPARISON OF THE RATE OF METABOLISM OF PARATHION TO PARAOXON AND TO DEPT EXPRESSED ON THE BASIS OF THE CYTOCHROME P-450 CONCENTRATIONS IN ROUGH-SURFACED, SMOOTH-SURFACED AND WHOLE MICROSOMES FROM POOLED LIVERS OF UNTREATED, PHENOBARBITAL-TREATED AND 3-METHYLCHOLANTHRENE-TREATED ADULT MALE NEW ZEALAND RABBITS*

	Enzyme source					
	Rough microsomes		Smooth microsomes		Whole microsomes	
Treatment	Paraoxon (nmoles formed/20 min/nmole P-450)	DEPT (nmoles formed/20 min/nmole P-450)	Paraoxon (nmoles formed/20 min/nmole P-450)	DEPT (nmoles formed/20 min/nmole P-450)	Paraoxon (nmoles formed/20 min/nmole P-450)	DEPT (nmoles formed/20 min/nmole P-450)
Untreated	6·90 ± 2·09	3·41 ± 0·44	8·49 ± 2·60	5·57 ± 1·75	13·48 ± 1·58	8·11 ± 1·28
Phenobarbital	9·46 ± 0·88	5·58 ± 0·72	7·13 ± 3·67	4·34 ± 2·54	10·83 ± 0·97†	4·50 ± 0·66†
3-Methylcholanthrene	10·62 ± 4·55	4·26 ± 2·40	7·49 ± 2·71	4·04 ± 2·43	13·80 ± 0·80	5·68 ± 1·20†

* These values are the means ± S.E.M. of three separate experiments in the case of the untreated and 3-MC-treated animals and two experiments using the PB-treated animals. In each experiment the microsomes were isolated from the pooled livers of three rabbits. The incubations were done in triplicate in each experiment and the rates of formation of each metabolite were calculated on the basis of the protein content of the microsomes or microsomal subfractions used in the incubation. After this the rate of formation of each metabolite per milligram of protein was divided by the nanomoles of cytochrome P-450 per milligram of protein in that sample of microsomes. From these data the means ± S.E.M. of the rates of formation of each metabolite were calculated.

† See Table 1.

Table 4 shows the rate of formation of paraoxon and DEPT using the various microsomal fractions and subfractions expressed on the basis of the cytochrome P-450 content of these microsomal fractions and subfractions. A statistical comparison of the rates of formation of each metabolite between treatment groups but using the same type of microsomal fraction reveals only a few differences. These are that the rate of formation of paraoxon per nanomole of cytochrome P-450 is less for whole microsomes from PB-treated animals than untreated or 3-MC-treated animals, and the rate of formation of DEPT using whole microsomes from PB- and 3-MC-treated animals is less than the rate using whole microsomes from untreated animals. These data suggest that whole microsomes from PB-treated animals, and in the case of DEPT formation, whole microsomes from 3-MC-treated animals contain additional cytochrome P-450 which is not metabolically active toward parathion. The reason for this may be that some of the cytochrome P-450 induced by pretreatment with PB and 3-MC is not combined with the other required enzymes into an active mixed function oxidase system.

Because the mixed function oxidase enzyme system catalyzing the formation of either paraoxon or DEPT may be selectively concentrated in the smooth-surfaced or rough-surfaced microsomal subfraction, the ratios of the rates of formation of paraoxon and DEPT in these subfractions and in whole microsomes from the various treatment groups were statistically compared using the method of Duncan.[16] There were no significant differences in the ratios between rough-surfaced, smooth-surfaced and whole microsomes within any treatment group (Table 5). This suggests that the

TABLE 5. RATIOS OF THE RATES OF FORMATION OF PARAOXON AND DIETHYL PHOSPHOROTHIONATE (DEPT) CATALYZED BY THE VARIOUS MICROSOMAL FRACTIONS AND SUBFRACTIONS OF THE LIVERS OF UNTREATED, PHENOBARBITAL-TREATED AND 3-METHYLCHOLANTHRENE-TREATED ADULT MALE NEW ZEALAND RABBITS*

	Microsomal fractions		
	Rough	Smooth	Whole
Treatment	Paraoxon / DEPT	Paraoxon / DEPT	Paraoxon / DEPT
Untreated	2·05 ± 0·65	1·53 ± 0·19	1·73 ± 0·30
Phenobarbital	1·71 ± 0·17	1·73 ± 0·21	2·52 ± 0·43
3-Methylcholanthrene	2·99 ± 1·13	2·10 ± 0·54	2·54 ± 0·65

* The rate of formation of paraoxon per milligram of microsomal protein in each incubation described in Table 1 was divided by the rate of formation of DEPT in the same incubation. From these data the means ± S.E.M. were calculated.

enzyme system catalyzing the formation of either DEPT or paraoxon is not selectively concentrated in any particular microsomal subfraction.

When the paraoxon to DEPT ratios in any microsomal fraction or subfraction between treatment groups are compared, there are suggestive increases in the ratio in the smooth-surfaced and rough-surfaced microsomal subfractions of 3-MC-treated

TABLE 6. COMPARISON OF THE RATIO OF THE RATE OF FORMATION OF PARAOXON AND DIETHYL PHOSPHOROTHIONATE (DEPT) WITH THE RATIO OF THE CONCENTRATIONS OF HIGH SPIN AND LOW SPIN CYTOCHROME P-450 IN ROUGH-SURFACED, SMOOTH-SURFACED AND WHOLE MICROSOMES FROM LIVERS OF UNTREATED, PHENOBARBITAL-TREATED AND 3-METHYLCHOLANTHRENE-TREATED ADULT MALE NEW ZEALAND RABBITS

	Enzyme source					
	Rough microsomes		Smooth microsomes		Whole microsomes	
Treatment	Paraoxon* / DEPT	High spin† / Low spin	Paraoxon* / DEPT	High spin† / Low spin	Paraoxon* / DEPT	High spin† / Low spin
Untreated	2·05 ± 0·65	0·32 ± 0·09	1·53 ± 0·19	0·32 ± 0·07	1·73 ± 0·30	0·39 ± 0·01
Phenobarbital	1·71 ± 0·17	0·24 ± 0·01	1·73 ± 0·21	0·23 ± 0·01	2·52 ± 0·43	0·19 ± 0·01
3-Methylcholanthrene	2·99 ± 1·13	0·54 ± 0·13	2·10 ± 0·54	0·45 ± 0·03	2·54 ± 0·65	0·92 ± 0·23

* See Table 5.

† The high spin to low spin cytochrome P-450 ratios were determined using the method of Jefcoate *et al.*[12] These values are the means ± S.E.M. of triplicate determinations in each of three separate experiments in the case of the untreated and 3-MC-treated animals and two experiments using the PB-treated animals.

animals as compared to untreated or PB-treated animals and in whole microsomes from PB- and 3-MC-treated animals as compared to untreated. However, because of large standard deviations, the ratios were not significantly different in any case.

Table 6 shows a comparison of the ratios of the rates of formation of paraoxon and DEPT using microsomes and microsomal subfractions from the livers of rabbits in the various treatment groups with the ratios of the concentrations of high spin and low spin cytochrome P-450 in these same microsomes and microsomal subfractions. The comparison of these ratios was made to determine if there were any correlation between the high spin or low spin cytochrome P-450 content of a particular sample of microsomes and the rate of formation of paraoxon or DEPT. For example, if high spin cytochrome P-450 were responsible for paraoxon formation, the paraoxon to DEPT ratio should increase after treatment with 3-MC, a compound which selectively induces high spin cytochrome P-450.[10] This selective induction of high spin cytochrome P-450 is reflected in the increases in the high spin to low spin cytochrome P-450 ratios in the microsome fractions and subfractions from the livers of rabbits pretreated with 3-MC (Table 6). The degree of association between the paraoxon to DEPT ratios and the ratios of the concentration of high spin and low spin cytochrome P-450 in each of the microsomal fractions and subfractions among the various treatment groups was statistically compared using the product moment correlation coefficient method.[20] In no case was there a statistically significant correlation between the variation of the ratio of the rates of formation of paraoxon and DEPT and the variation in the ratio of the concentrations of high spin and low spin cytochrome P-450 in the same type of microsomal fraction between treatment groups.

A further examination of the data in Table 6 indicates there was a significant decrease in the high spin to low spin cytochrome P-450 ratio in whole microsomes isolated from PB-treated rabbits as compared to untreated. At the same time there was no significant change in the paraoxon to DEPT ratio using these same microsomes. On the other hand, there was a significant increase in the high spin to low spin cytochrome P-450 ratio in whole microsomes from 3-MC-treated animals as compared to untreated, whereas again the paraoxon to DEPT ratio was not significantly different from untreated animals. Similar relationships were also seen in the rough-surfaced and smooth-surfaced microsomal subfractions. Thus, these data indicate there was no correlation between the concentration of high spin or low spin cytochrome P-450 in a particular sample of microsomes and the rate of metabolism of parathion to either paraoxon or DEPT.

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