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BRIEF COMMUNICATION

Differences in Cytochrome P-450 of Various Strains of Rats Following Chronic Administration of Pentobarbital[1]

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CREEL, D., D. E. SHEARER AND P. F. HALL. *Differences in cytochrome P-450 of various strains of rats following chronic administration of pentobarbital.* PHARMAC. BIOCHEM. BEHAV. 5(6) 705–707, 1976. – Cytochrome P-450 levels were analyzed in rats of two pigmented (black Long-Evans and ACI) and two albino strains (Fischer 344 and Sprague-Dawley) following the administration of pentobarbital sodium and physiological saline. Differences between the albino vs pigmented strains were observed following injections of saline. The Fischer 344 albino strains responded similarly to the pigmented strains following a progressively increasing dose schedule of pentobarbital sodium.

Strain differences Cytochrome P-450 Albino Pentobarbital

INDUCTION of microsomal enzymes in the liver is apparently the most important system involved in the metabolism of barbiturates [1,4,10]. The response of the liver to barbiturates is greatly influenced by a number of variables including species [4, 6, 16], strain within species [3, 7, 8, 9, 18], age [13] and sex [11,17].

It has been reported by Shearer *et al.* [18] and Collins and Lott [3] that the lethal dose (LD_{50}) for pentobarbital sodium administered intraperitoneally was significantly lower for several albino strains as compared to pigmented strains of rats, suggesting differing degrees of microsomal enzyme induction correlated with pigmentation. The majority of previous research concerning the ability of hepatic microsomal systems to metabolize barbiturates has been carried out using phenobarbital. The present study was designed to determine whether differences exist in levels of hepatic cytochrome P-450 between several albino and pigmented strains of rats following the administration of progressively increasing doses of pentobarbital sodium.

METHOD

The experimental animals were of two albino and two pigmented strains of rats. The albino rats were 34 animals of the Sprague-Dawley strain (Sim:SD) and 34 animals of the Fischer 344 strain (F344/Sim). Pigmented strains were represented by 33 rats of the solid-black-pelted Long-Evans strain [Blu:(LE)BR] and 35 rats of the ACI (ACI/Sim) strain. All animals were males in good health and without previous exposure to drugs. The rats were housed individually in a standard animal laboratory colony and maintained on feed and water ad lib. The studies were conducted so that different strains were kept in cages at random. We were cognizant of the possibility that intake of substances present in food and bedding might be capable of inducing hepatic microsomal P-450 [20] and efforts were made to reduce this possibility to a minimum. The age of the animals was approximately 90 days at the beginning of the drug-injection schedule.

The drug-treated groups of rats were injected intraperitoneally with pentobarbital sodium at the same time of day following six hours of food deprivation. The injections were given once per week for five consecutive weeks, increasing by 10 mg/kg each week from 10 mg/kg to 50 mg/kg. No infections of injection sites or behavioral changes were observed during the course of the progressive dosing schedule. The higher dose levels of pentobarbital used produced sedation for several hours but within 24 hours the rats appeared normal. The control group of rats were treated in the same way except that physiological saline was used instead of pentobarbital sodium.

Four hours following the fifth and final injection of pentobarbital (50 mg/kg) the rats were decapitated and the hepatic microsomes were prepared by an already established method [14]. Cytochrome P-450 was measured as the carbon monoxide complex described by Omura and Sato [15]. Protein was determined by the method of Lowry *et al.* [12]. The experiment was first conducted on half the animals of each strain and then repeated with the other half in a second experiment.

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TABLE 1

CYTOCHROME P-450 IN ALBINO AND PIGMENTED STRAINS OF RATS FOLLOWING SALINE INJECTION

Rats	Experiment	Cytochrome P-450 (n moles/mg Protein)	
Sprague-Dawley*	1	0.23 ± 0.04	
	2	0.29 ± 0.05	
Black Long Evans†	1	0.39 ± 0.06	
	2	0.41 ± 0.05	$p<0.01$
Fischer 344*	1	0.34 ± 0.03	
	2	0.31 ± 0.06	
ACI†	1	0.54 ± 0.02	
	2	0.51 ± 0.04	$p<0.05$

*Albino.
†Pigmented.
Rats receiving only saline injections were killed and cytochrome P-450 was measured as described in the text. The values for P-450 are means and standard errors of the mean; p values are computed on the combined values for the two experiments in each group.

TABLE 2

MAXIMAL RESPONSES OF CYTOCHROME P-450 TO PENTOBARBITAL SODIUM IN ALBINO AND PIGMENTED STRAINS OF RATS

Rats	Cytochrome P-450 (n moles/mg Protein)	Mean Increase Above Control
Sprague-Dawley*	0.40 ± 0.03	0.14
Black Long Evans†	0.75 ± 0.04	0.35
Fischer 344*	0.69 ± 0.06	0.36
ACI†	0.98 ± 0.07	0.46

*Albino.
†Pigmented.
Rats of all strains were treated with pentobarbital as described in the text. Values for P-450 are means and standard errors of the mean.

RESULTS

Statistical analysis of the data (group t-tests, standard error of the mean, and means) indicated differences in the amount of cytochrome P-450 (n moles/mg protein) between strains of rats.

Table 1 indicates the amounts of cytochrome P-450 measured in strains of rats following the saline-injection schedule. Reliable differences were obtained between the pigmented black Long-Evans (BLE) and the albino Sprague-Dawley (SD) rats ($p<0.01$). Reliable differences were also seen between the pigmented ACI and the albino Fischer 344 rats ($p<0.05$). On combining both pigmented (BLE, ACI) and albino (SD, 344) P-450 values, a reliable difference of $p<0.05$ was obtained. The results were verified in each strain when repeated with a second group of ainmals, i.e. Experiment 2.

Table 2 shows the maximal response of cytochrome P-450 as a result of the administration of a progressive dosing schedule of pentobarbital sodium. The response of the cytochrome P-450 to pentobarbital sodium also indicated possible albino vs pigmented differences. However, by comparing the P-450 values in Tables 1 and 2 it can be seen that this difference was not consistent. The Sprague-Dawley albinos showed less overall P-450 and an accompanying smaller increase following the administration of pentobarbital than the three other strains (BLE, ACI, and 344) and the Fischer 344 albino strain responded similarly to the pigmented strains.

DISCUSSION

The results which indicate differences between these strains in the cytochrome P-450 levels following administration of saline are consistent with existing reports of differences between rats of the Long-Evans and those of various albino strains. However, reliable differences between the albino and pigmented strains used in this study were not observed following the administration of pentobarbital sodium. The P-450 level in the rats of the Fischer 344 albino strain rose to approximately the same level as in rats of the pigmented strains (see Tables 1 and 2). This observation does not, however, exclude the possibility that strain differences correlated with melanin pigmentation may exist in the rates at which levels of P-450 increase in response to drugs since no measurements were made before the injection schedule had been completed.

Creel, Shearer and Wilson [5] reported differences in the median lethal dose of pentobarbital sodium and in the visually evoked responses of albino and pigmented strains of rats. They suggested that differences between strains possibly reflect a deficiency in the ability of Holtzman albino rats to metabolically cope with increasing doses of pentobarbital.

Shearer *et al.* [18] further reported significant differences in the lethal dose (LD_{50}) of pentobarbital sodium between several albino and pigmented strains of rats given a progressive dosing schedule. Differences in mortality may or may not be significantly correlated to pigmentation of the eye or pelt. In spite of this, there are apparent strain differences, especially when rats of the Long-Evans strain are compared to albino strains [3, 7, 8, 9, 18]. The hepatic metabolism of rats of the Sprague-Dawley, Holtzman and and Wistar albino strains appear to be less responsive to the administration of phenobarbital than that observed in the pigmented Long-Evans rat [19]. These differences between strains appear to be reliable. Jori *et al.* [9] have reported differences in reactivity to phenobarbital even between rats of the pigmented Long-Evans strain that were obtained from different sources.

Barbiturates, regardless of the route of administration, bring about the induction of microsomal enzymes, structural changes, proliferation of the smooth endoplasmic reticulum and gross enlargement of the liver in a strain of albino rat [2]. It would be of interest to determine whether these findings are generalized to other strains of rat. In this connection, studies performed with other less familiar strains of *Rattus rattus* and *Rattus norvegicus*, may prove rewarding.

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