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VARIATION IN THE ACTIVITY OF LIVER MICROSOMAL DRUG-METABOLIZING ENZYMES IN RATS IN RELATION TO THE AGE

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Abstract—The variation in the activity of the microsomal drug-metabolizing enzymes in rats in relation to age was studied. The systems used were the *in-vitro* metabolism of hexobarbital, pentobarbital, meprobamate, carisoprodol and strychnine, the *in-vivo* metabolism of pentobarbital, meprobamate and carisoprodol and the duration of pentobarbital hypnosis and carisoprodol paralysis and the toxicity of strychnine and OMPA (Octamethylpyrophosphoramide).

New-born rats showed only a very low metabolic activity, but this increased progressively until 30 days after which it gradually decreased with aging. In the metabolisms *in vitro*, 30-day-old rats showed about 2·7, 2·2, 1·7 and 1·5 times higher activity than those of 250, 150, 100 and 60 days old, respectively. Similar, though smaller, differences were observed *in vivo*. The duration of pentobarbital hypnosis, carisoprodol paralysis and strychnine toxicity was almost the same in immature (30 days old) and adult rats (60–150 days old). Possibly, the immature have a higher sensitivity to the drugs than the adult rats, but this is overshadowed by the higher metabolic activity in the immature rats. The possible mechanism for the marked difference in the activity *in vitro* of the drug-metabolizing enzymes among the differently aged rats is discussed in connection with the variation of *in-vivo* metabolism and activity of microsomal TPNH oxidase.

INTRODUCTION

IT IS WELL known that a variety of drugs are metabolized by the enzyme systems localized in the liver microsomes.

The important role of these enzyme systems for the duration and intensity of actions of a variety of drug has been widely recognized by several works carried out in the laboratory directed by Brodie and in our laboratory.[1–11]

There are only a few works on variations in drug action in differently aged animals, especially there are no reports concerning variations in drug action in differently aged animals in relation to their capacity of the drug metabolism. It is a well known fact that in the new-born animals there is a deficiency in certain drug-metabolizing enzymes in liver microsomes.[12, 13] The activity of these enzymes rapidly increases with age and in guinea-pigs the enzyme activities become the level of the adults at 20 days after birth.

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In a previous study we reported that immature rats can metabolize carisoprodol more quickly than adult rats.[8] The purpose of present studies is to investigate the activity of liver microsomal drug-metabolizing enzymes in differently aged rats in relation to the duration and intensity of drug action.

The detailed works give evidence that the maximum activity in the liver microsomal drug-metabolizing enzymes is found in rats of 30 days old and after this age the activity is progressively decreased. The activity of microsomal drug-metabolizing enzymes in 150-day-old rats is about 50% that of the 30-day-old rats.

EXPERIMENTAL

Female rats of the Sprague-Dawley strain, bred in our Institute were used.

Determination of the in vitro *metabolisms of the drugs*

The metabolism *in vitro* of hexobarbital, pentobarbital, meprobamate, carisoprodol and strychnine was determined by measuring the amount of the disappeared substrates in the microsomal preparation or in liver slices after an incubation of 1 hr.

The rats were killed by decapitation and the liver immediately removed and homogenized with a Potter-Elvehjem type homogenizer adding 3 vol. of 1·15% KCl. The nuclei and mitochondria were sedimented by centrifugation of the homogenate at 8,500*g* for 15 min and the microsomal preparation (5 ml) was made up adding the following to 3 ml of the microsome-containing supernatant: 20μ mole glucose-6-phosphate, 0·4 μmole TPN,50 μmole nicotinamide, 75 μmole $MgCl_2$ and 0·1 ml of 1 M KCl and more 1·3 ml of 0·1 M phosphate buffer (pH 7·4) or 0·1 M tris-buffer (pH 8·2) (for strychnine) and 0·2 ml of the substrates and water. The final concentration of hexobarbital, pentobarbital, mepromabate, carisoprodol and strychnine were 4×10^{-4}, 2×10^{-4}, 3×10^{-4}, 3×10^{-4} and 2×10^{-4} M respectively. The microsomal preparation was incubated in a Erlenmeyer flask (25 ml) which was shaken for 1 hr in an atmosphere of air at 37°, and at the end of the incubation period 2 ml of the reaction mixtures were used for the determination of the amount of the remaining substrates. In some experiments the liver slices were also used. The liver sliced with a microtome and the liver slices (500 mg) were suspended in a Warburg flask which contained 6 ml of Krebs phosphate buffered Ringer (pH 7·4 or pH 8·2 for strychnine) and 0·2 ml of the substrates and incubated in an atmosphere of oxygen at 37° for 1 hr with shaking. At the end of the incubation period the reaction mixture was homogenized and 2 ml of the homogenate were used for the determination.

Determination of the in-vivo *metabolism of the drugs*

Determination of the metabolisms *in vivo* of pentobarbital, meprobamate and carisoprodol was carried out by measuring the concentration of administered drugs in the serum and brain at different time intervals after the injection.

Determination of hepatic microsomal RNA and protein content

The livers were homogenized in 5 vol. of 0·25 M sucrose solution and centrifuged at 8,500*g* for 15 min. The supernatant fraction was then centrifugated at 105,000*g* for 5 min to sediment the microsomes. The microsomal fraction was twice washed with 0·1 M sodium phosphate buffer (pH 7·4) and the microsomes were suspended in the

phosphate buffer. The determination of the RNA and protein was carried out according to the methods of Schneider[14] and Lowry *et al.*[15]

Determination of hepatic microsomal TPNH oxidase activity

The activity of the hepatic microsomal TPNH oxidase was determined by measuring the decrease in optical density at 340 mμ in a model DU Beckman Spectrophotometer at 25° in a manner similar to that described by Gillette *et al.*[16]

A cuvette contained 0·3 μmole TPNH, 10 μmole nicotinamide and 20 μmole $MgCl_2$ and 2·0 ml of 0·1 M sodium phosphate pH 7·4 and more 0·1 ml of microsomal suspension which is equivalent to 60 mg of liver (total volume = 2·4 ml).

Chemical procedure. The determination of the concentration of hexobarbital, pentobarbital, meprobamate, carisoprodol and strychnine in the incubation mixture and in the serum and brain was carried out according to the methods of Cooper and Brodie,[17] Brodie *et al.*,[18] Hoffmann and Ludwig,[19] Kato *et al.*[20] and Kato *et al.*[10]

Determination of pharmacological action of the drugs

Hexobarbital and pentobarbital hypnosis and carisoprodol paralysis were determined by the duration of the loss of righting reflex. Strychnine toxicity was determined by the mortality and convulsion after the intraperitoneal or intravenous injections.

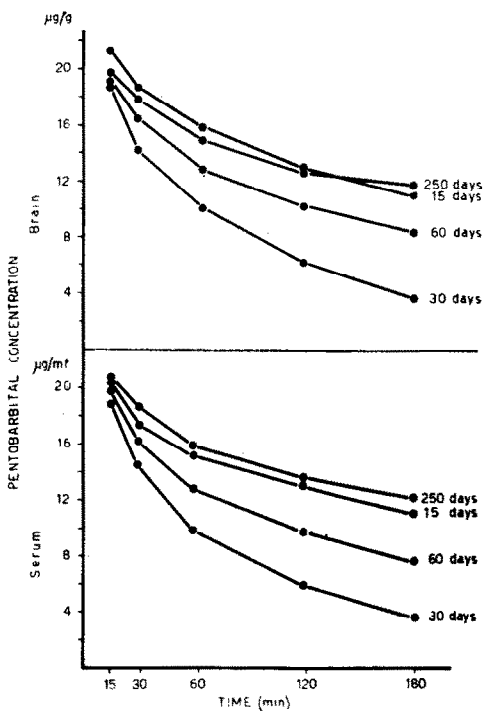

FIG. 1. *In-vivo* metabolism of pentobarbital in rats of 15, 30, 60 and 250 days old. 20 mg/kg of sodium pentobarbital were given intraperitoneally. The rats were killed 15, 30, 60, 120 or 180 min after injection and pentobarbital concentrations in brain and serum were determined. Pentobarbital concentration was expressed as sodium pentobarbital. The values given represent the averages obtained from at least six determinations.

OMPA (Octamethylpurophosphoramide) toxicity was determined by the time required for producing the toxic symptoms (ST_{50}), the clonic convulsion (CT_{50}) and death (LT_{50}) in 50 per cent of animals.

Sodium hexobarbital, sodium pentobarbital and strychnine sulphate were dissolved in 0·9% solution of sodium chloride, while meprobamate and carisoprodol were suspended in a 1% solution of carboxymethylcellulose and all drugs were given in a volume of 2 ml/kg or 4 ml/kg (for body weight).

RESULTS

Metabolism in vivo *of pentobarbital, carisoprodol and meprobamate in differently aged rats*

Marked differences among differently aged rats in the *in-vivo* metabolism of pentobarbital and carisoprodol are shown in Figs. 1 and 2. These results show that the

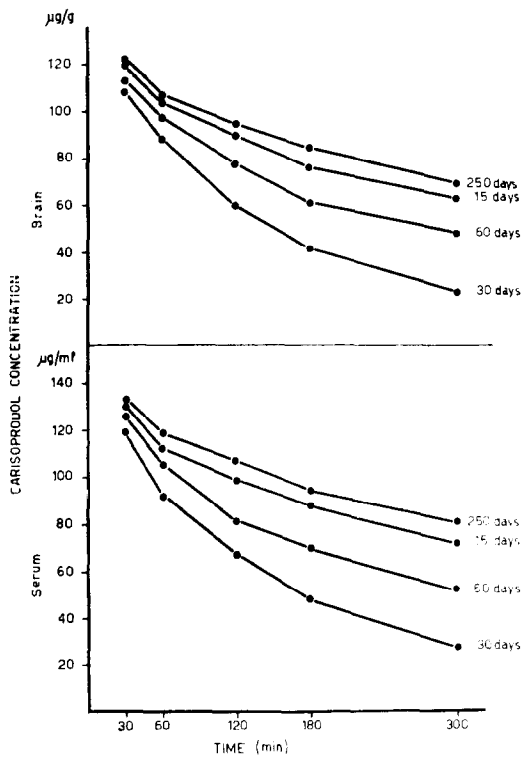

FIG. 2. *In-vivo* metabolism of carisoprodol in rats of 15, 30, 60 and 250 days old. 150 mg/kg of carisoprodol were given intraperitoneally. The rats were killed after 15, 30, 60, 120 or 180 min after the injection and carisoprodol concentrations in brain and serum were determined. The values given represent the averages obtained from at least six determinations.

30-day-old rats have higher capacity for the metabolism of pentobarbital and carisoprodol than 60-, 15- and 250-day-old rats. For example, the serum pentobarbital concentration in 30-day-old rats, which were received 20 mg/kg of sodium pentobarbital 60 min before, was 10·0 ± 0·6 μg/ml, while the concentration in 60-, 15- and 250-day-old rats was 12·9 ± 0·7 μg/ml, 15·2 ± 0·5 μg/ml and 15·9 ± 1·1 μg/ml respectively. The serum carisoprodol concentration in 30-day-old rats, which had

received 150 mg/kg of carisoprodol 3 hr before, was 48 ± 4·5 μg/ml, while the concentration in 60-, 15- and 250-day-old rats was 61 ± 4·3 μg/ml, 79 ± 6·7 μg/ml and 84 ± 5·8 μg/ml respectively. The biological half-life of pentobarbital in rats of 15-, 30, 60 and 250 days old was about 178, 65, 113 and 213 min, respectively and also the biological half-life of carisoprodol in the four groups of rats was about 270, 124, 182 and 295 min respectively.

The serum and brain concentrations of pentobarbital, meprobamate and carisoprodol in rats of 10, 15, 20, 30, 40, 60, 100, 150 and 250 days old are given in Figs. 3, 4 and 5.

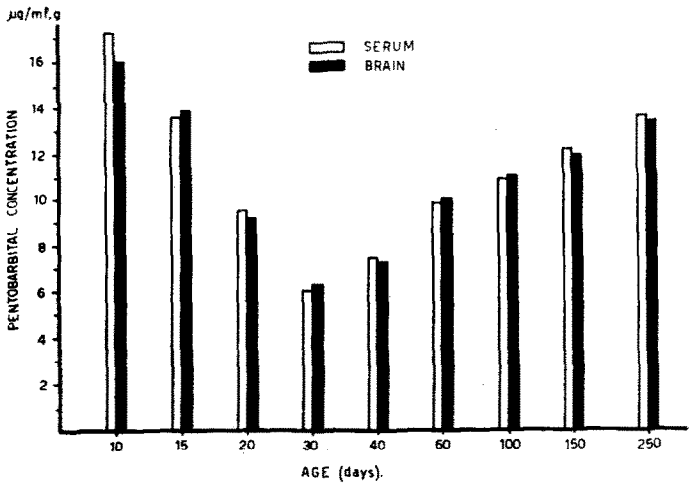

FIG. 3. *In-vivo* metabolism of pentobarbital in different aged rats. 20 mg/kg of pentobarbital sodium were given intraperitoneally 2 hr before the sacrifice. The values given represent the averages obtained from at least six determinations. In the case of 10- and 15-day-old rats the serum and brain pooled from three or two rats were used.

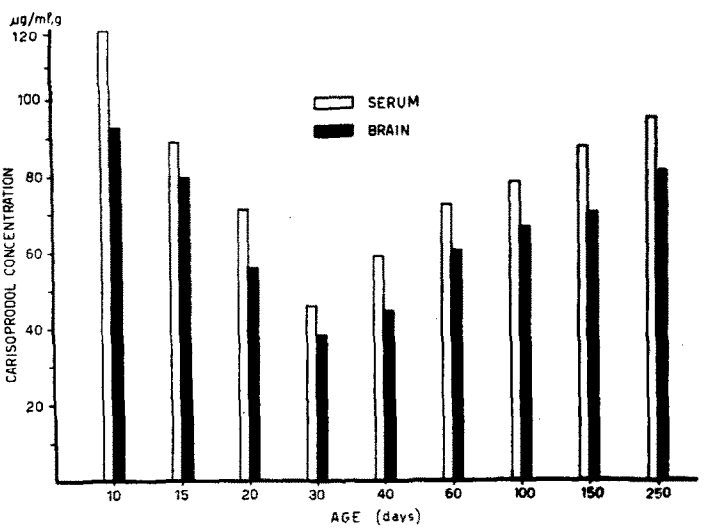

FIG. 4. *In-vivo* metabolism of carisoprodol in different aged rats. 150 mg/kg of carisoprodol was given intraperitoneally 3 hr before the sacrifice. The values given represent the averages obtained from at least six determinations.

These results indicate that the *in-vivo* metabolism of the drugs increases with age but 30 days after birth the rate of metabolism is the highest, after which it gradually becomes slow. It is of very great interest that the metabolic velocity of the drugs in young rats (100 days old) is about 60% of that in immature rats (30 days old).

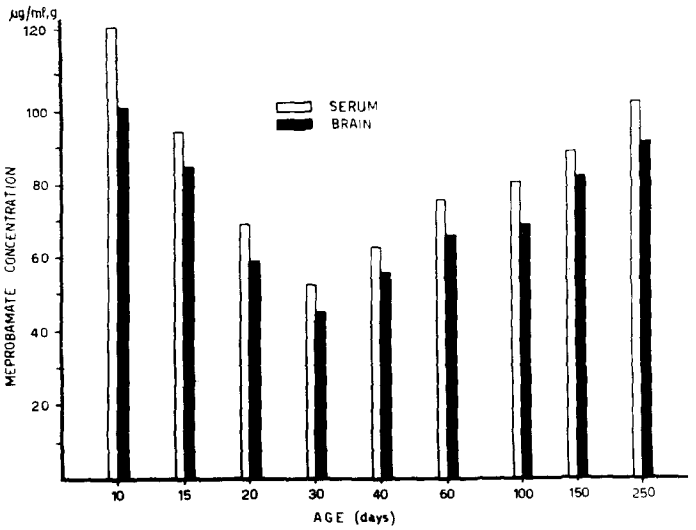

FIG. 5. *In-vivo* metabolism of meprobamate in different aged rats. 150 mg/kg of meprobamate was given intraperitoneally 3 hr before the sacrifice. The values given represent the averages obtained from at least six determinations.

Duration of pentobarbital and hexobarbital hypnosis, carisoprodol paralysis and toxicity of strychnine and OMPA in different aged rats

The duration of pentobarbital hypnosis and carisoprodol hypnosis in different aged rats is given in Fig. 6.

The duration of hypnosis by 23 mg/kg of pentobarbital was 234 ± 25 min in 10-day-old rats and duration of paralysis by 180 mg/kg of carisoprodol was 469 ± 49 min. The duration of the pentobarbital hypnosis and the carisoprodol paralysis was progressively diminished with the aging and the duration of the pentobarbital hypnosis was 53 ± 2·8 and the duration of the carisoprodol paralysis was 57 ± 4·1 in 30-day-old rats; but the duration of the pentobarbital hypnosis and of the carisoprodol paralysis was again gradually increased with the aging. For example, the duration of the pentobarbital paralysis in 60-, 100- and 250-day-old rats was 61 ± 5·2, 62 ± 4·8 and 76 ± 7·3 min respectively, and the duration of the carisoprodol paralysis in the same groups of rats was 75 ± 6·9, 83 ± 12 and 119 ± 14 min respectively.

These differences were more clearly observed when the doses of pentobarbital were increased. For example, 35 mg/kg of pentobarbital caused a hypnosis of 123 ± 6·4 min in 30-day-old rats, while it caused a hypnosis of 219 ± 15 min in 150-day-old rats. The similar results also observed in the duration of hexobarbital hypnosis. For example, the duration of hypnosis by 90 mg/kg of hexobarbital is 49 ± 3·5 for 30-day-old rats, while it is 74 ± 5·9 for 150-day-old rats.

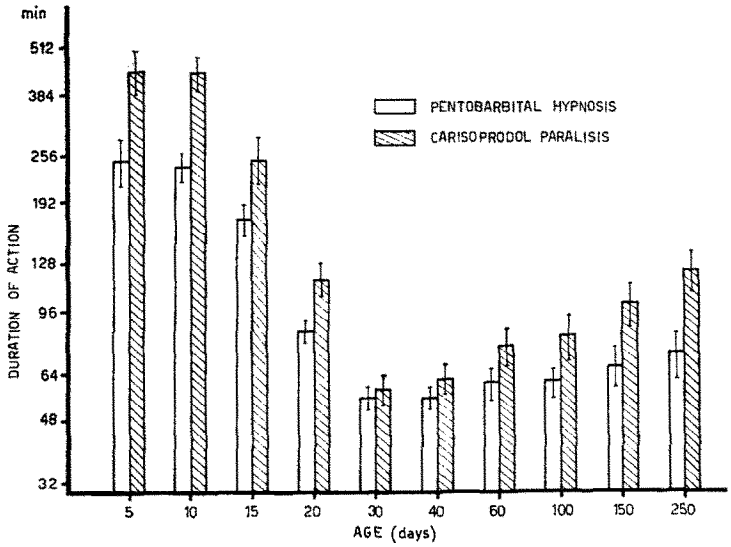

FIG. 6. Duration of pentobarbital hypnosis and carisoprodol paralysis in different aged rats. Rats were given intraperitoneally 23 mg/kg of sodium pentobarbital or 180 mg/kg of carisoprodol. The values given represent averages ± S.E. obtained from at least sixteen rats.

Table 1 shows a different toxicity of strychnine between adult rats (150 days old) and immature rats (30 days old). The strychnine toxicity (i.p.) is a little higher in the adult rats than the immature rats, and if strychnine (1·2 mg/kg or 1·4 mg/kg) is given again 1 hr after the injection of 1·2 mg/kg of strychnine produced a marked different

TABLE 1. TOXICITY OF STRYCHNINE IN ADULT AND IMMATURE RATS

Dose (mg/kg)	Adult rats (150 days old)		Immature rats (30 days old)	
	Convulsion (%)	Mortality (%)	Convulsion (%)	Mortality (%)
1. 1·7 (i.p.)	67	53	53	43
2. 1.4 (i.p.)	37	17	17	13
3. 1.2 (i.p.) and after 1 hr 1·2 (i.p.)	90	83	23	17
4. 1·2 (i.p.) and after 1 hr 1·4 (i.p.)	97	87	40	27
5. 0·55 (i.v.)	57	37	90	87
6. 0·50 (i.v.)	27	10	53	50

Each value given represents the average obtained from 30 rats.

toxicity between the adult rats and immature rats. For example, the second injection of 1·2 mg/kg of strychnine (1 hr after 1·2 mg/kg) caused 90% convulsion and 83% mortality in the adult rats, while it caused 23% convulsion and 17% mortality in the immature rats and injection of 1·4 mg/kg strychnine caused 97% convulsion and 87% mortality in the adult rats, while it caused 40% convulsion and 27% mortality in the immature rats.

On the contrary, the immature rats were more sensitive than the adult rats to strychnine injected intravenously. For example, the intravenous injection of strychnine (0·55 mg/kg) caused 57% convulsion and 37% mortality in the adult rats, while it caused 90% convulsion and 87% mortality in the immature rats. The strychnine metabolism in rats is fairly rapid and modification of strychnine metabolism caused clear modification of strychnine toxicity.[6, 9, 10] These results therefore strongly suggest that the immature rats have more rapid metabolism of strychnine than the adult rats, but the immature rats are more sensitive to strychnine than the adult rats.

Octamethylposphoramide (OMPA) is a compound producing a potent antichloinesterase action after being metabolized in the liver microsomes, therefore the toxicity of OMPA is clearly corresponding to the activity of microsomal drug-metabolizing enzymes.[6] Table 2 shows a clear difference of OMPA toxicity among the three groups of rats. For example, the onsets of symptoms in the half of animals (ST_{50}) after the injection of 20 mg/kg of OMPA is 25 ± 3 and 53 ± 8 min in 30-day-old (immature) and 60-day-old (young) rats respectively, while such doses of OMPA do not produce the symptoms in the half of 250-day-old rats.

TABLE 2. TOXICITY OF OMPA IN OLD, YOUNG AND IMMATURE RATS

	Dose (mg/kg)	No. of rats	Onset of symptoms in the half of rats (ST_{50}) (min)	Onset of convulsion in the half of rats (CT_{50}) (min)	Death in the half of rats (DT_{50}) (min)	Mortality (%)
1. Old rats (250 days old)	30	32	34±3	48±4	63±6	72
	25	32	46±7	63±5	*	41
	20	32	*	*	*	0
2. Young rats (60 days old)	30	32	26±4	34±3	45±7	91
	25	32	35±3	49±6	62±6	60
	20	32	53±8	*	*	22
3. Immature rats (30 days old)	30	32	18±3	20±2	23±4	100
	25	32	23±4	26±3	33±5	100
	20	32	25±3	30±4	39±5	91

The results are expressed by time required for the onset of the symptoms in the half of rats (ST_{50}), for the onset of the convulsion in the half of rats (CT_{50}) and for producing the death in the half of animals (DT_{50}). Each experiment consisted of a group of 8 rats and the experiments carried out four times. The results given represent averages ± standard errors obtained from the four experiments.

* In which the half of rats did not present the symptoms, the convulsion or did not die.

Metabolism in vitro *of hexobarbital, pentobarbital, meprobamate, carisoprodol and strychnine in different aged rats.*

The variation of the metabolism *in vitro* of hexobarbital, pentobarbital, meprobamate, carisoprodol and strychnine in different aged rats was studied by using the microsome-containing supernatants and liver slices. The enzyme activities progressively increased after birth and at 30 days the activities reach the maximum. After 30 days the activities begin to decrease gradually and 150-day-old rats have the activity of only 45–50 per cent of that of 30-day-old rats (Figs. 7 and 8). The variation of

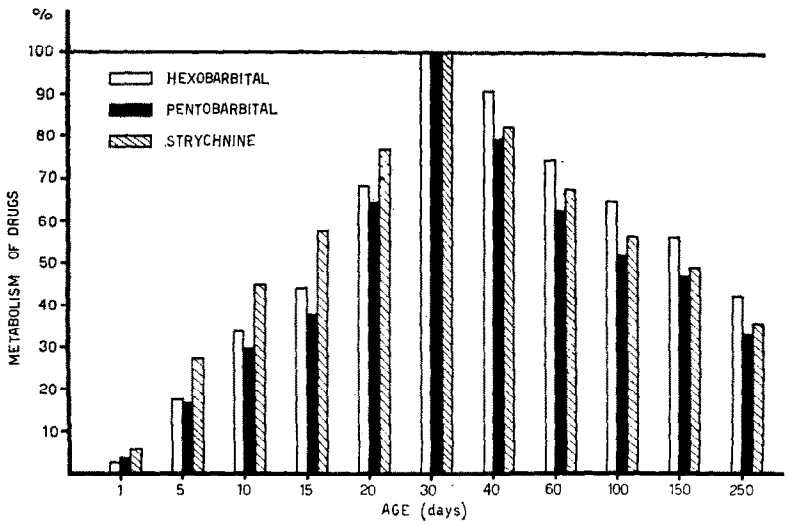

FIG. 7. Metabolism of hexobarbital, pentobarbital and strychnine in the microsomal preparation obtained from different aged rats. The enzyme activities of 30-day-old rats are expressed as 100. The average enzyme activities (± S.E.) of 30-day-old rats are following: Hexobarbital (252 ± 7·8 μg/g per hr), pentobarbital (82 ± 5·1 μg/g per hr) and strychnine (158 ± 7·6 μg/g per hr). The values given represent averages obtained from at least six determinations.

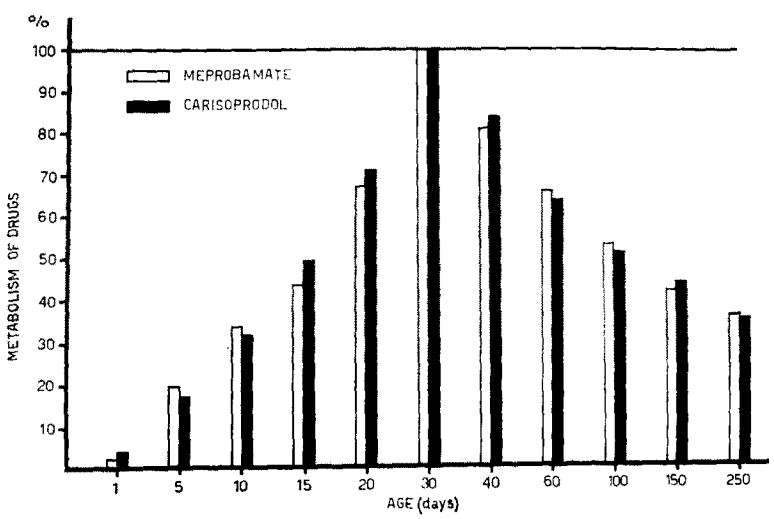

FIG. 8. Metabolism of meprobamate and carisoprodol in the microsomal preparation obtained from different aged rats. The metabolic activities of 30-day-old rats are expressed as 100. The average enzyme activities (± S.E.) of 30-day-old rats are following: Meprobamate (85 ± 7·3 μg/g per hr), carisoprodol (103 ± 7·5 μg/g per hr). The values represent averages obtained from at least six determinations.

activity was almost similar for the enzymes responsible for five examined drugs. By using the liver slices similar results were also obtained.

These results were in accordance with the results obtained in the *in-vivo* metabolism of the drugs, but the difference of the enzyme activities between 30-day-old and 100- to 150-day-old rats observed in the *in-vitro* experiments were more marked than that observed in the *in-vivo* experiments.

Figure 9 shows the *in-vitro* metabolism of hexobarbital in different periods of the incubation by the liver of 30-, 150- and 250-day-old rats.

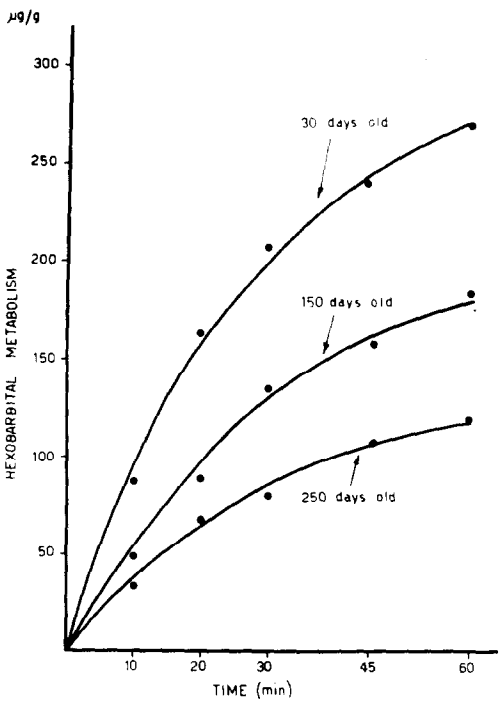

FIG. 9. *In-vitro* metabolism of hexobarbital in different aged rats at different periods of the incubation. Enzyme activity is expressed by metabolized hexobarbital by the microsomal preparation made from 1 g of liver. The values given represent averages obtained from four determinations.

These results indicate that the low metabolic activity in the adult and in the old rats is not due to a rapid loss of enzyme activity, and the different metabolic activity is also observed in early periods of the incubation.

Possible factor which may produce the marked difference in the in vitro *metabolic activity between immature and adult rats*

Since the marked difference in the metabolic activity in the *in-vitro* experiments between immature and adult rats was observed, a possible presence of factors, which may be responsible for producing such difference, is investigated for elucidating the mechanism of the metabolic difference due to the age.

Table 3 shows an influence of different methods for the preparation of the microsome-containing supernatant on the enzyme activity for hexobarbital metabolism.

TABLE 3. METABOLISM OF HEXOBARBITAL BY THE MICROSOME-CONTAINING SUPERNATANT PREPARED WITH DIFFERENT METHODS OF HOMOGENIZATION

Method of homogenization	hexobarbital metabolism (μg/g per hr)	
	30 days old	150 days old
1. Krebs phosphate buffer (3 vol.)	261±10·1	171±6·9
2. 0·1 M Na phosphate buffer (3 vol.)	84±4·5	40±3·7
3. 0·2 M Na phosphate buffer (3 vol.)	259±8·7	163±4·9
4. 0·1 M K phosphate buffer (3 vol.)	158±6·1	83±5·3
5. 0·2 M K phosphate buffer (3 vol.)	289±9·3	172±7·6
6. 0·25 M sucrose solution (3 vol.)	136±3·4	63±3·1
7. 1·15% KCl solution (2 vol.)	266±6·5	126±4·9
8. 1·15% KCl solution (3 vol.)	293±7·1	185±4·8
9. 1·15% KCl solution (4 vol.)	282±9·8	178±5·7

The results given represent the averages ± S.E. obtained from at least four determinations.
The pooled livers from three rats were used for one determination.

Isotonic KCl (1·15%) is the best solution for the preparation, while the preparation with isotonic (sucrose 0·25 M) markedly reduce the activity. In the overall case the activity of 150-day-old rats was remarkably lower than that of 30-day-old rats, and it is likely that in the inadequate preparation, such as the use of 0·1 M sodium or potassium phosphate buffer and 0·25 M sucrose solution, the difference may be exaggerated.

Table 4 shows the influence of different methods of the incubation on the enzyme activity for hexobarbital metabolism.

TABLE 4. METABOLISM OF HEXOBARBITAL BY THE MICROSOME-CONTAINING SUPERNATANT OF 30- AND 150-DAY-OLD RATS IN DIFFERENT METHODS OF INCUBATION

Methods of incubation	hexobarbital metabolism (μg/g per hr)	
	30 days old	150 days old
1. Normal	278±9·5	182±6·9
2. −TPN, +TPNH*	366±12·1	227±5·6
3. 1/5 TPN	255±9·8	164±7·8
4. $-MgCl_2$	78±5·1	43±2·3
5. +ATP (2 μ mole)	287±7·8	187±5·9
6. +Deoxycholic acid (5×10^{-4})	63±2·3	35±1·8
7. Exposed at 20° for 15 min before incubation	218±10·2	142±8·3

The results given represent the averages obtained from four determinations.
* 2 μ mole TPNH were added at zero time and 20 min later.

These results show that the low enzyme activity of the adult rat is not due to a low supply of TPNH or a deficiency of ATP or a rapid loss of the activity in the process of the preparation, but it may be due to a real low enzyme activity.

To detect possible presence of an inhibitor or an activator in the different microsome-containing supernatants, the microsome-containing supernatants obtained from 5-, 30- and 250- day-old rats were mixed and incubated. The results are given in

Table 5. There are no practical differences between the experimental values and calculated values, therefore these results indicate that there are no activator or inhibitor in the rats of the three groups.

Relationship among activity of the microsomal drug-metabolizing enzymes, microsomal TPNH oxidase and microsomal RNA and protein content

Figure 10 shows the activities of hepatic microsomal TPNH oxidase in 1-, 15-, 30-, 60- and 250-day-old rats. It is of interest that the activity of the hepatic microsomal TPNH oxidase is the highest in 30-day-old rats as observed concerning the microsomal drug-metabolizing enzymes.

TABLE 5. HEXOBARBITAL METABOLISM IN THE MIXTURES OF THE MICROSOME-CONTAINING SUPERNATANT; OBTAINED FROM DIFFERENT AGED RATS

Microsomes containing supernatant	Hexobarbital metabolism (μg/g per hr)	
	experimental value	calculated value
1. 5-day-old rats	21±3·8	—
2. 30-day-old rats	272±10·3	—
3. 250-day-old rats	128±8·7	—
4. 5 days + 30 days (1 : 1)	155±7·5	147
5. 5 days + 250 days (1 : 1)	81±5·2	75
6. 30 days + 250 days (1 : 1)	191±6·3	200
7. 5 days + 30 days (1 : 4)	209±9·8	222
8. 5 days + 250 days (1 : 4)	122±6·3	106
9. 250 days + 30 days (1 : 4)	242±10·5	244

The results given represent averages obtained from at least 4 determinations.
The pooled livers from three rats were used for one determination.
In each experiment total volume of 3 ml of microsomes containing supernatant were used.

The relationship among activities of the microsomal drug-metabolizing enzymes, microsomal TPNH oxidase and microsomal RNA and protein content is given in Fig. 11. In which it is easy to be observed that in new-born and infant rats the amount of the microsomes is small and the microsomal RNA and protein contents are very low. However the low enzyme activity of new-born and infant rats can not be accounted for only the low contents of microsomal protein. The amounts of the microsomes and the microsomal protein and RNA contents increased progressively with age and are likely constant after 60 days old.

On the contrary, the variation of the microsomal TPNH oxidase is almost completely the same as that of the microsomal drug-metabolizing enzymes.

DISCUSSION

The results reported in the present paper indicate that the new-born rats show only very little capacity to metabolize a number of drugs and the capacity progressively increases and 30-day-old rats have the maximum capacity. The metabolic capacity again decreases gradually with aging and adult rats (150 days old) show about 50 per cent of that of the immature rats (30 days old).

It is of interest that 30-day-old rats have a higher metabolic activity of the drugs than adult ones and the drug action likely more intense in adult rats than in immature

rats, although it is generally considered that immature rats are more sensitive to drugs than adult rats.

But the difference in the metabolic activity due to age was more marked than the difference in the drug action. Thus this discrepancy may be due to the following fact that the immature rats are more sensitive to the drugs than the adult rats, but this factor is overshadowed by the higher metabolic activity of liver in the immature rats.

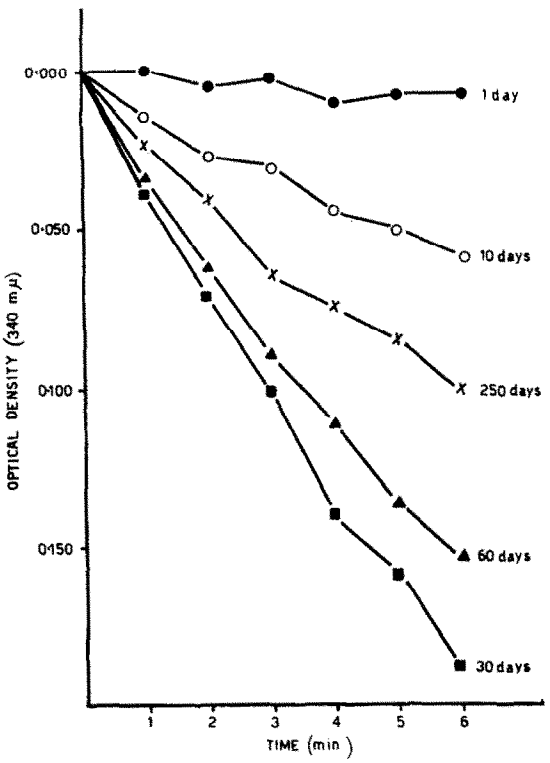

FIG. 10. Activity of hepatic microsomal TPNH oxidase in different aged rats. Enzyme activity expressed by the decrease of the optical density (340 mμ) during the incubation.

This hypothesis may be supported from the results obtained from the toxicity of strychnine and of OMPA. Strychnine convulsion occurs about 8 min and 6 sec after the intraperitoneal injection and the intravenous injection respectively. Therefore, in the case of the intraperitoneal injection strychnine toxicity are clearly influenced by the metabolic activity of the liver, while in the case of intravenous injection the toxicity mainly depends on the sensitivity of the tissue.[6, 9, 10, 11] In the immature rats the strychnine given intraperitoneally was less toxic than in the adult rats, on the contrary, the strychnine given intravenously was more toxic in the immature rats than in the adult. On the other hand, the OMPA was markedly toxic in the immature rats, than in the adult, because of the production of toxic agents are more rapid in the immature rats than in the adult and the sensitivity may be higher in the immature rats.

And the higher sensitivity of infant rats than adult rats is also observed from Figs. 3, 4 and 6. The metabolic activity in 15-day-old rats was about same with that in 250-day-old rats but the duration of pentobarbital hypnosis and carisoprodol paralysis are longer in 15-day-old rats than in 250-day-old rats. And the metabolic

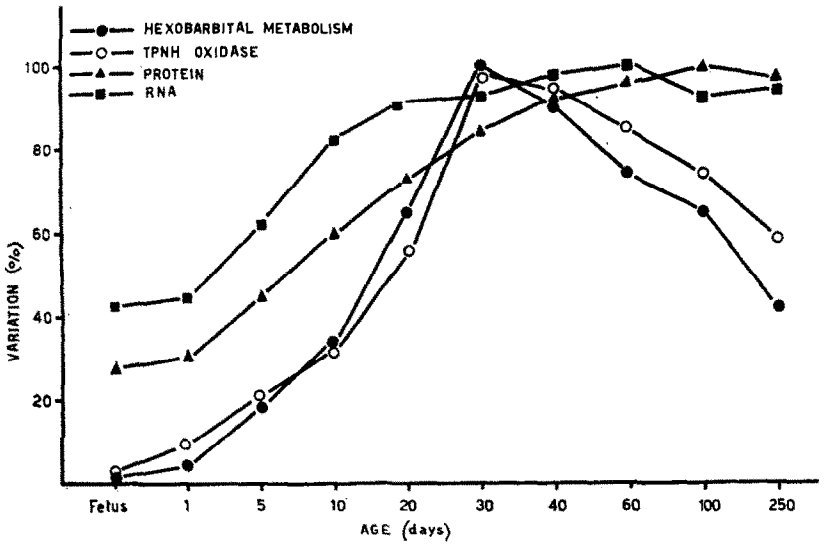

FIG. 11. Relationship among activity of microsomal-drug metabolizing enzymes and of microsomal TPNH oxidase and microsomal RNA and protein content. Microsomal drug-metabolizing enzyme activity was represented by the hexobarbital metabolism. The values are expressed as a per cent of the maximum values. The maximum value of hexobarbital metabolism, microsomal TPNH oxidase activity, microsomal RNA and protein content was 284 ± 11 μg/g per hr, 0·181 ± 0·012 — $\triangle$340 mμ/60 mg per 6 min, 2·28 ± 0·25 mg/g and 18·7 mg/g. The values given represent the averages obtained from at least four determinations. In the case of fetus (18 days), 1-, 5- and 10-day-old rats, the pooled livers obtained from five to two rats were used.

TABLE 6. PERCENTAGE OF LIVER WEIGHT TO BODY WEIGHT IN THE DIFFERENT AGED RATS

Age (days)	No. of rats	Body weight (g)	Liver weight (g)	Liver weight / Body weight (%)	Variation (30 days = 100)
5	20	15±0·9	0·47±0·04	3·13±0·07	68
10	24	26±1·4	0·72±0·07	2·68±0·07	58
15	25	36±1·8	1·23±0·09	3·42±0·08	73
20	22	49±2·0	2·10±0·11	4·29±0·05	92
30	43	73±3·1	3·39±0·22	4·65±0·06	100
40	33	107±4·2	4·93±0·25	4·61±0·06	99
60	40	166±5·8	7·42±0·41	4·48±0·08	96
100	27	238±7·9	9·88±0·57	4·15±0·09	89
150	18	293±11·5	11·54±0·52	3·94±0·07	85
250	24	344±13·0	12·07±0·01	3·51±0·08	74

activity in 20-day-old rats was a little higher than in 60-day-old rats, but the duration of the drug action are longer in 20-day-old rats.

The percentages of liver weight to body weight of different aged rats were shown in Table 6. 30-day-old rats have the highest percentage (4.65 ± 0·05) while in 60-, 150- and 250-day-old rats it is 4·48 ± 0·08, 3·94 ± 0·07 and 3·51 ± 0·08 respectively. The

difference in the *in-vivo* metabolism can not be ascribed to only the different percentage of the liver weight to the body weight. Taking this factor in the consideration it is noted that the difference in the *in-vivo* metabolism must be more marked than that in the *in-vitro* metabolism. However, on the contrary, the calculated carisoprodol metabolisms *in vivo* in 30-, 60-, 150- and 250-day-old rats were about 84, 66, 52 and 41 μg (100:78:62:49) per g body weight per 3 hr respectively, while the difference in the *in-vitro* metabolism of carisoprodol was a ratio of 100:64:44:35 and the difference in the liver weight percentage was ratio of 100:96:85:74.

To elucidate a possible mechanism by which produce the marked difference in the *in-vitro* metabolism of the drugs, several experiments were carried out and the results were given in Tables 3, 4 and 5. However the marked difference was consistently observed by the different preparation of the microsome-containing supernatant and by the different methods of the incubation, and more a presence of inhibitor or an activator could not be demonstrated. These results indicate that the observed difference in *in-vitro* metabolism may be due to a real difference in the activity of the enzymes among different aged rats. The discrepancy between the metabolic difference calculated from the *in-vitro* metabolism and from the liver weight percentage and the metabolic difference obtained from the *in-vivo* metabolism still remain in the question. The observed variation of the microsomal TPNH oxidase in the different aged rats may have some relation to the variation of the microsomal drug-metabolizing enzymes. Recently we also observed more markedly decreased activity of the drug-metabolizing enzymes in 400- and 600-day-old rats.

The results obtained in the present study give a typical example which demonstrates an importance of age factor in the studies of drug metabolisms and drug actions. This result also gives a typical example for demonstrating an importance of metabolic factor as much as tissue sensitivity in the intensity and duration of drug action.

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