Biochemical Pharmacology, Vol. 22, pp. 905–910. Pergamon Press, 1973. Printed in Great Britain.

THE INFLUENCE OF AGE AND BARBITAL TREATMENT ON THE CONTENT OF CYTOCHROME P-450 AND b_5 AND ON THE ACTIVITY OF GLUCOSE-6-PHOSPHATASE IN MICROSOMES OF THE RAT LIVER AND KIDNEY

DIETER MÜLLER, DIETER FÖRSTER, HOLGER DIETZE, RALF LANGENBERG and WOLFGANG KLINGER

Institute of Pharmacology and Toxicology, Friedrich Schiller University of Jena, German Democratic Republic

(*Received* 10 *August* 1971; *accepted* 25 *October* 1972)

Abstract—In liver and kidney microsomes of male control and barbital-treated Wistar rats aged 10, 18, 33, 63 and 123 days the content of cytochromes P-450 and b_5 and the activity of glucose-6-phosphatase have been studied.

The cytochrome P-450 content of the liver increases slightly during the first 4 weeks of life. Barbital enhances the amount of cytochrome P-450 in all age groups but in 10-day-old rats the induction was very poor. The changes of cytochrome P-450 due to ageing and to barbital are not correlated with changes in the various drug-metabolizing reactions. It is concluded that the amount of cytochrome P-450 is not the rate-limiting factor in drug oxidation. In connection with former investigations more than one monoxygenase system or cytochrome P-450 are assumed.

The cytochrome b_5 amount decreases slightly during ageing and was not influenced by barbital. The activity of glucose-6-phosphatase was greatest in young rats and then declined with increasing age. Barbital treatment does not change the activity.

INTRODUCTION

THERE is considerable evidence that the oxidation of various drugs in liver microsomes is mediated by cytochrome P-450.[1] Changes in hydroxylase activity have frequently been found to be correlated with changes in cytochrome P-450 content. It was demonstrated that the increase in cytochrome P-450 caused by phenobarbital pretreatment corresponded closely to the enhancement of *N*-demethylation,[2–4] pentobarbital hydroxylation[4] and progesterone-hydroxylation.[5] Remmer *et al.*[6] found the same time-course of induction for hydroxylase reactions, as for cytochrome P-450 content. However there are many investigations published in which drug metabolizing activities are not directly proportional to cytochrome P-450 concentration.[6–10]

In previous systematic investigations of rats of different ages, it was found that the activity and induction of amidopyrine-*N*-demethylase, phenazonehydroxylase and codeine-*O*-demethylase[11] as well as nitroreductase[12] by barbital depends to a large extent on age. Different results have been found about the age-dependence of cytochrome P-450. Kato[4] described a marked increase of cytochrome P-450 content in rat liver with increasing age, strongly corresponding to the development of oxygenase reactions. Short and Davies[13] reported similar findings in the age-dependent development of cytochrome P-450 in the pig liver. On the other hand Gram *et al.*[14] found only

a slight change in cytochrome P-450 content between 1- and 12-week-old rats. Eling *et al.*[15] did not detect any differences in the cytochrome P-450 concentration of 10- and 30-day-old rats.

In connection with the investigation of age-dependence and induction by barbital of four drug metabolizing reactions, we investigated the cytochrome P-450 content in the rat liver and kidney, under the same conditions as regards animals, age groups and pretreatment. Simultaneously we determined microsomal cytochrome b_5. The activity of glucose-6-phosphatase was evaluated in comparison with cytochrome P-450 as a microsomal reference protein.

MATERIALS AND METHODS

Animals. We used male Wistar rats (Jena) of our colony, fed with standard pellets (Arbeitsgruppe Versuchstierzucht Berlin) and water *ad lib.* The young rats were caged with their mothers until the age of 4 weeks. The animals were given 150 mg/kg barbital (1·5% in 0·9 % NaCl solution) i.p. daily for 3 days, the control rats received the same amount of 0·9% NaCl solution. Twenty-four hr or 8 days after the last pretreatment the animals were killed. At that time the rats were 10, 18, 33, 63 and 123 days old, or 1 week older, respectively.

Preparation of microsomes. After decapitation of the animals the livers were immediately excised, pooled and homogenized with 2 vol. of ice-cold 1·15% KCl (pH 7·4) in a Potter–Elvejhem homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min in a refrigerated centrifuge (K 24, Janetzki). The supernatant was decanted and centrifuged at 110,000 *g* for 1 hr in an ultracentrifuge (VAC 60, Janetzki). The sediment was resuspended with 1·15% KCl to give a final volume of 4·5 ml microsomal suspension per gram of liver.[16] Kidney microsomes were similarly prepared but with a final volume of only 2–2·5 ml/g kidney.

Analytical methods. 0·2 ml of the microsomal suspension were used for the assay of glucose-6-phosphatase (according to Swanson).[17] Protein was determined by the biuret method. For the assay of cytochrome P-450 and b_5 the microsomal suspension was diluted (2 + 1) with 0·1 M phosphate buffer containing 15 mg/ml sodium dithionite for reduction of microsomes. For further details of assay see Lange.[16]

RESULTS

The data presented in Fig. 1 indicate that the cytochrome P-450 concentration in liver increases by about 50 per cent between 10-day-old and adult rats. Barbital enhances the cytochrome P-450 content in all age groups. In 10-day-old rats, however, the induction effect is very poor. Since these animals were considerably affected by 150 mg/kg barbital, half the dose was administered in a further experiment. But this dose did not change the cytochrome P-450 content at all. Eight days after the barbital treatment the induction effect could not be demonstrated any longer in any age group. In some age groups the injection of NaCl solution causes a little increase of cytochrome P-450 content, made evident by the decline of P-450 from the first until the eighth day after NaCl administration. Moreover, in additional investigations, it was found that 33- and 63-day-old untreated rats have a smaller cytochrome P-450 content than NaCl-treated rats.

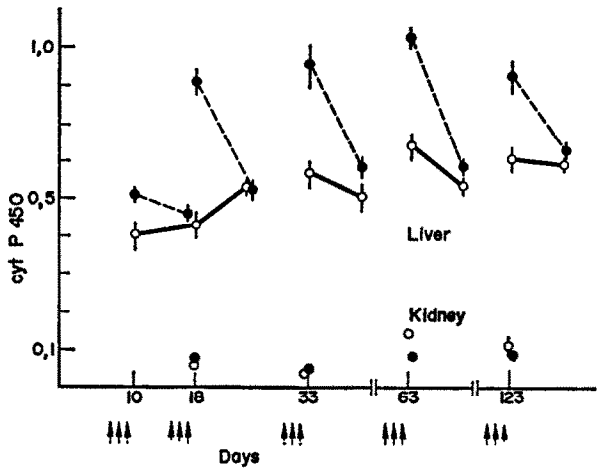

FIG. 1. Developmental changes in cytochrome P-450 content of liver and kidney microsomes of rats pretreated with NaCl (○) or 150 mg/kg barbital (●). Cytochrome P-450 content is expressed as $\Delta E_{450\ nm}-\Delta E_{500\ nm}$/10 mg of microsomal protein. Each point represents the mean ± S.E. of 6–12 determinations. ↑↑↑–Days of pretreatment.

In kidneys only a small amount of cytochrome P-450 was present (about 10–15 per cent of the concentration in liver). Developmental changes and induction by barbital was not significant.

In Fig. 2 the development of liver cytochrome b_5 is illustrated. In contrast to cytochrome P-450, cytochrome b_5 decreases with increasing age until the 63rd day of life. Barbital does not change the cytochrome b_5 amount.

Figure 3 shows the activity of glucose-6-phosphatase in relation to age. The highest values are obtained in 10-, 17- and 18-day-old rats. The activity decreases continually until the 40th day of life; thereafter no changes could be observed. After barbital pretreatment the activity is not changed.

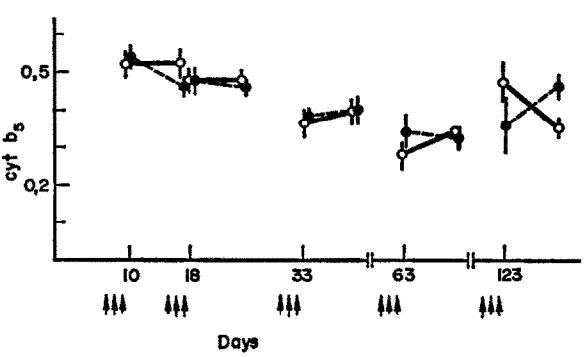

FIG. 2. Developmental changes in cytochrome b_5 content of liver microsomes of rats. For pretreatment and symbols see Fig. 1. Cytochrome b_5 content is expressed as $\Delta E_{425\ nm}-\Delta E_{500\ nm}$/10 mg of microsomal protein.

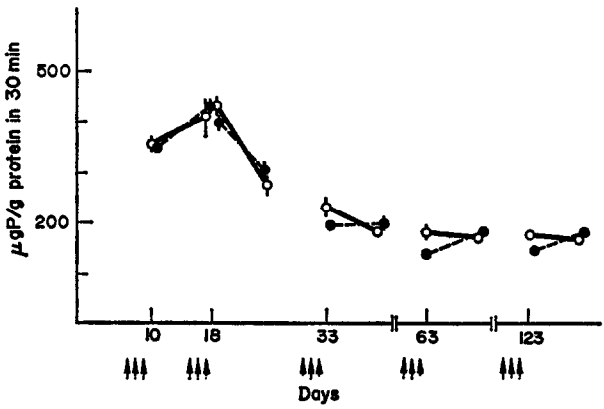

FIG. 3. Developmental changes in glucose-6-phosphatase in rat liver microsomes. For pretreatment and symbols see Fig. 1. Glucose-6-phosphatase activity is expressed as µg P/mg microsomal protein in 30 min. In 10–25-day-old animals the livers and kidneys of 2–6 rats were pooled for one determination. Sometimes the S.E. was smaller than the size of the symbols and was not then drawn.

DISCUSSION

Our results indicate that in the liver of male rats during the first 4 weeks of life the amount of cytochrome P-450 increased only slightly. This confirms the observations of Gram *et al.*[14] If the development of cytochrome P-450 content is compared with age-dependent changes in the activities of amidopyrine-*N*-demethylase, phenazone-hydroxylase, codeine-*O*-demethylase[11] and nitroreductase[12] no quantitative correlation can be demonstrated. Although the development of these enzymes is not uniform, it can be established that in any case the increase of enzyme activities during ageing is greater than the enhancement of cytochrome P-450 concentration. In addition, barbital influences drug metabolism and cytochrome P-450 differently. For instance, amidopyrine-*N*-demethylation can be enhanced by about 700 per cent in 18-day-old rats, the cytochrome P-450 amount by about 100 per cent. These findings support the assumption that the content of cytochrome P-450 cannot be the rate-limiting factor in drug metabolism.

Whether qualitative changes of cytochrome P-450 are responsible for development and induction of oxygenase reactions is not clear. Remmer *et al.*[6] revealed that in rats, sex differences in hexobarbital and amidopyrine oxidation are correlated to differences in maximal spectral changes (E_{max}) caused by addition of these substances. On the other hand Davies *et al.*[9] reported that marked species differences in both the V_{max} and K_m values in the demethylation of ethyl-morphine, do not reflect differences in the amount of spectral change. In addition, Guarino *et al.*[18] could not find any correlation between the increase caused by phenobarbital in spectral changes (E_{max} and K_s) for aniline, and the increase of V_{max} and K_m for aniline hydroxylation. Recent investigations of Eling *et al.*[15] indicate that changes in spectral characteristics with increasing age do not correspond to metabolism of aniline or benzphetamine.

These findings cannot explain the differences in either development or induction of the above-mentioned drug-metabolizing enzymes.

In connection with differences regarding other properties of these oxidative reactions[11,19,20] the assumption is supported that more than one monoxygenase system or cytochrome P-450 could exist.[21,22] Perhaps qualitative changes in membrane structures[23,24] or the microenvironment of cytochrome P-450 could be important in the induction or development with increasing age.

In rat kidneys no significant age dependence or induction by barbital of cytochrome P-450 was detectable. This agrees with investigations of Uehleke and Greim[26] who found no induction by phenobarbital in the kidneys of adult rats.

Our results indicate that cytochrome b_5 content decreases slightly with increasing age and is not enhanced by barbital treatment. This supports the assumption that cyt. b_5 is not directly involved in drug metabolism.[6]

The cytochrome P-450 content and the activity of glucose-6-phosphatase used as microsomal reference proteins, markedly differ in age-dependence and response to barbital. Our results are in accordance with those of Weber and Cantero,[27] Dawkins[28] and Dallner *et al.*[29] who found higher activities in livers of newborn or day-old rats than in adult ones, agreeing with the findings of Orrenius *et al.*[30] who demonstrated no induction in the livers of adult rats, even a decrease in glucose-6-phosphatase caused by phenobarbital.

REFERENCES

1. D. Y. Cooper, S. Levin, S. Narasimhulu, O. Rosenthal and R. W. Estabrook, *Science, N.Y.* **147,** 400 (1965).
2. S. Orrenius and L. Ernster, *Biochem. J.* **92,** 37P (1964).
3. S. Orrenius, *J. Cell Biol.* **26,** 713 (1965).
4. R. Kato, *J. Biochem., Tokyo* **59,** 574 (1966).
5. G. Lange and K.-J. Thun, *Naunyn-Schmiedeberg's Archs exp. Path. Pharmak.* **267,** 265 (1970).
6. H. Remmer, R. W. Estabrook, J. Schenkman and H. Greim, *Naunyn-Schmiedebergs Archs exp. Path. Pharmak.* **259,** 98 (1968).
7. H. Greim and H. Remmer, *Naunyn-Schmiedeberg's Archs exp. Path. Pharmak.* **255,** 16 (1966).
8. F. Kratz and H. Staudinger, *Hoppe-Seylers Z. physiol. Chem.* **348,** 568 (1967).
9. D. Davies, P. Gigon and J. R. Gillette, *Pharmacologist* **9,** 203 (1967).
10. T. E. Gram, A. M. Guarino, F. E. Greene, P. L. Gigon and J. R. Gillette, *Biochem. Pharmac.* **17,** 1769 (1968).
11. W. Klinger, T. Kusch, A. Neugebauer, F.-K. Splinter and H. Ankermann, *Acta biol. med. germ.* **21,** 257 (1968).
12. D. Müller, F. Reichenbach and W. Klinger, *Acta biol. med. germ.* **27,** 605 (1971).
13. C. R. Short and L. E. Davis, *J. Pharmac. exp. Ther.* **174,** 185 (1970).
14. T. E. Gram, A. M. Guarino, D. H. Schroeder and J. R. Gillette, *Biochem. J.* **113,** 681 (1969).
15. T. E. Eling, R. D. Harbison, B. A. Becker and J. R. Fouts, *Eur. J. Pharmac.* **11,** 101 (1970).
16. G. Lange, *Naunyn-Schmiedeberg's Archs exp. Path. Pharmak.* **259,** 221 (1968).
17. M. A. Swanson, in *Methods of Enzymology* (Eds. S. O. Colowick and N. O. Kaplan) Vol. 2, p. 541. Academic Press, New York (1955).
18. A. M. Guarino, T. E. Gram, P. L. Gigon, F. E. Greene and J. R. Gillette, *Molec. Pharmac.* **5,** 131 (1969).
19. W. Klinger, A. Neugebauer, F.-K. Splinter and K.-H. Chemnitius, *Biochem. Pharmac.* **19,** 2677 (1970).
20. W. Klinger, *Farmak. Toks.* **34,** 199 (1971).
21. W. L. Heinrichs and A. Colas, *Biochemistry* **7,** 2273 (1968).
22. A. H. Conney, W. Levin, M. Ikeda, R. Kuntzman, D. Y. Cooper and O. Rosenthal, *J. biol. Chem.* **243,** 3912 (1968).
23. G. Dallner, P. Siekevitz and G. E. Palade, *J. Cell. Biol.* **30,** 73 (1966)
24. G. Lange and Ch. Steubl, *Naunyn-Schmiedeberg's Archs exp. Path. Pharmak.* **263,** 235 (1968).
25. S. J. Yaffe, A. Rane, F. Sjöquist, L.-O. Boreus and S. Orrenius, *Life Sci.* **9,** 1189 (1970).
26. H. Uehleke and A. Greim, *Naunyn-Schmiedeberg's Archs exp. Path. Pharmak.* **259,** 199 (1968).

27. G. WEBER and A. CANTERO, *Cancer Res.* **17,** 995 (1957).
28. M. J. R. DAWKINS, *Br. med. Bull.* **22,** 27 (1966).
29. G. DALLNER, P. SIEKEVITZ and G. E. PALADE, *Biochem. biophys. Res. Commun.* **20,** 135 (1965).
30. S. ORRENIUS, J. L. E. ERICSSON and L. ERNSTER, *J. Cell. Biol.* **25,** 627 (1965).