Biochemical Pharmacology, Vol. 18, pp. 915–921. Pergamon Press. 1969. Printed in Great Britain

PERINATAL DEVELOPMENT OF RAT LIVER AND KIDNEY ESTERASES*

W. S. SCHWARK† and D. J. ECOBICHON

Department of Physiology and Pharmacology,
University of Guelph, Guelph, Ontario, Canada

(*Received* 4 *March* 1968; *accepted* 25 *October* 1968)

Abstract—The perinatal development of the esterases of rat liver and kidney was examined quantitatively by pH-stat titrimetric analysis and qualitatively by starch gel electrophoresis. Low aliesterase activity was found in prenatal tissues. At birth, the renal esterases showed a marked increase, a level 40 per cent higher than that observed in adult male kidney being detected at day 0. In contrast, hepatic esterase activity rose slowly from birth until day 14 of postnatal life when it increased rapidly, exceeding the adult level by 95 per cent at day 28. After the first month, the activity in both tissues dropped slowly to the usual adult level. Electrophoretic investigation of the perinatal development of hepatic and renal esterases showed that the increased enzyme activity was predominantly of microsomal origin.

THE PERINATAL period of the mammal is characterized by marked biochemical fluctuations which reflect the sudden change in environment and the rapid growth of this period.[1] A widely used biochemical parameter to investigate changes in the perinatal period is the enzymatic development of the individual.[2] The implications of developmental biochemistry in pharmacology can be appreciated when one considers the role of enzymes in the process of drug metabolism and, hence, the overall pharmacological response to a drug. The perinatal individual generally lacks the full enzymatic constitution of the adult and will respond differently to a drug.[3,4] The carboxylesterases may constitute one such group of enzymes which are involved in the metabolism of a variety of ester-type drugs.[5] Quantitative and qualitative techniques were used to study the perinatal development of the liver and kidney esterases of the rat, a laboratory animal commonly employed in pharmacological investigations.

METHODS

In order to examine the ontogenesis of rat hepatic and renal esterases, it was necessary to obtain tissue samples from animals at known stages of development. For this purpose, two female and one male adult Wistar rats were placed together in cages and daily vaginal smears were taken and stained with Rose Bengal as described by Herman and Madden.[6] The day of finding sperm cells in the vaginal smear was designated as day 0 of pregnancy. Considering the gestation period of the rat as being 21 days, it was possible to obtain foeti at various stages of prenatal development.

* The work reported in this paper formed part of a thesis submitted in partial fulfilment of the requirements for the degree of Master of Science, University of Guelph, April, 1967.

† Present address: Pharmacology Division, Research Laboratories, Food and Drug Directorate, Tunney's Pasture, Ottawa, Canada.

Following decapitation of the pregnant females, the foeti were removed from the uterus, killed by decapitation and the livers and kidneys were removed. In the case of postnatal animals, the pregnant females were allowed to give birth normally and infants were killed at selected stages up to 1 month after birth.

Because of the small amounts of tissues obtained from pre- and early postnatal rats, tissues from a number of animals were pooled in order to obtain adequate amounts for the extraction procedure. This necessitated using 15–20 foeti for samples at 3 days before parturition, with correspondingly fewer animals being necessary as age increased. No attempt was made to separate the tissues on the basis of the sex of the perinatal rat from which they were obtained. Tissues from adult male rats were used for comparison with the foetal tissues.

The pooled tissues or individual samples were weighed and the homogenates were prepared as described previously, employing a chilled, motor-driven glass Potter–Elvehjem homogenizer equipped with a Teflon pestle.[7] The method was modified in that the step involving the removal of the residual blood from the perinatal tissues was omitted. Twenty per cent homogenates in distilled water were prepared from liver, sufficient kidney tissue being available to prepare only 10 per cent homogenates. Homogenates of similar concentration were prepared from adult tissues. The homogenates were centrifuged in a refrigerated centrifuge at 0° for 1 hr at 14,500 *g*. The sediments were discarded and the supernatants were stored at 4° until used for enzymatic investigation.

The extracts were separated by vertical zone electrophoresis in starch gel by the procedure of Smithies.[8,9] The gel was prepared with 16% starch in 0·001 M borate buffer, pH 8·4. The bridge buffer was 0·004 M borate at the same pH. The sample slots held approx. 75 μl of solution. Electrophoresis was carried out for 16–18 hr at room temperature with a constant current of 14–16 mA passing through the gel. Following electrophoresis, the esterase activity was localized in the gel by the histochemical stain employing the substrate α-naphthyl acetate as described by Ecobichon and Kalow.[10] The gels were preserved by washing them in methanol:water:glacial acetic acid (5:5:1). The electrophoretically separated proteins were stained qualitatively in the gel using a saturated solution of Amido Black 10B in methanol:water:glacial acetic acid (5:5:1).[10]

The esterase activity in aqueous extracts of perinatal rat liver and kidney was detected by a pH-stat titrimetric technique as described by Ecobichon and Israel.[11] The volume of the reaction mixture was always 4·0 ml. A volume of 0·2 ml of the enzyme solution or a suitable dilution was pipetted into the jacketed reaction vessel, 2·8 ml of a 0·9% sodium chloride solution was added and the mixture was adjusted to a pH of 7·6. The substrate, α-naphthyl acetate, was added in a volume of 1·0 ml so that the final concentration in the reaction mixture was 2×10^{-3} M. Sodium hydroxide at a concentration of 0·005 M was used as the titrant. Continuous titration was carried out at 25° for 6 min, holding the pH constant at 7·6. Initial rates of hydrolysis in μmoles of NaOH per ml of extract per minute were determined from the titration curves.

RESULTS

Figure 1 is a graphical representation of the results of quantitative titrimetric analyses of the liver and kidney esterases activity during perinatal development expressed as a per cent of the activity in the adult tissue. Esterase activity in the

prenatal liver and kidney was only a fraction of that in adult tissue. The levels began to diverge at −1 day, the activity in renal tissue showing a distinct rise while the hepatic activity remained at a relatively low level. At birth, the total esterase activity of the kidney showed a marked increase, surpassing that of adult tissue by some 40 per cent.

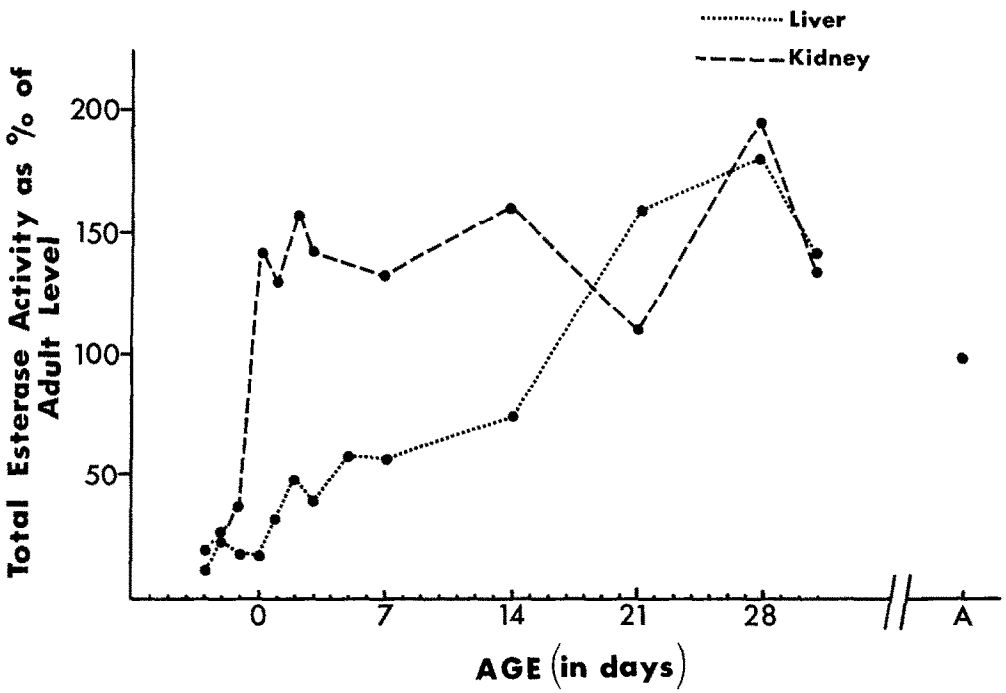

FIG. 1. The esterase activity of perinatal rat livers and kidneys expressed as a percentage of the activity found in corresponding adult tissues (A) and plotted against age in days. The esterase activity was determined by titrimetric assay employing α-naphthyl acetate as the substrate. Each point represents the mean of three or four different samples.

Fluctuations occurred in the esterase activity of the kidney throughout the first month of life but the activity was always greater than that found in adult tissue. Liver esterase activity showed a gradual increase, reaching a level of 60 per cent of the usual adult level by the second week. During the third and fourth weeks there was a rapid increase in hepatic activity, a level 75 per cent greater than the adult level being attained by the end of the third week. A peak of activity in both tissues was reached at 4 weeks of age and immediately began to descend to the normal adult level.

Figure 2 is a schematic diagram of a starch gel electropherogram of adult rat liver showing the subcellular localization of the esterases in the mitochondrial (Mt), microsomal (Mc) and supernatant (S) fractions as determined in an earlier report.[12] The bands, marked according to the convention suggested by Webb,[13] are shown to facilitate an understanding of the results below. The majority of the esterase activity was localized in the microsomal fraction, this fraction being composed of the slower migrating bands (bands 5–13). The esterases in the supernatant fraction comprised the

rapidly migrating bands (bands 1–4) as well as some activity in bands 8 and 9. These slow bands, as well as those present in the mitochondrial fraction (bands 9–11), probably represent microsomal contamination of these fractions.

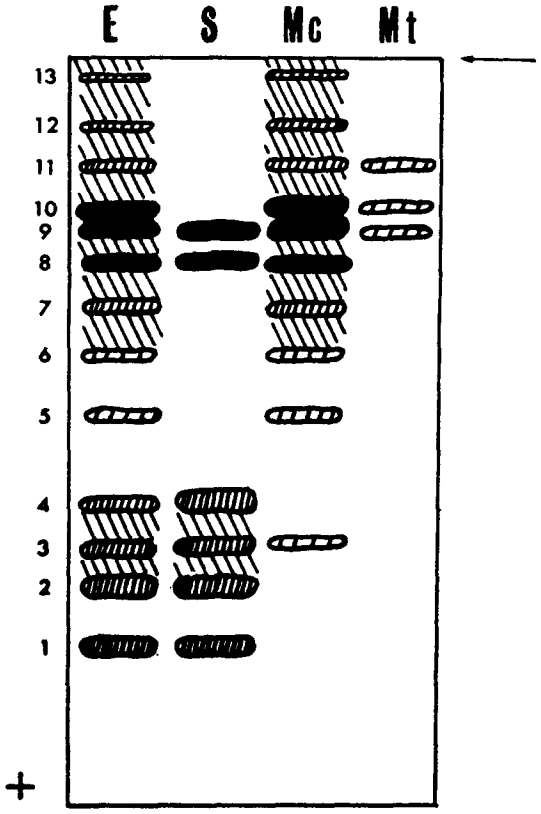

FIG. 2. The subcellular distribution of the esterase activity in the mitochondrial (Mt), microsomal (Mc) and soluble (S) fractions of adult male rat liver prepared by differential centrifugation in 0·25 M sucrose.[18] E represents the pattern of the supernatant of a homogenate centrifuged at 14,500 *g* for 1 hr. Enzymatic activity was detected by the substrate α-naphthyl acetate.

Figure 3A shows the electrophoretic patterns of the liver esterases from pre- and early postnatal animals when compared with the adult. There were few bands of esterase activity in the prenatal liver. Those present were of the slow-migrating type, although one rapidly migrating band was seen in the −2-days liver. By day 1 of postnatal life, some additional bands of the slow-migration type (bands 9–11) were observed, with an increase in the number and intensity of bands in the rapid-migration area (bands 2–8). There was increased activity at 2 and 3 days of postnatal life but the total activity of the bands, particularly in the microsomal region, was much less than that of the adult pattern. Figure 3B shows the pre- and early postnatal electrophoretic patterns of kidney esterases. In contrast to the liver, there was considerable esterase activity 2 days before birth, this increasing in a step-wise fashion with increasing age until at birth, the esterase activity, particularly in the microsomal region, was greater than that of adult tissue (A). There was a progression in the amount of activity with

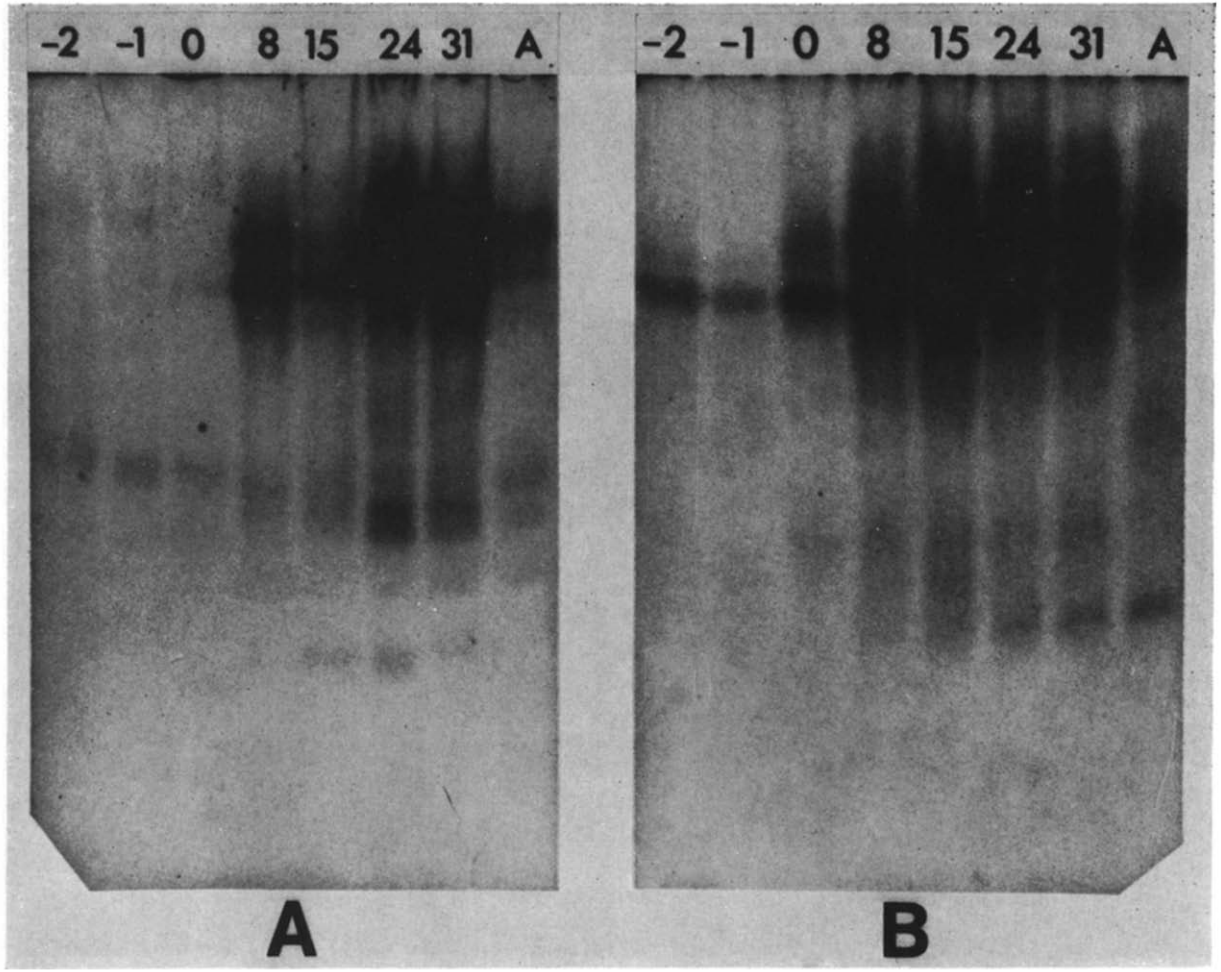

FIG. 4. The starch gel electrophoretic patterns of esterases extracted from the livers (A) and kidneys (B) of pre- and postnatal rats and compared to adult tissue (A). The postnatal period, indicated in days at the top of the photograph, covers the first month of life. The esterase activity was detected using

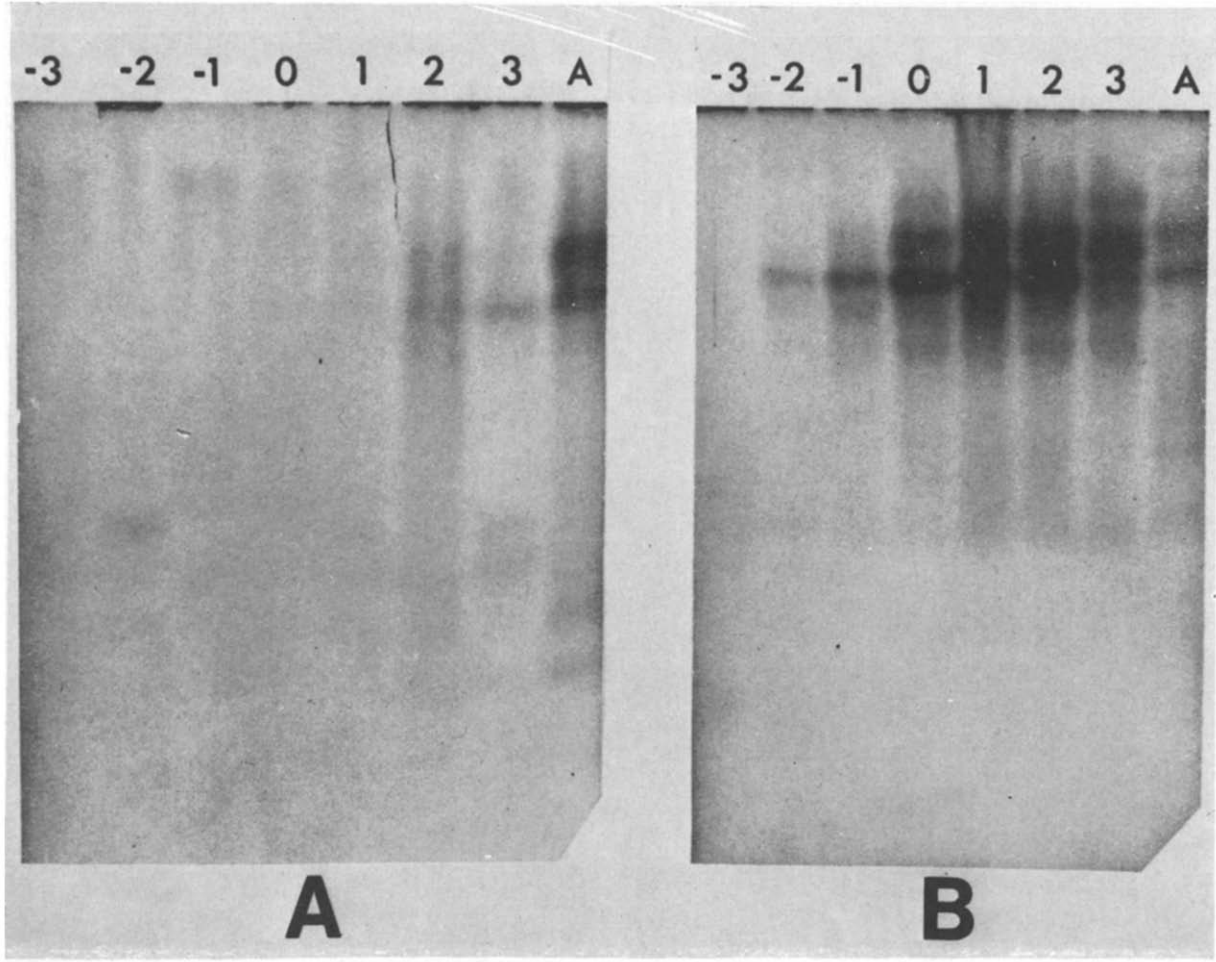

FIG. 3. The starch gel electrophoretic patterns of esterases extracted from the livers (A) and kidneys (B) of pre- and early postnatal rats as compared to adult tissue (A). The age of the animals (in days) is indicated at the top of the photograph. The esterase activity was detected by the substrate α-naphthyl acetate.

increasing age. The supernatant bands did not show the distinct resolution of the adult tissue, although there was esterase activity in this region.

Figure 4A shows liver esterase activity in pre- and late postnatal tissue, the postnatal period in these gels covering the first month of life. By 8 days after birth, an increase in the amount of hepatic microsomal esterase activity was detected (Fig. 4A). Also, the cell sap bands were of increased resolution and intensity. The same general pattern was observed at 45 days after birth; however, band 1 was observed to be markedly increased in intensity. At 24 and 31 days after birth both the microsomal and cell sap bands showed intense activity, surpassing the amount of activity seen in adult liver (A). Band 1 showed marked activity in these tissues as compared to the adult where it was virtually nonexistent. Figure 4B shows kidney esterase patterns over a similar period of time as in Fig. 4A. The initial high amount of esterase activity at birth was maintained or increased throughout the first month of life. However, increasing age led to less intense activity in the microsomal region. Coincident with this, the supernatant fraction bands began to show more distinct resolution and greater intensity, this being particularly noticeable in band 1.

DISCUSSION

The results of the present study indicate that the esterase activity of the perinatal rat liver and kidney is but another example of the marked enzymatic changes which characterize this period of development.[1,2] The esterase activity in both liver and kidney was low in the prenatal animals, these results being in agreement with histochemical studies of esterase activity in embryonic tissues.[14,15] Other workers have reported on the gradual increase of esterase activity with increasing age, Read *et al.*[16] reporting over thirteen times as much liver carboxylesterase in the adult female as in the newborn animal. Blanco and Zinkham have shown a gradual increase of esterase activity in various human tissues during development.[17]

Somewhat surprising was the marked increase in kidney esterase activity at birth compared to the slow increase in liver esterase activity from birth until between the second and third weeks of postnatal life when it also exceeded the adult level. At 1 month of age, both hepatic and renal activity was higher than that of adult tissues. Other workers have described this phenomenon of higher esterase activity in immature as compared to adult tissues.[17,18] The electrophoretic results differ from those of Paul and Fottrell[19] who found no differences in the esterase patterns of immature and adult tissues. Whereas Read *et al.*[16] observed little esterase activity following electrophoresis of newborn rat liver extracts, the present results showed bands of esterases to be present in the newborn and as early as 3 days before birth.

An earlier study showed the bands of renal and hepatic esterases to be electrophoretically similar and to have similar substrate specificity and inhibitor sensitivity.[7] The subcellular localization was also similar and showed that the esterases were predominantly of microsomal origin.[12] Bands of the slowly migrating group (Fig. 2. bands 9–13) were definitely microsomal in origin while certain bands of the rapidly migrating group (Fig. 2, bands 1–4) may be soluble enzymes. A comparison of the electropherograms of esterase patterns from tissues at different stages of development showed marked differences between adult and immature animals. In the pre- and early

postnatal liver there was a relative absence of activity in the slow (microsomal) region of the gel. This is in agreement with the known lack of endoplasmic reticulum, particularly the smooth variety, in the immature liver cell.[20–22] The marked increase in activity in this region at 3 weeks of age may reflect the rapid proliferation of the endoplasmic reticulum at this time or the activation of the esterases contained therein.

In contrast to the liver, the present observations indicate that the microsomal esterases of kidney are already present as early as 3 days before birth, with a rapid increase occurring in the immediate postnatal period. Increasing age led to more intense activity in the slow (microsomal) region of the gel. While no references could be found on ultrastructural changes in the perinatal kidney, endoplasmic reticulum and consequently esterase activity may develop at an earlier stage in kidney than in liver.

The difference in the rate of esterase development in the perinatal rat liver and kidney correlates remarkably well with what is known about the function and gross development of these tissues. The neonatal animal must have functional kidneys within a relatively short time of birth but does not need a highly functional liver since it is relying on the mother for nutrients which can be utilized easily. Oliver *et al.*[23] have shown that, while the rat doubles its body weight in the first 10 days of life, the liver weight increases only slightly. Examination of neonatal rats readily shows a more rapid growth of renal tissue in the first week of development.

Considering the reputed role of the esterases in metabolizing drugs with ester-linkages,[5] the present results should explain the altered sensitivity of newborn rats to these compounds. Few studies on the sensitivity of newborn animals to ester-type drugs have been performed. Read *et al.*[16] speculated that young rats with low esterase levels should be more susceptible to organophosphate insecticides than older animals. Brodeur and DuBois[24] compared the acute toxicity of certain anticholinesterase insecticides in weanling and adult Holtzman rats, finding the younger animals to be more sensitive to the compounds. According to the present results, however, weanling rats (approx. 3 weeks old) have greater liver and kidney esterase activity than comparable adult tissues and would be expected to show a greater resistance on this basis. However, other factors such as differences in the rate of absorption, distribution, excretion and in the sensitivity of receptors may also have some effect on the final response.[25] Furthermore, strain differences (Wistar rats were used in the present study) may account for this discrepancy. More recently, Brodeur and DuBois[26] have described the development of malathionase, a form of carboxylesterase, in neonatal rat liver as a determining factor of malathion toxicity. In agreement with the present results, low enzyme activity was found in the immediate neonatal period with a gradual increase as age progressed. Since a variety of other ester-type drugs such as atropine and acetylsalicylic acid have been found to be very toxic to newborn animals,[27,28] the low liver esterase activity in newborn animals could, at least in part, account for this observation.

Although the liver is usually considered the important organ with regard to drug metabolism,[29] the kidney may also have some importance in this respect. Regarding the dichotomy between liver and kidney esterase ontogenesis, perhaps the high activity in kidney immediately at birth could provide a protective mechanism in the neonatal individual by off-setting the low activity in the liver of early postnatal rats.

Acknowledgements—This work was supported in part by funds received from the Agricultural Research Institute of the Ontario Department of Agriculture and Food.

We thank Drs. H. G. Downie and F. Lotz, Department of Physiology and Pharmacology, for their interest and invaluable criticism of the manuscript.

REFERENCES

1. N. Kretchmer, R. E. Greenberg and F. Sereni, *A. Rev. Med.* **14**, 407 (1963).
2. H. Herrmann and M. L. Tootle, *Physiol. Rev.* **44**, 289 (1964).
3. A. K. Done, *A. Rev. Pharmac.* **6**, 189 (1966).
4. S. J. Yaffe, *A. Rev. Med.* **17**, 213 (1966).
5. W. Kalow, *Proc. First Int. Pharmac. Meet.* (Stockholm 1961) **6**, 137 (1962).
6. H. A. Herman and F. W. Madden, *The Artificial Insemination of Dairy Cattle. A Handbook and Laboratory Manual*, p. 28. Lucas Brothers, Columbia (1953).
7. W. S. Schwark and D. J. Ecobichon, *Can. J. Biochem.* **46**, 451 (1967).
8. O. Smithies, *Biochem. J.* **61**, 629 (1955).
9. O. Smithies, *Biochem. J.* **71**, 585 (1959).
10. D. J. Ecobichon and W. Kalow, *Biochem. Pharmac.* **11**, 573 (1962).
11. D. J. Ecobichon and Y. Israel, *Can. J. Biochem.* **45**, 1099 (1967).
12. W. S. Schwark and D. J. Ecobichon, *Can. J. Physiol. Pharmac.* **46**, 207 (1968).
13. E. C. Webb, *Nature, Lond.* **203**, 821 (1964).
14. R. L. Hunter, *Proc. Soc. exp. Biol. Med.* **78**, 56 (1951).
15. W. Buno, *Acta Anat.* **60**, 285 (1965).
16. S. I. Read, T. K. Murray and W. P. McKinley, *Can. J. Biochem.* **44**, 317 (1965).
17. A. Blanco and W. H. Zinkham, *Bull. Johns Hopkins Hosp.* **118**, 27 (1966).
18. G. J. Maletta, A. Vernadakis and P. S. Timiras, *Proc. Soc. exp. Biol. Med.* **121**, 1210 (1966).
19. J. Paul and P. Fottrell, *Biochem. J.* **78**, 418 (1961).
20. A. M. Jezequel, K. Arakawa and J. W. Steiner, *Lab. Invest.* **14**, 1894 (1965).
21. G. Dallner, P. Siekevitz and G. E. Palade, *J. Cell. Biol.* **30**, 73 (1966).
22. G. Dallner, P. Siekevitz and G. E. Palade, *J. Cell. Biol.* **30**, 97 (1966).
23. I. T. Oliver, F. J. Ballard, J. Shield and P. J. Bently, *Devl Biol.* **4**, 108 (1962).
24. J. Brodeur and K. P. DuBois, *Proc. Soc. exp. Biol. Med.* **114**, 509 (1963).
25. H. M. Nitowsky, L. Matz and J. A. Berzofsky, *J. Pediat.* **69**, 1139 (1966).
26. J. Brodeur and K. P. DuBois, *Can. J. Physiol. Pharmac.* **45**, 621 (1967).
27. A. K. Done, *Clin. Pharmac. Ther.* **6**, 432 (1964).
28. R. A. Yeary, R. A. Benish and M. Finkelstein, *J. Pediat.* **69**, 663 (1966).
29. B. B. Brodie, J. R. Gillette and B. N. La Du, *A. Rev. Biochem.* **27**, 427 (1958).