less than 2 per cent of the detected pyrrolidinone was accounted for as deuterated pyrrolidinone. This suggests that the brain pyrrolidinone was not derived from cyclization of GABA or [$^2H_2$]GABA during work-up of the tissue.

To further characterize the mouse brain pyrrolidinone, a derivative was formed by reaction of the pyrrolidinone fraction from the ion exchange column with pentafluoropropionic anhydride. Mass spectral analysis of the g.c. peak with the appropriate retention time showed the presence of the parent ion of *N*-pentafluoropropionylpyrrolidinone (M^+, m/e 231) and its major fragment ($M^+-C_2F_5$, m/e 112) in approximately the same ratio as found in authentic material.

As a means of quantifying pyrrolidinone in single whole brain, a second derivative and gas chromatography–mass spectrometry were utilized[10]. In this method, pyrrolidinone, which had been separated from GABA by ion exchange chromatography, was hydrolyzed in acid. The resulting GABA was treated with *N,N*-dimethylformamide dimethyl acetal[11] to form methyl 4-(*N,N*-dimethyl-*N'*-formamidino)butanoate (I). Compound I has suitable properties for gas chromatography–mass spectrometry quantitative analysis. [$^2H_2$]Pyrrolidinone served as internal standard. The molecular ions of the hydrolyzed and derivatized pyrrolidinone products I (m/e 172) and [$^2H_2$]-I (m/e 174) were monitored in the g.c. effluent from reconstructed ion current profiles derived from successive scanning[12]. The ratio of the peak area of m/e 172 and of the known amount of standard m/e 174 yielded a quantitative estimate of the quantity of pyrrolidinone in the sample. By this method the concentration of pyrrolidinone in single whole mouse grain was found to be 42 ± 9 nmoles/g wet tissue (mean of six mice ±S.E.M.).

While the biosynthetic source of the brain pyrrolidinone and a relationship between pyrrolidinone and the functioning GABA system have not been established, the possibility exists that a physiologically significant GABA–pyrrolidinone equilibrium may occur in mouse brain.

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Maternal transport of chlorophenoxyisobutyrate at the foetal and neonatal stages of development

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Administration of the widely used anti-hypercholesterolemic drug 'clofibrate' (ethyl -α-*p*-chlorophenoxyisobutyrate) to the rat has been shown to produce many changes in the liver. Mention, in this regard, may be made of hepatomegaly[1], proliferation of mitochondria[2,3] and increase in the activity of the mitochondrial enzyme α-glycerolphosphate dehydrogenase* (L-glycerophosphate (acceptor) oxidoreductase, EC 1.1.99.5)[3, 4]. Both mitochondria and GPD are known to increase post-natally in animals[5]. It was therefore, of interest, to know the effect of administration of clofibrate to the mother during pregnancy and lactation on the above-mentioned developmental changes. The results of such a study are presented and discussed in this paper.

Female albino rats weighing 120–150 g from the Central Animal Facility of this Institute were maintained on a stock diet obtained commercially. Experimental animals were administered with clofibrate orally (50 mg per rat per day) as an emulsion in water (0.2 ml) during the entire period of mating, gestation and lactation. Animals not receiving the drug served as controls. The offspring were weaned from their mothers after 21 days of lactation.

Offspring of age groups as indicated were killed and the serum and liver collected. The livers of 12–15 new born pups were pooled for subcellular fractionation which was done essentially as described earlier[3]. Mitochondrial GPD was assayed manometrically[3]. The level of chlorophenoxyisobutyrate in serum and liver was estimated spectrophotometrically[6] as well as by using methyl [^{14}C] CPIB. Protein was estimated using Folin's reagent[7].

Administration of clofibrate to the adult animal causes cessation of weight gain as well as enlargement of the

* Abbreviation used: GPD, α-glycerolphosphate dehydrogenase; CPIB, *p*-chlorophenoxyisobutyrate (free acid).

liver[4,8]. Pups born to drug-fed mothers did not show any significant difference from control pups, in weight at birth or weight gain during lactation (Fig. 1). However, their liver weight at birth was significantly higher ($P < 0.01$). This difference was maintained, though to a less degree, until towards the end of lactation (Fig. 1). In both groups, the liver weight (for 100 g body weight) decreased for the first two weeks of lactation, increased thereafter and reached the normal adult value at about weaning time. This would mean that, initially, the growth of the liver does not keep pace with that of the whole body. This is consistent with the observation[9] that in the liver of the new born rat mitotic activity is practically non-existent for the first one week and increases 20 fold at the end of the next week.

At birth, the protein content of liver was 10–15 per cent less than the adult value (220 ± 10 mg per g fresh weight) which was attained in less than a week. The protein recovered in the mitochondrial fraction from the livers of new born rats (19 ± 5 mg/g fresh weight) was only about half of that obtained from the adult animal (40 mg/g fresh weight of lover). This is in agreement with previous reports[10–12]. Administration of clofibrate is known to increase the content of mitochondria in liver[2, 3, 13, 14]. However, offspring born to drug-fed mothers had the same hepatic mitochondrial content (21 ± 6 mg protein/g fresh weight) as normal pups. The adult value 42 ± 5 mg protein/g fresh weight) was attained in a week's time.

Mitochondrial glycerolphosphate dehydrogenase develops in the rat neonatally[5,15]. The enzyme is low at birth and increases to the adult level in a few days[16,17]. The results presented in Fig. 2 are in general agreement with these observations. The activity at birth, which was about 20% of the adult value, increased progressively and reached the adult value in about 12 days (Fig. 2).

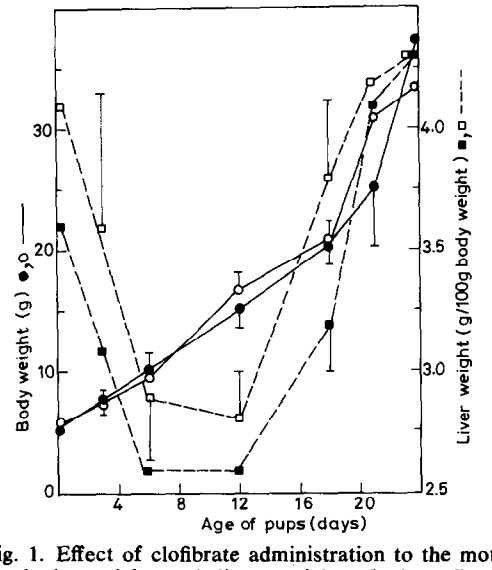

Fig. 1. Effect of clofibrate administration to the mother on body weight and liver weight of the offspring. Clofibrate (50 mg/rat day) was administered to the mothers in the experimental group (○, □) during mating, gestation and lactation. Mothers in control group (●, ■) received water. In the case of new borns, 12–15 offspring were killed at a time and the livers pooled to form one sample. The values for body weight (●, ○) and liver weight (■, □) given are the mean of at least 4 such samples. The standard deviation varied between 5 and 10 per cent of the mean. Some typical standard deviations are indicated. Day 0 pups were taken as soon as born. The young ones were weaned on 21st day.

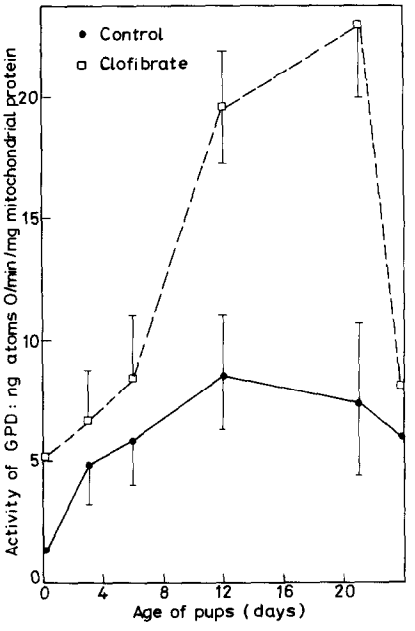

Fig. 2. Effect of clofibrate administration to the mother on hepatic mitochondrial glycerol phosphate dehydrogenase activity in offsprings. The enzyme activity in pups from mothers that did not receive clofibrate (●–●) and from those that received clofibrate (□---□) throughout the period of gestation and lactation are presented. Twelve to fifteen young ones were killed and the livers pooled to form one sample. The values are the mean of five such samples. Some typical standard deviations are also indicated. The enzyme in mitochondrrial samples was assayed manometrically as described earlier[3].

One of the effects of clofibrate administration to adult rats is a large increase (three to six-fold) in the activity of this mitochondrial enzyme[3,14,18]. The activity of the enzyme in hepatic mitochondria isolated from new born rats whose mothers were fed with the drug, was almost three times that obtained with control offspings ($P < 0.01$). The activity increased and was maintained at a higher level (two fold) during lactation. When the young animals were weaned from their drug-fed mothers the enzyme activity rapidly decreased to practically the same level as obtained in control animals (Fig. 2).

Results presented above show that some effects of clofibrate administration like hepatomegaly and increase in mitochondrial GPD activity are observed in pups born to and nursed by drug-fed mothers. This raised the possibility that the drug might find its way from the mother to the foetus. Also, the offspring might get a steady supply of the compound from mother's milk. To test this, the serum and livers of new-born as well as suckling pups were analyzed for the presence of the drug. The drug which is administered in the ester form is rapidly hydrolyzed by tissue and plasma esterases and only the free acid (CPIB) is detected in circulation[19].

The serum of new born pups (determined as soon as born) contained 93 nmoles of CPIB per ml. This decreased to 48 nmoles/ml on the 12th day and to 31 nmoles/ml at the time of weaning. The concentration of the compound in the liver at birth was 45 nmoles/g fresh weight. This increased to 71 nmoles in the first few days and decreased thereafter. Only traces could be

detected at the time of weaning. Both chemical estimation[6] and isotopic experiments revealed the same trend. It may be mentioned that the concentration of CPIB in the serum and liver of the offspring was only about a tenth of that found in the adult animal[6].

The above results show that the compound crosses the placental barrier and enters foetal circulation. Also, the drug may be transferred to the pup by way of mother's milk. In agreement with this, placenta collected before birth from clofibrate-fed mothers was found to contain about 80 nmoles of CPIB per g fresh weight. Milk collected from mothers fed with radioactive CPIB showed substantial amounts of radioactivity (data not given).

The passage of CPIB into the young ones resulted in increases in liver size and glycerolphosphate dehydrogenase activity at birth and during lactation, but did not lead to proliferation of hepatic mitochondria. We have also observed that hepatic catalase activity was higher in the newborn experimental pups, but the increase was not maintained during the suckling phase (data not given).

The reasons for the differential effects are not at once obvious. It is possible that adequate quantities of CPIB are not reaching the offsprings. It has been shown[20] that body weight and age of the animal influence the effect of the drug. Weanling rats do not readily respond to the action of the drug[21]. Even in the adult animal, all the lobes of the liver are not affected equally by the drug[22]. Another possibility is that the animal may not be in a state of 'competance' to respond to the stimulus. The secretion of throxine which has been implanted to mediate in the action of clofibrate[23] begins only at the late fetal stage[5] and this could also limit the response. Administration of the hormone to the mature foetus directly or to the pregnant mother increases foetal liver glycerolphosphate dehydrogenase activity[5]. The rapid decrease in the enzyme activity on weaning is consistent with our earlier observation[8] that where administration of the drug is discontinued the effects are reversed and that the compound practically disappears from the serum in 24 hr[6]. It is also consistent with the view that CPIB passes to the offspring *via* the mother's milk.

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Inhibition of cyclic nucleotide phosphodiesterase by FPL 55712, an SRS-A antagonist

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It has been suggested that the antiallergic drug disodium cromoglycate prevents allergic asthmatic attacks by inhibiting the release of chemical mediators of immediate allergic reactions through the inhibition of cyclic AMP phosphodiesterase[1] and the subsequent increase in intracellular cyclic AMP levels. In addition, the potential antiallergic compounds doxantrazole [3-(5-tetrazolyl)-thioxanthone 10,10-dioxide] and CTD (3-carboxythioxanthone 10,10-dioxide) inhibit the phosphodiesterase of human and guinea pig lung and beef heart, suggesting that their antiallergic activity may be related to their ability to elevate intracellular cyclic AMP levels by inhibiting phosphodiesterase[2, 3].

The present study explores the inhibition of cyclic