TABLE 4. MOUSE BLOOD LEVELS ($\mu$g/ml ± S.E.) OF OXAZEPAM AFTER I.V. ADMINISTRATION OF OXAZEPAM SUCCINATE HALF-ESTER (7·15 mg/kg)

| Time after administration (min) | Administered drug form (+) | (−) | (±) |
|---|---|---|---|
| 5 | 1·15 ± 0·03 | 0·35 ± 0·02 | 0·40 ± 0·02 |
| 30 | 0·87 ± 0·02 | 0·18 ± 0·01 | 0·30 ± 0·01 |
| 60 | 0·60 ± 0·02 | 0·12 ± 0·008 | 0·24 ± 0·01 |
| 180 | 0·43 ± 0·015 | 0·08 ± 0·005 | 0·18 ± 0·02 |
| 300 | 0·34 ± 0·01 | 0·07 ± 0·004 | 0·12 ± 0·008 |

Data not reported here in details indicate that the difference between the two isomeric forms is not present when the oxazepam succinate half-esters are given by oral route to mice or to rats even if the animals were pretreated for 6 days with neomycin (1·5 g/kg) by oral route to minimize a possible hydrolytic activity of the intestinal flora.

The fact that there is a correlation between the anticonvulsant activity and the blood levels of oxazepam suggests that the succinate half-esters of oxazepam act by making available oxazepam. It remains to be established why the two optic isomers release different concentrations of oxazepam. As a working hypothesis it is suggested that a stereospecific esterase present in blood or in liver may be responsible for the difference observed.

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## Effect of phenobarbitone on hepatic microsomal enzymes of the male rat*

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THERE have been contradictory reports of the effect of barbiturates on the activity of hepatic UDP-GT.[1–4]† This inconsistency may be due to the different species, substrates and enzymes preparations and incubations, as well as to the durations and doses of drug used. Small doses of phenobarbitone have been given to patients with hyperbilirubinaemia,[5] and the subsequent reduction of the plasma bilirubin levels may be due to an effect on UDP-GT. Doses in animals, however, have been much larger relative to body weight. In this paper we have compared in the male rat the effects of a dose

* A preliminary report of this work was presented at the meeting of the American association for the Study of the Liver, November, 1969.

† Abbreviations used: UDP-GT: UDP-glucuronate-glucuronyl transferase (EC 2.4.1.17).

TABLE 1. RATIO OF LIVER WEIGHT ×100 TO BODY WEIGHT, AND HEPATIC MICROSOMAL PROTEIN CONTENT OF RATS TREATED WITH PHENOBARBITONE

| Group | Liver weight × 100/body weight* | | | Protein concentration* (mg/g wet weight liver) | | | Total microsomal protein content* (mg/liver) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Phenobarbitone | Change (%) | Control | Phenobarbitone | Change (%) | Control | Phenobarbitone | Change (%) |
| I | 3·44(4) ± 0·13 | 3·56(4) ± 0·07 | +4 | 35·6(4)† ± 1·5 | 41·2(5)† ± 0·5 | +16 | 429(4) ± 14 | 525(4)† ± 15 | +22 |
| II | 3·02(3) ± 0·17 | 3·74(3) ± 0·24 | +24 | 40·9(3)‡ ± 4·9 | 53·0(3)‡ ± 1·3 | +29 | 415(3)§ ± 28 | 656(3)§ ± 61 | +58 |
| III | 3·28(3)† ± 0·08 | 4·01(3)† ± 0·05 | +22 | 51·9(3) ± 6·3 | 57·3(3) ± 1·5 | +10 | 496(3)§ ± 60 | 685(3)§ ± 37 | +38 |
| IV | 3·86(4)§ ± 0·04 | 4·36(4)§ ± 0·20 | +13 | 49·8(4) ± 4·8 | 47·8(4) ± 1·3 | −4 | 758(4) ± 8 | 797(4) ± 54 | +5 |
| V | 4·41(4)† ± 0·10 | 5·04(5)† ± 0·08 | +11 | 38·3(4) ± 2·1 | 43·4(5) ± 2·4 | +13 | 614 ± 49 | 763(4) ± 62 | +24 |

* Means ± S.E.M. Numbers of animals in parentheses.
† $P<0.01$.
‡ $P<0.001$.
§ $P<0.05$.

Phenobarbitone sodium was given daily in saline by intraperitoneal injection. Group I received a mean dose of 28 mg/kg body weight (range 27–31) for 10 days; II, 31 mg/kg (31–32) for 10 days; III, 101 mg/kg for 3 days; IV, 79 mg/kg (76–82) for 10 days; V, 86 mg/kg (82–94) for 10 days.

(30 mg/kg body wt.) smaller than that used in animal studies, with those of the more usual 75–100 mg/kg. We have tried to exclude within the experiments the known effects on the activities of hepatic enzymes, of sex[6,7] animal strain,[8] age,[9,10] previous exposure to inducing compounds,[11] and time of day. Concomitant change of liver weight, the protein content of the microsomal subcellular fraction and the concentration of cytochrome P450 were also studied.

*Materials and methods*

Male Wistar albino rats were bred in this laboratory and were never exposed to insecticides. They weighed 300–400 g, were approximately 3 months old, and were fed Medical Research Council diet 41.b with water *ad lib.* All were fasted for 16 hr overnight before being killed at 10.00 a.m. Doses of phenobarbitone are expressed per body weight at sacrifice.

*Preparations of microsomes and enzyme assays.* A 25% (w/v) homogenate from 2·5–3·0 g portions from the centres of hepatic lobes was prepared in 0·25 M sucrose, centrifuged at 10,000 *g* for 20 min, and the pellets rehomogenized with a volume of sucrose 4× (v/w) the weight of the portion of liver, and recentrifuged. The pooled supernatants (now 12·5% w/v) were centrifuged at 105,000 *g* for 60 min and the microsomal pellets suspended in 4·5 ml buffered sucrose solution (0·25 M sucrose: 0·1 M tris-HCl buffer (pH 7·4), 1:2 (v/v)), and stored in aliquots at −18°. Protein concentration of microsomal suspensions was determined by a modification[12] of the method of Lowry *et al.*[13]

P450 was assayed and calculated as described by Omura and Sato[14] on fresh microsomal suspensions after diluting 1 in 5 with 0·1 M tris-HCl buffer (pH 7·4). UPD-GT was assayed with bilirubin as substrate.[15] Bilirubin was dissolved in male human plasma such that its final concentration in the incubation mixture was 0·23 mM. No detergents were added. Incubation was for 30 min at 37° in air without shaking, and diazo coupling was for 20 min at 25°. The activity of UDP-GT with *p*-nitrophenol as substrate was also assayed.[16]

*Results*

Body weight was unchanged. Liver weight was variably, but consistently, increased, but the change reached significance only with the higher dosage (Table 1). By contrast, microsomal protein concentration was significantly increased only at the lower dosage; although when calculated for the whole liver it was more consistently increased at both doses (Table 1).

The specific content of P450 was approximately doubled by all dose regimens (77–169 per cent increase) and the absolute content was increased even more strikingly (112–248 per cent increase). The specific activity of bilirubin UDP-GT varied within and between groups (Table 2). At a dose of 30 mg/kg/10 days, or 100 mg/kg/3 days (Groups I–III), slight mean decreases were found, while at

TABLE 2. SPECIFIC AND ABSOLUTE ACTIVITIES OF HEPATIC MICROSOMAL UDP-GT OF RATS TREATED WITH PHENOBARBITONE

| Group | Specific activity* (nmoles bilirubin conjugated/min/mg protein) | | | Absolute activity* (nmoles conjugated/min/100 g body weight) | | |
|---|---|---|---|---|---|---|
| | Control | Phenobarbitone | Change (%) | Control | Phenobarbitone | Change (%) |
| I | 0·34(4) ± 0·03 | 0·32(5) ± 0·03 | −5 | 41·1(4) ± 4·3 | 44·3(4) ± 4·4 | +8 |
| II | 0·33(3) ± 0·05 | 0·27(3) ± 0·01 | −18 | 42·9(3) ± 2·1 | 56·9(3) ± 5·8 | +33 |
| III | 0·30(3) ± 0·05 | 0·28(3) ± 0·03 | −7 | 49·1(3) ± 3·3 | 63·2(3) ± 6·1 | +29 |
| IV | 0·22(4)† ± 0·04 | 0·30(4)† ± 0·02 | +38 | 41·0(4)† ± 3·0 | 61·3(4)† ± 2·2 | +50 |
| V | 0·39(4) ± 0·07 | 0·61(5) ± 0·10 | +56 | 65·4(4)‡ ± 10 | 133(5)‡ ± 21 | +103 |

* Means ± SEM. Numbers of animals in parentheses.
† $P<0·01$.
‡ $P<0·05$.

79 or 86 mg/kg/10 days (Groups IV–V), small increases were found. Calculated as absolute activities, however, increases were demonstrated even at the smaller dose, and reached significance at the higher dosage. In Group IV this was confirmed by demonstrating a mean increase of 77 per cent in the specific activity of *p*-nitrophenol UDP-GT (control 0·06 ± 0·001 nmoles, phenobarbitone 0·11 ± 0·004 nmoles conjugated/min/mg protein, $P < 0·001$).

*Discussion*

We have taken enzyme induction to mean the net increase of a microsomal enzyme protein relative to the protein of the microsomal cell fraction. This definition is arbitrary,[17] but the simultaneous hypertrophy of the whole cell produced by inducing drugs is thereby excluded. We have measured enzyme activity in this study and have referred the results to microsomal protein; reference of activities to wet weight of tissue is inadequate, for any change of activity is distorted when, as reported here, there also occur asynchronous changes in liver weight and microsomal protein per unit weight of liver. This is one reason for the differences between previous studies of the effect of phenobarbitone on UDP-GT activity.[1–4] Phenobarbitone has been reported either to increase,[19] or not to affect body weight.[20] In the present study there was no significant change in body weight, nor was it increased by dicophane in a previous study.[18] The ratio of liver weight to body weight was increased by both drugs, but the changes were not marked. Previous reports agree with this.[20,21] The total microsomal protein of the liver was increased in four out of five groups, but the increase of the protein concentration per unit weight of liver was small, and significantly increased only by a dose of 31 mg/kg. This may be because of the concomitant increase in liver size at the higher dosage due to hypertrophy and hyperplasia of liver cells. In support of this, changes in the absolute microsomal protein per liver were more marked. Barbiturates increase microsomal protein turnover,[22] but not always the concentration.[3,23,24]

Only a small increase of the specific activity of UDP-GT occurred with the longer and higher dose of phenobarbitone, although the absolute activity was again more consistently increased. The whole liver should therefore be able to conjugate *in vivo* more bilirubin even without induction, as defined here, of UDP-GT. Our results show that the activity of UDP-GT increases little compared with changes of P450 content, and then only with relatively prolonged and high dosage.[1–3] It could be that there are different UDP-GT enzymes for different substrates,[25–27] and bilirubin UDP-GT may be more slowly and less affected by inducing drugs than the others of these enzymes which are all relatively resistant to treatment.[1] The larger increase of *p*-nitrophenol UDP-GT activity in Group IV supports this. Even in our stable population of rats there were between the groups unexpectedly large variations in the control levels of UDP-GT activity. We used a high bilirubin concentration in the incubation medium since this is important to reveal drug-induced increases of UDP-GT activity.[29] The conditions of incubation[11] and detergents,[15,28] may also greatly influence UDP-GT activity *in vitro* and the apparent effect of inducing drugs on it, but would not explain our finding of an increase in specific activity at the high dose of phenobarbitone, and no change or decrease at the lower dose. The doses we used clearly have much less effect than the more usual experimental doses of 75–100 mg/kg. In agreement with our findings was a study[4] in rabbits in which a dose of 15 mg/kg/day was reported not to increase the specific activity of UDP-GT.

In man, only small doses of phenobarbitone (0·5–3·0 mg/kg/day) are necessary to reduce plasma bilirubin in unconjugated hyperbilirubinaemia.[5] Dicophane is also effective,[18] and we have shown it to have similar effects on the enzymes of the male rat as the lower dosage of phenobarbitone in this study—namely that there is no increase in the specific activity of bilirubin UDP-GT.[18] Both drugs, therefore, may have a clinical effect due not to true enzyme induction, but rather to increases of liver size and absolute enzyme activity. Further, recent work suggests that for bilirubin excretion the effect of phenobarbitone on canalicular bile flow[30] may be more important than the increase of hepatic microsomal enzymes,[31] although in a study in rats, bile flow was not increased by doses of less than 30 mg/kg.[21]

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## Inhibition of protein synthesis in brain subcellular fractions by the convulsant allylglycine*

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In a previous work we carried out a study of the cerebral cortex and cerebellum of rat convulsing after the administration of 2-amine 4-pentenoic acid (allylglycine). The neurochemical findings were an inhibition of glutamic acid decarboxylase and a decrease in the concentration of GABA; with the electron microscope, alterations of certain nerve endings, especially of those which probably are mediated by GABA, were found.[1,2] Since there is evidence in the literature that the nerve endings isolated from brain are able to carry on some protein synthesis,[3–6] we became interested in testing the effect of allylglycine on such a process. In the present communication we will describe the finding of a marked inhibition in the incorporation of L-[$^{14}$C]leucine into proteins by isolated subcellular fractions of the cerebral cortex and liver of allylglycine-convulsant rats. The addition *in vitro* of allylglycine to the same fractions isolated from control rats also inhibited the synthesis of proteins, except for the microsomal ones.

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