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Interaction of phenobarbitone and ethanol in mice studied from dose-response curves and drug concentrations in blood

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The interaction of phenobarbitone and ethanol given intraperitoneally to mice has been studied. Dose-response relations were established for incidence of death, sleeping time and time of onset of sleep. Concentrations of phenobarbitone and ethanol were assayed in whole blood samples. When the two drugs were administered together, sleep occurred more rapidly than calculated as likely on the basis of the effects of the drugs given individually. On the other hand, sleep was of shorter duration and the incidence of death was less than anticipated. These modifications of the expected effects related to drug concentration changes in blood. Isobolograms indicated the need for precision in describing the interactions as potentiation, addition or antagonism, as different terms could be applied depending on the effect measured.

Many interaction studies with depressant drugs and ethanol have been reported (Forney & Hughes, 1968; Polacsek, Barnes & others, 1972), but relatively few have included references to dose-response relations and drug concentrations in blood. A recent report from this department showed that changes in expected responses to phenobarbitone, glutethimide and ethanol in man can be induced by consumption of the drugs together, and that these changes relate at least in part to changes in concentrations of the drugs in blood (Mould, Curry & Binns, 1972). The present report concerns a study of the phenobarbitone-ethanol interaction in mice, with particular reference to the drug concentrations in blood, and to dose-response relations. The data were analysed from the point of view of potentiation, addition and antagonism of response.

METHODS

Log dose-response relations

Groups of 5–10 mice (Random bred Theiler's Original, all male) were given doses of phenobarbitone, ethanol or combinations of the two drugs by intraperitoneal injection as indicated in the figures. Solutions were of such a strength that the injection volume was 0·1 ml per 20 g mouse. Injections contained in isotonic saline: phenobarbitone alone, ethanol alone, or phenobarbitone and ethanol in mixtures to give dose ratios of 1:10, 1:20 and 1:30 of phenobarbitone and ethanol respectively. The mice were observed for: onset of sleep indicated by loss of righting reflex (tested every 30 s after each dose); duration of sleep (the time between loss of the righting reflex and when the animal first turned over from the prostrate position); for death and the time it occurred. The data from each group of mice were pooled, and the mean for each group was plotted (Figs 1–3).

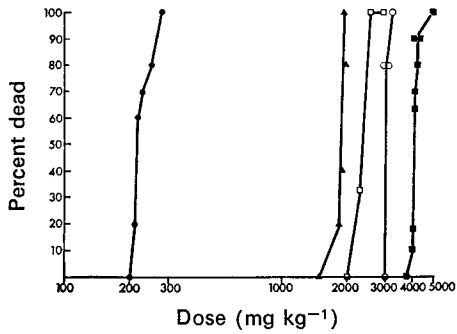

FIG. 1. Log dose/incidence of death relation for mice administered phenobarbitone, ethanol, or phenobarbitone and ethanol by intraperitoneal injection. Code: ●—● phenobarbitone alone (doses indicated by abscissae); ■—■ ethanol alone (doses indicated by abscissae); ▲—▲ phenobarbitone plus ten-fold amount of ethanol (doses indicated in terms of ethanol content on abscissae); □—□ phenobarbitone plus twenty-fold amount of ethanol (doses indicated in terms of ethanol content on abscissae); ○—○ phenobarbitone plus thirty-fold amount of ethanol (doses indicated in terms of ethanol content on abscissae).

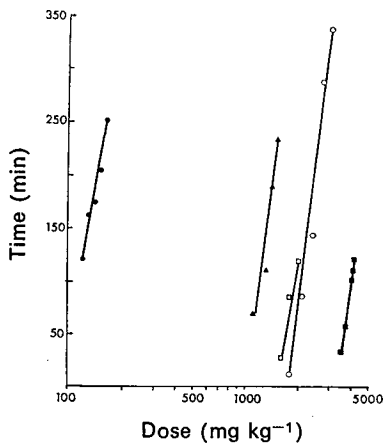

FIG. 2. Log dose/duration of sleep (min) relations. (Experiment and code as in Fig. 1.)

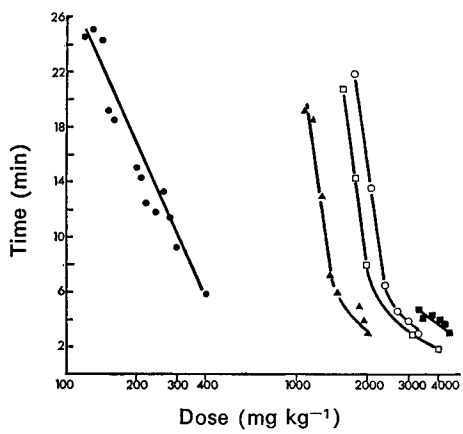

FIG. 3. Log dose/onset of sleep (min), relations. (Experiment and code as in Fig. 1.)

Mathematical assessment

Each dose-response line for death was used to calculate the LD50 with 95% fiducial limits (Litchfield & Wilcoxon, 1948). Each dose-response line for sleeping time was used to calculate the dose inducing sleep of 120 min duration, with 95% fiducial limits, by methods described by Snedecor & Cochran (1967). These methods were also applied to the onset times, but the log dose-response relations were not all linear. The relation was linear for ethanol. That for phenobarbitone required linear extrapolation to the 4 min region. For the drugs together, a linear transform was needed and a graph of reciprocal response *vs* reciprocal dose gave the best estimate, indicated by calculation of the best straight line and its statistical characteristics. This was obtained for each available linear transform, using a desk-top computer programmed for linear regression by least squares arithmetic. Once the linear transformation was complete, doses for 4 and 10 min onset times, with 95% fiducial limits, were calculated as described for sleeping time measurements.

Assay of phenobarbitone in blood

Blood from decapitated mice in groups of five was pooled. Phosphate buffer (pH 6·5; 4 ml) was added to 1 ml of the pooled sample. The aqueous mixture was shaken mechanically for 30 min with ether (5 ml), centrifuged, and 3 ml of the ether extract was shaken mechanically for 30 min with 0·5N NaOH (4 ml). The extinction of the alkaline extract was read at 240 nm ($E1\%$ 1 cm value 431). The reference cell contained a solution prepared similarly but with plasma from untreated mice from the same population. The instrument was calibrated with extracts from a series of standard solutions of phenobarbitone in samples from the same batch of plasma. The method was usable in the concentration range 0·01—1 mg ml^{-1}. In pilot experiments, individual readings from 5 mice were always within 10% of the mean values, so that individual readings in excess of 10 μg ml^{-1} which was three times the blank reading (approximately 3 μg ml^{-1}) were considered scientifically valid. Recovery was 100 $\pm$ 10% Ethanol did not affect the assay procedure for phenobarbitone.

Assay of ethanol in blood

Individual blood samples from decapitated mice in groups of five were assayed according to Curry, Walker & Simpson (1967). Phenobarbitone did not affect the assay.

RESULTS AND DISCUSSION

Log dose-response relations for incidence of death, sleeping time and onset of sleep are shown in Figs 1–3. Time of death was not dose-related with ethanol, so no analysis was made of the results obtained. In each family of log dose-response plots in Figs 1–3, there is a plot for phenobarbitone alone and for ethanol alone, together with plots for the three mixtures, which for convenience are indicated by their ethanol content, the phenobarbitone dose being 1/10, 1/20 and 1/30th of the ethanol dose. For example in Fig. 1 (incidence of death) the line second from the left shows that doses of ethanol in the range 1500–2000 mg kg^{-1} in combination with 150–200 mg kg^{-1} of phenobarbitone (ratio 10:1) caused incidences of death in a similar pattern to that found with phenobarbitone alone (200–300 mg kg^{-1}) or

ethanol alone (3800–5000 mg kg^{-1}). An analogous pattern is evident for the other dose ratios and effects. The plots in Figs 1–3 also indicate that the effect originally obtained with one of the drugs could, generally speaking, be obtained with a lower dose of that drug if the other drug was also given (but see specific comments that follow).

Data of this type can be interpreted in terms of potentiation, addition and antagonism by the preparation of isobolograms (Smith & Herxheimer, 1969), which are plots linking a set of equipotent doses of two drugs given alone and together on a graph which has the doses of the drugs as ordinate and abscissa. Fig. 4 shows the relevant data from Fig. 1 (LD50 with 95% confidence limits). Isobolograms were similarly prepared for the other two parameters (Figs. 5 and 6). Onset times of 4 and 10 min were chosen because one point was missing from the 10 min isobol. No hypnotic dose of ethanol alone had an onset time as great as 10 min. The 4 min point for phenobarbitone was obtained by extrapolation.

When the isobol is a straight line, the doses of the individual drugs contribute to the combination exactly the effects recorded for the drugs separately (addition). When the isobol bows towards the origin, potentiation is indicated since less than the calculated amount produces a standard effect. When the plot bows away from the

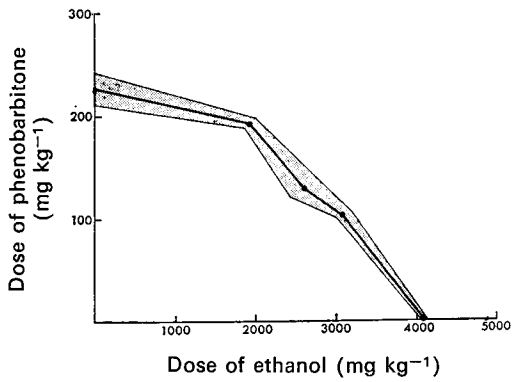

FIG. 4. Isobologram for death calculated from the date of Fig. 1. Dotted areas are 95% fiducial limits.

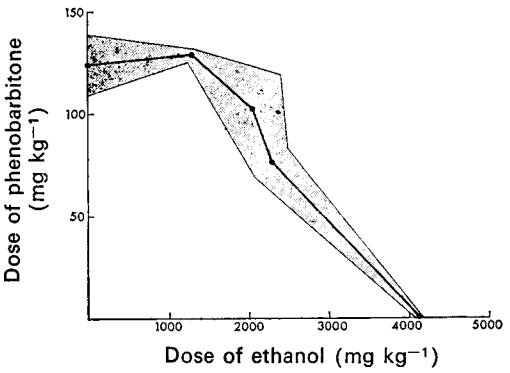

FIG. 5. Isobologram for duration of sleep calculated from the data of Fig. 2. Dotted areas are 95% fiducial limits.

origin antagonism is indicated. Thus Figs 4 and 5 indicate antagonism while Fig. 6 shows potentiation. In a confirmatory experiment, shorter sleep was obtained from 100 mg kg^{-1} of phenobarbitone +1000 mg kg^{-1} of ethanol, than from 100 mg kg^{-1} of phenobarbitone alone.

Thus the interaction of phenobarbitone and ethanol can be described, either as antagonism or potentiation, depending on the particular effect studied, a finding in contrast with the common view that the interaction is invariably an addition or a potentiation.

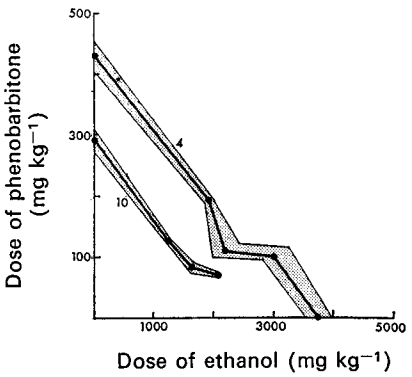

FIG. 6. Isobologram for onset of sleep at 4 and 10 min calculated from the data of Fig. 3. Dotted areas are 95% fiducial limits.

Our conclusions are based almost entirely on linear log dose-response relations. The full log dose-response relation in each case is, theoretically at least, a sigmoid curve. Clearly, it would be possible to choose non-hypnotic doses of both phenobarbitone and ethanol, and induce sleep by administration of the combination. This would presumably be a potentiation, regardless of the implications of the dose-response relations and isobolograms presented as it would involve induction of an effect not demonstrated with either dose alone. The task of interpreting non-linear relations or, for that matter, non-parallel relations, in terms of potentiation, addition and antagonism, would be immense. This further underlines the point we wish to stress, that when precision is applied to a system of the kind examined, a single term for the interaction becomes inadequate.

We have searched for the mechanisms of the interactions observed by measuring the concentrations of phenobarbitone and ethanol in blood. The whole-blood phenobarbitone concentrations alone and with ethanol are shown in Fig. 7. The areas under the two curves are nearly the same, but the times for which high concentrations existed are different. After phenobarbitone alone, the highest reading (320 μg ml^{-1}) was at 15 min. After the combinations, the highest reading (450 μg ml^{-1}) was at 5 min. Ethanol appears to accelerate the absorption of phenobarbitone without changing the overall degree of absorption.

The whole-blood ethanol concentrations alone and with phenobarbitone are shown in Fig. 8. Here, there is strong evidence that phenobarbitone delayed the absorption of ethanol. The format of Figs 7 and 8 is different because of the nature of the experimental data obtained, in that the phenobarbitone figures were from assays of pooled samples, while with ethanol no pooling was necessary.

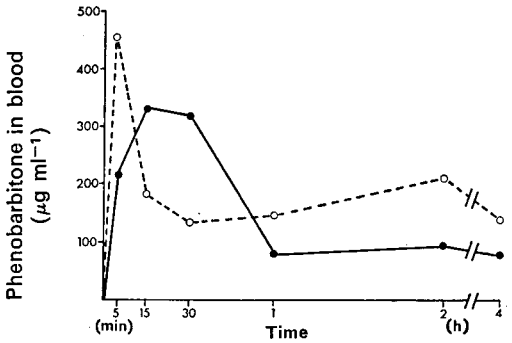

FIG. 7. Mean concentrations of phenobarbitone in whole blood at various times after administration of 100 mg kg^{-1} of phenobarbitone alone and 100 mg kg^{-1} of phenobarbitone in combination with 1000 mg kg^{-1} of ethanol. (Continuous line, phenobarbitone alone; dotted line, combination.)

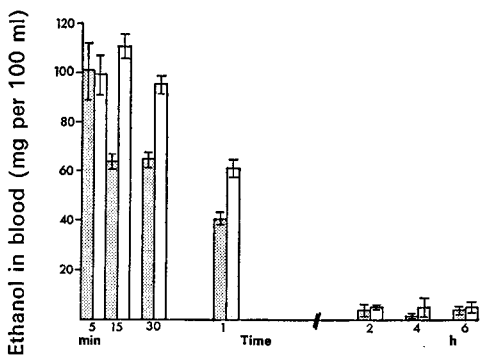

FIG. 8. Concentrations (mean ± s.e.) of ethanol in whole blood at various times after administration of 1000 mg kg^{-1} of ethanol alone and 1000 mg kg^{-1} of ethanol in combination with 100 mg kg^{-1} of phenobarbitone. (Dotted histogram, ethanol alone, open histogram, combination.)

The onset of drug effect relates closely to the appearance of pharmacologically active molecules in blood. When the two drugs were given together, the appearance of ethanol was delayed whilst that of phenobarbitone was accelerated. When onset of sleep was considered, potentiation occurred between the drugs. The potentiation was greater when the doses of phenobarbitone were relatively high, indicated by the greater bowing in the isobologram near the phenobarbitone axis. This potentiation is almost certainly a function of the phenobarbitone rather than the ethanol concentration. In contrast, with sleeping time as the parameter, antagonism occurred. However, hypnotic effects probably relate to a threshold drug concentration. The drugs together gave higher overall concentrations of ethanol for the time sleep was maintained, but lower overall concentrations of phenobarbitone. Sleep was shortened. The antagonism, like the earlier potentiation, was detected most particularly when the doses of phenobarbitone given were high. This antagonism probably related most clearly to a smaller proportion of the phenobarbitone being present in the blood at later times.

The LD50 measurements are more difficult to interpret. Death can result from a short-lived high concentration or a long exposure to a lesser amount of drug. We

found death occurred several hours after administration of the dose, when the drug concentrations had fallen from the peak values. It seems that the animals could withstand early sharp peaks, such as occurred after ethanol alone, or after phenobarbitone under the influence of ethanol, and that LD50 scores appeared to be related more to the time during which a moderately high concentration of phenobarbitone was maintained, so the antagonism seen in the isobol for LD50 (Fig. 4) relates to reduced phenobarbitone concentrations at the later times after administration of the drugs together.

That both antagonism and potentiation can be recorded within one system but at different times is not the expected pattern but it should be borne in mind that drug effects, and drug concentrations in plasma, show the growth and decay pattern typical of many biological systems. The area under the graph of effect or concentration against time is a function of the dose (Harris & Riegelman, 1969). If the dose is unchanged, the area is unchanged, but if the rate of rise of concentration, or the speed of onset of effect is changed by any secondary influence, then, for the area to remain the same, compensatory changes must occur in the time and height of the peak, and in the time a high concentration is maintained. Thus, any change in onset showing potentiation will result in antagonism of duration of effect. Additional factors will of course need to be considered if there is a change in the rate of metabolism of the drug, or in the rate of decline of effect.

There is therefore good reason to believe that the nature of the interaction between phenobarbitone and ethanol is largely a function of the concentrations of the drugs in blood. Changes which occur can lead to the recording of potentiation or antagonism, depending on the particular effects observed.

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