5. Isaacs, A., Adv. Virus Res., 1963, v10, 1.

6. Eckman, P. L., King, W. M., Brunson, J. G., Am. J. Path., 1958, v34, 631.

7. Dougherty, R. M., Groupé, V., Proc. Soc. Exp. Biol. and Med., 1957, v95, 593.

8. Nemes, M. M., Hilleman, M. R., ibid., 1962, v110, 500.

9. Wagner, R. R., Snyder, R. M., Hook, E. W., Luttrell, C. N., J. Immunol., 1959, v83, 87.

10. Gresser, I., Naficy, K., Proc. Soc. Exp. Biol. and Med., 1964, v117, 285.

11. Michaels, R. H., Weinberger, M. M., Ho, M., New Eng. J. Med., 1965, v272, 1148.

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Influence of Route and Concentration of Ethanol Upon Central Depressant Effect in the Mouse. (31524)

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Hiestand *et al*(1) reported that a fixed dose of ethanol, administered intraperitoneally to mice in increasing concentration, produced an increasing rate of mortality. Similar observations were made by Aston and Cullumbine(2), who found an inverse relationship between the concentration and the LD50 of ethanol solutions administered intraperitoneally to mice. The present report describes the relationship between the concentration of ethanol, administered orally, intraperitoneally and intravenously, and its hypnotic effect in mice, as measured in terms of potentiation of a fixed dose of hexobarbital.

Methods. Male albino mice of the Holtzman strain weighing from 19 to 40 g were housed in stainless steel cages with exterior dimensions of 24 × 19.5 × 19.0 cm in aggregates of approximately 8 per cage. Their diet consisted of Rockland Chow and water. The lighting of the animal quarters was automatically controlled to provide 12 hours of artificial light alternating with 12 hours of darkness. Room temperature was maintained at 74°F. The mice were kept in their quarters at least 3 weeks prior to experimentation to allow adequate environmental adaptation.

Hexobarbital sodium was administered in a dose of 75 mg/kg intraperitoneally *(i.p.)* as a 1% solution which was made isotonic with sodium chloride. Ethanol solutions of 30, 50, 70 and 90% (vol/vol) were made up in water with sodium chloride added to make the solutions isotonic. These solutions were administered by the oral *(p.o.)*, intraperitoneal *(i.p.)* or intravenous *(i.v.)* route in combination with the barbiturate and sleeping times were recorded. All doses of alcohol reported are expressed in terms of absolute ethanol.

The mice were divided into 3 major groups on the basis of the route of ethanol administration. One group received 3 g/kg ethanol *p.o*,. 5 minutes prior to hexobarbital. Another group received 2 g/kg ethanol *i.p.* simultaneously with hexobarbital. A third group received 1.5 g/kg ethanol *i.v. via* the dorsal tail vein, 4 minutes after barbiturate administration. Sleeping times were measured as the time elapsing between the loss of righting reflex and the point at which the anesthetized mice righted themselves.

Statistical procedures employed in this report were those outlined by Burn *et al*(3). The significance of differences between the mean sleeping times was evaluated by application of the 'Student' t-test. The letters S.E. in this paper refer to the standard error of the mean.

Results. The results are given in Table I. The 3 control series, in which hexobarbital was given by the *i.p.* route, provided mean sleeping times of 33.5, 36.0, and 41.5 minutes. These values were not statistically homogeneous, indicating that day-to-day variation in sleeping time was significant. An attempt to reduce this error was made by insuring that all treatments, within one group,

TABLE I. Sleeping Times (Min) ± S.E. of Male Mice Receiving Ethanol with 75 mg/kg Hexobarbital Sodium I.P.

Ethanol concentration (vol %)	Ethanol route		
	Oral	Intraperitoneal	Intravenous
0	33.5 ± 1.84* (16)†	36.0 ± 2.63* (13)	41.5 ± 1.35* (13)
30	74.0 ± 4.35 (17)	83.5 ± 2.84* (13)	72.5 ± 2.84 (12)
50	76.0 ± 5.35 (16)	97.0 ± 6.79 (13)	73.0 ± 3.00* (13)
70	78.5 ± 3.98 (16)	103.5 ± 3.48* (12)	96.0 ± 4.14* (10)
90	75.5 ± 5.51 (16)	84.0 ± 5.31 (12)	155.5 ± 8.55 (10)

* Significantly different (P = 0.01) from value following in the Table.
† No. of animals.

were given on the same calendar dates.

The groups of mice receiving ethanol *p.o.*, *i.p.*, or *i.v.*, in addition to hexobarbital *i.p.*, all showed mean sleeping times which were significantly greater than those observed in their respective barbiturate control groups (P = 0.01). The relationship between ethanol concentration and sleeping time differed, however, in the case of each of the 3 routes of administration.

In the oral ethanol series, the 4 mean sleeping times ranged from 74.0 to 78.5 minutes. These values do not differ statistically one from another, indicating the absence of a relationship between ethanol concentration and depressant effect by this route.

Mice to which ethanol was administered *i.p.* exhibited a positive correlation between alcohol concentration and mean sleeping time, ranging from 83.5 minutes with 30% to 103.5 minutes with 70%. When the concentration of *i.p.* ethanol was increased from 70 to 90%, however, a significant reduction (P = 0.01) in mean sleeping time of 19.5 minutes was observed. The relationship between alcohol concentration and depressant effect, by the *i.p.* route, therefore, appears to be a convex one with a maximum at, or near, 70%.

In the series of mice receiving ethanol *i.v.*, alcohol concentrations of 30 and 50% elicited mean sleeping times which were statistically equivalent. Increasing the concentration of ethanol to 70 and 90% produced progressive increases in mean sleeping time which were significant (P = 0.01). This indicates that *i.v.* alcohol concentrations greater than 50% show a direct relationship to depressant effect.

The mortality rate of animals receiving 1.5 g/kg ethanol *i.v.* in varying concentrations, without concomitant hexobarbital, was also found to be proportional to the concentration employed between 50 and 90%. All deaths were immediate. The percent mortality at 50, 70 and 90% was 18.75 in 16 mice, 52.38 in 21 mice and 68.75 in 32 mice respectively. Analysis of these data provided the regression equation: $y = 4.70 + 5.31x$, in terms of log concentration and probit. The LC50 (lethal concentration) ± S.E. was calculated to be 71.45 ± 1.08 with 95% confidence limits of 62.13 and 82.17.

Discussion. Combined administration of ethanol and hexobarbital, as an index of the central depressant effect of the alcohol, was employed in this study because it was found that, in attempting to produce anesthesia of about one hour's duration with ethanol alone, a discouragingly high mortality rate was encountered. It is assumed in this investigation that any differences in sleeping time, observed as the result of administration of varying dosages of ethanol with a fixed dosage of hexobarbital, must be due to the ethanol given.

Because of the method employed, it is impossible to make direct comparisons between routes of administration of ethanol and absolute sleeping time. This could be done only if one were certain that the peak blood levels of alchohol and hexobarbital were coincident in every animal tested, as has been pointed out by Gruber(4). The data, however, do exhibit trends in sleeping time in relation to ethanol concentration for each route of administration employed, and these trends may be meaningfully compared.

The results of the present study indicate that the central depressant effect of ethanol does not vary significantly when the agent is administered *p.o.* in concentrations ranging from 30 to 90%. This is in keeping with observations by Rasmussen(5) who found the blood level of ethanol in rats was inversely re-

lated to the oral concentration administered up to 21% and that in concentrations above 21% the blood level remained nearly constant. This may be due to irritation and hemorrhagic erosion of the gastric mucosa, as observed by Williams(6) in the guinea pig receiving ethanol *p.o.* in concentrations of 20% or more. It has been suggested that in man such irritation reduces gastric absorption of the drug and also initiates pylorosplasm, preventing the passage of alcohol into the duodenum, from which site it is rapidly absorbed (7).

When a constant dose of ethanol was administered *i.p.*, in this study, the depressant effect of the drug increased with the concentration up to 70%. In a concentration of 90%, *i.p.* ethanol showed a significantly reduced central depressant effect, compared to 70%. These observations are similar to those made by Hiestand *et al*(1), who found that mortality rate among mice, resulting from the *i.p.* administration of 120 mg ethanol per mouse, increased from zero to 88% as ethanol concentration was increased from 12 to 60%, but failed to increase further as ethanol concentration was increased beyond 60%. This was corroborated by Aston and Cullumbine (2) who observed that the *i.p.* LD50 of ethanol in mice was reduced from 6093 to 3745 mg/kg when the concentration was increased from 30 to 50%. It has been suggested by Hiestand *et al*(1) that the lack of correlation between mortality and higher concentrations of ethanol *i.p.* may be due to poor intraperitoneal absorption resulting from coagulation of protein in peritoneal surfaces by the more concentrated ethanol solutions.

Administered *i.v.*, the depressant effect of a fixed dose of ethanol proved to be proportional to the concentration in which it was employed, at least in concentrations greater than 50%. Similarly, a direct relationship was observed between mortality and the concentration of 1.5 g/kg ethanol *i.v.* This is likely a reflection of a positive correlation between blood concentration and central depressant effect, and corroborates the suggestion that the non-linear concentration response data obtained by the *p.o.* and *i.p.* routes is the result of alterations in the rate of permeation of the gastrointestinal and peritoneal barriers respectively.

Summary. The central depressant effect of ethanol has been assessed in male mice in terms of the extent of prolongation of hexobarbital sleeping time produced. The dosage of alcohol was varied in terms of both concentration (30, 50, 70 and 90 vol%) and route of administration (*p.o.*, *i.v.*, and *i.p.*). Correlation between concentration and depressant effect of ethanol was absent by the *p.o.* route and positive by the *i.v.* route. When administered *i.p.*, the depressant effect of ethanol increased with increasing concentration up to 70%, but showed a significant reduction at 90%.

1. Hiestand, W. A., Stemler, F. W., Wiebers, J. E., Quart. J. Studies Alc., 1952, v13, 361.

2. Aston, R., Cullumbine, H., Toxicol. Appl. Pharmacol., 1959, v1, 65.

3. Burn, J. H., Finney, D. J., Goodwin, L. G., Biological Standardization, Oxford Univ. Press, London, 1950.

4. Gruber, C. M., Arch. intern. pharmacodynamie, 1955, v102, 17.

5. Rasmussen, E. W., Biochem. Z., 1940, v304, 358.

6. Williams, A. W., Brit. Med. J., 1956, v1, 256.

7. Ritchie, J. M., The Pharmacological Basis of Therapeutics, Goodman, L. S., Gilman, A., eds., The Macmillan Co., New York, 1965, p143.

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