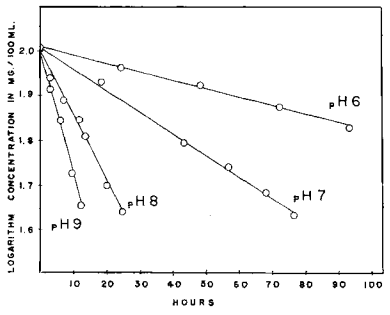

Fig. 1—Pseudo first-order hydrolysis of methylparaben at 85°.

for half of a given amount of methylparaben to decompose can be evaluated by dividing 0.693 by the velocity constant (14). Figure 1 is typical of the results obtained. The experimentally determined velocity constants and half-life periods are given in Table I.

As the hydrolysis appears to follow a first-order relationship, the energy of activation E_a was calculated using the logarithmic form of the Arrhenius equation between the limits of absolute temperature T_2 and T_1 (15).

$$\log \frac{k_2}{k_1} = \frac{E_a}{2.303\,R}\left(\frac{T_2 - T_1}{T_2 T_1}\right)$$

The apparent energy of activation is approximately 24 Kcal./mole. The value for the velocity constant for the reaction was calculated at 25 and 121°. The calculated velocity constants and half-life periods at 25° are listed in Table II.

As autoclaving may be required of certain pharmaceutical solutions, the per cent decomposition of a methylparaben solution in an autoclave was experimentally determined. After autoclaving for 30 min. at pH 6 and 9, there remained 94.5 and 58.0% of the initial concentration, respectively. Using $k_{121°} = 0.105$ hr.$^{-1}$ at pH 6 as calculated from the Arrhenius equation, the predicted decomposition was 5.13%; the experimental decomposition was 5.5%. Likewise, at pH 9 the predicted loss was 48.36%; the experimental decomposition was 42.0%.

TABLE I—HYDROLYSIS OF METHYLPARABEN AT VARIOUS TEMPERATURES AND pH'S

Temp.	pH		$k \times 10^2$	$t_{1/2}$
70°	6.0	±0.050	0.123 hr.$^{-1}$	565.2 hr.
	8.0		0.737	94.0
	9.0		1.842	37.6
80°	6.0		0.189	364.7
	8.0		2.303	30.0
	9.0		4.900	14.1
85°	6.0		0.485	142.9
	7.0		1.076	64.7
	8.0		3.290	21.0
	9.0		6.810	10.1

TABLE II—THEORETICAL VELOCITY CONSTANTS AND HALF-LIFE PERIODS FOR THE HYDROLYSIS OF METHYLPARABEN AT 25° AS CALCULATED FROM AN ARRHENIUS PLOT

pH	$k \times 10^5$	$t_{1/2}$
6.0	0.432 hr.$^{-1}$	6675 days
8.0	3.236	892
9.0	6.998	412

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Possible Ethanol-Induced Tolerance in Rats

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A tilting-plane technique was employed to determine the effects of alcohol on performance of rats with specific attention to development of possible tolerance. Significant differences were obtained only on the first day of treatment with possible tolerance developing in only 1 day. No significant learning effects could be shown over the 3-week period.

A VARIETY of tests have been employed for determining performance in humans and animals while under alcohol administration (1–6). The many variables in response to pretreatment, age, sex, strain, and stress confounds the study of tolerance to alcohol using these tests (7–16). Behavioral tolerance has been investigated (17) and was reported in monkeys following once daily administration, 2 Gm./Kg. i.v., for only 4 consecutive

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days (18). Metabolic tolerance has been indicated by a lower blood alcohol level as early as the third day in self-maintained intoxicated rats receiving increased alcoholic intakes (19), and it has been postulated that metabolic tolerance may serve as a limiting factor in alcohol consumption (20). The rate of metabolism of ethanol in humans has been shown to be increased after a 3 to 14-day period of induced intoxication (21). The same has been shown in rats along with fine structural changes in the liver (22, 23).

It was the purpose of this study to demonstrate the effect of alcohol in rats through the use of a tilting-plane technique with specific attention to the development of any degree of tolerance observed over a 2-week administration period.

EXPERIMENTAL

The technique, the tilting-plane technique, proposed by Arvola, Sammalisto, and Wallgren appeared most satisfactory for quantitating equilibrium and motor coordination (24). The test is suitable for differentiating certain degrees of alcohol intoxication. The rat is placed on a board, the board is tipped up, and the angle at which the animal slides is determined. The surface of the plane is rough and the rat clings with its claws to the surface. A deeply intoxicated rat cannot cling to the plane and slides down the plane at a lower angle than an unintoxicated rat. The method has been used and found satisfactory by others (24–27).

Twenty Wistar male rats, 200 Gm. average, were used. Ten served as a control group and 10 were used in the test group.

The first week without any drug administration both groups were put on the tilting-plane and 10 readings of each animal on each of 4 consecutive days were made for control, learning, pretreatment averages. The second and third weeks, the test animals received 2 Gm./Kg. body weight of a 10% solution of ethanol i.p. This dose, 2 Gm./Kg., was chosen as that maximally tolerated and in best agreement with that used in the literature. At this dosage, the animals at first appeared nearly unconscious, but activity after 60 min. was satisfactory for testing on the tilting-plane.

The controls were injected with an equivalent volume of normal saline thus subjecting them to the same handling (stress) as the test group.

Both groups were again placed on the tilting-plane and 10 readings of each animal, on each of 4 consecutive days, for the second and third weeks were made. Readings were taken 60 min. after injections. The room temperature was maintained at 24°.

The tilting-plane was made from rough pine wood with the dimensions being 23.5 × 44 cm. All animals were placed on the plane and raised through an angle of 90° in 5 sec. and the angle at which the animals slid was recorded.

The averages of all readings were determined and the t test of significance performed.

RESULTS AND DISCUSSION

After the first week of alcohol injections, approximately 50% of the rats appeared to enjoy the injection, they were much easier to handle, and would remain quiet during the injection. The same was not true for the saline injected rats. From the averages of the readings taken, the comparisons with t tests and standard deviations were calculated and are shown in Tables I, II, and III.

In the tables, the animals are referred to as control and test groups. A further delineation as to treatment periods within groups is indicated by "Control Learn, Control Test" and "Test Learn, Test Test." The first day control learn indicates the first day the control group was placed on the tilting-plane during the first week learning period. Likewise, the first day test learn indicates the first day the test group was placed on the tilting-plane during the first week learning period.

The first day control test indicates the first day the control group, receiving comparable volume saline injections, was placed on the tilting-plane during the first week of the test period, actually the second week in the test program. Likewise, the first day test test indicates the first day the test group receiving alcohol injections was placed on the tilting-plane during the first week of the test period, again, actually the second week in the test program.

Furthermore, the sixth day control test would indicate the sixth day the control group, receiving the saline injections, was placed on the tilting-plane during the second week of the test period, actually the third week in the test program. Likewise, the sixth day test test indicates the sixth day the test group receiving alcohol injections was placed on the tilting-plane during the second week of the test period, again actually the third week in the test program.

These results show that in the test animals there was a significant difference between the first day trial on the tilting-plane and the first day of test (t = 5.79), and also the fourth day of trial and the first day of test (t = 3.41). Likewise, the first and fourth day control learn animals showed a significant difference from the first day test test animals (t = 4.03 and t = 5.22, respectively). The first day control test animals were not significantly different from the first day test test animals at P 0.05 but were at P 0.1 (t = 2.09), a t value greater than 2.101 being required at P 0.05.

However, in a comparison of the second day of trial on the plane and the second day of test, in all cases of comparison, there were no statistically significant differences. The animals were apparently not as intoxicated the very next day after receiving the same dosage of alcohol. Therefore, it would appear possible to induce alcohol tolerance in 1 day as determined by this method. Behavioral tolerance has been reported to occur in monkeys in 4 days and a metabolic tolerance has been reported in rats in 3 days (18, 19).

Alcohol metabolism may have been altered very rapidly in this study, although Wistar rats have been shown to select less alcohol than G-4 rats when given free choice (9). What this would imply regarding the respective strains' metabolism is questionable. The animals used in this study were males, and males also have been shown to drink more alcohol than females, another possible factor influencing tolerance (11). Operant performance has also been shown to vary between alcohol-preferring and nonpreferring animals (12).

Statistical evaluation of control animals showed no significant difference in these animals over the

whole 3-week period. Apparently with this method, learning played no significant part in this study. It has been reported, however, that alcohol-treated rats appear to learn more slowly (2).

All of the animals' weights were followed; however, no significant differences were determined between the control and test animals.

TABLE I.—STANDARD DEVIATIONS AND *t* VALUES OF VARIOUS CONTROL AND TEST GROUP ANIMALS

	S.D.	*t*
First week test test *vs.* second week test test	5.08	1.64
First week control test *vs.* first week test test	5.46	0.89
800 Control test *vs.* 800 test test	4.66	1.07
First day control test *vs.* first day test test	6.03	2.09[a]
Second day control test *vs.* second day test test	6.36	0.40
Third day control test *vs.* third day test test	6.06	1.44
Fourth day control test *vs.* fourth day test test	5.70	1.09
Fifth day control test *vs.* fifth day test test	4.35	0.99
Sixth day control test *vs.* sixth day test test	4.46	1.15
Seventh day control test *vs.* seventh day test test	4.88	1.71
Eighth day control test *vs.* eighth day test test	4.57	1.45

[a] Significant at *P* 0.1.

TABLE II.—STANDARD DEVIATIONS AND *t* VALUES OF VARIOUS CONTROL AND TEST GROUP ANIMALS

	S.D.	*t*
First day control learn *vs.* first day control test	5.63	0.62
Second day control learn *vs.* second day control test	5.02	0.66
Third day control learn *vs.* third day control test	4.64	0.87
Fourth day control learn *vs.* fourth day control test	4.11	0.54
First day test learn *vs.* first day test test	3.50	5.79[a]
Second day test learn *vs.* second day test test	6.78	1.51
Third day test learn *vs.* third day test test	6.53	1.00
Fourth day test learn *vs.* fourth day test test	6.74	0.55
Fourth day test learn *vs.* first day test test	5.59	3.41[a]
Fourth day control learn *vs.* first day test test	4.36	5.22[a]
First day control learn *vs.* fourth day control learn	3.79	1.73
First day control learn *vs.* fourth day control test	3.71	0.42
First day control learn *vs.* eighth day control test	3.79	1.58
First day test learn *vs.* fourth day test learn	4.80	0.26
First day test learn *vs.* fourth day test test	5.13	0.48
First day test learn *vs.* eighth day test test	3.63	1.35

[a] Significant at *P* 0.05.

TABLE III.—STANDARD DEVIATIONS AND *t* VALUES OF VARIOUS CONTROL AND TEST GROUP ANIMALS

	S.D.	*t*
First day control learn *vs.* first day test learn	2.76	1.49
First day control learn *vs.* fourth day test learn	5.16	0.53
First day control learn *vs.* first day test test	3.99	4.03[a]
First day control test *vs.* first day test test	6.03	2.09
First day control test *vs.* fourth day test test	7.11	1.42
First day control test *vs.* eighth day test test	6.27	0.41

[a] Significant at *P* 0.05.

SUMMARY AND CONCLUSIONS

Male Wistar rats were subjected to a tilting-plane technique to determine the effects of alcohol on their performance. Subjected first to a 1-week learning period, this was followed by a 2-week test period. Control rats received normal saline and test rats received 2 Gm./Kg. of alcohol. All animals were placed upon the tilting-plane and the angle at which they would slide off was recorded. Significant differences were obtained between first day control learn *versus* first day test test, first day control test *versus* first day test test, first day test learn *versus* first day test test, fourth day test learn *versus* first day test test, and fourth day control learn *versus* first day test test. As measured by this method, tolerance would seem to appear after only 1 day. No significant learning effect could be shown by this method in the control animals over the entire 3-week period.

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