binations of fixatives and dehydrating agents appeared to introduce artifacts, some of these being visible in the photomicrographs.

No photomicrograph is submitted for ascites cells not exposed to tetracycline since at the particular wavelength employed there is no autofluorescence; hence, it is not possible to take a photomicrograph of the cells against the darkfield background. The first five photomicrographs were taken of cells before exposure to dehydrating agents. Figure 1 illustrates the appearance of tetracycline-containing tumor cells freshly removed from the host animal but having been subjected to no treatment other than their removal from the host. They may be considered as control preparations. In such cells the tetracycline seems to be located in the cytoplasmic portion of the cell, with concentration in the perinuclear zone. Figure 2 shows the effect of 4% formalin fixation for 2 min. There is an apparent loss of definition and diffusion of the tetracycline. Higher concentrations of formalin or longer fixation periods further exaggerated these observations. Figure 3 shows the effect of diethyl ether–alcohol fixation for 2 min. The tetracycline complex appears to be precipitated as judged by the altered pattern of fluorescence. The effect of acetone fixation is shown in Fig. 4. There is extensive blebbing with the suggestion that tetracycline has diffused into the karyoplasm. Figure 5 is a photomicrograph of cells fixed with glutaraldehyde. One sees an apparent intensification of the fluorescence. This was a consistent finding in all glutaraldehyde preparations.

The remaining photomicrographs demonstrate the effects of several dehydrating agents. Figure 6 illustrates the result of acetone dehydration following glutaraldehyde fixation. Even when the total dehydrating time is kept to 4 min. there appears to be a reduced fluorescence. The use of ethanol dehydration following glutaraldehyde fixation (Fig. 7) seems to reduce sharpness of detail of the preparation although there is no loss of fluorescence. Areas of bright fluorescence in some of the intercellular spaces suggests a possible leakage of fluorescent substance from the cells. The final photomicrograph (Fig. 8) shows the result of glutaraldehyde fixation followed by glycerol dehydration in accordance with the protocol described in this report. There is no loss of cellular detail, nor any apparent loss or distortion of fluoresence.

REFERENCE

(1) Cailleau, R., and Costa, F., *J. Natl. Cancer Inst.*, **26**, 271(1961).

Hydrolysis of Methylparaben

By N. N. RAVAL and E. L. PARROTT

The hydrolysis of methylparaben in aqueous solution at 70, 80, and 85° in pH range from 6 to 8 has been studied.

SINCE their introduction into the United States in the 1930's, the parabens have been widely used as preservatives in foods, drugs, and cosmetics (1–5). The lack of toxicity and the broad spectrum of preservative activity of methylparaben have been reported (6–8). The correlation of preservative activity with the binding of methylparaben to other substances has led to more effective and intelligent product formulation (9–11).

Methylparaben is stable in air. Aqueous solutions of methylparaben buffered at pH 3 and 6 showed no decomposition when heated for 2 hr. at 100° or for 30 min. at 120° (12). As the removal of the ester portion by hydrolysis yields *p*-hydroxybenzoic acid, which possesses insignificant preservative activity, this investigation was undertaken to study the extent of hydrolysis of methylparaben in a pH range utilized in pharmaceuticals.

EXPERIMENTAL

A 0.1-Gm. quantity of methylparaben[1] was accurately weighed into a 100-ml. volumetric flask and dissolved in a sufficient amount of buffer to bring to a volume of 100 ml. of solution. The solution was filled and sealed into 10-ml. ampuls. The ampuls were placed in appropriate constant-temperature baths, and after thermal equilibrium was attained, an ampul was removed and, by means of iced water, was cooled to 25°. This solution was analyzed for zero-time concentration of methylparaben.

Five milliliters of the solution was withdrawn, adjusted to pH 7, and sufficient distilled water was added to bring the volume to 100 ml. A 10-ml. aliquot was extracted with four 10-ml. portions of anhydrous ether. The ether was evaporated and the residue was dissolved in 100 ml. of distilled water. The absorbance of the solution was measured at 255 mμ using a Beckman DU spectrophotometer. By means of a standard absorption curve, the concentration of methylparaben was determined. Ampuls were removed at definite intervals of time and analyzed by this procedure, which is essentially that of Aalto *et al.* (1).

All chemicals were reagent grade. McIlvaine's buffer was used for pH 6 and 7; Palitzsch's buffer was used for pH 8 and 9 (13). All buffer solutions were adjusted at and the pH determined at the temperature of hydrolysis by means of Beckman Zeromatic pH meter.

RESULTS AND DISCUSSION

The rate of hydrolysis of methylparaben was followed at pH 6, 8, and 9 at 70, 80, and 85°. A plot of the logarithm of concentration against time produced a straight line. The observed velocity constant k can be evaluated by multiplying the slope of the line by 2.303. The half-life or time required

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[1] The methylparaben used was Methyl Parasept, Heyden Newport Chemical Corp.

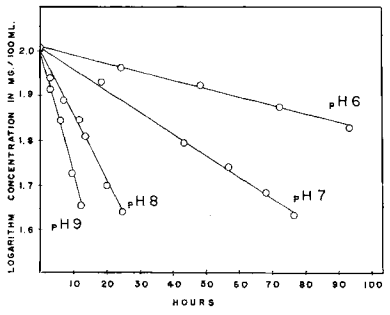

Fig. 1—Pseudo first-order hydrolysis of methylparaben at 85°.

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

REFERENCES

(1) Aalto, T. R., Firman, C. C., and Rigler, N. E., *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 449(1953).
(2) Neidig, C. P., and Burell, H., *Drug Cosmetic Ind.*, **54**, 408(1944).
(3) Gottfried, N. S., *Am. J. Hosp. Pharm.*, **19**, 310(1962).
(4) Sokol, H., *Drug Std.*, **20**, 89(1962).
(5) Wedderburn, D. L., "Advances in Pharmaceutical Sciences," Academic Press Inc., New York, N. Y., 1964, p. 195.
(6) Matthews, C., Davidson, J., Bauer, E., Morrison, J., and Richardson, A., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 260(1956).
(7) Jones, P. S., Thigpan, D., Morrison, J., and Richardson, A. P., *ibid.*, **45**, 268(1956).
(8) Lawrence, C. A., *ibid.*, **44**, 457(1955).
(9) Pisano, F. D., and Kostenbauder, H. B., *ibid.*, **48**, 310(1959).
(10) Blaug, S., and Ahsan, S., *J. Pharm. Sci.*, **50**, 441 (1961).
(11) Guttman, D., and Higuchi, T., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 659(1956).
(12) Phares, C., Goldschmidt Chemical Corp., personal communication.
(13) Britton, H. T. S., "Hydrogen Ions," 4th ed., Chapman and Hall Ltd., London, England, 1955, vol. I, pp. 356, 361.
(14) Hamill, W. H., and Williams, R. R., Jr., "Principles of Physical Chemistry," Prentice-Hall, Inc., Englewood Cliffs, N. J., 1959, Chap. XII.
(15) Daniels, F., and Alberty, R. A., "Physical Chemistry," 2nd ed., John Wiley & Sons, Inc., New York, N. Y., 1961, p. 315.

Possible Ethanol-Induced Tolerance in Rats

By THEODORE H. EICKHOLT, LEO J. SCHILLACI*, and S. ALAN SEARCY*

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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