cylic acid is presented for the study of liver aldehyde oxidase. By the use of this method it was observed that aldehyde oxidase is inhibited by epinephrine and other adrenergic amines as well as by adrenergic blocking agents. It was observed that inhibition by the first group of substances is reversible and competitive while inhibition by adrenolytic drugs is irreversible and non-competitive.

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Ionization Constants of Basic Dyes in Relation to Gastric Secretion.* (20286)

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Since Ingraham and Visscher(1) have shown that basic dyes are secreted by the stomach, a study was undertaken to see whether or not there was a relation between the ionization constants of the dyes and their ability to be secreted by the stomach. Ray and Jung(2) have determined the ionization constants of basic dyes from titration data in unbuffered 50% alcoholic solutions, and attempted to correlate them with their tendency to be secreted by the stomach. However, Ray and Peters(3) noticed certain discrepancies in their data. In the hope of finding an explanation for these discrepancies, Woislawski (4) redetermined the ionization constants of the dyes, which Ray and Jung have used, by titration in aqueous medium. He also noticed discrepancies between his values and those found by Ray and Jung.

The present study was undertaken because it was hoped that the determination of the ionization constants of the dyes by a different method(5), namely, from spectrophotometric data in buffered 50% alcoholic solutions, might shed some light on these discrepancies.

Method. A stock solution was prepared by dissolving 1 x 10^{-4} M of the dyes in 95% alcohol. To 10 ml of the stock solution in a 100 ml volumetric flask was at first added either 50 ml N HCl, N/10 HCl, phthalate buffer pH 3.0, acetate buffer pH 5.0, phosphate buffer pH 6.7, borax-HCl buffer pH 8.2, N/10 NaOH or N NaOH, which was then diluted to the mark with 95% alcohol. The final concentration of the dyes was thus 1 x 10^{-5} M. In both the buffered and unbuffered solutions the pH shifted toward the alkaline region upon dilution with alcohol. The absorption spectra were measured using a Beckman Model DU spectrophotometer. Spectra were measured from 400-700 mμ at 24 $\pm$ C. For the determination of pH a Beckman pH meter (model G) with glass electrode 1190T and calomel electrode 1170 was used. When the pH rose above 10 a Beckman glass electrode 1190E was used. Because the pKa cannot be converted to pK_B with any degree of accuracy in 50% alcoholic solutions, only pKa values are given in this paper. The pKa of the dyes was determined by the formula of Flexser *et al.*(6), $pKa = pH_m + \log E_B\text{-}E_m/$

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TABLE I. Comparative Values of Basic Ionization Constants Obtained by Different Methods.

Compound	pKa (buffered 50% alcohol)	pKa* (water)	pKa† (unbuffered 50% alcohol)	Buffer* Benzene	Stomach‡ Blood
Nile blue A	1.6	3.1	2.4	§	.8
Bismarck brown Y	4.5	4.8	5.0	§	26.5
Rhodamine B	4.6	3.7	3.2	¶	6.1
Acridine red	5.1	8.1	3.1	¶	8.4
Chrysoidin Y	5.5	5.0	5.3	§	75.0
Neutral red	6.8	6.7	6.5	¶	26.4
Pyronine B	7.1	6.9	7.7	1.96	5.7
Neutral violet	7.3	6.6	3.2	.68	27.2
Methylene green	9.7	4.1	3.2	2.50	13.6
Brilliant cresyl blue	9.9	4.6	3.2	2.70	11.3
Toluidine blue O	10.8	7.9	7.5	2.90	36.4
Thionin	11.2	6.8	6.9	11.60	36.8
Safranin O			6.4	5.60	27.3
Methylene blue			3.8	‖	15.6

* Ref. 7. † Ref. 4. ‡ Ref. 1. § 100% in benzene. ‖ 100% in buffer.
¶ Colorless.

E_m-E_{BH+} where E_B is the optical density of the uncharged molecule of the dye, E_{BH+} that of the charged ion, and E_m represents the density of the mixture of the 2 species; pH_m is the pH of the solution containing a mixture of the 2 species. For very strong bases it was not possible to determine the pKa because the dyes changed color near or above pH 13 which is beyond the limit of the pH meter. In those regions where the concentrations of the uncharged molecule and charged ion were approximately equal, additional spectra for solutions which differed only by a few tenths of a pH unit in either direction were determined.

Results and discussion. In Table I, column 2, the pKa values of the dyes are arranged in the order of increasing basicity in buffered 50% alcoholic solutions as determined from spectrophotometric data. In column 3 are listed the pKa values as determined from titration data in aqueous medium by Woislawski(4). In column 4 are listed the pKa values as determined by Ray and Jung(2) from titration data in unbuffered 50% alcohol. In column 5 are listed the distribution ratios between buffer pH 6.73 and benzene as determined by Woislawski(4). In column 6 are listed the Stomach/Blood ratios as determined by Ingraham and Visscher(1). Fairly good agreement for the ionization constants is obtained by all methods for Nile Blue A, Bismarck Brown Y, Rhodamine B, Chrysoidin Y, Neutral Red and Pyronine B. With the exception of Acridine Red, these are all the weaker bases. The spectrophotometric value is, however, close to the average of the other two. Higher values were found for the other dyes by the spectrophotometric method. The greatest discrepancy is the position of Bismarck Brown Y. With a low pKa it was a high rate of secretion by the stomach. Pyronine B, on the other hand, has a rather high pKa but a low rate of secretion. It seems probable that other factors are important in secretion. The three xanthenes: Pyronine B, Rhodamine B and Acridine Red, are secreted by the stomach in low concentrations (C_s/C_B = 5.7-8.4); their pKas are all low. The 3 azines: Safranin O, Neutral Violet and Neutral Red are much better secreted, and approximately to the same extent (C_s/C_B = 26.4-27.3); this in spite of the fact that Safranin O seems to be a much stronger base. Of the 4 thiazines, Methylene Blue and Methylene Green are secreted to a lesser extent than Thionin and Toluidine Blue O. Methylene Blue is known to undergo hydrolytic cleavage. It is probable that the dialkylimino group more readily undergoes this reaction *in vivo* with the formation of less basic oxazones. This decrease in basicity may account for their lessened secretion by the stomach. Methylene Green differs from Methylene Blue by an ortho nitro group which should further facilitate the hydrolytic cleavage of a dimethylimino group. The two oxazines Brilliant Cresyl Blue and Nile Blue A differ greatly in basicity, the former being a much stronger

base. This alone could explain the greater secretion of the former dye. Nile Blue A is also known to undergo hydrolytic cleavage with the formation of a less basic oxazone. The two azo dyes Chrysoidin Y and Bismarck Brown Y are secreted by the stomach to a much greater extent than one would expect from the pKa values. It is quite possible that some other property plays an equally important role in secretion. Both of these compounds form chelates. While reducing the basicity this molecular configuration may be favorable to gastric secretion. We notice that the spectrophotometric pKa values largely parallel the buffer/benzene distribution ratios, column 5. The slight discrepancy between Neutral Violet and Pyronine B may well be within the margin of the error. Neutral Violet has a slightly larger pKa value than Pyronine B, but a larger buffer/benzene ratio. A more serious discrepancy is that Safranin O appears to be more basic than Thionin from spectrophotometric data, whereas the distribution ratios point to the reverse order. This discrepancy may be due to the limitations of the spectrophotometric method for very strong bases because the meso phenyl group of Safranine O should reduce the basicity. The fact that the ionization constants of the basic dyes have different values in different media is not surprising. It is obvious that the ionization of a base will be repressed in a more basic medium (pancreas) and increased in a more acid medium (stomach). This would result, however, in the ionization constants maintaining the same relative order. The data show that this is not the case. Woislawski(7) has shown that the order of diffusion also changes in different media.

Although the ionization constant of a compound undoubtedly plays an important role in diffusion one should be careful not to extrapolate ionization constants as determined in water to biological media. It was not possible to correlate satisfactorily the secretion of dyes in a biological system with their ionization constants in aqueous or buffered 50% alcoholic solution.

Summary. The ionization constants of 14 basic dyes belonging to 5 chemical classes, azo, azines, xanthenes, oxazines and thiazines, each containing 2-4 compounds, were determined spectrophotometrically in buffered 50% alcoholic solutions. The dyes were those which Ingraham and Visscher have found to be secreted by the stomach. Fairly good agreement for the ionization constants by 3 different methods (spectrophotometric in buffered 50% alcohol, titration in water and in 50% unbuffered alcohol) is obtained for Nile Blue A, Bismarck Brown Y, Rhodamine B, Chrysoidin Y, Neutral Red and Pyronine B. With the exception of Acridine Red these are all the weaker bases. Higher values were found for the other dyes by the spectrophotometric method. The spectrophotometric pKa values paralleled the buffer/benzene ratios. The order of basicity varies in different media. It was not possible to correlate satisfactorily the secretion of dyes in a biological system with their ionization constants in aqueous or buffered 50% alcoholic solution.

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