

first 2 or 3 weeks. There was marked eczema in 2 cases. Some alopecia occurred in a few of the rats. Alkaline phosphatase⁶ in the blood serum was markedly reduced but it was not affected in bone and kidney. The concentration of bone ash was decreased. In the experimental rats the average value was 55.8% and in the controls it was 62.2. There appeared to be some hemoconcentration. Carbonic anhydrase^{7,8} of blood was slightly raised, but the enzyme activity per unit of red cells was unchanged. This is of special interest in relation to the finding that the purified enzyme contains 0.32% Zn.⁹ The average concentration of Zn in the femurs of Zn-deficient rats was 94.7 μg per g of ash and in the controls it was 236.6 μg per g of ash. The method of Caughey, Holland and Ritchie¹⁰ was used to estimate Zn. Detailed histological studies of the tissues will be published subsequently.

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**Influence of H Ion Concentration on Depressant Effect of
Nicotine Solutions on Ciliated Epithelium.**

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Ellisor and Richardson¹ report a considerable difference between the toxicity of nicotine sulfate and the free alkaloid for gold fish. While they did not actually measure the ratio between these toxicities, they were able to derive it from calculations based on their own data, and found the base to be about 6 times more toxic than the salt. They also observed that the base penetrates into gold fish from 5 to 7.5 times more rapidly than the salt. In view of these observations it seemed desirable to ascertain whether or not a similar difference is to be observed in the action of nicotine and its salt on a relatively simple physiological system such as the ciliated epithelium. This tissue was selected because it may be obtained readily from the esophagus of

⁶ Wiese, A. C., Johnson, B. C., Elvehjem, C. A., Hart, E. B., and Halpin, J. G., *J. Biol. Chem.*, 1939, **127**, 411.

⁷ Roughton, F. J. W., *Physiol. Rev.*, 1935, **15**, 241.

⁸ Lambie, C. G., *Edinburgh Med. J.*, 1938, **45**, 373.

⁹ Keilin, D., and Mann, T., *Nature*, 1939, **144**, 442.

¹⁰ Caughey, R. A., Holland, E. B., and Ritchie, W. S., *J. Assn. Off. Agric. Chem.*, 1938, **21**, 204.

¹ Ellisor, L. O., and Richardson, C. H., *J. Cell. and Comp. Physiol.*, 1938, **11**, 377.

the frog; and ciliary activity may be considered a fair index of the physiological state of the tissue.

Experimental Methods. Ciliated epithelium used in these experiments was obtained from *Rana pipiens*. The nicotine was specially prepared, and known to be of a high degree of purity. The salt (acetate) was prepared by the addition of glacial acetic acid until the desired pH was obtained. The ratio between free base and salt in solutions of known pH was obtained by application of the formula $\text{pH} = \text{pK} + \log \frac{\text{base}}{\text{salt}}$, in which pK was found experimentally to be 8.07. At pH 8.07 approximately 50% of the total nicotine is present as the free base, whereas at pH 6.5 only about 2% exists as base, the remainder being in the form of the salt. Solutions at higher or lower pH values cannot be used because of the H-ion effects on the tissue.

The experimental methods are similar in many respects to those described by Richardson² and by Perrine, Thronson, and Tainter;³ although certain modifications were found advisable. The method is as follows: A section of the esophagus 5 to 8 mm in length was removed and divided lengthwise into 2 strips. Each strip was pinned to a piece of cork so that it lay relatively smoothly without being under undue tension. Mucus was removed as completely as possible, usually by gentle mopping with a small pledget of cotton. The tissue was moistened with frog Ringer's solution of approximately the same pH as the nicotine solution being investigated. Ciliary activity was ascertained by measuring the length of time required for a small piece of cork to be swept across a certain distance on the surface of the tissue. For these observations a small lens having a magnification of about 4X was used; and to the under side of the lens were attached 2 parallel hairs about 2 mm apart. Time required for the cork to pass between the 2 hairs was measured with a stop-watch. Two groups of 5 observations each were made on each strip to indicate normal or control conditions. The piece of cork bearing the tissue was then placed, tissue down, into the solution being investigated, and allowed to remain for 5 minutes. It was then removed, and another series of 5 observations made. The average of these 5 observations was then compared with the average of the 10 observations previously made to indicate the change in ciliary activity. Observations on several hundreds of strips, both control and experimental, indicate that changes of less than 25% are of doubtful significance. In most in-

² Richardson, A. P., *J. Pharmacol. and Exp. Therap.*, 1937, **59**, 101.

³ Perrine, R. L., Thronson, A. H., and Tainter, M. L., *J. Dental Research*, 1939, **18**, 81.

TABLE I.
Influence of pH on Depression of Ciliary Activity Caused by Solutions of Nicotine

| pH | Concentration % | Control time, sec | Experimental time, sec | Control | | Remarks |
|------|--------------------|-------------------------|---------------------------|--------------|---------|--------------------------|
| | | | | Experimental | Control | |
| 6.5 | 1.0 | 4.43 | 5.02 | 0.882 | | |
| " | 2.0 | 4.27 | 8.62 | 0.495 | | |
| " | 4.0 | 5.27 | 12.92 | 0.408 | | |
| " | 6.0 | 4.91 | | | | All sections stopped |
| 8.0 | 0.5 | 4.36 | 5.76 | 0.757 | | |
| " | 1.0 | 4.69 | 13.02 | 0.360 | | |
| 8.10 | 1.5 | 4.45 | 15.60 | 0.285 | | 5 of 10 sections stopped |
| 8.05 | 2.0 | 4.39 | | | | All sections stopped |

stances 10 strips were used for each concentration of nicotine at each of the hydrogen ion concentrations.

Results. In Table 1 are shown the effects of nicotine solutions of different concentrations at pH 6.5 and pH 8.0-8.1. As stated above, at pH 8.07 approximately 50% of the total nicotine is present as the free base, whereas at pH 6.5 only about 2% exists as the base, the remainder being in the form of salt. Thus, a threefold increase in total nicotine was necessary to produce the same physiological effect (stoppage of ciliary motion) at pH 6.5 as at pH 8.05. At the same time there was a decrease in free nicotine content from 50% to 2%. At pH 10 more than 99% of the nicotine is present as the free base, but such a solution could not be used because of the OH-ion effect. Therefore, it was not possible to make a direct comparison between solutions containing practically no free base and others containing practically no free salt. However, since an increase in free base from 2% to 50% increases toxicity threefold, a further increase in free base to 100% might reasonably be expected to cause a proportionate increase in strength of action to about six times that of the salt, a ratio in satisfactory agreement with that reported by Ellisor and Richardson.¹

Conclusion. A change in the physiological activity of a nicotine solution accompanies a change in pH of the solution when the phenomenon of transport across membranes is involved. An increase in pH causes an increase in the proportion of free base present, and, as pointed out by Ellisor and Richardson,¹ an increase in the rate at which nicotine passes through membranes. This is believed to account for the fact that a solution containing equal parts of nicotine acetate and the free base is about three times more effective in inhibiting ciliary activity than a solution containing about 98% of the acetate.