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Influence of pH on Absorption of Nicotine from Urinary Bladder and Subcutaneous Tissues.

JANET TRAVELL.*

From the Department of Pharmacology, Cornell University Medical College, New York City.

It has been shown that the pH plays an important rôle in the absorption of alkaloids from the stomach.[1] So far as we know, no studies are available as to the influence of this factor on the absorption of these compounds from the urinary bladder. It seemed likely, however, that this membrane might behave like the gastrointestinal mucosa and absorb alkaloids quite rapidly from alkaline solutions. Nicotine was selected for the study because of the possibility that its reabsorption from the urinary bladder may be a source of poisoning in heavy tobacco smokers when the urine becomes alkaline.[2]

Experiments were carried out, therefore, to determine the influence of the pH on the absorption of nicotine from the urinary bladder. Under light ether anesthesia the ureters were ligated in 10 cats and a cannula inserted in the urethra for injection. The bladder was washed with physiological salt solution, and nicotine was injected in a buffered solution. Phthalates, phosphates and sodium bicarbonate were used as buffers. The volume of fluid injected in each experiment was 5 cc per kg. The pH of the solution injected into the bladder and of that recovered from the bladder 4 hours later or at death was determined electrometrically (glass electrode).

The results are shown in Table I. Figures are given for the time of onset and duration of the advancement of the nictitating membrane; the duration of this effect parallels closely the degree of nicotine poisoning. It may be seen that at pH levels of about 4.5 to 6.0, doses of 10 and 20 mg of nicotine produced no effects. Similar doses at pH levels of about 7.0 to 7.9 produced fairly marked poisoning; advancement of the nictitating membrane appeared in 20 to 30 minutes after injection and was present for an average of 53 minutes. At pH levels of about 8.0 to 9.0, 10 mg caused very severe

* With the assistance of Charles Salzman, B.S.

1 Travell, J., *J. Pharm. and Exp. Ther.*, 1940, **69**, 21.

2 Travell, J., Bodansky, O., and Gold, H., *J. Pharm. and Exp. Ther., Proc.*, 1940, **69**, 307.

TABLE I.
Influence of pH on Absorption of Nicotine from Ligated Urinary Bladder.

Cat No.	pH of solution injected into bladder	pH of solution recovered from bladder*	Dose of nicotine injected into bladder† mg per kg	Advancement of nictitating membrane onset, min	Advancement of nictitating membrane duration, min	Degree of nicotine poisoning	Calculated concentration of undissociated nicotine base in solution injected
1	4.6	4.7	10	—	0	None	1:1,250,000
2	5.4	5.8	20	—	0	"	1:83,000
3	7.2	7.0	10	24	19	Moderate	1:3,300
4	7.9	7.4	10	21	50	Marked	1:1,000
5	7.0	6.8	20	30±	54	"	1:2,500
6	7.9	7.0	20	20	90	"	1:520
7	8.6	8.1	10	5	180	Very marked	1:630
8	8.7	8.1	10	10	148	" "	1:590
9	9.0	9.0	20	5	—	Fatal 17 min	1:270
10	9.0	8.9	20	6	—	" 40 "	1:270

*4 hours after injection or at death.
†Injected in a volume of 5 cc per kg.

nicotine poisoning; advancement of the nictitating membrane appeared much sooner and persisted for a much longer period of time, 2½ to 3 hours. At these latter pH levels 20 mg proved fatal. These results indicate that nicotine may be absorbed by the urinary bladder and that within a certain range the higher the pH, the more rapid is its absorption.

In Table I a correlation is shown between the degree of nicotine poisoning and the concentration of undissociated nicotine base. The latter was calculated in accordance with the formula which we have previously derived,[1] taking the dissociation constant of nicotine[3] as 9×70^{-7}.† At the pH values used in these experiments, the percent of total alkaloid present as undissociated base varied through a wide range: approximately 0.04% at pH 4.5, 0.2% at pH 5.0, 1% at pH 6.0, 10% at pH 7.0, 52% at pH 8.0, and 92% at pH 9.0. Taking into account the dissociation and the dose of nicotine and the volume of the solution, the concentration of undissociated nicotine base in the solution injected into the bladder was found to range from 1:1,250,000 in Cat 1 to 1:270 in Cats 9 and 10. At the lowest concentrations of undissociated base, 1:83,000 or lower, no nicotine effects were observed. At concentrations of about 1:3,000 to 1:1,000, moderate to marked poisoning occurred; the average duration of advancement of the nictitating membrane was 41 minutes. At a concentration of about 1:600, nicotine effects were more

[3] Craig, L. C., and Hixon, R. M., *J. Am. Chem. Soc.*, 1931, **53**, 4367.

† Thus, $\frac{[\text{Undissociated nicotine}]}{[\text{Nicotine ions}]} = \frac{1 \times 10^{-14}}{(9 \times 10^{-7})\ (1 \times 10^{-\text{pH}})}$

marked and the average duration of the nictitating membrane advancement was 139 minutes. At a concentration of 1:270, death occurred. These results indicate that the rate of absorption of nicotine from the urinary bladder depends to a large extent on the concentration of undissociated nicotine base.

Two experiments were reported[1] in which nicotine was injected into the ligated stomach of the cat. Twenty-four hours after the injection of 50 mg per kg in acid solution (pH 1.2), the pH of the gastric fluid was 4.2, and nicotine effects had not been observed. Injected at a pH of 8.6, 20 mg per kg was fatal in 41 minutes. In these experiments the volume of fluid was 15 cc per kg, and the calculated concentration of undissociated nicotine base in the solution injected was approximately 1:15,000,000 in the case of the acid medium, and 1:900 in the case of the alkaline medium. As we have seen, concentrations of the base of about 1:500 to 1:1,000 injected into the urinary bladder caused severe poisoning, but did not prove fatal. However, if allowance is made for the greater absorbing surface of the stomach as compared with that of the bladder, the rates of absorption of nicotine by the membranes of these 2 organs appear to be of the same order.

The influence of the hydrogen ion concentration on the speed of absorption of an alkaloid from the subcutaneous tissues has not heretofore been studied directly, but Moore and Row[4] found nicotine base to be 5 times as active as its hydrochloride when injected subcutaneously in the frog. Haag and Neale[5] more recently reported that the toxicity of nicotine‡ injected subcutaneously in mice was only slightly greater than that of nicotine sulfate or salicylate. We have found that when nicotine is injected subcutaneously (within the inguinal fold of skin) in cats in a buffered solution in a constant volume of fluid, 5 cc per kg as employed in the bladder, the pH plays a rôle in the speed of absorption as indicated by the interval to death, and by the M.L.D. (Table II). When the pH was reduced from an alkaline to an acid value and at the same time the dose of nicotine was increased by 33%, the average interval to death was prolonged from 32 minutes to 102 minutes. When the pH was similarly reduced from 9.0 to 4.8 but the dose of nicotine increased by 66%, the average interval to death was the same, namely, 32 minutes at the acid and alkaline pH. The dose of nicotine which proved fatal in the majority of animals when injected at an alkaline

4 Moore, B., and Row, R., *J. Physiol.*, 1897, **22**, 273.

5 Haag, H. B., and Neale, R. C., *J. Pharm. and Exp. Ther., Proc.*, 1940, **69**, 289.

‡ We have determined that a 1% solution of nicotine in the form of the base has a pH of about 10.1, and in the form of the sulfate, a pH of about 4.3.

TABLE II.
Influence of pH on Absorption of Nicotine from Subcutaneous Tissues.

Dose of nicotine* mg per kg	pH of solution injected	No. cats	No. died	Avg time injection to death, min
6	9.0	6	0	—
7.5	9.0	6	0	—
9	9.0	6	5	32 (25-36)
9	4.8	6	0	—
12	4.8	6	2	102 (50-304)
12	6.4	6	3	
15	4.8	6	4	32 (24-50)

*Injected in a volume of 5 cc per kg.

pH was 9 mg per kg, and at an acid pH was 15 mg per kg; 9 mg per kg injected at an acid pH was fatal in none. Thus within a certain critical range of pH and dosage both the M.L.D. of nicotine and the interval to death may be materially increased by lowering the pH of the solution injected. The use of a buffer in our experiments might explain the apparent discrepancy between these results and those of Haag and Neale.[5]

These experiments indicate that the absorption of nicotine is greater from subcutaneous tissues than from the urinary bladder, when injected at similar pH values and in the same volume of fluid. In the case of the urinary bladder, the amount of fluid injected remained unchanged, and hence as nicotine was absorbed, its concentration necessarily decreased. This fact would result in a diminishing rate of absorption, and provided the initial concentration was not high enough to cause death, nicotine effects would disappear while appreciable amounts of nicotine still remained in the bladder. When given by the subcutaneous route, the fluid was rapidly absorbed; this fact would tend to maintain the original concentration of nicotine, to prevent the slowing down of absorption and to insure virtually complete absorption of the alkaloid. That in the latter case there may actually be a rising curve of absorption is shown by the fact that in one experiment 20 mg per kg injected subcutaneously at a pH of 7.0 caused death after as long as 5 hours. In this case the increasing rate of absorption could scarcely be explained by a changing pH. It is obvious that owing to the number of variable factors a comparison cannot be made as to the influence of the pH alone on the speed of absorption of nicotine from subcutaneous tissues and that from the urinary bladder.

Summary. These experiments show that the hydrogen ion con-

centration plays an important rôle in the absorption of nicotine from both the urinary bladder and the subcutaneous tissues. The toxicity of nicotine given by these routes is appreciably decreased when the pH of the solution is reduced within a certain range. At similar pH levels nicotine is absorbed from the urinary bladder and from the stomach at essentially similar rates. Absorption of nicotine from the subcutaneous tissues is greater than from the urinary bladder when the alkaloid is injected at similar pH values, but the impossibility of controlling the factors which influence absorption from the subcutaneous tissues precludes direct comparison as to the rôle of the pH alone.

A correlation is shown between the rate of absorption of nicotine from the urinary bladder and the calculated concentration of undissociated nicotine base in the solution injected.

The ligated urinary bladder provides a technic for the study of factors influencing absorption which is in some respects more satisfactory than the ligated stomach or intestinal loop, owing to the greater ease of controlling in the bladder certain variable factors such as the volume of fluid and the pH.

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Effect of N'-Dodecanoylsulfanilamide and of Sulfapyridine on Experimental Tuberculosis in Rabbits.*

CARL MUSCHENHEIM, CLAUDE E. FORKNER AND DOROTHY RHOADES DUERSCHNER.

From the New York Hospital and Department of Medicine, Cornell University Medical College, New York City.

N'dodecanoylsulfanilamide was synthesized by Crossley, Northey, and Hultquist[1] and proposed as a possible chemotherapeutic agent against tuberculous infections. Climenko[2] has recently shown it to be capable of inhibiting the growth of human tubercle bacilli *in vitro*. He also reported that it exerts an inhibitory effect against the development of experimental tuberculosis in guinea pigs infected with

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1 Crossley, M. L., Northey, E. H., and Hultquist, M. E., *J. Am. Chem. Soc.*, 1939, **61**, 2950.

2 Climenko, D. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 624.