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.

11747

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

* This study was aided by a gift from the late Mr. George L. Bourne. The dodecanoylsulfanilamide and the sulfapyridine used in this experiment were supplied by the Calco Chemical Company through the courtesy of Dr. D. A. Bryce.

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.

a human strain of tubercle bacilli. Steinbach and Duca,[3] however, failed to demonstrate an inhibitory effect by this drug in experimental tuberculosis in guinea pigs. In both of the investigations the H-37 strain of human tubercle bacilli was used and daily doses of 100 mg N-dodecanoylsulfanilamide in 2% olive oil solution were administered to the animals for from 30 to 45 days. Sulfapyridine in olive oil was also administered in the same dosage by Steinbach and Duca and this drug was reported by them to be equally ineffective.

The present communication records the results of the administration of N-dodecanoylsulfanilamide and sulfapyridine in olive oil suspension to rabbits infected with the Ravenel strain of bovine tubercle bacilli. The infecting dose of bacilli was 0.001 mg inoculated intravenously. A daily dose of 1.6 g of either drug was administered in divided doses of 400 mg suspended in 2 cc of olive oil and fed by means of a dropper. Sixty male albino rabbits weighing from 2 to 3 kg each were infected and divided into groups for treatment as follows:

Group A. 24 animals, given 400 mg N-dodecanoylsulfanilamide 4 times daily for 57 days.

Treatment started 1 day after infection—6 animals
" " 10 " " " 6 "
" " 20 " " " 6 "
" " 35 " " " 6 "

Group B. 24 animals, given 400 mg sulfapyridine 4 times daily for 57 days.

Treatment started 1 day after infection—6 animals
" " 10 " " " 6 "
" " 20 " " " 6 "
" " 35 " " " 6 "

Group C. 12 animals, untreated controls.

One animal of the control group was discarded on the first day after infection because of severe diarrhea. A second animal of Group C was sacrificed 64 days after infection. With the exception of one animal in Group A which lived until 326 days after infection when it was sacrificed the remainder died within a period of from 14 to 272 days after infection. The control animal sacrificed at 64 days revealed no gross or microscopic evidence of tuberculosis and all of the animals which died within 65 days revealed no evidence of tuberculosis or, at most, a few isolated small tubercles. There were 9 animals which died within this period. The cause of death in some of those may have been the toxic effect of the drug as paralytic manifestations were frequently the precursors of death. All of the animals which lived for more than 65 days showed gross evidence of

[3] Steinbach, M. M., and Duca, C. J., PROC. SOC. EXP. BIOL. AND MED., 1940, 44, 133.

tuberculosis except the one animal in Group A which survived for 326 days when it was sacrificed and revealed no gross or microscopic evidence of tuberculosis. The explanation of this exception is not clear, and because of its marked disparity with the general results it probably should be regarded as not significant. The majority of both the treated and the control animals that lived for more than 65 days after infection had generalized tuberculosis of sufficient extent to be the cause of death. In many there was massive caseation of the lungs, the organ which was usually the most extensively involved. Table I shows the probable causes of death of the animals in the 3 groups which survived for longer than 65 days after infection. The percentage of those in each group which apparently died of tuberculosis is shown in the table with the average survival period. It is apparent that there is little difference between the treated groups and the controls. The somewhat lower percentage of deaths caused by tuberculosis in the controls and the somewhat longer average survival period may be due to the absence of toxic effects of the drugs which were given in very large doses to the treated groups. The relatively small number of controls, however, makes the possible significance of the difference uncertain.

The extent of tuberculous involvement of the separate organs and of the total disease in each animal was graded on a scale varying from + to ++++. This served as a basis for the determination of the probable causes of death. The grading also gave a means of comparison between the sub-groups of 6 animals in Groups A and B, whose treatments were started at varying intervals after infection. In this comparison there appeared to be no significant differences attributable to the time when treatment was begun.

TABLE I.

		Probable causes of death						
			Non-tbc causes. Extent of tbc insufficient to cause death	Tuberculosis. Massive tbc of lungs or extensive generalized tbc sufficient to cause death				
	No. of animals	Sacrificed. No evidence of tbc		Massive	Extensive	Total	%	Avg duration of life
Group A	20	1 (326th day)	4	12	3	15	75.0	188 days
Group B	21	0	6	10	5	15	71.4	176 "
Group C	8	0	3	5	0	5	62.5	213 "

Analysis of causes of death of animals which lived longer than 65 days following intravenous inoculation with bovine tubercle bacilli. Group A—treated with N'-dodecanoylsulfanilamide; Group B—treated with sulfapyridine; Group C—untreated controls.

The concentration of drug in the blood was determined by Marshall's method[4] in a number of animals of Groups A and B. The values varied from 1.2 mg % to 3.6 mg % of the free drug calculated as sulfanilamide. Those animals which exhibited higher blood levels appeared to develop as extensive disease as those which exhibited low levels. Calculi were found in the kidney pelves of several of the animals in Group B and in the ureters of one of these animals. No renal calculi were found in any of the animals treated with N'-dodecanoylsulfanilamide, or in the controls.

Conclusions. N'-Dodecanoylsulfanilamide and sulfapyridine administered in large doses failed to exert any demonstrable inhibitory effect on experimental tuberculosis in rabbits infected with a bovine strain of tubercle bacilli.

11748

Electrophotometric Determination of Phosphorus by the Method of Fiske and Subbarow.

D. J. McCUNE AND A. A. WEECH.

From the Department of Pediatrics, College of Physicians and Surgeons, Columbia University, New York City.

The substitution of the filter photometer for the visual matching of solutions directs attention forcibly to the fact that many of the reactions employed in quantitative colorimetric microanalysis yield colors that are highly variable with respect to their rates of intensification and fading. An attempt to carry out Bodansky's directions[1] for the determination of serum phosphatase activity with a filter photometer* demonstrated the procedure of Kuttner and Lichtenstein[3] for the determination of phosphorus to be particularly troublesome in this respect. The essay was made during an excessively hot and humid period of the summer when the daily fluctuations of

[4] Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

[1] Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 197; 1933, **101**, 93; 1937, **120**, 167.

* The instrument was constructed in this laboratory. It employs a Lange selenium photocell, type S 50.[2] The absorption tubes are matched test tubes, 20 x 175 mm. The source of light is an automobile headlight bulb; a current of approximately 1.6 amperes is drawn from a storage battery that is under a continuous trickle charge. A Lange "Multiflex" galvanometer is used.

[2] Lange, B., translated by St. John, A., *Photoelements and Their Application*, New York, Reinhold Publishing Corporation, 1938.

[3] Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1932, **95**, 661.