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A New Class of Tuberculostatic Substances.

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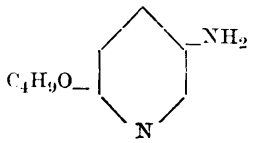
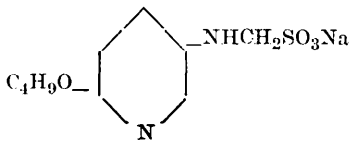
The search for substances having tuberculostatic activity has in recent years engaged increasing interest in many laboratories. Using an *in vitro* technic in which a rapidly growing avirulent strain of "human" tuberculosis organism is subjected to various concentrations of compounds under test makes feasible the "screening" of large numbers of substances in a comparatively short time. This method, the details of which are essentially the same as described earlier,[1] has brought to attention 5-amino-2-butoxypyridine



which was investigated in some detail. Several hundred derivatives of this substance were synthesized† one of which, the sodium formaldehyde bisulfite derivative



relatively among the least toxic,[2] is included in this report.

The growth of 607, the rapidly growing strain of the tubercle bacillus, was inhibited by these compounds in dilutions in excess of 1 to 3 million. Other strains, virulent and recent isolations, were inhibited in dilutions as high as 1 to 100 million. A remarkable feature associated with this high tuberculostatic effect is that while several bacterial genera have been investigated only Mycobacterium was susceptible to the action of these substances in significant dilution. The data presented in Table I are typical of the results obtained in many-time repeated experiments. The specificity for Mycobacterium is illustrated by the lack of inhibition of growth of Staphylococcus, Streptococcus, Pneumococcus, *B. mycoides, E. coli,* and others, even in what may be termed high concentrations. This specificity contrasts sharply with the lack of specificity shown by sulfones and sulfonamides[1] which are active against the rapidly growing avirulent *Mycobacterium tuberculosis 607* (line No. 1) as well as *E. coli* in a synthetic medium (line No. 10). Comparison is also made with promin and its parent substance p,p'-diaminodiphenylsulfone.

It is important also to note that while the more active sulfonamide and sulfones are capable of inhibiting the growth of the avirulent strain of tubercle bacillus in comparatively high dilution (line No. 1), these compounds are relatively ineffective against other more virulent strains (lines 2, 3 and 4).

The antagonistic effect of para-aminobenzoic acid[3] and methionine[4] for sulfonamides is well known. As suspected these substances did not inhibit the activity of I or II, as shown by lines 1, 14 and 15 in Table I. The bacteriostatic activity of our compounds against the tubercle bacillus is likewise not antagonized by the incorporation of adequate quantities of riboflavin, calcium pantothenate, adenine, guanine, thiamine, uracil, nicotinic acid, biotin, culture filtrates from staphylococci, pneumococci and tubercle bacilli, pus from streptococcal lymphadenitis, constituents from beef culture media, peptone (which

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[1] Fitzgerald, R. J., and Feinstone, W. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 27.

† 5-Amino-2-butoxypyridine and its derivative were synthesized by the chemical staff of the Pyridium Corp. under the supervision of Drs. E. T. Tisza and H. L. Friedman.

[2] To be reported elsewhere.

[3] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[4] Harris, J. S., and Kohn, H. I., *J. Pharm. and Exp. Therap.*, 1941, **73**, 383.

TABLE I.
Comparative Bacteriostatic Effect of 5-Amino-2-Butoxypyridine and Certain Sulfonamides and Sulfones.

	Organism	Culture medium	Lowest concentration (mg per 100 cc) showing bacteriostasis. Compounds					
			I†	II§	Sulfanilamide	Sulfathiazole	Promin§	p,p′-Diaminodiphenylsulfone‡
1	*M. tbc.* 607‖	Dorset's Synthetic	0.031	0.062	2.0	0.062	4.0	0.062
2	*M. tbc.* H37RV	Proskauer Beck	0.0039	0.0078		0.5	64.0	0.5
3	*M. tbc.* G2¶	" "	0.00099	0.00099	64.0	2.0	16.0	2.0
4	*M. tbc.* Benton**	" "	0.0019	0.00099	32.0	0.5	16.0	2.0
5	*M. tbc.* bovine‖	" "	0.0078	0.015				
6	*M. avium*‖	" "	0.156	0.0312				
7	*M. phlei*‖	" "	0.0039	0.0039				
8	*M. stercoris*‖	" "	0.0039	0.015				
9	*M. lepræ*‖	" "	0.031	0.031				
10	*E. coli*	Sayhun Synthetic	64.0	64.0	2.0	0.031	10.0	1.0
11	*Strep. hem.* C203	Beef Infusion Broth	32.0	64.0	32.0	16.0		10.0
12	*B. mycoides*	Peptone Dextrose Broth	8.0	32.0		8.0		
13	Pneumococcus SV1	" " "	64.0	64.0	8.0	0.5		
14	*M. tbc.* 607	Dorset's + 0.1 mg % para-aminobenzoic acid	0.031	0.062	64.0	8.0		8.0
15	*M. tbc.* 607	Dorset's + 1.0 mg % methionine	0.031	0.062	16.0	0.25		1.0

* Complete or almost complete visible inhibition of growth for 72 hours following inoculum or in the case of slow growing tubercle bacillus for 30 days. Tubercle bacilli inoculum either by small pellicle or suspension of about 20,000 organisms per cc.

† I—5-Amino-2-butoxypyridine as the hydrochloride.

‡ II—Sodium formaldehyde bisulfite of 5-amino-2-butoxypyridine.

§ Promine and p,p′-diaminodiphenylsulfone supplied through the kindness of Dr. L. A. Sweet, Parke, Davis & Co.

‖ Obtained from The American Type Culture Collectioon. *M. lepræ* is of course of questionable identity.

¶ Obtained through the courtesy of Dr. Guy P. Youmans, Northwestern University Medical School.

** Obtained through the courtesy of Dr. William H. Feldman, Mayo Foundation.

antagonizes sulfonamide activity[5] and whole blood and serum up to 25%.

The antimycobacterial activity of 5-amino-2-butoxypyridine and its derivative is primarily bacteriostatic rather than bactericidal. (We hold the term bactericidal in its strictest sense, *i.e.*, complete sterilization of a sizable inoculum in a culture medium which, when free of inhibitory compounds, supports vigorous growth). Concentrations of 5-amino-2-butoxypyridine 1,000 times the minimum tuberculostatic concentration do not consistently render the system free of viable organisms.

The fact that these compounds are bacteriostatic rather than bactericidal and strikingly specific for the acid-fast group of organism suggest a mechanism of action involving the interference with some essential metabolic process common to species of Mycobacterium but which is lacking, has readily available alternatives or is nonessential in other genera of organisms.

[5] Lockwood, J. S., and Lynch, H. M., *J. A. M. A.*, 1940, **114**, 935.

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Bilateral Nephrectomy in Rats: Blood Chemistry, Longevity and the Effect of Aluminum Hydroxide.

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In the hypocalcemic tetany of the uremic patient aluminum hydroxide reduces the tendency towards convulsions by elevating the serum calcium[1] presumably by preventing the absorption of the phosphate ion.[2] Subsequently, these studies were initiated in order to observe the effect of aluminum hydroxide gel on the survival time and on the blood levels of sulphates, phosphates, calcium, urea and cholesterol in rats following bilateral nephrectomy.

Method and Material. A total of 108 rats were used in this study. Fifty-one rats were employed in the longevity study of which 26 (16 males and 10 females) served as controls and 25 (15 males and 10 females) as experimental animals. The males weighed 400 to 500 g and the female rats 250 to 300 g. Both kidneys were removed by a transperitoneal approach through a midline ventral incision under ether anaesthesia. Sterile technic was utilized throughout. Each of the animals in the experimental group was given 5 ml of 4% aluminum hydroxide gel by stomach tube. An analgesic dose of ether was administered prior to the passage of the stomach tube in order to permit intubation without the use of a gag. The aluminum hydroxide was given once daily for 2 days prior to nephrectomy, then 4 to 6 hours after surgery, and thereafter once every 24 hours until the animals expired. The survival time of these rats was compared with the control group.

For the study of variations in blood chemistry, 28 non-nephrectomized male rats were anaesthetized with ether after which the abdominal aorta was exposed through a midline ventral incision. Blood was then aspirated from the aorta by means of a 26-gauge needle and a 2-ml hypodermic syringe. Seventeen male rats were nephrectomized and 48 to 50 hours postoperatively blood was withdrawn from the abdominal aorta as described above. In addition, 11 male rats were nephrectomized but were given aluminum hydroxide by stomach tube. These animals were also sac-

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[1] Personal observation, unpublished data.

[2] Evans, G. T., and Flink, E. B., *Modern Med.*, 1945, **13**, 63; Fauley, G. B., Freeman, S., Ivy, A. C., Atkinson, A. J., and Wigodsky, H. S., *Arch. Int. Med.*, 1941, **67**, 563.