

and when both were severed the inhibitory reflex was abolished as well as the sensation of pain arising from a spastic caecum.

Various modifications of these experiments indicate that the 2nd lumbar roots of the coeliac ganglia as well as the lumbar sympathetic trunks are not pathways primarily involved in regulation of the biliary tract. Severance of the coeliac branch of the right vagus, in one instance, was followed by retardation of emptying.

The implications of these experiments are that evacuation of the extra hepatic biliary tract is inhibited by the sympathetic nervous system as mediated through the splanchnic nerves, and that the reason, perhaps, why severance of the specific nerves to the cholecho-duodenal junction does not relax this junction is that such nerves carry both vagus and sympathetic fibers and also innervate both the sphincter of Oddi and the contiguous portion of the duodenum.

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Bacteriostatic Effect of Paranitrobenzoic Acid on Pneumococci *in Vitro*.*

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Mayer and Oechsli¹ reported that paranitrobenzoic acid and certain derivatives were effective in pneumococcal infections in mice although they showed little activity *in vitro*.

Gruhzit² found that paranitrobenzoate protected mice infected with *Streptococcus viridans*. He found little activity against beta hemolytic streptococci and none against Type I pneumococci. Miller³ recently reported that *Streptococcus viridans* is inhibited *in vitro* by sodium paranitrobenzoate.

Barlow⁴ finds the acid and ester effective in pneumococcal infections in mice.

* Aided by grants from the Medical Research Fund, Graduate School, the University of Minnesota, and the Department of Medical Research, Winthrop Chemical Co., Inc. Assistance in the preparation of these materials was furnished by the Work Projects Administration, official project No. 65-1-71-140, sub-project No. 237, and by the National Youth Administration. Drugs supplied by the Winthrop Chemical Co., Inc.

¹ Mayer, R. L., and Oechsli, C., *Compt. Rend. Soc. de Biol.*, 1939, **130**, 211.

² Gruhzit, O. M., *Arch. Path.*, 1940, **20**, 732.

³ Miller, J. K., *J. Pharm. and Exp. Therap.*, 1941, **71**, 14.

⁴ Barlow, O. M., unpublished data.

We have studied the effect of the acid and sodium salt on pneumococci in the tissue culture medium and with the methods which we used earlier in studies on Streptococci.⁵⁻⁹

Pneumococcus cultures were grown in rabbit serum extract of 7-8-day chick embryos. Cultures were incubated at 37.5°C for approximately 5 hours.

Drugs were dissolved in Tyrode's solution and sterilized by Berkefeld filtration. Stock solutions of sodium salts were 400 mg % concentration; the acid 40 mg %.

Dilution of the bacterial culture was made rapidly through Tyrode into the serum extract. The usual dilution was 10⁻⁸ or 10⁻⁹.

The drugs were then added after pH adjustment (except as noted below). Buffer standards were checked with the glass electrode. The colorimetric method was used in making the adjustments. An appropriate amount of Tyrode was added to the control medium to compensate the dilution caused by the drug-Tyrode mixture. When drug-Tyrode mixtures of different concentrations were used, the stronger mixture was diluted to the strength of the weaker by addition of Tyrode before adding to the culture medium.

The cultures were planted on 22 mm round cover slips. Each

TABLE I.
Bacteriostatic Effect of Paranitrobenzoic Acid, and the Sodium Salt, Compared with Sodium Sulfathiazol; Type II Pneumococcus; 15 mg%.

Organisms per cc	Control	Thiazol	P-NO ₂ BA	Inhibition %	
				Thiazol	P-NO ₂ BA
958	23.9*	6.7	11.5	72.0	51.9
940	27.2	8.1	12.0	70.3	55.9
254	65.9	10.8	14.4	83.6	78.1
3090	17.6	8.2	9.3	53.4	47.2
342	42.4	11.7	12.8	72.4	69.8
234	162.1	95.8	94.0	40.9	42.0
402	109.2	49.9	57.5	54.3	47.3
1128	71.8	40.0	43.7	44.3	39.1
468	52.1	30.1	33.6	42.3	35.5
252	124.7	38.1	54.3	69.4	56.4
368	101.1	21.2	25.7	79.0	74.6
108	99.6	29.7	39.9	70.2	59.9

*Eyepiece units.

⁵ King, J. T., Henschel, A. F., and Green, B. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 810.

⁶ King, J. T., Green, B. S., and Henschel, A. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 812.

⁷ King, J. T., and Henschel, A. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 208.

⁸ King, J. T., Henschel, A. F., and Green, B. S., *J. A. M. A.*, 1939, **113**, 1704.

⁹ King, J. T., and Henschel, A. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **44**, 268.

culture was made up of 1 drop of heparinized rabbit plasma and 3 drops of bacterial suspension. Clotting occurs promptly. Calibrated pipettes were used to ensure cultures of the same volume in each group. Cultures were incubated as Maximow double cover-slip preparations at 37.5°C. Details have been described in previous papers.

Readings were made at 22-24 hours. The diameter of the colonies was measured with ocular micrometer and mechanical stage at 60X. (114 eyepiece units equal to 1 mm).

Each series is composed of 18 cultures, 6 of each drug and 6 controls.

The results of 12 series on pneumococcus, Type II (Bieter), are given in Table I.

The average inhibition of colony diameter for the 12 series was 61.0% for sulfathiazol; 54.5% for paranitrobenzoic acid and the salt.

There is no significant reduction in the number of colonies with either drug.

The first 5 series were done with the acid; the last 7 with the sodium salt. In the first 2 series the pH of the Tyrode-drug mixture was not adjusted before adding to the culture medium. In the 3rd series the two drugs were adjusted to the same pH. In the 4th and 5th series the drugs were adjusted to the pH of the Tyrode added to the control. In the last 7 series the sodium salt of paranitrobenzoic acid was used. In these, both drugs and the Tyrode added to the control were adjusted to pH 7.6 before adding to the culture medium.

Since the sodium salt of sulfathiazol is more alkaline than the salt of paranitrobenzoic, we have used sterile 1 N HCl for adjusting the former and 0.1 N HCl for adjusting the latter in order to avoid unequal dilution of drug solutions. There was, however, a slightly greater dilution of the benzoic compound than of the thiazol. On the average 3 drops of acid were required to adjust 10 cc of thiazol and 5 drops to 10 cc of benzoic.

Studies in progress on higher and lower concentrations show increasing inhibitory effect with increasing concentration of both drugs.

These findings on Type II pneumococci are in contrast with those of Mayer and Oechsli on Type I where they obtained little effect *in vitro*.

Summary. Paranitrobenzoic acid and the salt compare favorably with sulfathiazol in their bacteriostatic power against Type II pneumococci *in vitro*.