11517

Effect of Azosulfamide (Neoprontosil) on Experimental *Welchii* Infection in Mice.

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Bliss and Long[1] report that sulfanilamide has a curative effect in mice infected with *Clostridium welchii.* Carpenter and Barbour[2] report that oral administration of Neoprontosil prevented death in mice given the toxin of *Clostridium welchii.* We decided to study the effect of Neoprontosil (Winthrop Chemical Co.)* on experimental *welchii* infection in mice.

The strain of *Cl. welchii* used in the following experiments was isolated from a human case of gas gangrene and is similar, in its biological characteristics, to the classical *Cl. welchii* described in textbooks. The culture was passed through Swiss mice before culturing in glucose broth in order to retain its virulence. Its M.L.D. for mice under these conditions was 0.1 cc of a 24-hour glucose broth culture.

Experiment I—Ninety mice, separated into 3 groups of 30 each, were inoculated intramuscularly with ascending doses of a 24-hour glucose broth culture of *Cl. welchii.* The first group was injected with 0.05 cc, the second with .075 cc, and the third with 0.1 cc. Seventy-nine of these mice, or 87.7%, died before 72 hours—20 (66%) in the first group, 29 (96.6%) in the second, and all (100%) in the third.

Ninety additional mice were similarly grouped and inoculated, but besides the corresponding dose of the organisms, each was given 1 cc of Neoprontosil intramuscularly at the time of inoculation. Eighty-five (94.4%) mice died before 72 hours—25 (83.3%) in the first group, and all (100%) in the second and third groups (Table I, Exp. I).

Ten mice were similarly inoculated with 1 cc of Neoprontosil only, and all survived.

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1 Bliss, A. L., and Long, P. H., *J. Am. Med. Assn.*, 1937, **109**, 1524.

2 Carpenter, C. M., and Barbour, G. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 255.

* The Neoprontosil used was 5% solution, kindly supplied by Mr. Rassow, local representative of the Winthrop Chemical Company.

TABLE I.

Groups studied	No. of mice inoculated	No. of mice that died	% of mice that died
	Experiment I.		
Control			
1	30	20	66
2	30	29	96.6
3	30	30	100
Totals	90	79	87.7
Experimental			
1	30	25	83.3
2	30	30	100
3	30	30	100
Totals	90	85	94.3
	Experiment II.		
1	30	4	13.3
2	30	7	23.3
3	30	27	90
4	30	27	90
5	30	0	0

In the above experiment, Neoprontosil did not protect mice from *Cl. welchii* infection.

Experiment II—A 24-hour glucose broth culture was centrifuged at high speed. The supernatant was set aside and the sediment was repeatedly washed with saline. After repeated centrifugations and washings, enough saline was added to the cells to restore the original volume of the culture, and 0.1 cc of this suspension was used for the inoculations.

One hundred fifty mice were separated into 5 groups of 30 mice each. Group 1 was inoculated intramuscularly with 0.1 cc of washed cells of *Cl. welchii.* Group 2 was inoculated intramuscularly with 0.1 cc washed cells and 0.1 cc filtrate from the same culture. Group 3 was inoculated with 0.1 cc washed cells and 0.1 cc filtrate, plus 1 cc Neoprontosil, Group 4 with 0.1 cc washed cells plus 1 cc Neoprontosil, and Group 5 with 0.1 cc filtrate plus 1 cc Neoprontosil.

Four mice (13.3%) died in Group 1, 7 (23.3%) died in Group 2, 27 (90%) in Group 3, 27 (90%) in Group 4, and none in Group 5. (Table, Exp. II.) In this case, the injection of Neoprontosil with washed cells of *Cl. welchii* led to the development of the corresponding infection, while the washed cells alone showed little tendency to develop in the tissues after inoculation.

In repeating the above experiment, the dose of Neoprontosil was lowered, using 0.75 cc, 0.50 cc and 0.25 cc, and in every case, when the dose of Neoprontosil was given with washed cells of *Cl. welchii,*

the animal succumbed to infection. On examining the lesions produced, one could observe normal phagocytic activity and numerous organisms within the tissue of the lesions produced. Feinstone, Bliss, Ott and Long[3] present evidence indicating that the activity of Neoprontosil depends on its reduction to sulfanilamide *in vivo*.

Gye and Cramer[4] found that ionizable salts of calcium inoculated together with washed spores of *Cl. welchii* or *Cl. tetani* led to the development of the corresponding infections in their fatal form, while washed spores alone did not lead to death. Fildes[5–7] thinks that there must be some definite stimulus to vegetation in the tissues injected by calcium salts, and suggests that this stimulus is probably the result of diminished oxygen tension. He showed further that the injection of solutions of calcium chloride lead to the production of localized areas of oxygen deficiency. We do not know if Neoprontosil in this case acts in a similar way.

Summary—We were unable to protect mice from M.L.D. of *Cl. welchii* by intramuscular injection of Neoprontosil. The intramuscular injection of washed cells of *Clostridium welchii* with Neoprontosil in mice led to the development of a fatal infection.

11518

Blood Progesterone During Sexual Cycle of *Macaca rhesus;* Quantitative Assay.

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At the suggestion of Professor George W. Corner, the presence of progesterone in the blood during the menstrual cycle of the monkey, *Macaca rhesus,* has been studied and an effort made to

3 Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hospital,* 1938, **62**, 565.

4 Gye, W. E., and Cramer, W., *Sixth Sci. Rep. Imp. Cancer Res. Fund,* 1919, pp. 40-57.

5 Fildes, P., *Brit. J. Exp. Path.,* 1927, **8**, 387.

6 *Ibid.,* 1929, **10**, 151.

7 *Ibid.,* 1929, **10**, 197.

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