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Destruction of Sulfonamide Inhibition Present in Sera by Soil Bacillus (Mirick)*

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Mirick[1] described a Gram negative soil bacillus which was capable of oxidizing 10 mg of *p*-aminobenzoic acid in 100 ml of medium in 24 hr. Because *p*-aminobenzoic acid strongly inhibits the action of sulfapyridine, it was deemed important to determine whether the bacillus would destroy the inhibitor found in some human serums.

A culture of this organism was generously supplied by Doctor George S. Mirick, and was tested against several human serums which inhibited sulfapyridine and the highest dilution in which they reacted against 5 mg of sulfapyridine per 100 ml was determined. Previous experiments showed that 0.2 to 0.3 mg % of *p*-aminobenzoic acid is necessary to inactivate 5 mg % of sulfapyridine, so that the concentration of inhibitors may be translated into mg % of *p*-aminobenzoic acid activity.

Tubes containing inhibitor free beef heart infusion broth and varied amounts of inhibiting serum with *p*-aminobenzoic acid were set up. One set of tubes seeded with 0.5 ml of an 18-hr culture of the soil bacillus culture was incubated over night at 37°C. Next morning the tubes containing the soil bacillus as well as a similar set of sterile tubes were heated for 2 hr at 56°C. The dead organisms were removed by rapid centrifugation. All the tubes were then seeded with an 18-hr culture of our standard pneumo-

TABLE I.
Effect of Soil Bacillus (Mirick) on Human Serum Inhibition of Sulfapyridine 5 mg per 100 cc.

No.	Sulfapyridine 5 mg% and serum	Inoculated with soil bacillus	Growth of pneumococcus T 3-1	
			24 hrs	48 hrs
1	M	+	±	0
2	M	0	++++	++++
3	C	+	±	0
4	C	0	++++	++++
5	S	+	±	++++
6	S	0	++++	++++
7	D	+	+	++++
8	D	0	++++	++++
9	Kle*	+	±	0
10	Kle*	0	0	0
11	Kau*	+	0	0
12	Kau*	0	0	0
13	Broth + 3 mg% PABA	+	±	0
14	Broth + 3 mg% PABA	0	++++	++++
15	Broth + 0.2 ml horse serum	+	0	0
16	Broth + 0.2 ml horse serum	0	0	0

The initial inoculum of Pneumococcus T 3-1 was 1000 org/ml.
*Serums Kle and Kau contained no inhibitors and were included as controls.
+ indicates that the media was inoculated with soil bacillus.
0—tube was not inoculated.
PABA—*p*-aminobenzoic acid.
± cloudiness, small amount of growth present.
++++ abundant growth.

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[1] Mirick, G. S., *J. Clin. Invest.*, 1941, **20**, 434.

coccus III to contain 1000 organisms per ml. Each tube also received enough sulfapyridine to make its concentration 5 mg % and all were incubated over night at 37°C. The table shows that sulfonamide inhibiting action was removed from serum by the soil bacillus.

Conclusion. It may be inferred from these experiments that soil bacillus (Mirick) interferes with the activity of the substance in serum which inhibits sulfapyridine.

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Experimental Transmission of *Lymphogranuloma venereum* Virus Through the Placenta.

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In view of the recent emphasis by Goodpasture[1] on transmission of viral infections to the fetus, and because of the possibility of applying experimental study to human cases with eventually a clearer understanding of virus pathogenesis, an investigation dealing with the experimental transmission of the lymphogranuloma virus through the placenta of mice was undertaken. This animal host was selected because of its well-known susceptibility by intracerebral route to the agent under investigation. Two lines of study were followed: (1) injection of pregnant mice by non-infecting routes and (2) infection of the host by the usual experimental portal of entry, the brain. In both types of experiments a fixed mouse strain of lymphogranuloma virus was injected in dilutions of 1:10 into pregnant mice. These animals were sacrificed 24, 48, 72 and 96 hr after the injections, or after they had gone to term and kindled. At these periods, emulsions from the brains of both mother and fetus were injected into other mice. Part of the material so injected was made up into antigens in the usual manner (heating 3 hr at 58°). The antigens were then injected intradermally into human beings and compared with human antigen or Lygranum.[1] As has been recommended,[2,3] a skin test was considered positive if a papule with a diameter of 5 mm appeared within 48 hr. Normal mouse brains (maternal and fetal) treated similarly to the antigens were used as controls. Fifty-three human cases were tested. The presence or absence of virus in fetal tissue was also studied by intracerebral injections of tissue emulsions into normal mice.

Human and mouse antigens in 37 cases with positive Frei tests (human antigen) gave the following results (Table I). In 30, fetal brain antigens produced positive skin reactions. In 12 negative cases, the same type of antigen yielded 10 negative skin tests. The table shows also strong reactions to antigens prepared from the brains of infected mothers (11 positive and 9 indefinite). Again, in agreement with previous investigators,[4] the limitations of the use of mouse brain antigens are apparent by the number of non-specific reactions attendant upon injection of such animal tissue into the human skin.

In one experiment 96 hr after subcutaneous injection of virus, the mother was sacrificed and fetal brain emulsion was inoculated intracerebrally into 4 normal mice. Seven days post-inoculation all were sick but the illness did not progress and the ani-

1 Goodpasture, Ernest W., *Science*, 1942, **95**, 391.

2 Ormsby, Oliver S., *A Practical Treatise on Diseases of the Skin*, Lea and Febiger, Philadelphia, 1937, 978.

3 Harvard School of Public Health Symposium, *Virus and Rickettsial Diseases*, Harvard Univ. Press, Cambridge, Mass., 1940, **372**, 371.

4 Reider, Reuben Frank, and Cañizares, Orlando, *Arch. Dermat. and Syph.*, 1938, **38**, 930.