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**Active Immunity to Pneumococci in Rabbits Protected by Sulfapyridine.\***

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The results of experiments on the protection of rabbits, infected with pneumococci, by oral administration of sulfapyridine have been reported previously.<sup>1</sup> The purpose of this communication is to present data obtained in the study of the immunity in animals that had recovered from the infection produced under such circumstances.

Rabbits weighing approximately 2 kilos were inoculated intracutaneously with 0.3 cc of a 1/100 dilution of an 8-hour culture of a pneumococcus type II. The inoculum contained an average of 300,000 organisms as determined by plate-count. The virulence of the organism is such that an average of 1.5 organisms are necessary to cause an infection in 66% of a statistically sized group of mice weighing 20 g. This we have taken to be our minimal lethal dose. Its virulence for rabbits is such that 0.3 cc of a 1/1000 dilution of the original culture, containing 30,000 organisms, will kill all of the rabbits inoculated.

The drug-treated animals were given sulfapyridine orally in a 10% acacia suspension. Each animal received a total of 5 g of the drug, 0.5 g 1 hour before infection, 0.5 g 3 hours after the initial dose, and 0.5 g every 12 hours thereafter until the full dosage was administered.

Daily record was made of the temperature, and the size and nature of the skin-lesion. Rabbits recovering from the primary infection were reinoculated intracutaneously in the same general areas as previously, but not at the site of the original lesion if a scar had persisted. Only normal areas of the skin were used for reinoculation.

*Type-specific Immunity.* Twelve rabbits reinoculated intracutaneously with 0.3 cc of the whole 8-hour culture of the homologous type II pneumococcus 30 days after the original infection, showed no rise in temperature, and no local reaction around the point of inoculation. Five rabbits reinoculated 62 days after the original infection, and 5 after 83 days, showed no reaction. Of three rabbits

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<sup>1</sup> Larson, W. P., Bieter, R. N., and Levine, M., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 703.

reinoculated after 92 days, one showed a slight inflammation around the point of inoculation, while 2 showed no reaction. The rabbit with the local reaction ran a temperature for one day, then returned to normal. The controls (1 for each treated animal) all developed the typical lesions and died in from 4 to 10 days.

Six of the 12 animals given a second inoculation at the end of 30 days were injected a third time with the whole culture of the type II organism 94 days after the original infection, and again showed no reaction. These animals were reinoculated monthly thereafter, and at the end of 6 months showed no reaction to intracutaneous inoculation of the whole culture of the type II organism.

*Species-specific Immunity.* Eighteen of the animals recovered from the type II infection were inoculated intracutaneously with 0.3 cc of the whole 8-hour type II culture. This was done to raise the protective powers of the animals to a uniformly high level. There was no reaction to this inoculation. Ten days later these rabbits were reinoculated with 0.3 cc of the whole 8-hour culture of the type I pneumococcus; 15 controls were inoculated at the same time. The controls all died in from 5 to 10 days, showing typical lesions with edema, hemorrhage and inflammation. The virulence of the type I strain has not been determined, although the whole culture killed mice regularly on intraabdominal injection, as well as the rabbits which were injected intracutaneously. The results of reinoculation of the 18 rabbits previously recovered from the type II infection are shown in Table I.

We next used a type III and a type VIII pneumococcus which were lethal for mice on intraabdominal injection, but which produced a non-fatal infection in rabbits. Sixteen rabbits that had recovered from the type II infection were reinoculated intracutaneously with 0.3 cc of a whole 8-hour culture of a type III pneumococcus. Fourteen controls were inoculated at the same time. Twelve rabbits,

TABLE I.  
Rabbits Recovered from Type II Infection and Reinoculated with Type I.

No. of rabbits	No. of days temp. above 103.5°	Type of skin lesion	Result
5	0	No reaction	Lived
4	1-3	Slight inflammation in the immediate area of inoculation	"
3	4-5	More pronounced inflammation but no edema or hemorrhage	"
2	6	Inflammation and edema	"
4	Till time of death	Inflammation, edema and hemorrhage in the skin	Died

Of 18 rabbits, reinoculated, 14 lived; 15 controls all died.

which had recovered from a type II infection, and 12 normal controls, were inoculated with 0.3 cc of the whole 8-hour culture of the type VIII pneumococcus.

In both types, the control and reinoculated animals all lived. An examination of the lesions in the rabbits, as well as the temperature readings, revealed some striking differences in the reactions of the control and reinoculated groups. The lesions in the skin of the control groups were mild, showing little edema, but a widespread area of inflammation 3 to 4 inches in diameter. This inflammation persisted for from 5 to 7 days, then dried up leaving no scar. The lesions of the reinoculated group were localized at the point of inoculation, and presented on the second or third day a marked abscess-like induration which ulcerated, and in some cases formed a fistula through the skin so that the outer peritoneal wall was visible. No organisms could be recovered from these ulcers.

The temperatures obtained in these groups reveal that the infection persisted for a longer period of time in the control than in the treated animals, and this despite the formation in the reinoculated animals of ulcers which lasted in some cases as long as one month.

The average temperature of 18 rabbits recovered from infection with type II and then inoculated with type III was 105.7, 103.9, 103.3, and 102.9 on the 1st, 2d, 3d, and 4th days after inoculation. The averages for 15 control rabbits were 105.3, 104.2, 105.5, 105.1.

For 12 rabbits recovered from type II and then inoculated with type VIII the figures were 106.1, 103.8, 103.7, 103.6, 103.3, 102.8, 102.8, and for 12 control rabbits 105.7, 105, 105.7, 106.3, 105.2, 104.7, 103.7.

Two hundred readings on the rectal temperatures of normal rabbits indicate that 103.5°F is the upper limit of normal variation, and 102.4° the lower limit. Thus the temperatures of the control group inoculated with type III pneumococci remained up for more than 4 days whereas the reinoculated group showed a rise for one day and then a drop to about normal on the second day. Control rabbits inoculated with type VIII had abnormally high temperatures for 7 days, whereas the reinoculated group showed a drop on the second day, with an isolated rabbit having a temperature higher than normal on each of the following 3 days.

It is evident from the above data that in addition to a pronounced type-specific immunity present in rabbits recovered from type II infections, there is also a distinct species-specific immunity, as shown by the protective action against infection with the heterologous type I. The results obtained with the avirulent types III and VIII show

a degree of protection due to the original type II infection, in that the lesions produced were limited in extent, and the temperatures of reinoculated animals were lower on the average than those of control animals. The ulcers formed may be indicative of an allergic condition set up coincident with a state of partial immunity.

### 10960 P

#### Conductive Cooling of Living Human Tissue.\*

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In the course of studies of temperature changes in the posterior male urethra, I investigated the effect of running cold water through a hollow metal rectal applicator. I found (as Table I indicates) that this procedure caused a definite fall in the temperature of the posterior urethra.

The posterior urethral temperatures were determined by means of a thermocouple (made of iron-constantan) with its tip protruding through an opening at the end of a silk woven catheter. The catheter was pushed beyond the cut-off muscle. The thermocouple was permitted to remain in the posterior urethra during the course of the experiment. The greatest fall in urethral temperature was 23.8°F, occurring in experiment No. 5. Before and after each experiment the accuracy of the thermocouple was checked by comparing its readings with standard thermometers.

Because of the marked effect of cold when applied conductively in this region, investigations of the influence of this measure were made in several other parts of the human body.

TABLE I.

Experiment No.	Urethral temperature °F		Temperature of water passing through rectal applicator	Duration of experiment	
	At start	At end		Hr.	Min.
1	99.1	95.2	70	1	15
2	99.1	93.3	62	1	10
3	99.	95.9	64		30
4	98.8	77.7	44	1	30
5	98.4	74.6	44	1	25

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