THE PRODUCTION OF INFECTION WITH PNEUMOCOCCI IN RABBITS BY INTRADERMAL INOCULATION OF TYPE III PNEUMOCOCCI, THE TREATMENT WITH SPECIFIC ANTISERUM, AND A COMPARISON WITH TYPE I INFECTIONS

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The very favorable results from treatment of Type I pneumonias and the encouraging although less favorable results in Type II led the clinicians who were aiding in the general pneumonia investigation* to try serum treatment in Type III infections. As the observations and statistics of Bullowa,[1] Rosenbluth,[1] Park, Bullowa and Rosenbluth,[2] Cecil and Sutliff[3] of the treatment of patients with Type III pneumonia with specific antiserum did not indicate very favorable results, it seemed to us that it would be interesting to study the effect of the Type III antiserum in the treatment of pneumonia in experimental animals. The progress of the disease could be compared with that of Type I pneumonia in which serum treatment of patients has proved to be of value. In case the animals could be infected and cured, the relative amount of serum necessary could be found and might furnish data on which to base a plan of dosage for further clinical trial of the antiserum.

The production of an infection by intradermal inoculation and the subsequent intravenous inoculation of a single dose of antiserum was carried out by a technique slightly modified from that used by Good-

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[1] *Jour. Am. Med. Assn.*, 1928, **90**, 1354, 1351.

[2] *Jour. Am. Med. Assn.*, 1928, **91**, 1503

[3] *Jour. Am. Med. Assn.*, 1928, **91**, 2035.

ner[4] in his Type I intradermal pneumonia experiments. Goodner showed that by the injection of a small amount of Type I pneumococcus culture intradermally a local lesion and a bacteremia could be produced and that the infection was fatal for about 96 per cent of the rabbits inoculated. He showed that the bacteremia and the development of the lesion could be checked and that recovery took place when suitable amounts of Type I antiserum were given. He suggested that this method be used for the standardization of Type I antipneumococcus serum.

For our experimental work in treating the Type III infections a concentrated Type III antiserum was used which was estimated by mouse protection tests to have 1000 units per cubic centimeter against the stock Type III strain. This strain was the one used for immunization of the horse from which the antiserum used for the therapeutic preparation was obtained. This antiserum, however, by the mouse protection test had only 1 or 2 units per cubic centimeter protection against the freshly isolated Type III strain, "Schultz," which was used for one of the series of intradermal infection tests reported below. The Type III "Schultz" strain was recovered from the lung of a postmortem case February 17, 1930. The three other Type III strains used to produce infection in rabbits were "R_3," the stock laboratory strain, "Cartell," a strain isolated from the meninges December 19, 1929, and "Challenger" isolated from the blood of a fatal case April 9, 1930. A comparative study of the development of the skin lesion and bacteremia and the therapeutic treatment with antisera was made using the Type III strains and a Type I strain.

Method

The cultures were grown in beef heart broth (pH 7.5) to which small amounts of sterile defibrinated horse blood had been added. All cultures were of such a virulence that 0.000,000,01 cc. of an 18 hour standardized culture given intraperitoneally killed mice in 48 to 72 hours. The virulence was tested by injecting the high dilutions of the culture into mice about ½ hour before the intradermal injection was made in the rabbits.

Healthy gray rabbits, male or female, weighing 1525 to 2750 gm. were selected. The abdominal area of each rabbit was shaved and depilated the day previous

[4] *Jour. Exp. Med.*, 1928, **48**, 1 and 413.
Jour. Immunology, 1929, **17**, 279.

to the inoculation. Inoculation was made 6 to 7 cm. lateral to the rabbit's ventral midline. Each animal received an intradermal injection of 0.001, 0.0005 or 0.0002 cc. of pneumococcus culture.

Eighteen hours after inoculation, 1 cc. of blood was withdrawn from the marginal vein of the ear, by means of a sterile syringe, and expelled into a tube containing 1 cc. of 2 per cent citrated beef heart broth. Previous experiments had proved that 2 per cent sodium citrate in rabbit blood, the smallest amount that could be depended upon to prevent coagulation, did not very appreciably inhibit the growth of pneumococci. As soon as possible 0.1, 0.3 and 0.6 cc. were pipetted from the citrated blood broth suspension into sterile petri dishes and melted agar, cooled to 45°C., to which 2 per cent horse blood had been added, was poured into the petri dishes. The citrated rabbit blood and agar were gently rotated until the mixture was uniform. The plates were incubated for 24 to 48 hours at 37.5°C. The sum of the colony count of the three plates is approximately the number of diplococci per 0.5 cc. of blood.

The remaining 1 cc. of citrated rabbit blood dilution was added to beef heart blood broth. Occasionally pneumococci will grow in the liquid medium when the poured plates for a given blood culture are negative. Blood broth cultures were made from the heart's blood of animals which died during the course of an experiment, or which were killed at the conclusion of an experiment.

Preliminary experiments were carried out to determine the extent and character of the lesion produced by Type III strains and their power of invading the blood stream (Table I).

A stock strain "R_3" which has been passed through mice at least once a week during its cultivation in the laboratory for many years and a freshly isolated strain, "Cartell," were inoculated intradermally into rabbits. Four rabbits received the "R_3" strain and two rabbits received the "Cartell" strain in 0.001 cc. doses. The stock strain in a dose estimated to contain 130,000 diplococci stimulated lesions of different severity in individual rabbits which lasted from 2 to 10 days. The freshly isolated strain "Cartell," in a dose containing 350,000 diplococci, produced severe lesions lasting until death of the animals. Later, because of the early death of the rabbits in the first experiment, the "Cartell" strain, after preliminary passage through a rabbit, was injected into six rabbits in duplicate doses of 0.001, 0.0005 and 0.0002 cc. One of the animals which received 0.001 cc., 120,000 organisms, had a slight lesion lasting only 3 days. The other developed a lesion of moderate severity which lasted until its death on the ninth day. One of the rabbits inoculated with one half as many diplococci, 0.0005 cc., developed a severe lesion and died on the sixth day. The other rabbit had a slight lesion on the second day and survived. The rabbits which received 0.0002 cc., 24,000 organisms, developed moderate lesions which lasted in one instance until the death of the animal on the tenth day and in the other more than 14 days.

The Type III lesion was found to be definitely established at 18 to 48 hours as a slightly swollen area 1 to 5 cm. in diameter, regardless of whether the strain used was a stock or freshly isolated one. The redness and edema increased to 3 to 10 cm. with parts of the surface of the lesion hemorrhagic after 2 to 3 days. In our experiments Type III strains did not produce necrosis, nor did the lesions tend to spread extensively over the abdomens of the rabbits. The development of the lesion in rabbits inoculated with Type I culture was much more rapid than in those inoculated with Type III. Redness and edema of 5 to 10 cm. with hemorrhage was not unusual 24 hours after intradermal inoculation. Necrosis with seepage of exudate at central area of lesion was pronounced in 95 per cent of the untreated Type I rabbits in 48 to 72 hours.

The comparative power of the different Type III strains to invade the blood stream was as follows: The stock strain used in four rabbits was not found in the blood stream over a period of 13 days. Three of the four animals survived. One rabbit died on the seventh day but no pneumococci were recovered from the heart's blood at autopsy. On the twenty-eighth day the remaining animals were killed and autopsies made. No pneumococci were found in cultures made from the heart's blood of these animals. The freshly isolated strain "Cartell," inoculated into two rabbits, caused their death within 48 hours. Plate counts of the blood of these animals 18 hours after inoculation showed 186 pneumococcus colonies per cubic centimeter in one and in the other, numbers too great to count in the amount examined. Pneumococci were recovered from the heart's blood at autopsy.

The results with the rabbits inoculated with Type III, "Cartell," in doses of 0.001, 0.0005 and 0.0002 cc. were as follows: one of the rabbits inoculated with 0.001 cc. culture, actually one-third the number of organisms injected in the first "Cartell" experiment, died after a bacteremia of 8 days duration. The other rabbit had a positive blood culture on the sixth, seventh and eighth day and died on the ninth day. It is interesting that pneumococci were recovered as late as the fourteenth day from the blood stream of two rabbits which survived after the inoculation with 0.0005 cc. and with 0.0002 cc. culture. In two instances pneumococci were found to be present in the blood in 24 hours after injection. In one rabbit pneumococci were found after

48 hours, in another after 72 hours and in a third after 96 hours. Two of these rabbits died. The rabbit which survived had over 1660 organisms per cubic centimeter at 72 hours, 126 organisms per cubic centimeter at 96 hours and later four negative blood cultures with subsequent occurrence of small numbers of organisms in the blood for 5 days.

The invasive power of a Type I stock strain inoculated intradermally in a dose of 0.001 cc. into eight rabbits is given for comparison (Table II) with the results with Type III. Pneumococci were recovered from the blood stream of all the rabbits 18 hours after the intradermal injection of the culture. The dose of diplococci introduced into the rabbits varied from 130,000 to 400,000 but there was no correlation between the severity of lesion or the day of death and the number of diplococci injected. All animals died in 3 to 6 days after inoculation of the culture. Pneumococci were recovered from the heart's blood at autopsy.

The effect of treatment of rabbits infected with Type III pneumococci with Type III antiserum was investigated (Table III).

The freshly isolated "Cartell" strain, which was used in the experiments reported above, and a still more recently isolated Type III strain, "Schultz" were used for these tests. 0.001 cc. of these strains was inoculated intradermally into two series of rabbits. All animals responded with definite lesions and bacteremia in 18 hours. Two animals of each series received no antiserum and served as controls and the other four received a single intravenous injection of Type III antiserum 24 hours after the intradermal inoculation of the cultures. Relatively large amounts of serum were tried because the results in mice led us to believe that in Type III infections large amounts would be necessary. Those inoculated with the "Schultz" strain were protected by 20 cc. of serum, 20,000 units, and the blood cultures remained negative after treatment. The blood cultures of the rabbit receiving 10 cc., 10,000 units, were found to be negative after treatment and until after the death of the animal when pneumococci were found in the heart's blood cultures at autopsy. The rabbit receiving 5000 units died 1 day after treatment. No pneumococci however, were recovered from the heart's blood at autopsy. It is probable that this rabbit did not die because of the failure of the serum to protect but from some other cause. Of the first series of rabbits injected with the "Cartell" strain, the blood cultures of those receiving 10,000 and 20,000 units were found negative after treatment and the animals survived. The blood of the rabbit receiving 5000 units was found positive on the first day, negative on the second day, 24 hours after treatment and positive again on the third day; but

TABLE I

Development of Local Lesion and Bacteremia in Rabbits Inoculated Intradermally with Stock or Freshly Isolated Type III Strains

Strain and number of diplococci in dose	Dose	Rabbit number		Degree of lesion development and number of pneumococci per cc. in blood after the following days											Survival or day of death	Blood cultures at autopsy
				1	2	3	4	6	7	8	9	10	11	13	14	
	cc.															
R_3	0.001	6-84	Lesion	+	++	+++	++	++								7	Negative
Stock			Pneumo	0	0	0	0	0								
130,000	0.001	6-76	Lesion	+++	+++	+++	+++	+++	+++	++	+	−	−	−		S	Negative
			Pneumo	0	0	0	0	0	0	0	0	0	0	0		
	0.001	6-14	Lesion	++	++	−	−	−	−	−	−	−	−	−		S	Negative
			Pneumo	0	0	0	0	0	0	0	0	0	0	0		
	0.001	6-51	Lesion	+++	+++	+++	+++	++	++	++	++	+	−	−		S	Negative
			Pneumo	0	0	0	0	0	0	0	0	0	0	0		
"Cartell"	0.001	6-92	Lesion	+++												2	Positive
350,000			Pneumo	∞													
	0.001	6-83	Lesion	+++	+++											2	Positive
			Pneumo	186													
"Cartell"	0.001	3-73	Lesion	+	+	+	++	++	+++	+++						9	Positive
120,000			Pneumo	0	0	0	0	42	26	68							
	0.001	27	Lesion	+	+	+	−	−	−	−						8	Positive
			Pneumo	∞	∞	∞	∞	6,000	226	170							
"Cartell"	0.0005	29	Lesion	+	++++	++++	++++									6	Positive
60,000			Pneumo	0	0	0‡	430										

	0.0005	25	Lesion	−	+	−	−	−	−	−	−	−	−	−	−	S	Negative
			Pneumo	0	0	1,660	126	0	0	0	0	40‡	48	16	0‡		
"Cartell"	0.0002	26	Lesion	+++	++++	++++	++++	+++	+++	++	++	++	++	+	+	S	Negative
24,000			Pneumo	20	3,500	∞	5,500	540	6	6	74	86	230	80	10*		
	0.0002	24	Lesion	++	++	+++	+++	++	++	++	++					10	Positive
			Pneumo	86	106	100	146	14	6	2	8						

− Negative. + Slight lesion. ++ Fair lesion. +++ Moderate lesion. ++++ Maximum lesion. ∞ Too many to count in the amounts which were plated.

Rabbits receiving 0.001 cc. culture were killed 26 days after initial inoculation of culture.

Rabbits receiving 0.0005 and 0.0002 cc. of culture were killed 27 days after initial inoculation of culture.

* The blood culture of Rabbit 26 negative after the fourteenth day.

‡ Blood broth positive.

TABLE II

Development of Local Lesion and Bacteremia in Rabbits Inoculated Intradermally with a Type I R1 Strain

No. of diplococci per dose	Rabbit number	Lesion development after the following days						No. of pneumococci in the blood after the following days						Day of death	Blood culture
		1	2	3	4	5	6	1	2	3	4	5	6		
400,000	3-96	+++	++++	++++				120	∞	∞				4	Pos.
	3-71	+++	++++					540	∞					3	Pos.
130,000	6-95	+++	+++	+++				1,500	9,000	∞				4	Pos.
	6-94	+++	+++					410	6,000					3	Pos.
150,000	3-87	+++	+++	+++				1,220	∞	∞				4	Pos.
	3-70	++++	++++	++++				∞	∞	∞				4	Pos.
400,000	11-36	+++	++++	++++	++++	++++	++++	6,300	6,500	180		350		6	Pos.
	11-35	+++	++++	++++	++++	++++		6,200	63,000	100,000				5	Pos.

after that was found negative and the rabbit survived. No end point of protection was found in this experiment.

Another series of rabbits injected intradermally with the "Cartell" strain was treated with smaller doses of serum. The blood cultures of those receiving 5000 and 1000 units were found negative after treatment and the rabbits survived. The blood cultures of one of the rabbits receiving 500 units was positive on first, negative on the second day, 24 hours after treatment, positive again on the third and fourth day and the rabbit survived. The other rabbit which was killed on the tenth day because of a severe diarrhoea had positive blood cultures for 8 days. Apparently the dose of 500 units of serum did not check the infection sufficiently to clear the blood at once of pneumococci but delayed its development long enough to aid the rabbits to recover.

The treatment of two series of rabbits inoculated with another freshly isolated Type III strain, "Challenger," was carried out. The "Challenger" strain was isolated from the blood of a fatal case of pneumonia. In the first series the control rabbits died on the fifth and sixth days. The rabbits receiving 1000 units or more of Type III antiserum survived. The rabbits receiving 300 and 500 units of serum died on the second and the sixth day, respectively. It is uncertain how far the overwhelming bacteremia developed in these last two rabbits before treatment influenced the determination of the end point of protection as all the other rabbits at that time had only a few or no organisms in the blood.

Therefore, we tested another series of rabbits with the purpose of determining the end point of protection, more certainly. Our results in this series were not fully satisfactory as the plates made from the blood of the rabbits to determine the extent of the bacteremia before inoculation were so badly contaminated with *Streptococcus viridans* which was present as a contamination in the horse blood used for the blood agar, as to render it impossible to count the pneumococcus colonies accurately. One of the rabbits receiving 150 units of serum died, the other had a bacteremia for 8 days and recovered. With one exception, all the rabbits which received 300 units or more had no positive blood cultures after treatment and survived; one rabbit receiving 500 units either had an overwhelming bacteremia before treatment or developed it shortly afterwards and died on the fourth day of the infection. Protection occurred in this series of rabbits with a smaller amount of serum than we had expected.

Results obtained with rabbits inoculated with Type I pneumococci and treated with Type I antiserum or concentrated antiserum are given for comparison (Table V).

A series of six rabbits received by intradermal injection 0.001 cc. dose of Type I culture containing 200,000 diplococci. Eighteen hours after inoculation all the rabbits were found to have positive blood cultures. Six hours later four of the rabbits received a single injection of serum containing one of the following amounts:

TABLE III

The Effect in Rabbits of Intravenous Injection of Type III Concentrated Antiserum Given Twenty-Four Hours after Intradermal Inoculations with Freshly Isolated Type III Strains

Pneumococcus strain and No. of diplococci per dose	Rabbit number	Concentrated Type III serum. Lot 7 (Felton)		Degree of lesion development after the following days								No. of pneumococci per cc. in the blood after the following days								Survival or day of death	Blood cultures at autopsy
		Cc.	Units	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8		
"Schultz"	14	5	5,000	++								54								2	Negative
100,000	8	10	10,000	+	+	+						50	0	0						4	Positive
	13	20	20,000	+	+	++	+++	+++	+++	+++	++	384	0	0	0	0	0	0	0	S	
	93	20	20,000	+	++	++	+++	++	++	+	+	50	0	0	0	0	0	0	0	S	
	12	0	0	+	+++	+++						186	∞							3	Positive
	99	0	0	+	++	++	++					28	16	8	10					5	Positive
"Cartell"	3-95	5	5,000	+	+	+	−	−	−	−	−	66	0	44	0	0	0	0	0	S	
180,000	3-60	10	10,000	++	++	+	−	−	−	−	−	94	0	0	0	0	0	0	0	S	
	3-53	20	20,000	++	−	−	−	−	−	−	−	244	0	0	0	0	0	0	0	S	
	3-72	20	20,000	++	−	−	−	−	−	−	−	38	0	0	0	0	0	0	0	S	
	3-49	0	0	++	+++	+++						10	∞							3	Positive
	3-88	0	0	++	+++	+++						16	∞							3	Positive
"Cartell"	1-00	0.5	500	+	+	+	−	−	−	−	−	28	0	8	4	0	0	0	0	S	
200,000	3-83	0.5	500	++	+++	+++	+++	+++	+++	++	+	40	10	12	6	16	4	2	8	*	Negative
	2-97	1.0	1,000	+	++	+	+	+	+	−	−	14	0	0	0	0	0	0	0	S	
	2-07	1.0	1,000	+	+	−	−	−	−	−	−	16	0	0	0	0	0	0	0	S	
	3-50	5.0	5,000	+	+	+	+	−	−	−	−	16	0	0	0	0	0	0	0	S	
	4-00	5.0	5,000	+	+	−	−	−	−	−	−	24	0	0	0	0	0	0	0	S	
	94	0	0	+	+	+	+	+	+			20	16	34	270	48				6	Positive
	3-94	0	0	++	++	+++						14	207,000	∞						4	Positive

* Animal discharged from series on the tenth day because of diarrhea and snuffles. No pneumococci were recovered from the heart's blood.

TABLE IV

The Effect in Rabbits of Intravenous Inoculation of Type III Concentrated Antiserum Twenty-Four Hours after the Intradermal Injection of Type III Pneumococcus (Challenger Strain)

Pneumococcus strain and No. of diplococci per dose	Rabbit number	Concentrated Type III. Lot 7 (Felton)		Degree of lesion development after the following days							No. of diplococci per cc. in the blood after the following days							Survival or day of death	Blood cultures at autopsy
		Cc.	Units	1	2	4	6	8	10	12	1	2	4	6	8	10	12		
"Challenger"	4-50	0.3	300	+							∞							2	Positive
180,000	2-00	0.5	500	+	+	−	−				∞	∞	∞	∞				6	Positive
	3-74	1.0	1,000	+	+	−	−	−	−	−	6	0	0	0	0	0	0	S	
	3-86	5.0	5,000	+	+	−	−	−	−	−	0	6	0	0	0	0	0	S	
	4-87	10.0	10,000	+	+	−	−	−	−	−	16	0	0	0	0	0	0	S	
	3-98	20.0	20,000	+	−	−	−	−	−	−	10	0	0	0	0	0	0	S	
	3-70	0	0	++	+++	+++					6	∞	∞					5	Positive
	4-96	0	0	++	+++	+++					0	10	∞	∞				6	Positive
"Challenger"	60	0.15	150	++	+++	+++	+++	−	−	−	Pos.	900	88,500	5,100	4	0	0	S	
200,000	63	0.15	150	++	+++	+++	+++				Pos.	∞	∞	4,200				7	Positive
	65	0.3	300	++	++	+++	+++	+	+	+	Pos.	0	0	0	0	0	0	S	
	66	0.3	300	+	+++	+++	++	+	+	+	Pos.	0	0	0	0	0	0	S	
	68	0.5	500	+	+	−	−	−	−		Pos.	0	0	0	0	0		11	Negative
	61	0.5	500	+	++						Pos.	∞						4	Positive
	67	1.0	1,000	+	+++	+++	++	+	+		Pos.	0	0	0	0	0		11	Negative
	69	1.0	1,000	+	+	+++	+++	+	+	+	Pos.	0	0	0	0	0	0	S	
	62	0	0	+++	+++	+++	+++	+			Pos.	∞	∞	6,000	20			9	Positive
	64	0	0	++							Pos.							2	Positive

TABLE V

The Effect in Rabbits of Intravenous Injection of Type I Antiserum Given Twenty-Four Hours after Intradermal Inoculation of a Type I Pneumococcus Strain

Pneumococcus strain and No. of diplococci per dose	Rabbit number	Type I antiserum[1]		Degree of lesion and development after the following days							No. of pneumococci per cc. in the blood after the following days							Survival or day of death	Blood cultures at autopsy
		Cc.	Units	1	2	3	4	5	6	7	1	2	3	4	5	6	7		
Type I R 1	6-34	1.0	150	+	++	++					800	882	2,400					4	Positive
200,000	6-49	2.0	300	++	+++	++++	++++		+++		120	0	150	0		0		S	
	6-70	4.0	600	++	++++	++++	+++				400	0	0					4[2]	Positive
	6-57	8.0	1,200	+++	++++	++++	++++		++		500	0	0	0		0		S	
	6-78	0	0	+++	++++	++++	+++				200	3,000	200,000	100,000				4	Positive
	6-69	0	0	+++	++++	++++	++++				1,200	5,000	100,000					4	Positive
Type I R 1	6-92	2.0	300	++	+++		++++	++++			500	600			800	1,000		6	Positive
200,000	6-89	2.0	300	+	++		+++	++++			1,000	500			250	100		6	Positive
	6-58	4.0	600	+++	+++		++++	++++	++++	+++	300	0			0	0	0	S	
	6-32	4.0	600	+++	+++		+++	+++	+++	+++	5,000	500			500	0	0	S	
	6-66	0	0	+++							1,000							2	Positive
	6-73	0	0	+++							600							2	Positive
Type I R 1	82	0.1	150	+++	++++	++++		++++	++++		980	600				0	0	7	Positive
150,000	2-87	0.2	300	+++	++++	++++		+++	+++	+++	1,400	0				0	0	S	
	5-06	0.5	750	++	+++	++++		++++	+++	++	2,000	0				0	0	S	
	11-37	0	0	+++	++++	++++		++++			2,800	800						5	Positive
	15-54	0	0	++++	++++	++++		++++			50,000	100,000						5	Positive

[1] The first series of rabbits was treated with regular therapeutic Serum 7A, the second with experimental therapeutic Serum K, and the third with refined, concentrated Serum 23-868 Banzhaf.

[2] Death due to injury to heart in bleeding.

150 units, 300 units, 600 units or 1200 units. The rabbit receiving 150 units like the control rabbits died on the fourth day. The rabbits receiving 300 and 1200 units survived. The one receiving 600 units had two negative blood cultures after treatment but died on the fourth day after an injury to the heart inflicted in making a bleeding. Pneumococci were recovered from the heart's blood.

Another series of six rabbits after inoculation with a Type I culture were injected 24 hours later with Type I antiserum as follows: two of the animals received 2 cc., 300 units of antiserum, and two received 4 cc., 600 units of antiserum. The other two rabbits were held for controls. The animals developed slight to moderate lesions within 18 hours after the inoculation of the culture. The blood of one of the rabbits receiving 300 units of antiserum, was estimated to contain 500 diplococci per cubic centimeter at 18 hours; the bacteremia continued to progress until the sixth day, at which time the animal died. Pneumococci were recovered from the heart's blood at autopsy. The other rabbit receiving 2 cc., 300 units of antiserum, developed an initial bacterial count of 1000 diplococci, but there was a gradual decrease in numbers each day until the fifth day only 100 colonies were estimated to be present in 1 cc. of blood. The rabbit died on the sixth day. Pneumococci were recovered from the heart's blood at autopsy. The antiserum given in a 4 cc. dose sterilized the blood of one rabbit, while the blood of the other remained positive for 72 hours before becoming sterile. Both animals survived the pneumococcus infection.

Another group of five rabbits was inoculated with the Type I stock strain and treated with Type I concentrated antiserum. They received intradermal inoculation of 0.001 cc. of Type I culture, which was estimated to contain 150,000 diplococci per cubic centimeter and 24 hours later intravenous injection of a single dose of the following amounts of the concentrated antiserum: 0.1 cc., 150 units; 0.2 cc., 300 units or 0.5 cc., 750 units. A 0.1 cc. dose of the preparation, 150 units, was not sufficient to neutralize the pneumococcus infection in a rabbit which died the seventh day of the disease. A culture made from the heart's blood at autopsy was positive. In rabbits receiving 0.2 and 0.5 cc. doses the bacteremia was checked after treatment. Both rabbits survived the infection.

The control animals of both the Type I series just discussed developed maximum lesions and a progressive bacteremia and died in 2 to 5 days.

DISCUSSION

The virulence of freshly isolated Type III strains for rabbits was apparently somewhat less than that of Type I as shown by the slighter lesion and the later development of the bacteremia. However, all the untreated rabbits, eighteen in number, inoculated with Type III in doses of 100,000 diplococci or more, died. Two of the rabbits inoculated with smaller doses survived. If these are excluded, and only those taken into account which received 0.001 cc., the amount given

to the animals which were treated, the mortality with this dose was 100 per cent. If the rabbits receiving the smaller amounts are included, twenty rabbits of twenty-two died making a mortality of 90 per cent.

Although the number of rabbits treated is too few to allow a close comparison of the relative amounts of serum required, the results indicate that the amounts of Type III serum required to protect rabbits injected intradermally with freshly isolated Type III cultures were not appreciably, if any, greater than the quantity of Type I serum necessary to protect rabbits inoculated with Type I culture. As Type III apparently is slightly less virulent for rabbits than Type I and it has been found by clinical observation that Type III is more virulent for man than Type I, it is not possible to draw inferences from these experiments as to the relative amounts of serum required for treatment of Type I and Type III pneumonia in man.

In spite of this it has been encouraging to find Type III serum potent for protecting rabbits against infection with freshly isolated Type III strains because it was so little effective in protecting mice against these strains. The Type III antiserum used in the above experiments had by the mouse protection tests only 1 to 10 units of protection against freshly isolated strains and 100 to 1000 times as much serum was required to protect mice inoculated intraperitoneally with these strains as with the fully virulent (see above) stock strain. Clinical trial has indicated that Type I therapeutic serum should have a potency somewhat higher than the early Hygienic Laboratory standard (100 units per cubic centimeter) and preferably should have 500 units or more per cubic centimeter. It is reasonable to believe that Type III therapeutic serum should be of equal or greater potency than Type I. If the potency standards in mouse protection units for Type I sera were applicable to Type III, then the Type III therapeutic sera having only 1 to 25 units protection per cubic centimeter against freshly isolated Type III cultures would be of little use. However, while the mouse protection test probably furnishes a fairly satisfactory basis for comparing the relative potency of sera of the same type, it is doubtful whether it is an adequate basis for comparing the strength of the sera of one type with those of another for treatment of pneumonia in man or in animals other than the

mouse. Our experiments in treating intradermal pneumonia in rabbits support this view. It is probable that the suitable dosage of homologous serum for pneumonias caused by each type will have to be determined by clinical trial of serum standardized arbitrarily in mouse protection units as to its relative potency.

Unless it is shown that the determination of the strength of sera by titration of their protective effect in the intradermal pneumonia of rabbits gives more accurate or additional information as to their therapeutic value, we believe the method to be impractical as a routine method for standardization because it is more laborious and more expensive than the mouse method.

SUMMARY

Intradermal lesions and subsequent bacteremia were produced in rabbits by the intradermal inoculation of Type III strains freshly isolated from pneumonia cases. Slight intradermal lesions were produced but no bacteremia was found in rabbits inoculated with an old stock Type III strain.

The intradermal lesions and the bacteremia produced by the inoculation of Type III cultures in rabbits were usually less severe than those produced by the inoculation of Type I culture.

The bacteremia in rabbits was checked and the rabbits protected by suitable amounts of Type III concentrated antiserum given intravenously 24 hours after the infecting dose.

The amount of Type III antiserum necessary to protect rabbits inoculated intradermally with virulent Type III cultures was not appreciably greater than the amount of Type I antiserum required to protect against Type I.