

ORAL IMMUNIZATION AGAINST PNEUMOCOCCUS TYPES
II AND III AND THE NORMAL VARIATION IN RESIST-
ANCE TO THESE TYPES AMONG RATS

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A preliminary report on the production of an increased resistance to Types II and III pneumococcus by feeding the organisms to rats has been published elsewhere (1). Maeji, working with Type III, has confirmed this result (2). In the first communication it was stated that rats develop a natural immunity to Type II with increasing age and that this makes it difficult to demonstrate the immunizing effect of feeding the organism. At that time it appeared that a similar natural immunity was not built up against Type III. Since then, it has been found that an analogous resistance to Type III does occur, though it is less extensive and less common than in the case of Type II. This natural, increased tolerance for Types II and III pneumococcus appears at different ages in different rats. It can be observed at weaning time or even sooner toward Type II, and somewhat later in life toward Type III. Several strains of each of these two types, highly virulent for mice, have been used in the experiments reported here.

Variation in Resistance to Types II and III

The resistance to Type II reaches such a height in adult rats of 150 gm. that 10^{-8} cc. or more of a culture highly virulent for mice, may fail to kill. Very often 10^{-8} cc. may kill a very young rat, though it has been observed that equally young rats may not infrequently survive 10^{-5} and 10^{-4} cc. 10^{-8} and 10^{-7} cc. have rarely been observed

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to kill an adult rat. In the case of Type III, very young rats also generally succumb to 10^{-8} cc., and with increasing age an increased resistance also takes place, but with the strains so far employed adult rats have failed to survive the excessively large quantities that they

TABLE I
Variation in Resistance of Rats to Type II Pneumococcus (Rockefeller Institute Strain)

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.		gm.	cc.	
23	10^{-8}	D 4	*41	10^{-8}	S	**17	10^{-8}	D 2
24	10^{-7}	D 4	47	10^{-8}	S	**19	10^{-7}	D 2
24	10^{-6}	D 4	48	10^{-4}	S	**20	10^{-6}	D 2
177	10^{-2}	S	49	10^{-8}	S			
184	10^{-1}	D 2				*28	10^{-8}	D 2
197	5x 10^{-1}	D 2	*23	10^{-7}	S	33	10^{-7}	D 2
			22	10^{-8}	S	33	10^{-6}	D 3
*37	10^{-8}	S	22	10^{-8}	S	36	10^{-8}	D 3
39	10^{-7}	S	25	10^{-4}	S	38	10^{-4}	D 2
39	10^{-6}	S				134	10^{-8}	S
203	10^{-3}	D 3	*139	10^{-4}	S	134	10^{-7}	S
229	10^{-2}	D 3	143	10^{-8}	S	140	10^{-6}	D 3
231	10^{-1}	D 2	144	10^{-2}	S	165	10^{-6}	D 3
232	5x 10^{-1}	D 2	171	10^{-1}	S	172	10^{-4}	S
						182	10^{-8}	D 2
*61	10^{-7}	S	*29	10^{-7}	S	180	10^{-2}	S
72	10^{-6}	S	30	10^{-8}	S	182	10^{-1}	D 2
73	10^{-6}	S	30	10^{-8}	S	221	10^{-8}	S
73	10^{-4}	S	191	10^{-8}	S	235	10^{-7}	S
			198	10^{-2}	S	235	10^{-6}	D 2
*99	10^{-2}	S	199	10^{-1}	S	238	10^{-5}	S
94	10^{-2}	D 2	205	5x 10^{-1}	D 2	243	10^{-4}	D 2
104	10^{-1}	D 2				252	10^{-2}	D 2
			*44	10^{-8}	S	275	10^{-2}	D 2
			47	10^{-8}	S	295	10^{-1}	D 3
			48	10^{-7}	S			
			48	10^{-7}	S			
			49	10^{-6}	S			
			48	10^{-6}	S			
			50	10^{-6}	D 2			
			51	10^{-4}	D 2			

S = survived. D = died, —days.

* New series. ** Mice.

do in the case of Type II. It is often observed, particularly in the case of Type II, that when a number of animals of approximately the same age are injected with increasing doses, there is no sharp demarcation dividing the dose which kills from that which does not, but that

quantities of 10^{-6} and 10^{-5} cc. may kill, whereas the rats receiving 10^{-4} and 10^{-3} cc. may live and that finally, those injected with 10^{-2} and 10^{-1} cc. may succumb.

Table I shows results obtained at different times with the Rockefeller Institute strain of Type II. It can be seen that all the facts noted above are illustrated here, namely (1) some very small rats succumb to 10^{-8} cc., others may survive doses thousands of times as large, (2) large rats are not killed by 10^{-8} and 10^{-7} cc. but generally require thousands of times this quantity, (3) the need for large quan-

TABLE II
Comparative Resistance of Mice, and Young and Adult Rats to Pneumococcus Type II (Rockefeller Institute Strain)

Mice			Young rats			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
25	10^{-8}	D 2	50	10^{-8}	S	126	10^{-8}	S
25	10^{-8}	D 2	51	10^{-7}	D 3	118	10^{-8}	S
26	10^{-7}	D 2	57	10^{-8}	S	144	10^{-7}	S
26	10^{-7}	D 2	55	10^{-8}	D 2	152	10^{-7}	S
27	10^{-6}	D 2	61	10^{-4}	D 2	152	10^{-6}	S
27	10^{-6}	D 2	62	10^{-3}	D 3	152	10^{-6}	S
28	10^{-5}	D 2	66	10^{-2}	D 3	157	10^{-6}	S
28	10^{-5}	D 2				161	10^{-6}	S
30	10^{-4}	D 2				157	10^{-4}	S
30	10^{-4}	D 2				165	10^{-4}	D 3*
						171	10^{-3}	S
						169	10^{-3}	S
						197	10^{-3}	S

* Pneumococcus in heart blood.

tities to kill a rat is not owing to a lack of virulence for mice, (4) the irregularity of resistance among rats of approximately the same age (size). Tables II, III, IV, V and VI (using Rockefeller Institute and other strains) further illustrate the facts that, in general the increased resistance appears with increasing age, that the large doses required to kill adult rats are not owing to a diminished virulence for mice in any of the strains used, and that the increased resistance does not appear in rats of approximately the same size at the same time.

The variation in resistance to Type III is less common and appears

later in life than in the case of Type II. Also adult rats have more frequently been found to succumb to smaller doses of the former.

TABLE III
Comparative Resistance of Mice, and Young and Adult Rats to Pneumococcus Type II (Rockefeller Institute Strain)

Mice			Young rats			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
26	10 ⁻⁹	D 2	36	10 ⁻⁸	S	109	10 ⁻⁸	S
*28	10 ⁻⁹	D 5	35	10 ⁻⁸	S	115	10 ⁻⁸	S
29	10 ⁻⁸	D 3	38	10 ⁻⁷	D 2	115	10 ⁻⁷	S
29	10 ⁻⁸	D 2	40	10 ⁻⁷	S	118	10 ⁻⁷	S
30	10 ⁻⁸	S	42	10 ⁻⁶	D 3	118	10 ⁻⁶	S
31	10 ⁻⁷	D 2	46	10 ⁻⁶	D 3	119	10 ⁻⁶	S
32	10 ⁻⁷	D 2	52	10 ⁻⁵	S	120	10 ⁻⁵	S
53	10 ⁻⁷	D 2	52	10 ⁻⁵	S	121	10 ⁻⁵	S
33	10 ⁻⁶	D 2	54	10 ⁻⁴	S	121	10 ⁻⁴	S
33	10 ⁻⁶	D 2	58	10 ⁻⁴	D 3	143	10 ⁻⁴	S
33	10 ⁻⁶	D 2	58	10 ⁻³	D 2	**143	10 ⁻³	D 2
34	10 ⁻⁶	D 2	60	10 ⁻²	D 4	146	10 ⁻³	S
34	10 ⁻⁵	D 2				148	10 ⁻²	S
35	10 ⁻⁵	D 2				180	10 ⁻²	S
36	10 ⁻⁴	D 1						
36	10 ⁻⁴	D 2						

* Contaminant in heart blood.

** Pneumococcus in heart blood.

TABLE IV
Resistance of Mice, and Young and Adult Rats to Pneumococcus Type II (Gill Strain)

Mice			Young rats*			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
17	10 ⁻⁹	S	35	10 ⁻⁸	S	142	10 ⁻⁷	S
17	10 ⁻⁸	S	38	10 ⁻⁷	D 2	146	10 ⁻⁶	S
18	10 ⁻⁸	S	38	10 ⁻⁶	D 2	147	10 ⁻⁵	S
18	10 ⁻⁸	D 1	38	10 ⁻⁵	D 1	149	10 ⁻⁴	S
18	10 ⁻⁷	D 2	39	10 ⁻⁴	D 1	152	10 ⁻³	D 2
18	10 ⁻⁷	D 2	42	10 ⁻³	D 1	165	10 ⁻²	S
18	10 ⁻⁶	D 2						
19	10 ⁻⁶	D 2						

* Single litter.

Table VII shows results obtained with the Rockefeller Institute strain of Type III and indicates in general the greater resistance of adult

rats. There is a suggestion of a loss of this immunity later in life, (in the data of the last group) but this may be a matter of chance. The very young rats succumb to the same small doses (10^{-7} and 10^{-8} cc.) which kill mice. The test with the strain Harris (Table VIII)

TABLE V
Resistance of Mice and Young and Adult Rats to Pneumococcus Type II (Weathers Strain)

Mice			Young rats*			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.		gm.	cc.	
15	10^{-9}	D 2	30	10^{-8}	S	105	10^{-7}	S
16	10^{-9}	S	34	10^{-7}	D 2	111	10^{-8}	S
17	10^{-8}	D 2	34	10^{-8}	D 2	115	10^{-8}	S
18	10^{-8}	S	35	10^{-8}	D 2	119	10^{-8}	D 2
19	10^{-7}	D 2	36	10^{-8}	D 2	128	10^{-8}	S
19	10^{-7}	D 2	36	10^{-8}	D 2	132	10^{-8}	D 2
19	10^{-8}	D 2						
19	10^{-8}	D 2						

* Single litter.

TABLE VI
Resistance of Mice, and Young and Adult Rats to Pneumococcus Type II (Boone Strain)

Mice			Young rats*			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.		gm.	cc.	
17	10^{-9}	S	34	10^{-8}	D 2	143	10^{-7}	S
17	10^{-9}	S	35	10^{-7}	D 3	143	10^{-8}	S
18	10^{-8}	D 2	36	10^{-8}	D 2	147	10^{-8}	S
19	10^{-8}	D 2	37	10^{-8}	D 2	151	10^{-8}	S
19	10^{-7}	D 2	38	10^{-8}	D 2	154	10^{-8}	S
19	10^{-7}	S	38	10^{-8}	D 2	166	10^{-8}	D 2
19	10^{-8}	D 2						
20	10^{-8}	D 2						

* Single litter.

shows further that adult rats possess an increased tolerance toward Type III, whereas very young ones die of doses no larger than are required to kill mice. The results with the strain Fink (Table IX)

illustrate an instance of a relatively early appearance of the resistance to Type III, 10^{-4} cc. failing to kill a rat weighing only 70 gm.

The experiments with the Challenger and Abaca strains (Table X) illustrate the beginning of the appearance of immunity to Type III

TABLE VII

Resistance of Young and Adult Rats to Pneumococcus Type III (Rockefeller Institute Strain)

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
27	10^{-8}	S	*32	10^{-8}	D 2
29	10^{-8}	D 2	33	10^{-7}	D 2
29	10^{-7}	D 2	36	10^{-8}	D 2
29	10^{-8}	D 2	36	10^{-8}	D 2
			42	10^{-4}	D 2
138	10^{-8}	S			
157	10^{-4}	S	130	10^{-7}	S
162	10^{-8}	D 6	139	10^{-8}	S
186	10^{-8}	D 2	162	10^{-8}	D 2
			164	10^{-4}	D 2
*36	10^{-8}	D 2	181	10^{-8}	D 2
40	10^{-7}	D 2	184	10^{-8}	S
41	10^{-8}	D 2	187	10^{-8}	D 2
			195	10^{-1}	D 1
108	10^{-7}	S	230	10^{-8}	S
107	10^{-8}	S	285	10^{-7}	D 7
113	10^{-8}	D 3	237	10^{-8}	D 2
			243	10^{-8}	D 2
*38	10^{-8}	D 2	245	10^{-4}	D 2
40	10^{-7}	D 2	262	10^{-8}	S
54	10^{-8}	D 1	285	10^{-8}	D 2
			305	10^{-1}	D 1
126	10^{-7}	S			
129	10^{-8}	S			
131	10^{-8}	D 2			
*51	10^{-8}	S			
55	10^{-7}	D 2			
58	10^{-8}	D 2			
122	10^{-7}	S			
125	10^{-8}	S			
129	10^{-8}	S			

* New series.

with increasing age, in the former by the survival of one of each of 2 rats injected with 10^{-7} and 10^{-6} cc. respectively, and in the latter by the survival of the rats injected with 10^{-8} and 10^{-7} cc. It can be seen that the virulence for mice is high. The strain Small (Table XI)

seemed at first to be equally toxic for mice, and young and adult rats, killing all 3 in a dose of 10^{-7} cc. (upper half of table). Another group of animals was tested and it was found that although 10^{-8} cc. killed both young rats, 1 survived 10^{-7} cc. and 1 adult rat failed to die of

TABLE VIII

Resistance of Mice and Young and Adult Rats to Pneumococcus Type III (Harris Strain)

Mice			Young rats*			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.		gm.	cc.	
16	10^{-9}	S	29	10^{-8}	D 2	132	10^{-8}	S
17	10^{-9}	S	30	10^{-8}	S	135	10^{-7}	S
17	10^{-8}	S	32	10^{-7}	D 2	138	10^{-6}	S
19	10^{-8}	D 2	32	10^{-6}	D 2	138	10^{-5}	S
19	10^{-7}	D 2	34	10^{-5}	D 2	145	10^{-4}	D 2
19	10^{-6}	D 2	35	10^{-4}	D 2	148	10^{-3}	D 2
20	10^{-6}	D 2						

* A single litter.

TABLE IX

Resistance of Mice, and Young and Adult Rats to Pneumococcus Type III (Fink Strain)

Mice			Young rats*			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.		gm.	cc.	
17	10^{-9}	S	59	10^{-9}	S	143	10^{-8}	S
20	10^{-9}	S	66	10^{-8}	S	141	10^{-7}	S
22	10^{-8}	S	60	10^{-8}	S	157	10^{-6}	S
21	10^{-8}	S	67	10^{-7}	S	165	10^{-5}	S
24	10^{-7}	D 2	68	10^{-6}	S	170	10^{-4}	D 4
23	10^{-7}	D 2	68	10^{-5}	S			
25	10^{-6}	D 2	70	10^{-4}	S			

* A single litter.

10^{-6} cc., showing that rats may develop the characteristic immunity towards this strain as well. This is substantiated by the data in Table XXIII obtained in a feeding experiment where several controls survived 10^{-5} and 10^{-6} cc., although others died of 10^{-8} and 10^{-7} cc.

TABLE X

Resistance of Mice and Rats to Pneumococcus Type III (Challenger and Abaca Strains)

Challenger						Abaca					
Mice			Rats			Mice			Rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
14	10 ⁻⁸	S	79	10 ⁻⁸	D 3	15	10 ⁻⁸	D 3	68	10 ⁻⁸	S
14	10 ⁻⁸	D 2	90	10 ⁻⁷	S	16	10 ⁻⁸	D 3	77	10 ⁻⁷	S
13	10 ⁻⁸	S	91	10 ⁻⁷	D 2	16	10 ⁻⁸	D 2	80	10 ⁻⁷	S
14	10 ⁻⁷	D 2	97	10 ⁻⁸	D 2	16	10 ⁻⁷	D 2	95	10 ⁻⁸	D 2
14	10 ⁻⁷	D 2	94	10 ⁻⁸	S	16	10 ⁻⁷	D 2	93	10 ⁻⁸	D 2
14	10 ⁻⁷	D 2	101	10 ⁻⁸	D 2	17	10 ⁻⁷	D 2	110	10 ⁻⁸	D 2
15	10 ⁻⁸	D 2	111	10 ⁻⁸	D 3	17	10 ⁻⁸	D 2	113	10 ⁻⁸	D 2
15	10 ⁻⁸	D 2	117	10 ⁻⁴	D 2	17	10 ⁻⁸	D 2	115	10 ⁻⁴	D 2
15	10 ⁻⁸	D 2	116	10 ⁻⁴	D 2	17	10 ⁻⁸	D 2	142	10 ⁻⁴	D 2
14	10 ⁻⁸	D 2	133	10 ⁻⁸	D 2	18	10 ⁻⁸	D 2	191	10 ⁻⁸	D 2
15	10 ⁻⁸	D 2	124	10 ⁻⁸	D 2	18	10 ⁻⁸	D 1	150	10 ⁻⁸	D 2
15	10 ⁻⁸	D 1				18	10 ⁻⁸	D 1			

TABLE XI

Resistance of Mice, and Young and Adult Rats to Pneumococcus Type III (Small Strain)

Mice			Young rats*			Adult rats		
Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
**16	10 ⁻⁸	S	34	10 ⁻⁸	S	132	10 ⁻⁸	S
18	10 ⁻⁸	S	37	10 ⁻⁷	D 2	133	10 ⁻⁷	D 2
20	10 ⁻⁷	D 2	51	10 ⁻⁸	D 1	149	10 ⁻⁸	D 2
21	10 ⁻⁸	D 1	53	10 ⁻⁸	D 1	157	10 ⁻⁸	D 1
21	10 ⁻⁸	D 2				160	10 ⁻⁴	D 2
**13	10 ⁻⁸	S	32	10 ⁻⁸	D 2	127	10 ⁻⁷	D 2
16	10 ⁻⁸	S	32	10 ⁻⁸	D 2	128	10 ⁻⁸	S
16	10 ⁻⁸	D 3	35	10 ⁻⁷	D 3	136	10 ⁻⁸	D 1
17	10 ⁻⁷	D 3	37	10 ⁻⁷	S	140	10 ⁻⁸	D 2
19	10 ⁻⁷	D 3	39	10 ⁻⁸	D 2	155	10 ⁻⁸	D 1
22	10 ⁻⁸	D 3	39	10 ⁻⁸	D 1			

* A single litter.

** Two tests done on different days.

Immunity to Pneumococcus Types II and III after Ingestion of the Acid-Killed or of Bile Salt-Dissolved Organisms

The high degree of natural immunity of all but very young rats to Types II and III, particularly to the former, makes it more difficult to

demonstrate clearly the increased resistance which follows feeding of these types than when Type I is employed. If one takes a group of rats at random for example, one may find that the control and treated rats survive equally large doses especially in the case of Type II. This is so because a change from a resistance equal to 1000 M.L.D. to that of 2000 M.L.D., which may have been the effect of feeding the bacteria, is not detectable by the method employed. Even if one chooses very young rats of different litters and mixes them, this difficulty is avoided only fairly well in the case of Type III, and not in the case of Type II.

By using single young litters and dividing into controls and experimental animals it has been possible to show that the ingestion of Type II pneumococci is followed by a definitely increased resistance to this type. This precaution is not necessary in the case of Type III, though desirable, and by using young rats (60 gm.) of different mothers, mixing them and dividing into controls and experimentals, an analogous result is obtained.

When the first experiments were begun with Types II and III, 15 feedings were employed. At the end of this time part of the natural immunity would appear even if young animals were used. After it was found that a single feeding was sufficient for producing an immunity against Type I, the same or a slightly larger number were tried for Types II and III. This removed another interfering factor.

In all the experiments described here the cultures were killed by adding N HCl to make N/12 and leaving at room temperature for 2 hours, and then centrifuging. The media used were either beef heart broth or 0.3 per cent glucose meat extract broth. Wherever bile salt-dissolved organisms were employed they were grown only in beef heart broth. The cultures for injection were grown in beef heart broth enriched with blood, and injections were made intraperitoneally in a volume of 0.20 cc., dilutions being made in beef heart broth.

Experiments with Type II

Experiment 1, Table XII.—Each of 3 litters of 8 was divided into 4 controls and 4 treated rats. The organism was grown in glucose meat extract broth and the bacteria were mixed with cracker meal. Controls were fed the cracker meal alone. The deaths are very regular among the controls, with the exception of 10^{-6} cc. in 1st litter. There were 6 deaths among the untreated and only 1 among the treated rats.

Experiment 2, Table XIII.—Each of 2 litters was divided into controls and treated rats. Organisms were grown in beef heart broth, and mixed with cracker meal. In the 1st litter controls were killed by 10^{-8} , 10^{-7} , 10^{-6} and 10^{-5} cc.

TABLE XII

Resistance to Pneumococcus Type II Following Ingestion of the Acid-Killed Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C28	10^{-8}	S	C49	10^{-8}	S	C32	10^{-8}	S
C37	10^{-7}	D 2	C53	10^{-7}	*	C33	10^{-7}	S
C42	10^{-6}	S	C55	10^{-6}	D 2	C36	10^{-6}	D 2
C43	10^{-5}	D 2	C65	10^{-5}	D 2	C37	10^{-5}	D 1
E37	10^{-8}	S	E48	10^{-8}	S	E30	10^{-8}	S
E38	10^{-7}	S	E53	10^{-7}	S	E32	10^{-7}	S
E42	10^{-6}	S	E56	10^{-6}	S	E32	10^{-6}	S
E43	10^{-5}	D 2	E60	10^{-5}	S	E34	10^{-5}	S

C = control. E = treated rat. S = survived. D = died,—days.

Each group of C and E rats is a litter. Each E rat received bacteria from 5 cc. growth on each of 2 successive days. Test was done 3 days after 2nd feeding.

* Missing, probably died.

TABLE XIII

Resistance to Pneumococcus Type II Following Ingestion of the Acid-Killed Organisms

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C38	10^{-8}	D 2	C34	10^{-8}	S
C40	10^{-7}	D 2	C35	10^{-7}	S
C40	10^{-6}	D 2	C36	10^{-6}	D 2
C49	10^{-5}	D 2	C41	10^{-5}	D 2
E38	10^{-8}	S	E35	10^{-7}	S
E38	10^{-7}	S	E40	10^{-6}	S
E39	10^{-6}	S	E41	10^{-5}	S
E42	10^{-5}	S			

Each group of C and E rats is a litter. Each E rat received bacteria from 5 cc. growth on each of 3 successive days. Test was done 48 hours after 3rd dose.

Treated animals survived these quantities. In the 2nd litter 10^{-7} and 10^{-6} cc. proved fatal for controls but not for treated animals.

Experiment 3, Table XIV.—Each of 3 litters was divided into 4 controls and 4 treated rats. The organisms were grown in beef heart broth and were suspended

in milk. The suspension was administered by medicine dropper. Controls died regularly of 10^{-7} , 10^{-6} and 10^{-5} cc., with one exception in the 3rd litter. The treated animals survived 10^{-7} and 10^{-6} cc. in all 3 litters but succumbed to 10^{-5} cc. There were 8 deaths among controls as against 3 among the treated rats.

TABLE XIV

Resistance to Pneumococcus Type II Following Ingestion of the Acid-Killed Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C39	10^{-8}	S	C36	10^{-8}	S	C30	10^{-8}	S
C41	10^{-7}	D 2	C38	10^{-7}	D 1	C32	10^{-7}	D 1
C44	10^{-6}	D 2	C42	10^{-6}	D 2	C33	10^{-6}	S
C50	10^{-5}	D 1	C45	10^{-5}	D 1	C34	10^{-5}	D 2
E42	10^{-8}	S	E37	10^{-8}	S	E28	10^{-8}	S
E43	10^{-7}	S	E38	10^{-7}	S	E30	10^{-7}	S
E43	10^{-6}	S	E39	10^{-6}	S	E31	10^{-6}	S
E43	10^{-5}	D 2	E40	10^{-5}	D 2	E31	10^{-5}	D 2

Each group of C and E rats is a litter. Each E rat received bacteria from 5 cc. growth on each of 3 successive days. Test was done 1 day after last feeding.

TABLE XV

Resistance to Pneumococcus Type II Following Ingestion of the Acid-Killed Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C37	10^{-8}	S	C34	10^{-8}	D 2	C27	10^{-8}	D 1
C41	10^{-7}	D 2	C31	10^{-7}	S	C29	10^{-7}	D 2
C45	10^{-6}	D 1	C34	10^{-6}	D 1	C32	10^{-6}	D 1
C46	10^{-5}	D 3	C35	10^{-5}	S	C33	10^{-5}	D 1
E38	10^{-8}	S	E31	10^{-8}	D 2	E26	10^{-8}	D 1
E39	10^{-7}	S	E33	10^{-7}	S	E28	10^{-7}	D 2
E42	10^{-6}	S	E33	10^{-6}	S	E31	10^{-6}	S
E48	10^{-5}	S	E34	10^{-5}	S	E34	10^{-5}	S

Each group of C and E rats is a litter. Each E rat received the bacteria from 5 cc. growth on each of 3 successive days. Test was done 48 hours after 3rd feeding.

Experiment 4, Table XV.—Each of 3 litters was divided into 4 controls and 4 treated rats. The organisms were grown in beef heart broth and were mixed with cracker meal. There were 9 deaths among the controls and only 3 among the treated rats.

Three experiments were done in which the Berkefeld V filtrate of sodium glycocholate-dissolved organisms was fed. For Type I such filtrates were found to immunize as well as the entire cell (3). Powdered sodium glycocholate was added to the centrifuged living Type II organisms and after solution was complete water was added, the whole allowed to stand for a while and then filtered. Cracker meal was added to this until the mass was of pasty consistency, and each rat as usual was fed in an individual cage.

TABLE XVI

Resistance to Pneumococcus Type II, Following Ingestion of Berkefeld Filtrate of the Sodium Glycocholate-Dissolved Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C32	10 ⁻⁸	S	C36	10 ⁻⁸	S	C37	10 ⁻⁸	D 2
C34	10 ⁻⁷	D 2	C40	10 ⁻⁷	D 3	C39	10 ⁻⁷	D 2
C37	10 ⁻⁸	D 2	C41	10 ⁻⁸	S	C40	10 ⁻⁸	D 2
C40	10 ⁻⁸	D 2	C42	10 ⁻⁸	D 2	C41	10 ⁻⁸	D 2
E27	10 ⁻⁸	S	E36	10 ⁻⁸	S	E40	10 ⁻⁸	S
E33	10 ⁻⁷	S	E38	10 ⁻⁷	S	E39	10 ⁻⁷	S
E34	10 ⁻⁸	D 2	E42	10 ⁻⁸	D 2	E40	10 ⁻⁸	S
E38	10 ⁻⁸	D 2	E42	10 ⁻⁸	S	E43	10 ⁻⁸	S

Each group of C and E rats is a litter. Each E rat received the equivalent of 10 cc. growth on each of 3 successive days. Test was done 1 day after last feeding.

Experiment 5, Table XVI.—Each of 3 litters was divided into control and treated rats. Deaths were regular among controls with the exception of 10⁻⁶ cc. rat in the 2nd litter. There were 9 deaths among the controls and 3 among the treated animals.

Experiment 6, Table XVII.—Each of 2 litters was divided into controls and treated rats. In each litter 10⁻⁶ and 10⁻⁵ cc. were fatal for controls while all the treated rats lived. 2 deaths in which a contaminating organism was found at autopsy are not counted (1 C and 1 E).

Experiment 7, Table XVIII.—Each of 3 litters was divided into controls and experimental animals. There were 7 deaths among the former and 1 among the latter.

It is evident from these experiments that rats develop an increased resistance to Pneumococcus Type II, following the ingestion of either the dead whole organisms or the Berkefeld filtrate of the bile salt-dissolved bacteria.

In order to learn whether cross-protection existed between Types I and II, three experiments were done, one in which Type II was fed and two in which Type I was administered.

TABLE XVII
Resistance to Pneumococcus Type II, Following Ingestion of Berkefeld Filtrate of Sodium Glycocholate-Dissolved Organisms

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C31	10 ⁻⁸	S	**C36	10 ⁻⁸	D 3
C32	10 ⁻⁷	S	C39	10 ⁻⁷	S
*C39	10 ⁻⁸	D 1	*C44	10 ⁻⁸	D 2
C40	10 ⁻⁸	D 2	C47	10 ⁻⁸	D 1
E28	10 ⁻⁸	S	E37	10 ⁻⁸	S
E29	10 ⁻⁷	S	E41	10 ⁻⁷	S
**E35	10 ⁻⁸	D 4	E42	10 ⁻⁸	S
E35	10 ⁻⁸	S	E46	10 ⁻⁸	S

Each group of C and E rats is a litter. Each E rat received the equivalent of 10 cc. growth on each of 3 successive days. Test was done 2 days after last feeding.

* Pneumococcus in heart blood.

** Contaminant in heart blood. Both of these rats were ill before the injection.

TABLE XVIII
Resistance to Pneumococcus Type II, Following Ingestion of Berkefeld Filtrate of Sodium Glycocholate-Dissolved Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C37	10 ⁻⁸	S	C33	10 ⁻⁸	S	C41	10 ⁻⁸	S
C40	10 ⁻⁷	D 1	C34	10 ⁻⁷	D 2	C43	10 ⁻⁷	D 2
C44	10 ⁻⁸	D 1	C43	10 ⁻⁸	S	C55	10 ⁻⁸	D 3
C44	10 ⁻⁸	D 2	C43	10 ⁻⁸	D 1	C57	10 ⁻⁸	S
E39	10 ⁻⁸	S	E33	10 ⁻⁸	S	E31	10 ⁻⁸	S
E42	10 ⁻⁷	S	E34	10 ⁻⁷	D 2	E42	10 ⁻⁷	S
E45	10 ⁻⁸	S	E35	10 ⁻⁸	S	E49	10 ⁻⁸	S
E46	10 ⁻⁸	S	E37	10 ⁻⁸	S	E54	10 ⁻⁸	S

Each group of C and E rats is a litter. Each E rat received the equivalent of 10 cc. growth on each of 2 successive days. Test was done 2 days after the 2nd feeding.

Experiment 8, Table XIX.—Large quantities (see table) of HCl-killed Type II organisms grown in glucose meat extract broth were fed to rats but it is obvious that little or no increased resistance to Type I resulted.

Experiment 9, Table XX.—Each of 3 litters was divided into controls and

TABLE XIX

Resistance to Pneumococcus Type I, Following Ingestion of Pneumococcus Type II

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C117	10 ⁻⁸	D 2	*C142	10 ⁻⁸	D 2
C125	10 ⁻⁷	D 2	C143	10 ⁻⁷	D 2
E130	10 ⁻⁸	D 2	C144	10 ⁻⁸	D 2
E151	10 ⁻⁸	D 2	C158	10 ⁻⁸	D 2
			E142	10 ⁻⁷	D 2
*C121	10 ⁻⁸	S	E156	10 ⁻⁸	D 2
C127	10 ⁻⁷	S	E143	10 ⁻⁸	D 2
C130	10 ⁻⁸	D 2	E165	10 ⁻⁸	D 2
E112	10 ⁻⁷	D 2			
E131	10 ⁻⁸	D 2			

Each treated rat was fed the equivalent of 50 cc. acid-killed growth per day. In the first group the 2 E rats received 16 such feedings over a period of 21 days. The 2 E rats in the second group received 17 feedings over a period of 22 days and the 4 E rats in the last group received 19 feedings over a period of 25 days. In each group the test was done on the day following the last feeding.

* New series.

TABLE XX

Resistance to Pneumococcus Type II, Following Ingestion of Pneumococcus Type I

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C32	10 ⁻⁸	S	C26	10 ⁻⁸	S	C30	10 ⁻⁸	S
C34	10 ⁻⁷	D 2	C34	10 ⁻⁷	D 2	C31	10 ⁻⁷	S
C35	10 ⁻⁸	D 2	C34	10 ⁻⁸	D 2	C35	10 ⁻⁸	D 2
C35	10 ⁻⁸	S	C37	10 ⁻⁸	D 2	C41	10 ⁻⁸	D 2
E31	10 ⁻⁸	D 2	E34	10 ⁻⁸	S	E29	10 ⁻⁸	S
E36	10 ⁻⁷	D 1	E36	10 ⁻⁷	*	E31	10 ⁻⁷	D 2
E37	10 ⁻⁸	D 1	E38	10 ⁻⁸	D 4	E37	10 ⁻⁸	S
E38	10 ⁻⁸	S	E42	10 ⁻⁸	D 1	E38	10 ⁻⁸	D 1

Each group of C and E rats is a litter. Each E rat was fed the bacteria from 10 cc. growth on each of 2 successive days. Test was done 2 days after 2nd feeding.

* Missing, probably dead.

treated animals. Type I organisms grown in beef heart broth and killed by contact with N/12 HCl were fed. There was a total of 7 deaths among controls and 7 among the treated animals.

Experiment 10, Table XXI.—Each of 3 litters was divided into controls and treated animals. Type I organisms, grown in glucose meat extract broth, killed with N/12 HCl, centrifuged and mixed with cracker meal were fed. There were 8 deaths among the controls and 9 among the treated rats.

TABLE XXI
Resistance to Pneumococcus Type II, Following Ingestion of Pneumococcus Type I

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C36	10 ⁻⁸	S	C36	10 ⁻⁸	D 2	C28	10 ⁻⁸	S
C38	10 ⁻⁷	S	C37	10 ⁻⁷	S	C37	10 ⁻⁷	D 2
C40	10 ⁻⁸	D 1	C42	10 ⁻⁸	D 1	C40	10 ⁻⁸	D 3
C42	10 ⁻⁸	D 1	C47	10 ⁻⁸	D 1	C47	10 ⁻⁸	D 1
E37	10 ⁻⁸	D 2	E36	10 ⁻⁸	D 2	E33	10 ⁻⁸	D 2
E40	10 ⁻⁷	D 2	E39	10 ⁻⁷	S	E35	10 ⁻⁷	D 2
E46	10 ⁻⁸	D 2	E41	10 ⁻⁸	D 3	E39	10 ⁻⁸	S
E50	10 ⁻⁸	D 1	E46	10 ⁻⁸	D 1	E44	10 ⁻⁸	S

Each group of C and E rats in a litter. Each E rat was fed the bacteria from 10 cc. growth on each of 3 successive days. Test was done 2 days after the last feeding.

It is plain that no evidence for any increased resistance against Type II following ingestion of Type I was obtained.

Experiments with Type III

Experiment 1, Table XXII.—22 rats of approximately the same age were divided equally into controls and treated groups. Organisms were grown in beef heart broth, centrifuged and mixed with cracker meal. Of the 11 controls only 3 survived, 1 each with 10⁻⁸, 10⁻⁷ and 10⁻⁶ cc. Of the 11 treated rats only 1 died.

Experiment 2, Table XXIII.—22 rats of approximately the same age were divided equally into controls and treated animals. In order to learn whether desiccation of the bacteria and feeding together with other types would interfere with the immunizing effect, Types I, II, III, IV and V were grown in glucose meat extract broth and administered as a powder. The powder was suspended in water and mixed with cracker meal. In spite of the irregularity in resistance to Type III among control rats it seems clear that the treated animals, with only 1 death among the 11 injected, were immunized. The results seem to show no noticeable loss of immunizing value owing to desiccation, or simultaneous feeding with other types.¹

¹ A similar experiment in which resistance against Type I was tested showed no loss of immunizing action.

TABLE XXII

Resistance to Pneumococcus Type III, Following Ingestion of the Acid-Killed Organisms

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C78	10 ⁻⁸	D 2	E77	10 ⁻⁸	S
C80	10 ⁻⁸	S	E86	10 ⁻⁸	S
C80	10 ⁻⁷	D 2	E88	10 ⁻⁷	S
C88	10 ⁻⁷	S	E92	10 ⁻⁷	S
C87	10 ⁻⁷	D 2	E94	10 ⁻⁷	S
C95	10 ⁻⁸	S	E95	10 ⁻⁸	S
C90	10 ⁻⁸	D 2	E95	10 ⁻⁸	S
C98	10 ⁻⁸	D 2	E95	10 ⁻⁸	S
C107	10 ⁻⁸	D 1	E98	10 ⁻⁸	D 1
C101	10 ⁻⁸	D 2	E119	10 ⁻⁸	S
C110	10 ⁻⁸	D 2	E111	10 ⁻⁸	S

Each E rat received the organisms from 10 cc. growth on each of 3 successive days. Test was done 1 day after last feeding.

TABLE XXIII

*Resistance to Pneumococcus Type III, Following Ingestion of the Acid-Killed Organisms of Types I, II, III, IV and V**

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C92	10 ⁻⁸	D 2	E90	10 ⁻⁸	S
C94	10 ⁻⁸	S	E85	10 ⁻⁸	S
C94	10 ⁻⁷	D 2	E95	10 ⁻⁷	S
C100	10 ⁻⁷	D 2	E101	10 ⁻⁷	S
C101	10 ⁻⁷	D 2	E99	10 ⁻⁷	S
C108	10 ⁻⁸	D 2	E108	10 ⁻⁸	S
C107	10 ⁻⁸	S	E110	10 ⁻⁸	S
C111	10 ⁻⁸	S	E107	10 ⁻⁸	S
C115	10 ⁻⁸	D 2	E121	10 ⁻⁸	S
C125	10 ⁻⁸	S	E114	10 ⁻⁸	D 2
C113	10 ⁻⁸	S	E115	10 ⁻⁸	S

A desiccated powder was fed. This contained HCl-killed Pneumococcus Types I, II, III, IV and V. Each E rat received the bacteria from 5 cc. growth of each type on each of 3 successive days. Test was done 2 days after last feeding.

* Type numbers are those used at Bureau of Laboratories, Department of Health. See Cooper, G., Edwards, M., and Rosenstein, C., *J. Exp. Med.*, 1929, 49, 461.

Experiment 3, Table XXIV.—The details of this experiment are like those in the preceding one. There is less irregularity in resistance among the controls than in Experiment 2. There were 7 survivors among the treated animals and 4 among the controls.

Experiment 4, Table XXV.—The details are the same as for Experiment 1, except for the quantity of bacteria fed and the time of the test. With a single

TABLE XXIV
Resistance to Pneumococcus Type III, Following Ingestion of the Acid-Killed Organisms of Types I, II, III, IV and V

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C72	10 ⁻⁸	S	E75	10 ⁻⁸	S
C77	10 ⁻⁸	D 2	E75	10 ⁻⁸	S
C78	10 ⁻⁷	S	E78	10 ⁻⁷	S
C79	10 ⁻⁷	D 2	E79	10 ⁻⁷	S
C79	10 ⁻⁷	S	E80	10 ⁻⁷	S
C82	10 ⁻⁸	S	E82	10 ⁻⁸	S
C85	10 ⁻⁸	D 2	E81	10 ⁻⁸	S
C84	10 ⁻⁸	D 2	E82	10 ⁻⁸	D 2
C86	10 ⁻⁸	D 2	E86	10 ⁻⁸	D 2
C89	10 ⁻⁸	D 2	E89	10 ⁻⁸	D 2
C85	10 ⁻⁸	D 2	E90	10 ⁻⁸	D 2

A desiccated powder was fed. This contained the acid-killed organisms of Types I, II, III, IV and V. Each E rat was fed the bacteria from 5 cc. growth of each type on each of 3 successive days. Test was done 3 days after last feeding.

TABLE XXV
Resistance to Pneumococcus Type III, Following Ingestion of the Acid-Killed Organism

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C83	10 ⁻⁸	D 2	E80	10 ⁻⁸	S
C81	10 ⁻⁸	S	E85	10 ⁻⁸	S
C90	10 ⁻⁷	D 2	E85	10 ⁻⁷	S
C85	10 ⁻⁷	D 2	E88	10 ⁻⁷	S
C88	10 ⁻⁷	D 2	E95	10 ⁻⁷	S
C90	10 ⁻⁸	S	E95	10 ⁻⁸	S
C90	10 ⁻⁸	D 2	E93	10 ⁻⁸	S
C90	10 ⁻⁸	D 2	E100	10 ⁻⁸	D 2
C93	10 ⁻⁸	D 2	E100	10 ⁻⁸	S
C103	10 ⁻⁸	D 2	E103	10 ⁻⁸	D 2
C98	10 ⁻⁸	D 2	E102	10 ⁻⁸	S
			E101	10 ⁻⁸	S

Each E rat received the bacteria from 5 cc. growth on each of 3 successive days. Test was done 3 days after last feeding.

exception (10⁻⁸ cc.) the controls died uniformly in this group. There were 2 survivors among the 11 controls and 10 among the 12 immunized animals.

Experiment 5, Table XXVI.—There were 3 litters in this experiment, each being divided equally into controls and treated animals. The organisms were grown in beef heart broth, centrifuged, suspended in milk and fed by medicine dropper. Deaths among the controls are quite regular, all but 1 of 12 dying (10^{-8} cc.). Among the treated rats 3 out of 12 died.

TABLE XXVI
Resistance to Pneumococcus Type III, Following Ingestion of the Acid-Killed Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.		gm.	cc.	
C63	10^{-8}	D 2	C73	10^{-8}	D 2	C49	10^{-8}	S
C71	10^{-7}	D 1	C80	10^{-7}	D 2	C50	10^{-7}	D 2
C77	10^{-8}	D 1	C82	10^{-8}	D 2	C61	10^{-8}	D 1
C79	10^{-8}	D 1	C92	10^{-8}	D 1	C68	10^{-8}	D 1
E51	10^{-8}	S	E75	10^{-8}	S	E50	10^{-8}	S
E61	10^{-7}	S	E77	10^{-7}	S	E51	10^{-7}	S
E75	10^{-8}	S	E78	10^{-8}	D 1	E54	10^{-8}	S
E77	10^{-8}	D 1	E83	10^{-8}	D 1	E60	10^{-8}	S

Each E rat received the bacteria from 5 cc. growth on each of 3 successive days. Test was done 1 day after last feeding. Each group of C and E rats is a litter.

These data substantiate the earlier results and show that an immunity can be obtained in rats by oral administration of dead Type III pneumococci. Additional experiments were done with the Berkefeld filtrate (V) of sodium glycocholate-dissolved bacteria. The manner of preparing this filtrate was the same as described above for Type II.

TABLE XXVII
Resistance to Pneumococcus Type III, Following Ingestion of Berkefeld Filtrate of the Sodium Glycocholate-Dissolved Organisms

Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.	
C39	10^{-8}	S	E44	10^{-8}	S
C40	10^{-7}	D 3	E46	10^{-7}	S
C42	10^{-8}	D 2	E50	10^{-8}	S
C46	10^{-8}	D 2	E51	10^{-8}	D 1
C52	10^{-8}	D 2	E53	10^{-8}	D 1
			E50	10^{-8}	D 2

Each E rat received the equivalent of 20 cc. growth 48 hours before the test.

Experiment 6, Table XXVII.—6 young rats were given a single feeding of the filtrate mixed with milk and containing 2 mg. of sodium glycocholate. The controls were fed sodium glycocholate alone in equal amounts in a similar manner. 1 control and 3 treated rats survived.

TABLE XXVIII

Resistance to Pneumococcus Type III, Following Ingestion of the Berkefeld Filtrate of the Sodium Glycocholate-Dissolved Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C33	10 ⁻⁸	S	C39	10 ⁻⁸	S	C55	10 ⁻⁸	D 2
C33	10 ⁻⁷	S	C41	10 ⁻⁷	D 2	C61	10 ⁻⁷	S
C34	10 ⁻⁶	D 2	C45	10 ⁻⁶	D 2	C70	10 ⁻⁶	D 3
C37	10 ⁻⁶	D 5	C49	10 ⁻⁶	S	C74	10 ⁻⁶	D 2
C38	10 ⁻⁶	D 2	C53	10 ⁻⁶	D 2	C85	10 ⁻⁶	S
E30	10 ⁻⁸	S	E45	10 ⁻⁷	S	E64	10 ⁻⁷	S
E35	10 ⁻⁷	S	E45	10 ⁻⁶	S	E65	10 ⁻⁷	S
E36	10 ⁻⁶	S	E54	10 ⁻⁶	D 2	E71	10 ⁻⁶	S
E41	10 ⁻⁶	S	E60	10 ⁻⁶	S	E79	10 ⁻⁶	S
E36	10 ⁻⁵	D 2	E62	10 ⁻⁵	S	E83	10 ⁻⁵	D 3

The test was done 4 days after a single feeding, equivalent to 20 cc. growth, per rat.

TABLE XXIX

Resistance to Pneumococcus Type III, Following Ingestion of the Berkefeld Filtrate of the Sodium Glycocholate-Dissolved Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C10	10 ⁻⁸	D 3	C24	10 ⁻⁸	D 1	C17	10 ⁻⁸	S
C12	10 ⁻⁷	D 2	C24	10 ⁻⁷	D 1	C20	10 ⁻⁸	D 1
						C20	10 ⁻⁷	D 1
E11	10 ⁻⁸	S	E20	10 ⁻⁸	S	C21	10 ⁻⁸	D 1
E13	10 ⁻⁷	S	E26	10 ⁻⁷	S			
E12	10 ⁻⁷	D 2	E27	10 ⁻⁶	D 1	E22	10 ⁻⁸	S
						E23	10 ⁻⁷	S
						E23	10 ⁻⁶	S
						E24	10 ⁻⁵	D 1

A single feeding equivalent to 5 cc. growth was given by medicine dropper 48 hours previously. Each group of C and E rats is a litter.

Experiment 7, Table XXVIII.—A group of rats were divided into 3 parts and each of these was subdivided into controls and treated animals of approximately the same range of weights. The Type III pneumococcus was grown in beef heart broth, centrifuged and dissolved in sodium glycocholate (0.75 mg. for 20 cc.).

Deaths among controls were fairly regular and 9 out of 15 succumbed as against 3 out of 15 among the treated animals.

Experiment 8, Table XXIX.—Each of 3 litters was divided into controls and treated animals. The pneumococcus was grown in beef heart broth, and dissolved in sodium glycocholate (0.5 mg. for organisms from 5 cc.). The animals were fed the Berkefeld filtrate mixed with milk, by medicine dropper. 7 out of 8 controls and 3 out of 10 treated rats died.

Experiment 9, Table XXX.—Each of 3 litters was divided into control and experimental animals. The Type III pneumococcus was grown in beef heart broth, dissolved in sodium glycocholate (1 mg. per day per rat) and mixed with cracker meal. There was only 1 death among the treated rats as against 7 among the controls.

TABLE XXX

Resistance to Pneumococcus Type III, Following Ingestion of Berkefeld Filtrate of the Sodium Glycocholate-Dissolved Organisms

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
gm.	cc.		gm.	cc.		gm.	cc.	
C35	10 ⁻⁸	S	C36	10 ⁻⁸	D 2	C31	10 ⁻⁸	S
C38	10 ⁻⁷	S	C38	10 ⁻⁷	D 2	C33	10 ⁻⁷	D 2
C38	10 ⁻⁸	D 2	C42	10 ⁻⁸	S	C36	10 ⁻⁸	S
C42	10 ⁻⁸	D 1	C45	10 ⁻⁸	D 2	C38	10 ⁻⁸	D 2
E35	10 ⁻⁸	S	E36	10 ⁻⁸	S	E26	10 ⁻⁸	S
E36	10 ⁻⁷	S	E41	10 ⁻⁷	S	E29	10 ⁻⁷	S
E38	10 ⁻⁸	S	E43	10 ⁻⁸	S	E31	10 ⁻⁸	S
E40	10 ⁻⁸	D 2	E45	10 ⁻⁸	S	E33	10 ⁻⁸	S

Each group of C and E rats is a litter. Each E rat was fed the bacteria from 10 cc. growth on each of 2 successive days. Test took place 2 days after 2nd feeding.

It is clear from the data presented in Tables XXII to XXX that the ingestion of the intact dead cell or of the Berkefeld filtrate of the sodium glycocholate-dissolved organism produces an increased resistance to Pneumococcus Type III. It also seems as if the dose and the time of appearance of the immunity are approximately the same as for Type I. A single feeding of the bacteria from 5 cc. growth is sufficient and the effect is evident in 48 hours.

In order to learn whether the immunity thus produced is type-specific, rats were fed Pneumococcus Type III and tested for resistance to Type I. Others were fed Type I and tested with Type III.²

² Earlier experiments, performed before it was known that rats exhibit a variation in resistance to Type III, indicated that feeding Type I did not protect against Type III.

Experiment 10, Table XXXI.—The Type III organism was grown in glucose meat extract broth. The centrifuged bacteria were suspended in water and mixed with cracker meal. 1 of 2 rats injected with 10^{-8} cc. and 1 of 2 injected with 10^{-6} cc. survived among the treated animals, giving the impression that there was some protection. The experiment was repeated.

TABLE XXXI
Resistance to Pneumococcus Type I, Following Ingestion of Pneumococcus Type III

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C81	10^{-8}	D 2	E65	10^{-8}	D 3
C61	10^{-8}	D 3	E72	10^{-8}	S
C82	10^{-7}	D 3	E88	10^{-7}	D 2
C84	10^{-7}	D 2	E74	10^{-7}	D 2
C89	10^{-8}	D 3	E101	10^{-8}	D 2
C86	10^{-8}	D 5	E95	10^{-8}	S
C94	10^{-8}	D 2	E101	10^{-8}	D 1

Each E rat received the bacteria from 5 cc. growth, killed with $N/12$ HCl on each of 2 successive days. Test was done 3 days after 2nd feeding.

TABLE XXXII
Resistance to Pneumococcus Type I, Following Ingestion of Pneumococcus Type III

Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C78	10^{-8}	S	E79	10^{-8}	D 2
*C78	10^{-8}	D 5	E65	10^{-8}	D 2
C81	10^{-8}	D 2	E83	10^{-7}	D 2
C88	10^{-8}	D 2	E80	10^{-7}	D 2
C97	10^{-7}	S	E91	10^{-8}	D 2
C90	10^{-7}	D 2	E90	10^{-8}	D 2
C98	10^{-8}	D 2	E93	10^{-8}	D 2
C104	10^{-8}	D 2	E98	10^{-8}	D 2

Details same as in Table XXXI.

* Pneumococcus in heart blood.

Experiment 11, Table XXXII.—The details are the same as in Experiment 10 except that there were 3 feedings and the test took place 2 days after the last of these. All 8 treated rats succumbed. Among the controls, 1 of 2 animals injected with 10^{-7} cc. survived. It can be seen therefore that very little if any immunity against Type I resulted from the ingestion of Type III organisms.

Experiment 12, Table XXXIII.—Each of 3 litters was divided into controls and experimental rats. The Type I pneumococcus was grown in glucose meat extract

broth. The survival of the 2 treated rats injected with 10^{-8} and 10^{-7} cc. respectively in the 3rd litter, possibly suggests some cross-protection. In view of the variation in resistance to Type III discussed above, and further illustrated by the survival of both controls (in Litters 1 and 2) injected with 10^{-6} cc. in the present

TABLE XXXIII
Resistance to Pneumococcus Type III, Following Ingestion of Type I

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C49	10^{-8}	D 2	C64	10^{-8}	D 2	C70	10^{-8}	D 2
C50	10^{-7}	D 2	C67	10^{-7}	D 2	C74	10^{-7}	D 2
C50	10^{-6}	S	C72	10^{-6}	S	C74	10^{-6}	D 2
C64	10^{-6}	D 1	C78	10^{-6}	D 1	C81	10^{-6}	D 2
E42	10^{-8}	D 2	E66	10^{-8}	S	E69	10^{-8}	S
E55	10^{-7}	D 2	E68	10^{-7}	D 2	E71	10^{-7}	S
E57	10^{-6}	D 2	E73	10^{-6}	D 2	E72	10^{-6}	D 2
E59	10^{-6}	D 1	E76	10^{-6}	D 1	E83	10^{-6}	D 2

Each E rat was fed the bacteria from 5 cc. growth on each of 2 successive days. Test was done 3 days after 2nd dose. Each group of C and E rats is a litter.

TABLE XXXIV
Resistance to Pneumococcus Type III, Following Ingestion of Type I

Weight	Dose	Result	Weight	Dose	Result	Weight	Dose	Result
<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>		<i>gm.</i>	<i>cc.</i>	
C35	10^{-8}	S	C32	10^{-8}	D 2	C33	10^{-8}	D 2
C41	10^{-7}	S	C33	10^{-7}	D 1	C33	10^{-7}	D 1
C43	10^{-6}	D 1	C34	10^{-6}	D 1	C33	10^{-6}	D 1
C45	10^{-6}	D 1	C36	10^{-6}	D 1	C38	10^{-6}	D 1
E40	10^{-8}	D 2	E34	10^{-8}	S	E32	10^{-8}	D 2
E40	10^{-7}	S	E34	10^{-7}	D 1	E34	10^{-7}	D 1
E41	10^{-6}	S	E35	10^{-6}	D 1	E34	10^{-6}	D 2
E50	10^{-6}	D 1	E39	10^{-6}	D 1	E39	10^{-6}	D 1

Each E rat received the bacteria from 7 cc. Type I growth, killed by N/12 HCl, on 1 day, followed by 13 cc. the next day, suspended in milk and given by medicine dropper. Test was done 3 days after 2nd dose. Each group of C and E rats is a litter.

experiment, the results are probably to be considered as indicating no cross-protection.

Experiment 13, Table XXXIV.—Each of 3 litters was divided into control and treated rats. The organism was grown in beef heart broth. There were 10 deaths among the controls and 9 among the treated animals, indicating the absence of any appreciable cross-protection.

DISCUSSION

The marked difference in resistance among rats to both Types II and III pneumococci is apparently the reflection of a natural partial immunity. This partial immunity which appears at a considerably earlier age in some animals than in others, and which is quantitatively greater for Type II than Type III, contrasts markedly with the equal and great susceptibility of young and old rats to Type I pneumococcus. No experimental data are available which explain the appearance of this partial immunity toward these types in the rat. It is possible that the rat harbors these types of pneumococcus normally in the same way that many human beings do. It is also conceivable that among its intestinal flora are some microorganisms sufficiently closely related antigenically to Types II and III pneumococci to give protection against them. This necessarily assumes that such organisms are antigenically more closely related to these types of pneumococci than the latter are to Type I, and that they also can immunize by their presence in the intestine.

Avery, Heidelberger and Goebel (4) have found a close immunological relationship between Type II pneumococcus and a certain strain (E) of Friedländer's bacillus extending to reciprocal protection of the antisera, and which is probably based on the marked resemblance, both chemical and immunological, which they demonstrated existed between the purified specific substances of these organisms.³ Sugg and Neill (5) have demonstrated a similar close resemblance between a variety of *Saccharomyces cerevisiae* and Type II pneumococcus as determined by immune serum reactions, and which they thought was based on an S-anti-S relationship. Sugg, Richardson and Neill (6) have actively immunized mice against Type II pneumococcus by vaccination with this yeast. It would seem from these observations that a partial natural immunity to some types of pneumococci by fortuitous infection with a biologically distantly related microorganism is possible.

It is now evident that the successful immunization of rats against

³ The part which the polysaccharide of the pneumococcus plays in oral immunization is discussed in the next paper, as well as in the papers entitled "The rôle of the soluble specific substance in oral immunization against Pneumococcus Types II and III," and "The fate of orally administered soluble specific substance of Pneumococcus Types I, II and III."

Type I pneumococcus can be duplicated in the case of Types II and III. The difficulties encountered, owing to the natural partial immunity discussed above, are avoided by using young rats to demonstrate the effect of feeding Type III, and by dividing single litters into controls and experimental animals for the Type II experiments. The initial resistance is then low and in many instances rather uniform, so that the immunity created by feeding is not lost sight of as it would be if larger rats already possessed of a marked resistance were used. The dosage and the interval between feeding the vaccine and the appearance of the increased resistance are approximately the same as for Type I. The retention of the immunizing action by the dissolved bacteria further extends the similarities in the results obtained with Types I, II and III. The earlier statement that a constituent of the cell rather than the intact organism is responsible for the immunity produced by feeding the Type I pneumococcus can now be made for Types II and III as well. The mode of action would also seem to be the same, judging by the several analogies.

SUMMARY

1. Considerable variation in the resistance of different rats toward Type II pneumococcus has been demonstrated. In general, older rats survive much greater doses than young ones, illustrating the acquisition of a natural partial immunity. The same is true for Type III but the immunity appears somewhat later in life and does not reach the same height.

2. An active immunity can be created against Types II and III in rats by feeding the dead organisms or the Berkefeld filtrate of the bile salt-dissolved cells. This immunity resembles that obtained against Type I in several respects.

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