THE FATE OF ORALLY ADMINISTERED SOLUBLE
SPECIFIC SUBSTANCE OF PNEUMOCOCCUS
TYPES I, II AND III

By VICTOR ROSS,* PH.D.

*(From the Department of Biological Chemistry, College of Physicians and Surgeons,
Columbia University, and the Bureau of Laboratories, Department
of Health of the City of New York, New York)*

(Received for publication, July 29, 1931)

The writer has reported elsewhere (1, 2) that the ingestion of the soluble specific substance of Pneumococcus Type I by rats is followed by an increased resistance to the homologous organism. The similarities between this effect and the one produced by feeding the whole organism are rather striking. In both cases, (a) 1 feeding is sufficient, (b) the immunity appears within 48 hours, (c) when the immunity is exhausted it can be renewed by a single feeding, (d) the action is type-specific. In general, however, the percentage of animals protected and the degree of increased resistance are greater when the organisms are fed. When the specific polysaccharide of Type III is fed, the immunizing effect is less pronounced than in the case of Type I. No clear evidence of protection following the oral administration of the specific substance of Type II was obtained.

The fact that the Type I specific substance, when orally administered, immunized white rats against the pathogenic organism from which it was isolated, created an interest in its fate after ingestion. Also, since it had been suggested in an earlier communication that the feeding of the vaccine in the very early stages of human pneumonia might have a beneficial effect on the course of the disease (based on the promptness with which an immunity is created in animals), it became necessary to learn whether any of the contained polysaccharide might enter the circulation. The appearance of this substance in the blood

* Wm. J. Gies Fellow in Biological Chemistry, Columbia University, 1930-31. The author wishes to thank Mrs. Lawrance Harriman for her kindness in providing funds in aid of this work.

in more than traces, following the ingestion of pneumococci, would be reason for avoiding such a suggested therapeutic measure since it has been shown that the pneumococidal action of a normal serum-leucocyte mixture is inhibited by the presence of the type-specific polysaccharide (3). The resistance of this substance to the action of trypsin made it probable that it would either be absorbed as such, or eliminated unchanged. Its immunizing action made the former alternative seem a necessary prerequisite, but its polysaccharide nature appeared to make the latter very likely. These considerations would, of course, apply to the soluble specific substances of Types II and III pneumococcus as well.

Analyses of blood, urine and feces were consequently made in order to learn something about the fate of the specific substances of these three types of pneumococci in the animal body.¹ The results obtained in the fecal analysis for Types I and II, and in the preliminary blood and urine examinations for Type I, have been briefly reported elsewhere (4). The work has since been extended to include (1) analyses of the feces following ingestion of the specific substance of Type III and (2) analyses (with the aid of a more refined technique than that used at the beginning) of the blood and urine of rats fed the polysaccharides of Types I, II and III. The present paper gives a detailed description of the methods employed and the experimental data obtained.

Methods

Feeding.—The soluble specific substance of Type I was dissolved either in $N/15$ HCl or in Sørensen's phosphate buffer solution of pH 7.7, that of Type II in water, and of Type III in $N/10$ NaOH followed by neutralization with $N/10$ HCl. The amount administered varied from 0.25 mg. to 6 mg., but in most of the experiments was 1 mg. The desired quantity, generally in a volume of 0.2 to 0.5 cc., was placed in a small dish having a slight concavity to avoid spreading of the fluid, and cracker meal was added to make a pasty mixture. In many cases milk was substituted for cracker meal. The animals were fasted for 8 to 18 hours before feeding and only an occasional one failed to eat the material almost completely in a few minutes. Food was again given 2 or 3 hours later and consisted of bread, water, milk and lettuce.

Cages for Collection of Feces.—The rats were kept in individual cages having two

¹ The results of the chemical analyses of these three substances are given in References 2 and 5.

wire mesh bottoms, the lower a permanent one of $\frac{1}{4}$ inch mesh, the upper removable and of $\frac{1}{2}$ inch mesh, with a space of about 1 inch between them. The feces always dropped through the upper gauze and generally through the lower one as well, resting on filter paper placed in the tray underneath and separated by a distance of about 1 inch from the cage bottom. In the experiments with mice, the cage was made with only one bottom, of $\frac{1}{4}$ inch mesh wire, also raised above the tray. The feces were collected for 3 days following the final feeding, or longer. From 4 to 11 animals comprised each such experiment.

Cages for Collection of Urine.—Each rat was kept in an individual cage measuring about 9 x 9 x 9 inches having two removable wire mesh bottoms, and the corners of which rested almost on the outer edge of a dinner plate. The upper gauze was of $\frac{1}{2}$ inch mesh and the lower of $\frac{1}{4}$ inch. These were 1 inch apart. The feces were retained by the lower gauze and the urine passed through to the plate. Contamination of the urine by the feces as the former passed through the lower gauze was possible, but judging by experience did not take place. One possible exception is discussed below. On the other hand, feces occasionally dropped through the lower gauze into the plate. Such contaminated samples were discarded. The urine was collected at intervals. The longest period of collection was 3 days, when the intervals were 0–18 hours, 24–42, 42–48, 48–66 and 66–72 hours. 5 cc. of N/30 HCl were kept in the plate for the short-period collections and 10 cc. for the longer ones. Each experiment consisted of 1 control and 2 experimental animals.

Examination of the Serum.—The early experiments with Type I specific substance showed that it could be detected if present in a concentration of 1 part in a million of serum. It was thought desirable to avoid the slight extraneous precipitate that formed on incubating rat serum with undiluted antiserum at 37°C. for 18 hours. It was found that if equal volumes of the sample and of a 1:5 dilution of antiserum were used, and the mixture left in an ice box overnight, very satisfactory positive tests were obtained when the soluble specific substance of Types I, II and III were added to rat serum to make a concentration of 1 part in 2 millions. This was the procedure employed in the blood tests recorded below.

Examination of Urine.—The individual specimens were centrifuged, the supernatant fluid made alkaline to phenolphthalein and allowed to stand for from $\frac{1}{2}$ to 1 hour. Following centrifugation, the supernatant was drawn off, its color discharged with N/5 HCl and 0.5 cc. quantities were mixed with 0.5 cc. of a 1:5 dilution of the corresponding antiserum and with 0.5 cc. of a 1:5 dilution of an antiserum of another type to serve as a control. These were left in an ice box overnight. The urines of the control rat and of the 2 fed rats were examined simultaneously in each experiment. By adding known amounts of the specific substance to urine, it was found that 1 part in 3 millions of urine, inclusive of acid, could be detected (Types I, II and III). This is the concentration in which the polysaccharides can be detected in water.

Examination of Feces.—The combined feces of each rat were put into a 50 cc.

graduated centrifuge tube and normal HCl added to bring the volume to the 35 cc. mark. After standing overnight, the pieces were broken up, acid added to the 40 cc. mark, the whole stirred well, allowed to stand for 1 hour and then centrifuged for 1 hour at about 2000 R.P.M. Some of the supernatant fluid was transferred to a 15 cc. centrifuge tube for further clarification, after which 3 cc. of the new supernatant fluid were removed to another 15 cc. centrifuge tube, a drop of phenolphthalein solution put in and 10 per cent solution of NaOH added until distinctly alkaline. At this stage a voluminous precipitate appeared. This was allowed to stand for 1 hour. Enough water was added to make the volume 5 cc., and the whole stirred and centrifuged. The supernatant was generally clear. 4 cc. were transferred to another centrifuge tube and N/5 or N/10 HCl was added until the color disappeared and the solution had become very slightly acid. The liquid was then made up to 4.8 cc. with water, and the whole stirred and left in an ice box overnight. Occasionally a slight sediment formed; this was removed by centrifugation. Most samples remained clear. The concentration of the soluble specific substance was now one-half that in the fluid portion of the original 40 cc. to which the feces and HCl were made up. When 0.5 mg. was fed this concentration would be 1 part in 160,000,² assuming complete elimination in the feces and a uniform distribution of the S.S.S. in the fecal and fluid portions of the original mixture. To the collection of feces of the control rats was added a quantity of polysaccharide equal to that fed, and the extraction was carried out simultaneously with that of the feces of the experimental animals.

Serial dilutions were made with 0.9 per cent salt solution; 0.5 cc. of each dilution was mixed with 0.5 cc. of a 1:5 dilution of antiserum corresponding to the type of the polysaccharide fed. Antiserum of another type was mixed with the extract and with one of its serial dilutions to observe any foreign sediment which might form. These mixtures were left in an ice box for 48 hours in the early experiments. This was subsequently reduced to 24 hours. The tubes were shaken and an arbitrary value of 10 assigned to the precipitates in the tubes representing the control rats, to the feces of which polysaccharide had been added. The tubes representing the fed rats were given numerical values based on this value. If 2 controls were used, the precipitates for the 2nd one were assigned values in terms of the 1st. There were thus a series of numbers for each rat equal to the number of dilutions of the extract which had been set up with serum. These numbers were averaged to obtain a final value representing the amount eliminated in the feces of each rat. It was considered that a more accurate result would be secured by setting up a series of dilutions for each extract than if only the undiluted extract were examined. The obvious difficulties associated with the extraction of such small quantities of material from feces, and the errors inherent in the method as a whole, resulted in considerable variation in the values obtained for different rats in a single experiment, as well as for the individual tubes representing each animal.

² Or less, in those experiments where the original volume was greater than 40 cc.

There was some variation in the procedure described, *e.g.*, where the quantity of feces for several of the rats in an experiment was large, 3 extractions were made with HCl, and the fluid was made up to 100 cc.³ These are mentioned in the description of the experiment.

The resistance of rats fed Types I and II specific substances to the homologous organisms was tested, and is recorded either in this paper or in Reference 2 or 5.

RESULTS

Blood.—In those experiments in which the incubation of the serum took place at 37°C., quantities up to 6 mg. were fed, and in no case was a positive test obtained, thus showing that if present at all in the serum, the polysaccharide was there in a concentration of less than 1 part in a million. Only Type I was fed in such large quantities and tested for at this temperature of incubation. Subsequently, as described above, incubation took place in an ice box, and the tests were extended to include Types I, II and III. 1 mg. of each was fed to each rat and the animals were bled from the heart once, and then sacrificed. Blood was drawn 2, 3, 4, 5 and 6 hours after the ingestion of the polysaccharide, which took place after an 18 hour fast. A total of 90 rats (30 for each type) was examined and no evidence for the presence of the specific substance in the serum was found. This indicates that, if present at all, the concentration must have been less than 1 part in 1 or 2 millions of serum, up to 6 hours after the feeding.

Urine.—The quantity of urine, including acid, collected from the plates for any one period, varied between 3 and 10 cc., the variation having been due to different degrees of evaporation and to differences in length of the periods. This means that from 0.001 to 0.003 mg. of the specific substance would have to be present in the urine before any could be detected. Since 1 mg. was fed, it would also follow that if between 0.1 per cent and 0.3 per cent of the amount fed were present in any one sample of urine, it could be detected. If spread over 2 or

³ It was found that the ratio of the volume of supernatant HCl to that of the fecal sediment after centrifuging should be between 3:1 and 4:1. A smaller ratio appears in some way to inhibit the final precipitation, and a larger one takes out much pigment.

more samples, it would escape detection. Somewhat larger quantities than this might also escape detection if distributed over several collections of urine.

It was also found that 0.01 mg. is the smallest quantity of the polysaccharide of either Type I, II or III, which, when injected subcutaneously or intraperitoneally into a rat, can be detected in the urine. Such an injection is followed by the appearance of from 0.001

TABLE I

Results of Urine Examinations Following Ingestion of Soluble Specific Substance of Pneumococcus Types I, II and III

Hours after ingestion.....	0-18			18-24			24-42			42-48			48-66			66-72		
Type S.S.S. fed...	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Rat No.																		
1	-	-	-	-	°	-	-	-	-	-	-	-	-	*	-	-	-	-
2	-	-	-	-	°	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	*	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	°	*	-	-	-	-	-	-	-	-	-	-	-
10	-	*	-	-	-	°	*	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = absent, + = present.

° Sample of 18 to 42 hours, negative.

* Feces present; sample discarded.

to 0.003 mg. in the urine secreted in the first 18 hours.⁴ After this time none can be found, so that one may conclude that the remainder is probably excreted at so slow a rate as to yield amounts beyond the limits of the test.

Of the urines of 32 rats fed 1 mg. each of either Types I, II or III specific substance, only 1 urine gave a positive test. This was urine

⁴ No attempt was made to learn whether the urine of less than 18 hours had any.

of Rat 3, fed Type III (Table I). The 18 hour sample gave a faintly positive test. Subsequent samples gave a negative result. Although no feces were observed in this sample, it is possible that a minute quantity was present and accounted for the positive precipitin test.

It appears therefore that less than 0.1 per cent to 0.3 per cent of the amount ingested was present in any one urine up to 72 hours, and that

TABLE II
Per Cent of Orally Administered Soluble Specific Substance of Pneumococcus Types I, II and III Found in the Feces

Experiment No.	Type I						Type II		Type III				
	1	2	3	4	5*	6*	7	8	9	10	11	12	13
Control A	100	100	100	100	100	100	100	100	100	100	100	100	100
Control B		94		84		100	102	93	85	133	80	88	113
Rat No.													
1	66	80	99	93	73	98	74	80	48	75	80	22	93
2	69	89	103	—	95	103	91	90	55	30	—	34	70
3	71	86	110	56	85	95	63	77	63	33	90	24	78
4	46	81	80	84	78	80	79	97	85	40	—	—	72
5	59	75	86	67			90	79	43	77	107	30	45
6	130	85	97	79			87	101	60	47	63	36	55
7	91	76	43				96	89	49	30	50	76	68
8	111	83					87	95	73	33	110	—	55
9	—						—	80		60	67	—	58
10										77	50	52	72
11										20	41	34	97
12										—	—		—
Average, per cent...	80	82	88	76	83	94	83	88	60	48	73	38	69
		87		90		94	81	95	71	37	90	43	61

— = sample not done.

* Mice.

it is unlikely that there was as much as 0.01 mg. present at one time in the body.⁵ The tests did not exclude the possibility that this quantity, or somewhat more, was absorbed and excreted over a longer period than 18 hours, but at such a slow rate as to prevent discovery.

Feces.—The failure to find the specific substance in either the serum

⁵ That is, outside the gastrointestinal tract.

or urine in the early experiments, suggested an examination of the feces. It was quickly learned that a large proportion of the ingested material could be demonstrated there. In the following descriptions

TABLE III

Resistance to Pneumococcus Type I, Following Feeding of the Soluble Specific Substance

Dose	Experiment 2				Experiment 3				Experiment 4			
	C		E		C		E		C		E	
	Weight	Result	Weight	Result	Weight	Result	Weight	Result	Weight	Result	Weight	Result
cc.	gm.		gm.		gm.		gm.		gm.		gm.	
10^{-9}	69	S			90	S			77	3		
	65	S							76	S		
10^{-8}	71	2	67 ³	S	92	3	91 ³	S	78	2		
	75	2	60 ⁷	2	94	2	90 ⁷	S	78	2		
10^{-7}	77	2	69 ³	2	101	2	92 ⁵	S	80	2	81 ⁵	2
	78	2	70 ⁵	2	95	S*	93 ²	S	101	2	78 ⁶	2
10^{-6}	101	2	72 ¹	S	117	2	95 ⁶	3			82 ⁴	2
	75	2	75 ²	S	105	2	96 ⁴	S			95 ²	S
10^{-5}	101	2	76 ⁴	2	141	2	98 ¹	S			98 ³	3
			81 ⁶	S	127	7						
10^{-4}											95 ¹	1
	Test was done 72 hrs. after 2nd of 2 feedings				Test was done 72 hrs. after 2nd of 2 feedings				Test was done 5 days after last of 5 feedings			

The exponent appearing above the E weights identifies the rat in the corresponding experiment in Table II where the elimination of the S.S.S. in the feces is recorded.

These three experiments were done with Lot 2 of Type I specific substance described in Reference 2.

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

* Became ill but recovered.

the largest and smallest quantities recovered, as well as the average for all the rats comprising the experiment, are given. Table II gives a summary of all the results.

Experiment 1.—0.5 mg. S.S.S. Type I, dissolved in N/15 HCl, to each of 8 rats. Maximum and minimum values were 130 per cent and 46 per cent, with a final average of 80 per cent. The resistance of these rats to Type I pneumococcus appears in Table V, of Reference 2. 4 out of 9 rats survived.⁶

Experiment 2.—0.5 mg. S.S.S. Type I, dissolved in N/15 HCl, to each of 8 rats on each of 2 successive days. Same quantity of S.S.S. was added to feces of each of 2 controls. Maximum and minimum values were 89 per cent and 75 per cent, with a final average of 82 per cent, using Control A, and of 87 per cent for Control B. 4 out of 8 rats survived an injection of Type I pneumococcus (Table III).

Experiment 3.—0.5 mg. S.S.S. Type I, dissolved in phosphate solution, pH 7.7, to each of 7 rats on each of 2 successive days. Maximum and minimum values were 110 per cent and 43 per cent, with a final average of 88 per cent. The 5th day's feces were collected and examined separately, but no polysaccharide was found. 6 out of 7 rats survived an injection of Type I pneumococcus (Table III).

Experiment 4.—0.5 mg. S.S.S. Type I, dissolved in phosphate solution, to each of 5 rats on each of 5 successive days. Feces were collected in 24 hour periods. Only the 4th day's collection was analyzed. The maximum and minimum values were 93 per cent and 56 per cent, with final averages of 76 per cent and 90 per cent, based on the 2 controls. Only 1 rat out of 6 survived the Type I pneumococcus injection (Table III).

The specific polysaccharide in the feces (8 days collection) of the rats of this experiment was extracted by the same method as that used for its analysis, and then precipitated with antiserum. The recovered material was fed to a new group of rats with the object of learning whether it would immunize them. The results are given in Table IV, Part 1, and show that 7 out of 10 rats survived an injection of Type I pneumococcus.

This experiment was repeated. The soluble specific substance was extracted from the feces of a group of rats which had been tested for cross-protection against Type III. The total quantity originally fed was 12 mg. The recovered polysaccharide was fed to 10 rats on 2 successive days. In order to avoid the possibility that any part of the antiserum used for precipitating the polysaccharide and retained with it, was the cause of the increased resistance, several rats were fed antipneumococcus serum alone. This had no effect. 7 out of 10 rats fed the extracted material survived varying amounts of Type I pneumococcus (Table IV, Part 2).

It is evident from these experiments that a very large proportion of the orally administered Type I polysaccharide can be recovered in the feces and that, when administered by mouth to a new group of rats, it protects them against the homologous organism.

⁶ The discrepancy between the number of rats appearing in the resistance test and the number described as having been given the specific substance is owing to the fact that the latter includes only the animals whose feces were examined.

In view of the fact that mice could not be immunized by feeding the specific substance of Type I, it was desirable to learn whether destruction of the substance takes place in the gastrointestinal tract of this animal. The method of analysis was the same as for rat feces.

TABLE IV

Resistance to Pneumococcus Type I, Following Ingestion of Soluble Specific Substance Recovered from Feces of Rats to Which It Had Been Previously Fed

Dose	Part 1				Part 2					
	C		E		C		C		E	
	Weight	Result	Weight	Result	Weight	Result	Weight	Result	Weight	Result
cc.	gm.		gm.		gm.		gm.		gm.	
10 ⁻⁹	82	S			78	S				
	83	S			78*	5				
10 ⁻⁸	101	2	81	S	81	2	80	2	82	S
	98	2	85	S	88	2	78	2	70	S
10 ⁻⁷	106	2	93	2	97	S	83	2	90	S
	103	2	91	S	90	2	86	4	87	2
			98	S					85	S
10 ⁻⁶	108	2	107	S	98	2	89	2	91	S
			104	S			90	2	92	S
			105	S			88	2		
10 ⁻⁵	111	2	121	2	104	2	94	3	98	2
			133	3			100	2	95	2
							101	2	97	S
	Test was done 4 days after the 2nd of 2 feedings				Test was done 3 days after the 2nd of 2 feedings. The C rats in the 1st column received nothing. Each C rat in the 2nd column was fed 4.25 cc. Type I antiserum on each of 2 successive days					

Parts 1 and 2 of this table give the data of two experiments done at different times.

* Pneumococcus in heart blood.

Experiment 5.—0.25 mg. of S.S.S. Type I, dissolved in a 1:10 dilution of phosphate solution, pH 7.7, was fed to each of 4 mice, and the same quantity added to the feces of a control mouse. Maximum and minimum values were 95 per cent and 73 per cent, with a final average of 83 per cent (Table II).

Experiment 6.—The experiment was repeated under the same conditions. 2 controls were used. The maximum and minimum values were 103 per cent and 80 per cent, with a final average of 94 per cent as compared with both controls (Table II).

Judging from these data, it seems that the quantities recovered are about the same as in the case of rats, and there is consequently no reason to believe that destruction of the material in the gastrointestinal tract plays a part in the failure to immunize mice.

Two experiments were done in which the specific substance of Type II pneumococcus was fed to rats.

Experiment 7.—0.5 mg. S.S.S. Type II, to each of 8 rats on each of 2 successive days. On account of the large bulk, the feces were extracted with 100 cc. normal HCl. There were 2 controls. The maximum and minimum values were 96 per cent and 63 per cent, with final averages of 83 per cent and 81 per cent (Table II).

Experiment 8.—0.5 mg. S.S.S. Type II, to each of 9 rats on each of 2 successive days. There were 2 controls. Maximum and minimum values were 101 per cent and 77 per cent, with final averages of 88 per cent and 95 per cent.

These figures show that Type II polysaccharide is eliminated in the feces of rats in the same proportion as Type I.

Values for the excretion of Type III polysaccharide were lower than for Types I and II, as the following experiments illustrate.

Experiment 9.—0.43 mg. S.S.S. Type III, to each of 8 rats on each of 2 successive days. The maximum and minimum values were 85 per cent and 43 per cent, with a final averages of 60 per cent and 71 per cent⁷ (Table II).

Experiment 10.—0.5 mg. S.S.S. Type III, on 1 day followed by 0.45 mg. the next day to each of 11 rats. The maximum and minimum values were 77 per cent and 20 per cent, with final averages of 48 per cent and 37 per cent (Table II).

Experiment 11.—0.5 mg. S.S.S. Type III, to each of 9 rats on each of 2 successive days. The maximum and minimum values were 110 per cent and 41 per cent, with final averages of 73 per cent and 90 per cent (Table II).

Experiment 12.—0.5 mg. S.S.S. Type III, on each of 2 successive days to each of 8 rats. Maximum and minimum values were 76 per cent and 22 per cent with final averages of 38 per cent and 43 per cent (Table II).

Experiment 13.—0.5 mg. S.S.S. Type III, on each of 2 successive days to each of 11 rats. Maximum and minimum values were 97 per cent and 45 per cent, with final averages of 69 per cent and 61 per cent (Table II).

⁷ The resistance of the fed rats in this and succeeding Type III experiments is recorded in Table II of Reference 5.

DISCUSSION

Judging from the results obtained in these experiments, it can be concluded that at no time, up to 6 hours after the ingestion of 1 mg. of soluble specific substance of Types I, II and III pneumococcus, was there present in the serum a quantity sufficient to make a concentration of 1 part in 2 millions. This fact, coupled with the negative results obtained with the urines up to 72 hours after the feedings, makes it probable that none of the material was absorbed from the intestinal tract. This opinion is strengthened by the fact that quantities up to 84 per cent of Type I, 87 per cent of Type II and 59 per cent of Type III specific polysaccharide could be recovered in the feces.⁸ In spite of this evidence, however, it is conceivable that quantities too small to be detected were absorbed. The lower value for Type III substance is difficult to explain. An unsuitability of the general method used for this particular type of polysaccharide is not likely to be the cause since this would apply to the control feces as well.

There does not appear to be any direct relation between the quantity of polysaccharide found in the feces and the survival or death of the animal when tested with the virulent organism of any of these three types.

The available data suggest the tentative conclusion that certain cells in the gastrointestinal tract are active in the production of the increased resistance which follows the ingestion of the specific substances of Types I and III.⁹ The apparently unchanged state in which this material leaves the intestinal tract, as determined by its activity when fed to new groups of animals, arouses curiosity as to its mode of action.

One of the reasons for making the present investigation was to learn whether it would be contraindicated to undertake the suggested therapeutic measure of feeding a pneumococcus vaccine during the early stages of human pneumonia. If the human being also fails to absorb the polysaccharide of ingested pneumococci, such a procedure loses the principal element of risk which first seems to characterize

⁸ Average of all final averages for Types I (only rats), II and III.

⁹ Regarding Type II, see Reference 5.

it. Healthy persons apparently suffer no ill effects following ingestion of large doses of acid-killed pneumococci (6).

SUMMARY

1. Rats, when fed the soluble specific substance of Types I, II and III pneumococcus, excrete a very large proportion in the feces.

2. Following ingestion of these substances, tests sensitive enough to detect 1 part in 2,000,000 of serum, and 1 part in 3,000,000 of urine, were negative.

3. The polysaccharide of Type I, when recovered from the feces, is as active an immunizing agent as it was originally.

REFERENCES

1. Ross, V., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 658.
2. Ross, V., *J. Exp. Med.*, 1931, **54**, 899.
3. Sia, R. H. P., *J. Exp. Med.*, 1926, **43**, 633.
4. Ross, V., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 274.
5. Ross, V., *J. Exp. Med.*, 1932, **55**, 1.
6. Ross, V., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 822.