THE PROTECTIVE ACTION OF TYPE I ANTIPNEUMOCOCCUS SERUM IN MICE

II. The Course of the Infectious Process

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A preceding paper (1) dealt with the general quantitative aspects of the mouse test for evaluating the protective properties of specific antipneumococcus serum. The present paper is a report of studies concerning the nature of pneumococcus infection in the mouse, and of the modifications of the infectious process induced by the protective action of immune sera.

Although the intraperitoneal infection of mice with pneumococci has long been a standard of laboratory practice, little is known of the nature and course of events in the infective process in these susceptible animals. It was of interest, therefore, to carry out a detailed study covering the following topics: (*a*) the rate of development of the infection as judged by the multiplication of bacteria; (*b*) the nature of the cells in the peritoneum and their influence on the course of the infectious process; (*c*) the changes in these developments induced by the introduction of specific immune serum.

EXPERIMENTAL

The details of the general experimental method are given in the preceding paper (1). They may be briefly summarized as follows:

Mice.—Female mice of the Rockefeller Institute stock were used. The weight range was 17–21 gm.

Culture.—Neufeld strain of Type I Pneumococcus. Virulence for mice such that 0.000,000,01 cc. given intraperitoneally invariably produced fatal infection.

Sera.—A single lot of Type I antipneumococcus horse serum was used throughout these experiments.[1]

[1] This serum was kindly supplied by Dr. Augustus Wadsworth of the Division of Laboratories of the New York State Department of Health.

General Method of Infection and Study.—Mice were injected intraperitoneally with 1 cc. of fluid containing the desired amount of culture and serum, the culture being diluted in broth, the serum in saline. At determined intervals thereafter individual animals were sacrificed by deep ether anesthesia and immediately after death the peritoneal cavity of each animal was thoroughly washed out with 1 cc. of saline. Smears were at once made of these fluids. White cell counts were made by the method commonly used for blood. The smears were stained by the Gram technic and examinations made of a large number of contiguous microscopic fields. The numbers of extracellular and intracellular pneumococci were recorded, and also in the same fields the numbers of white cells showing phagocytosis and those which were negative in this respect were noted. From these data the number of bacteria per cubic millimeter of peritoneal washings was calculated on a proportional basis with reference to the determined number of white cells. It is obvious that information obtained by this method is inaccurate inasmuch as the technic of washing cannot be thoroughly controlled.

Qualitative blood cultures were made by first dipping the tail of the mouse into tincture of iodine, allowing this to dry, and then snipping off the tip with sterile scissors. The blood that exuded was streaked directly on blood agar.

Counts of cells normally present in the peritoneal cavity of control mice of this weight showed considerable individual variation. If, however, one considers the mean count derived from several animals, a fair degree of consistency is obtained.

Course of Intraperitoneal Pneumococcus Infection in Mice

The first experiment was designed to study the course of events following the intraperitoneal injection of virulent pneumococci. No immune serum was administered. Under these circumstances the infected mice die in 12–24 hours. The findings with one experiment of this type are shown in Table I.

Each mouse received 0.1 cc. of Type I pneumococcus broth culture in 1.0 cc. of fluid intraperitoneally. This constitutes more than 10,000,000 minimal fatal infective doses. The method of study was that which has been previously described.

From the data concerning the numbers of cells in the peritoneal cavity, it will be noted that there was no considerable variation until the 2nd hour, at which time a slight increase occurred. At the 4 hour period a marked increase had occurred. Subsequently the number of cells diminished.

A slight decrease in numbers of pneumococci in the peritoneum was noted during the first few minutes but the lag phase of growth was

actually very short. The log phase of growth was demonstrable at the end of the 1st hour, and the increase in numbers was exceedingly rapid up to and including the 4th hour. At the 6th hour the average number of bacteria was somewhat lower, a finding presumably related to the increased numbers of leukocytes present at 4 hours. At 10 hours, however, when the animals were invariably very ill or moribund, the number of bacteria had increased to an exceedingly high figure.

TABLE I

Course of Intraperitoneal Pneumococcus Infection

A series of white mice were each injected intraperitoneally with 1.0 cc. of fluid containing 0.1 cc. of 18 hour blood broth culture of Type I Pneumococcus. At designated intervals thereafter pairs of mice were sacrificed by abrupt ether anesthesia and the peritoneal cavities immediately washed with 1 cc. of saline. The various determinations were made by methods described in the text. Each of these figures represents the average of determinations on two animals.

Time elapsing after infective inoculation	White blood cells per c.mm. of peritoneal washings	Calculated extracellular pneumococci per c.mm. of washings	Calculated total pneumococci per c.mm. of washings	White cells showing phagocytosis	Pneumococci intracellular	Phagocytic index
				per cent	*per cent*	
1 min.	3725	15,120	15,370	1	1	4
15 min.	3925	11,310	11,615	2	3	3
30 min.	3900	11,770	13,150	7	10	4
1 hr.	4050	18,940	21,051	10	10	5
2 hrs.	5125	44,450	51,850	18	13	7
4 hrs.	22,135	193,500	220,400	16	10	16
6 hrs.	7825	164,400	192,800	23	10	11
10 hrs.	9570	1,114,700	1,187,700	33	6	20

All control mice died in 12–24 hours.

Blood cultures showed that pneumococci were invariably present in the blood stream in detectable numbers as early as 15 minutes after intraperitoneal injection.

The figures regarding the per cent of pneumococci intracellular and the per cent of white cells active are possibly very inaccurate, for it is often difficult to determine whether organisms are actually within the cells or merely superimposed. There is no doubt, however, that a considerable degree of phagocytosis does take place even in the absence of specific immune serum. Thus, throughout the course of

this particular experiment an average of almost 10 per cent of all organisms was considered to be within the cells. Since the numbers of bacteria increased with extreme rapidity, more and more cells became active until at 10 hours one-third of all the white cells were participating in the phagocytic reaction. The number of pneumococci taken up by the individual cells was, however, low, averaging only 4 per active cell during the 1st hour, and thereafter approximately 12. These findings are somewhat contrary to the general impression that phagocytosis of virulent pneumococci does not occur in the absence of specific immune serum.

Even though a considerable amount of phagocytosis was demonstrated, there was no evidence that the bacteria were destroyed as a consequence or that the phagocytes were even capable of halting the growth of those bacteria which they had ingested. Microscopic examinations gave ample evidence that the bacteria continued to multiply within the cells and finally brought about their rupture. It is possible, therefore, that the high phagocytic index after the 1st hour may be due to intracellular growth rather than to the actual number of pneumococci phagocyted.

Course of Pneumococcus Infection in Mice Protected by Specific Immune Serum

In order to learn what changes might be brought about by the administration of immune serum, a series of mice was each given 1 cc. of fluid containing 0.1 cc. of culture together with 0.025 cc. of immune serum, an amount known to protect two out of three mice against fatal infection. It was necessary to use relatively large amounts of culture in order to facilitate the counting of the organisms in the peritoneal fluid. The results of a typical experiment of this order are presented in Table II.

From the data presented in Table II it will be noted that the numbers of white blood cells in the peritoneum varied in much the same manner as did those in mice which received no immune serum. The number of pneumococci had increased at the 30 minute period, but subsequently dropped rapidly. Had the animals received no serum the log phase of bacterial growth would have been apparent at 1 hour. However, in the serum protected mice it was found that at the end of the 1st hour some 67 per cent of the observed pneumococci had been

taken up by phagocytes. The per cent of pneumococci intracellular rose to 100 at the 4 hour period.

In this particular series, somewhat at variance with the findings in other experiments, the numbers of white cells showing phagocytosis in the earlier phases of the infection were not materially different from those at corresponding periods in unprotected mice. However, the number of pneumococci taken up by each cell was much greater in the presence of immune serum. Thus, during the 1st hour in the unprotected mice the average was only 4 per cell, whereas in the animals which received serum the average was 21. This increased efficiency in the

TABLE II

Course of Intraperitoneal Pneumococcus Infection in Mice Which Had Received Specific Immune Serum

Each mouse received an intraperitoneal injection of 1 cc. of fluid containing 0.1 cc. 18 hour blood broth culture of Type I Pneumococcus and 0.025 cc. of specific antipneumococcus horse serum.

Time elapsing after infective inoculation	White blood cells per c.mm. of peritoneal washings	Calculated extracellular pneumococci per c.mm. of washings	Calculated total pneumococci per c.mm. of washings	White cells showing phagocytosis	Pneumococci intracellular	Phagocytic index
				per cent	*per cent*	
1 min.	3350	18,290	19,540	4	7	
15 min.	2850	16,550	19,750	2	16	
30 min.	4150	36,450	39,200	5	4	21
1 hr.	3600	2610	7930	10	67	
2 hrs.	3850	1168	4410	5	73	
4 hrs.	9850	0	1667	3	100	5
6 hrs.	7400	0	121	1	100	

67 per cent of control mice receiving this combination of serum and culture survived.

latter instance is apparently due to the fact that the pneumococci have been agglutinated by the action of the immune serum, and that clumps of bacteria instead of individual diplococci are engulfed.

In all animals of the control series in which the infection subsequently terminated fatally, pneumococci were demonstrated in the blood as early as 15 minutes after the injection of serum and culture. These findings suggest that the initial reaction in the peritoneal cavity is important in determining the subsequent fate of the animal.

Comparative Analyses

By means of this technical procedure the course of pneumococcus infection has been studied under six different conditions, and a comparison will be made of the characteristic findings. However, before enumerating these conditions it will be necessary to define certain terms and procedures.

For each lot of antipneumococcus horse serum there is one definite amount which affords mice the maximum degree of protection against large amounts of culture. This is termed the optimal protective amount of serum. For the particular serum used in these experiments the optimal protective amount was 0.025 cc.

TABLE III

Survival Rates in Pneumococcus Infections under Various Conditions

Nature of material injected	Normal mice		Mice injected with sodium nucleinate 18 hrs. prior to infection	
	Group	Survival rate	Group	Survival rate
		per cent		*per cent*
0.1 cc. of Type I pneumococcus broth culture	A	0	D	0
0.1 cc. of culture plus 0.025 cc. of immune horse serum (optimal protective amount)	B	66	E	92
0.1 cc. of culture plus 0.4 cc. of immune horse serum (prozoning amount)	C	5	F	90

With amounts of serum greater than this optimum, less protection is obtained. This effect is termed the prozone and an amount of serum chosen to demonstrate this phenomenon is termed a prozoning amount. In the following experiments 0.4 cc. of serum was employed.

Certain of the mice in the following experiments had been given an intraperitoneal injection of 0.5 cc. of a 5 per cent solution of sodium nucleinate 18 hours previous to the injection of serum and culture. These animals are hereafter referred to as prepared, indicating that a cellular reaction had been elicited by the previous injection of the irritant. These terms have been defined in detail in a previous paper (1).

The six conditions under which pneumococcus infection of the mouse was studied are listed in Table III together with the survival rates among non-sacrificed controls.

All mice in these series received in the same infective inoculum: 0.1

cc. of Type I pneumococcus broth culture. Group A is made up of mice which received the culture only. Group B comprises those which received an optimal protective amount of antipneumococcus horse serum. Group C includes animals which received a large or prozoning amount of serum. Groups D, E, and F are made up of nucleinate prepared mice similarly treated.

From the survival rates shown in Table III, it will be noted that in the absence of specific immune serum all mice succumbed. With the normal mice the addition of an optimal protective amount of serum led to a survival rate of 66 per cent.

TABLE IV

Relative Total White Cells in Peritoneal Fluid

Time elapsing after infective inoculation	Normal mice			Prepared mice		
	A	B	C	D	E	F
	No serum	Optimal protective amount of serum	Prozoning amount of serum	No serum	Optimal protective amount of serum	Prozoning amount of serum
1 min.	4160*	5352	5130	8678	9290	7390
15 min.	3775	3162	3400	4540	5360	7610
30 min.	5488	3308	4209	8425	6410	7590
1 hr.	3488	2777	3860	11,888	11,905	8590
2 hrs.	4950	4771	2960	9740	11,970	12,340
4 hrs.	14,020	8794	5680	20,263	11,890	9486
6 hrs.	7450	8557	5540	12,533	7903	6040
10 hrs.	9570	6089	5225	8200	4290	3350

* Each figure in this and the following tables represents the mean of determinations on four or more individual animals.

With this amount of serum the survival rate in nucleinate prepared mice was 92 per cent. The use of the prozoning amount of serum in normal mice gave a survival rate of 5 per cent, but this same amount led to the survival of 90 per cent of prepared mice. Although the previous administration of sodium nucleinate had no effect on the survival rate in animals which had received no serum, it markedly enhanced the survival rates in animals which received immune serum.

The data relating to the findings in these six groups will be presented under the following headings: (*a*) total cells in peritoneal fluids, (*b*) white cells active as phagocytes, (*c*) phagocytic indices, and (*d*) proportions of pneumococci found within cells.

Total Cells in Peritoneal Fluids.—The data on the mean numbers of peritoneal cells of mice infected under these several conditions are presented in Table IV.

The following points are worthy of attention in these results.

1. Unprepared mice show the first general increase in numbers of cells at 4 hours, the height of this response being apparently inversely related to the amount of serum injected. Thus the highest mean count in animals which received 0.4 cc. of serum was 5680, in animals which received 0.025 cc. of serum 8794, while in animals which received no serum the peak was 14,020.

TABLE V

Mean Per Cent of White Cells Active as Phagocytes during Various Periods after Infective Inoculation

Group	Character of mice	Immune serum	Mean per cent of white cells active as phagocytes		
			During 1st hr.	From 2nd to 4th hr.	6th to 10 hr.
			per cent	*per cent*	*per cent*
A	Normal	No serum	5	17	25
B	"	Optimal protective amount of serum	16	11	5
C	"	Prozoning amount of serum	5	22	40
D	Prepared	No serum	7	20	43
E	"	Protective	7	9	7
F	"	Prozone	4	16	4

2. In mice prepared by the previous injection of sodium nucleinate the total number of cells was almost twice that found in normal mice. The results with the prepared mice are not as regular as those with the normal animals, but it is apparent that the first general increase in cell count occurred 2 to 3 hours earlier than it did with normal animals.

White Cells Active as Phagocytes.—The data are shown in a condensed form in Table V.

It will be noted that in normal mice during the 1st hour the per cent of cells actively phagocytic was highest in those animals which received the so called protective amount of serum. Certain other experimental observations aid in the interpretation of these results.

1. If normal mice are injected with a similar number of rough pneumococci, 30 per cent of the white cells in the peritoneal cavity show phagocytosis at 15 minutes. This finding may be compared to 5 per cent with smooth virulent pneumococci. It would appear that some factor must act as an inhibitor of phagocytosis in the case of the smooth organisms. Most obvious are the capsule and the soluble specific substance derived from it. The specific capsular polysaccharide of Type I Pneumococcus was therefore injected in a dilution of 1–100,000 together with rough pneumococci derived from a strain of Type III Pneumococcus. Samples of peritoneal fluid taken at 15 minutes showed that on the average only 16 per cent of the cells were actively phagocytic as contrasted to 30 per cent in controls. The mode was much below this figure. These observations suggest that under the conditions of the experiment the specific capsular polysaccharide has the capacity of inhibiting the phagocytic activity of the cells.

2. A second observation which must be taken into consideration in the explanation of these results is the fact that the immune horse serum itself has a similar inhibitory effect. The data presented in Table V show that although with 0.025 cc. of serum 16 per cent of the cells were active during the 1st hour, with 0.4 cc. only 5 per cent showed phagocytosis. Further experiments on the inhibition of phagocytosis have shown that various sera, protein solutions, and lipoids in certain concentrations have a similar inhibiting effect.

In the light of these observations, the reduced phagocytic activity of the cells of mice infected but not receiving immune serum can be explained largely on the inhibitory action of free and fixed capsular polysaccharide. With the addition of an optimal protective quantity of serum, however, this inhibitory influence has been to some extent neutralized. The reduction of phagocytic activity found in mice which received a prozoning amount of serum is explained by the inhibition caused by foreign protein and other substances contained in the serum.

It will be noted from the results shown in Table V that after the 1st hour the phagocytic activity decreased in the group of mice which received optimal protective amounts of serum, but rose sharply in each of the other series. This may be in part explained as follows:

(*a*) In the mice which received an optimal protective amount of serum the number of extracellular pneumococci became progressively smaller so that under these conditions the number of cells showing phagocytosis is not an accurate index of their potential capacity.

(*b*) In the other instances, in which phagocytic activity increased sharply, the interpretation is not so obvious. In a later section, however, it will be shown

that there occurs during the course of the infection a shift in the character of the cell picture from one in which the phagocytes are predominantly mononuclear to one in which polymorphonuclear cells are present in great numbers. It may be that the latter cells are not as sensitive as are the mononuclears to the various substances which inhibit phagocytosis. In the instance of the prozone group a further possibility is that some of the serum may have been absorbed, thus lowering the concentration in the peritoneum and thereby lessening the inhibitory effect.

With the nucleinate-prepared mice, the results are somewhat different. It must be recalled that in these animals the total number of cells was originally much higher, and, as will be shown in a later section, many polymorphonuclear cells are initially present. In the group which received no serum the phagocytic activity was somewhat greater than in the corresponding group of normal mice. The proportion of cells active as phagocytes within the serum groups may be explained on the basis of the same factors which are operative in normal mice.

Of particular interest is the fact that the cells of the normal mice show a high rate of phagocytic activity. In spite of this it is known that the injection of a single pneumococcus will bring about death of the animal. Thus, phagocytosis is in itself not an adequate means of defense. It has already been pointed out that without previous specific sensitization the pneumococci tend to multiply within the cell and finally bring about its rupture. It is suggested that immune sera possess the property of rendering the pneumococci sensitive to digestion by the intracellular enzymes of the leukocytes.

Phagocytic Indices.—The average number of pneumococci taken up by each active cell is termed the phagocytic index. The results of studies on this point are condensed in Table VI.

It will be noted from the results given in Table VI that during the first 4 hours the actively phagocytic cells of mice which received serum took up significantly greater numbers of pneumococci than did those in mice receiving no serum. Thus during the 1st hour in the normal group there was an average intake of 5 microorganisms per cell when no serum was present. In the protective series the phagocytic index was 18. On the other hand, in the prozone group each active cell took up an average of only 11 pneumococci. Similar results were

obtained in the series of mice prepared by the previous injection of sodium nucleinate.

It is believed that these results may be in part explained on the following basis. The non-inhibited phagocyte apparently takes up particles rather indiscriminately. In serum injected animals these particles consist of agglutinated groups of bacteria rather than of single diplococci. Obviously, in these instances the phagocytosis of clumps of bacteria increases the phagocytic index without indicating an increased activity on the part of the individual cells.

These results show that the phagocytic index is lower in animals which received an excess or prozoning amount of serum than in animals which received an optimal protective amount. Studies with moist

TABLE VI

Mean Phagocytic Indices

Group	During 1st hr.	From 2nd to 4th hr.	From 6th to 10th hr.
A Normal—no serum	5	13	23
B " protective	18	20	12
C " prozone	11	27	15
D Prepared—no serum	4	10	13
E " protective	15	16	11
F " prozone	9	19	11

preparations after the method of Etinger-Tulczynska (2) have given a clear explanation of this difference. When a small amount of serum is used the agglutinated mass is extremely compact, the cell bodies proper being in close contact. When, however, large amounts of serum are used, the clumps are made up of cells separated by swollen capsules—that is, these pneumococci show the *Quellung* phenomenon of Neufeld (3). Thus, in a given mass of agglutinated organisms there are fewer bacteria and the efficiency of the system is thereby somewhat diminished.

Proportions of Pneumococci Found within Cells.—The end-result in a protection test in terms of the death or survival of the animal is obviously more or less a summation of the combined force of many positive and negative factors. A somewhat more sensitive index,

however, is to be found in the proportion of pneumococci present within cells at various times after infective inoculation. In Table VII are presented the results of studies dealing with the per cent of pneumococci found within cells.

Only in the three groups showing a high survival rate (B, E, F) had all organisms been taken up by cells at 10 hours. In mice with protective amounts of serum, rapidly increasing numbers of bacteria were taken up. In normal mice this reaction had been almost completed at 4 hours, in prepared mice at 1 hour. This difference is not entirely accounted for by the greater numbers of cells in the latter instance, since the initial absolute number of active cells did not differ

TABLE VII

Pneumococci Intracellular at Various Time Periods

Figures indicate the mean percentage of observed pneumococci found within cells.

Group	Time after infective inoculation							
	1 min.	15 min.	30 min.	1 hr.	2 hrs.	4 hrs.	6 hrs.	10 hrs.
A Normal—no serum.....	2	2	11	8	24	7	16	6
B " protective.....	13	15	34	57	66	99	**100**	**100**
C " prozone.....	5	3	4	3	7	17	57	14
D Prepared—no serum.....	28	7	9	9	15	28	21	19
E " protective.....	13	49	61	99	**100**	**100**	**100**	**100**
F " prozone.....	2	4	22	25	63	78	97	**100**

materially. It may be recalled, however, that the conditioned increase in numbers of cells occurred much earlier in nucleinate prepared mice, and it may be that this was a material factor in bringing about complete phagocytosis. Some explanation of these differences may also be found in the types of cells initially present.

With normal mice which had received a large or prozone amount of serum, the highest per cent of pneumococci intracellular was observed at 6 hours, but the previously unchecked growth of pneumococci undoubtedly presented at this time an insurmountable barrier. With the corresponding group of nucleinate prepared mice, on the other hand, complete phagocytosis had occurred at 10 hours. There is no

readily available explanation for these differences except that of possible differences in types of cells initially present.

Types of White Cells Found in the Mouse Peritoneum.—The white cells in the mouse peritoneum have been generally referred to as mononuclears and cannot be differentiated readily in fixed smears. It therefore seemed important to attempt a classification of these cells and a comparison of the cell types of the normal mouse with those in nucleinate prepared animals. These differential studies were carried out by means of the supravital technic of Sabin (4). By this technic the cells usually designated as mononuclears may be easily differentiated into lymphocytes and monocytes. This distinction is important, since the monocytes are potential phagocytes.

TABLE VIII

Differentiation of the Types of Cells in the Peritoneal Fluids of Normal as Compared with Nucleinate Prepared Mice

Mice of same age; weight 18 ± 0.5 gm.		
Cell type	Normal mice	Prepared mice
	per cent	*per cent*
Polymorphonuclear leukocytes	0	15
Eosinophiles	1.5	1
Lymphocytes	37	39.5
Monocytes	61.5	44.5

The monocytes of the peritoneal fluid of the mouse when stained by the supravital method present certain morphological characteristics by which they are easily distinguished from the lymphocytes in the same preparations. The unevenness of the surface films and the irregularity or scalloping of the cellular outline are characteristic of the cells of the monocytic series. The nucleus, which is usually round or oval and rarely indented, is almost invariably centrally situated. Surrounding the nucleus is a zone containing numerous granules and mitochondria, whereas the cytoplasm of the periphery is clear. Considerable variation in the size of these cells has been observed, the smaller ones being no larger than the intermediate lymphocytes in the same preparation, while the largest ones are often three to four times this size. The lymphocytes in the peritoneal fluid closely resemble the lymphocytes of the peripheral blood.

The results of differential studies concerning the types of cells in the peritoneal fluids of normal as compared with nucleinate prepared mice are shown in Table VIII.

From these results it will be noted that polymorphonuclear leukocytes were absent in the peritoneal fluids of normal mice although they constituted 15 per cent of the cells in the case of the prepared mice. Monocytes made up 61.5 per cent of the cells in the peritoneal fluid in normal mice, as compared to 44.5 per cent in prepared animals.

Under these experimental conditions the average total white cells per c. mm. of peritoneal washings of normal mice was 4600, while that of nucleinate prepared mice was 8450. From these figures it is apparent that the injection of the nucleinate brought about an increase in the absolute number of lymphocytes and of monocytes. The most characteristic change, however, is that polymorphonuclear cells, normally absent, have appeared in considerable numbers.

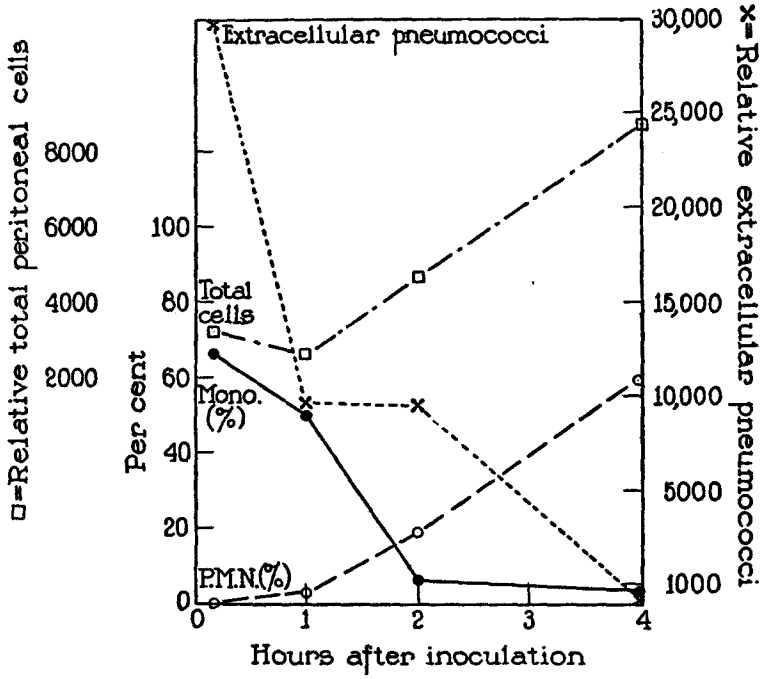
The initial presence of the polymorphonuclear cells undoubtedly contributes to the apparently enhanced protective action obtained with mice which had received a previous injection of sodium nucleinate.

Cellular Participation in the Protective Process.—It is of some interest to consider the sequential changes in the proportion of the various cells occurring in the course of the infectious process, and the part which these different types of cells play in the removal of the infectious agents. Supravital differential counts of the cells in the peritoneal fluid have been made at various times after infective inoculation on a group of mice which had received the usual protective amount of serum together with 0.1 cc. of culture. The findings of this study together with certain mean determinations obtained by the previous method are shown in Text-fig. 1.

From these results it will be noted that during the 1st hour the proportion of monocytes fell slightly. After that time, however, there occurred an abrupt drop in the number of these cells. On the other hand, the proportion of polymorphonuclear cells steadily increased from 0 per cent at 15 minutes to 60 per cent at 4 hours. There was at 1 hour a slight decrease in total numbers of white cells. This was followed by a steady increase corresponding roughly to the increase of the polymorphonuclear cells.

The participation of the monocyte in the infectious process, and the reason for the abrupt fall in the number of these cells, are matters of considerable interest. At the 15 minute period it was observed that

some of these cells were already taking up pneumococci. At 1 hour it was observed that marked phagocytosis had occurred, but more significant was the fact that the monocytes were massed in huge clumps about agglutinated pneumococci, and that within these masses the organisms were being rapidly ingested. At 2 hours, however, these masses or large groups of pneumococci and cells were not found. This



TEXT-FIG. 1. Composite chart showing the changes in the proportions of cell types in the peritoneal fluid after injection of serum and culture, correlated with the number of extracellular pneumococci at various times.

disappearance coincided with the disappearance of the monocytes from the peritoneal fluid. It is therefore rationally presumed that the massing of these cells had formed particles too large to remain suspended in the peritoneal fluid. It would appear that the observed decrease in the proportion of monocytes gives a false impression, and that these cells probably continue active in the destruction and elimination of the bacteria.

With the appearance of large numbers of polymorphonuclear cells, marked phagocytosis was again observed.

It is of some interest to note that the number of extracellular pneumococci had dropped abruptly during the 1st hour. During the 2nd hour, when the proportion of monocytes in the fluid was apparently decreasing and the number of polymorphonuclear cells had not yet reached any considerable level, there was little appreciable change in the number of free pneumococci. This probably represents a balance between two processes, phagocytosis on the one hand and bacterial growth on the other. From the 2nd to the 4th hour there apparently occurred a second wave of elimination of free pneumococci, this being associated with the inrush of polymorphonuclear cells.

DISCUSSION

An attempt has been made to study the sequence of events following the intraperitoneal injection of mice with pneumococci and to learn in what manner this sequence might be altered by the administration of immune serum. As in any infectious process of this order, the number of forces or factors involved in the production of the end-result is very large. An infection represents a constant state of flux, and the tendency in either direction toward a state of equilibrium is continuously influenced by numerous opposing forces often confusingly interrelated. The complexity of this succession of events is so great as to forbid any complete simulation by *in vitro* methods. Infection can only be studied by direct observation, and the results interpreted in the light of isolated and carefully controlled experiments. The data presented in this study, although inadequate, have permitted the figurative reconstruction of certain phases of the infectious process and a rough evaluation of some of the factors which influence its course.

The number and nature of the cells present in the peritoneum at the time of injection of serum and culture have been shown to have an important bearing on the subsequent infectious process. These studies point to the importance of the monocyte in the defense reaction of the host. This cell appears to form the first line of defense of the tissues, for it is the actively phagocytic element normally present and

serves to hold the infectious agent in check until the polymorphonuclear cells of the blood have responded to the stimulus.

The present studies support the view that antipneumococcus serum owes its protective properties to three specific actions: (*a*) the neutralization of the capsular polysaccharide and the consequent elimination of its inhibition of phagocytosis; (*b*) the agglutination of bacteria, permitting a greater efficiency in the phagocytic action in that clumps of bacteria rather than single diplococci may be engulfed; and (*c*) the sensitization of the pneumococci which favors subsequent intracellular enzymatic digestion, a process to which virulent organisms are resistant in the unsensitized state.

SUMMARY

Observations are reported which concern the nature of the infectious process resulting from the intraperitoneal injection of mice with virulent pneumococci.

The course of the infection has been figuratively reconstructed on the basis of the following data: The rate of bacterial multiplication, the numbers of cells present in the peritoneal cavity, the character of these cells at various stages, and the rate of phagocytosis.

The significant alterations in this infectious process brought about by the administration of type specific immune serum are described, and the general significance of the findings discussed with reference to the functions of the immune serum and the rôle of phagocytes in protection.

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