STUDIES ON HOST FACTORS IN PNEUMOCOCCUS INFECTIONS

II. The Protective Action of Type I Antipneumococcus Serum in Rabbits

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The analysis of the experimental data presented in the preceding paper dealing with the curative action of specific antipneumococcus horse serum in Type I dermal pneumococcus infection in rabbits showed a dominance of two factors in determining the outcome of the infection; *viz.*, the degree of bacteriemia, and the white blood cell count at the time of serum administration (1). There were, however, a large number of instances the results of which were not predictable on the basis of these two factors alone, and, although an exhaustive analysis was made of all available data, no other significant factors were found. As previously pointed out the complexity of this type of experiment admits of many technical errors. A further objection, in view of the obvious significance of the number of infecting organisms, was that the primary infection was focal and hence the estimation of the number of pneumococci in the blood stream could hardly be an adequate appraisal of the total number involved in the infection. It also became apparent as the work progressed that it would be absolutely essential to control genetic factors by the use of a single breed of rabbits.

These objections were overcome by the adoption of an experimental method consisting of a protection test in rabbits of a single breed, both antiserum and infecting organisms being injected at the same time intravenously. By this technic the variable genetic factors have been reduced, and the initial bacteriemia held constant.

EXPERIMENTAL

Rabbits.—Throughout this series of experiments healthy male rabbits of the breed known as the New Zealand Red have been used. These animals are uni-

formly susceptible to Type I pneumococcus infection. Constant dietary conditions were maintained throughout these experiments.

Culture.—The strain of Pneumococcus Type I was of such virulence that 0.000,000,01 cc. of an 18 hour blood broth culture, given intradermally produces a fatal infection in normal rabbits. About 100 times this amount is required to establish infection if given by the intravenous route. Unless otherwise stated the culture was diluted 1–10 in broth and 0.5 cc. of this dilution used as the infective inoculum.

Serum.—A single lot of Type I antipneumococcus horse serum was used throughout the experiments. Unless otherwise stated the amount injected was 0.5 cc. per rabbit.

Infective Inoculation.—Immediately after taking blood for the white cell count each rabbit received the serum intravenously in the right ear and the culture intravenously in the left ear, in the order named. The infective inoculation was invariably carried out at the same hour of the day.

Observations.—Since there is no visible external lesion following this infective procedure it was necessary to determine by the body temperature whether or not an infection had been established. Temperature determinations were made daily at the same hour on each rabbit. A rectal temperature of 104.0°F. or over was regarded as significant.

On the basis of the previous work it was expected that with this technically improved approach and a properly balanced combination of antiserum and microorganisms, all animals having a low white cell count would die while those with higher white counts would survive. This proved not to be the case, although it was found that 70 per cent of the individual animals having white counts below 10,000 died whereas 59 per cent of those with counts of 14,000 or over survived. On further analysis it was determined that the weight of the animal possessed considerable significance, since 65 per cent of animals under 2,000 gm. died whereas the death rate among the larger rabbits was only 39 per cent. The next step in the consideration of the results was to determine the relation between these two variables and the outcome.

For the purposes of this analysis all animals have been divided into three groups as follows:

Group A.—Rabbits which survived and which failed to show evidence of infection at any time.

Group B.—Those rabbits which survived but only after a febrile course indicative of the presence of an infective process.

Group C.—Those animals which developed an infection and died as a consequence thereof.

TABLE I

Results of Protective Tests with Reference to Body Weights and White Blood Cell Counts

Each of the following rabbits received 0.5 cc. of Type I antipneumococcus serum and 0.5 cc. of a 1–10 dilution of an 18 hour blood broth culture of Type I Pneumococcus intravenously.

Group	Rabbit No.	Body weight	White blood cell count
		gm.	
A	1	2,200	15,260
Completely protected	2	2,780	11,100
against infection	3	2,200	16,600
	4	2,680	18,550
	5	3,380	12,000
	6	2,260	13,640
	7	2,480	27,250
	8	2,140	10,080
	9	2,740	18,900
	10	2,720	15,040
	11	2,660	12,300
	12	2,560	16,100
	13	1,890	12,100
	14	1,720	19,400
	15	2,330	10,150
	16	1,970	6,450
	17	2,490	12,350
	18	2,320	15,240
	19	2,460	17,840
	20	2,200	17,050
B	1	1,810	23,800
Animals which developed	2	2,290	7,680
an infection but eventually survived	3	1,910	13,850
	4	1,690	11,250
	5	1,950	7,400
	6	1,880	20,100
	7	1,930	6,850
	8	1,750	7,500
	9	2,060	11,520
	10	1,870	9,200
	11	1,870	11,600
C	1	2,200	13,550
Animals which developed an infection and	2	2,200	11,900
	3	2,170	11,760
died as a consequence	4	1,440	13,400
thereof	5	1,480	16,720

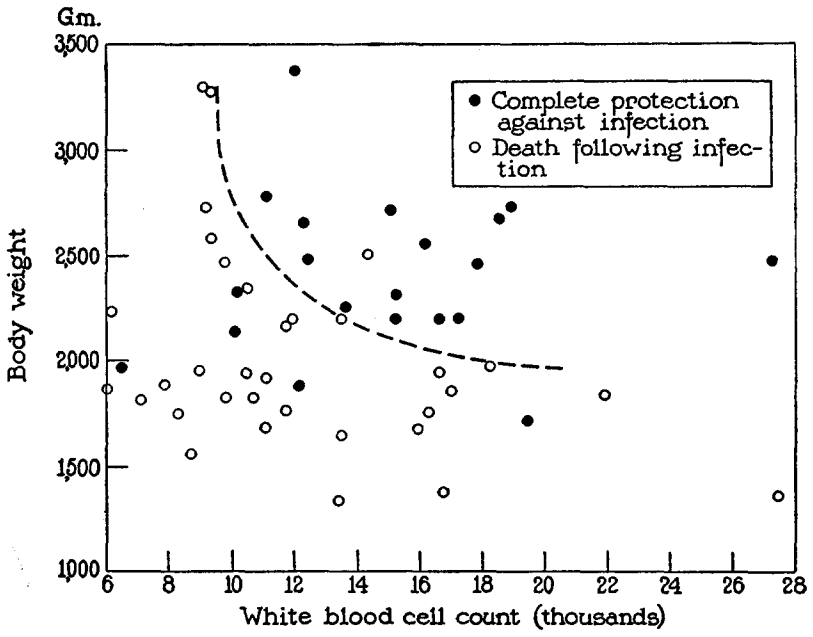
TABLE I—*Concluded*

Group	Rabbit No.	Body weight	White blood cell count
		gm.	
C—*Concluded*	6	1,980	18,250
Animals which developed an infection and died as a consequence thereof	7	2,470	9,750
	8	3,280	9,350
	9	1,950	16,600
	10	1,860	17,000
	11	1,370	27,400
	12	3,300	9,050
	13	2,230	6,140
	14	1,830	10,750
	15	1,960	8,950
	16	1,890	7,850
	17	2,580	9,300
	18	1,650	13,450
	19	2,510	14,320
	20	1,920	11,050
	21	1,680	15,960
	22	1,570	9,700
	23	1,770	11,750
	24	1,760	8,350
	25	1,950	10,500
	26	2,730	9,240
	27	1,820	7,050
	28	1,870	6,000
	29	1,690	11,050
	30	1,760	16,040
	31	1,830	9,700
	32	1,840	21,860
	33	2,220	4,300
	34	2,340	10,950

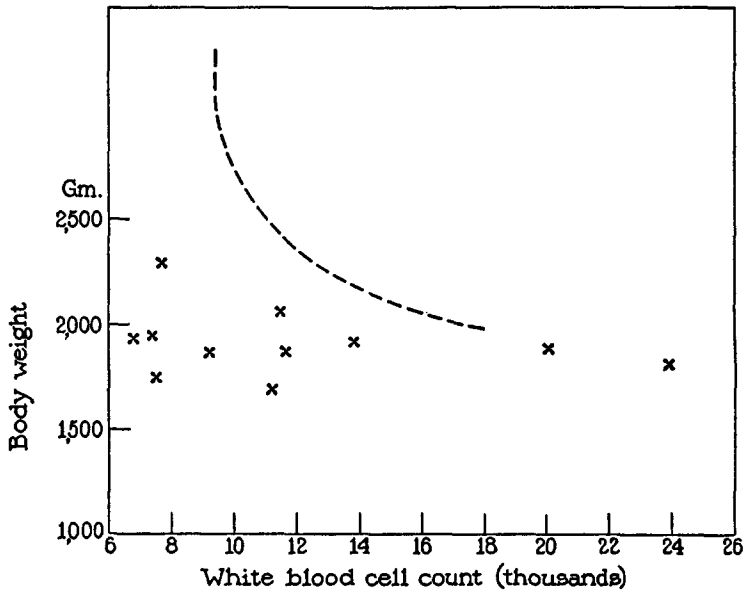
The results of the protection tests in the rabbits of these groups are listed in Table I together with the data which proved to be of the greatest significance as a result of the analysis.

By definition Groups A and C contrast the failure to establish any infection on the one hand against infection and death on the other. The results are plotted in Text-fig. 1 against the weight and the white blood cell count at the time of infective inoculation.

A consideration of Text-fig. 1 shows that, in general, the members of Group A occupy one section of the figure while those of Group C are oppositely placed. A

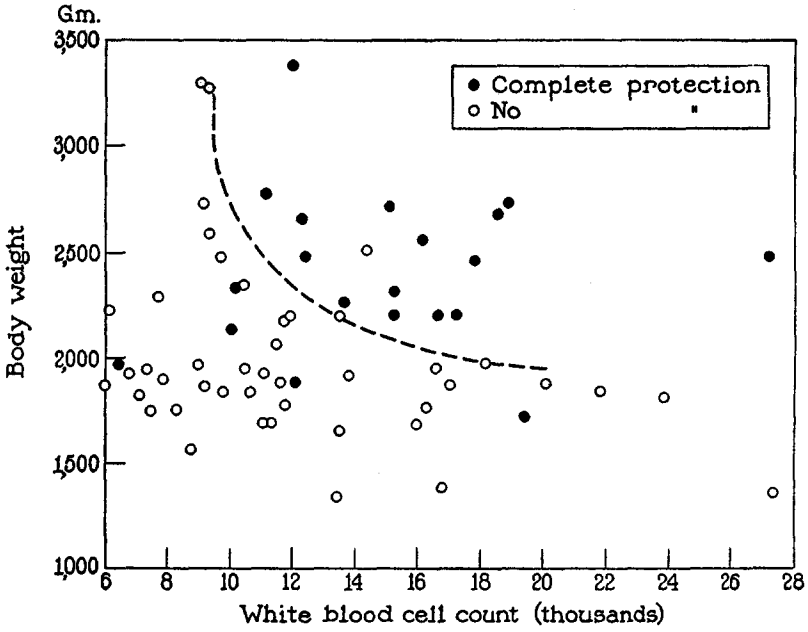


TEXT-FIG. 1. Results of protection tests on rabbits in Groups A and C. The broken line has been inserted to show a possible boundary between the two areas.



TEXT-FIG. 2. Results of protection tests on animals in Group B. All rabbits survived but only after a febrile course indicative of infection. The broken line is that of Text-fig. 1.

broken line has been inserted to show a possible boundary between these two zones. Six of the 65 points do not follow the general trend of distribution but the results are sufficiently definite to permit the conclusion that the heavier animals having white counts above the average seem to be completely protected against infection.



TEXT-FIG. 3. Text-figs. 1 and 2 are combined to show protection as against lack of protection. A broken line has been inserted to show a possible boundary between the two areas.

There remain for consideration the animals in Group B, all of which developed a definite infection but eventually recovered. The results have been plotted in Text-fig. 2 on the same scale as that used in Text-fig. 1 and the same broken line has been inserted in order to show the boundary that existed between Groups A and C. From a consideration of this figure it will be seen that all animals in Group B occupy positions in the area corresponding to those of Group C in Text-fig. 1. The natural division appears to be between animals which were protected against the development of infection as opposed

to those which became infected. An analogy is to be found in a previously reported series of tests for active resistance in which it was shown that an animal which survived after a long febrile course could be considered as differing little from one which eventually died (2).

Hereafter in this paper the distinction will be made between protected as opposed to non-protected animals. On this basis Text-figs. 1 and 2 have been combined in Text-fig. 3, in which the animals fully protected are shown by closed circles and those not protected by open circles.

From these results it would appear that the weights and white cell counts serve as indices of two intrinsic variables which, under the present experimental conditions, appear to be significant when certain factors such as breed, amount of antiserum, and number of pneumococci are controlled. It appears that the heavier animals having the higher white cell counts are much more likely to be protected against infection. These results are somewhat surprising in view of the fact that the amount of serum injected was the same in all rabbits irrespective of the body weight. Under these circumstances the antibody concentration would be much greater in the smaller animals than in the larger. It seems reasonable to assume that the serum should be more effective as its concentration increases. This, however, proved not to be the case. Consequently one must conclude that the larger animals possess a physiological capability which makes possible the more effective utilization of the protective properties of the specific antiserum.

Significance of the White Blood Cell Count

Type of Cells Involved.—Differential counts have demonstrated that the only type of cell with which the results can be correlated is the polymorphonuclear leukocyte. Since the mechanism of the destruction of the pneumococcus undoubtedly involves phagocytosis this finding is not unexpected.

Variability of the Numbers of White Blood Cells in Rabbits.—It is quite generally recognized that the numbers of white blood cells vary widely in rabbits from time to time. A large variety of environmental and physiological conditions appear to influence the count of individual animals to such an extent that within a single hour a considerable

shift may take place. In the present series the counts reported have been made immediately before the infective inoculation in each individual animal. Other counts made previous to this time and 1 hour, 4 hours, and 24 hours after the infective inoculation have shown little or no direct correlation. The results on a number of animals in the critical weight zone (2,000 gm. and over) are listed in Table II

TABLE II

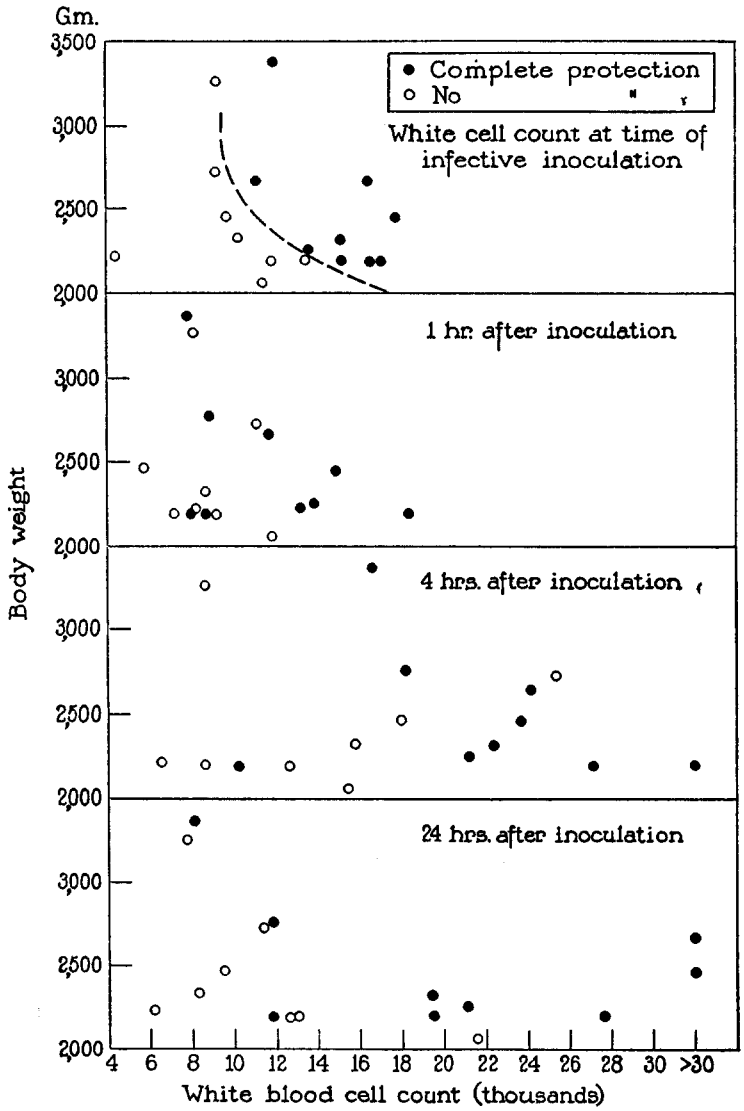
White Cell Counts at Various Times after Infective Inoculation

Each of the following rabbits received 0.5 cc. of Type I antipneumococcus serum and 0.5 cc. of a 1–10 dilution of an 18 hour blood broth culture of Type I Pneumococcus intravenously.

Rabbit	Body weight	White blood cell counts				Results in terms of protection
		Initial	After infective inoculation			
			1 hr.	4 hrs.	24 hrs.	
	gm.					
a	2,060	11,520	11,900	15,400	21,600	−
b	2,200	13,550	9,200	12,600	12,600	−
c	2,200	11,900	7,260	8,600	13,000	−
d	2,470	9,750	5,800	18,000	9,500	−
e	3,280	9,350	8,100	8,600	7,800	−
f	2,730	9,240	11,260	25,460	11,400	−
g	2,220	4,300	8,060	6,500	6,300	−
h	2,340	10,280	8,700	15,800	8,300	−
i	2,200	17,050	18,400	30,700	27,600	+
j	2,460	17,840	14,900	23,704	74,800	+
k	2,320	16,240	13,200	22,400	19,460	+
l	2,260	13,640	13,900	21,200	21,100	+
m	3,380	12,000	7,940	16,600	8,100	+
n	2,680	18,550	11,800	24,200	31,000	+
o	2,200	16,600	8,700	27,200	11,800	+
p	2,780	11,100	8,900	18,200	11,800	+
q	2,200	15,260	8,000	10,200	19,500	+

− = no protection. + = complete protection.

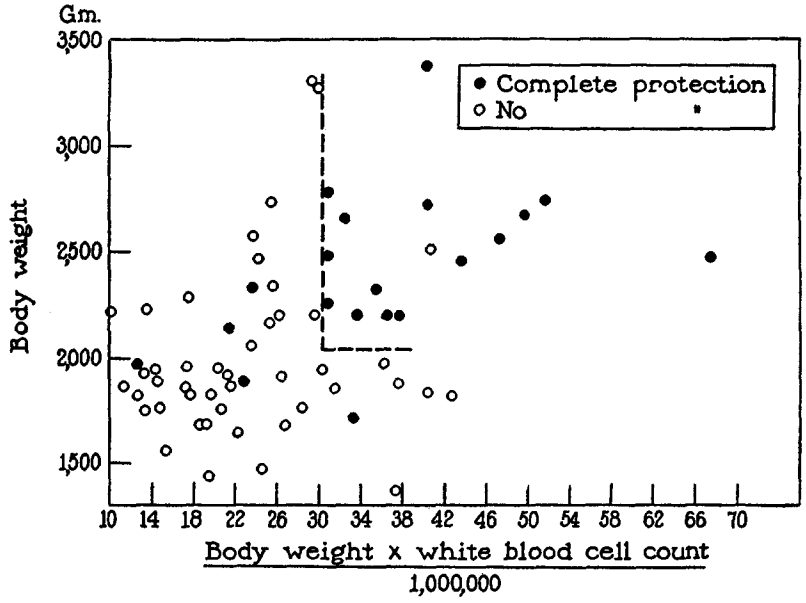
and plotted in Text-fig. 4. From these results it will be seen that after the time of infective inoculation there is little apparent organization of the points into definite characteristic areas. The possible significance of these findings will be discussed later, but it may be stated that the results indicate that the only white cell count of significance is that made just at the time of infective inoculation.



TEXT-FIG. 4. Results in relation to white cell counts at the time of infective inoculation and at various times thereafter.

The Total Number of White Blood Cells.—From the data shown in Text-fig. 3 it appears that an animal of 3,000 gm. is protected if the

white count is over 10,000 but that an animal of 2,000 gm. requires a much higher count for protection. Obviously a large rabbit actually has a much greater total number of circulating white blood cells than a smaller animal having the same count. This suggests that the result may actually be determined by the total number of white cells. This, of course, cannot be absolutely determined but if one multiplies the cell count by the body weight in each instance, one arrives at a figure representing a relative value for the total number of white cells



TEXT-FIG. 5. Results plotted against weight on the basis of relative total white blood cells.

in the blood stream. This calculation has been carried out for the animals shown in Text-fig. 3 and the results plotted against the weight are shown in Text-fig. 5. From a consideration of the data it would appear that the results are divided sharply into two divisions, and that the curve of Text-fig. 3 has been replaced by straight lines, one representing a minimum absolute number of white blood cells and the other a minimum weight. The significance of these minimums will be discussed later.

Induced Changes in the White Blood Cell Count.—Since the white blood cell count at the time of infective inoculation appeared to be a significant factor it seemed desirable to learn if experimentally induced changes in the leukocyte count might materially alter the status of any individual animal.

For this purpose intravenous injections of a heat-killed suspension of *B. coli* were given to several animals. A suitable amount of the suspension was judged to be one which would produce a considerable

TABLE III

Induced Leukocytosis in Reference to the Protective Action of Antipneumococcus Serum

Each of the following rabbits received an intravenous injection of a heat-killed suspension of *B. coli* representing 0.2 cc. of a broth culture. 18 hours thereafter each animal received 0.5 cc. of Type I antipneumococcus serum and 0.5 cc. of an 18 hour blood broth culture of Type I pneumococcus intravenously.

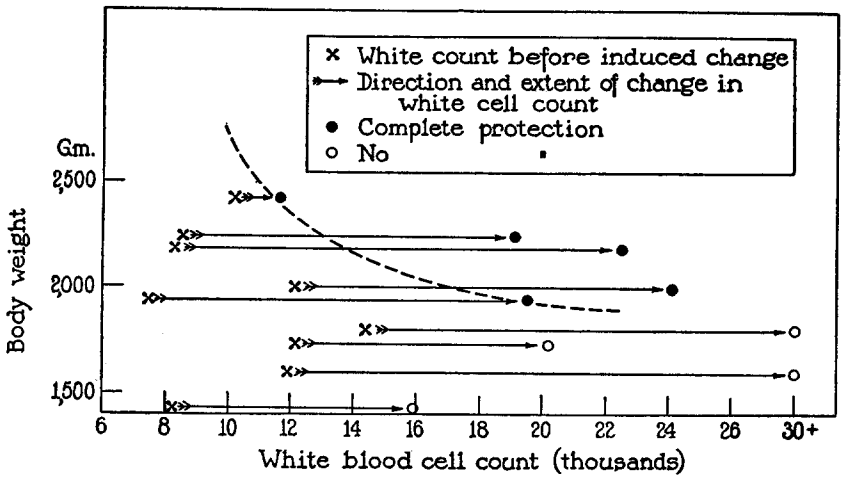
Rabbit	Body weight	Preliminary white count	*B. coli* suspension injected intravenously	Interval of 18 hrs.	White cell count at time of infective inoculation	Results in terms of protection
	gm.					
A	2,540	7,150			13,520	+
B	2,420	10,300			11,540	+
C	2,240	8,700			19,060	+
D	2,180	8,300			22,650	+
E	2,000	12,150			24,100	+
F	1,940	7,340			19,450	+
G	1,800	14,460			35,860	−
H	1,730	12,100			20,400	−
I	1,600	11,900			50,400	−
J	1,430	8,200			15,850	−

− = no protection. + = complete protection.

leukocytosis yet not prove fatal. Preliminary counts were taken on a number of animals and the bacterial suspension then administered intravenously. 18 hours thereafter the regular infective procedure was carried out. The results of these experiments are listed in Table III and are plotted in Text-fig. 6. In this figure the counts before the change induced by the injection of the heat-killed suspension of *B. coli* are indicated by crosses while the counts at the time of the infective inoculation are shown by the circles. These points are joined by

lines in order to show the direction of the shift in count. The curve is that shown in Text-fig. 3.

It is apparent from these results that the non-specific stimulation produced in all cases a leukocytosis and that if the shift was sufficient to place the individual within the so called protective zone the result was complete protection. This treatment did not, however, induce any other apparent change since animals which lacked the required weight were not protected. These data support the previous deduction that the outcome is directly related to the cellular status at the time of infective inoculation.



TEXT-FIG. 6. Induced leukocytosis and the effect on the capacity of the animal to be protected against infection. The broken line is that of Text-fig. 3.

It is impossible at the present time to construct a hypothesis which will explain these findings. A rough calculation shows that the proportional number of white cells is about 200 for each pneumococcus injected. This ratio is surprising for in *in vitro* experiments many polymorphonuclear cells often take up several pneumococci each. It has been suggested that the ratio may be determined by the chances of collision between the cells and the pneumococci in the circulating blood but due to the probable participation of fixed or stagnant phagocytic cells this hypothesis is difficult to substantiate. It has also been

suggested that the relative level of the white cell count may be a reflection of the general state of cellular reactivity.

Significance of the Weight Factor

It is a general impression that weight is an expression of age. Unfortunately, the exact age of none of these animals is accurately known and consequently this hypothesis, although probably correct, cannot be definitely supported. If this were true one might more properly use the term physiological maturation.

The results of experiments now in progress indicate that the weight factor is associated with some passively transferable component of the serum. Although our information is as yet too meager to warrant definite conclusions this factor appears to be heat-labile but not identical with the so called hemolytic complement.

Interrelationships of Intrinsic and Extrinsic Factors

It would seem important to learn in what manner the various dominant factors might be mutually related, but even by maintaining the genetic elements constant this is a difficult task. The situation is one in which four variable factors may be related each to the other.

With the amount of antiserum and the number of infecting organisms constant it has been shown that an increase of white cells does not compensate for a lack of weight and that an excess of weight does not compensate for a lack of white blood cells. In so far as our experience extends, there is no reason to believe that the weight and white cell count are mutually compensatory or related in any way except through the mediation of the two extrinsic factors.

The further approach to this question is through the experimental variation of one of the extrinsic factors, keeping the other and one of the intrinsic elements as nearly constant as possible. This approach is not difficult but the results are confusing since a variation in one of the extrinsic factors immediately upsets the delicate balance that had been created. Nevertheless the results that have been obtained are presented and the possible significance considered briefly.

Variations in the Amount of Specific Antipneumococcus Serum in Relation to the Body Weight Factor.—If the amount of specific antipneumococcus serum is varied and the white count maintained a con-

stant by the selection of animals showing counts within a narrow range, it is possible to obtain a result showing the interrelation of serum and weight. Thus in a range of white cell counts of 11,000 to 12,500, if 0.5 cc. of the serum was employed, the minimal weight at which protection was obtained was about 2,300 gm., with 0.4 cc. of serum it was approximately 2,900 gm., while with 0.6 cc. of serum the minimal

TABLE IV

The Relation of Number of Pneumococci in the Infective Inoculum to the Number of White Blood Cells in Reference to the Protective Action of Antipneumococcus Serum

Infective inocula					
0 05 cc.			0.005 cc		
Weight	White count	Result	Weight	White count	Result
gm.			*gm.*		
			2,060	8,760	−
			2,160	9,960	−
2,140	10,080	+	2,080	10,400	−
2,060	11,520	−	2,060	11,000	+
2,170	11,760	−	2,050	11,550	+
2,200	11,900	−			
			2,140	12,300	+
2,200	13,550	−			
			2,100	14,080	+
			2,010	14,520	+
2,200	15,260	+			
2,200	16,600	+			
2,200	17,050	+			
			2,140	20,600	+

− = no protection. + = complete protection.

weight was 1,700 gm. These experiments, too extensive to be reported here in detail, have conclusively shown that increasing amounts of serum can compensate for lack of weight and *vice versa*.

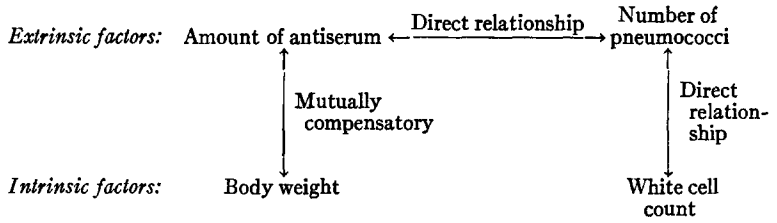
Variations in the Amount of Specific Antipneumococcus Serum in Relation to the White Cell Count Factor.—No evidence has as yet been obtained which would indicate that increasing amounts of serum compensate in any way for a comparative lack of white blood cells.

Thus even when 2.5 cc. of the serum, or 5 times the standard amount was used, the white cell minimum remained the same with the standard number of infecting microorganisms.

Variations in the Number of Pneumococci in Relation to the White Blood Cell Factor.—In another series of experiments the amount of serum was maintained constant but the amount of culture was decreased to 0.005 cc., or one-tenth the amount of culture previously injected. Then by a comparison of results in a selected weight range, the relation of this change to the white counts can be determined. The results of such an experiment are shown in Table IV together with data on animals of comparable weight in the previous series. From the table it would appear that a decrease in the number of pneumococci permits the protection of those animals with lower white counts. Hence the number of white blood cells necessary is definitely related to the number of pneumococci, more cells being required with increasing numbers of infecting organisms, although not in the same ratio.

Variations in the Number of Pneumococci in Relation to the Weight Factor.—From the data now available it seems certain that, as in the previous experiment, when relatively few pneumococci are injected, animals with lower white cell counts and also those of lower weights are protected. Under such circumstances all rabbits with white cell counts above the average, no matter how small they themselves were as individuals, appeared to be protected. This is, we believe, not due to any direct correlation between the number of bacteria and the weight of the rabbit but to the fact that the intimate balance between number of pneumococci and the amount of serum has been disturbed. It has already been shown that changes of as little as 0.1 cc. in the amount of serum make a considerable difference with regard to the minimal weight. It is not surprising therefore that with the decreased amount of culture, corresponding proportionally to a tenfold increase in the amount of serum, the smaller animals were protected. The result is believed to be a reflection of the altered serum-culture ratio rather than a direct effect.

In so far as can now be determined the relationship of these variable factors, the breed being constant, may be illustrated by the following diagram:



The intrinsic factors appear to be related in this manner to the extrinsic factors and through these to each other.

DISCUSSION

The purpose of these experiments has been to arrange critical combinations of controllable variables in order to learn, if possible, whether the results might be in any way related to one or more determinable host factors. As the work progressed it has been possible to make certain technical improvements and thereby bring out certain more dominant factors. In the first paper (1) experiments were reported which sufficed to point to the present more successful method of approach. It should also be mentioned that the normal rabbit is completely lacking in any form of specific antipneumococcus antibodies so that the quantity of antiserum administered represents the complete amount of specific immune substances present. Furthermore the rabbit is so susceptible to Type I pneumococcus infection that the results are sharply defined.

The experiments appear to have demonstrated the existence of two host factors which have a direct bearing on the capacity of the animal to utilize specific antipneumococcus antibodies. These two intrinsic factors find expression in terms of body weight and white blood cell count; but no evidence has been obtained to show that either the weight or the number of cells is other than a reflection of a more complex physiological system. They serve, however, as indices and, as such, they express the quantitative aspects of non-specific host resistance. Under the circumstances of the present experiments it has been possible to demonstrate that heavier animals with high white cell counts are in a physiological condition favorable to the utilization of passively conferred specific antibodies. It would appear that those animals which are physiologically mature and possess a condition of

high cellular reactivity as evidenced by the number of circulating white blood cells have a considerable advantage in the utilization of antibacterial antibodies over animals less mature and with a lower cellular activity.

SUMMARY

The power of specific antipneumococcus serum to protect rabbits against infection with Type I Pneumococcus has been studied with reference to the capacity of the animal to utilize the specific antibodies. Under conditions ensuring relatively controlled genetic factors it was found that heavier animals and those with high white blood cell counts are much better able to utilize the passively conferred immune principles. The interrelationships of the extrinsic and intrinsic factors responsible for immunity have been discussed.

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