ANTIBLASTIC IMMUNITY.

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As a result of the study of the natural or acquired resistance of animals against infection with living microorganisms, many previously unknown properties of the cells and fluids of the body have been discovered. The value of these properties as resistance factors is dependent upon the degree of antagonism which they manifest to invasion with living foreign elements. On the other hand, virulence of bacteria may be said to be proportional to the strength of defence which they possess against such antagonistic forces. In the face of such opposition, however, the bacterium must not only maintain its life, but in order to increase must be able to obtain a proper food supply. The mechanism by means of which bacteria extract from living tissue the substances essential to their nutrition is not well understood, nor can we say to what extent the animal body opposes this appropriation of its own supplies. It is in the hope of throwing light upon this obscure problem that the following study has been undertaken.

The microorganism used was the pneumococcus, not because it is especially suitable, but because many of the materials necessary for the experiments were easily obtainable. The hypothesis upon which our efforts are based is that bacteria do not assimilate all of their foodstuffs in the condition in which they exist in the medium of their environment, but that certain changes must be effected before absorption occurs. The preparation of nutritional substances may take place upon the surface of the bacterial cell or in its immediate neighborhood, and this function, when carried on within the substance of a living animal, may be opposed by certain inhibitory forces. The interaction of these phenomena may play an important part in resistance and immunity to infection.

By immunization of the horse to pneumococcus, a serum can be prepared which protects susceptible animals against many times the fatal dose of virulent pneumococci. A complete understanding of the mode of action of this serum is still lacking. However, the fact has been observed and confirmed that antipneumococcus serum possesses neither bactericidal nor bacteriolytic action *in vitro*. Indeed, pneumococcus is known to grow in considerable concentration of its homologous immune serum. In the following experiment (Table I) is shown the rate of growth of pneumoccoccus in homologous and in heterologous antipneumococcus serum and in normal horse serum. The amount of growth was determined by the plate method.

TABLE I.

Experiment I. Inhibition of Growth of Pneumococcus by Antipneumococcus Serum.

Serum 0.1 cc.	Culture 0.00001 cc.	Immediately.	After 3 hrs.
Antipn. serum Type I.....	Pn. I	230 colonies.	216 colonies.
" " " II.....	" I	200 "	630 "
Normal.....	" I	200 "	1,400 "

This experiment shows clearly that a marked inhibition of the growth of pneumococcus occurs in antipneumococcus serum, as compared with normal horse serum. The inhibitory effect of homologous immune serum is greater than that of heterologous immune serum; in fact, in the former serum no increase in the number of pneumococci has taken place in three hours. The inhibition of growth may be explained in four ways: first, that agglutination occurs in immune serum, and that the failure to increase is apparent only; second, that the formation of threads is responsible for the apparent inhibition; third, that we are dealing with the bacterial lag of freshly planted cultures; and fourth, that active interference with the growth phenomena of the organisms has occurred. That the first two reasons are entirely responsible for the inhibition of growth is disproven by the fact that a marked delay in development occurs in heterologous immune serum in which no agglutination whatever takes place and in which thread formation is not more extensive than in normal serum. The latent period that marks the growth of freshly planted

cultures of bacteria cannot explain the inhibition, inasmuch as this would affect the culture in normal serum as well as that in immune serum, since the same culture of pneumococcus was used for seeding and the conditions of cultivation were similar in each tube. We are forced, then, to conclude that these various phenomena do not entirely explain the inhibition of growth, and that such inhibition as occurs is largely dependent upon some property of immune serum which adversely affects the circumstances of multiplication. The inhibitory influence of immune serum is manifest only for a relatively short period of time, for if plates are made at the end of twenty-four hours, the pneumococcus is found to have overcome the inhibition, and innumerable colonies develop from comparable amounts of all the cultures.

Carbohydrate and protein form the main food supply of most bacteria. In our hypothesis we have suggested that these substances may not be absorbed unchanged and that some form of preparation may occur at the surface of the bacterial cell. If immune serum is added to the medium in which the cell is growing, the changes necessary for the development of food substances appropriate for assimilation would be subject to the influence of any inhibitory bodies present in the immune serum. In the following experiments evidence is brought that antipneumococcus serum possesses the power to inhibit both the splitting of protein and the fermentation of sugar by pneumococcus. The organisms were grown in serum broth for twenty-four hours. The amount of protein splitting has been estimated by the increase in amino nitrogen determined by the method of Van Slyke.

The following experiment is one of a series showing the degree of protein splitting that occurs when pneumococci are grown in broth containing normal horse serum, and the effect of substituting antipneumococcus serum for normal serum in the mixtures. The estimations of the increase in amino nitrogen were made after twenty-four hours' incubation at 37° C., a period at which marked growth of the pneumococcus had occurred both in the tubes containing normal serum, and in those containing immune serum. In those tubes in which the pneumococcus has grown in its homologous serum, agglutination has occurred. There has been, however, no agglutination either in normal serum or in heterologous immune serum.

TABLE II.

Experiment II. Inhibition of Digestion of Protein by Pneumococcus with Antipneumococcus Serum.

										Increase in amino nitrogen per cc.
Pn.	Type	I	+ 1 cc.	normal horse serum	+ 4 cc.	broth	.....			0.21 cc. nitrogen.
"	"	I	" 1 "	antipn. serum Type	I	+ 4 cc.	broth	.....		0.02 " "
"	"	I	" 1 "	" " "	II	" 4 "	"	.....		0.06 " "
"	"	II	" 1 "	normal horse serum		" 4 "	"	.....		0.21 " "
"	"	II	" 1 "	antipn. serum Type	I	" 4 "	"	.....		0.14 " "
"	"	II	" 1 "	" " "	II	" 4 "	"	.....		0.06 " "

The figures of this experiment (Table II) make it strikingly evident that the addition of antipneumococcus serum to growing cultures of pneumococcus markedly diminishes, in some instances almost to the point of extinction, the production of amino-acids by the organism. The formation of amino-acids in mixtures of broth and normal horse serum has been found generally equivalent to the amount found in broth alone. Certain other normal animal sera have, however, been observed which inhibit the process in varying degrees. Inhibition by homologous immune serum is most complete, although, as in the experiment showing inhibition of growth of pneumococcus, heterologous immune serum possesses considerable inhibitory power. Whether the increase in amino-acid is due to endogenous or exogenous metabolism cannot, of course, be definitely determined. We believe, however, that, in the utilization of protein, the pneumococcus effects a splitting of the protein before absorption, and that the increase in amino-acid represents the excess of protein split, over that used up in the process of growth.

Carbohydrate, as is well known, is an important part of the food supply of most bacteria, and the great variety of sugars that many organisms ferment is extraordinary. That such a splitting of sugars is in some way associated with the nutrition of microorganisms seems a logical assumption. We have found that the addition of antipneumococcus serum to cultures of pneumococcus containing such sugars as glucose, saccharose, lactose, and inulin inhibits in varying degrees the fermentation of the sugars by the organism. Inhibition of fermentation of inulin is most complete, and an example of this inhibition is given in Experiment III (Table III). Pneumococcus, when added to serum water media containing inulin, and litmus as

an indicator, ferments the inulin which results in acidification of the medium and coagulation of the serum.

TABLE III.

Experiment III.

	24 hrs.	48 hrs.	72 hrs.	5 days.
Pn. Type I + inulin.....	++	++	++	++
" " I " antipn. serum Type I + inulin.	−	−	−	−
" " I " " " " II " "	Sl. ac.	Ac.	++	++

++ indicates complete acidification and coagulation; + indicates acid and incomplete coagulation; ± indicates acid and beginning coagulation; V. sl. ac. indicates very slight acidification; Sl. ac. indicates slight acidification; Ac. indicates slight acidification and no coagulation; − indicates no acidification or coagulation.

Experiment III shows that the addition of homologous immune serum to a culture of pneumococcus in inulin completely suspends fermentation of the inulin. Heterologous immune serum delays the reaction, but does not entirely inhibit it. Fermentation of the sugars more actively attacked by pneumococcus, such as glucose, lactose, and saccharose, is not inhibited to the same degree as that of inulin. Determination of the rate of production of acid in cultures containing such easily fermentable sugars shows that, in the early hours of growth, the formation of acid is markedly delayed by the presence of immune serum. After twenty-four hours, however, the acid concentration may reach the same degree in all tubes and, in general, represents the grade of acidity at which pneumococcus ceases to grow. The splitting of carbohydrates by bacteria probably occurs at the surface of the bacterial cell, as is thought to be the case in fermentation of sugar by yeast, and the anti-enzymotic forces of immune serum in all probability exert their antagonistic action at this point.

A study of human blood serum obtained at intervals during the course of an attack of lobar pneumonia shows that bodies having an anti-enzymotic action similar to that of immune serum develop during the period of recovery from the disease. The tests were made in the same manner as those in which an artificially prepared immune

serum was used. The two following experiments are typical of the results obtained (Tables IV and V).

TABLE IV.

Experiment IV. Inhibition of Digestion of Protein by Pneumococcus with Human Serum in Lobar Pneumonia. Infection with Pneumococcus Type II.

	Increase in amino nitrogen per cc.
Pn. Type II + 2 cc. broth + 0.5 cc. serum, 5 days before crisis.....	0.16 cc. nitrogen.
" " II " 2 " " " 0.5 " " at crisis.....	0.02 " "
" " II " 2 " " " 0.5 " " 9 days after crisis.....	0.02 " "

TABLE V.

Experiment V. Inhibition of Fermentation of Inulin by Pneumococcus with Human Serum in Lobar Pneumonia. Infection with Pneumococcus Type I.

	24 hrs.	48 hrs.	72 hrs.	5 days.
Inulin + Pn. Type I.....	++	++	++	++
" " " " I + serum 8 days before crisis...	±	++	++	++
" " " " I " " 6 " " " " ...	+	++	++	++
" " " " I " " just after crisis.....	−	V.sl.ac.	Sl.ac.	Ac.
" " " " I " " 7 days " "	Sl.ac.	Ac.	±	±

These experiments show clearly that at the crisis of lobar pneumonia substances appear in the serum either for the first time, or in greatly increased amount, which have the power of inhibiting the proteolytic and glycolytic activities of the pneumococcus. The period of development of these substances corresponds in time with that of other immune bodies which have been recognized in the serum of individuals with lobar pneumonia.

DISCUSSION.

The series of experiments presented in this paper demonstrate the following facts. Antipneumococcus serum possesses the power of inhibiting for a certain period of time the multiplication of pneumococci. In conjunction with this capacity, it has also the power of inhibiting in varying degree the proteolytic and glycolytic functions of pneumococci. This power is present to a limited extent in the

sera of certain normal animals and absent in others, and in human sera during the course of an attack of lobar pneumonia it appears for the first time or increases markedly at the critical period of the disease. From these facts we are led to assume that retardation of growth is, in part at least, dependent upon inhibition of metabolic function. The observation that immune serum possesses in high degree the powers described, suggests that these properties play an important part in resistance and immunity to infection with pneumococcus. Investigators have demonstrated previously that certain other immune sera possess analogous qualities; such as the inhibition of pigment production by *Bacillus pyocyaneus* (1), the liquefaction of gelatin by *Staphylococcus pyogenes aureus* (2), and the formation of methemoglobin by pneumococcus (3). We have chosen the term "antiblastic immunity" as descriptive of this phenomenon, in order to indicate that the forces at work are antagonistic to the growth activities of the organism. Ascoli (4) coined the term several years ago (*βλαστειν*, to grow). From his studies in anthrax immunity, he was led to suppose that the latter was in part dependent upon the inhibition of formation by *Bacillus anthracis* of a capsule which is a prerequisite for its successful development in the animal body, and he ascribed to anti-anthrax serum an antiblastic action, directed against the metabolic activity of this organism. A concrete interpretation of this phenomenon as applied to the growth of pneumococcus and the inhibitory influence of immune serum is as follows: Pneumococcus, in order to grow, must obtain a sufficient supply of protein and carbohydrate; these substances are furnished by the environmental medium, but probably require, to render them suitable for absorption, preliminary preparation in the nature of digestion. This change is effected at the surface of the bacterial cell and the integrity of this digestive zone is essential to the growth of the bacterium. Anti-enzymotic bodies such as have been demonstrated in immune serum act at the point of contact of the cell with its environment, and influence in an unfavorable manner the nutritional processes there carried on, and the consequence of such action is retardation or inhibition of growth. It is possible that capsule formation represents on the part of the organism an attempt to protect the function of the digestive zone. Should the foregoing prove

to be a correct explanation of the phenomenon observed, considerable light would be thrown on the obscure mechanism by means of which parasitic bacteria establish themselves in animal tissues, and on the forces mobilized by the animal body in opposition to such invasion.

CONCLUSIONS.

1. Antipneumococcus serum possesses the power of inhibiting for a certain period of time the multiplication of pneumococci.

2. It also has the capacity of inhibiting the proteolytic and glycolytic functions of pneumococci.

3. This power is acquired for the first time or appears in increased amounts in human serum at the time of crisis in lobar pneumonia.

4. The retardation of bacterial growth is thought to be dependent upon the inhibition of metabolic function due to the presence of anti-enzymotic substances in antipneumococcus serum. To this phenomenon we have applied the term antiblastic immunity.

BIBLIOGRAPHY.

1. Ghéorghiewsky, *Ann. de l' Inst. Pasteur*, 1899, xiii, 298.
2. von Dungern, *Centralbl. f. Bakteriol., Ite Abt., Orig.*, 1898, xxiv, 710.
3. Cole, R., personal communication.
4. Ascoli, A., *Centralbl. f. Bakteriol., Ite Abt., Orig.*, 1908, xlvi, 178.