

THE GENETICS OF PATHOGENIC ORGANISMS

Publication of the American Association
for the Advancement of Science
No. 12

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Published for the American Association for the Advancement of Science by
THE SCIENCE PRESS

1940

PROBLEMS IN THE VARIATION OF PATHOGENIC BACTERIA

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FROM the early days of Pasteur and Koch down to the last decade and a half, pathogenic bacteriology was dominated by the dogma of *monomorphism*. With few exceptions, the earlier studies were more concerned with establishing the fixity of species than with variation. But during the last 15 to 18 years the literature of the subject has been flooded with evidences of variability.

It has become increasingly apparent that pathogenic species of bacteria may pass through thousands of generations under laboratory conditions or in the animal body without detectable change in their characteristics. At the same time, it is equally evident that most, and probably all, pathogenic species at times undergo variation. The fact that bacteria do vary has repercussions in almost every field of the study of infectious diseases. I should like to call attention to problems connected with variations in antigenic composition and virulence and to suggest some hypothetical considerations concerning the causes of variations.

VARIATIONS IN ANTIGENIC COMPOSITION

From the early work, especially that of Arkwright (1920), it has been apparent that certain variations are generally correlated. The most frequently observed variation, the Smooth to Rough (S to R) colony form, is not merely a change in the structure of the colony; there is usually associated a change in virulence, in antigenic composition and other characteristics, such as cell morphology, sensitivity to bacteriophage, and hydrophobe or hydrophile properties of the cell. The variation is generally discontinuous and the variants are frequently highly stable. In many species the S to R change occurs readily, but reversion or R to S occurs only rarely. The significance of this type of variation is not apparent,

but it seems safe to assume that it represents a relatively major change in the genetic makeup of the organism.

The pathological importance of the change is primarily due to variation in the antigenic composition which appears to be a fundamental consideration in virulence as well as in antibody stimulating and reacting mechanism. The S to R variation ordinarily involves a loss in the heat-stable somatic antigen that characterizes the surface of the normal virulent type. This loss may further result in the uncovering of somatic antigens which then dominate the variant type. The somatic antigen, probably most frequently concerned in this loss variation, we know, from the initial work of Heidelberger and Avery, is a polysaccharide. In chemically pure form the polysaccharides are haptens or partial antigens—they react with antibodies but do not stimulate antibody formation. In the cell, however, they act as antigens. In the case of the *Pneumococci*, the polysaccharide is contained in the capsule. In the course of the S to R variation the capsule and the specific polysaccharide antigen are lost. There remains, however, in the R organisms an antigenic protein component common to all types of *Pneumococci*. It has been shown that a similar series of changes accompany S to R variation in several, possibly all, pathogenic species. This loss of surface antigen means that the variant lacks antigenic specificity and lacks the ability to stimulate the formation of specific antibodies in animals. It has not been possible to put all antigenic differences between bacterial species or variants into chemical terms or to interpret antigenic variation in this precise manner, but the fact that certain major changes can be so interpreted tends to clarify the situation.

Knowledge gained from a study of variation in the antigenicity of bacteria, during the last dozen years, has resulted in many modifications in methods of immunization and diagnosis by immunological means. We now know that in the preparation of vaccines for immunization against typhoid, paratyphoid, and other infections of this group, consideration must be given to the complex antigenic composition of the organisms and the ease with which variation in these components occurs under laboratory conditions. Similar problems are met with to a greater or lesser degree in the preparation of vaccines from all types of pathogenic bacteria. Similarly, in the preparation of antisera, as against *Pneumococci* and *Meningococci*, possible variation in antigenic composition is a fundamental problem. In such a simple procedure as the Widal and other agglutination reactions used in the identification of antibodies for diagnosis of infections, due regard must be given to the antigenic structure and possible variation of the organisms used as antigens. Such problems appear in almost every phase of immunology.

It will be recalled that the French group—Calmette *et al.*—obtained from an old culture of tubercle bacilli a strain, BCG, of exceedingly low virulence, a type capable of producing only localized lesions which are fairly rapidly absorbed. It was shown by Petroff (1927), Begbie (1930), Sasano and Medlar (1931), Seiffert (1932), and ourselves (1934) that this organism is probably an R variant of the normal tubercle bacillus. It has been found that the strain generally is highly stable like most R forms. In the hands of a few observers, sufficient variation has, however, occurred to permit recovery of the fully virulent or normal S form (Reed, Orr, and Rice, 1934).

The French group and others have long claimed that the introduction of BCG cultures into infants results in a considerable degree of immunity against ordinary tuberculous infection. This vitally important problem has been seriously questioned on statistical grounds. We have approached it differently by examining the antigenic

structure of the BCG organisms in contrast to normal tubercle bacilli.

In earlier papers (Rice 1931; Rice and Reed 1932) it was shown that, if rabbits are immunized with whole cultures of normal S forms and other rabbits with R variants of mammalian tubercle bacilli, a distinction can be made in the antibody content of the two lots of animal sera. The examination procedure was an indirect one involving complement fixation with antigens prepared from S and R types of organisms. The anti-S immune serum fixed, on the average, twice as much complement with the S antigen as was fixed with R antigen. The anti-R serum fixed, on the average, the same amount of complement with both S and R antigens. These observations were interpreted to mean that the S organisms contain an S-specific and a species antigen, and that the R organisms lack the S-specific antigen but contain the species antigen. Correspondingly, the anti-S serum contains the two antibodies; the anti-R serum, the species antibody, while it lacks the S-specific antibody.

The same examination procedure was followed with the ordinary BCG cultures and the S type recovered from them (Reed, Orr, and Rice 1934). Rabbits were immunized with the two types and the antisera tested against antigens prepared from S and R type mammalian tubercle bacilli. The results are summarized in Table I. It is apparent from the column on the left that antisera prepared from several cultures of BCG contain antibodies which fix complement in approximately equal amounts in the presence of S and R antigen. The antisera prepared from S variants of the BCG (which are virulent), as indicated on the right of Table I, fixed approximately twice as much complement in the presence of the S antigen as in the presence of the R antigen. The simplest explanation of the observations is that the BCG organism, like other R tubercle bacilli, lacks an antigenic component which characterizes normal virulent tubercle bacilli. Lacking this antigen, the corresponding antibody is lacking in the serum of BCG immunized animals.

TABLE I

A COMPARISON OF THE REACTIVITY OF TYPICAL S AND R MAMMALIAN TUBERCLE BACILLUS ANTIGENS WITH THE SERUM OF RABBITS IMMUNIZED WITH ORDINARY BCG-R ORGANISMS AND WITH DISSOCIATED S ORGANISMS AS MEASURED IN CUBIC CENTIMETERS OF COMPLEMENT FIXED SPECIFICALLY AT FIFTY PER CENT HEMOLYSIS

Antiserum	Antigen	Specific fixation	Antiserum	Antigen	Specific fixation
BCG-R Montreal (1)	S	.0041	BCG-S Trudeau	S	.0064
	R	.0035		R	.0030
BCG-R Montreal (2)	S	.0080	BCG-S Alberta (1)	S	.0032
	R	.0074		R	.0014
BCG-R Montreal (3)	S	.0147	BCG-S Alberta (2)	S	.0143
	R	.0139		R	.0087
BCG-R Montreal (4)	S	.0110	BCG-S Alberta (3)	S	.0143
	R	.0101		R	.0066
BCG-R Montreal (5)	S	.0073	BCG-S Ottawa (1)	S	.0057
	R	.0069		R	.0028
BCG-R Paris	S	.0090	BCG-S Alberta (4)	S	.0150
	R	.0080		R	.0087
BCG-S & R Alberta (5)	S	.0100			
	R	.0082			

It seems probable that the BCG organism has arisen as a variant from "normal" tubercle bacilli and that one feature of the variation was a loss of a specific antigenic substance. This adds support to the doubt raised from the statistical analysis based upon the use of the material as an immunizing agent in man and cattle. It is in line, too, with general findings with other species of pathogenic bacteria, such as the *Pneumococci*, members of the typhoid coli group, that the S to R type of variation is accompanied by a loss of a specific antigen which renders the variant ineffective as an immunizing agent.

VARIATION IN VIRULENCE

A long recognized type of variation is change in virulence or change in ability to produce toxins. This change occurs in many species under laboratory conditions, and we know that avirulent or atoxogenic variants may frequently be isolated from man (Wadsworth and Sickles 1927; Okell 1929; Gunn and Griffith 1928). Variation in the animal body is probably related to the interaction of antibodies with the surface antigens of the organisms.

An attractive explanation of the rise and fall of epidemics has been based, with some degree of experimental support, upon these findings. The extent to which host immunity and bacterial variation contribute to the course of epidemics has never been determined, but there seems to be ample evidence from both the study of epidemic disease in man and experimental herd infections in animals that variation in the causal bacteria is at least a factor of importance, even though it be not a simple and obvious relationship.

In a long series of studies of herd epidemics in mice, Webster and colleagues (1930, 1933) have shown that different strains of the same bacterium recovered at intervals during the course of one epidemic may possess an approximately equal virulence. This they regularly found to be the case. They, therefore, conclude that changes in virulence play no part in the fluctuations in mortality frequently observed in a long continued epidemic. They have, however, reported different degrees of virulence in the same bacterial species recovered in different epidemics (1930). At the same time, they demonstrated that in one species of

Pneumococcus (Webster and Clow 1933), a strain of high virulence, as judged by intraperitoneal and intranasal infection, might as a result of nose-to-nose passage, lose its intranasal virulence without affecting its intraperitoneal virulence.

On the other hand, Topley, Greenwood, and associates (1928) have considered a second factor. They find that virulence, as measured by direct inoculation, and the power to induce severe epidemics by contact infection are not always correlated. Recent experiments with a species of *Pasteurella* (Greenwood *et al.* 1936) seem to afford proof that changes in infectivity may occur during the epidemic spread of a bacterial parasite. They, therefore, suggest that an "epidemic strain" is characterized by two attributes, high virulence and high infectivity. Loss of either through variation may result in loss of ability to cause an epidemic. It seems safe to conclude from these herd studies that variation in the invading bacteria may constitute a major factor in the spread of human infections. There is also much supporting evidence to be found in a study of human infections.

THEORETICAL CONSIDERATIONS

The similarity of the variation pattern in many species of bacteria and especially the variation of correlated characteristics, as just noted, have contributed largely to the theory of cyclic change in the life history of the bacteria. But variation by no means always occurs simultaneously in several characteristics. In a recently published study (Reed 1937) of the saprophytic species, *Serratia marcescens* Bizio, it was shown that variation may occur independently in several characteristics.

In this species, as is generally the case, colony structure, cell form, and specific somatic agglutinin are always associated: S colonies are composed of short rods and coccoid cells containing specific somatic antigen, while the R colonies are composed of long rods and filaments without the specific somatic antigen. Where variation occurs from S to R or in the reverse direction, the associated characteristics vary syn-

chronously. This may arise from an inseparable linkage of the inheritance factors or these three characteristics may result from the transmission of a single gene. What is measured as somatic antigen may, for instance, determine the cell form and cell form may determine the colony topography.

Several other characteristics exhibited independent variation. Capsulated cells with the resulting mucoid structure of colonies occur in both S and R forms. Variation may proceed in the direction of either gain or loss in capsulation, and this variation may occur irrespective of variation in S or R colony form. It is evident, therefore, that the factor for the inheritance of mucoidness or its absence is independent of the factor or factors for colony structure. Again, variation in pigmentation from red to orange-red or white, or from orange-red to white, occurs regardless of variation in colony structure or capsulation of the cells. Color factors must, therefore, be inherited independently of factors for colony structure or for capsulation.

Independent variation in three sets of characteristics does not appear to be consistent with any theory of cyclic change in the organisms. It seems more likely that, in these cases at least, the inheritance of individual characteristics has been subject to some irregularity. It is more in line with current genetical opinion to assume that this is the result of change in individual genes or "gene mutation." A study of *Staphylococci* now in progress adds additional evidence in support of this hypothesis.

There are, however, certain well known variations in pathogenic bacteria which possibly permit a simplification of the gene hypothesis, a simplification which may be applicable to all inheritance and variation in the bacteria, and perhaps in a wider field.

Since the work of Neufeld in 1909, we have been familiar with the existence of antigenically different types of *Pneumococci*. Three types and a heterogeneous group were first distinguished, and quite recently the unclassified group has been differentiated into some 27 additional types. There is ample evidence that the antigenic difference

TABLE II
CHARACTERISTICS OF THE TYPE-SPECIFIC POLYSACCHARIDES OF PNEUMOCOCCI
(After Avery and Heidelberger)

Type	Optical rotation	Per cent nitrogen	Substances obtained on hydrolysis	Dilution giving specific precipitation
I	300°	5	Galacturic acid and amino-sugar derivatives	1: 6,000,000
II	74°	0	Glucose	1: 5,000,000
III	-33°	0	Glucose and glucuronic acid	1: 6,000,000

between at least the original three types depends upon chemical differences in the polysaccharide, which is the principal component of the capsule surrounding the organism, as indicated in Table II.

As just noted in another connection, variation frequently occurs in the strain of this species in which the capsule and type-specific polysaccharide is lost. Such variants, therefore, do not belong to a specific type; they have lost their type characteristic. It was shown by Griffith (1928) and recently confirmed particularly by Dawson and Sia (1931) that, if a non-capsulated, non-type-specific variant arising from type I is grown in a menstruum including type II polysaccharide, reversion to a capsulated type-specific form may occur. And, very significantly, the new form will be not type I from which the non-capsulated strain arose but type II, the type of the polysaccharide present in the menstruum in which the variation occurred. Once this new type II has arisen, it continues to breed as a characteristic type II; that is, the newly gained ability to synthesize the type II polysaccharide is passed on from generation to generation.

The fact that the type-specific polysaccharide is taken up from the environment and continues to be formed in subsequent generations suggests that it may be a self-propagating substance like Stanley's virus protein. If so, the type-specific inheritance may be regarded simply as a cutting off, in the fission division, of a portion of the substance sufficient to permit the propagation of more similar substance in the developing daughter cell. A loss variation is then

simply a cell division in which the polysaccharide fails to divide. If this is true of specific polysaccharide, possibly there are in the cell other self-propagating substances. Such a simple hypothetical mechanism probably makes it unnecessary to postulate genes, although it is conceivable that the genes are just such substances.

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