Bacteriology. — *Antigenic structure and specificity.* By Prof. J. J. VAN LOGHEM. (Communicated by Prof. W. SCHÜFFNER.)

(Communicated at the meeting of November 24, 1928.)

§ 1. *Introduction.*

The antibodies which appear in answer to the resorbtion of albuminoid substances fit so well into the corresponding antigens that they are used for the determination of these antigens.

The foregoing does not prove that a serological reaction is always a specific reaction. We have to consider the possibility that various species have in common the same antigen, so that experience only can show whether a serological reaction is also a specific reaction.

In pathological bacteriology especially most workers are little inclined to relinquish the great expectations, which were fostered from the serological determination of bacterial species. Others, meanwhile, recognise that the immunological reaction often fails as a specific reaction.

The first dificulty arises from bacteria, which belong to different species and cause the appearance of the same antibody. A well studied example of this kind may be found in the genus Vibrio.

Another experience which lessens the specific value of the serological reactions, one makes with bacteria of a same species which produce different antibodies. The atoxic dysentery-bacilli, the coli bacillus and the proteus-bacillus are well studied examples of this.

The third difficulty is caused by the variability of the serological results, which one may meet with in one and the same strain. Many authors do not give up the specific meaning of such results. For this reason they accept an instability of species which implies that the limits between certain bacterial species are not fixed.

Viewing the practical and theoretical signification of a sharp limiting of the notion : species, I will try to discuss the specific value of immune reactions.

§ 2. *The complex antigenic structure of bacteria.*

Already on the occasion of my first work on Proteus, I made experiments, which prove that the species Proteus anindologenes contains several antigens. The antigenic structure of many strains of this species, however, is far from complete, being only characterised by one component. So one

may meet with two strains, the specific identity of which can only be proved by the aid of a third strain.

As a scheme we may accept that there are at least two Proteus antigens : A and B. An A-strain (which contains principally the A-antigen) will give a serum, which will not agglutinate a B-strain in a typical way. So it is impossible to recognise the specific identity of strain A and strain B without the help of a strain, which contains both the antigenic components.

I will illustrate this by an example. In Amsterdam I isolated from a case of Pneumaturia a strain "Pneum", which proved to be serologically identical to a strain 22 from an absces. A few years later I isolated three Proteus anindologenes from faeces in Sumatra. Their antigenic structure, however, was different to the Amsterdam-strains. Then I received a strain from Leyde (isolated from urine). The serum, prepared by the strain-Leyde agglutinated both the Amsterdam and the Sumatra-strains.

So it is clear that the Amsterdam-strains principally contained A-antigen; the Sumatra-strains principally B-antigen ; the Leyde-strain both A- and B-antigens.

ANDREWES and others compared the complex antigenic structure of bacteria with a mosaic or a kaleidoscope ; and they have given a further analysis of this structure. The possibility of such an analysis is afforded by CASTELLANI's method. We also used this method in our work on Proteus [1]) (with BOELMAN). We proved the antigenic structure of Proteus to contain at least three components. In some strains we found all these three components, in others only 1 or 2 of them.

This comparative analysis I extended also to Proteus indologenes, which biochemically differs from Proteus anindologenes.

It was already known to us that anindologenes-sera generally do not agglutinate the indologenes-strains, or agglutinate them less well than indologenes-sera.

The positive results in a few cases meanwhile proved their relationship. The absorption tests showed that indologenes-strains and anindologenes-strains have indeed, at least, one component in common ; this means that in a given case, if this component prevails, two strains, belonging to two different species, appear as serologically identical.

At the same time it is clear that some antigens have a more "special" character, while others seem to belong more to the genus.

§ 3. *The genus-antigen prevails ; the species-antigen remains in the background.*

I have mentioned already the serological identity of different species of the genus Vibrio. Many bacteriologists do not pay any attention to the differences between Vibrio cholera and Vibrio El Tor. Yet, the points of difference between them are so important — production of an exo-toxin

[1]) Ned. Tschr. v. Geneeskunde, 1925, I. p. 1314,

and an exo-haemolysin by Vibrio El Tor [1]), where this production is negative in V. cholerae — that there is no doubt of the necessity of recognizing them as two species.

Their serological identity is only explicable by accepting in their antigenic structure a so preponderent position of the genus-components, that the species-components do not manifest themselves. Meanwhile their presence — in a latent way — is quite possible.

Often, indeed, one sees co-agglutination by cholera serum in toxic and haemolytic watervibrios. In these organisms too the genus-components remain often in the fore-ground.

§ 4. *Constant intraspecial differences in antigenic structure.*

The Proteusstrains, mentioned in § 2, illustrate not only an idea of complex antigenic structure, but they lead also to the conception that within the species difference in antigenic structure exists.

With a view to the uncertainty whether Proteus represents only one species and with a view to the variability observed in the antigenic structure in Proteus, it is preferable to study these "intraspecial differences" in species, which are less subject to variability.

Our first thoughts go to meningococcus and pneumococcus, the types of them mostly are understood as races within species. The types are different in antigenic structure; so they are examples of intraspecial difference.

On the other hand, the specific identity of these types still being in discussion, I prefer to lay stress on the fact that intraspecial differences even occur in bacterial species, about the homogeneous character of which there is no doubt at all.

A bacterium of a very constant character is the typhoidbacillus and yet, it is possible to show also in this organism an intraspecial difference in antigenic structure. It is necessary however, as I proved years ago, to use agglutinating sera of a very low titer. I will illustrate this by giving the results of an experiment. Rabbits are immunized by one injection of the strain "Ty. lab.", after one week their sera agglutinate bacilli of the same strain to 1 : 1000 and in the same dilution bacilli of the strain Str. On the other hand these sera have not the least influence on other strains of our collection neither on a very agglutinable suspension of typhoidbacilli, the diagnosticum of FICKER.

After a few weeks, during which the rabbits received several injections of the strain "Ty. lab." this "intraspecial" difference disappeared. All the above-mentioned strains and also the FICKER-suspension are now agglutinated in the same dilution.

[1]) J. J. VAN LOGHEM, Exo-haemolysine en Endo-haemolysine bij Vibrio Tor en Vibrio cholerae, Ned. Tschr. v. Geneeskunde. 1924, II, p. 773. Bakteriophage und hämolytisches Endotoxin des Cholera vibrio. Centralbl. f. Bakt. 1926. Orig. I, Bd. **100**, p. 19.

Studying the literature at this point one does not doubt that intraspecial differences in antigenic structure are quite normal. The question whether the types of meningococcus, pneumococcus, diphtheriabacillus, Salmonella, tubercle-bacillus, tetanusbacillus, etc. are to be considered as representatives of one species is in my opinion to be confirmed.

§ 5. *Intraclonar variations in antigenic structure.*

Yet, there are differences in antigenic structure of another nature. They may appear as "intraspecial", but in reality they are variations within the clone or the individuality of the bacterium.

In accordance with an earlier publication [1]) on variations of bacteria, I remember the fact that all the bacterial cells, representing the offspring of one cell are to be considered as a clone or individual line.

It is on this individual line, that the outerworld has its effects. The influence of this outer world is partly normal and causes physiological adaptative changes of the bacteria ; it is partly abnormal and leads to an abnormal bacterial regression, characterized by a diminution, a loss or a degeneration of functions.

As an example of atrophy I mention the loss of the property to liquify gelatine or to produce spores.

An example of degeneration is the production of indol by the typhoid-bacillus.

Such variations of the clone during its development I propose to call *intraclonar variations*. They also apply to the antigenic structure.

Both the regressive and the adaptative intraclonar variations are met with intraclonar regressive variations of the antigenic structure.

As a typical example of these variations I consider the characteristic disorganisation of the antigenic structure which I have studied in old and inveterating strains of salmonella [2]).

Clones of Paratyphoid-B-strains which, immediately after their isolation, show an homogeneous structure, may after a certain time splice themselves in two individual lines of different antigenic structure.

One of these lines remains in possession of the antigenic structure of the motherstrain and contains a genus-component (co-agglutination by a typhoid-serum) ; the other line is less complex and showed a.o. the loss of the genus-component [3]).

Not long before my own work on the change in antigenic structure of inveterating Paratyphus-B-strains, others published very important findings on the variation of the antigenic structure of Proteus-strains.

I am alluding to the so well known observations of WEIL and FELIX.

---

[1]) Ned. Tschr. v. Geneeskunde, 1921, II, p. 2981, and Centralbl. f. Bakt., 1922, I, Origin, Bd. **83**, p. 257

[2]) Ned. Tschr, v. Geneesk., 1919, II and Centralbl. *f.* Bakt. I, Origin., Bd. **83**.

[3]) Ned. Tdschr. v. Geneesk. 1919, II en Centralbl. f. Bakt. I Origin.. Bd. **83**.

In 1917 they obtained from complete Hauch-strains incomplete Ohne-Hauch strains the latter differ also in agglutination-tests.

The Hauch-strains possess — just as I found in the above mentioned Paratyphoid-B-strains — two antigens ; the Ohne Hauch-strains only one.

WEIL, FELIX, ANDREWES, SAVAGE, BRUCE WHITE and others examined from this stand-point first the typhoid-paratyphoid group and proved the "double type" of receptors to exist also in these bacteria.

In my opinion not all the facts, which have since been collected on the variability of the antigenic structure, may be considered as identical. The symptoms of the variability of the Paratyphoid-B-bacillus and the coli bacillus — compared with the typhoid bacillus — are partly connected with the less developed parasitic faculties of both. For the atoxic dysentery-bacillus also, we showed the adaptative changes in the antigenic structure after growth in the liquid of living tissues.

On the other hand there is an immense mass of evidence relating to variations of strains which have been examined under unfavourable conditions. Cultures of old strains, intoxicated and starved cultures form the material, in which for the last years the variability of bacteria has been studied by preference.

Agglutinative and sedimentary growth, rough and irregular colonies (change from "smooth" into "rough"), loss of virulence, loss of biochemical functions, loss of pigmentproduction, loss of capsule, spores, flagella, change of the normal shape of the bacterium cells — in short a long series of variations has been observed which — in my opinion — may be taken for regressive variations of the bacterial individuality.

The variations represent reactions on abnormal incitations from the outer-world ; they are symptoms of disease during the individual life of the bacterial clone. Sometimes they end by dying, sometimes by recovering (if the abnormal conditions are changed into normal ones), sometimes they lead to irreparable loss : to a mutilation or an atrophy, which will characterise the bacterium for the rest of its existence.

To this regressive variations — which is called dissociation by a group of American bacteriologists — I also attribute certain changes of the antigenic structure.

I think, the fact that the antigenic structure loses its genuscomponent — as was shown by me in inveterated paratyphus-B-bacilli and which has been traced also by others in the Proteusgroup as well — test explained as a fact of regression.

Although the facts of variability in bacteria are well established, the discussion as to the significance of these facts is not yet at an end. Since the publication of MASSINI on the variability of B coli it is a common practice to speak about mutants, about inheritance of acquired functions, about "Dauer-modifikation" (lasting modification) etc. In short, the variability of the bacteria is generally considered as a chapter of Genetics.

Consequently, many bacteriologists accept an instability of the bacterial species; they accept the possibility that bacteria of one species change themselves into bacteria of another species; the possibility that under the direct influence of the outer world or of the experiment, new species originate.

EISENBERG [1]) concluded his review of the bacterial variations by the words: "Eins geht aus dem vielen besprochenen Befunden zweifellos hervor, das ist die Erblichkeit erworbener Eigenschaften bei Bakterien und anderen Mikro-organismen." E. GOTSCHLICH [2]) recognises: "Tatsachen prinzipieller Bedeutung für die Ueberschreitung des Artbegriffes" and HADLEY [3]) in his otherwise so able study on Microbic Dissociation refers to "an ever increasing mass of evidence pointing to the instability of species".

In my opinion bacteriology has no right to draw conclusions on the nature and the variability of the genotypic construction of bacteria which lead to revolutionary consequences for biology.

Herewith I do not deny the possibility of real mutations — i.e. genotypic variations — in bacteria.

The words "es handele sich bei der Variabilität der Bakterien überhaupt nicht um Vererbungs- sondern nur um entwicklungsphysiologische Vorgänge" [4]) — in this way JOLLOS has formulated my view in his report in the 10th meeting of the Deutsche Vereinigung für Mikrobiologie — give an incorrect summary of my conceptions. Literally I wrote [5]): Die Erblichkeitslehre der Unizellulären kann sich nur entwickelen und ein Teil der Genetica werden, wenn sie gesäubert ist von allem, was nicht zu ihrem Objekt gehört, aber systematisch damit verwechselt wird.

b. Intraclonar adaptative variations of the antigenic structure.

In contrast with the regressive variations one may meet with changes in a bacterial individual, best understood as a normal reaction on a normal influence from the outerworld.

According to the conception given by me these variations belong to the physiology of the bacterial individuality and represent its adaptation It is especially these adaptative variations that give rise to so much misunderstanding.

The antigenic structure of many bacteria shows also the adaptative variability as a function.

Within the typhoid-coli-group there is a good opportunity to study the adaptative property of the antigenic structure. In some coli-strains one

[1]) WEICHARDT's Ergebnisse, 1914, p. 136.
[2]) Centralbl. f. Bakt., 1924, I, Orig. Bd. **93**.
[3]) Journal of Infectious Diseases, 1927, Vol. **40**.
[4]) Centralbl. f. Bakt., 1924, I. Orig. Bd. **93**.
[5]) Centralbl. f. Bakt., 1922, I. Orig. Bd. **88**, p. 257.

is struck by the fact that the characteristic function of this organism to adapt itself to natural conditions is represented by a very mobile structure.

I have shown [1]) that in such cases it is not possible to prepare an agglutinating serum ; at the moment of the agglutination test the antigenic structure is changed in such a way, that antibody and antigen do not fit into each other.

It is also possible that a coli-strain of such a mobile structure, after having been cultivated under special conditions, changes the character of its structure. Then, the antigenic composition seems to fix, enabling one to prepare an agglutinating serum.

The adaptative change in antigenic structure may be accompanied by change in biochemical function (f.i. temporary loss of production of lactase).

We found something of this kind in the atoxic dysentery bacillus and thereby explained why so many workers tried in vain to find a serological or a biochemical classification of the dysentery-bacilli.

If one examines carefully some strains under the conditions of the laboratory it may be possible to observe, by agglutination reaction with an preserved serum, how the antigenic structure of certain strains changes its type. In some cases the biochemical functions of the strain vary at the same time ; in other cases they do not.

These findings were made at first by me with KORTHOF [2]) and BOCHARDT [3]) ; later I repeated and extended them.

The dates on adaptative variations, studied in the laboratory, bring us to the question whether the types, (as they have been studied especially in meningococcus and pneumococcus), are to be considered as adaptative stages of the antigenic structure, which have been fixed under natural conditions.

Herewith we come also back to the intraspecial differences in antigenic structure, mentioned in § 4 ; differences which even may be found in a species of such a constant character as the typhoid bacillus.

In all these dates together we again see a contribution to the knowledge of variability as a function ; and to the knowledge of the loss of this function in case of the increasing of the parasitic properties.

The parasitic typhoid bacillus shows an almost immovable antigenic structure and its intraspecial functions are only slightly indicated.

The organisms which are more inclined to commensalism — f.i. meningococcus, pneumococcus, atoxic dysentery bacillus, paratyphus-B-bacillus, diphtheria-bacillus — show a smaller or greater number of serological types, as the manifestation of a movability of the antigenic structures, which leads to the fact that certain antigenic components come to the foreground.

---

[1]) Ned. Tschr. v. Geneeskunde, 1921, II, p. 1966.

[2]) Thesis, University Amsterdam, 1918.

[3]) Ned. Tschr. v. Geneeskunde, 1923, II. p. 144.

In these organisms we meet with characteristic fixations of the antigenic structure which characterise the intraspecial types of these organisms.

The greatest movability of the antigenic structure we meet with in B. coli. Yet, fixation is also possible in this species; very important is the experience that the fixed coli-bacilli are often met with under pathological circumstances and that we obtain them by cultivating normal strains in the living fluids of an animal. For more particulars I refer to the thesis of TER POORTEN [1]).

§ 6. *Summary.*

Bacteria possess a complex antigenic structure.

Some of the components belong to the genus (or the family); others have a special or specific character.

Genus-antigens may prevail in such a way that a serological reaction in order to distinguish two species from each other may fail.

On the other hand there exist also differences in antigenic structure in representives of the same species. These differences may be called intraspecial differences. They are very common and it is even possible to demonstrate their existence in very homogeneous species such as the typhoid-bacillus. In other species we meet with them as serological types, which have been recognized as pneumococcus, meningococcus, diphtheria-bacillus, tubercle bacillus, tetanusbacillus, etc.

We know further the intraclonar or individual changes of the antigenic structure and we distinguish them as adaptative or regressive ones.

The adaptative variations are physiological reactions of the clone or the individuality on normal influences from the outer-world; the various atoxic dysentery-bacilli (Flexner, Y, etcetera) are examples of adaptations of antigenic structure and biochemical functions within one and the same species. The adaptative changes of the coli-bacillus, which are very often regarded as mutation, represent another example. The adaptative intra-clonar variations may be fixed for a shorter or longer time, and they may lead to the origin of fixed intra-special differences, i.e. serological types.

The regressive variations are pathological manifestations of the clone, caused by noxious (abnormal) influences from the outerworld. They occur also in the antigenic structure. Many variations in the form of cell and colony, in growth, in biochemical function and antigenic structure — which are classified as "mutations", "Dauermodifikationen", "dissociations" etc. and which are taken, from a genetical point of view, as proof of the instability of bacterial species — are better understood as degeneration and atrophy of ill-treated clones.

Institute of Hygiene, University, Amsterdam.

[1]) Thesis, University Amsterdam, 1920.