Some Aspects of the Chemistry of the Vibrios

By Richard W. Linton

(*Department of Pathology, Cornel University Medical College, New York*)

I PROPOSE to review in this paper a series of researches on the cholera vibrio which were carried out in India under my direction between 1931 and 1938. They were supported by grants from the *Indian Research Fund Association*, and the material upon which the paper is based was published in the *Indian Journal of Medical Research* during those years.

When the study commenced in 1931 we were faced with a heterogeneous mass of vibrios, some named and some numbered, some isolated years before and some newly isolated, from highly fatal epidemics or mild cases, some from the beginning and some from the end of epidemics, others from carriers or from water. With the information then available it was impossible to make any useful differentiation of these vibrios or to say which was dangerous and of epidemiological importance and which was harmless.

Another aspect of interest in the cholera problem at that time was the question of variation. Workers in India were then much interested in bacteriophage, which was being widely used as a therapeutic agent, and under the influence of 'phage, in the laboratory at least, the vibrio appeared capable of an extraordinary range of variation; and these variants were themselves objects of speculation as to their relation to cholera.

At that time we did not have the advantage of any useful knowledge of vibrio serology, such as has been obtained during more recent years through the work of Gardner and Venkatraman and Bruce White, although this work itself, as is now becoming evident, was too narrowly based to stand up under accumulating field experiences. In our own work, it was accordingly necessary to choose strains at random, since there was only the slightest information, at first, as to what their potentialities might be in the cholera problem.

Our first work was on the isolation of polysaccharides from the vibrios; later as the work expanded we undertook the study of vibrio proteins, of vibrio metabolism and finally of the chemical basis of vibrio variation.

Leaving aside the technical details of the isolations, I may point out that of over 300 vibrio strains from all sources, three polysaccharides were obtained. As far as we were able to carry the analysis, these appeared to be made up of the following constituents: (1) Galactose plus an aldobionic acid consisting of galactose and glucuronic acid; (2) Arabinose plus an aldobionic acid consisting of galactose and glucuronic acid; (3) Glucose alone, no aldobionic acid. The first two of these were reported in 1932 and the last in 1935, although it is probable that this polysaccharide was identical with one isolated by Jermoljewa and Bujanowskaja in 1930, who tentatively identified glucose in the hydrolysis products. These polysaccharides vary in nitrogen content between 3% and 6%, with about 0·6% of amino nitrogen. Landsteiner and Levine found a nitrogen content of 4·3% in the vibrio polysaccharide which they isolated. These structures are acetylated in the cell, and have distinctly different specific rotations.

In the same large group of vibrios, a study of the proteins was next undertaken. The well-known complexity of the proteins seemed to afford an opportunity for an almost unlimited capacity to break down into a number of fractions. Rather than do this, however, we chose to take the protein as a whole, and see if any differences could be found between proteins from various vibrios from different sources. We applied Woodman's technique to these proteins, and found that no matter how many vibrios we studied we obtained only two curves, and that these curves correlated with the source in nature, or one might say, with the epidemiological source of the organisms.

We had thus three polysaccharides and two proteins among the vibrio group as a whole. Each individual vibrio appeared to be made up of one polysaccharide and one protein. We accordingly had the possibility of six groups among these organisms,

and over the course of several years, all six of these groups were found. For example, after three of the groups had been formed, it was possible to predict the existence of a fourth group, and several months afterwards upon the analysis of some carrier strains from El Tor in Egypt, vibrios were found which conformed to this chemical composition. In the same way, the discovery of the glucose-containing polysaccharide, which came after the work had been in progress for three years, permitted us to predict the existence of the sixth group, and eighteen months later on analysing some vibrios from China, this group was found.

Having thus been led to form groups and divide the vibrios according to their chemical structure, it was important for our hypothesis to pursue other lines to see if we could strengthen our work, or if we should modify our conception. These further researches, which in large part went on at the same time as the chemical analyses, and were an integral part of them, followed three lines: evidence from epidemiology; evidence from the metabolic activity of the vibrios; and evidence from variation.

The evidence from source or epidemiology showed us that vibrios having the chemical structure of groups I or II invariably came from cholera cases, in recent isolation; that is, not from old laboratory strains which might have come from cases years previously; also in these groups were vibrios from people who had been in recent contact with cases, in the same house for example. Group II vibrios are rather rare in our series, and hardly appear in Calcutta during the annual cholera epidemic at all; on the other hand, their isolation from highly fatal epidemics in Assam indicates that they have an important part in some places in the disease. Strains from water were generally found to have the group III structure, although, as one would expect, since cholera is often a water-borne disease, vibrios of the first two groups were occasionally found. Strains from chronic carriers, from El Tor and from carriers in India and China were found to have the group IV or V structure; specifically, the El Tor strains were of group IV structure. It was of interest to study these strains and to show that they were related to the cholera vibrios of group I by having the same polysaccharide, and to the water vibrios by having the same protein. Our analysis showed that chemically the strains were the same in the earlier isolations at El Tor thirty-five years ago, and the isolations of 1930–32. Old laboratory strains from cases, or case strains which had been made to vary artificially generally were found to possess the group VI structure. Accordingly before the work was ended, it was possible to predict with a good deal of accuracy just what chemical structure a strain would possess, when its source was known. In short we had a constancy of protein and polysaccharide in relation to source which strengthened the validity of the groups.

The evidence from metabolism consisted in brief of differences which were constantly present between the different groups, although the separation was not as complete as it was on the basis of chemical structure. Anaerobic glycolysis appeared to be constant in all the vibrios, but there existed regular differences in respiration and aerobic glycolysis. It was interesting to find that the group IV carrier strains did not show any aerobic glycolysis.

Altogether, something over two hundred strains were studied by metabolism methods, and it was found that 98% of the case strains and 100% of the contact strains fell into the same group and chemical analysis showed that these were groups I and II of the chemical classification, or, if the strain had been for some time in the laboratory, group VI. Strains with another level of metabolism were carrier strains, and with a third were water vibrios. It appeared again that we had differences between vibrios consistent with the groups which appeared on the basis of the chemical analyses.

The subject of variation in the vibrios and its chemical basis is a large one, and occupied us for several years. In general, the method was to take a strain of known chemical composition, metabolism, colony form, biochemical reactions, serological reactions, etc., and either study its spontaneous dissociants, or force dissociation by any of the well-known methods and then study these dissociants in the same way. As an example of spontaneous dissociation, I may give the following: In April 1935, chemical analysis of strain 1200, which had been isolated in the previous December, showed that it belonged to group VI, *i.e.*,

its polysaccharide was of the glucose-containing type. In May 1936, thirteen months later, after undergoing routine sub-cultures, a second analysis was made and indicated the presence of a large amount of galactose accompanied by some glucose in the hydrolysate of the polysaccharide. A third analysis in January 1937, twenty-five months after isolation, showed only galactose in the hydrolysate. At the same time, the strain had shifted its serological reaction and now agglutinated only with an antiserum to another galactose-containing organism, whereas on first isolation it had reacted only with antiserum to a glucose-containing organism. In other words, the shift in its chemical structure had been reflected in its serological reactions.

Very many experiments of this type, and also with single-cell cultures and forced or spontaneous dissociation showed us that variation had a chemical basis in the vibrios. In the case of single-cell cultures, we began with a culture descended from a single cell and having a certain set of characteristics: biochemical, cultural, serological; and a certain chemical structure. At the end of the experiment we had produced from this culture a new strain having another set of biochemical, cultural and serological characteristics, and a different chemical structure. It followed that the new strain fell into a different chemical group than the old, and I wish to emphasize that all the characteristics of the new strain were similar to those of other strains in the chemical group into which it now fell. In other words, the changes in chemical composition, biochemical reactions, cultural and serological properties are correlated.

It is of interest to note further that while the vibrios vary in this correlated way, they always remain within the framework of the six chemical groups, that is, within the framework of the two proteins and the three polysaccharides. Within these limits the powers of synthesis and variation are considerable, but the organisms appear incapable of giving rise to any other chemical constituents. The conception is that of a strictly limited capacity for transformation.

To return, in closing, to the present situation in the vibrio group in contrast to that which I outlined at the beginning of this paper, we have been able to divide the vibrios into a small number of groups, which correlate with their epidemiology and metabolic activity. This result is in contrast to the large number of heterogeneous groups which have always resulted from purely serological attempts at classification, a number which varied directly with the number of strains which were being studied. For recent examples of this statement, the papers of Taylor, Pundit and Read, and of Mertens and Mochtar may be consulted. The former found that 33 "O" antisera were insufficient to classify 558 vibrio strains, while the latter could not classify 32 strains using 17 antisera. Finally, some order has been brought into the subject of vibrio variation, the limits of this variation appear to have been established, and transformation of vibrio strains has been shown to occur in the laboratory.

Any account of our research would be incomplete without mention of those whose unceasing efforts over a period of years enabled the facts to be elucidated, and I accordingly take pleasure in adding to this paper the names of Dr. D. L. Shrivastava, Dr. B. N. Mitra, Dr. S. C. Seal, Jemadar Harwant Singh, and Messrs. S. P. Mookerji and D. N. Mullick.

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