

in this country but it is understood that definite evidence of a considerable degree of protection is presented. The possession of an inoculation certificate does of course ensure complete freedom from risk.

## Bacteriological and Immunological Aspects of Cholera.

[Summary]

By P. BRUCE WHITE, B.Sc., F.R.S.

IN the nineteen-thirties an international reinvestigation of *Vibrio cholerae*, its serology and relation to cholera, was undertaken, which culminated in the extensive field trials and investigations which Sir John Taylor organized in India.

At this time two items of information were waiting general application to laboratory practice. On the one hand, first Balteanu, then Shousha, Abdoosh and Gohar in succession had successfully applied to the vibrio the receptor analysis methods of Weil and Felix and on the other Kabeshima and a series of later Japanese observers had pointed out that the classic vibrio occurred in the closely related but serologically different forms which they termed the Original, Intermediate, and Variant types.

As participants in the International Investigation of 1934-35 Dr. A. D. Gardner and I confirmed in detail the findings of all these workers on an extended series of vibrio strains of varied provenance, added certain novelties of our own and were able to present the whole in one picture and to offer some suggestions as to the best methods of utilizing the newer knowledge in practice.

The essential points established were the relative specificity of the O agglutination reaction and the existence of Inaba and Ogawa subtypes of cholera and El Tor vibrios. The reason for many past discrepancies in the identification of cultures was that those who had read their agglutination tests early had recorded the specific reactions of the O antigen; those who had read them late registered the wide range of H non-specificity.

Gardner with his Indian colleague, Venkatraman, armed with the new knowledge, began what Greig had formerly attempted, a serological classification of the vibrio world. They succeeded in classifying the majority of the available vibrio strains from cholera patients, healthy subjects and water sources into six O agglutination groups of which *V. cholerae* and *V. El Tor* with their subtypes formed Group I.

At a meeting arranged by the Ministry of Health at which Sir John Taylor was present Dr. Gardner and I took occasion to urge that a pure O anti-cholera serum, or preferably the separate O antisera of the Inaba and Ogawa types, formed the proper reagent or reagents for the recognition of *V. cholerae*. The result was that at Sir John's request I undertook to prepare, not pure O antisera, but pure O antigens, which might be supplied to India and all others interested for the local preparation of valid diagnostic antisera. Dr. Gardner undertook to check the properties of my vaccines—which we rather flamboyantly called “standard O antigens”—to issue them as required from the Standards Laboratory, Oxford, and to collect and analyse any reports that might come in. From that time the O-Inaba and O-Ogawa antigens have been dispensed as requested from the original bulk preparations amounting in each case to about an ounce of dried, alcohol-extracted, steam-treated, ether-washed powder of which the bulk still remains in hand.

It is sufficient to say that, whatever other problems remained unsolved, the extensive trials which Sir John Taylor organized in India completely vindicated the hope that the sera raised against these and like antigens would give to the search for *V. cholerae* a precision hitherto lacking. The results have been set out in detail in the *Indian Journal of Medical Research*, 35, 3, July 1947.

At their first differentiation there was some suspicion that the Original or Inaba type of *V. cholerae* might be the organism of epidemic cholera, the Variant or Ogawa type that associated with the sporadic case. This was not supported by later Japanese observations; nevertheless there clung the suggestion that the Ogawa type might be a *mitis* form responsible for outbreaks of relatively mild disease. There does not seem to be any evidence either from the Japanese or later Indian investigations that this is really the case. The doctrine grew up that the type concerned in any epidemic was fixed though there might be mixed epidemics due to collateral spread of more than one type of the vibrio. For instance in the great Shanghai epidemic of 1932, 12 of 15 strains isolated in the early phase and 48 of 53 strains isolated in the later phase were of the Hikojima or middle type, the remainder being Ogawa. It was argued that the epidemic had kept its balance and Kuroya and Ono wrote of the “inalterable character of the serological types of vibrios”. In India, where Sir John arranged for the distribution of type monospecific antisera, it seemed that the Inaba

and Ogawa types each had their more or less distinct zones of influence though each made occasional epidemic incursions into the territory of the other; in some outbreaks the singleness of the epidemic type seemed absolute, in others a few cases due to the sister type were encountered; some outbreaks were frankly mixed.

In his first description of the Original and Variant subtypes Kabeshima had stated that they were transmutable and he gave an instance in which the Variant type had yielded the Original type during transfer in media containing its homologous antiserum. Later workers, probably believing that in 1917 Kabeshima could not possibly have known enough about vibrio serology to understand his observations, have brushed this statement aside and have assumed the distinctness and fixity of the subtypes. I myself made several attempts to check his view without success, obtaining only, from the type cultures exposed to their antisera, the salt-agglutinable "rough" variants in which the O specificity of the organism is lost. But during a recent visit to India, Dr. D. L. Shrivastava, Biochemist to the I.R.F.A. Cholera Inquiry, and I applied to the type strains, not their full sera, but their monospecific antisera from which the common antibody had been removed, so focusing the serous influence on the type-specific antigen. Without difficulty we converted every one of 13 Ogawa type cholera and El Tor vibrios to the Inaba persuasion; and though we were unable so to alter Inaba type strains to the Ogawa type we found it possible to induce 4 of 8 strains of the Intermediate type, stemming from an outbreak in Calcutta, to make this change. Quite clearly the types are not separate species or subspecies but phases of a single species—something like the specific and non-specific phases of the Salmonella. Incidentally the instance is interesting from the point of view of basic serology in that, while such changes and the modifying influence of serum are well known in the case of the presumably protein flagella antigens, this would seem to be the first in which they have been observed to affect the polysaccharide-lipoid O complex.

I rather suspected that when platings from cholera cases were carefully examined we should find that in quite a number more than one type would be found as a result of spontaneous variation during pathogenicity. In the careful examination of about 100 cases of the spring epidemic at Calcutta, shared equally between the Ogawa and Inaba types we were, however, unable to discover a single instance of "mixed" infection. What bearing the now known transmutability of the types has on the importance to be attached to their differentiation in the field and what bearing it has on the proper composition of cholera vaccine it is difficult to say.

To pass on: following up Gardner and Venkatraman's classification by O groups and Heiberg's classification based on fermentation and non-fermentation of mannose, arabinose, and saccharose, and once more applying the cholera red and V.-P. tests of the old masters, Sir John Taylor and his co-workers were able:

(a) To show that a vibrio which ferments mannose and saccharose but not arabinose and is at the same time cholera red positive and V.-P. negative and non-hæmolytic is in all probability a true cholera vibrio of O group 1.

(b) To classify 311 of 558 vibrio strains inagglutinable with O group 1 serum and drawn from cholera cases, healthy people and water sources into 33 O groups, so making it possible to argue the role of these in the causation of cholera by consideration of the origins of their several component strains. The conclusion reached by Sir John Taylor in assessing the collected results of this and other series of examinations was that, with one possible exception, no case for the choleric activity of any of these could be made out.

In comment on this conclusion, to which I cannot but in general concur, it must be admitted that in India many experienced cholera workers are by no means so convinced, and for one reason or another cling to the view that vibrios other than *V. cholerae* may from time to time contribute to cholera.

Dr. Melville Mackenzie described the measures taken to deal with the Egyptian outbreak on the international level. The Interim Commission of the World Health Organization offered help to the Egyptian Government and on their request undertook to purchase cholera vaccine for them. This method of purchase had been satisfactory but in the absence of any standards the Egyptian authorities had been asked to test the antigenic efficacy of the vaccines received. The Commission's Expert Committee on Quarantine held an emergency meeting at which the Egyptian Under-Secretary of State for Quarantine gave an account of the epidemic. The Committee were impressed by the extent and thoroughness of the steps taken by the Egyptian authorities. It appeared that some countries had imposed restrictions on travellers from Egypt which greatly exceeded the provisions of the International Sanitary Code. These measures were inspired by fears which largely arose from insufficient knowledge of the true epidemic situation; in order to allay them infected countries should provide prompt information to the WHO for transmission to national

health authorities. The Committee gave advice on precautions against the spread of cholera in aircraft by infected water, passengers or flies.

In England and Wales all M.O.s.H. were informed of the occurrence of the outbreak and of its course. Advice was given about dealing with ships and aircraft arriving from an infected port. Travellers arriving by air were not kept under surveillance until the occurrence of suspected cases in Italy and Australia when it was applied to those who had, in the five days before arrival, slept one or more nights in Egypt. Cholera inoculation prior to arrival was not insisted upon. Other countries had done so and in some countries inoculated persons had been exempted from surveillance but this seemed to place excessive confidence in the protective value of the vaccine. The Ministry of Transport had warned ships calling at Egyptian ports that as far as possible they should not allow those on board to land or take on water, fresh fruit or vegetables. The import of baled cotton, sacked rice, rags and dates had not been prohibited.

Arrangements had been made for Sir John Taylor and Mr. Bruce White to visit Egypt on behalf of the Ministry of Health and the Medical Research Council in order to study the results of the use of sulphur drugs and the value of DDT spraying and to carry out researches on the cholera vibrio. There was need for scientific investigation of the length of the cholera vibrio's viability (1) on flies particularly when carried by aircraft, (2) in sewage both crude and during treatment, and (3) on fresh fruit and other commodities. The Expert Committee on Quarantine was interested in these questions and in the standardization of cholera vaccine and the Commission had been offered some 6,000,000 French francs by the Office International d'Hygiène Publique for epidemiological research with cholera as a first objective. Medical supplies to the value of £5,000 were being flown to Egypt within the next few days.

Sir Leonard Rogers showed maps illustrating the spread of cholera epidemics from India to Europe during the nineteenth century. With regard to the present outlook the speaker said that cholera incidence might be expected to decline in Egypt during the latter part of November and to die down in December and January, but there was a serious danger of its breaking out again in that country in 1948 in the spring or early summer with rising temperature and absolute humidity, but the geographical conditions were favourable to its control by modern methods and to the prevention of its spread over South Europe such as occurred in 1883-84. There was, he thought, grave danger of the present epidemic in the troubled and chaotic conditions of the Punjab being carried by the invading North-West Frontier tribes to Afghanistan, and recurring there in the spring of 1948 with spread by the usual overland route to Southern Russia, whose very lengthy land frontier would be most difficult to protect against invasion by the disease in spite of medical advances in Soviet Russia. Overcrowding in Russian towns would be favourable to the spread of cholera if it gained a firm foothold in that vast country. That line of spread might prove to be more dangerous than from Egypt, but it was to be hoped that modern medical science would prove equal to the occasion.

Lieut.-General Sir Bennett Hance observed that in checking the epidemic in Egypt the principles of attack were the same as those holding in India—namely attention to environmental hygiene and inoculation.

In rural India at any rate attention to environmental hygiene could be summed up in the disinfection of water supplies since, outside large towns or organized cholera camps, nothing like efficient conservancy or protection of food supplies was possible. Where piped water supplies or wells existed disinfection by chlorination was comparatively simple, but where, as in parts of India, the water supply was the village "tank" or, as in Egypt, a sweet water canal, such measures became difficult or impossible. It followed, therefore, that all the more reliance would have to be placed upon inoculation, and it was satisfactory to know that, at last, a controlled and statistically authoritative evaluation of the efficacy of inoculation was available (*see Ind. J. med. Res.*, 35, 3, July 1947, by R. Adishesan, C. G. Pandit and K. V. Venkatraman). This would appear to establish the efficacy of the measure upon which doubts had been thrown by the various authorities. Such experience as the speaker had had in handling outbreaks of cholera had certainly tended to confirm the efficacy of the measure and, in consequence, he personally had never doubted it; but the confirmatory evidence of the survey referred to was most satisfactory. A combination of environmental hygiene and inoculation had removed most of the dangers of the great Pilgrimages in India and, in areas where the water supply was "tanks", the speaker personally knew of only one method of holding the spread of cholera and that was "at the point of the needle".