SOME PRESENT-DAY PROBLEMS OF TUBERCULOSIS RESEARCH

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I. FILTERABILITY OF TUBERCULOUS VIRUS

The discovery of the tubercle bacillus by Koch seemed at first to solve the riddle of tuberculosis. It was, however, realized later that by this discovery, great as the advance was, not all the problems of tuberculous infection in human beings had been solved. Now even the question of the tuberculous organism does not seem to have been settled, as since 1922 the phenomenon of the filterable virus, first observed in 1910 by Fontès in Rio de Janeiro in the Institute of Oswaldo Cruz, has been investigated with great industry by such French workers as Calmette, Nègre, Boquet, and Saenz, by Vaudremer and Hauduroy, and also by Arloing, Dufourt, and others. These authors obtained ultra-filtrates from special cultures, or from the organs of infected guinea-pigs, after passage through Chamberland L₂ candles or Berkefeld N filters, which produced, when injected into guinea-pigs, localized lesions or enlargement of the lymphatic glands, but no generalized tuberculosis. These glands sometimes contained a very few tubercle bacilli with or without characteristic histological alterations, while according to Nègre and Valtis calcification could be produced by filtrates when acetone extracts of tubercle bacilli were added to the filtrates, even when these were from the ineffective B.C.G.

These facts have been confirmed by many publications in other countries, while in this country the results obtained were mostly negative. Some advances in the investigation have been made by using the method of Ninni for the intraglandular injection of the neck, excluding as it does contamination and spontaneous infection, and facilitating observation. The method of Alessandri and Santanelli of using dialysis bags in the peritoneum for filtration has not been greatly developed.

TUBERCULOUS "GRANULES"

It is a well-known fact that some strains of tubercle bacilli, especially when under special conditions, differ from others in having a granulated bacillus, and seem to consist of a chain of small granules (Spengler's splitters, or Much's granules), which were noticed first by Babes (1885) and by Koch himself, who considered them to be spores. These granules were found to be partly acid-fast (as are the ordinary bacilli), partly Gram-positive (Much, 1908), or stainable only by Giemsa (Pagel, 1934). It may be assumed that these granules could pass filter membranes, as described by the French authors, especially as by other methods it has been shown that the life-cycle of the tubercle bacillus can include stages in which the virus has the nature of small granules or very small bacilli (Lominski). This investigation has been confirmed by single-cell cultures in micro-droplets by Morton Kahn, in which he observed various types of organisms: diplococcoid units of 0.1 to 0.38 μ , breaking down to dust-like particles which develop later into very small bacilli of a size of 0.3 to 1.2 μ and, later still, to mature tubercle bacilli. Mellon has shown that of sixteen strains used only two were found to be filterable.

We have used for our own purposes a strain, "bovine Vallée,"* alleged by the workers of the Institut Pasteur to possess a filterable virus. Employing the method of Ninni to inject the glands of the neck, we compared filterability through Chamberland L₂ filters with pores of a size of 0.5 μ with filterability through collodion membrane filters of a similar size, and with pores of a known maximum size, under comparable conditions of pressure and time. In this way we obtained some positive results with floating cultures of the strain, and a sufficient number of positive results from organs of infected animals after filtration through membrane filters. Altogether we secured seven positive results after filtration through membrane filter 0.75 μ , three positive results after passage through L₂ candles, and two positive results with membrane filter 0.3 μ . We have sometimes produced tuberculous lesions not only in the lymphatic gland of the neck, where the material was injected, but also some large tuberculous nodules in the spleen. In other cases only enlargement of the injected gland, with or without very rare acid-fast tubercle bacilli, has been observed. When tubercle bacilli were not found in the gland by means of smears or sections, we could sometimes demonstrate their presence by a culture on Loewenstein medium or by injection into another guinea-pig, producing a generalized tuberculosis.

Our findings confirm to some extent those of the French authors, and indicate that the filterability of the tuberculous virus, which is not always obtainable in a strain known to possess a virus, would seem to depend largely upon the size of the pores of the filter employed. It can therefore be assumed that the tubercle virus is not filterable in all stages of its life: this could be assumed from the findings of Lominski and Morton Kahn, as at one time only the large tubercle bacillus can be found. In addition, the smallest particles seen in the micro-droplet culture of Morton Kahn might not be able to infect guinea-pigs, even with the mild localized infection that was sometimes produced. This fact is, of course, important from the point of view of the immunity experiments which have been performed by Arloing and his collaborators, and by Nègre, Boquet, and Valtis. Careful experiments on the subject of immunity must be carried out in the future with various filtrates, but the method of filtering through filter membranes with pores of a standardized size is for this purpose quite indispensable.

Other questions, such as the subject of heredity of tuberculosis, arise from the investigation of the filterable virus. It has been shown by Calmette, together with Valtis, Boquet, and Nègre, that the injection of filtrates into pregnant female guinea-pigs infected the foetus through the placenta, and gave rise to enlarged glands and a state of "decomposition," but to no real tuberculous lesions, although typical Koch bacilli were sometimes found in the glands. These findings, confirmed in animal experiments by Arloing and Dufourt, von Beneden and Rabinowitsch, have been confirmed in the human pathology by Valtis and Lacomme and by Monckeberg and Vergara. Other clinical investigations suggest that diseases such as rheumatic purpura (Paisseau, Boegner, and Vaillard) or bronchial asthma (Sergent, Durand, Kourilsky, Benda) might be due to an infection with the filterable virus. It would, of course, be of great importance if the work of Doyle, Orr, and Purchase, who obtained a filterable virus from the milk and blood of tuberculous cows, were to be confirmed. It must be pointed out that Ninni has obtained filterable viruses from bovine, human, avian, and other types of tubercle bacillus.

* We are much indebted to the late Professor Calmette and Drs. Boquet and Saenz of the Tuberculosis Research Department of the Institut Pasteur in Paris for supplying us with these cultures.

II. ALLERGY AND TUBERCULOSIS

The investigations of Friedberger and Mita suggest that rise in temperature and other symptoms in infectious diseases are due to anaphylactic or allergic reactions in the body. In tuberculosis the allergy towards tuberculin is a well-known fact from the clinical investigations of von Pirquet. The hypersensitivity can be shown in general by rise in temperature and by reactions, both locally at the site of the injection and in the infective focus. Recently the question has arisen whether there is any relation between other allergic conditions, such as bronchial asthma, and tuberculosis (E. Fraenkel, Hamann, and Küpper). It was a general belief that tuberculosis and bronchial asthma exclude each other, because in statistical records of tuberculosis cases bronchial asthma was seldom mentioned. Thus Schroeder found in 4,700 cases of tuberculosis only 30 cases of bronchial asthma. Some clinicians considered that infection with tuberculosis afforded protection against bronchial asthma. From this clinical observation it has been shown by Seligman, Pagel, and Friedberger that tuberculosis in guinea-pigs afforded protection, to some extent, from anaphylactic shock in animals sensitized with horse serum.

B.C.G. AND ALLERGY

We have carried out some experiments in that direction with Calmette's B.C.G., because we thought that this method could perhaps be applied as a prophylactic or therapeutic measure in allergic cases. In twenty guinea-pigs injected with frequent large doses of B.C.G. over a long period, both before and after sensitization, we observed two instances of shocks with death, two of recovery, and two of mild shocks. In twelve controls we observed ten typical shocks with death and two typical shocks with recovery. It could, however, be demonstrated that a single injection or treatment with killed B.C.G. did not afford any protection, which was only obtained when the injection produced local foci. The protection was not afforded when large doses of desensitizing serum were employed. These findings correspond with those of Pagel and Garcia-Frias that in tuberculous guinea-pigs the protection from anaphylactic shock is due not to a specific anti-allergy, and not only to the spread of the foci, but to the whole condition of the infection in the body ("Durchseuchung"). Thus we cannot hope to get an effective and specific protection with B.C.G. in allergic cases. We had thought that this treatment might be used in place of the tuberculin treatment recommended by Storm van Leeuwen as a non-specific treatment for asthma.

On the other hand, we were able to show that in cases of bronchial asthma tuberculosis was not as rare as had been assumed before. As reported in an earlier communication, among 369 cases of bronchial asthma, all examined for tuberculosis by means of x rays and sputum smears, we found sixty-two cases with signs of past or present tuberculosis, sixteen of them with active tuberculosis, four with tubercle bacilli in the sputum, and four others with cavities in the lungs. In twenty-six cases inactive tuberculosis was present, and in twenty cases healed tuberculous foci. In a recent paper Küpper reports on fourteen cases of tuberculosis with asthma.

In all these instances the asthma was observed before the tuberculosis, and he assumes that the allergic condition of the body created a reaction towards tuberculosis. We must remember the fact that from a clinical point of view tuberculosis of the lungs has been classified by Ranke from the point of allergy or conditions of immunity. We must further bear in mind that the

anergic condition of the body after measles creates in addition a predisposition to the onset of tuberculosis. It is of course possible that the mechanism of allergy, which has a tendency to spread out from one allergen to another, may lead from the first specific allergy towards a specific allergen, such as pollen, to a hypersensitivity towards the tubercle bacillus either under the symptoms of real asthma, as observed by Fraenkel, Hamann, and Küpper, or under the aspect of an exudative and active tuberculosis.

It has, however, been observed that the onset of asthma followed a so-called influenzal infection, which when investigated with x rays proved to be tuberculous. It is possible that the allergy towards tubercle bacilli in these cases was the first feature of allergy, and other hypersensitivities developed from this. It is, however, possible that the infection was the predisposing cause for the hypersensitivity towards the allergens.

In most of the cases of asthma and tuberculosis healed tuberculous lesions and inactive tuberculosis have been discovered. It may thus be seen that according to the animal experiments a species of antagonistic mechanism would seem to exist between the two conditions.

Pagel has pointed out that allergy in tuberculosis is an important factor according to the histological evidence, especially in the case of exacerbation of calcified lesions and in the breaking down (liquefaction) of the tissue prior to the formation of cavities. The experiments of Römer in producing cavities by the superinfection of allergic animals confirm that impression. In addition, the allergy of the whole animal towards tuberculin and the onset, spread, and form of tuberculosis must be kept in mind. These factors might be altered not only, as Pagel assumes, by the influence of the endocrine glands, but also by alterations provoked by other allergens.

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The Central Association for Mental Welfare has published the report of the conference held in the British Medical Association House last November, to which reference was made at the time in these columns. The various papers read are reproduced. These include: boarding out patients from mental hospitals, by Dr. W. Brooks Keith; a historical survey of the development of mental welfare work, by Miss Evelyn Fox, the honorary secretary of the Central Association; the modern institution, by Dr. Noel H. M. Burke; community care, by Miss Sybil G. Nugent; research, by Dr. L. S. Penrose; the voluntary provisions of the Mental Treatment Act, by Dr. C. Davies Jones; the functions of an educational psychologist, by Miss Lucy G. Fildes; and the work of an educational psychologist in a Scottish county. A verbatim record of the discussion following each paper is appended. The report is published at 3s. 6d., and may be obtained from the honorary secretary of the Central Association for Mental Welfare, 24, Buckingham Palace Road, S.W.1.