REVIEWS

Experimental Tuberculosis and Leprosy*

In October 1954 an international group of chemists, pharmacologists, pathologists, bacteriologists, immunologists and tissue-culture experts engaged in tuberculosis research met in conference in London. They were the guests of the Ciba Foundation, who had organised a symposium on experimental tuberculosis and leprosy.

The meetings lasted two and a half days, two days being devoted to tuberculosis and the last half day to papers on leprosy. Twenty-two papers were read on the former disease and four on the latter, each paper being followed by discussion. These papers and discussions have now been published in a volume of about 400 pages, and should be of immense value to all experimental workers in both diseases.

(1) Of the four papers on leprosy the first is by M. B. Lurie on the *Pathogenic Relationship between Tuberculosis and Leprosy.*

Tuberculosis has a " symbiotic phase " when there is an early diffuse accumulation of monocytes with coarsely vacuolated cytoplasm harbouring many bacilli in symbiosis with uninjured host cells. This is followed by a " nodular phase " in which mature epitheloid cells with very finely vacuolated cytoplasm are collected in tubercles and at the same time there is destruction of many intracellular mycobacteria. This latter phase is characterised by cell death and caseation and tuberculosis sensitivity. Lepromatous leprosy corresponds to the former of these phases and tuberculoid leprosy to the latter. Thus the pattern of tissue response to different mycobacteria in different species, including those of human leprosy, appears as variants of a common theme. This theme varies with the predominating growth or destruction of the bacteria and the meagre or marked development of allergic sensitivity.

(2) J. Lowe in his paper on *The Leprosy Bacillus and the Host Reaction to it,* mentions a " curious dichotomy in the manifestations of leprosy infection." In the two main types there is sensitization and immunity on the one hand and complete lack of these on the other; while in tuberculosis there is an " interplay

* Experimental Tuberculosis, Bacillus and Host, with an Addendum on Leprosy. Edited for the Ciba Foundation by G. E. W. Wolstenholm and M. P. Cameron. J. & A. Churchill Ltd., 42s. shillings net.

of findings, some indicating the invasive powers of the infection and some indicating sensitization and resistance of the host tissues." In the lepromatous case with abundant bacilli but no cellular reaction to them, circulating antibodies are easily demonstrated, but no sensitization, no cellular antibodies revealed by the lepromin test, and no resistance to infection. While in the tuberculoid case, with limited lesions and very few bacilli, but intense cellular reaction to these few bacilli, circulating antibodies are difficult to demonstrate, and there is apparently a high degree of sensitization and immunity to the infection.

Protein desensitization may be effected without impairing cellular response to the whole bacillus, as shown by the delayed Mitsuda reaction, so that the response to the whole bacillus and not to any fraction appears to be the main factor in immunity.

(3) The paper by R. G. Cochrane is a short review of the *Reaction of the Host Tissue to Myco. leprae.* He describes the tissue reaction in the various types of leprosy, discussing particularly two views regarding the nature of the lepromin reaction: (a) that the allergic response determines the type of leprosy, (b) that the type of leprosy determines the allergic response. A permanent cure depends on the ability of macrophage cells to develop an environment which prevents re-multiplication after the number of bacilli has been sufficiently reduced by sulphone therapy.

(4) J. H. Hanks writes on *Immunological and Physiological Basis of Immunization in Tuberculosis and Leprosy.* Diseases like tuberculosis and leprosy are chiefly confined to those in whom immunological response is slow or poor. Therefore attempts at immunization should be confined to identifying and immunizing the poor responders. The difficulty of the tuberculoid patient is not that he cannot make an immune response, but that he achieves this response rather slowly. Had he been previously immunized he might not have had leprosy at all. The question of a mixed vaccine of B.C.G. and dead *Myco. leprae* is mentioned. It is suggested hypothetically that when B.C.G. is injected in a more resistant patient the bacilli may be more promptly destroyed; while in the more suspectible persons (requiring more antigen) the bacilli may not be destroyed so quickly and may thus produce more antigen.

Among the papers and discussions on tuberculosis there were many points raised which may suggest lines of investigation in leprosy: some of the more important of these are abstracted below.

(5) M. Stacey, writing from the chemist's point of view on *The Proteins of the Tubercle Bacillus*, begins with the observation: " The tubercle bacillus is an amazing chemical factory producing in the living cell and in culture fluids a wide variety of chemical substances. Other members of the acid-fast mycobacteria are no less prolific or complex." He then goes on to describe a new method for obtaining a biologically active protein, termed U.C., from live tubercle bacilli by urea extraction in a manner whereby extensive degradation is avoided. He expresses a hope of obtaining even better protein products of great selective diagnostic value.

(6) J. Asselineau and E. Lederer write on *The Structure and Activity of Mycolic Acids.* The number of these varies in different mycobacteria from 2 to 4. They have high molecular weight and contain about 88 carbon atoms. Most of them are lipopolysaccharides (wax D). Fractions of these vary in different strains of mycobacteria. Mycolic acid is the only definitely acid-fast compound isolated from mycobacteria, but this rôle is believed to be an " indirect one, by participating in the formation of an impermiable film round the cell." It is suggested that fuchsin is probably linked as a complex salt to the carbonyl and hydroxyl groups of mycolic acids. Virulent and avirulent strains of tubercle bacilli can be differentiated by shaking up with a buffered solution of neutral red, virulent bacilli acquiring a red colour, and avirulent or saprophytic bacilli only a yellow colour. Injection of mycolic acids has been found to cause persistent necrotic lesions with formation of giant cells.

(7) F. L. Rose and G. A. Snow write on *Mycobactin: a Growth Factor for Acid-fast Bacilli.* This substance is prepared from *Myco. phlei* with the object of providing a growth factor for mycobacteria on the lines of the early work of Twort and Ingram in growing *Myco. johnei.*

(8) M. Stacey in his paper on *Polysaccharide Components of the Tubercle Bacillus* describes two polysaccharides present in most bacteria, the capsular and the somatic. The capsular, which often diffuses into the culture medium, varies chemically and serologically with the type of bacterium, while the somatic is more a common component to all. In *Myco. tuberculosis* the polysaccharide components are unusually complex, eight different structures being known. Three polysaccharides present in the culture filtrate are described. The importance of polysaccharides associated with lipids is being more recognised in regard to eliciting of skin and

cellular reactions and in immunity generally. Attention is drawn to the fact that the " lipid-bound " polysaccharide of mycobacteria bears a superficial resemblance to that of streptomycin, which latter could be imagined as a possible blocking group inhibiting the biosynthesis of certain of the tuberculosis polysaccharides. There are new prospects opened up in attempting to decide how streptomycin inhibits *Myco. tuberculosis* growth, and why resistance can be so easily gained to streptomycin.

(9) A paper by J. Ungar on *Granuloma-producing Properties of Synthetic Fatty Acids* was followed by an interesting discussion on various substances that produce caseation and granuloma, the remarkable fact being that something so small as a single tubercle bacillus can cause a tubercle, whereas comparatively colossal amounts of fatty acids are necessary to produce the same effects.

(10) A. A. Miles writes on the *Early Tissue Reactions to Tubercle Bacilli and their Products.* He shows that tubercle-inoculated animals with very early tissue responses show high survival value in rapidly growing pathogens, such as staphylococci and this is so to a lesser degree in defence against mycobacteria. His method is to follow bacterial inoculation with injection of adrenalin or Liquoid, which have the effect of temporarily inhibiting defence reactions. The sizes of the lesion diameters are then compared with controls without an inhibiting agent. He shows that decisive responses may be established very early before visible histological changes take place.

(11) J. G. Hirsh discusses the *Biochemical Factors which may influence the Fate of Tubercle Bacilli.* Various substances may be contained in certain organs of the body in some animals as compared with others and determine the growth of bacilli in these organs. Retention of carbon-dioxide and accumulation of organic acids may exert an effect on acid-fast bacteria. Spermine and basic peptides are among the substances mentioned as unfavourable to tubercle bacilli.

(12) H. Bloch, in *Bacterial Components concerned in the Early Phase of Infection,* discusses the " cord factor " which is partially responsible for the virulence of tubercle bacilli, and is present in various amounts in the bacilli. The physico-chemical properties are mentioned. Injection of this substance within 72 hours prior to infection turns a mild into a severe form of the disease, and when mice with chronic stationary tuberculosis are injected it makes the infection flare up and become rapidly progressive.

(13) S. V. Boyden and E. Sorkin write on the *Serological Activity of various Fractions of Culture Filtrates of the Tubercle Bacillus.* After chemical fractionation of the filtrates, the method of Middlebrook-Dubos was used for haemagglutination, and tests were made with tanned red cell haemagglutination and haemagglutination by mixtures of antisera and antigen. Precipitation in agar was practiced by Ouchterlony's method. The results obtained stress the great complexity of these culture filtrates, but no attempt has been made to attribute any of the serological activities of the various fractions described to any one single substance, nor is it known whether the serological differences between the various antigenic constituents of culture filtrates are of any special significance in relation to tuberculous disease or type specificity.

(14) C. N. Iland and D. B. Peacock read a paper on the *Serology of Tubercle Polysaccharides.* The results of their own experiments, and those of Dr. Pound, " suggest that the haemagglutination test is either an enhancement of a non-specific reaction or it is a complex phenomenon. In either case it does not provide evidence of the presence of anti-polysaccharide antibody, or of any antibody-antigen reaction until further proof is available."

(15) S. Raffel, J. Asselineau and E. Lederer write on *The Chemical Nature of the Lipoidal Factor of the Tubercle Bacillus responsible for the Induction of Tuberculous Hypersensitivity.* They conclude that it is likely that the peculiar ability of these lipids to take part in the induction of tuberculin sensitivity when injected into guinea pigs along with tuberculoprotein resides in the esterified mycolic acids. Wax D, the most active of the fractions, is a mycolic acid-polysaccharide ester.

(16) A paper by R. L. Mayer on *Tubercle Bacilli as Immunological Adjuvants* leads to the conclusion that various adjuvants, including tubercle bacilli, may *increase* the number and degree of sensitizations of the delayed type, but that they *do not modify* the type of sensitization towards a particular antigen or following a particular mode of sensitizing procedure. An interesting discussion followed this paper on possible reasons for the differences in allergies of the delayed and immediate types.

(17) E. Suter read a paper on the *Relation between Growth Inhibitory Property of Monocytes for Tubercle Bacilli and Hypersensitivity to Tuberculin: An in Vitro Study.* He shows that, although growth inhibition by monocytes and delayed hypersensitivity to tuberculin appear at approximately the same time

after vaccination, the indication is that the growth inhibitory property resulting from vaccination is independent of demonstrable hypersensitivity, and that hypersensitivity can have a depressive effect on growth inhibition by monocytes. In an *in vitro* system there is a delicate balance between phagocytes and tubercle bacilli. Hypersensitivity, as well as large numbers of bacilli within the cells, seems to favour multiplication of tubercle bacilli, whereas small numbers of bacilli and hypersensitivity allows full effect of the inhibitory power of the cells. Unfortunately, there is as yet no way to control consistently all these variables *in vitro*. The tubercle bacillus seems to exert a toxic action only from within the cell and not from without, as cells outside bacilli in infected tissue cultures were not affected.

(18) G. Brownlee and D. G. Madigan write on *Tuberculous Hypersensitivity and Desensitization.* Three groups of subjects were chosen: (a) 4 Mantoux-negative nurses, (b) 4 patients who had been desensitized with graduated daily intramuscular injections of OT and bacillary emulsion, (c) Mantoux positive tuberculous patients. In all these groups 0.1 ml. BCG vaccine was injected and they were challenged with 1 in 10 Old Tuberculin 51 days later. The results are shown in photographs. The BCG nodule was similar and moderate in the first two groups, but there was severe ulceration in the third group. The results are shown clearly in photographs. The authors believe that complete and effective desensitization to skin-challenge has been achieved.

(19) E. M. Brieger's paper on *Tubercle Bacilli in Infective Tissues grown on Tissue Culture* raises the problem of the discrepancy between the small number of bacilli found in early lesions and the extent and severity of the lesions. Why should there be an apparent suppression of growth *in vivo,* while in tissue cultures there is immediate intra-cellular growth? The question is raised whether more bacilli are present in the tissues during the early period after infection than can be shown by ordinary staining methods. Can the bacillus take a form other than an acid-fast rod? Evidence is given of a granular form similar in size to Much's granules, and that non-bacillary structures isolated in the parasitic stage from infected tissues require the living cell for the restitution to the bacillary form.

(20) M. Bloch writes on *The Rôle of Bacterial Multiplication in the Establishment of Immunity to Tuberculosis.* He found that when living preparations of different strengths of BCG were com-

pared, the resulting immunity they produced was proportional to the number of living bacteria contained in the vaccine. The experiments mentioned show that multiplication of the bacilli in the host is not essential for a vaccine to be effective, non-multiplying H37Ra being as effective as multiplying BCG. Immunity in animals was noticeable 3 to 6 days after BCG vaccination, and reached its peak in 3 to 12 weeks, almost completely disappearing 9 months after vaccination.

(21) M. B. Lurie and P. Zapparodi write on *The Mode of Action of Cortisone on the Pathogenesis of Tuberculosis and its Implications for the Nature of Genetic Resistance to the Disease.* When virulent human type bacilli are inhaled by hormone-treated rabbits, three or four times as many primary tubercles are produced as in controls of the same litter without hormone. But the tubercles are smaller and swarm with more bacilli than in the controls. There is excessive accumulation of bacilli within the phagocytes of the treated rabbits, and a corresponding modification of their tissue response. It appears that the digestive capacity of the phagocytes of the treated rabbits is impaired as the maturation of epithelioid cells (a sign of intracellular disintegration of bacilli) is impaired, and there are many bacilli within immature coarsely vacuolated cells. The phagocytic power of the cells is not impaired but their power to limit bacillary multiplication. Whereas in the untreated controls epithelioid cells mature more readily as indicated by the extremely fine cytoplasmic vacuoles, and the persistence of very few bacilli within them. It is concluded that resistant animals are *ab initio* a poorer medium for the growth of bacilli before specific antibody formation is demonstrable; this greater initial inhibitory property of the macrophages of the resistant animal is followed by a more rapid development of acquired specific resistance; acquired resistance is superimposed on and determined by native resistance. Cortisone reduces the fragility of capillaries and protects them against agents which increase the permeability of their walls; the inflammatory response to tuberculin is markedly reduced, and a barrier is interposed between the injured cells and the circulation. Possibly the altered hormone balance, by interfering with the internal metabolism of the macrophages and by reducing antibody formation, affects the site of antigen-antibody interaction, the caseous focus, so that the latter fails to proceed to its completion

(22) *The Mechanism involved in Acquired Immunity to Tuberculosis* by S. Raffel. Evidence of three kinds is brought forward,

all of which have failed to reveal any indication that antibodies or other humeral factors serve as instruments of defence in tuberculosis. All available serological tests have failed to show more activity in sera from immune animals than in sera from animals treated with bacillary components, and which failed to show immunity. Infected animals receiving either immune serum or immune whole blood derived no benefit from these derived substances. A third line of experimentation was to place plastic capsules with a Gradacol membrane at the end, and containing virulent tubercle bacilli, in the peritoneal cavities of normal and immune guinea pigs. There was failure to find any distinction between the multiplication of bacilli in capsules implanted in immune animals and subjected to its humours, and those implanted in normal animals. There was also found to be no distinction under reduced oxygen tension. Thus, although it seems quite probable that immunity to *Myco. tuberculosis* is not dependent upon an antibody-leucocyte relationship, the nature of this mechanism is still unresolved.

(23) *Human Lung Tissue Reactions to the Tubercle bacillus in Relation to Chemotherapy.* In this paper G. Canetti describes histological appearances found in 70 out of 117 cases of pulmonary tuberculosis in lung tissue excised surgically. There was an apparently paradoxical appearance in that in patients who had been treated with chemical agents, particularly isoniazid, there was a temporary increase in the specific cellular metaplasia (epithelioid and giant cell formation). In patients who had not been treated with these chemical agents the lesions were comparatively discrete. In the former there were very few or no bacilli. The explanation given is that the specific cellular metaplasias are due to disintegration of the tubercle bacilli in the centre of the macrophages caused by the bacteriostatic or bactericidal action of the chemical agents.

(24) P. D'Arcy Hart and R. J. W. Rees write on *Influence of Certain Surface-Active Agents on the Host-Parasite Relationship in Experimental Tuberculosis.* Various non-ionic surface-active agents are described, the starting material of which is *p-tert*-octyl phenol. Three of these used were known respectively as D_2—, LOC—, and HOC—. These were found to have an anti-tuberculous effect using members with 10 to 20 ethylene oxide units; but when the ethylene units were increased to 25 to 30 therapeutic activity was abolished; when increased to 45 to 90 there was a protuberculous effect. The hypothesis is that these surface-active agents influence

tuberculous infection by modifying the surface lipids of the tubercle bacillus within the monocytes. Whatever their mechanism, it seems clear that they enter the monocytes and probably provide a means of artificially influencing the operation of the cellular defences in tuberculous infection.

(25) *The Relationship between the Growth Requirements and the Pathogenicity of Isoniazid-resistant Mutants of Tubercle Bacilli: A Study of the Role of Host Physiology in Susceptibility to Infectious Disease,* by G. Middlebrook. It is suggested that INH in adequate dosage achieves " physiological imprisonment " of tubercle bacilli in the tuberculous-allergic animal in a three-fold fashion. It inhibits the multiplication of drug-susceptible organisms and may sterilize them. It loses this direct anti-microbial activity only when the parasite suffers genetic loss of a physiological function which is apparently essential for its full pathogenicity. Its continued administration not only attacks the natively susceptible parasite, which may at any time be given an opportunity to multiply after long periods of metabolic quiescence in necrotic lesions; it also attacks any drug-susceptible reverse mutants which may make their appearance from drug-resistant populations.