bunden werden und auf diese Weise diffusionsecht festgelegt sind. Die Anordnung ist diese: Die oberste, blauempfindliche Schicht enthält die Purpurkomponente, dann folgt die rotempfindliche Schicht mit der Blaugrünkomponente und schließlich die grünempfindliche Schicht mit der Gelbkomponente. Das Auflösungsvermögen dieses Materials übersteigt 100 Linien je mm.

Schon diese kurze Übersicht zeigt, daß sich die Fortschritte der Farbenphotographie gerade jetzt wieder in lebhaftem Fluß befinden, nachdem ihr durch das Prinzip der Farbentwicklung erneut ein starker Anstoß zuteil wurde.

Summary

In the early days of photography daguerrotypes were stained with specific pigments (ISENRING). LIPPMANN'S "interference procedure", working with the pure colours of the spectrum is of great interest theoretically, but cannot be used practically. Only after the division of the visible spectrum into 3 regions: 400–500 mμ (blue), 500–600 mμ (green) and 600–700 mμ (red) and the discovery of sensitizers by VOGEL did the modern colour photography become possible for practical purposes. DUCOS DU HAURON recommended several different ways for colour photography based on that principle and even followed some of them up by himself; however, only a few have proved valuable. The method of microscopic-coloured mosaic filters and the lenticular film method work with an additive process. For coloured prints in books and journals one first produces separate exposures from the original by the aid of filters that are transparent in the before-mentioned regions of the spectrum. By means of these separate negatives the print is produced with printing stains that are complementary to the filter stains, i.e. yellow, magenta and cyan. The Technicolor-method now widely used in cinematography works under similar conditions. Beside that only the method of colour development in different varieties and ways of application is in use today.

Chemical Factors Involved in the Induction of Infectious Allergy[1]

By SIDNEY RÄFFEL,[2] Stanford, Cal.

The allergy which accompanies tuberculosis is an outstanding example of the hypersensitivity of "infectious type", termed also "tuberculin-type" or "delayed" allergy. The studies to be discussed here are mainly concerned with tuberculous allergy and with the hypersensitivity which may be induced to unrelated antigens by the use of a constituent of the tubercle bacillus. However, for the sake of a more general orientation it has seemed desirable to include in this review a discussion not only of allergy, but of the other basic responses of the host to the tubercle bacillus as well. We may then see how far our current information permits us to match up these host responses with individual chemical components of the bacillus.

I. *Basic Responses of the Host to the Tubercle Bacillus*

The fundamental responses of the infected host to the tubercle bacillus include (a) tubercle formation, (b) the development of allergic reactivity, and (c) the acquisition of specific resistance. The various aspects of the disease process will stem from the interplay of the latter two factors especially.

II. *The Chemical Constituents of the Tubercle Bacillus*

Only a brief survey can be given here of the detailed investigations of the past thirty years relative to the chemical composition of the tubercle bacillus, and this must be kept pertinent to the factors which are to be discussed. The accompanying diagram charts these substances.

(a) *Proteins.*—SEIBERT[1] especially has made detailed studies of the protein constituents of the human tubercle bacillus. These are not homogeneous in their physico-chemical properties nor entirely so in their biological properties[1,2], but for immunological purposes, as discussed below, the proteins may be considered as a single substance in respect to their qualitative relationship to host responses.

(b) *Lipids.*—Tubercle bacillary lipids have been extensively studied by R. J. ANDERSON and his associ-

[1] Based upon a lecture delivered on March 22, 1950, to a meeting of the Naturforschende Gesellschaft of Basle.

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[1] F. B. SEIBERT, Chem. Rev. *34*, 107 (1944).

[2] E. B. BEVILACQUA and J. R. MCCARTER, J. Exp. Med. *87*, 229 (1948). – J. R. MCCARTER and E. B. BEVILACQUA, J. Exp. Med. *87*, 245 (1948).

ates[1] following the work of T.B. JOHNSON and his group[2] in America. In France also this subject has received considerable attention. Our interest here will center upon the current studies of LEDERER and his coworkers[3] in Paris.

Anderson's work has identified in the human and other varieties of bacillus a series of substances extractable by organic solvents. Preliminary extraction with ether-alcohol mixture yields a *phosphatide* containing fatty acids, sugar acids, and a still unidentified nitrogenous base. A series of acetone-soluble liquid and solid fatty acids are also removed by this procedure. When the residual bacilli from this extraction are subsequently treated with chloroform, a wax-like substance is extracted, referred to as *crude wax*[4]. This substance, which is chemically not a wax but resembles beeswax in appearance and consistency, is a complex mixture which may be easily segregated into *purified wax* and *soft wax*. When purified wax is further broken down[5] by saponification it yields various fatty acids, an unsaponifiable wax fraction, and a specific polysaccharide, different from that obtained from the culture filtrate or from the bacillary bodies by simple extraction. This we shall refer to as "polysaccharide W"

The unsaponifiable wax is composed of several substances, including *mycolic acid*, an acid-fast substance for which ANDERSON *et al.*[6] have proposed the formula $C_{86}H_{171}\begin{cases}OCH_3\\OH\\COOH\end{cases}$, and a molecular weight of almost 1300. These data have been confirmed by LEDERER[7] who has added the observation that α and β isomers may be separated by chromatographic adsorption. In addition, unsaponifiable wax contains tuberculostearic and mycocerosic acids, all three acids presumably in combination with phthiocerol, a dihydric higher alcohol.

The polysaccharide W which splits from the lipids on hydrolysis of purified wax was thought by ANDERSON to be in combination with mycolic acid and other fatty acids, and this has been verified by ASSELINEAU[8]. In the accompanying diagram LEDERER's fractionation of purified wax by means of chromatographic adsorption on alumina columns is also shown, since the lipopolysaccharide by this procedure remains intact, and has been employed in the studies to be described.

Following the chloroform extraction, treatment of the bacillary residue with a mixture of alcohol, ether and hydrochloric acid releases *firmly bound lipids*, a mixture of unsaponifiable wax, fatty acids and polysaccharide.

ANDERSON's figures indicate a wide variation in the quantities of these lipoidal fractions from different strains of human-type bacilli. In the widely employed H37 strain the total lipids represent about 35 per cent of the bacillary weight, of which the phosphatide is 6·5 per cent, the crude wax 11 per cent, and the firmly bound lipid about the same.

(c) *Polysaccharide.*—From the bacillary bodies after removal of phosphatide and wax, polysaccharides (denoted by *A* in the diagram) may be obtained by extraction with 25 per cent alcohol. These are similar to or the same as the polysaccharides in tuberculin[1].

III. *Relationship of the Chemical Constituents of the Tubercle Bacillus to Responses of the Host*

(a) *The Tubercle.*—SABIN[2] has demonstrated that the isolated *phosphatide* of the tubercle bacillus when injected into the tissues of normal animals induces the formation of the tubercle, and that this activity is in the main due to the *phthioic acid* component. Monocytes which ingest phosphatide disperse this through their cytoplasm in very fine particles, and thus become epithelioid cells. These usually arrange themselves in a fashion similar to the epithelioid cells in the "hard" tubercle of infection, and through nuclear division epithelioid giant cells—the Langhans cells—are formed. Other bacillary constituents, for example the proteins, may also cause the formation of epithelioid cells, but apparently not so readily nor so generally as does the phosphatide. Since, as the chart shows, phthioic acid occurs in other fractions than the phosphatide, these may also be expected to induce cellular changes in the tissues. The phosphatide, however, is most effective in this activity, presumably because phthioic acid occurs here in greatest concentration.

(b) *Allergic Reactivity.*—The author's studies have been largely concerned with the allergic response of the host to the tubercle bacillus[3]. As was stated earlier, the allergy of tuberculosis is a prime example of hypersensitivities of the "infectious" or "delayed" type, as distinguished from those of the "anaphylactic" or "immediate" type. Allergies of both kinds are based upon the fact of altered responsiveness of the sensitized body to antigenic substances. Aside from this basic agreement however, certain marked differences are

[1] R. J. ANDERSON, Chem. Rev. *29*, 225 (1941).
[2] T. B. JOHNSON, Nat. Tuberc. Assoc. Trans. *23*, 233 (1927).
[3] J. ASSELINEAU and E. LEDERER, Bull. Soc. Chim. biol. *31*, 492 (1949).
[4] J. A. CROWDER, F. H. STODOLA, M. C. PANGBORN, and R. J. ANDERSON, J. Amer. Chem. Soc. *58*, 636 (1936).
[5] R. J. ANDERSON, J. Biol. Chem. *74*, 525 (1927); *83*, 505 (1929).
[6] F. H. STODOLA, A. LESUK, and R. J. ANDERSON, J. Biol. Chem. *126*, 505 (1938).
[7] J. ASSELINEAU and E. LEDERER, C. r. Acad. Sci. *228*, 1892 (1949).
[8] J. ASSELINEAU, C. r. Acad. Sci. *229*, 791 (1949).

[1] M. HEIDELBERGER and A. E. O. MENZEL, J. Biol. Chem. *118*, 79 (1937); F. E. HOOPER, A. G. RENFREW, and T. B. JOHNSON, Amer. Rev. Tuberc. *29*, 66 (1934); F. SEIBERT, Amer. Rev. Tuberc. *59*, 86 (1949).
[2] K. C. SMITHBURN and F. R. SABIN, J. Exp. Med. *68*, 641 (1938); F. R. SABIN, Amer. Rev. Tuberc. *44*, 415 (1941).
[3] S. RAFFEL, Amer. Rev. Tuberc. *54*, 564 (1946); S. RAFFEL, J. Inf. Dis. *82*, 267 (1948); S. RAFFEL and J. E. FORNEY, J. Exp. Med. *88*, 485 (1948); S. RAFFEL, L. E. ARNAUD, C. D. DUKES, and J. S. HUANG, J. Exp. Med. *90*, 53 (1949).

known to differentiate these hypersensitivities, both with regard to *induction* of the allergic state and the *nature of the response elicited* in the already sensitized subject.

For its *induction*, the anaphylactic type of hypersensitivity requires only that an antigenic substance, for example a bland protein, gain access to the tissues. Within a period of perhaps ten days it will be found that the subject no longer treats the antigen as a bland substance, for if it be reinjected certain general or local reactions to the protein will occur, depending upon the route of injection, the species of animal, and other considerations. In contrast, "infectious" hypersensitivities cannot be induced by antigenic substances alone. It is necessary that the infectious organism be present in the tissues, either as an actual agent of disease or in the form of a killed vaccine. In addition to tuberculin allergy many examples of this type of hypersensitivity are well known amongst bacterial, viral and fungal diseases. Instances include the classical response to vaccinia virus in the previously vaccinated subject, the allergy to the *Brucellas*, and the reactivity to *Coccidioides immitis*. These are the more striking and familiar examples, but it is probably safe to assume that this kind of hypersensitivity is induced by all infectious agents, though with considerable variation in degree, so that in many instances its presence may be appreciated only through special and careful tests. In most cases the allergy is not so intense as to play a role in the pathogenesis of the disease caused by the particular agent concerned.

There are differences also in the *nature of the responses* of sensitized subjects to antigen in anaphylactic and infectious hypersensitivities. These differences are in (1) rapidity of response, (2) the kind and extent of cellular involvement, and (3) the relationship of humoral antibody to the response.

(1) *Rapidity of response.*—The anaphylactic type of response usually occurs immediately after contact with antigen in the already sensitized subject. As is well known, anaphylactic shock itself may become apparent within seconds after injection of antigen. In contrast, the responses in infectious allergy are delayed, whether these be systemic or limited to the skin. Manifest skin reactions appear after perhaps six hours, but reach their peak at 24 to 48 hours. Systemic reactions may become discernible in about the same interval, although subjectively the sensitive individual may feel the effects of exposure to antigen within about 3 hours. From these considerations the terms "immediate" and "delayed" have been applied to the two types of allergy.

(2) *Kind and extent of cellular involvement.*—The tissues and cells which take part in anaphylactic reactions are chiefly involuntary muscle and blood vessel walls. In recent years it has become appreciated also that collagen fibers may undergo focal degeneration as seen in the lesions accompanying serum sickness and rheumatic fever[1]. Whatever the manifestations of the reaction however, smooth muscle and vascular tissues are chiefly concerned, and changes in these may cause the bronchiolar and other contractions in anaphylactic shock as well as the edematous changes seen in the local wheal. In infectious hypersensitivity on the other hand various kinds of cells—perhaps all cells of the body—may be affected by exposure to antigen. Thus, epidermal and dermal cells may be injured by the injection of antigen into the skin, and if cells of the spleen or bone marrow are explanted into tissue culture to which the antigen has been added, these may degenerate, as will be described below in regard to tuberculosis. There seems here to be an individual responsiveness of cells of many types in contrast to the special "shock tissues" which take part in anaphylactic reactions.

(3) *Relationship of humoral antibody to the response.*—In anaphylactic sensitivity the "shock tissues" described above appear to be acted upon by a product of antigen-antibody union, in the nature of a histamine-like substance. The reaction between antigen and antibody may take place in or upon the tissues affected—for this detail the available evidence is not clear—but the antibody concerned is the same as that which circulates in the blood. This is apparent from the fact that a normal subject may be rendered sensitive by transfer of serum from a sensitive one, and that isolated tissues of the kind mentioned, e.g. smooth muscle suspended in a bath *in vitro*, may be sensitized by the addition of humoral antibodies to the bath so that it will contract on subsequent addition of antigen.

Again a contrast presents itself when we turn to delayed hypersensitivity. Humoral antibodies may be formed against the antigenic constituent of the infectious agent which has induced the sensitivity, but these antibodies have no relationship to the hypersensitive state. Thus, this type of hypersensitivity cannot be transferred from a sensitive to a normal subject by means of serum, nor can isolated normal cells be so modified by humoral antibodies as to react with antigen. The transfer of this type of hypersensitivity has been effected only by means of cells[2].

(4) *Studies of tuberculous allergy.*—We return now to tuberculosis as a specific example of infectious hypersensitivity. The induction of tuberculin sensitivity requires the presence of living or killed tubercle bacilli in the body. The specific antigen of the bacillus responsible for the allergic state is the protein; this has been appreciated for many years because the isolated bacillary protein *elicits reactions* in sensitized subjects.

[1] A. R. RICH, *The Harvey Lectures 42*, 106 (1947); P. KLEMPERER, in: *Allergy in Theory and Practice* by R. A. Cooke, (Saunders Co., Philadelphia, 1947), p. 69.

[2] F. A. MCJUNKIN, J. Exp. Med. *33*, 751 (1921). – K. LANDSTEINER and M. W. CHASE, Proc. Soc. Exp. Biol. Med. *49*, 688 (1942).

But, conforming to what was said above concerning the inability of isolated antigens to induce infectious hypersensitivity, it is found that tuberculoprotein, despite its good qualities as an antigen, cannot when separated from the bacterial cell *induce* the allergic state.

Chemical constituents of the bacillus responsible for the induction of tuberculous allergy.—During the course of a study of the relationship of tubercle bacillary components to host responses, the author and his associates have had occasion to administer to animals many of the isolated substances described earlier, as well as bacillary cells from which certain components had been extracted[1]. In some instances combinations of these substances were employed, and it was observed that those animals which received the combination of bacillary protein with the wax-developed sensitivity to Old Tuberculin. As a corollary to this was the further observation that animals treated with bacillary cells from which the wax had been extracted did not develop tuberculin sensitivity, though the protein in these bacilli had remained antigenically intact. It became apparent then that the wax fraction of the organism determines the development of the delayed type of allergy to the protein. So far as we have been able to determine the wax itself is immunologically inert, causing no detectable response in animals treated with it. But if it is injected together with protein as a mixture, or separately within a period of one or two hours, the result of this combined administration is the development of the typical hypersensitivity of tuberculosis. For this statement fuller evidence will be presented below.

From the chemical standpoint we have wished to know precisely which ingredient of the purified wax might be responsible for this directive effect in the induction of hypersensitivity. For this purpose R. J. ANDERSON kindly supplied us with the substances which, as shown in the chart, he has segregated from purified wax by hydrolysis. Apparently no one of these, including mycolic acid, polysaccharide W, phthioic acid, mycocerosic acid, tuberculostearic acid, phthiocerol or unsaponifiable wax has the same directive activity in the induction of tuberculous allergy as has the purified wax itself. Recently, however, we have been supplied by E. LEDERER with wax components isolated by chromatographic methods on alumina columns[2], and one of these, a lipopolysaccharide, appears to be the active ingredient of the wax. This substance is made up of mycolic acid bound through its carboxyl radical with the polysaccharide W as the major components, with about 5 per cent of other fatty acids also bound to the carbohydrate. There are present in the lipopolysaccharide also small amounts of three amino acids[1], alanine, glutamic acid and α, ε diaminopimelic acid, the last recently isolated as well from the diphtheria bacillus by WORK[2]. It appears probable then that an ester of mycolic acid with polysaccharide W is the essential factor in determining the nature of the response of the body to tuberculoprotein, but further studies are being carried out to establish this point[3].

It is of interest to attempt to correlate these findings with other current investigations along related lines. CHOUCROUN[4] has described a sensitizing substance obtained from the human tubercle bacillus by extraction with paraffin oil, but a similarity to the wax component is not apparent in view of its insolubility in chloroform and other organic solvents. In respect to tubercle bacillary virulence, recent work by LEDERER and his associates[5] and possibly also by BLOCH[6] may relate the lipopolysaccharide to this, though the status of this relationship is yet too fresh for detailed interpretation.

Criteria employed in evaluating the allergy induced by protein and wax.—The author's studies have required that so far as possible objective criteria be met in evaluating the type of hypersensitivity established by the injection of tuberculoprotein and wax. Skin reactivity to Old Tuberculin is itself a good criterion, but it is open to the objection that an intense reaction of the local anaphylactic type might be sufficiently protracted to masquerade as a reaction of the delayed type. Accordingly, experiments were carried out as described below.

Based upon the knowledge discussed earlier that individual cells of the animal with infectious hypersensitivity are responsive to contact with antigen, RICH and LEWIS[7] some years ago carried out studies with tissue cultures of splenic cells of tuberculous animals, and found that in the presence of tuberculin the cells in such cultures ceased to migrate and underwent disintegration. This observation has been confirmed by others[8]. We[9] have applied this method to the problem at hand, employing the bone marrow of guinea pigs which had been previously treated in three different ways: by injection of tuberculoprotein alone, with wax, or by infection. The bone marrow explants were then exposed to a suitable concentration of dialyzed Old Tuberculin, and the results are shown in

[1] S. RAFFEL, Amer. Rev. Tuberc, *54*, 564 (1946).

[2] J. ASSELINEAU and E. LEDERER, Bull. Soc. Chim. biol. *31*, 492 (1949); J. ASSELINEAU, C. r. Acad. Sci. *229*, 791, (1949).

[1] J. ASSELINEAU and E. LEDERER, C. r. Acad. Sci. *230*, 142 (1950).

[2] E. WORK, Nature *165*, 74 (1950).

[3] S. RAFFEL, TH. FRIIS, E. LEDERER, and H. C. ENGBAEK, work in progress.

[4] N. CHOUCROUN, Amer. Rev. Tuberc. *56*, 203 (1947).

[5] J. ASSELINEAU and E. LEDERER, C. r. Acad. Sci. *230*, 142 (1950); J. ASSELINEAU, H. DEMARTEAU, and E. LEDERER, C. r. Acad. Sci. *230*, 877 (1950).

[6] H. BLOCH, J. Exp. Med. *91*, 197 (1950).

[7] A. R. RICH and M. R. LEWIS, Bull. J. Hopk. Hosp. *50*, 115 (1932).

[8] J. D. ARONSON, J. Exp. Med. *54*, 387 (1931); J. Immunol. *25*, 1 (1933); J. K. MOEN and H. F. SWIFT, J. Exp. Med. *64*, 339 (1936).

[9] S. RAFFEL, J. Inf. Dis., *82*, 267 (1948).

the accompanying figures (Fig. 1 to 6). It is first seen that normal bone marrow is not injured by the tuberculin preparation employed (Fig. 1 and 2). Secondly, it is apparent (Fig. 3 and 4) that the cells from animals sensitized with tuberculoprotein *alone* are not injured by the presence of tuberculin in the culture, because the "shock tissues" for anaphylactic reactivity are not present here, although the animals from which these cells were obtained were highly subject to anaphylactic shock. Thirdly, it is seen that the cells from those animals sensitized by injections of tuberculoprotein with wax are highly vulnerable to tuberculin (Fig. 5 and 6), precisely as are those from animals with tuberculous infection.

Analogous studies have been made *in vivo*, employing the corneas of intact animals rather than cells transferred to tissue cultures[1]. The cornea, a tissue without blood vessels or involuntary muscle, is incapable of taking part in an anaphylactic type of response no matter how highly sensitized the animal may be. But the corneal cells can become involved in the infectious type of hypersensitive response on the basis of the sensitivity of corneal cells *per se* to antigen. Since it is possible then to demonstrate a corneal reaction only in the animal with the infectious type of hypersensitivity, the elicitation of a reaction is itself evidence that the animal has this kind of allergy. Again, as in the tissue culture studies, the results were obtained from animals prepared in three different ways, and tested by the deposition of small volumes of tuberculin in their corneas. In a normal group it was found that no serious injury was evident 24 to 48 hours after such an injection (Fig. 7). In a group anaphylactically sensitized by means of tuberculoprotein alone, and highly sensitive to this antigen when injected intravenously or into the skin, there was similarly no response in the cornea (Fig. 8). In contrast, animals sensitized by the administration of tuberculoprotein with wax responded to the intracorneal injection of protein with marked damage to the corneal fibers, and with secondary edema and inflammation (Fig. 9). These last changes were identical with those elicited in the corneas of tuberculous animals. The photographs used here for illustration were actually made from the corneas of animals sensitized to another antigen (egg albumin) with tubercle bacillary wax, since no photographs of the groups under discussion are presently available. The reactions are entirely analogous however.

In addition to these methods of study other procedures, including tests of KOCH reactivity, of systemic responses to tubercle bacilli and tuberculin, and of the non-transferability of the sensitivity by serum, agreed in pointing to the conclusion that the allergy induced by tuberculoprotein and wax is identical with that which occurs in tuberculous infection.

[1] S.W. HOLLEY, Amer. J. Path. *11*, 937 (1935), A.R. RICH and H.R. FOLLIS, Jr., Bull. J. Hopk. Hosp. *66*, 106 (1940).

Activity of wax in "directing" the allergic response of the body to other antigens.— The fact that tubercle bacillary wax functions as it does with tuberculoprotein

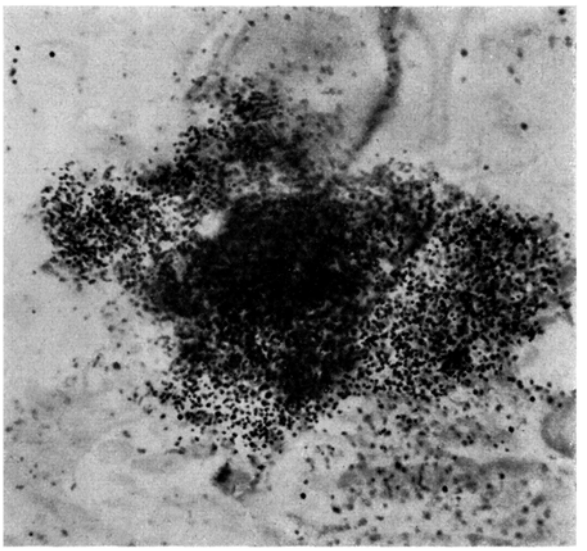

Fig. 1. – Bone marrow culture of a normal guinea pig after 24 hours of exposure to dialyzed O.T. 1:30. 165 ×. Harris hematoxylin stain.

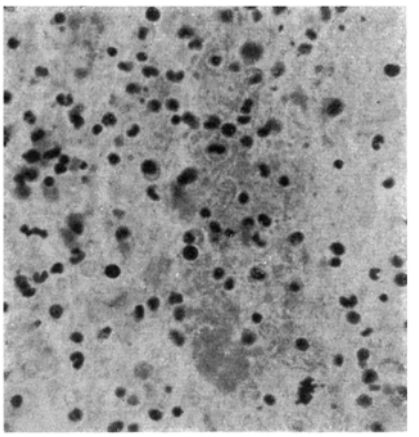

Fig. 2. – Same explant, 400 ×.

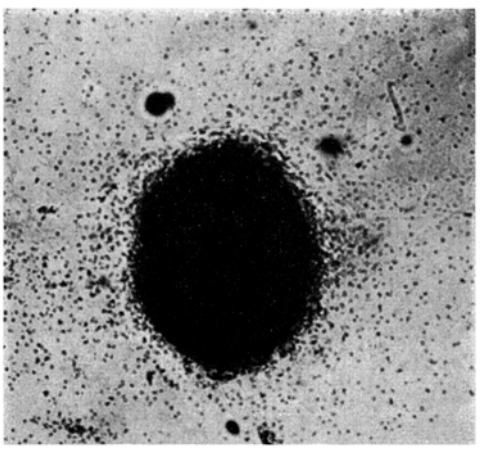

Fig. 3. – Bone marrow of a guinea pig sensitized with tuberculoprotein alone, after 24 hours of exposure to dialyzed O.T. 1:30. 165 ×. Harris hematoxylin stain.

suggested that this might be a general property of this lipid with other antigenic substances also, entirely unrelated to the tubercle bacillus. This suggestion receives considerable support from the finding by DIENES[1] and HANKS[2] some years ago that in guinea pigs infected with tubercle bacilli, or injected with killed bacilli, it was possible to establish the tuberculin-type of reactivity to various antigenic substances, such as for example egg white. To investigate this possibility with the purified wax of the bacillus we employed two antigens, one a protein of non-infectious source, egg albumin, the second a simple chemical substance, picryl chloride, which becomes antigenic after injection because of its marked ability to combine with body proteins which then function as "schleppers"[1].

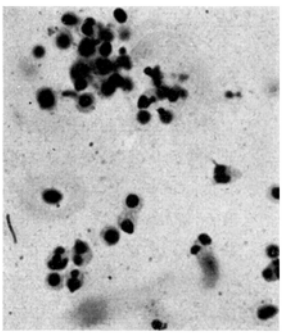

Fig. 4. – Same explant, 900 ×.

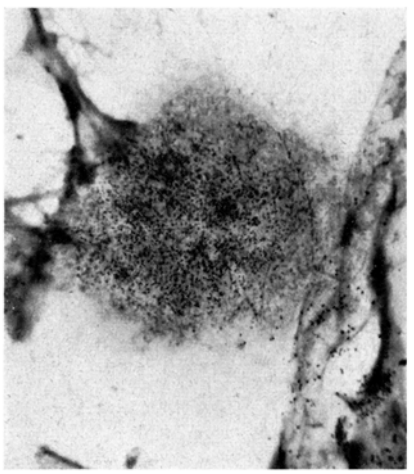

Fig. 5. – Bone marrow of a guinea pig sensitized with tuberculoprotein and wax, after 24 hours of exposure to dialyzed O.T. 1:30. 165 ×. Harri's hematoxylin stain.

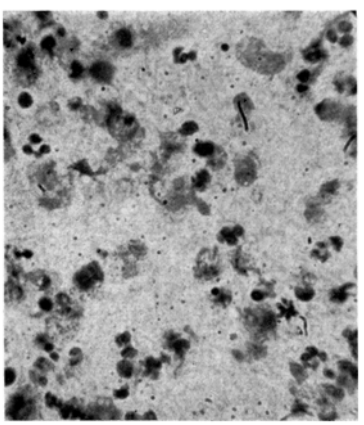

Fig. 6. – Same explant, 900 ×.

Figs. 3, 4, 5 and 6
(Taken from S. RAFFEL, J. Infect. Diseases, *82*, 267 [1948])

[1] L. DIENES and E.W. SCHOENHEIT, J. Immunol. *14*, 9 (1927); L. DIENES, J. Immunol. *15*, 153 (1928); *20*, 221 (1931).
[2] J.H. HANKS, J. Immunol. *28*, 105 (1935).

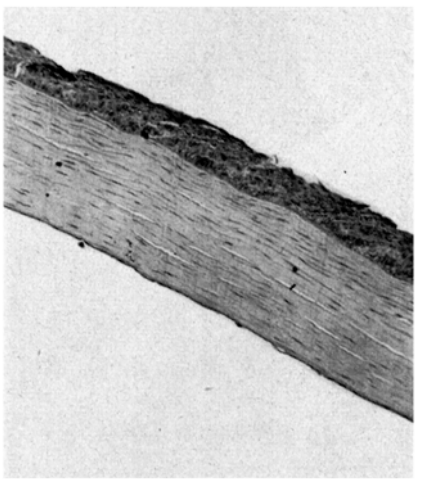

Fig. 7. – Cornea of a normal guinea pig, 48 hours after injection of egg albumin. 85 ×. Hematoxylin-eosin stain.

Egg albumin when injected alone into animals induces the formation of humoral antibody and of a high degree of anaphylactic sensitivity. It has been possible by the simultaneous injection of bacillary wax to invoke in addition the tuberculin-type of reactivity to this protein. This has been evidenced by the character of the skin reactions to egg albumin and as well by the behavior of explanted tissues in culture and by corneal cells *in vivo*. Fig. 7 to 9 are illustrations of the last-mentioned effect employing egg albumin as the intracorneal antigen. This result in the guinea pig sensitized with egg albumin and wax and injected later intracorneally with albumin (Fig. 9) is as striking as that seen in tuberculous animals injected intracorneally with tuberculin[2].

The irritating properties of picryl chloride for tissues has not permitted its use in tissue culture or corneal studies, but another means has been available for evaluating the effect of tubercle bacillary wax upon the response of the animal to this simple substance. LANDSTEINER and CHASE[3] found that picryl chloride injected intraperitoneally into guinea pigs causes the

[1] K. LANDSTEINER and J. JACOBS, J. Exp. Med. *61*, 643 (1935); *64*, 625 (1936); P.G.H. GELL, C.R. HARINGTON, and R.P. RIVERS, Brit. J. Exp. Path. *27*, 267 (1946).
[2] S. RAFFEL, L.E. ARNAUD, C.D. DUKES, and J.S. HUANG, J. Exp. Med., *90*, 53 (1949).
[3] K. LANDSTEINER and M.W. CHASE, J. Exp. Med. *66*, 337 (1937); *73*, 431 (1941).

establishment of both antibodies and anaphylactic reactivity, but no *contact reactivity* of the skin to application of the substance. This last type of response, a characteristic one in the human being with respect to

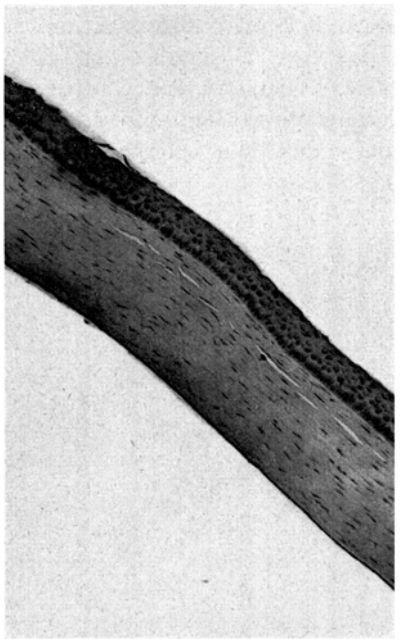

Fig. 8. – Cornea of a guinea pig sensitized with egg albumin alone, 48 hours after injection of egg albumin. 85 ×. Hemotoxylin-eosin stain.

(Taken from S. RAFFEL *et al.*, J. Experiment. Med. *90*, 53 [1949])

various industrially employed chemical substances and certain plant resins (primula, poison oak, poison ivy), is a form of delayed allergic response and exists independently of anaphylactic reactivity to the same antigen[1]. LANDSTEINER and CHASE[2] were able to cause contact reactivity by the intraperitoneal injection of picryl chloride provided that killed tubercle bacilli were injected with it, a finding reminiscent of those of DIENES and HANKS mentioned earlier.

When we employed purified wax with picryl chloride we were able to induce an intense degree of epidermal contact reactivity to the chemical itself, and it was possible to abolish the concomitant anaphylactic sensitivity of such animals by desensitization, leaving this delayed form of allergy intact[3].

From the broader biological standpoint it would of course be of utmost interest to know whether in other infectious agents there may exist a chemical basis for the delayed hypersensitive state analogous to that found in the tubercle bacillus. In several instances we know that the antigenic moiety is analogous, i.e. a protein. Whether a lipoidal factor may also be involved has been the subject of study in the author's laboratory for some time, and our observations, though incomplete, indicate that there may be lipids in other infectious agents with a biological property in respect to sensitization similar to that of the wax of the tubercle bacillus.

Nature of the activity of wax in modifying hypersensitive response.—We do not as yet have evidence as to the mechanism through which tubercle bacillary wax alters the channel of hypersensitive response of the animal body. The effect is not that of an immunologic *adjuvant*, by which is meant a substance which, while not specifically participating in inducing an immunologic response, causes a more marked response to the antigen with which it is injected. Such an activity is analogous to that of a chemical catalyst, and is possessed by a number of substances, e.g. the alum commonly employed in the preparation of toxoids. Such precipitated toxoids are better immunizing agents than the toxoids in solution, although the antibody response is in no wise directed against the alum.

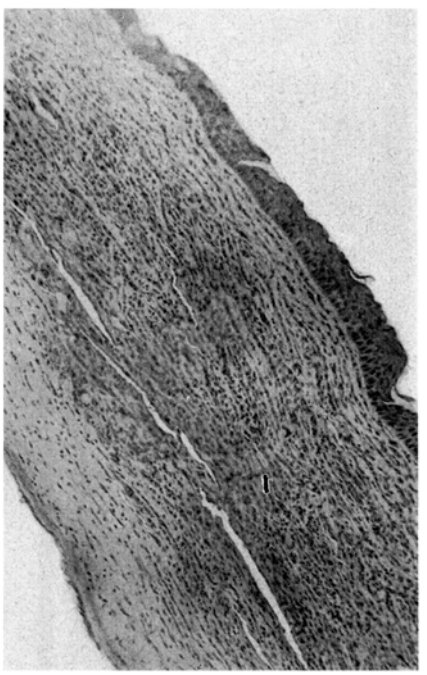

Fig. 9. – Cornea of a guinea pig sensitized with egg albumin plus wax, 48 hours after injection of egg albumin. 85 ×. Hematoxylin-eosin stain.

(Taken from S. RAFFEL *et al.*, J. Experiment. Med. *90*, 53 [1949])

The bacillary wax has no such effect. With three antigens, tuberculoprotein, egg albumin and picryl chloride, we have been able to discern no indication that the addition of wax increases antibody responses or intensifies anaphylactic sensitivity. These remain the same as in animals receiving the antigens alone. The wax causes *in addition* the appearance of the infectious type of hypersensitivity.

It is provocative to speculate that the ability of the wax to cause foreign body giant cell formation and

[1] K. LANDSTEINER and W. M. CHASE, J. Exp. Med. *66*, 337 (1937); *73*, 431 (1941).

[2] K. LANDSTEINER and M. W. CHASE, J. Exp. Med. *71*, 237 (1940).

[3] S. RAFFEL and J. E. FORNEY, J. Exp. Med., *88*, 485 (1948).

Chemical constituents of *M. tuberculosis* var. *hominis*
(Compiled from the work of ANDERSON, SEIBERT, LEDERER and others. References are given in the text)
Italics (e. g. Phthioic Acid) indicate that the substances possess activity as described in the text

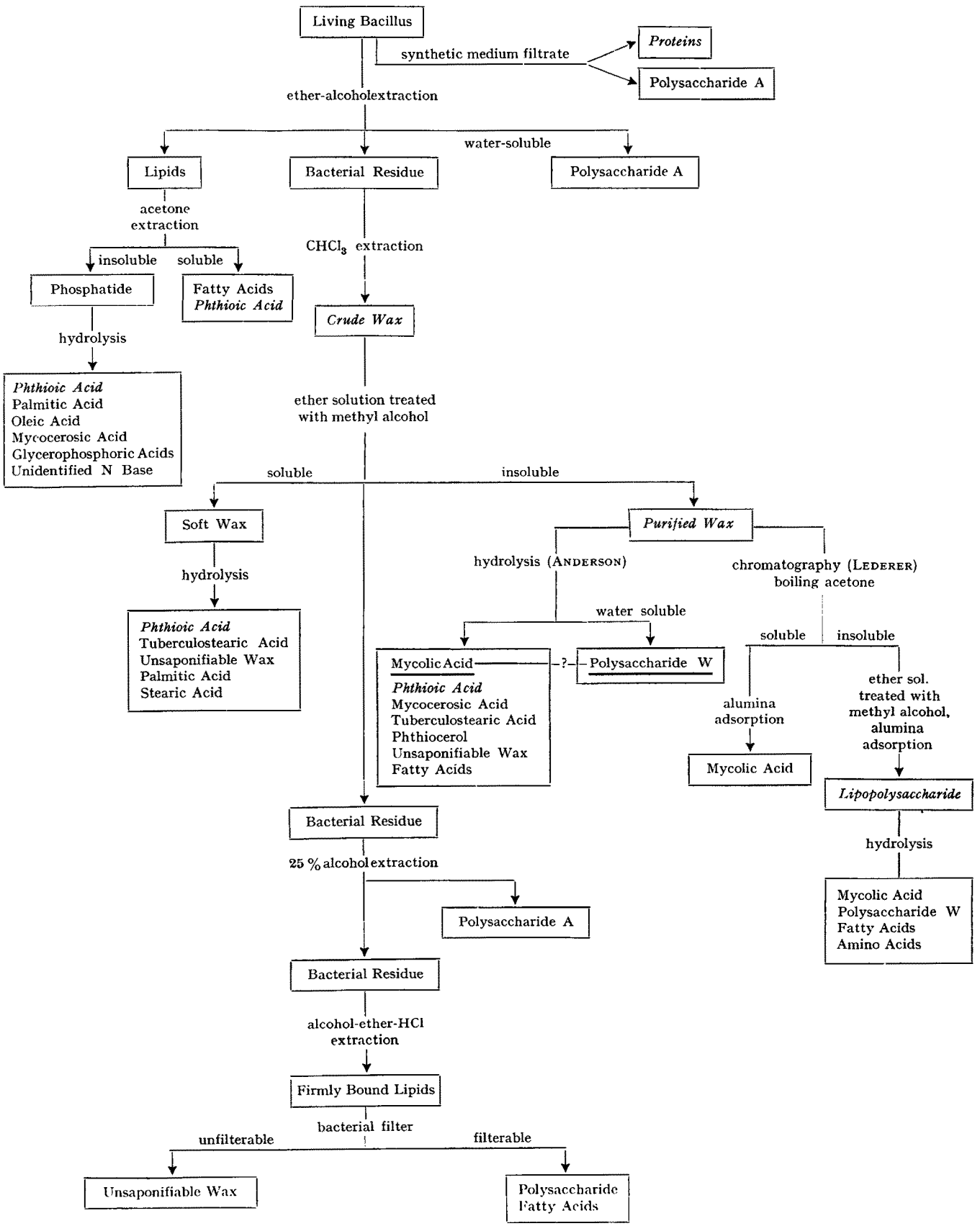

Table I

Comparison of Allergic and Immune Responses in Guinea Pigs Sensitized with B.C.G. and with Tuberculoprotein-Wax

Group No.	Treatment of animals	Average reactions to O.T.	Average Koch reactions	Tuberculous involvement of organs				
				Liver	Spleen	Lungs	Tr. br. nodes	Ing. nodes
1	B.C.G.	13·0 2·0*	6·0 0·9*	0	0	±	±	+
2	Protein-wax	12·8 1·9	4·1 0·6	2+	3+	2+	3+	4+
3	None	0 0	0 0	2+	4+	3+	3+	4+

* The first figure represents mm diameter of the reaction, the second figure estimated mm raised.

other striking cellular alterations[1] may be related to its influence in hypersensitivity. This possibility does not appear to be well founded however, since another tubercle bacillary constituent, the phosphatide, has an even more marked effect in altering cells, to the extent of simulating tubercles, without in any way influencing the hypersensitive response[2]. Furthermore, many infectious agents with no analogous cytologic influence whatever are nevertheless capable of inducing allergy of the same type.

(c) *Acquired Resistance.*—The last of the host responses to be considered is the acquisition of relative resistance to the tubercle bacillus as the result of its residence in the body. There is a good deal of evidence for such an acquisition in man as well as in laboratory animals[3]. There is however as yet no answer as to the nature of the bacillary substance which may be responsible for its induction, nor whether this may be a fixed bacillary constituent or a diffusible product of bacterial metabolism. We have unsuccessfully attempted to induce resistance with all the constituents shown in the chart, alone and in various combinations, as well as with substances obtained from the bacillary cells in other ways[4] (and unpublished data). Nor do we have a definitive reply to the question of the mechanism of this resistance, although LURIE's experiments[5] indicate that alterations in humoral as well as cellular (macrophagic) properties may both contribute to acquired defense.

Although our own experiments have supplied no positive information on this question, there is certain negative information which may warrant brief description. For many years it has been contended that the allergic state itself is the chief instrument of acquired resistance (discussed in ref. 3). The arguments in favor of this view have been rational ones; first, that animals when they acquire resistance concomitantly develop allergy, and secondly, that the intensified inflammation which occurs in the tissues of the sensitive subject as the result of contact with bacilli endows the body in higher than usual degree with the localizing mechanism generally associated with the inflammatory reaction. This view infers that a response which can be protective under ordinary circumstances should be even more effective when it is extraordinarily intensified, as at the site of the allergic reaction.

This concept has been seriously questioned in the past 20 years by the studies of RICH[1] and others[2]. Thus, it has been shown that an intense inflammation may lose the ability to localize foreign particles because of the rapid sweep of fluids through the tissues, and indeed may serve to disseminate bacteria from an initial focus. More specifically, it has been demonstrated[2] that *desensitization* of the animal resistant to tuberculosis does not diminish its immunity, although certain peculiar events may occur to interfere with the clear interpretation of such experiments[3].

Upon purely immunological grounds it seems logical to put the question of the relationship of allergy to resistance in this way: Does the immunological response to tuberculoprotein and wax endow the body with resistance as well as allergy to the tubercle bacillus? The use of these isolated components permits an appraisal of the question unencumbered by responses to other bacillary components as is the case when entire bacilli are employed for vaccination. We have carried out several repeated experiments to answer this question with results of the kind described below[4].

Three groups of guinea pigs were chosen for these observations. One group was immunized with B.C.G. vaccine, the second received at the same times appropriate doses of tuberculoprotein and purified wax, and the third remained without treatment. At the end of the vaccination period the three groups of animals were tested with Old Tuberculin. The results of such

[1] K.C. SMITHBURN and F.R. SABIN, J. Exp. Med., *68*, 641 (1938); F.R. SABIN, Amer. Rev. Tuberc., *44*, 55 415 (1941).

[2] S. RAFFEL, J. Inf. Dis., *82*, 267 (1949).

[3] A.R. RICH, *The Pathogenesis of Tuberculosis* (Charles C. Thomas, Springfield, Ill., 1944); M. PINNER, *Pulmonary Tuberculosis in the Adult* (Charles C.Thomas, Springfield, III, 1945).

[4] S. RAFFEL, Amer. Rev. Tuberc., *54*, 564, (1946); S. RAFFEL, J. Inf. Dis., *82*, 267 (1948).

[5] M.B. LURIE, J. Exp. Med. *75*, 247 (1942); M.B. LURIE, J. Exp. Med. *69*, 555 (1939).

[1] A.R. RICH and H.A. MCCORDOCK, Bull. J. Hopk. Hosp. *44*, 273 (1929); A.R. RICH, Bull. J. Hopk. Hosp. *47*, 189 (1930).

[2] H. ROTHSCHILD, J.S. FRIEDENWALD, and C. BERNSTEIN, Bull. J. Hopk. Hosp. *54*, 232 (1934).

[3] C.E. WOODRUFF and R.G. KELLY, J. Immunol. *45*, 79 (1942).

[4] S. RAFFEL and W. HANNS, manuscript in preparation.

tests in one experiment are shown in Table I. Immediately after the 48-hour readings had been made, all groups were challenged, in exactly the same way, with virulent human tubercle bacilli injected in part subcutaneously, in part intracutaneously, in the region of the groin. The intracutaneous injections were made in order to facilitate observations of the possible occurrence of KOCH reactions, and these readings are also shown in Table I. It is apparent that at the time of infection, the B.C.G. immunized and the protein-wax treated animals showed very similar levels of tuberculous allergy, as judged by cutaneous reactivity to Old Tuberculin and to living virulent bacilli as well. Eight weeks after infection all the guinea pigs of the three groups were sacrificed for pathological examination. At this point the similarity between the two vaccinated groups ceased. The protein-wax sensitized group which had been in a class with the B.C.G. vaccinated in respect to allergic reactivity, is seen to be, in respect to resistance, in a class with the untreated infection controls. We have concluded from such experiments that the bacillary constituents which engender allergy are not the same as the bacillary factors which induce resistance to tuberculosis, and as a corrollary, that tuberculous allergy and tuberculous resistance are not synonymous.

Zusammenfassung

Die drei grundsätzlichen Reaktionsweisen des Wirtes auf den Tuberkelbazillus, nämlich die Tuberkelbildung, der allergische Zustand und die erworbene Resistenz, werden im Hinblick auf die chemischen Bestandteile des Tuberkelbazillus erörtert.

Die Zellveränderungen, die zur Tuberkelbildung führen, können durch das isolierte Phosphatid des Bazillus hervorgerufen werden. Der aktive Bestandteil dieses Stoffes ist die Phthiolsäure, mit der Formel $C_{26}H_{52}O_2$.

Der «verzögerte», durch den Tuberkelbazillus verursachte Allergietypus kann auch durch andere Infektionserreger erzeugt werden. Bei der Tuberkulose wird diese Wirtsreaktion durch ein bazilläres Antigen, ein Protein, bestimmt, welches zusammen mit einer aus dem Organismus extrahierten Fraktion aus «gereinigtem Wachs» wirkt. Der spezifische, in diesem Sinn aktive Bestandteil des Wachses scheint ein Lipopolysaccharid zu sein. Die Merkmale der «verzögerten» Allergie, die sie von der «anaphylaktischen» Überempfindlichkeit unterscheiden, werden beschrieben. Mit diesen Unterschieden läßt sich beweisen, dass die durch Tuberkelprotein und Wachs hervorgerufene Allergie mit derjenigen der Tuberkuloseinfektion identisch ist. Der Wachsbestandteil des Bazillus kann von nicht tuberkulösen bazillären Antigenen dazu benützt werden, um analoge «verzögerte» Überempfindlichkeiten hervorzurufen.

Die erworbene Resistenz gegen den Tuberkelbazillus kann heutzutage keinem der bekannten chemischen Bestandteile des Bakteriums zugeschrieben werden. Es werden jedoch Beweise angeführt, daß die Resistenz nicht gleichbedeutend mit dem allergischen Zustand ist. Dieser kann durch Behandlung von Versuchstieren mit isoliertem Tuberkelprotein und Wachs hervorgerufen werden. Die auf diese Weise sensibilisierten Tiere zeigen aber keine erhöhte Immunität gegenüber virulenten Bazillen.

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

Recherches sur la biosynthèse des caroténoïdes chez un Microorganisme

Mise en évidence des précurseurs

Les Champignons filamenteux, et particulièrement certaines Mucorinées contiennent des quantités élevées de caroténoïdes et se prêtent bien à l'étude de la biosynthèse de ces pigments. On ne sait que peu de chose à ce sujet.

En 1935, nous avons étudié les conditions de formation de ces pigments chez *Phycomyces Blakesleeanus*[1]. Le β-carotène s'est révélé être le caroténoïde le plus abondant, sinon le seul; l'extrait de thalle manifeste une activité vitaminique A nette chez le rat carencé[1]. KARRER et KRAUSE-VOITH confirment que le β-carotène est le caroténoïde essentiel de *Phycomyces*[2]. D'ailleurs celui-ci a été obtenu à l'état cristallisé[3]. Récemment GARTON, GOODWIN et LIJINSKY[4] apportent quelques

[1] W.H. SCHOPFER, C. r. Soc. Biol. Paris *118*, 3 (1935).

[1] W.H. SCHOPFER et A. JUNG, *id.* *120*, 1093 (1935).

[2] P. KARRER et E. KRAUSE-VOITH, Helv. chim. acta *802*, 31 (1947).

[3] W.H. SCHOPFER et V. KOCHER, Actes Soc. helv. sci. nat. 320 (1936).

[4] G.A. GARTON, T.W. GOODWIN, and W. LIJINSKY, Biochem. J. *46*, no. 5, 35 (1950).