

FACTORS IN RESISTANCE TO TUBERCULOSIS *

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When the literature dealing with the tuberculin reaction and immunity phenomena in general in relation to tuberculosis is surveyed it is rather surprising to note the decided skepticism which has found expression in recent times concerning the more or less current immunologic conception of the mechanism of the tuberculin reaction. Kraus, Landmann, Löwenstein and Volk, Aronson, Bessau, to mention only a few, have published observations which have led them to doubt the adequacy of the antigen-antibody conception. The fact that up to the present the major part of experimental work in tuberculosis has been along strictly immunologic lines, without apparently resulting in any substantial advance in our knowledge of the disease process or its therapy, is possibly the underlying reason that this skepticism has developed, for as yet no other adequate explanation has been put forward by the various workers which might serve as a basis for the diverging views. The existing confusion is due in part to the fact that immunologic ideas and terms have been maintained and used to express phenomena concerned with anaphylactic reactions as well as certain ferment changes; in part it is due to the fact that a large share of the literature is clinical in character and the observers have reiterated fallacious immunologic theories until the repetition has of itself seemingly carried the weight of uncontrovertible authority; perhaps, too, the fact that the effort is made to explain the skin reaction (von Pirquet) and the general reaction (subcutaneous) on one and the same basis, while as a matter of fact they are dissimilar in many important respects, has contributed in no small measure.

The whole trend of the study of tuberculosis has focused about the resistance to an established infection. The fundamental ideas concerning the factors involved in the establishment of the infection itself have been largely accepted, while the forces that protect the individual against infection are, from a practical standpoint, so largely social and economic that less interest has been attached to them from the experimental side. Until recent times, the idea that resistance to infection

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need not depend wholly on specific immune bodies has been largely ignored in experimental work, although the clinician was often compelled to rely solely on this uncertain factor in his treatment of the disease.

The data presented in this paper represent observations along experimental lines not immunologic in the usual sense, but having to do rather with some of these nonspecific reactions on the part of the host. If presented at all, it is with the hope that, however inadequate, they will be of interest in the interpretation of clinical problems as yet obscure.

For the time being it may be well to keep in mind certain facts accepted by recent workers as fundamental to the discussion to follow. These are the following: (1) the cutaneous reaction appears to be specific and is related to a definite sensitization; the resistance to this reaction need not be specific; (2) the subcutaneous reaction has no relation whatever to antibody concentration of either serum or cells; when the disease focus (tubercle) is removed the reaction becomes negative; the reaction can be elicited by nonspecific methods; (3) tuberculin treatment has no specific significance and its beneficial effects have no relation to an active immunization. The actual demonstration of an increase in antibody concentration in tuberculosis is not necessarily associated with favorable clinical results; recovery does not depend on the presence of specific antibodies.

CASEATION

The tubercle bacillus differs from most other organisms in its abundant fat and wax content, some 35 to 45 per cent. of the total dry weight consisting of lipid bodies, including waxes, fatty acids and neutral fats. When such bacteria undergo disintegration in the tissues this relatively resistant waxy material remains in situ for a considerable period of time. It happens that these lipoids, being unsaturated, provided their state of dispersion be great enough, act as antiferments against tryptic and leukoproteolytic ferments. This property of checking proteolysis depends, therefore, on both a chemical configuration — the number of unsaturated carbon bonds available, and on a physical basis — the ultimate state of division or dispersion. These fat and wax bodies probably do not exist free as such either in the living bacilli or in the infected tissues after the death of the organisms, but most probably as an intimate protein-lipoid combination, that is, a combination in the physical rather than in the chemical sense.

As the tubercle bacillus finds lodgment and multiplies in the tissues it entails the destruction of a certain number of tissue cells. This is brought about through the excretion of toxic metabolic products, or

possibly through the medium of extracellular bacterial ferments. Under ordinary pathologic conditions, tissue death is followed by autolysis and the removal of the fluid end-products through the vascular channels. It is apparent that this does not take place in caseation; autolysis is in some way prevented and the necrotic debris accumulates. It is true that the cellular reaction about the tubercle does not include polymorphonuclear leukocytes, which, because of their abundant proteolytic ferment content, liberated when they disintegrate, hasten autolytic processes; instead we find lymphocytes, the lipase carrying cells. But tissue autolysis does not depend on the presence of polymorphonuclear leukocytes, and the absence of autolysis in caseous foci indicates some inhibitory factor. The explanation for this is found in the presence of the unsaturated lipoids derived from the tubercle bacilli. These are able to bind and inhibit the action of any autolytic ferments that may be present and thereby prevent autolysis. That this is actually the case is readily demonstrated by the fact (*a*) that caseous material when extracted by the lipid solvents will become digestible by trypsin; (*b*) that the lipoids extracted will act as antiferments and will, when injected into normal tissues, cause typical caseous foci. Of equal importance is the fact that when caseous material is treated with iodine, which presumably saturates some of the unsaturated carbon bonds, tryptic digestion can take place.

In general terms, we can consider the tubercle as a necrotic mass consisting of native proteins and of lipoids derived partly from the cells and partly from the tubercle bacilli, together with some of the higher and less diffusible protein split products. Bounding this necrotic mass we have to consider connective tissue, endothelial cells, lymphocytes and a few polymorphonuclear leukocytes; the whole permeated with the tissue fluid, which in man contains only a moderate amount of lipase, some protease and peptidase, and a large amount of antiferment, the latter in an amount quite sufficient to overbalance any ordinary extracellular proteolytic activity. The quiescent tubercle represents a balance between the digestive and digestion inhibitory forces; that is, it serves as a potential source of toxic split products derived from the necrotic material, potential rather than actual, because the active autolysis and removal into the circulation of the products of autolysis is prevented by the antiferment. Any factor that will alter the conditions of this delicate balance so that autolysis can occur will bring about a toxic reaction, that is, a tuberculin reaction. This may be brought about if we increase the ferments of the serum, or decrease the antiferment of the necrotic focus or of the serum. It is apparent that such an alteration need have no relation to specificity.

THE RELATIONS OF THE SERUM ALTERATIONS OF PREGNANCY AND MENSTRUATION ON THE TUBERCULOUS FOCUS

During the past few years we have become familiar with certain of the serum changes that take place during pregnancy. With the aid of this knowledge we can follow the changes that occur in the tuberculous process as a result of these definitely understood serum alterations of pregnancy, in order to throw some light on the reactions involved.

One of the first things that occurs after the onset of the pregnancy is a reduction of the blood and tissue lipases, the fat splitting ferments. We have, as yet, no knowledge as to the source of these ferments, although there is evidence (Stuber) that points to glands of internal secretion as involved in the regulation of the production. As a sequel of this lipase reduction an accumulation of fats and lipoids takes place in the blood stream. This change in the blood lipoids is both quantitative and qualitative, as determined by numerous analyses.¹ Inasmuch as the serum antiferment also consists of these lipoids, those that are unsaturated, we can expect an increase in the antiferment titer of the serum.² This increase is so constant in pregnancy that the test for this antiferment rise has been repeatedly advocated as a test for pregnancy. The increase comes on early, reaches the maximum at the time of labor and then rapidly — from five to seven days — returns to a normal level (Chart 1).

This antiferment rise is probably of decided physiologic importance in raising the threshold of protein metabolism. In 1915, Jobling³ showed that the rate of nitrogen excretion in the starving rabbit was almost inversely proportional to the level of the antiferment titer; that is, if the antiferment was high the rate of protein metabolism was low; if the antiferment was reduced, on the other hand, much nitrogen was excreted. Wilson, in a paper published last year,⁴ describes the metabolism of the pregnant woman in a way that leads one to believe that the relation that Jobling noticed in the rabbit holds good for the pregnant woman, and for a like reason, that is, the increase in antiferment brings about a positive nitrogen balance.

In the particular case charted (Chart 1, after Wilson) a total storage of about 420 gm. of nitrogen took place in the five-months' period of observation. Even if the nitrogen of the birth products

1. Herrmann, E., and Neumann, J.: *Wien. klin. Wchnschr.*, 1912, **25**, 1557. Fränkel, S.: *Wien. med. Wchnschr.*, 1913, **63**, 2198.

2. Franz, R.: *Arch. f. Gynäk.*, 1914, **102**, 79. V. Graff, E., and V. Zubrzycki, J.: *Ztschr. f. Geburtsh u. Gynäk.*, 1912, **72**, 303. Gammeltoft, S. A.: *Gynäk. Rundschau*, 1913, **7**, 543.

3. Jobling, J. W., and Petersen, W. F.: *Ztschr. f. Immunitätsforsch., Orig.*, 1915, **24**, 219.

4. Wilson, Karl: *Bull. Johns Hopkins Hosp.*, 1916, **27**, 121.

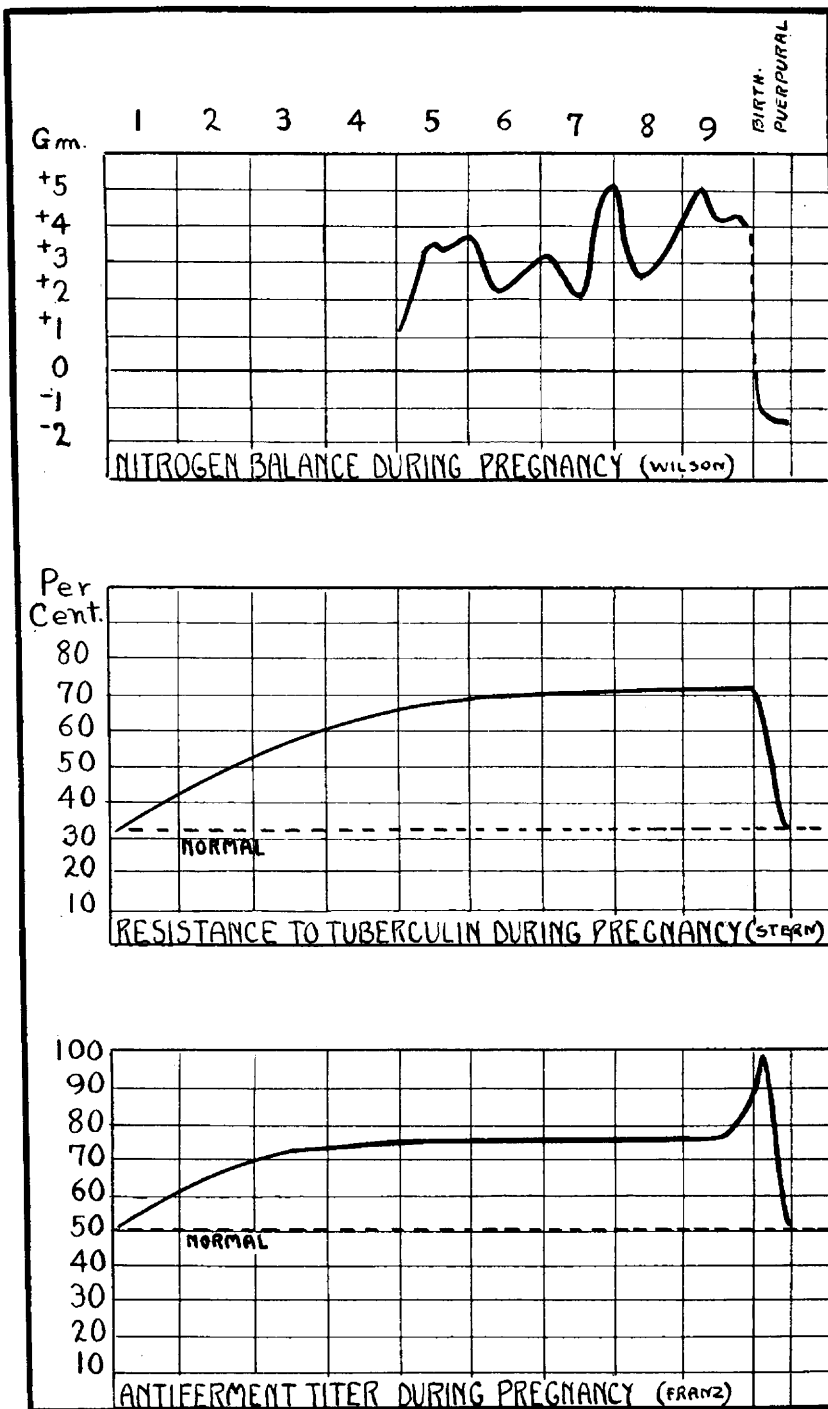


Chart 1.—Relation of the antiferment during pregnancy to the nitrogen metabolism and resistance to tuberculin.

and that estimated for the hyperplasia of the uterus and breasts is subtracted, Wilson considers that fully 285 gm. of nitrogen were actually stored by the maternal organism in this case.

Immediately after birth the nitrogen balance, as indicated in the chart, changes to the negative side, corresponding to the period in the metabolism when the antiferment titer rapidly falls, a time too, when the uterus must be digested back to its normal size.

The sequence of events — lowering of the lipase activity, accumulation of lipoids in the serum, increase in the antiferment titer, checking of protein metabolism, the retention of nitrogen — seems simple and logical.

Coincident with these changes, certain definite alterations occur in the titer of the proteolytic serum ferments: (a) the ereptase or peptidase is increased to from two to four times the normal, throughout pregnancy, and remains at a constant level during delivery and the puerperal period, when uncomplicated; (b) the protease is increased to a small extent early in pregnancy and reaches a maximum immediately after delivery. In the following table the average values for a series of pregnant women gives an idea of this relation (protease).

SERUM DIGESTION (CHLOROFORM METHOD AT 47 C., 24 HOURS)

Before delivery	0.03 mg. per c.c.
1st day following.....	0.08 mg. per c.c.
10th day following.....	0.00 mg. per c.c.

This corresponds in general to the findings of the Abderhalden reaction, which is also augmented immediately after the delivery.

Do these serum changes influence a coexisting tuberculous process? It is an accepted clinical observation that pregnancy, and particularly parturition, are decidedly detrimental to the tuberculous woman, the most pronounced activity developing as a rule immediately after delivery or shortly after the puerperium. Even early in the pregnancy, cases which have been arrested are apt to give evidence, on careful examination, of focal activity while the general symptoms may remain suppressed.

The reaction involving the balance that has been discussed as obtaining in the tubercle may be described somewhat as follows: During the early stages of pregnancy the protease begins slowly to attack the fibrous connective tissue wall of the tubercle, small amounts of toxic material are liberated from the caseous focus, this in turn causing some local reaction on immediately adjacent tissues. This effect of the protease is counterbalanced to some extent by the coincident increase in the antiferment, and also by virtue of the increase in the ereptase as a result of which the body is able to take care of a certain amount of toxic split products. The net result of the altera-

tion of the ferment balance is a condition in which we may have a focal activation but also a more or less complete detoxication of the patient and feeling of well being. This may continue until the time of delivery. At this period two fundamental changes occur: (1) the antiferment is diminished, and (2) a marked mobilization of protease takes place, both changes that favor proteolysis in the body. The more resistant connective tissue is now rapidly digested, partly auto-lyzed toxic split products as well as bacteria are in consequence released and absorbed in some quantity, and the conditions are most

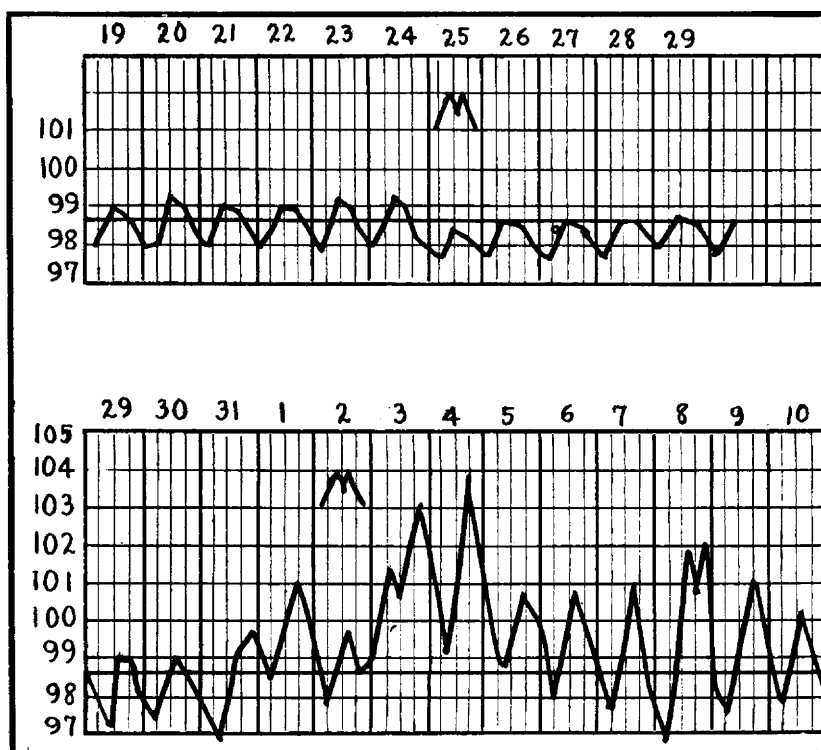


Chart 2.—Effect of menstruation on the temperature curve of the tuberculous woman (after Pottenger). M— day of menstruation.

favorable for the rapid progress of the disease. During this time the ereptase has not increased, and an accumulation of the toxic products may take place because their destruction is delayed. In other words, the ferment-antiferment balance of the serum has been altered to such a degree that the local process is influenced unfavorably.

This explanation along lines of ferment observation coincides exactly with clinical observation and the underlying facts have been established in a number of laboratories.

We may now take another example, less complicated, because in it the antiferment changes do not take place to any extent and we have to deal solely with an increase in the proteolytic ferment. It is known that the Abderhalden reaction becomes positive in every female for a period varying from three days to one week before menstruation, then promptly becomes negative again.⁵ The effect of the menstrual cycle on the temperature of the tuberculous woman is also a well known clinical phenomenon. In incipient cases we usually find a slight increase in temperature just preceding menstruation; in advanced cases a stormy febrile reaction lasting a week or more may be ushered in. The two charts (Chart 2) which are from Pottenger's book,⁶ illustrate such cases. Here again, the proteolytic ferments are evidently able to attack connective tissue, expose the necrotic foci and with the coincident hyperemia wash into the circulations some of the toxic split products that have accumulated.

These two examples — pregnancy and the menstrual cycle — have been made use of here simply to make clear the one fact that we are dealing with conditions in which immunologic factors, as usually understood, are not concerned, so that these temperature reactions and activation of latent foci need bear no relation to antibody reactions, and we may dispense with them for the time being in the discussion of these special conditions.

THE TUBERCULIN REACTION

When Koch introduced tuberculin in the therapy of tuberculosis he did so with the clear-cut idea of inducing an active immunization. Under the method of administration as recommended by him, active immunization did occur. That is, a demonstrable increase in antibodies — bacteriolysins, agglutinins and precipitins — was noted, but despite this augmentation patients frequently went to a fatal termination under circumstances that warranted the belief that the tuberculin treatment had harmed them.

We have at present no clear-cut evidence that resistance to an established tuberculosis is related to antibody concentration of the serum or tissues. Titze⁷ as a result of extensive animal experiments says "nothing speaks with any certainty for the fact that the organism destroys the invading tubercle bacilli through the agency of antibodies

5. Baumann, E.: *Monatschr. f. Geburtsh. u. Gynäk.*, 1915, **42**, 199. Van Waasbergen, G. H.: *Monatschr. f. Geburtsh. u. Gynäk.*, 1915, **42**, 230. Kjaergaard, S.: *Zentralbl. f. Gynäk.*, 1914, **38**, 264. Engelhorn, E., and Wintz, H.: *München. med. Wchnschr.*, 1914, **61**, 689.

6. Pottenger, F. M.: *Pulmonary Tuberculosis*, 1908, William Wood & Co., New York.

7. Titze: *Berl. Tierärztl. Wchnschr.*, 1912, No. 30.

or of phagocytosis . . . healing is rather to be sought in the fixation of the bacilli by the tissues." Haupt⁸ Schürr,⁹ Citron¹⁰ and other investigators have come to the conclusion that resistance to tuberculosis does not parallel the antibody concentration.

If, then, tuberculin therapy is a valuable therapy, its effect probably depends on factors that are not related to the specific antibodies. Bessau¹¹ has recently reviewed the subject in a helpful manner. He calls attention to the fact that the tuberculin reaction is not related to our ordinary conception of specificity or immunity, because, in the first place, we cannot sensitize animals to tuberculin; secondly, that tuberculin contains no native protein — only polypeptids — and finally, that it has been practically impossible to transfer passively the sensitization of tuberculous animals to normal animals (Klopstock,¹² Landmann¹³). Hamburger had recognized the resistance that follows the repeated injections of tuberculin as an antianaphylactic phenomenon; nonspecific, independent of dosage, but depending rather on the reaction induced. Bessau rightly emphasizes the differences between the general and local reactions to tuberculin; in progressive tuberculosis, for instance, the general reaction may become more pronounced while the local reactions may be extinguished, and vice versa. While supposedly a method of active immunization, Bessau declares that the only effect is to produce a tuberculin resistance, which in a nonspecific way may be of benefit.

Aronson¹⁴ concludes as a result of the extensive investigations that he has carried out that the tuberculin reaction does not depend on antibodies; that the general reaction is not specific but is related to the disintegration of leukocytes about tuberculous foci; he concedes that there is an element of specificity in the cutaneous reactions. This latter deduction he draws from his experiments with tuberculin digested with pepsin-hydrochloric acid. This preparation was still able to induce a general but not a local reaction.

To summarize the conclusions of the more recent workers the following would seem to be the concensus of opinion: (a) the clinical course need not be influenced by the antibody concentration; (b) the tuberculin reaction does not depend on the antibody titer of the serum; (c) the general and local reactions are not comparable; (d) and tuberculin does not immunize, but the resistance to tuberculin established

8. Haupt, H.: *Ztschr. f. Tuberk.*, **22**, 209, 363, 463.

9. Schürr, J.: *Deutsch. Arch. f. klin. Med.*, 1912, **109**, 112.

10. Citron, J.: *Deutsch. Arch. f. klin. Med.*, 1913, **110**, 184.

11. Bessau, G.: *München. med. Wchnschr.*, 1915, **62**, 323.

12. Klopstock, F.: *Ztschr. f. exper. Path. u. Pharmacol.*, 1914, **15**, 13.

13. Landmann: *Deutsch. med. Wchnschr.*, 1912, **38**, 1245.

14. Aronson, H.: *Deutsch. med. Wchnschr.*, 1914, **40**, 487.

during tuberculin treatment may possibly influence the existing tuberculosis in a nonspecific manner.

Two further experiments firmly establish these deductions. Bail showed that if a tubercle is implanted in a normal animal it at once reacts to tuberculin with a general reaction, that is, before either sensitization or immunization could possibly occur. More recently Klemperer¹⁵ has definitely excluded both the cellular and humoral antibody complex in the tuberculin reaction when he demonstrated that, when in animals the single tubercle is extirpated, the tuberculin reaction is extinguished at the same time. This experiment is undoubtedly of primary importance.

Before entering into a theoretical discussion of the possible mechanism involved it may be well to briefly review certain facts in regard to tuberculin. The first of these concern the question as to the specificity of the reaction.

SPECIFICITY

Feistmantel extracted an acid-fast streptothrix and obtained an active tuberculin. On the other hand, leprous and actinomycotic patients are said to react strongly to tuberculin. The tuberculous individual will react to many of the following substances injected either subcutaneously or intravenously with a typical tuberculin reaction and constitutional symptoms, while the nontuberculous individual will tolerate equal doses without reaction; these include the following: Hypertonic salt solution; distilled water; iodids; some colloidal metals; protein split products; ferments; immune (tuberculous) serum; heterologous serums; exudates; photodynamic insults (heliotherapy, roentgen rays, deep red rays, etc.).

Nor must it be supposed that this nonspecificity is limited solely to the general tuberculin reaction. Tenzer,¹⁶ for example, noted that in 73 children, of whom 48 reacted positively to the von Pirquet, 22 gave a comparable reaction with Witte peptone. In another series of 69, of whom 34 reacted positively to the von Pirquet, 19 gave a reaction with cholera vaccine used in place of the tuberculin. These nonspecific reactions occurred only in the von Pirquet positive children. There is here distinct evidence that an increased susceptibility of the skin obtains which is wholly independent of specific factors. Petersen¹⁷ also calls attention to this fact, and Burnet¹⁸ has studied the relation of the skin and general reactions in the lower monkeys, noting that while the general reaction might be positive in these animals during all stages of

15. Klemperer, F.: *Beitr. z. klin. d. Tuberk.*, 1914, **30**, 431.

16. Tenzer, E.: *Monatschr. f. Kinderh.*, 1911, **10**, 131.

17. Petersen, H.: *Hospitalstid.*, 1912, **5**, 421.

18. Burnet, E.: *Compt. rend Soc. de biol.*, 1912, **72**, No. 28.

the disease, the skin reactions were negative throughout all stages of sensitization and disease. Observations such as these led Torrenson¹⁹ to use Witte peptone instead of tuberculin in the treatment of tuberculosis of the skin; his results, carried out on a limited number of patients, were satisfactory.

Experiments carried out by Matthes²⁰ more than twenty years ago were along similar lines. Matthes injected small amounts of albumoses into tuberculous guinea-pigs and found that a violent focal reaction occurred about each tubercle, followed by profound intoxication, loss of temperature and death in a short time. In the normal animal only a slight and transient rise in temperature was observed. These studies, really of the utmost importance in the study of the mechanism of the reaction, have been generally ignored.

Probably of equal importance is the fact that the von Pirquet reaction is never augmented by immune tuberculosis serum when it is added to the tuberculin immediately before, or when incubated with the tuberculin before inoculation (the serum may be derived from immune animals or from patients in the various stages of the disease, with or without tuberculin treatment), but is invariably delayed when such serums are added. (Petrova,²¹ Aronson,¹⁴ etc.) The only activating substances are lipoidal in nature as described by Bing and Ellermann.²²

We are probably justified in concluding that the general reaction to tuberculin rests on factors largely nonspecific and that a nonspecific element enters also into the cutaneous tests. When we turn to survey the factors that render the tuberculin reaction negative we find that this resistance is wholly nonspecific.

INHIBITION OF THE TUBERCULIN REACTION

Von Pirquet observed that during measles and streptococcus infections the tuberculin reactions became negative; Brandenburg²³ found this to be true for scarlet fever, and Krannhals²⁴ as well as Glintschikow²⁵ observed the same condition in typhoid, pneumonia and acute articular rheumatism. Cozzolino,²⁶ working with pertussis and Moltchanoff²⁷ with diphtheria and serum sickness, also observed this phenomenon. The resistance to tuberculin during and following serum

19. Torrenson, E. G.: *Abst., Ztschr. f. Immunitätsforsch.*, 1912, **5**, 1020.

20. Matthes, M.: *Deutsch. Arch. f. klin. Med.*, 1894-1895, **54**, 39.

21. Petrova, M. K.: *Abst., Ztschr. f. Immunitätsforsch.*, 1914, **6**, 1014.

22. Bing, H. J., and Ellermann, V.: *Biochem. Ztschr.*, 1912, **42**, 289.

23. Brandenburg, F.: *Deutsch. med. Wchnschr.*, 1910, **36**, 561.

24. Krannhals: *München. med. Wchnschr.*, 1910, **57**, 836.

25. Glintschikow, W. J.: *Abst., Ztschr. f. Immunitätsforsch.*, 1916, **8**, 509.

26. Cozzolino, O.: *Abst., Ztschr. f. Immunitätsforsch.*, 1914-1915, **8**, 310.

27. Moltchanoff, W. T.: *Jahrb. f. Kinderh.*, 1912, **75**, 434.

reactions in children was confirmed by Luithlein.²⁸ We are evidently dealing here with a general state of resistance to the local and in part the general tuberculin reactions during practically all the acute infections, during certain of the cachectic conditions, and following protein shock reactions (serum reactions).

In order to examine this relation to the problem before us we may revert to the second figure or curve in Chart 1 and study the material collected by Stern.²⁹ Stern determined that 65 per cent. of the women at his clinic (nonpregnant) gave a positive tuberculin reaction, that this percentage in the pregnant women decreased progressively to term and that immediately following delivery the percentage reacting positively began to increase until in women in the fifth and sixth day of the puerperium 67 per cent. gave the reaction. We have here definite evidence that a progressive resistance occurs during pregnancy and that this resistance vanishes in a surprisingly short period of time after delivery. It will be observed in the chart how closely this relation parallels the rise in the antiferment titer. Stern was familiar with the increase in the blood lipid content during pregnancy¹ and suggested that the resistance was due to a binding of the antibodies to these lipid bodies. It is apparent from these observations that the resistance to the tuberculin reaction (both general and local) need not depend on specific factors as might be surmised were we to observe it exclusively following repeated tuberculin injections.

EFFECT OF TUBERCULIN ON THE NONTUBERCULOUS ANIMAL

We are more or less familiar with the effect of tuberculin on the tuberculous organism and are apt to overlook the fact that tuberculin may develop marked reactions in the metabolism of the normal animal. That this is the case has been demonstrated by Mircoli.³⁰ Mircoli used tuberculin and also tubercle bacilli (killed) and injected these in small doses into normal experimental animals. The injections were followed by a short negative phase (especially when the dose was large) during which time the animals lost weight as compared to the controls; this was followed by a longer period during which the weight of the treated animals increased over that of the control animals. When repeated small doses were given, resistance developed, and the negative phase diminished, the animals showing a decided gain in weight as compared with the untreated animals.

28. Luithlein, F.: *Wien. klin. Wchnschr.*, 1914, **27**, 493.

29. Stern, R.: *Ztschr. f. Geburtsh. u. Gynäk.*, 1910, **66**, 532.

30. Mircoli, St.: *Pathologica*, 1914, **5**, 118.

THE DIRECT EFFECT OF TUBERCULIN ON AUTOLYSIS

Finally, it has been observed that tuberculin can influence autolytic processes directly. Pesci³¹ added tuberculin (and other toxins) to autolyzing organ emulsions and found that the rate and magnitude of the process was increased in a degree proportional to the amount of tuberculin added. This effect seems to have some relation to the lipoids of the substrate. It is known that the lipid solvents, including alcohol, will, when added to autolyzing organs, reduce the latent period of autolysis, presumably by destroying a lipid-protein combination that normally resists autolysis (Chiari³²), and which, under ordinary conditions, is only destroyed when a sufficient degree of acidity has developed. Pesci observed that in his tuberculinized substrate the neutral fats and fatty acids tended to decrease within fifteen minutes while the soaps increased, as compared to the normal autolysis. Barlocco³³ observed a similar lipid rearrangement during autolysis when diphtheria toxin had been added to the substrate.

THEORETICAL CONSIDERATIONS

The facts which have accumulated as a result of both experimental and clinical investigation make it apparent that we must seek an explanation along lines not necessarily associated with the usual conception of immunity. In the first part of this paper evidence has been presented which indicates that the proteolytic ferment and the anti-ferment exercise a considerable influence on the tuberculous process. Is it possible that changes in the ferment-antiferment balance may be responsible for some of the phenomena observed in the tuberculin reaction?

We must keep in mind that tuberculin is not a native protein, but consists largely of polypeptids and must essentially be a toxic substance; as Turban says: "A preparation which is nontoxic and yet potent, is unknown." There is evidence that the activity of the various tuberculins is in proportion to their surface tension, which, in so far as tissue effect is concerned, simply means that the more diffusible products are the more toxic.³⁴

In its essentials the tuberculin injection might be supposed to lead to the changes which we associate with the typical protein "shock" reaction, that is, it would result in (*a*) a mobilization of the proteolytic ferments, (*b*) in a primary reduction followed by a subsequent increase in the antiferment titer, and (*c*) in the development of a non-

31. Pesci, G.: *Pathologica*, 1911, **3**, 144, and *Zentralbl. f. Bakt.*, Part 1, Orig., 1911, **59**, 186.

32. Chiari, Rich.: *Arch. f. exper. Path. u. Pharmacol.*, 1908-1909, **60**, 257.

33. Barlocco, A.: *Pathologica*, 1911, **3**, 7.

34. Kollert, Victor: *Beitr. z. Klin. d. Tuberk.*, 1914, **30**, 173.

specific resistance comparable to the "antianaphylaxis" or the "desensitization" of the immunologist. Graphically, these changes can be illustrated as in Chart 3; that is, following the injection we might expect a period when proteolytic activity is favored — lowering of the anti-ferment and increase in the protease — followed by a zone of several days' duration during which time the reverse holds true. During this latter time the ereptase might be augmented, which theoretically would aid in detoxication by eliminating the higher split products.

We know that the use of small doses of tuberculin at definite or varied time intervals has been established in routine as the result of a vast accumulation of clinical experience, largely empirical, and we are

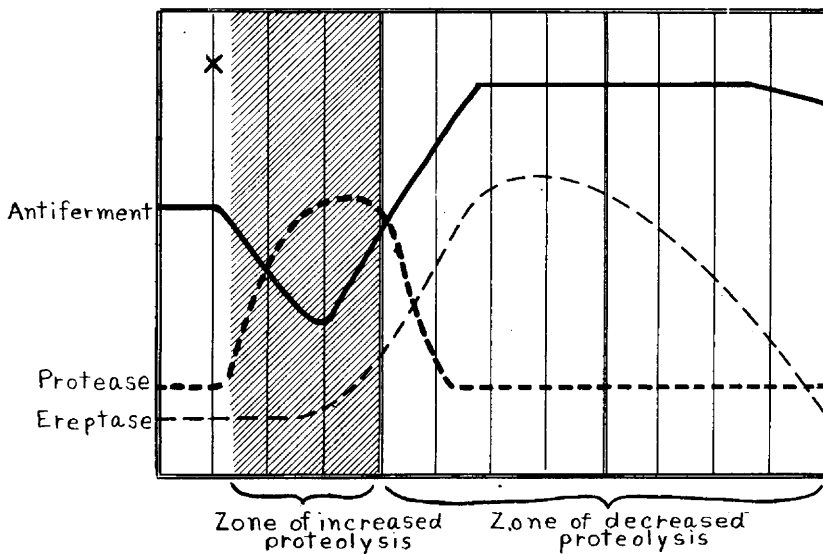


Chart 3.—Schematic relation of serum ferment and anti-ferment following tuberculin injection.

justified in believing that it represents the most favorable method of its use. With this method we can undoubtedly produce a tuberculin resistance or tolerance in the tuberculous individual; that is, as the result of repeated stimulation of this kind the organism becomes more and more resistant to intoxication. It has been the general impression that, because anaphylactic phenomena are so strikingly specific, the resistance following shock is equally specific. This, however, is not true. The most recent work, especially that of Bessau and his associates,³⁵ has shown that antianaphylaxis is practically nonspecific. The state of desensitization, or the refractory period following anaphy-

35. Bessau, G., Opitz and Preusse, O.: *Zentralbl. f. Bakt.*, Part 1, Orig., 1914, **74**, 162, 310.

lactic shock, occurs also following the injection of anaphylatoxins and similar protein poisons; Vaughn noted the same general phenomenon when injecting his protein split products; repeated sublethal doses increasing the resistance until one or possibly two times the fatal dose could be tolerated.

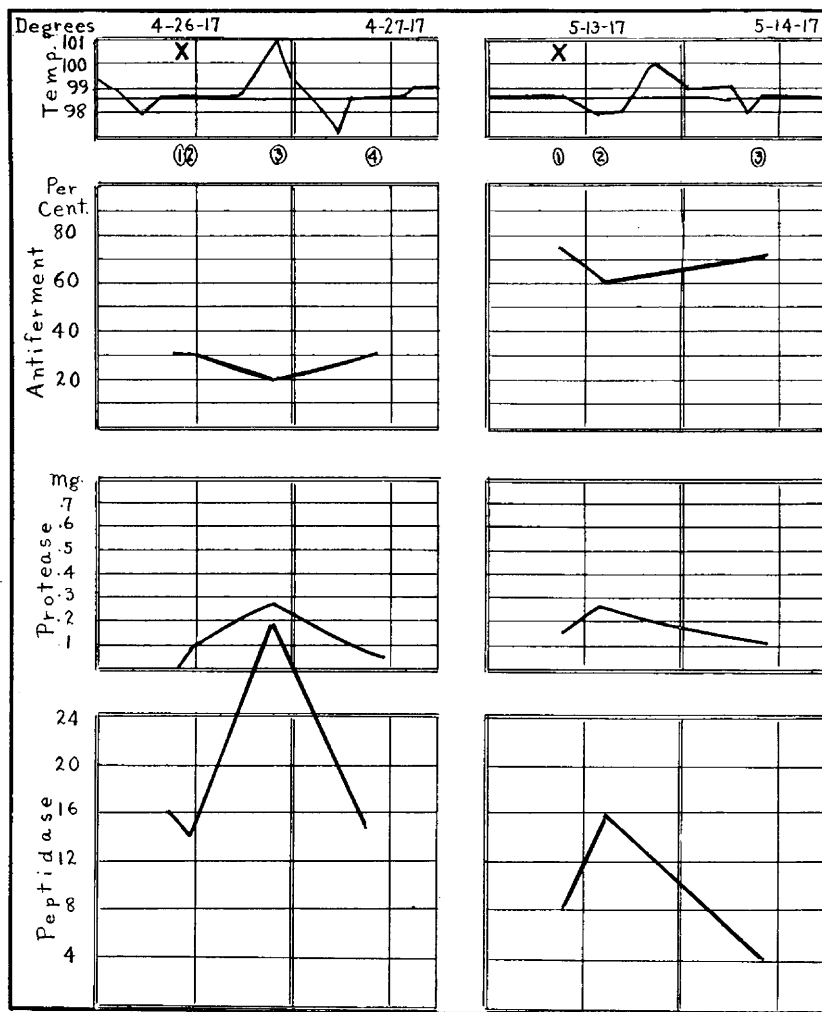


Chart 4.—Effect of tuberculin injection on serum ferment and antiferment.

It had been observed, first by Rusznjak³⁶ that the serum antiferment titer was markedly augmented after anaphylactic shock, to which fact Rusznjak in part attributed the increased resistance to the refractory

36. Rusznjak, S.: *Deutsch. med. Wchnschr.*, 1912, **38**, 168.

period. Jobling³⁷ made similar observations following a variety of protein shock poisons and showed, furthermore, that when the antigen was mixed in vitro with an antiferment before injection into the animal, anaphylactic shock could in many instances be averted. It seems reasonable at any rate to assume that where we are dealing with an intoxi-

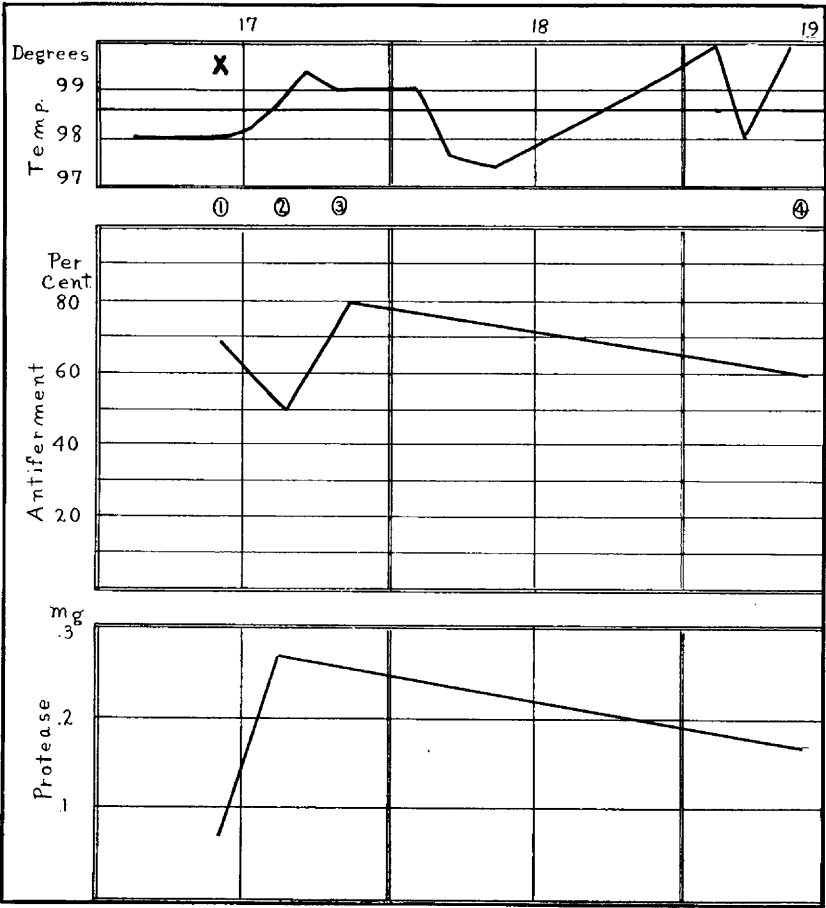


Chart 5.—Serum alteration following tuberculin injection.

cation having its origin in protein splitting in the body, an increase in the antiferment, that is, in those substances which inhibit or delay proteolytic activity, would be one of the factors involved in this resistance.

We could assume that the tuberculin reaction takes place somewhat as follows: When tuberculin is injected subcutaneously a certain number of cells are injured and some protease is liberated. This, just as it

37. Jobling and Petersen: Jour. Exper. Med., 1914, **20**, 468.

takes place physiologically during the menstrual period, is carried to the tuberculous focus, and, by digestion there, produces the typical focal reaction and with it a liberation of toxic material and general malaise. We must keep in mind, too, the possibility of the direct activating influence of the tuberculin on autolytic processes. At any rate we deal not with a condition of sensitization of the tissues of the body as a whole, but solely with the slight breaking down of tuberculous tissue at some point, the degree depending on the relative amount of ferment mobilized, on the relative vascularization, and on the amount of connective tissue that protects the focus. Thus, the reaction would be negative in the nontuberculous, for here, when a ferment mobilization does occur it finds no pathologic focus to attack, no protein is broken down and no febrile reaction occurs. In the tuberculous patient we have, furthermore, a cumulative effect when the ferment-antiferment balance is altered, because the liberated toxic material will add a secondary protein shock which is lacking in the normal person.

While it is apparent from these considerations that we may be dealing with a reaction that is nonspecific, in that the means used to elicit it are not specific, it is immediately apparent that the reaction itself resulting from the stimulation of the tuberculous focus implies a specific stimulation in the true sense of the term, in that disintegrating bacilli, and possibly even living bacteria, are absorbed from the focus, whether that focus is stimulated as a result of heliotherapy, of immune serum, of iodine or of tuberculin. As far as immunologic investigation has gone it seems certain that immunization brought about as the result of the incorporation of whole organisms, either living or dead, has given the most satisfactory results in protection experiments. It is essential at any rate to keep in mind this dual effect of the tuberculin reaction, the specific phenomena following in the wake and as a result of the effect of the nonspecific reaction.

REPORT OF EXPERIMENTAL CASES

CASE 1.—F. L., male, white, aged 18 years, had tuberculosis of the kidney (kidney removed six months previously). Cystoscopic examination revealed tuberculosis of the bladder. There was no active pulmonary involvement. One mg. old tuberculin was given subcutaneously at 10 a. m. Serum taken at 10 and 11 a. m., 10 p. m. and 10 a. m. the following day. The examination of the serum revealed the changes in the ferment titer charted in Chart 4, A.

It will be noted that the antiferment titer was diminished by evening, when the maximum temperature was recorded. The protease increased from 0 to 0.27 mg. per c.c. during this time, while the peptidase, which was originally fairly strong, increased to about twice the original titer. This increase was not maintained the following day. Two weeks later a second injection (2 mg. O. T.) was made and similar serum alterations followed this injection (Chart 4, B).

CASE 2.—H. V., man, white, aged 52, had unilateral pleurisy with a large amount of hemorrhagic exudate, which was withdrawn by puncture. The clinical course was afebrile, with occasional periods of temperature to 100 F. for one or two days. He was given 1 mg. O. T. subcutaneously. No increase

of temperature was observed, but considerable malaise and headache followed. The serum alterations have been recorded in Chart 5. There was a distinct decrease in the antiferment for several hours after the injection, then a slight increase in the evening. The protease increased following the injection. No clinical effect was observed from the small dose used.

For comparative purposes the following two cases are of interest:

CASE 3.—J. R., a white man, aged 45 years. Physical findings revealed bilateral pulmonary involvement with cavity formation, but absence of tubercle bacilli in the sputum. There was a considerable range of temperature, marked cough and expectoration. He was given 1 mg. O. T. subcutaneously. This

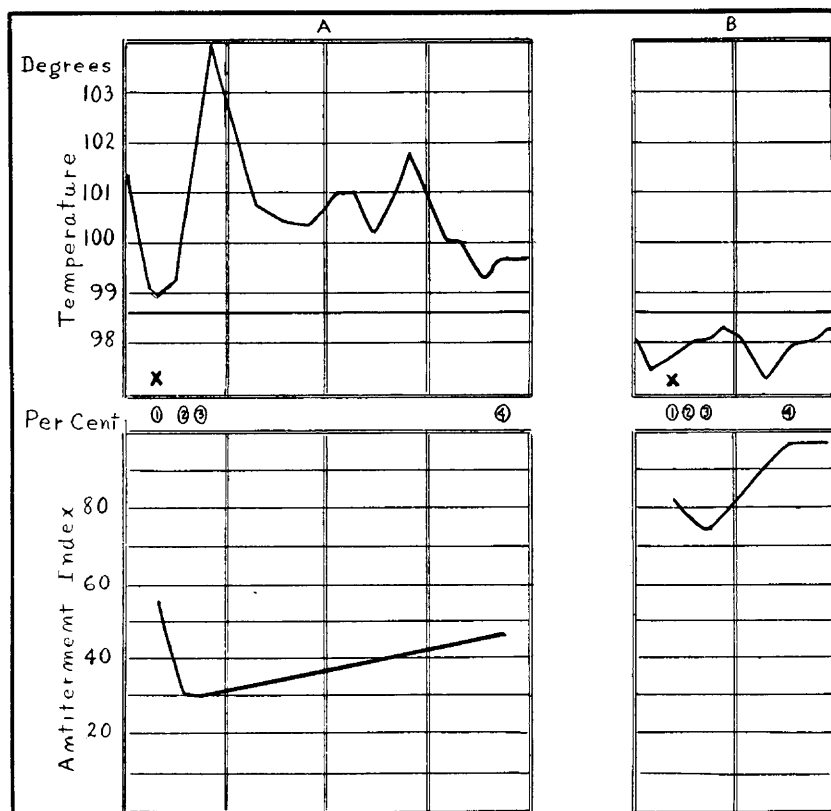


Chart 6.—Antiferment alteration in case unfavorably (a) and favorably (b) influenced by tuberculin.

was followed by a rise in the temperature as well as physical findings of focal activation, which continued for several days. Clinically this patient was not improved after the injection; indeed, the general feeling of malaise and discomfort seemed increased.

When the antiferment curve is examined (Chart 6, A) it will be observed that the decrease persisted for a considerable time and even after three days had not returned to the original titer. When contrasted with the following case this fact is of interest.

CASE 4.—F. B., a white man, aged 40, with arrested bilateral apical lesions and general visceroptosis, entered the hospital because of gastric distress. The temperature curve was persistently subnormal. He was given 2 mg. O. T. subcutaneously, followed by no temperature or constitutional effect. It will be noted that the antiferment (Chart 6, B) after the initial decrease, reached a level that was considerably higher than before the injection.

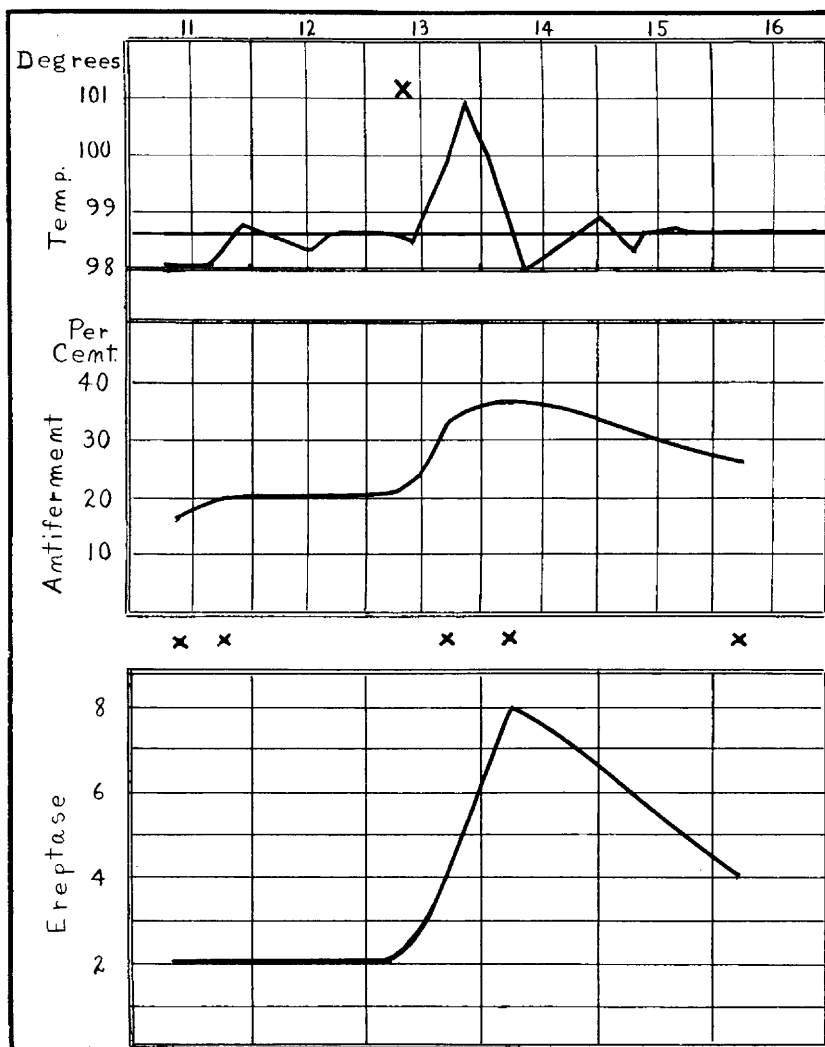


Chart 7.—Serum alteration following tuberculin injection.

The serum changes that occur following the injection in a fairly active case with well defined clinical improvement following the injection are illustrated in Chart 7.

CASE 5.—O. V., a white man, aged 24, had tuberculosis of the peritoneum. He entered the hospital with a considerable febrile temperature range, emaciated, and with a large exudate in the peritoneal cavity, which was removed at two separate times. With rest the patient improved progressively until the temperature remained practically normal. At this time he was given 1 mg. O. T. subcutaneously which was followed by a sharp temperature reaction (101 F.). Blood samples had been collected at several periods previous to the injection; they were also taken in the evening of the day of injection, the following morning and on the third day following.

It will be observed that the antiferment increased considerably and that the rise persisted for at least three days after the injection. The protease was not determined but the ereptase increased after the injection and continued at a high level for the day following. The patient said he felt improved, and objectively seemed so.

In several syphilitic cases the serum alterations following the tuberculin injection (2 to 5 mg. O. T.) have been similar in character.

In fifteen cases in which the serum reaction has been studied following tuberculin injection, of which the charted cases are representative, either one or more of the ferment-antiferment changes suggested as possibilities in the theoretical discussion, have been observed. The decrease in the antiferment has been found to be the most constant alteration; in the cases unfavorably influenced by the tuberculin no increase followed the original decrease; in the cases clinically improved a well-marked rise usually follows. The changes in the ferments — protease and ereptase — are less constant, but usually at least one of these ferments makes its appearance, the ereptase in particular being increased in the cases which give evidence of benefit from the reaction.

It might seem warranted to consider the tuberculin reaction as a two-phase phenomenon in the tuberculous individual. The primary alteration of the ferment-antiferment balance brings about a medium favorable for proteolysis in and about the tubercle. Digestion and the liberation of toxic material result and are reflected in the constitutional effects. In the nontuberculous individual it is probable that the primary serum alterations also occur, but the digestive ferments, finding no focus to attack, liberate no toxic material and no general reaction is elicited.

CORRELATION

From the evidence presented it seems probable that the ferment mobilization, however produced, will influence the tuberculous focus and bring about a general reaction if the digestion be of sufficient degree. From this point of view we can understand the undeniable lack of specificity associated with the general reaction; any agent that will bring about a ferment-antiferment balance favorable for proteolysis will effect a general reaction provided the focus be sufficiently unstable. That the various vaccines, protein split products and even

inert physical agents will do this has been demonstrated.³⁸ Similarly, Pfeiffer³⁹ observed such changes following burns of varying intensity, making it apparent why, when a patient during the course of heliotherapy burns to a moderate degree, a tuberculin reaction with focal and general effects may result. Conversely we can understand that in any infectious granulomas in which a balance exists similar to that obtaining in the tubercle, the injection of tuberculin will be followed by a marked febrile reaction.

If we assume that proteolytic factors enter also into the local cutaneous reactions, then an increase in the antiferment would operate to oppose such a reaction and render it negative. This seems evident when we keep in mind the increase in the antiferment that takes place during the course of a physiologic process (pregnancy), during disease (acute infections) and following induced protein "shock" or tuberculin reactions, and the inhibition of the tuberculin reactions during these conditions. In the late stages of tuberculosis this same increase in antiferment is observed (coincident with the decrease in the lipases Marutaew,⁴⁰ Bauer⁴¹), and the local reactions to tuberculin become less evident while the general reaction may become more severe, because of the lability of the numerous foci. The participation of the proteolytic ferments in the local reactions is also made probable in that any antiferment added to the tuberculin before its local application (sodium oleate and serum) will invariably delay the reaction. The recent work of Sherrick,⁴² of Stokes⁴³ and of Burroughs and Neymann⁴⁴ will, however, probably materially modify our present conception of skin reactions, so that any discussion at present is out of question in this particular domain. Sherrick observed that he could obtain a positive luetin reaction in all iodized patients, and that the injection of agar and starch gave a reaction as well. He noted that a patient who under normal conditions reacted to the luetin with a diffuse areola went on to complete pustule formation under iodids. This work has been repeatedly confirmed.⁴⁵ For reasons given early in this paper we assume that the iodine and iodide act therapeutically by lowering the antiferment of the blood and tissues when given in gradually increas-

38. Jobling, Petersen and Eggstein: *Jour. Exper. Med.*, 1915, **22**, 597.

39. Pfeiffer, H.: *Ztschr. f. Immunitätsforsch., Orig.*, 1915, **23**, 473.

40. Marutaew, A. S.: *Abst., Ztschr. f. Immunitätsforsch.*, 1913, **7**, 90.

41. Bauer, J.: *Wien. med. Wchnschr.*, 1913, **36**, 2197.

42. Sherrick, J. W.: *The Effect of Potassium Iodide on the Luetin Reaction*, *Jour. Am. Med. Assn.*, 1915, **65**, 404.

43. Stokes, J. H.: *Jour. Infect. Dis.*, 1916, **18**, 402.

44. Burroughs, M. T., and Neymann, C. A.: *Jour. Exper. Med.*, 1917, **25**, 93.

45. Kolmer, J. A., Matsunami, T., and Broadwell, S.: *The Effect of Potassium Iodide on the Luetin Reaction*, *Jour. Am. Med. Assn.*, 1916, **67**, 718. Kolmer, J. A., Matsunami, T., and Immermann, S., and Montgomery, C. M.: *Jour. Lab. and Clin. Med.*, 1917, **11**, 401.

ing doses,⁴⁶ and from this point of view the observations made by Sherrick and others are readily understood, in that a lowering of the threshold of proteolysis would bring about a condition when instead of the luetin producing a simple inflammatory reaction, the process would go on to complete pustule formation and necrosis when the anti-ferment "brake" was released. The observation of Burroughs and Neymann is of equal importance in that they have demonstrated that amino-acids in sufficient concentration may be toxic for cells. Inasmuch as the rate of diffusion in the cutaneous tissues is slow, it is readily understood that a sufficient concentration of such lower split products might accumulate and result in toxic manifestations quite different from those observed when dealing with the organism as a whole.

The alterations following tuberculin therapy probably bring with them two general effects: (a) the rate of nitrogen metabolism is decreased and a storage of nitrogen may result, as indicated in the work of Mircoli; (b) the reaction, even if local and without apparent constitutional effect, increases the anti-ferment gradually and in this way not only increases the resistance to following injections, but increases the resistance at the focus against digestion and intoxication.

With this idea in mind one can understand why many clinicians have, as a result of clinical experience alone, come to realize that in tuberculin therapy they are not dealing with a specific effect and are not primarily immunizing the patient against the tuberculous infection. The nonspecificity of the reaction that follows tuberculin injections has recently been taken advantage of by Browning⁴⁷ in dealing with patients highly susceptible to tuberculin, who nevertheless insisted on tuberculin treatment. In such cases Browning has found that by interpolating several doses of typhoid and other vaccine, he was able to increase the following tuberculin doses very materially without undue reaction on the part of the patient.

It is interesting to recall in this connection that tuberculin can be used interchangeably, to a certain degree, with other agents that bring about an alteration in the ferment-anti-ferment balance and thereby induce therapeutic effects. One needs but mention the use of tuberculin in syphilis (Biach⁴⁸) and the effect said to be noted in paresis (von Wagner⁴⁹). The results of von Wagner have been confirmed from many sources (Döllken,⁵⁰ Battistessa⁵¹ and Jukow⁵²). We have, fur-

46. Jobling and Petersen: The Therapeutic Action of Iodin, *THE ARCHIVES INT. MED.*, 1915, **15**, 286.

47. Browning, C. C.: Los Angeles (Personal communication).

48. Biach, M.: *Wien. klin. Wchnschr.*, 1915, **28**, 1345.

49. Von Wagner: *Wien. med. Wchnschr.*, 1909, **59**, 2125.

50. Döllken: *Berl. klin. Wchnschr.*, 1913, **50**, 962.

51. Battistessa, P.: *Riv. ital. di Neuropatol. psichiat. ed elettroter.*, 1912, **5**, 117.

52. Jukow, N. A.: *Abst., Ztschr. f. Immunitätsforsch.*, 1913, **7**, 558.

thermore, to keep in mind the well-known phenomenon of the activation of tuberculous lesions in diseases associated with an increased amount of proteolytic ferments in the serum, as, for instance, in dementia praecox and in carcinoma, Lubarsch having called attention to this latter fact.

ANIMAL EXPERIMENTATION

Inasmuch as practically all of the experimental work in tuberculosis has been done on the smaller experimental animals (guinea-pigs and rabbits) it may be well to discuss briefly the relation of the serum ferments in these animals and the effect on the tuberculosis problem.

Guinea-pig serum (and also rabbit serum, to a lesser degree) differs widely from the human in containing much less antiferment, more lipase and vastly more proteolytic ferment — both protease and ereptase. Under such conditions we should expect that the connective tissue fixation and encapsulation would be made more difficult because of the pronounced digestive effect of the serum. On the other hand, abscess formation might be expected to lead to caseation even with nontuberculous processes (the leukocytes of the guinea-pig contain no leukoprotease) if the permeability of the focus is not sufficient to permit the free entrance of serum. These are of course the actual conditions to be observed in pathologic conditions in these animals and constitute one of the chief reasons why the results of animal experimentation cannot be applied with any degree of certainty to the tuberculosis of man.

It is only when serum alterations are produced in the guinea-pig so that it resembles the condition in man that the picture of the tuberculous process offers a similarity to that of the human form. Helen Baldwin and Elise L'Esperance⁵³ have recently published observations that are of interest in this connection. By producing an occasional protein shock (with typhoid vaccine) they found that the treated animals gained in weight over the control animals, and that the tuberculous lesions showed a marked fibrosis, an appearance quite unusual in the tuberculosis of these animals. The results are probably to be accounted for by the decided increase in the antiferment following such typhoid injections, which would tend to preserve the connective tissue encapsulation and thus aid in the fixation of the bacteria.

Experiments such as these serve to bring out the fact that a sharp difference exists between an immunity against the establishment of an infection and the resistance to an infection already established. The guinea-pig, practically immune to spontaneous tuberculosis, possibly just because of the abundance of the serum ferments, by this very fact is enabled to offer little resistance once the infection is established,

53. Baldwin, Helen, and L'Esperance, Elise: *Jour. Immunol.*, 1917, **2**, 283.

because the unfavorable serum balance prevents the fixation of the bacteria by connective tissue. Under such conditions immunization per se will have no practical effect on the development of an established infection, whereas a protein "shock" reaction as carried out by Baldwin and L'Esperance does seem to influence the pathologic picture to a considerable degree, offering one of those instances in which therapeutics along nonspecific lines may possibly offer more benefit than therapy carried out with strictly specific end objects.

THE RELATION TO THERAPEUSIS

The probable basis for the therapeutic effect of tuberculin has been discussed along the lines of the ferment alterations. Clinical use has varied from overindulgence to underindulgence, with the final establishment of the small dose in ordinary routine. There is, however, some evidence that occasional larger doses, with constitutional effects, may in certain instances be of greater benefit than the continual use of the very minute doses commonly in use. Such authorities as Bandelier and Roepke,⁵⁴ for instance, call attention to the feeling of well being and the objective clinical improvement that may follow such a general reaction in some individuals. The difficulty in predetermining just which cases may so react probably makes the current method of administration more suitable for routine use.

It may be of interest to note in how far more or less established empirical therapeutics has followed along lines that influence the ferment-antiferment balance. This can be divided into two general groups, the first having to do with fats and lipoids of various kinds. Czerny⁵⁵ in a very interesting paper has presented evidence that the immunity of the nursing infant to many of the ordinary infections is not due to immune bodies furnished in the milk of the mother, but is closely related and dependent on the fat constituents of the milk, which in some manner augment the resisting power of the infant. Among empirical remedies used in tuberculosis, fats have played a large rôle, including the highly unsaturated fish oils, milk, cream and the yolk of eggs; the use of phosphorus must be included in this category.⁵⁶ In the consideration of the value of just these substances it is interesting to recall that Fermi⁵⁷ noted the antiferment property of milk and eggs many years ago, and it can be demonstrated experimentally that the antiferment of the lymph is gradually increased after a meal of milk.

54. Bandelier and Roepke: *Lehrbuch der spec. Diag. u. Therap. d. Tuberk.*, Ed. 8, Würzburg, 1915.

55. Czerny: *Med. klin.*, 1913, 895.

56. Frank, L., and Schloss, E.: *Jahrb. f. Kinderh.*, 1914, **79**, 539.

57. Fermi, C.: *Centralbl. f. Bakteriöl.*, Part 1, Orig., 1909, **50**, 225.

Braunstein and Kepinow⁵⁸ found that phosphorus increased the anti-ferment, and the increase in the anti-ferment following the continued use of the unsaturated oils seems logical considering the probable constitution of the anti-ferment lipoids. The feeding experiments of Weigert⁵⁹ are also of interest in this connection.

It is not within the range of this paper to discuss the relation of the lipases to the tuberculous infection, although the subject is intimately bound up with this particular phase of therapy and as such has been discussed by the Russian workers,⁶⁰ who seem to have devoted considerable study to the therapeusis of tuberculosis by means of the fat substances. Briefly, this therapy might be described as a method to increase the anti-ferment, to check proteolysis at the focus, and aid in the conservation of the connective tissue of the scar.

The second school, the foremost advocates of which we find among the French clinicians, has placed greater reliance on iodine and its compounds, but chiefly on free iodine.⁶¹ Apparently such therapy has decided value in glandular tuberculosis, and with apparent reason. By this method of therapy the anti-ferment is probably reduced⁴⁶ and the connective tissue reaction about the focus lessened so that a slow and gradual exposure of the focus follows. If this focus be in lymphatic tissue where lipolytic activity is provided by the lymphocytes, the tubercle bacilli are probably destroyed when they are exposed to such activity; if the focus is in pulmonary or other nonlymphatic tissue the effect of the iodine may simply be in the nature of an autotuberculinization, in which digestion is first aided, then checked by the anti-ferment reaction that follows the digestion. Theoretically, this seems ideal therapy in the early case in which the body is able to withstand a certain amount of intoxicating material gradually absorbed.

If in this paper certain of the ferment and anti-ferment changes have been dwelt on, it has not been done with the idea that they represent the exclusive factors that effect the tubercle in its relation to the host. The digestive factors are at best but one of the many complex balances that have a rôle in the adjustment of the infected organism to the parasite, but having such a rôle, it may be well to keep the effect of these ferments and the anti-ferment in mind when we wish to study the effect of therapeutic measures on the pathologic process.

58. Braunstein and Kepinow: *Biochem. Ztschr.*, 1910, **27**, 170.

59. Weigert: *Berl. klin. Wchnschr.*, 1907, **44**, 1209.

60. Metchnikow, S. J.: *Ztschr. f. Immunitätsforsch., Orig.*, 1914, **22**, 235.

61. Barbier: *Ztschr. f. Tuberk.*, 1914, **22**, 433.