On the nature, action, and therapeutic value of the active principles of tuberculin : an investigation / by William Hunter.

With the Author's Compliments

9.

ON THE

Nature, Action, and Therapeutic Value of the Active Principles of Tuberculin.

AN INVESTIGATION.

BY

WILLIAM HUNTER, M.D., M.R.C.P., F.R.S.E.,

Assistant Physician to the London Fever Hospital;
Research Scholar of the Grocers' Company.

(FROM THE LABORATORY OF THE ROYAL COLLEGE OF SURGEONS OF ENGLAND.)

[Reprinted from the "British Medical Journal," July 25th, 1891.]

London:
JOHN BALE & SONS,
87-89, GREAT TITCHFIELD STREET, OXFORD STREET, W.

1891.

On the Nature, Action, and Therapeutic Value of the Active Principles of Tuberculin.

An Investigation

By WILLIAM HUNTER, M.D., M.R.C.P., F.R.S.E.

Assistant Physician to the London Fever Hospital; Research Scholar of the Grocers' Company.

(From the Laboratory of the Royal College of Surgeons of England.)

Koch's remedy for tuberculosis is a glycerine extract of pure cultivations of the tubercle bacillus. The remarkable properties possessed by it, unfortunately for evil as well as for good, are, according to Koch, due to one active principle present in such small quantity as to constitute probably little more than a fraction of 1 per cent. of the whole. The following is his description of the chemical properties and behaviour of this substance.

Koch's Statements as to the Nature of the Active Principle.

It can be precipitated from the impure extract by absolute alcohol—still, however, impure and associated with various other insoluble extractive substances which are also alleged to be present along with it. By suitable methods it can be freed from these latter, so that it is possible to obtain it in a comparatively pure condition. As these other substances have, it is stated, no action at all,

still less any injurious action on the organism, this process of purification has, he alleges, no practical advantage and only adds unnecessarily to the expense of its preparation.

As regards the nature of this active principle, its complete precipitation by absolute alcohol points to it being of albuminous nature, perhaps belonging to the class of bacterial poisons of that nature recently described by Brieger and Fraenkel under the name of "toxalbumins." It differs from these, according to Koch, in two important respects—first, in being able to withstand high temperatures without losing its poisonous properties; secondly, *in dialysing quickly and easily through parchment membrane.* Koch concludes therefore that it is not a toxalbumin; that its nature must be left for the present a matter for conjecture. He prefers to describe it as "a derivative of albuminous bodies."

Comments on the Foregoing.

This definition of it as a "derivative" of albuminous material leaves, in truth, the question of the chemical nature of this substance entirely open. I need hardly point out that it is as fitly applicable to any of the more remote derivatives of albuminous metabolism, such as tyrosin, leucin, or urea, as it is to the more immediate derivatives such as albumoses, or peptones; and that it is equally applicable to such widely different classes, chemically speaking, of bacterial products as toxalbumins on the one hand, and alkaloids or toxines on the other.

I do not think that the description given by Koch of the behaviour of this hypothetical principle can possibly apply to any one chemical substance yet known to us. It is easy to understand an albuminous body being precipitated by absolute alcohol. But to no albuminous body, with the single exception of peptones, can the further statement possibly apply—that it is easily dialysable through parch-

ment membrane. This behaviour is more that of a crystalloidal, as distinguished from a colloidal substance; of a crystalline as distinguished from an albuminous body. If we assume for the moment that it is a product of bacterial action, its behaviour as thus described is more that of a toxine (poisonous alkaloid) than of a toxalbumin (poisonous proteid).

There is, however, another class of bodies standing in a different and even closer relation to bacteria than the above-mentioned products, namely, the proteins. These are albuminous substances derived from the plasma of the bacteria themselves, and are thus to be distinguished from toxines and toxalbumins, which are products of the fermentative action of bacteria on the surrounding tissue. First described by Nencki in 1880, these bodies have recently received great attention, and have been shown by Buchner and others to possess properties even more remarkable than those of toxalbumins. In particular, in this relation they have a remarkable power of resisting the action of heat. The behaviour of the active principle of tuberculin in this respect conforms, therefore, to that of a protein. On the other hand, such a body would not be dialysable in the way ascribed by Koch to his active principle.

The only substance capable of fulfilling, although still with difficulty, all the conditions laid down by Koch for his active principle would, in my opinion, be one of crystalloidal nature—presumably a toxine. Such a body might be (1) in sufficiently close association with one of proteid nature as to be carried down when the latter was precipitated by absolute alcohol, and yet (2) at the same time sufficiently loosely attached to be dialysable away. Further, it might (3) be able to resist the action of high temperature without losing its poisonous properties. On the

other hand, a protein substance which would readily fulfil the first and third conditions could not possibly fulfil the intermediate one of dialysing easily through parchment membrane. And, lastly, a poisonous peptone which could fulfil the condition as regards precipitation by alcohol, and might also fulfil the one as regards easy diffusibility, could with difficulty fulfil the third one of being unaffected by heat.

Actions of Tuberculin.

Before describing my own observations, which will be found in some important respects to be directly opposed to those of Koch, I would touch upon one other point having reference more to the action than to the alleged properties of this active principle. *Koch expressly states that the various substances present in tuberculin other than its active principle have no action at all on the organism.* All the activity of tuberculin is, on this view, bound up with the presence of this one body. If this be so, I conclude, then, that this activity must manifest itself in very diverse ways: (1) partly in inducing local inflammation around tuberculous areas, (2) partly in producing a general constitutional disturbance, the chief feature of which is fever, and (3) partly in favouring the growth of tubercle bacilli, and thus favouring the recurrence of the disease. For such I regard as the three chief effects of tuberculin, established both by clinical and pathological observation. They vary considerably both in their character and intensity, according, first, to the extent, character, and seat of the tuberculous lesion; and secondly, independently of all these, according to what can only be described as individual idiosyncrasy.

Both the local and the general action of this substance carries with it its own peculiar dangers. The dangers con-

nected with its local inflammatory action are those incidental to the softening and absorption of tuberculous tissue, more especially the danger which Virchow's observations, amply confirmed as they are by clinical observation, have conclusively proved, in my opinion, to be a real, although fortunately apparently a rare, one—namely, the increased activity and further dissemination of active bacilli.

The general effects include, in addition to fever, such general phenomena as headache, drowsiness, feelings of sickness, and *malaise*, aching pains in back and limbs, and occasionally severe depressant action on heart, indicated by weak and rapid pulse. Most of these unpleasant effects are as frequently noteworthy by their absence as by their presence, if the dose be small, so that the dangers connected with them might well be passed over as of relatively little importance. These general effects have, however, in my opinion, a significance of their own, apart from any dangers that may accompany them ; they indicate, in my opinion, in a special manner, the action of an albuminous poison. This is particularly true of the nausea, aching pains in back, and weak action of heart, which are perhaps the most characteristic. These symptoms find their closest parallel, in my experience, in the similar effects occasionally seen to follow the intravenous injection of defibrinated blood—blood, that is to say, containing various albuminous bodies, including certain of Wooldridge's "tissue fibrinogens."

The *fever* is, however, by far the most constant and characteristic phenomenon next to the local inflammation itself. It is so striking as at first sight to appear to have an altogether special relation to the more specific local action of the substance. This is, I judge, the view held by Koch, for he attaches an altogether special importance to

it; the main details of his treatment being regulated by him according to its presence or absence. According to this view, then, any active principle, possessing all the active properties of tuberculin would need to possess, in a special degree, the power of inducing as two of its most characteristic effects the local inflammation and fever characteristic of tuberculin.

One result of the present investigation is to show that no such principle exists, and that both the fever and the inflammation can be produced independently of each other by different constituents of tuberculin.

My Own Observations.

My own observations, commenced early in January of the present year, have had for their object:

1. To isolate the chief constituents of tuberculin, and to determine their chemical nature.

2. To ascertain their action with special reference to their power of inducing the two most characteristic effects of tuberculin—namely, local inflammation and fever.

3. To ascertain how far it was possible to eliminate all substances having an injurious action, and thus to obtain its remedial, without any of its injurious, effects.

The results I have up to the present obtained I shall describe under the three headings of—I. Composition; II. Action; III. Therapeutic Value.

I. COMPOSITION.

The fluid is a concentrated glycerine extract of golden brown colour, possessing a well-marked odour recalling that of meat extract. Its reaction, when diluted, is faintly alkaline. In any dilution above 1 in 10 it is liable, unless suitable precautions be taken, to undergo ordinary putrefactive decomposition, in which ordinary putrefactive pro-

ducts, such as indol and skatol, are recognisable by their characteristic odour.

The following are the results of my observations with regard to its composition.

Serum Albumin.

On acidulating slightly with acetic acid and boiling, no coagulation occurs. No serum albumin is therefore present.

Albumoses.

The extract is nevertheless very rich in proteids, these constituting, indeed, its chief solid constituent. On adding it drop by drop to absolute alcohol, a heavy white flocculent precipitate at once falls. The greater part of this presently takes the form of viscid masses, which float to the top or adhere to the sides of the vessel, leaving the fluid turbid. Within a short time, varying in different instances from half an hour to twenty-four hours, the remaining finer precipitate which occasions the turbidity is likewise deposited, leaving a clear, yellowish, supernatant fluid. If the latter be decanted or filtered off, and the precipitate be taken up in distilled water—in which it is, with few exceptions to be presently noted, readily soluble —a solution is obtained having much the colour of the original solution. This gives the following reactions:

Nitric acid, added to dilute solutions, produces no change. When added to more concentrated solutions, or, better, if the solution be poured down the sides of a test tube so as to form a layer on the top of the acid, a well-marked cloudiness ensues, the precipitate being soluble in excess of acid, or on heating.

Picric acid in saturated solution causes a well-marked cloudiness even in dilute solutions, and brings down a heavy flocculent precipitate in stronger solutions. This is readily dissolved on adding a few drops of dilute caustic

soda, or on applying heat. In the latter case the cloudiness rapidly reappears on cooling.

Biuret Reaction.—On adding a slight excess of strong caustic soda solution, and then allowing a single drop of dilute cupric sulphate to fall on to the top of the mixture, a well-marked rose-violet colour appears; the presence of albumoses or peptones, or both, is thereby indicated.

Ammonium Sulphate. — On saturating with neutral ammonium sulphate in the cold, a dirty viscid precipitate of considerable bulk soon falls out, followed by a further precipitate on allowing the fluid to stand over the salt for twenty-four hours. On filtering off this precipitate, and taking it up in distilled water, a solution is got which gives the same biuret reaction, and almost to the same degree, as the original solution. The clear filtrate, however, also gives a similar, although a much fainter biuret reaction. On acidulating this slightly with dilute acetic acid, adding more ammonium sulphate, and heating, a further cloudiness ensues. On removing this further precipitate by filtration, the clear filtrate obtained no longer gives any biuret reaction.

I conclude then that no peptones are present in tuberculin, since all proteids, with the single exception of peptones, are precipitated on complete saturation of their solutions with ammonium sulphate after the addition of acetic acid (Kühne).

In respect of its action towards heat (absence of coagulation), nitric acid (precipitation), picric acid (precipitate disappearing on heating and reappearing on cooling), ammonium sulphate (complete precipitation), and, lastly, the rose-violet biuret reaction, *the chief proteid constituent of tuberculin, has all the characters of an albumose.*

Certain of the above reactions I would note a little more in detail.

The absence of cloudiness on adding a drop of nitric acid to a dilute solution of albumoses is noted by Neumeister[1] as specially characteristic of these bodies. According to this observer, who has recently made a special study of the reaction of albumoses and peptones with various reagents, the whole group of primary albumoses are not precipitated by nitric acid unless they are present in a greater degree of concentration than 1 to 300; while deuteroalbumose is not precipitated by this reagent even in strong solutions. If, however, a trace of salt (NaCl) be added to such a dilute solution, precipitation at once ensues. In the case of the primary albumoses, this is necessary when the dilution is greater than 1 in 300, while with deuteroalbumose it is essential with any strength of solution.

In the case of the albumoses of tuberculin this behaviour was very marked, addition of a crystal of NaCl sufficing almost at once to produce cloudiness where the previous dilution had been too great to allow of it occurring at once on adding nitric acid.

The reaction with picric acid, which I had noted early in my observations, I subsequently found was regarded both by this same observer and Dr. Sidney Martin as also peculiar to albumoses, namely, complete precipitation, dissolving on heating, and reappearing again on cooling.

Nature of the Albumoses.

The albumoses present are chiefly protoalbumose and deuteroalbumose, with a variable proportion of heteroalbumose. Occasionally, also, a trace of the least soluble of all the albumoses—namely, dysalbumose—is to be found. These three albumoses are distinguished from deuteroalbumose in being precipitated from their solutions on satura-

[1] "Ueber Albumosen und Peptonen," *Zeits. f. Biol.*, 1890.

tion with ammonium sulphate or chloride of sodium, whereas addition of a few drops of acetic acid with the aid of heat is necessary to bring down the whole deutero-albumose.

Protoalbumose.—This forms, I conclude, the chief bulk of the albumoses present. On saturating with chloride of sodium in the cold the great proportion—I should judge not less than nine-tenths—of the total albumoses present were thrown down, and the precipitate proved—with the exceptions presently to be noted—easily soluble both in distilled water and in saline solutions of all degrees of strength short of the point of saturation. Solutions freed from salts by dialysis gave a distinct cloudiness with HNO_3, provided, as already described, they were not too dilute.

Deuteroalbumose.—The precipitation on saturation with ammonium sulphate was, as we have seen, incomplete. A trace remained in solution, and was not thrown down until a few drops of acetic acid were added, and the solution was further saturated with ammonium sulphate with the aid of the heat.

Heteroalbumose and Dysalbumose.— (1) I have stated that in a few cases the precipitate thrown down by absolute alcohol did not prove entirely soluble in distilled water. A slight insoluble residue remained on the filter. This proved, however, to be easily soluble in $\frac{3}{4}$ per cent. NaCl solution ; and on adding the solution so obtained to absolute alcohol a fine flocculent precipitate fell out. Furthermore, on saturating a portion of it with NaCl the solution became cloudy and threw down a finely granular precipitate.

(2) In another instance (Observation IV.) a portion of the precipitate thrown down on saturation with ammonium sulphate was taken up in distilled water, placed in a sausage-skin dialyser, and dialysed for an hour against

a running stream of water. At the end of this time the solution was found neutral in reaction, and was evaporated to dryness over a water bath, the residue taken up in a few drops of distilled water, and precipitated in two portions by absolute alcohol. The precipitate from one portion on being filtered off proved to be only partially soluble in ½ per cent. carbolic acid solution. A slight insoluble residue remained on the filter. In the second portion the albumose precipitate was allowed to lie under alcohol for two months. It was then filtered off, and, like the other, proved only partially soluble in distilled water. A slight insoluble residue remained on the filter. This was readily taken up by ¾ per cent. NaCl solution, and could be again precipitated from its solution on adding more sodium chloride.

(3) In another instance (Observation XX.) 5 cubic centimetres of tuberculin were thrown into 50 cubic centimetres of absolute alcohol. The alcoholic filtrate was rapidly evaporated over a water bath at a temperature of 70° C. The residue which remained was taken up in 10 cubic centimetres of a 1 per cent. carbolic acid solution and proved only partly soluble. On standing for twenty-four hours a granular flocculent precipitate of considerable bulk fell down. This was filtered off, and proved to be soluble in ¾ per cent. NaCl solution, from which it could be again thrown down on saturation with chloride of sodium.

(4) Lastly, if instead of being subjected to the action of alcohol alone as in (1), or alcohol and heat as in (3), or to the combined effects of alcohol, dialysis, and heat as in (2), the fluid were subjected simply to dialysis, a granular precipitate occasionally fell out with the same characters as above, namely, insoluble in distilled water, almost completely soluble in ¾ per cent. solution, insoluble in

concentrated solutions of some salt. Thus (Observation XIII.), 1 cubic centimetre of tuberculin diluted with 7 cubic centimetres of distilled water, placed in a sausage-skin dialyser, a few crystals of thymol being added to prevent putrefaction, dialysed for two hours against a running stream of water, then for eighteen hours against distilled water. On being removed no appreciable precipitate was visible. Bulk of fluid made up to 10 cubic centimetres and a crystal of thymol added, and placed in glass stoppered bottle. Three days later the solution looked a little cloudy and opaque, but still no definite precipitation. This change steadily went on till finally, after several weeks, a very distinct granular precipitate fell out, leaving the supernatant fluid perfectly clear. This change on dialysis I soon satisfied myself was in no way a putrefactive one, as I was at first inclined to think it might be. It never went any further than the granular precipitation I have described. Moreover, an entirely similar change occurred in solutions containing 1 per cent. of carbolic acid, while other solutions equally albuminous could be preserved for an indefinite period by adding carbolic acid in the proportion of $\frac{1}{2}$ per cent. On filtration, the filtrate passed through cloudy and opaque.

The granular precipitate proved to be partly soluble, although not completely, in $\frac{3}{4}$ per cent. NaCl solution; its solution was helped by gentle heat. On saturating with NaCl it again fell out as a cloudy precipitate.

In all the above cases it is to be noted that following on certain procedures an insoluble proteid substance was obtained having much the same characters—namely, insolubility in distilled water, solubility in $\frac{3}{4}$ per cent. NaCl solution, insolubility in saturated NaCl solutions. In all these respects its behaviour was that of a globulin, and as such I was at first inclined to regard it, more especially as dialysis appeared to have an important action in bring-

ing this body down. If a globulin, however, it was necessary, in order to explain its behaviour under different circumstances, to assume that it was present in exceedingly small quantity, since no appreciable coagulation occurred on boiling after slight acidulation with acetic acid. The alternative view to it being a globulin was that it was a modified albumose. Heteroalbumose in particular shares with globulins the properties of being insoluble in distilled water, soluble in dilute saline (NaCl) solutions, and insoluble in saturated saline solution. Under certain circumstances—for example, prolonged contact with absolute alcohol or after drying—it may lose its property of being soluble in saline solutions of any strength, and it then becomes, according to Kühne and Chittenden,[1] indistinguishable from the substance termed dysalbumose. The only point as regards solubility in which heteroalbumose differs from globulins is that while the former is soluble in saline (NaCl) solutions of any strength from $\frac{1}{2}$ per cent. up to and short of the point of saturation, and is best soluble in solutions of 10 per cent. strength, globulins are only soluble in dilute salines, and are precipitated when the concentration rises above 15 per cent.

On testing the substance I had obtained from tuberculin with special reference to this point, I found that it was not precipitated from its solutions (in $\frac{3}{4}$ per cent. NaCl) by chloride of sodium till the saturation point was nearly reached.

My conclusion, then, with regard to this body is that it is heteroalbumose, with a trace of dysalbumose adhering. As further confirming this conclusion, I would note the following:

1. I found it impossible to obtain a clear filtrate in cases (for example, after dialysis) in which it had been thrown

[1] Ueber Albumosen, *Zeits., f. Biol.*, 1884, p. 39.

down. The filtrate had a whitish slightly milky appearance, and this is one of the characters of solutions of heteroalbumose specially noted by Kühne.

2. Its appearance after prolonged dialysis is also readily explained, since the effect of dialysis would be to remove the small proportion of salts necessary to retain it in solution.

3. Its precipitation under the influence of prolonged exposure to alcohol, of heat, and other agents, is in my opinion to be ascribed partly to the conversion of heteroalbumose into the more insoluble dysalbumose, partly, perhaps, to a change of protoalbumose into heteroalbumose. It is noted by Kühne as a point peculiar to protoalbumoses that while they are rarely if ever affected by dialysis, their solutions always remaining clear, it is not uncommon for deuteroalbumoses under such circumstances to throw down a trace of heteroalbumose.

This sensitiveness of albumoses to outside influences, marked as it is in the case of their physical properties, is still more marked in their physiological relations. An albumose previously soluble in dilute saline solution becomes for some reason or other insoluble. In the same way an albumose previously active may for some reason or other lose its poisonous properties without any recognisable change in its physical characters. *This peculiarity of these bodies is one of prime importance in considering to what extent they can ever be extensively used as ordinary safe therapeutic agents.*

Peptones.—For the reasons already stated I conclude that no peptones are present in tuberculin; if present at all it must be in the minutest trace, since the quantity is too small to give any appreciable biuret reaction.

Mucin.—This is present in quantity sufficient to give a distinct cloudiness on adding acetic acid to strong solutions

of tuberculin. The precipitate is insoluble in excess of the reagent, and is not affected by heating; it dissolves readily on adding a few drops of caustic soda solution.

Other Substances Present in Tuberculin.

In addition to *glycerine* and the *colouring matter* which gives it its peculiar colour, tuberculin also contains a considerable proportion of salts and other substances whose precise nature it is not so easy to ascertain. Thus, on dialysing it, the dialysate sometimes has a peculiar odour recalling that of meat extract, and probably due to soluble *extractive substances* both of solid and volatile nature similar to some of those present in such extracts. As regards the *salts*, these are both of organic and inorganic nature. I have not thought it worth the trouble to ascertain by chemical analysis what the latter are, since in no case could they possibly be regarded as lending to tuberculin its characteristic properties. Salts are present, however, in considerable quantity. On evaporating the substance to such a degree of concentration as the presence of the glycerine will permit, a considerable crop of a salt crystallises out, having very definite crystalline characters, namely, in the form of truncated octohedral crystals. These dissolve but slowly in absolute alcohol; hence if the residue be extracted with absolute alcohol, a considerable proportion of these crystals may be carried down in the precipitate. If taken up in water and allowed to recrystallise they take the form of large square crystals with concentric markings, and at the same time smaller crystals of irregular rhomboidal or of ellipsoidal form make their appearance. I attempted to obtain the hydrochlorate salts of any bases present in tuberculin by removing, so far as possible, all the proteids by precipitation with absolute alcohol after previous acidulation with

dilute hydrochloric acid, and then allowing the remaining solution slowly to crystallise out. No crystalline forms other than the above-mentioned were obtained.

Alkaloids.—The alcoholic filtrate, after removal of all the proteids, so far as that was possible by repeated extraction with absolute alcohol, yielded a heavy precipitate with platinum chloride. This was filtered off, washed with rectified spirit till the filtrate passed through colourless, and then taken up in a minimal quantity of warm water. On cooling, an amorphous deposit at first fell out. The remaining solution on crystallising yielded definite and constant crystalline double platinum salts. Of these, two could be recognized—one in very small quantity, which crystallised out in form of colourless prisms of small size; the other, when perfectly pure in form of elongated prisms or beautiful clusters and rosettes of irregular prisms of a deep golden brown colour. On being broken up with H_2S this platinum salt yielded a hydrochlorate salt, which was only partially soluble in absolute alcohol, and easily soluble in rectified spirit. This substance could be again made to yield a platinum salt whose crystalline characters were identical with those obtained in the first instance, only that the coloured double salt crystallised out in rhombs instead of elongated prisms or rosettes. I have not as yet ascertained further the nature of these salts, the material available for this purpose being too small.

Summary of Results.

In their order of importance, as well as of amount, the chief substances present in tuberculin are, according to these observations:

1. *Albumoses;* chiefly protoalbumose and deuteroalbumose, along with heteroalbumose, and occasionally a trace of dysalbumose.

2. *Alkaloidal substances;* two of which can be obtained in the form of the platinum compounds of their hydrochlorate salts.

3. *Extractives;* small in quantity, and of unrecognized nature.

4. *Mucin.*

5. *Inorganic* salts.

6. *Glycerine* and *colouring matter.*

So far as my observations go, the following substances are absent from tuberculin, namely, serum albumin, globulin, peptones.

II. ACTION.

The result of my chemical investigations was thus to show that the only substances present in tuberculin with which its active properties could be connected were albumoses, organic bases of alkaloidal nature, and probably various extractives. My next object, therefore, was to ascertain to which of these bodies, or groups of bodies, tuberculin owed its characteristic properties, remedial and otherwise. Observations to this end were made in the first instance on animals—mice and guinea-pigs. Having in this way satisfied myself that the various modifications of tuberculin I had prepared were physiologically active—that their active properties had not been destroyed by the methods I had employed in preparing them—I suggested to Professor Watson Cheyne that the results I had obtained might be utilised in the treatment of tuberculous patients. He agreed to this suggestion, and since the end of April has carried out treatment with this modified tuberculin in King's College Hospital and in Paddington Green Children's Hospital, using it in place of the parent material—tuberculin.

The patients treated have up to the present time been comparatively few in number, and have been mostly cases

of lupus, of joint disease, and of bone disease; it has not yet been used in internal tuberculosis.

Mr. Watson Cheyne will next week record our conjoint experiences and opinion as to the therapeutic value of this modified tuberculin as compared with that of the parent substance. In the present communication I propose merely to describe its nature and methods of preparation, and to deal with certain points relating to its mode of action which have come out in the course of the treatment.

The modifications used have been four in number, and improvement more or less marked was recorded with each of them. With certain of these, as will be seen by the accompanying charts (Chart II.), a considerable degree of fever occurred in the first instance, similar to that met with in the early stages of treatment with tuberculin. Since our object, however, was not only to get the remedial, but at the same time to get rid of the injurious action of the substance, these modifications were discarded in favour of the others which seemed better to fulfil these requirements. In attaining this most desirable object we have had a considerable amount of success—so far at least as regards the avoidance of the constitutional disturbances so commonly met with in the course of treatment with tuberculin. To what extent the success applies to the more remote dangers as well—dangers of the recurrence of the disease—the limited period (three months) during which the treatment has been carried out does not permit us to state. Up to the present time, however, no indications of any such occurrence have been observed.

MODIFICATIONS OF TUBERCULIN AND THEIR METHODS OF PREPARATION.

The following are the modifications of tuberculin I have prepared, with regard to the value of which it is possible

to speak. I shall, for convenience sake, continue to designate them in the same way (by letters) as I have done throughout.

Modification A.

1. This contained the total precipitate thrown down from tuberculin by the action of absolute alcohol. It was prepared by adding tuberculin to absolute alcohol (specific gravity, ·795) in the proportion of 1 cubic centimetre of tuberculin to 10 cubic centimetres of alcohol. As regards the composition of this precipitate, I found that its solutions contained every constituent of tuberculin itself: albumoses, mucin, glycerine, colouring matter and salts. The proportion of these substances present were, however, altered, albumoses being relatively increased, the salts relatively diminished in amount. I estimated that about nine-tenths of the albumoses in tuberculin were contained in it. The albumoses were thus, even more than in tuberculin itself, its chief constitutent. I found it impossible to free them from the other substances present by repeated precipitation with absolute alcohol. Traces of glycerine, colouring matter, salts still adhered. If, however, weaker solutions of alcohol were employed, for example, 80, 70, 60, or 50 per cent., an increasing proportion of these impurities passed into the filtrate, along with, however, a correspondingly larger and increasing proportion of the albumoses. Fifty per cent. alcohol threw down, in fact, a very small proportion of the albumoses present.

Solution A_2.—I found that this purification could *for all practical purposes* be efficiently effected—that is, with least loss of albumose, and greatest removal of other substances—by precipitating with 70 per cent. alcohol, the tuberculin being added to alcohol in the proportion of 1 cubic centimetre of tuberculin to 7 cubic centimetres of absolute alcohol and 2 cubic centimetres of water. The

solution of albumoses obtained in this way (Solution A_2) was almost colourless; whereas, if 80 per cent. alcohol was used, the solution still retained a good deal of colouring matter. The action of Solution A_2 proved in certain important respects to be different from that of A. In particular, as will afterwards be seen, it had lost its power of causing the slight degree of fever usually produced by A.

Modification C.

2. This was the counterpart of A, and contained all the constituents of tuberculin not present in the latter. It was prepared by evaporating the alcoholic filtrate (after removal of A) over a water bath at a temperature preferably not exceeding 40° C. The residue was then taken up in $\frac{1}{2}$ per cent. carbolic acid solution. This preparation likewise contained all the constituents of tuberculin, but in reverse proportion to that in A; the salts and other soluble substances being relatively in excess, while the amount of albumose was relatively small. The composition neither of A nor C, as regards salts, was absolutely constant. This arose from the circumstance that, as has been stated, certain of the salts were only slowly taken up in solution by absolute alcohol. If therefore the precipitate (A) was filtered off quickly (within a quarter of an hour), it was liable to contain a greater proportion of salts, and the filtrate a correspondingly less proportion, than if it were allowed to stand for twenty-four hours. It was best therefore to allow a fixed period to elapse before separating A from C. I found reason to believe that the action of the two solutions varied in certain particulars if this rule were departed from.

The most interesting contrast was afforded by the action of these two modifications, A and C. Both of them had remedial properties, causing absorption and disappearance of tuberculous tissue; *but while in the case of A this was*

attended by decided and occasionally severe local inflammatory reaction, even with small doses, accompanied by comparatively little, sometimes no, fever (Chart I.), *in the case of C the improvement was attended with no local inflammation and nevertheless high fever* (Chart II.).[1]

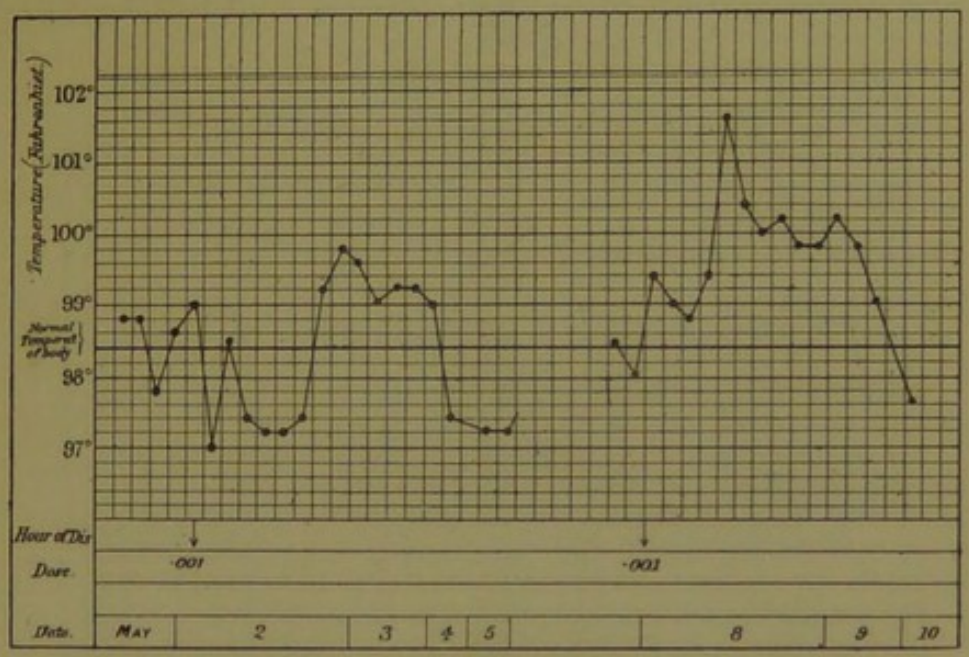

Chart I. *Case of Lupus.*—Woman aged 22. *Action of Modification* A: Severe local reaction after first injection of 1 milligramme; fever slight and delayed, probably purely inflammatory; no constitutional disturbance. After second injection of same dose five days later, general disturbance more, reaction less than before; fever more marked, of the tuberculin type, namely, reaching its maximum eight to ten hours after the injection; well marked constitutional disturbance, including headache, feeling of illness, severe aching pains, and restlessness during the night; pulse 130. A fortnight later the brown discoloration around the affected area as the result of the first inflammatory congestion was still well marked.

These facts seemed to point to the existence of a fever-producing substance in tuberculin independent of the one that had the remedial action. The chief difference between A and C as regards their composition was one of degree rather than of kind, the former containing most of the

[1] For fuller description of the action of these modifications see Mr. Watson Cheyne's Paper on "The Future of Tuberculin," in *British Medical Journal*, August 8th, 1891.

albumoses, the latter most of the salts. This seemed to point to the salts rather than the albumoses as the substances responsible for the fever. This conclusion was confirmed in the following way: On removing from A the small proportion of salts it contained, it was found to have lost its former power of inducing fever; solution A_2 proved,

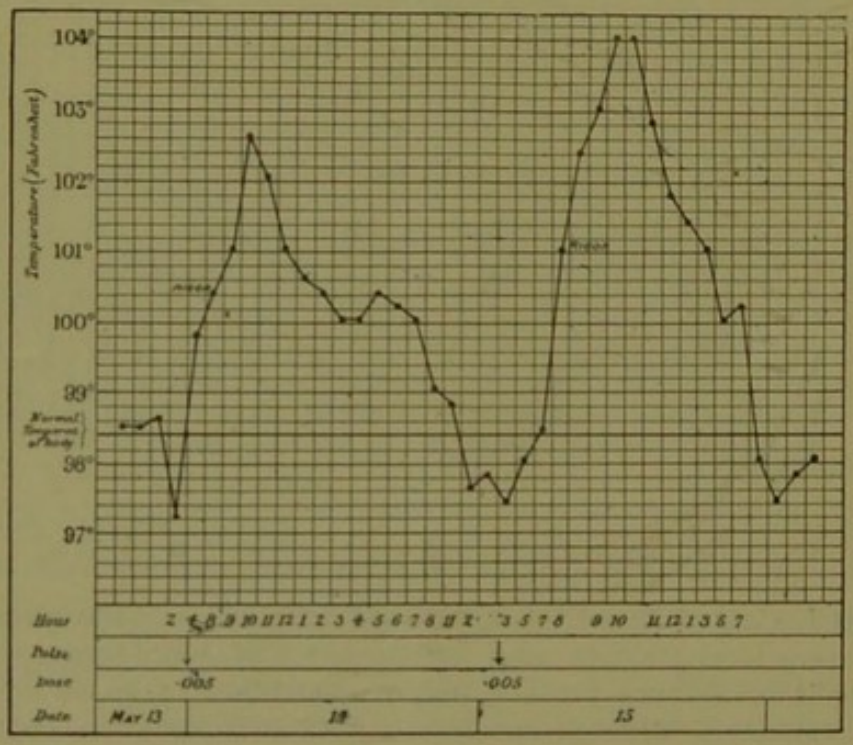

Chart II. *Lupus.*—Man aged 24. Extensive lupus of face involving upper and back part of ear, and causing great œdema of lobe of ear; latter swollen and pendulous, reaching as low down as the level of the angle of the jaw. *Action of Modification* C: Temperature reactions after third and fourth injections, the maximum in each case being reached between the 8th and 10th hour; fever, preceded each time by a rigor; typical tuberculin fever; tolerance rapidly acquired; subsequent action of this substance unattended with any fever. At no time was there ever the slightest evidence of local reaction other than increased scaling, and rapid improvement.

in fact, when tested, to have none of the initial slight fever-producing action of A. With this fact in mind, my next endeavour was to remove the salts and at the same time retain if possible the whole of the albumoses present in A or C.

Modification B.

3. To this end I precipitated the whole of the albumoses of tuberculin by means of ammonium sulphate, so far at least as that was possible (*v. a.*) without the aid of heat or the addition of acid. The precipitate was taken up in distilled water, placed in a sausage skin dialyser, and dialysed for 24 hours, first in running water (2 hours)

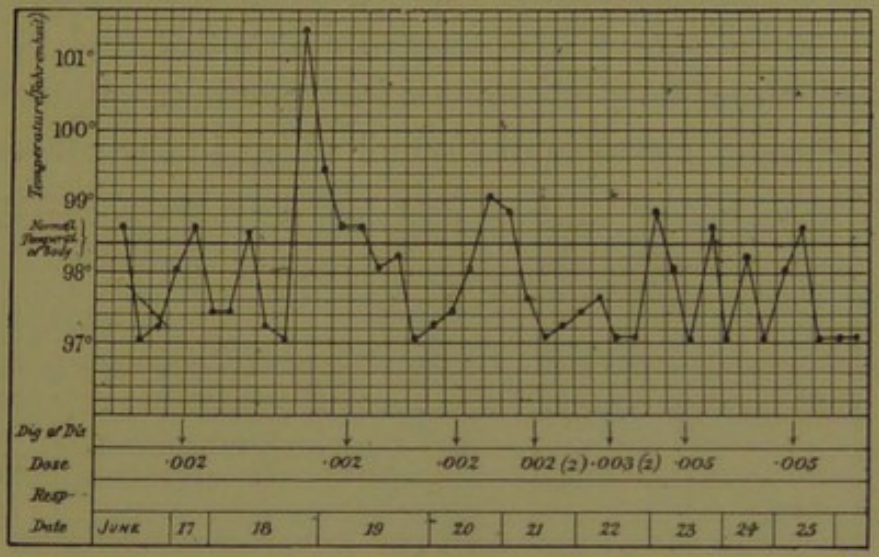

Chart III. *Lupus.*—Woman, aged 47. Extensive lupus of face and neck, with ulcers on right cheek, below this, and on back of neck. *Action of Modification* B: Temperature reactions of the first eight injections. Marked local reaction after first injection, the face and neck being red and swollen, and discharging freely. Temperature after first injection delayed (twenty-four to thirty hours), probably, therefore, purely inflammatory. Afterwards, notwithstanding that a considerable degree of local reaction was maintained, temperature had tendency to be subnormal. Dose since increased up to 10 milligrammes twice daily. Condition now, a month after injection, obviously improved: ulcers healed; cicatricial tissue of face and neck softer. Not the slightest constitutional disturbance from first to last.

then in distilled water (22 hours). At the end of this time it contained the merest trace of ammonium sulphate.

The solution thus prepared I found to possess in an eminent degree the power of inducing local reaction, followed by healing change around tuberculous lesions, unaccompanied by any constitutional disturbance whatever. (See Charts III. and IV.)

The following are the details of the preparation of this material as regards the exact proportions of tuberculin employed : 1 cubic centimetre of tuberculin, 5 cubic centimetres of distilled water, saturation with preferably large crystals of ammonium sulphate for 24 hours in the cold, the precipitate filtered off and freed so far as possible from any crystals of ammonium sulphate, taken up in solution, placed in small sausage skin dialyser (*previously carefully tested*) and dialysed first in running water and then in distilled water until all trace of ammonium sulphate has disappeared. Crystals of thymol added to the solution to

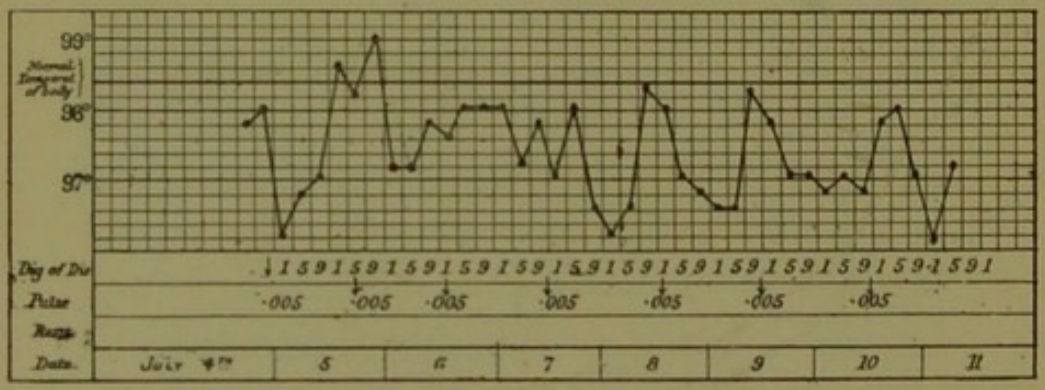

Chart IV. *Hip Disease with Sinus.*—Boy, aged 10. Case of hip-joint disease with breaking down of wound after arthrectomy. *Action of Modification* B: Temperature reaction after first eight injections, commencing at once with 5 milligrammes. Entire absence of constitutional disturbance. After first injection distinct and well-marked local reddening and swelling extending 1½ inch beyond margin of unhealthy wound. The wound put on at once a healthy appearance, and since then healing has gone on steadily.

prevent any putrefactive change, the remaining solution then made up to such bulk that 10 cubic centimetres shall correspond to each cubic centimetre of tuberculin employed. *(Title—B 10 per cent.)*

Up to the present this modification has, in my opinion, yielded the most constant and satisfactory results both in lupus and in joint disease with open sinuses.

Modification C B.

4. The healing action of C differed from that of A or B in one important respect, namely, that it was unattended

with any local inflammation or any sign of local reaction other than the scaling followed by steady improvement in the affected area. This difference I regarded to the advantage of C, since the chief drawback to the use of the substance in internal tuberculosis is its tendency to produce inflammation. I therefore next endeavoured to isolate the remedial apart from the fever-producing substances present. At first I had regarded C as free from any trace of albumoses, assuming, erroneously as it turned out, that all the albumoses would be precipitated by the absolute alcohol. I soon found that this was not the case, a very considerable proportion, which I have estimated as high as one-tenth of the whole, always passing into the filtrate. I first discovered the presence of this material in the following way :

On driving off the alcohol from the filtrate, I found that the residue, on further extraction with absolute alcohol, yielded a further flocculent precipitate of considerable bulk. I removed this by filtration, and believed at first that I had a comparatively pure albumose in hand. I soon found that this was not so. The behaviour of this substance towards absolute alcohol was peculiar, in so far as after separating out in flocculent masses, it was liable to become redissolved, not the slightest trace of it remaining. This I judged to be due to the glycerine and traces of mucin still remaining in the residue. To obtain it at all by this method it was therefore necessary to filter it off at once. This procedure was, however, attended with the disadvantage that in so doing I obtained also a considerable proportion of the less soluble salts, which it was my aim to get entirely rid of. These salts were also naturally thrown down by the absolute alcohol, and remained adherent to the precipitate.

As the most effective way of getting rid of these salts,

and at the same time obtaining the albumose material in a sufficiently pure condition, I ultimately resorted to dialysis. *My experiences in preparing B, as well as other experiences which I need not here record, satisfied me fully that Koch was entirely in error in his statement as to the easy diffusibility of the alleged remedial principle of tuberculin.*

I hoped by dialysis to be able to remove, in great part at least, the substances responsible for the fever. The

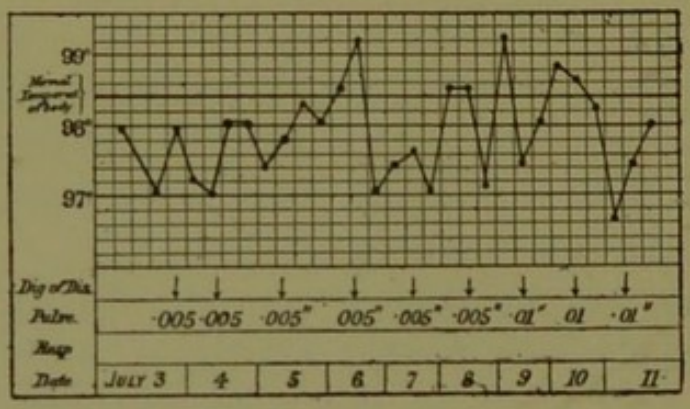

Chart V. *Lupus.*—Woman, aged 40. Lupus involving nose and both cheeks; septum partly gone, oval ulcer on left side of nose $\frac{3}{4}$ in. by $\frac{5}{8}$ in.; soft ulcer over edge of septum and on upper lip; right ear scaly behind, with small ulcer on lower edge. *Action of Modification* C B: Temperature reactions of the first fifteen injections, commencing with 5 milligrammes. Twenty-four hours after first injection felt slight burning sensation in face, with slight headache; slight degree of reddening of face. Since then improvement steady. A fortnight later ulcer on nose healed and improvement elsewhere; no constitutional disturbance whatever, although dose raised to 5 milligrammes twice daily on third day, and 10 milligrammes twice daily on sixth day.

result more than justified my hopes. I prepared in this way from C a solution (C B) which gave all the reactions characteristic of albumoses (*v. a.*). This solution seemed to fulfil the necessary requirements, since *it proved to be capable of inducing very distinct local improvement unattended with fever, and, still more markedly, accompanied by scarcely any noticeable inflammatory reaction* (Chart V.)

The following are the exact details of the method employed in preparing this fluid: 2 cubic centimetres of tuberculin dropped into 20 cubic centimetres absolute alcohol; heavy precipitate filtered off in a quarter of an hour; filtrate evaporated over water bath at a temperature preferably not exceeding 40° C., and just sufficiently long to drive off all the alcohol; residue taken up in 12 cubic centimetres distilled water; placed in sausage-skin dialyser, and dialysed for two hours in a running stream of water. At the end of this time its previous yellow colour much lessened. Quantity made up to 20 cubic centimetres, including 2 cubic centimetres of pure glycerine used for preservative purposes. A few crystals of thymol also added. *(Title—C B 10 per cent.)*

I have stated that C B as thus prepared "seemed" at first to fulfil all the requirements—remedial action without inflammation and without constitutional disturbance or fever. Up to the present the statement holds true as regards the remedial action without local inflammation. If pushed very rapidly, however, as in the case of a child with lupus of the thigh now under treatment, there is a slight tendency to fever, the temperature rising as high as 100° F. or 100.5°. In this case improvement at first was slow, and the dose was raised in the course of the first week of treatment from 5 milligrammes twice daily up to 20 thrice a day. The improvement since then is distinct, and has been unattended with any noticeable inflammation. This rapid increase of dose in a child aged 10 was, however, a very severe test of the temperature-producing properties of the preparation, and might fairly be regarded as an unfair one. I am nevertheless disposed to believe that the slight degree of fever indicates the presence of a trace of the fever-producing substance. I propose, therefore, to modify the method of preparation of C B as

follows, namely, to dialyse for at least four hours instead of two, and to use $\frac{3}{4}$ per cent. NaCl solution instead of distilled water. In this way I hope to remove more completely all the injurious substances, and at the same time prevent by means of the saline solution any alteration in the physiological characters of the albumoses, or any precipitation of heteroalbumose.

Summary.

I shall conclude my remarks at present by summarising very briefly the conclusions I have arrived at, based on the foregoing and many other observations, with regard to the nature of the various active substances in tuberculin, and their distribution amongst the various modifications just described.

1. Tuberculin owes its activity not to one principle but to at least three, and probably more, different substances.

2. Its action in producing local inflammation, fever, and general constitutional disturbance is not a simple but an extremely complex one.

3. Its active ingredients are of the nature of albumoses, alkaloidal substances, and extractives. *The action of these is in certain instances antagonistic.*

4. Its remedial and inflammatory actions are connected with the presence of certain of its albumoses, while its fever-producing properties are chiefly associated with substances of non-albuminous nature.

5. The albumoses are not lost by dialysis, the latter are. By the adoption of suitable methods it is thus possible to remove the substances which cause the fever, while retaining those which are beneficial in their action.

6. The fever produced by tuberculin is thus absolutely unessential to its remedial action.

7. The same holds true, I would fain believe, with regard to the inflammation produced by it. Inflammation

is certainly not absolutely necessary for the manifestation of its remedial action. It is in some cases, however, beneficial, and helps, under certain circumstances, the action of the remedial substance.

8. The difference between the substance which causes the inflammation and the one which possesses the remedial properties as regards their action is not merely one of degree. The local reaction which occurs with small doses of the former is much greater than that producible by large doses of the latter.

9. Nevertheless the remedial substance has as truly a local action as the one which tends to produce inflammation. This is evidenced in two ways—first, by distinct and obvious shrinking of the tuberculous tissues; and, secondly, increased scaling, pointing to the existence of a certain amount of deep local congestion. In some cases this congestion becomes obvious by slight superficial reddening.

10. The power of favouring recurrence, which tuberculin possesses, is connected partly with the non-albuminous substances removable by dialysis, *possibly also with a portion of the albumoses present.*

11. The remedial substance resists the action of high temperatures. Its action is, however, lessened if exposed to a temperature above 70° C. in a dry condition. Its properties, moreover, can be altered in other ways, sometimes, for example, materially by dialysis. *This change is not due to the passage of the substance through the membrane* as stated by Koch, but to changes occurring in it within the dialyser. Its sensitiveness in this respect I regard as one of its chief features, and causes its preparation to be attended with special difficulties.

12. Both it and the other albumoses present probably belong to the class of proteins—albuminous substances

derived from the plasma of the bacilli themselves, and not merely formed by the action of these bacilli on the surrounding tissues.

III. THERAPEUTIC VALUE.

The main object of the investigation was to ascertain to what extent it was possible to obtain the remedial without any of the injurious action of tuberculin. It will be noted that I have throughout employed the term "remedial" in contradistinction to "curative" to signify the therapeutic action of the various modifications described. I have done so purposely. When one considers how many factors are usually concerned in the production of disease, some connected with heredity, others relating to the individual, others to his surroundings, to apply the term curative to the action of any remedy, least of all in connection with so complex a disease as that denoted as phthisis, is utterly misleading. It is not, in my opinion, essential that the action of any substance should be curative in order that it may be classed amongst the list of our other remedial substances. Any substance which claims to be so classed should however, I consider, fulfil the following simple requirements:

1. Its action must in a suitable proportion of cases be beneficial.

2. In competent hands its action must not at the time produce any immediate ill effects.

3. Its action must not be followed by remote ill consequences directly traceable to it, still less by consequences that may endanger the life of the patient.

As examples of remedial substances that fulfil these conditions I would mention, among more acute diseases, salicylate of sodium in acute rheumatism; for more chronic diseases, iodide of potassium in syphilis.

Judged by the above standard, *tuberculin* cannot, in my opinion, be regarded as a safe remedy. It fulfils in a large number of cases the first condition, so far as its immediate action is concerned; it does not completely fulfil the second, and it sometimes fails to fulfil the last. Any modification of tuberculin must be subjected to the same test, and fulfil the same requirements before it can be regarded as a safe remedial agent.

As regards the *Modification* A above described, its action differs but slightly from that of tuberculin. It has the same uncertainty both as regards its immediate effects and the ultimate beneficial improvement. Its remedial action is, if anything, lessened, and the only advantage it offers over tuberculin is that it produces less fever. It partially fulfils the first, incompletely the second, and probably, also—although as to that there has as yet been no indication—behaves like tuberculin as regards the last. For these reasons it is not, in my opinion, to be recommended for use.

Modification C differs from tuberculin in being almost completely freed from the substance which gives rise to local inflammation. It contains, however, in a special degree the fever-producing agents. Its remedial properties are interfered with by the presence of the latter substances, which, there is reason to believe, from observations on animals, have a favouring rather than a retarding influence on the growth of the bacilli. At least this is their action when freed from the remedial substance (C B). On this account I cannot recommend this modification for use.

Modification C B.—This contains the remedial substance present in C, freed from the fever-producing and other bad properties of the latter. It fulfils the first condition in having a remedial action, the second in its action

being, so far as yet observed, free from any ill effects, and for the reasons above stated its power of favouring recurrence of the disease is materially lessened—I would fain hope abolished.

Its remedial action is unattended by any local reaction other than a slight degree of reddening, and that not always. It is slower and weaker probably for this reason than that of B. This absence of inflammation gives it, however, great advantages if it be employed for internal tuberculosis. My investigations with regard to this modification are not yet complete. I hope to strengthen its remedial properties without adding to its very slight power of produciug local reddening.

Modification B.—This contains the remedial properties of C B, with the additional property of inducing local inflammation. Its action is free, *so far as has yet been seen*, from any ill effects. It contains, in addition to albumose C B a considerable amount of albumose (A_2) which is either inert remedially or from certain observations now being made on animals may possibly prove to be injurious. This can be removed, whether however, without at the same time impairing the remedial action of B, I cannot yet say. My investigations with regard to this modification are not yet complete. It fulfils the first condition; so far as yet observed the second also, with the above-mentioned exception of producing local reaction, which may be injurious or beneficial according to circumstances.

To what extent as at present prepared it fulfils the third I cannot yet say. There has been no evidence of failure in this respect up to the present date. As at present prepared it has, in my opinion, advantages over CB in certain cases.

The crucial condition which must be fulfilled by this and every other modification of tuberculin is, I consider,

this third one. Should they fail in this respect they cannot, in my opinion, be considered as being safe remedial agents. Supplementary observations with regard to these and other points will follow in a later communication.

I would merely add one word in conclusion with regard to the *dosage* employed. In all cases the doses given are not those of the substances themselves, but are those of the original tuberculin. In most cases, therefore, I conclude that the action of the various substances has been materially intensified by the process of purification, since to produce the same remedial action, the doses have been much smaller than those required in the case of tuberculin.

For convenience I shall continue for the present to refer to these substances by the titles I have hitherto used, speaking thus not of "the albumose of tuberculin," as if only one albumose were present, but of Preparations B and C B of tuberculin, or, more shortly, Tuberculinose B and C B.

This nomenclature will serve, I trust, to avoid confusion arising in any further investigations which may be carried out either by myself and others with regard to the actions and properties of these substances.

Remarks in the Discussion following Prof. W. Watson Cheyne's Paper on Tuberculin.[1]

By WILLIAM HUNTER, M.D., F.R.S.E.,

Assistant-Physician to the London Fever Hospital.

I gladly avail myself of the opportunity afforded by the discussion on Mr. Watson Cheyne's paper to supplement in a few particulars the observations I have already recorded.

In the first place, I desire to endorse every word of caution contained in his paper. Tuberculin shows remarkable iodiosyncrasies in its action; and I consider it not unlikely, although as to that I have as yet no information, that this peculiarity will be found to apply to the modifications I have described. It has certainly been found to apply to the *Modification* A, and also, although to a less extent, to C. The experience gained with these two preparations is longer than that with B and C B, and I am quite prepared to meet the contingency that the behaviour of B and C B may be found to vary when tried over an extended series of cases.

In recording the results obtained with these latter, I have therefore described the exact method of preparation I employed, so that my results may be controlled by the

[1] From the *British Medical Journal*, August 8th, 1891.

observations of others. I consider these methods to be still imperfect (except for the preparation of small quantities), and I am now engaged in modifying them in such a way as to render them suitable for the preparation of the modified material in large quantities. One of the chief difficulties to be contended with is the instability of albumoses as compared with substances of crystalline nature. In this connection I may note one point which has forced itself on my attention during the course of my investigations—namely, the constancy in composition of different samples of tuberculin. With the exception of slight differences in consistency and colour of different samples, I have found nothing to suggest that the composition is not fairly constant. Considering the nature and source of the material, this seems to me a remarkable circumstance, and one that reflects not a little credit on its discoverer for the care he has bestowed on the details of its preparation.

As regards the therapeutic value of the modifications I have prepared I can add nothing to what Mr. Cheyne has said in the account he has just given, embodying as that does our conjoint experiences. (Vide *British Medical Journal*, August 8th, 1891, "The Future of Tuberculin.")

I wish, however, to direct attention to certain points on which my own results differ from those of Koch.

Instead of the remarkable properties of tuberculin—of inducing fever, constitutional disturbance, inflammation, etc.—being all connected with one substance, as Koch alleges, the observations now recorded show that several substances are responsible for the production of these various effects. *In particular, the one which causes the characteristic fever is not the same as that which causes the equally characteristic local inflammatory reaction.*

The results go even further, and tend to show that the substance in tuberculin which possesses the specific re-

medial action is not the same as that which causes the local inflammation. According to my observations both these latter (the remedial and the inflammatory agents) are of albumose nature, the remedial substance constituting but a small proportion of the whole. In my opinion Koch has been led into error by attaching too much importance to the fever of tuberculin. It is only in this way that I can explain his statement that his single active principle passes easily and quickly through parchment membrane. My observations show that this description applies to the substances which are mainly responsible for the fever, but certainly not to the albumoses, which are responsible for the remedial and inflammatory properties of tuberculin. I always employed dialysis to obtain the albumoses in their purest condition, and to free them from the fever-producing agents.

The practical outcome of these observations is to establish that it is possible to free tuberculin, at least to a very great extent, from its fever-producing properties, and thus remove one of the barriers to its more extended application.

To what extent it is also possible to dissociate its specific remedial action from its power of inducing local inflammation is a point not so clearly established. I have expressed the opinion that the two actions are dissociable from each other, and are distinct in their character. Up to the present this is an opinion based solely on the differences observed clinically. Whether the distinction I have thus, for the present, drawn between the two actions will be confirmed pathologically, it must remain for future observations to determine. So far as I can yet judge, the difference in the action of the two substances is probably of this nature—that while the remedial substance acts mainly on cells, and only to a slight extent on vessels (possesses chimiotactic properties independent of, or in excess of,

inflammatory properties), the substance which causes the inflammation acts more directly on the diseased vessels, causing not only diapedesis, but also exudation. The slight degree of congestion which is occasionally observed to accompany the action of the remedial substance during the first day or two appears to be different from the reddening, swelling and exudation (often extensive) which mark the action of the inflammatory agents.

It is not only, however, with regard to the *nature* and *properties* of the remedial substances, but also with regard to the other substances present, that my observations differ from those of Koch. The active principle which Koch describes is a purely hypothetical substance. All substances other than this hypothetical principle he describes as harmless. So far from this being the case it is clear from the observations now recorded by Mr. Cheyne and myself, that certain of them cause the fever and can be got rid of without impairing in any way the beneficial action of tuberculin.

I conclude that the power possessed by tuberculin of causing wide-spread constitutional disturbance, as well as favouring the recurrence of the disease, are also to be ascribed to substances other than those possessing the beneficial action, and that these also can be got rid of.

With regard to the latter point, more particularly, observations were made on tuberculous guinea pigs, as Mr. Watson Cheyne has mentioned. Two of these were inoculated with *Modification* C (which contained, it will be remembered, the greater portion of the soluble salts, extractives, etc., with a small trace of albumose) from which the albumose had been removed by repeated extraction with absolute alcohol. At the same time two other tuberculous guinea pigs were inoculated daily with the same dose (corresponding to 0·02 gramme of the crude

tuberculin) of *Modification* A (which contained, it will be remembered, the great mass of the albumose with only a small proportion of the other constituents).

In the first two the glands underwent a steady enlargement, while in the latter they underwent an equally steady diminution. The enlargement in the former case was more rapid and marked than would have occurred in the natural course of the disease. *I conclude for this reason, that by removal of this material from tuberculin* (and it has been removed in great part, both from B and CB, by the process of dialysis) *a distinct gain has been achieved*, since one of the chief drawbacks to the use of tuberculin is, as Mr. Cheyne has clearly shown, the power it undoubtedly possesses of favouring recurrence of the disease.

In one of the other animals the dose of A was raised from 0.02 to 0.1 gramme daily. The course of the disease did not appear to be materially affected thereby, since the animal died five weeks after the commencement of treatment and three months after its original inoculation with tuberculous material. *Modification* A, therefore, although capable of causing diminution in the size of the affected glands, seems to possess no power of conferring immunity.

In this respect it agrees with tuberculin, since all observers who have tried have hitherto failed to confer immunity against tuberculosis by means of tuberculin. This result is of practical as well as scientific importance; for I consider that the idea that it is possible to confer immunity against the disease underlies much of Koch's system of treatment. It is probably this idea which led Koch to push the treatment as rapidly as possible with increasingly large doses. If, as seems to be the case, it is impossible to confer immunity against the disease, the use of these large doses is unnecessary, if not harmful; and for

my own part I conclude from a study of the action of C B as well as of B, that the good results of tuberculin can be obtained with smaller doses, administered preferably according to Mr. Cheyne's plan, twice or thrice daily. In this way the dose (of the modified material) need rarely exceed 20 milligrammes at a time.

The last point I would notice is the mode of action of the remedial substance on tuberculous tissue. As regards the marked affinity it shows for tuberculous tissue, its action is I consider a truly specific one. It has been thought by some that any remedial action tuberculin possesses is due to its power of exciting local inflammation, and some support is lent to this view by the experience of Mr. Cheyne and many others, that the amount of improvement is proportional to the degree of inflammation initially set up. Some have even ascribed part of the beneficial result to the fever. The observations now recorded by Mr. Watson Cheyne and myself conclusively dispose of both these views, since they show that improvement—sometimes indeed to a very marked degree—may occur independent of either fever or inflammation.

As to the nature of its action, Koch has described it as a "necrosis," the tuberculous tissue being so acted upon as to become "necrotic." I consider the use of these terms most unfortunate and inapt. The changes in lupus after the administration of large doses are certainly sufficiently pronounced to be well described by these terms. I do not regard these changes, however, as representing the normal mode of action of tuberculin, any more than I should regard convulsions as representing the action of medicinal doses of strychnine. With tuberculin, as with strychnine and other poisons, there is a medicinal as well as a poisonous action according to the dose given, and I regard necrotic changes to be amongst the effects of

poisonous doses. As regards the "medicinal" action of the remedy, this could be well studied in the course of our observations, since with the various modifications I have prepared it was possible at will to produce remedial change unaccompanied by any fever or any inflammation.

The action of the remedial substance as thus observed I consider to be mainly on the cells around the affected part; and the chief result of their increased activity is to cause absorption of the tuberculous tissue. If the tuberculous material has blocked up lymphatics and led to local œdema, as in one of the cases treated, this absorptive action of the substance becomes very evident in the rapid subsidence of the œdema. The absorption of nodules is also sometimes very evident, the skin falling in over them and becoming wrinkled.

While these changes point to increased activity of leucocytes, just as a rapid disappearance of a blood clot points to a similar activity, the specific action of the substance on cells is evidenced in another way in open tuberculous wounds. The previously red, protuberant, unhealthy granulations soon assume at first a more dusky and then paler appearance, and become covered over rapidly with healthy epithelium. The surface loses at the same time its unhealthy look and becomes paler—a circumstance in itself which supports the view I have already advanced, that the action of the substance is no way necessarily an inflammatory one.