A CONTRIBUTION TO THE STUDY OF THE COMPLEMENT FIXATION REACTION IN TUBERCULOUS ANIMALS.

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(With 12 Charts.)

Experience gained from a previous enquiry on this subject in human tuberculosis led to the belief that much information might be derived from an investigation of the complement fixation reaction in animals experimentally infected with tubercle bacilli. Accurate dosage can be measured and the true path of infection is definitely known. Various enquiries were suggested and investigated by the detailed examination of rabbits and guinea-pigs; the latter class of rodents were used in batches of six to twelve in number, as otherwise the individual differences between animals in the same group of experiments are entirely overlooked. My cultures of the human tubercle bacillus were obtained by inoculating guinea-pigs with the sputum[1] from typical cases of pulmonary tuberculosis at the Brompton Hospital Sanatorium at Frimley. Pure cultures of the bacillus were obtained from the infected guinea-pigs and cultivated on Dorset's egg medium, so that within a period varying from 14 to 21 days an abundant growth was obtained. The culture of the bovine bacillus was supplied to me by Professor Delépine who obtained it directly from the tissues of an infected cow, and subcultures were kept going on Dorset's egg medium. In every experiment without exception the animals were infected with definitely known quantities of the human or bovine bacillus. These were obtained by carefully scraping the growth off the surface of the egg medium and weighing it

[1] N.B. I am indebted to Dr W. O. Meek, Director of the Sanatorium, for supplies of the tuberculous sputum.

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on sterile platinum foil, while in some cases (for comparison) a portion of the growth was dried in a desiccator before it was weighed. The untreated or dried bacilli were then shaken in a known quantity of sterile saline, so that a perfect emulsion free from clumps was obtained. The bacilli were kept in the dark in brown stoppered bottles and were always employed within a few days of their preparation.

The rabbits were weighed once a week during the observations and as far as possible animals of the same size and weight were employed in each group of experiments, but as regards guinea-pigs, only a rough method was adopted for their assortment.

The rabbits were always tested to ascertain whether the blood reaction was negative before inoculation, but it was only necessary to discard one animal out of the entire series. These animals were bled at least once a week during the period they were under observation, while in some cases it was necessary to bleed them much more frequently. The guinea-pigs were bled from the axillary artery at the end of the experiments. The serum was heated in the usual way and always tested within forty-eight hours, but comparisons were also made with "stored serum."

Observations on the blood in man can be made at definite periods in relation to certain clinical phenomena, or at rest, so as to avoid any question of the much discussed subject of auto-inoculation. In rodents, however, similar advantages cannot be seriously considered.

A complete post-mortem examination was carried out on every animal, and the various tissues were microscopically examined, as it is well recognised at the present day that naked eye observation alone can be disregarded.

Antigens.

Dudgeon, Meek, and Weir[1], in a preliminary communication on the complement fixation reaction in tuberculosis, described the preparation and the merits of certain antigens which they had employed; in their opinion the most satisfactory antigen contained the bodies of the tubercle bacillus. A vast amount of work on this subject has been completed since their paper was published with the result that this bacillary antigen has been practically abandoned. It is of undoubted value for determining a positive reaction but for titration purposes it cannot be regarded as ideal.

[1] Dudgeon, L. S., Meek, W. O., Weir, H. B. "A Preliminary Inquiry on the Value of the Complement Fixation Reaction in Tuberculosis." *Lancet*, Jan. 4th, 1913.

Experience has shown that this reaction is beset with difficulties, even greater in tuberculosis than in other conditions, but these can be largely overcome when a thoroughly satisfactory antigen is obtained. The first useful bacillary free antigen employed was made by extracting for long periods of time human tubercle bacilli cultivated on Dorset's egg medium from cases of pulmonary tuberculosis. These bacilli were weighed, then frozen and thawed over solid CO_2 and finally shaken with glass beads daily for some weeks, by which means a definitely specific saline antigen is procured, but if the bacilli are dried previously *in vacuo* and then extracted with distilled water instead of normal saline an antigen is obtained which possesses more active properties.

Extracts were also made with chloroform, benzene, acetone and ether, but finally a most satisfactory antigen was obtained, which is now used entirely for this reaction. It is prepared by a special extraction of living tubercle bacilli with absolute alcohol[1]. It gives a beautiful mixture with normal saline, absolutely free from bacillary bodies and when carefully titrated leads to most satisfactory results, but a quantity of fresh and active tubercle bacilli is required for the preparation of this antigen and it is not fully active for some weeks. I have compared it with an unlimited number of others prepared by various methods and I consider it to be vastly superior to any other. No standard of activity can be given, as each supply must be judged on its own merits by most careful titration.

The Haemolytic Mixture.

A highly potent haemolytic immune-body of at least three times the strength of the minimum lethal dose was always employed, and a 5 per cent. suspension of thoroughly washed sheep red cells in normal saline.

Complement.

Fresh guinea-pig complement was diluted with normal saline on each occasion until the greatest dilution of the complement which acted efficiently was obtained.

It is absolutely essential for satisfactory work in the complement fixation test in tuberculosis to employ a powerful antigen, for preference a bacillary-free antigen, active fresh complement suitably diluted, thoroughly washed red cells and a powerful haemolytic immune-body,

[1] Deycke and Much (*München. med. Wochenschr.* 1913, No. 3) found that a neutral fat could be extracted from tubercle bacilli which could be employed with good results for complement fixation experiments on tuberculin and tubercle immunity.

while titration experiments should always be carried out with a tuberculous immune serum of known activity.

Each series of experiments were undertaken for the purpose of throwing some light on the question of immunity in tuberculosis and, if possible, to trace a definite relationship between human and experimental tuberculosis.

It will be noticed by referring to the individual charts of the inoculated animals that doses with wide variations have been employed for these inoculation experiments, although the resulting lesions in the

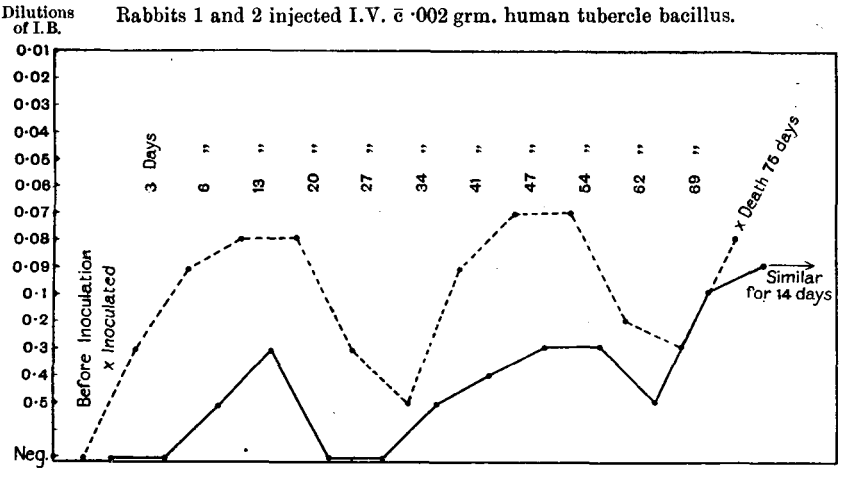

Chart 1.

Rabbit 1 represented by continuous line. Rabbit 2 represented by dotted line.

Weights:	Rabbit 1, before inoculation	2·610 grms.	Rabbit 2,	2·610 grms.
	on the 27th day	2·480 ,,	on the 34th day	2·050 ,,
	on the 69th day	2·190 ,,	on the 75th day	2·115 ,,

Result of post-mortem and microscopical examination.

Lungs: General caseous tuberculosis. Bacilli abundant in caseous areas.

Kidneys: Scattered areas of caseous tuberculosis with numerous bacilli present, but chiefly affecting pelvic portion.

Spleen: Rabbit 1. Very limited evidence of disease present.

rabbits appear to depend more upon individual characters of the host and the special properties of the individual bacillus isolated from a case of pulmonary tuberculosis in man than upon the actual doses employed.

We may perform as many control experiments as necessary, inoculate animals of similar size and weight by the same route, with the same

material, and with a similar dose, and, yet, the blood reaction may be widely different in each case, although the obvious clinical and pathological records are similar. Each rabbit requires special consideration, just as is necessary in the case of human beings, a fact which is strongly emphasised in the following experiment. Two rabbits were injected intravenously and two intraperitoneally with similar doses of the same emulsion of living human tubercle bacilli obtained from a case of pulmonary tuberculosis. The inoculations were made on the same dates and the blood was tested in an identical manner at the same intervals of time. Each rabbit received 1 c.c. of the emulsion which contained ·002 grm. of bacilli suspended in normal saline.

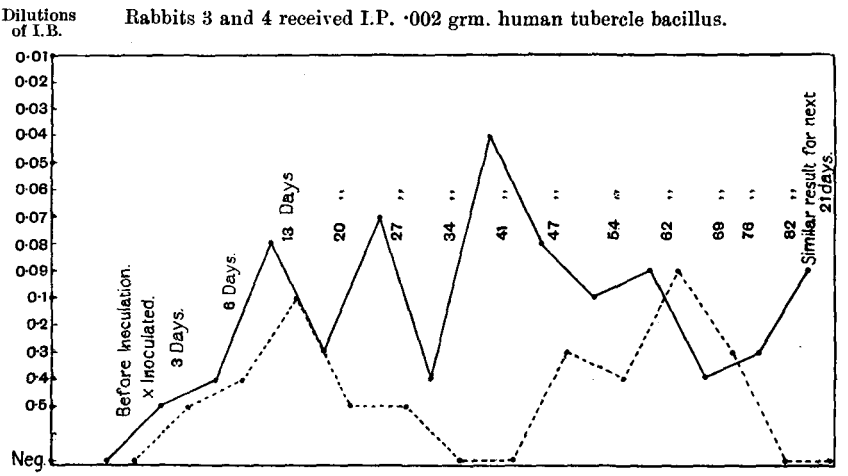

Chart 2.

Rabbit 3 represented by continuous line. Rabbit 4 represented by dotted line.

Weights:	Rabbit 3, before inoculation	2·960 grms.	Rabbit 4	2·530 grms.
	on the 34th day	2·750 ,,	41st day	2·600 ,,
	on the 96th day	2·290 ,,	82nd day	2·570 ,,

Result of post-mortem and microscopical examination.

Lungs: General caseous tuberculosis.
Peritoneum: Tubercles all through peritoneum.

When does the reaction first appear?

The earliest period at which the reaction was found to be positive occurred within four days from the time of inoculation. It is generally stated that 10 to 15 days are required for the development of the

allergic state from the time of the primary infection[1], previously little response to the infection has taken place, but then follows an inflammatory stage; fever develops, and local changes announce the fact that tubercle bacilli have produced a specific effect.

Three rabbits were inoculated intravenously with the same human bacillary emulsion and on the same date. One rabbit received 0·03, another 0·003, and the third animal 0·0015 grm. The rabbits which were inoculated with 0·03 and 0·003 grm. developed the reaction within a period of six days, while the rabbit injected with 0·0015 grm. did not react until after a period of fourteen days, but the final result in each case was similar. That the actual amount injected does not necessarily explain the difference, is shown by another experiment. Two rabbits of identical weight received the same dose intravenously, but there was a considerable difference between the periods when the reactions first developed. It is unnecessary to dwell further on this question, we will merely remark that there is abundance of evidence to show that the reaction period is not entirely dependent on dosage or virulence of the organism when injected intravenously or intraperitoneally.

Variations in the strength of the Reaction.

Attention has already been drawn to the technical details of the reaction, more especially to the fact that fresh guinea-pig complement suitably diluted was always employed, and that the same antigen was used week by week, while control observations were made with standardised immune-body so that every known factor in the titration experiments should remain constant during the whole course of the observations with various infected animals. The most satisfactory period in this work was reached when the antigens containing the bacillary bodies were replaced by the watery extract and finally by the most valuable antigen of all—the alcoholic. The most conclusive proof that constant results could be obtained by this method was demonstrated by titrating the sera each week, storing them, and repeating the whole series of observations at the end of the animal's life, six or eight weeks from the commencement of the experiment. It was then found that similar results were obtained, provided the sera were not contaminated.

[1] N.B. Allergy is a term used by Baldwin* and others to include all forms of "tuberculin" reaction, whether local or general, or any inflammatory process caused by tubercle bacilli or their products after infection has been established.

* Baldwin, Edward R. "Allergy and Re-Infection in Tuberculosis." *Johns Hopkins Hospital Bulletin*, July 1913.

It is, however, of considerable importance to realise that a strong positive reaction may be followed by a period when the reaction is absent, only to be succeeded by a reaction as strong as that which occurred before the negative period. Alterations in the character of the blood reaction observed at weekly intervals in this manner are of considerable importance, more especially in connection with the study of the blood in human pathology. It proves—if further proof is needed —that a negative reaction obtained as a result of a single blood examination may be totally disregarded. Two experiments will be cited to emphasise this point. A rabbit was inoculated intravenously with the human bacillus—a positive reaction developed in a few days and remained so for a period of one month, only to be succeeded by a negative period of 14 days duration, and then the reaction was again positive. A rabbit which had been injected intravenously with the bovine bacillus rapidly developed a powerful reaction which gradually declined, reaching the negative stage a month from the time of inoculation. This was followed by a short positive period just previous to the death of the animal. It only remains to point out that active immunisation of a tuberculous rabbit with a specific preparation may give rise to a negative reaction, as will be referred to later. In tuberculous animals, whether the disease is progressing slowly or rapidly, there is no appreciable alteration in their condition sufficient to explain these seralogical changes. It was thought that fluctuations in the weight of the infected animals might prove a clue to this particular point, but experience has proved that this is not so.

The Blood Reaction in Relation to Anaphylaxis.

The question which concerns us here is whether there is any relationship between the blood reaction and the hypersensitiveness of the infected animal. Sata, quoted by Austrian[1], claims to have shown a certain relationship between hypersensitiveness and the production of the anti-bodies. "Where hypersensitiveness develops, there immunity appears." Romer[2] found from a series of experiments upon animals that a primary infection was in itself protective against small re-infecting doses, but this immunity diminished as the second re-infecting dose was increased, finally reaching a state of hypersensitiveness to

[1] Austrian, C. E. "The Effect of Hypersensitiveness to a Tuberculo-Protein upon subsequent infection with Bacillus tuberculosis." *Johns Hopkins Hospital Bulletin*, Jan. 1913.

[2] Romer, P. K. (1908). *Beiträge z. Klinik d. Tuberkulose*, XI. p. 79.

large doses of tubercle bacilli. Romer considers that hypersensitiveness to tubercle bacilli is quite distinct from hypersensitiveness to tuberculin.

Austrian's work on hypersensitiveness of the rabbit to tubercle bacilli was carried out by employing for the sensitising dose a tuberculous extract made by a modification of Baldwin's method; while the living bacillus isolated from human sputum was injected 21 days later. By this means rapidly fatal tuberculosis was set up such as occurs when the bovine bacillus is injected into rabbits. No observations, however, were made on the blood condition during this period of anaphylaxis.

Rabbits 1 and 2 inoculated I.P. c 0·1 grm. of dried dead human tubercle bacilli and 21 days later with living human tubercle bacilli.

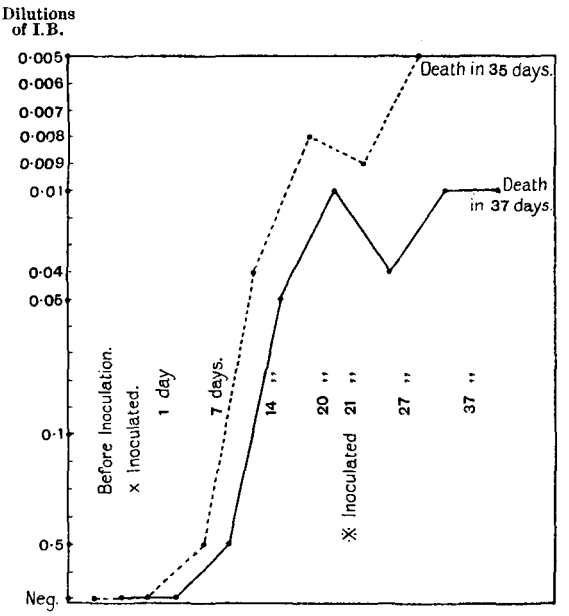

Chart 3.

Rabbit 1 represented by continuous line. Rabbit 2 represented by dotted line.

* Rabbit 1 received I.V. ·0027 grm. living bacilli from human sputum, while Rabbit received I.V. ·0013 grm.

Weights:	Rabbit 1, before inoculation	2·320 grms.	27 days later	2·100 grms.
	Rabbit 2, ,, ,,	2·560 ,,	27 ,,	2·290 ,,

Post-mortem and microscopical examination.

Fibro-caseous tuberculosis of the peritoneum.
General caseous tuberculosis of the various viscera.
Tubercle bacilli abundant in the caseous areas.

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In my experiments rapidly fatal tuberculosis was produced by the infecting dose which was administered about 20 days after a sensitising intraperitoneal injection of dead tubercle bacilli. The main object was to incite a rapidly fatal disease by means of tubercle bacilli isolated from human tuberculous sputum, and to observe the blood reaction while the animals were suffering from rapid tuberculosis as compared with the period of disease incited by dead bacilli.

If we take as examples rabbits 1 and 2 of this series—each animal was of somewhat similar weight and each received 100 mgrms. of dead

Two Rabbits, *X* and *Y*, inoculated I.P. c̄ 0·1 grm. dead human bacilli and 22 days later c̄ living human bacilli.

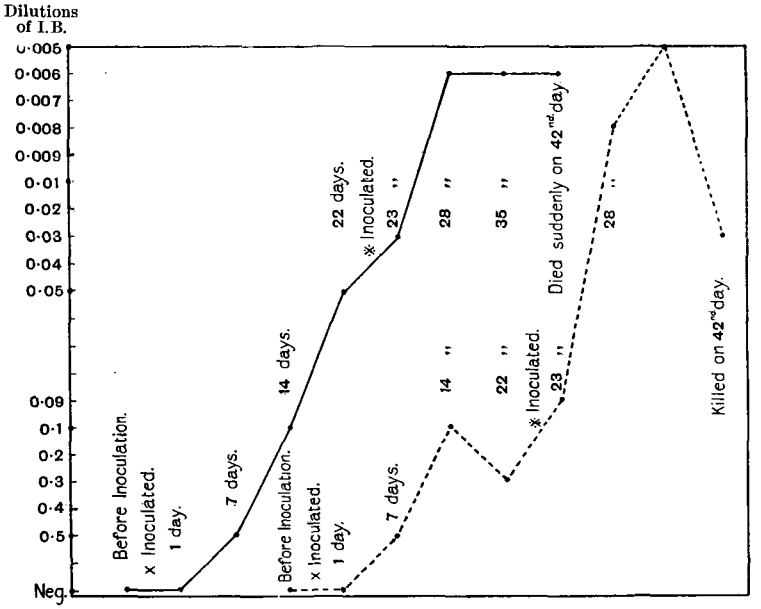

Chart 4.

Rabbit *X* is represented by the continuous line. Rabbit *Y* is represented by the dotted line.

* Rabbit *X* received 0·003 grm. living human bacilli, while *Y* received ·0021 grm.

Weights:	Rabbit *X*, before inoculation	3·230 grms.	Rabbit *Y*	3·410 grms.
	37 days later	2·630 ,,	42 days later	3·000 ,,

Post-mortem and microscopical examination.

Abdomen: Very extensive fibro-caseous tuberculosis throughout peritoneal cavity. Great omentum greatly thickened. Tubercle bacilli very abundant. Tuberculosis extended down the cord on each side. Caseous tuberculosis of both testicles. Tubercle bacilli very abundant.

Liver, Spleen and Kidneys: Areas of caseous tuberculosis.

Lungs: Diffuse caseous tuberculosis. Tubercle bacilli very numerous.

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tubercle bacilli. In each case a positive blood reaction developed which soon reached a high degree of activity. Twenty days after the first injection, each animal was inoculated intravenously with tubercle bacilli isolated from human sputum. One animal (R. 1) received 0·0027 grm., while the other (R. 2) was injected with 0·0013 grm. Both animals died a fortnight later from general tuberculosis, but during this period of fulminating tuberculosis, the blood reaction remained much the same as before the second dose was administered. Although this bacillus possessed active infective properties for the rabbit, as the control animal showed, yet, the sensitising dose had greatly increased the effect, while there was no reduction in the blood reaction in spite of the rapidly disseminated disease which was incited. The next three rabbits, to which full reference will be made, do not illustrate true anaphylaxis, but merely the effect of injecting a virulent bacillus into already sensitised animals. The control animal proved that the effect produced was not pure anaphylaxis. Rabbits *X* and *Y* received 100 mgrms. of dead human tubercle bacilli by the intraperitoneal route. A positive blood reaction occurred in each case within a week, and the strength of the reaction increased as time advanced. Twenty-two days later one animal (Rabbit *X*) and also a control rabbit, received 0·003 grm. of the bacillus isolated from human sputum, while rabbit *Y* was injected with a much smaller dose amounting to 0·002 grm. of the same bacillus. Although the second inoculation in each case was widely different, yet both animals developed general tuberculosis within a period of three weeks. While the active process was rapidly spreading, the blood reaction increased and reached a high degree of activity before death, and further it was found that each animal rapidly lost weight. The control rabbit had much less advanced tuberculosis than the rabbit *X*, which had been injected with a similar dose of the same strain of the living bacillus, and while rabbit *X* died of tuberculosis within three weeks of the second inoculation, the control animal was never *in extremis*.

The last rabbit (R. 22) which will be referred to serves to illustrate the effect of three intraperitoneal inoculations with dead human bacilli at intervals of about 17 days, while ten days after the last injection 0·006 grm. of living human bacilli was inoculated intravenously. The blood was examined each week and the effect carefully noticed. A highly active serum developed as a result of the inoculation with dead bacilli and still further increased after the intravenous injection of living bacilli, but became less marked in the final stages of the animal's

life. At the autopsy there was active and diffuse tuberculosis of the lungs, limited disease of the kidneys and spleen and chronic tuberculosis of the peritoneum.

True anaphylaxis set up in sensitised animals by the injection of a small dose of living bacillus occurred, but the blood reactions were similar to those recorded in the experiments referred to above. In conclusion these results emphasise that anaphylaxis and strong blood

Rabbit 22 inoculated on four separate occasions with human tubercle bacilli, (1) 0·04 grm. dead tubercle bacilli, (2) 16 days later 0·02 grm. of same emulsion, (3) 0·005 of same emulsion 35 days later, (4) 0·006 grm. of *living* tubercle bacilli I.V. 43 days later.

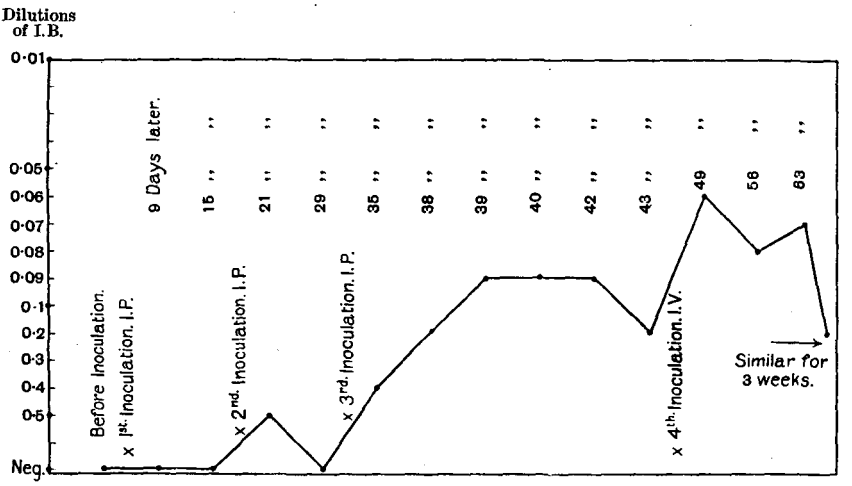

Chart 5.

Post-mortem and microscopical examination.

Lungs: Very oedematous. Diffuse tuberculosis. Caseation marked. Tubercle bacilli abundant.

Spleen: General tuberculosis.

Kidneys: Ditto.

Liver: Scattered tubercles present.

Peritoneum: Chronic fibrotic peritonitis.

reactions occur at one and the same time in the rabbit experimentally infected with tuberculosis—that there may be no alteration in the blood reaction in rabbits observed during the reaction period incited by the dead bacillus and the later stage of actively spreading tuberculosis.

Effect of Nuclein Injections on the Blood Reactions.

Various rabbits suffering from tuberculosis were injected subcutaneously with nuclein, the blood was collected at various intervals, and titration experiments were made with the serum. The total number of leucocytes and of polynuclear cells was estimated at the same periods as the blood examinations were made. It was not found, however, that nuclein caused any increase in the strength of the immune-serum, although the leucocytes and polynuclear cells showed the usual changes known to occur as the result of the injection of this substance. The blood was examined some days after the nuclein injections, but still no definite alteration had occurred which could be placed to the credit of the nuclein.

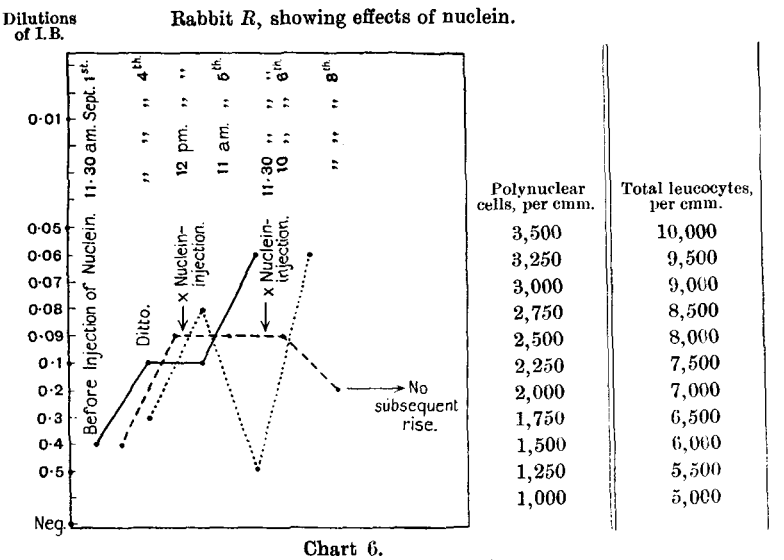

Chart 6.

(1) *Dotted line* = Curve of polynuclear cells per cmm. (2) *Interrupted line* = Immune body content. (3) *Continuous line* = Curve of total leucocytes per cmm.

* Nuclein injections I.P. (1) 1 c.c., (2) 2 c.c.

One experiment (R. *R*) is cited in detail showing the effect on the leucocytes and blood serum as the result of the injection of nuclein intraperitoneally in a rabbit suffering from tuberculous peritonitis due to the injection of ·04 grm. of dead human tubercle bacilli. As will be seen from the accompanying chart, no alteration was noted in the strength of the immune-serum, as measured by these methods of observation, although the usual leucocytic effects occurred.

On the Results of the Injection of Tubercle Bacilli previously "Treated" with Immune Tuberculous Serum.

Four rabbits were injected intraperitoneally with living human tubercle bacilli which had been subjected to the following treatment. Human tubercle bacilli were cultivated on Dorset's egg medium from the spleen and liver of a guinea-pig which had been inoculated with the

Rabbits 1 and 2 were inoculated I.P. c̄ 0·018 grm. of *treated* human tubercle bacilli.

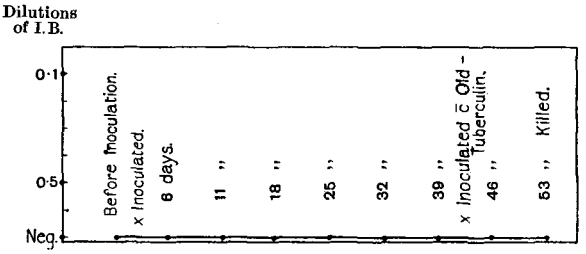

Chart 7.

Post-mortem and microscopical examination.

Nothing abnormal detected.

Rabbit 4 inoculated I.P. c̄ ·0037 grm. of *treated* human tubercle bacilli and thirty days later I.V. with the same emulsion.

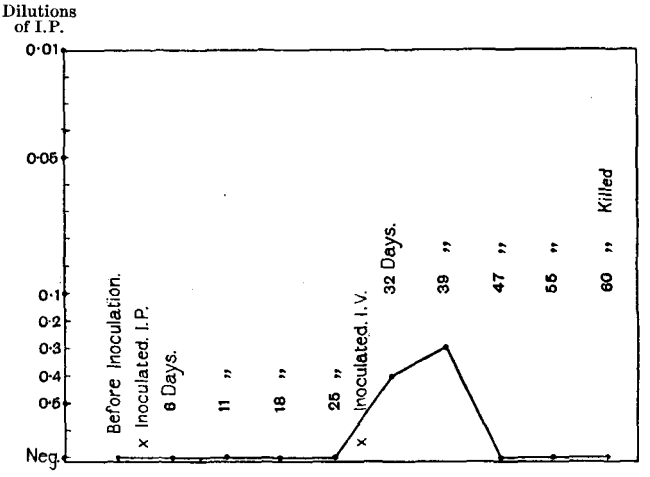

Chart 8.

Post-mortem and microscopical examination.

Lungs alone affected.

Scattered miliary tuberculosis of both lungs. Areas of consolidation are small and caseation is slight.

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sputum from a case of pulmonary tuberculosis. An abundant growth was obtained in three weeks at 37° C., and of this 0·185 grm. was mixed with sterile saline and shaken for one hour to break up the solid clumps. The saline was then pipetted off and the solid residue was thoroughly mixed in 2 c.c. of sterile saline, so that a very turbid suspension of bacilli was obtained. Two cubic centimetres of immune tuberculous rabbit serum was added and the total mixture incubated at 37° C. for 20 hours, then kept at room temperature for four hours. The bacilli were then thoroughly washed in sterile saline and finally suspended in that medium.

These results are of very great interest as we found that all four rabbits were free from active disease of the peritoneal cavity which was the seat of the primary inoculation, although the active pathogenicity of the bacillus[1] was proved by subcutaneous injection into guinea-pigs, as already referred to. Rabbits 1 and 2 received a large dose of the bacillus, but the blood examinations were negative without exception as can be readily seen from the accompanying charts. A month after the injection of living bacilli 10 mgrms. of Koch's old tuberculin were administered to both rabbits, but without any effect on the blood, and no alteration of the clinical condition in either case.

Rabbits 3 and 4 were injected on the first occasion with a much smaller dose of the bacillus than rabbits 1 and 2, but without any effect for a period of one month. Each animal was then inoculated with 0·0009 grm. of the same bacillary emulsion intravenously. In one instance (R. 4) a feeble blood reaction was obtained five days later, followed by a slightly stronger reaction, which, however, was succeeded by a negative period until the death of the animal. In the case of the other rabbit, the reaction was negative from first to last. Both rabbits were found to have early tuberculosis of the lungs at the post-mortem examination without any recognisable changes elsewhere, while rabbits 1 and 2 were apparently quite free from disease as already mentioned.

These results are of considerable interest as they were the only rabbits inoculated with treated bacilli. Three out of four failed to give a blood reaction at any period of examination while one did react for a very short period. Still further of importance is the fact that there was no tuberculous disease of the peritoneum which was the seat of injection, although both rabbits which had also been inoculated intravenously developed limited tuberculosis of the lungs.

[1] Both the untreated and the treated bacillus was found to be actively pathogenic to guinea-pigs.

The Results of the Examination of the Blood in Rapidly Fatal Tuberculosis in Rabbits.

The study of the blood in rapidly fatal tuberculosis in rabbits is a matter of comparative ease owing to the virulence of the bovine bacillus for this animal. In most cases of rapidly fatal tuberculosis in rabbits the reaction develops early and may be persistent during the short life of the animal, while little or no loss of weight may occur during the whole period of infection.

Rabbits 1 and 3 inoculated c̄ bovine tubercle bacilli. (1) R. 1, injected I.V. c̄ ·0015 grm. (2) R. 3, injected I.P. c̄ ·0015 grm.

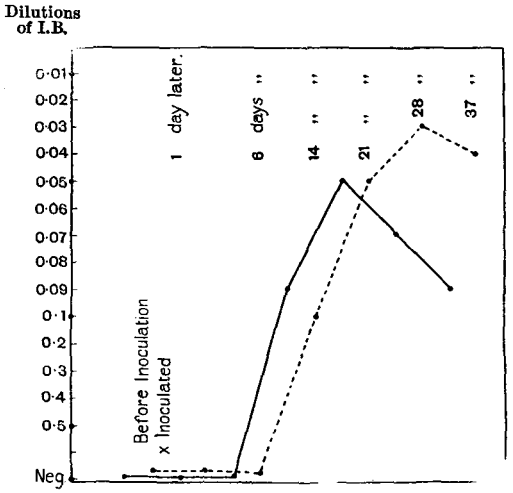

Chart 9.

Rabbit 1 represented by continuous line. Rabbit 3 represented by interrupted line.

Weights: R. 1, before inoculation 2·050 grms. R. 3, ... 2·850 grms.
34 days later ... 1·920 ,, 37 days later 2·797 ,,

Rabbit 1. *Result of post-mortem and microscopical examination.*

Lungs: General tuberculosis. Both organs greyish white in colour and no obvious healthy tissue visible. Bacilli abundant in caseous areas.

General tuberculosis of *Liver* and both *Kidneys*.

Spleen: Tuberculous on microscopical examination.

Rabbit 3. *Result of post-mortem and microscopical examination.*

General tuberculosis of the *Peritoneum*, especially of the great omentum and extending along the cord.

Testicles: Caseous tuberculosis. Bacilli abundant.

Spleen: General tuberculous foci.

Liver: Scattered tubercles present.

Lungs: Early tuberculous change detected. No bacilli seen.

Three rabbits, 1, 2 and 3, were injected intravenously with 0·0015 and 0·0057 grm. respectively of the bovine bacillus, while the third animal received 0·0015 grm. intraperitoneally. The doses were large and the disease was rapid, with the result that death of all the animals occurred within a period of five weeks from the time of inoculation.

If we compare the bovine results with those obtained by the inoculation of a bacillus isolated from a case of pulmonary tuberculosis which was highly pathogenic to rabbits even in minute doses, we find that here also the reaction developed early.

The life of the animals inoculated with this bacillus was limited to a period of 3–4 weeks from the time of the inoculation, and in every instance miliary tuberculosis was recorded at the post-mortem examination.

The Effect of Tubercle Bacillary Emulsion and Tuberculin on the Blood Reactions and on the Progress of the Disease.

A certain number of animals infected with tuberculosis were subjected to inoculation with dead tubercle bacilli or their products, so as to determine as far as possible what effect was produced on the blood reactions. In some instances the dose employed for this purpose was determined by a comparison of the weight of man and rabbit, but in other experiments a very much larger dose was given than in cases of human tuberculosis.

A large number of experiments were completed on these lines and as useless repetition is unnecessary, a few examples will serve to illustrate the chief points which have been noted during these observations.

Two rabbits, *A* and *B*, had been inoculated intravenously with 0·03 and 0·0015 grm. of living human tubercle bacilli. A month later subcutaneous injections of tubercle bacillary emulsion were commenced and continued in increasing amounts at regular weekly intervals. It must be stated at the outset that the doses were distinctly large, but similar results were obtained with smaller doses. One rabbit lost weight, as will be seen by reference to the chart, the other also lost weight, but gradually recovered it. It was found at the post-mortem examination that both rabbits had advanced tuberculosis of the lungs and also tuberculosis of the kidneys. In neither case was the blood reaction increased by the injection, but on the contrary, as shown by the charts (*A* and *B*), a distinct reduction in the activity occurred. Numerous experiments have proved that as a rule tubercle bacillary

emulsion causes no increase in the blood reaction, while it not infrequently gives rise to a rapid fall or may be a completely negative result.

Still further no healing process or arrest in the course of the disease was detected in any animal whatever preparation was employed for the subcutaneous inoculations. Among those who administer tuberculin or allied preparations in cases of pulmonary tuberculosis in man some recognise its importance in dealing with the febrile state and in controlling the quantity of the sputum. In my opinion the *clinical*

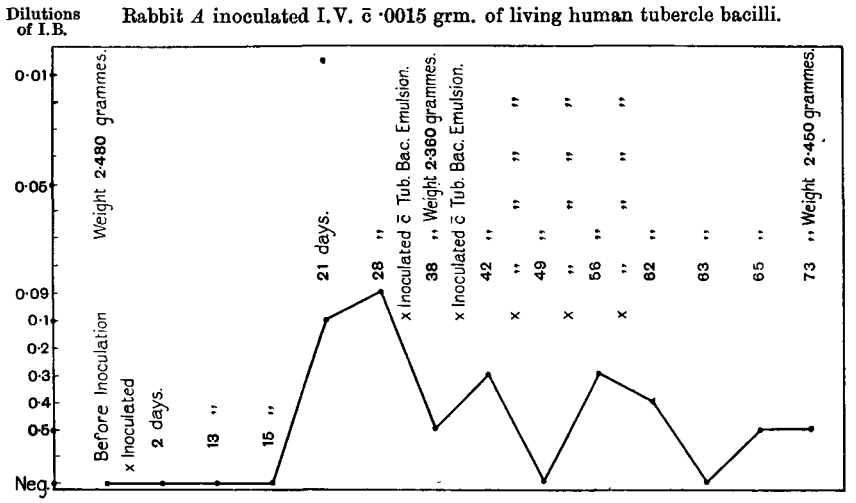

Chart 10.

Post-mortem examination.

Lungs: General tuberculosis, but posterior border more especially affected. Scattered tubercles all over both pleurae.
Kidneys: Tubercles seen on surface of left kidney.

Microscopy.

Lungs: Numerous scattered areas of caseation. Tubercle bacilli abundant.
Kidneys: Scattered tuberculous foci throughout both kidneys.

aspect of this disease in man and in infected rabbits has little in common and, therefore, it is unwise, because anti-tuberculous preparations are of little value in the treatment of tuberculosis in the lower animals, to condemn the use of specific preparations for the relief of certain symptoms of pulmonary tuberculosis in man. The charts of rabbits *C* and *D* show the effect of injecting two animals with dead

bovine bacilli intravenously and then treating the animals with subcutaneous doses of old tuberculin. Here we find a reduction in the immune body content of the serum as may occur as a result of this method of inoculation. The blood was frequently examined and the injections of old tuberculin were made at intervals of two and five days.

Both animals were killed three weeks from the time of the inoculation of the dead bacilli intravenously and the lungs showed a type of chronic pneumonia.

Rabbit *B* inoculated I.V. c̄ ·03 grm. of living human tubercle bacilli.

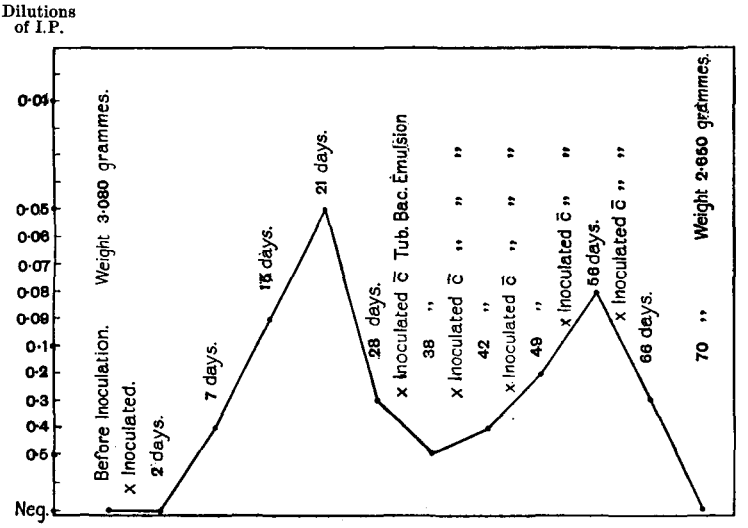

Chart 11.

Post-mortem and microscopical examination.

Similar results to Rabbit *A*, but disease more advanced, and *Spleen* shows general tuberculous foci.

Results obtained with Pleural and Peritoneal Exudates.

In certain instances effusions have been found at the post-mortem examinations on the infected animals. In nearly every instance the effusion has given a positive reaction, although the blood reaction may have been negative at the corresponding period, and also there was evidence to show that an effusion may be much more strongly positive than the blood serum. Dudgeon[1] pointed out in his Croonian Lectures

[1] Dudgeon, Leonard S., Croonian Lectures. "The Pathology of Immunity as concerns the Agressins." *Lancet*, June and July, 1912.

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that the exudate which collects in the peritoneum of immunised animals at the end of one hour may have a greater power to excite phagocytosis than the blood serum.

Rabbits *C* and *D* inoculated I.V. c̄ ·0025 grm. of dried dead bovine bacilli.

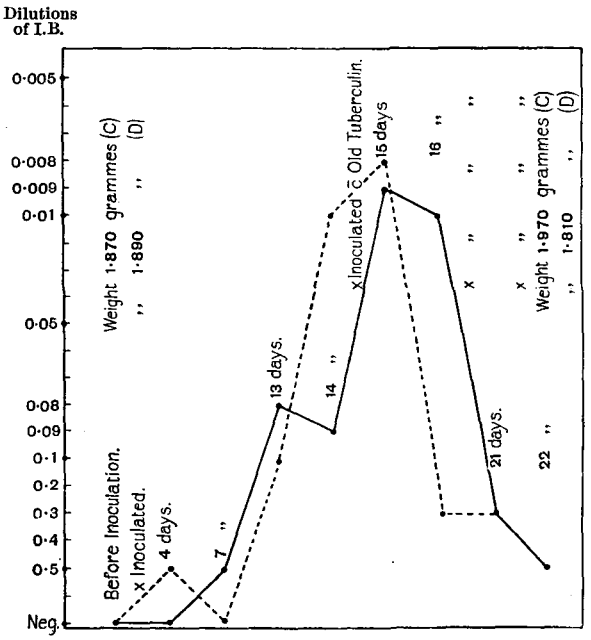

Chart 12.

Rabbit *C* represented by continuous line. Rabbit *D* represented by interrupted line.

Post-mortem and microscopical examination.

P.-M. Nothing abnormal detected.

Microscopy: Chronic indurative pneumonia present. Alveolar walls very much thickened. Patches of consolidations showing endothelial cells and giant cells present. Tubercle bacilli scarce.

An Examination of the Blood of Infected Guinea-Pigs.

These animals are not especially suitable for investigations of this kind, owing to the impossibility of obtaining sufficient blood for accurate observations at regular weekly intervals or may be even more frequently. For this reason single or duplicate experiments are quite valueless, but observations made on batches of these animals, so that individual anomalies can be recognised, afford the most satisfactory method.

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Sixteen guinea-pigs were inoculated subcutaneously with 0·001 grm. of virulent human tubercle bacilli. Three were killed ten days later and all gave a positive reaction, while in most instances those killed at longer intervals when the disease was much more advanced, gave a negative reaction; although occasional guinea-pigs killed four or five weeks from the time of inoculation at a period when they were suffering from general tuberculosis not only gave a positive, but a strong reaction.

A batch of guinea-pigs were inoculated subcutaneously with 0·00275 grm. of dried living human tubercle bacilli. The animals were killed at varying intervals so as to determine when the reaction first appeared. It was generally found that this occurred about the tenth day of the disease, at a time when the obvious post-mortem appearances are mainly confined to the area of inoculation and to local glands. By no means all guinea-pigs give the reaction at this period, but some out of every batch will generally be found to react.

A series of guinea-pigs were inoculated intraperitoneally with human tubercle bacilli in doses of 0·002 or 0·001 grm. These animals were also killed at varying intervals to determine the periods at which the reaction first appears. In one case the peritoneal fluid gave a positive reaction seven days from the time of inoculation, although the blood of the animal failed to react. The period at which the blood reaction most commonly develops is about the tenth to the fourteenth day.

Animals inoculated with dead bacilli develop the reaction usually at a later period of infectivity than those infected with the living organism. It appears that quite a high percentage of guinea-pigs with very advanced disease, in fact, animals dying of general tuberculosis, may quite fail to give the blood reaction.