

many villages suffering badly, and as a survey of the infected villages is about to be undertaken it is of the utmost importance that those placed on special kala-azar duty in connection with this survey should be on the look-out for this anomalous type of the disease. It may, and probably does, occur in a very small percentage of those attacked, but it is just as capable of transferring the disease and is consequently a much greater source of danger to the healthy community, as this particular type is so difficult to recognize.

ON THE PRESENCE OF AN EASILY PRECIPITABLE ANTI-COMPLEMENTARY GLOBULIN-LIKE SUBSTANCE IN HUMAN SERUM AND ITS IMPORTANCE IN THE DIAGNOSIS OF KALA-AZAR.

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WHEN human serum is diluted with excess of distilled water it becomes cloudy owing to partial precipitation of the serum globulin. Under certain circumstances, a copious precipitate forms instead of a mere cloudiness. This precipitate is due to a globulin-like substance as is evident from the following facts:—

(1) It is soluble in normal saline, in dilute acids, and in dilute soda bicarb. solution. It is also soluble in sodium hydrate solution.

(2) It is precipitated from its solution in normal saline when the solution is treated with equal parts of a saturated solution of $(\text{NH}_4)_2\text{SO}_4$ or when it is saturated with MgSO_4 or NaCl .

(3) It is not precipitated by NH_4OH from its solution in dilute acids.

(4) It is insoluble in distilled water.

On chemical analysis, this substance is found to contain C, N, H, O, but so far I have not been able to detect in it the presence of S, P, or any halogens. After being thoroughly washed in distilled water it can be collected as a white precipitate having a granular appearance under the microscope.

If further investigations confirm the observation that this substance does not contain any phosphorus or sulphur, then it will be found to be different from serum-globulins in chemical composition.

We hope to enter, at a future date, into the chemical nature of this substance and at present shall content ourselves by assuming that it is globulin-like in nature.

In the *Indian Medical Gazette* last September, I pointed out that this copious precipitate is frequently observed when the serum of a kala-azar case is mixed with excess of distilled water. I also pointed out that a precipitate apparently

similar to this has sometimes been observed in other diseases, e.g., chronic malaria, phthisis, cancer of the liver, etc. I was not, therefore, then able to state whether the presence of this precipitate was of any diagnostic importance in kala-azar. Further observations lead to the conclusion that, if instead of using an excess of distilled water (which in my original experiments consisted of 15 to 20 times the amount of serum used) one uses two or three volumes of distilled water then the precipitate appears almost exclusively in kala-azar. Thus in a series of 20 cases of kala-azar, the following results were obtained:—

One part of serum plus two parts of distilled water produced a copious precipitate.

(In some cases one part of serum plus one and-a-half parts of distilled water gave rise to a distinct precipitate.)

Similar experiments were made with the serum of a series of cases suffering from other diseases and a negative result was always obtained.

	One part of serum plus two parts of distilled water:
(1) Phthisis ...	No pp.
(2) Malarial fever ...	No pp.
(3) Cirrhosis of the liver ...	No pp.
(4) Enteric fever ...	No pp.
(5) Brights' disease ...	No pp.
(6) Anchylostomiasis ...	No pp.
(7) Pernicious anæmia ...	No pp.
(8) Dengue ...	No pp.
(9) Dysentery ...	No pp.
(10) Pneumonia ...	No pp.
(11) Catarrhal jaundice ...	No pp.
(12) Broncho-pneumonia with enlarged spleen (No L. D. bodies in the spleen)	No p.

In a few cases with enlarged spleen in which no L. D. bodies were found on spleen puncture, a similar precipitate was obtained, though clinically they looked like kala-azar.

Whether these are cases of kala-azar in which the parasites could not be found on spleen puncture, as sometimes is the case as pointed out by Leishman or whether some of them are cases of spontaneous cure from kala-azar can not be definitely stated in the present state of our knowledge.

I have also found that if distilled water is gently poured on to the top of the serum of a kala-azar case a distinct white ring is formed at the junction similar to the ring of albumen that is found on addition of nitric acid to a solution of albumen. This test also appears to be of diagnostic importance in kala-azar. A similar test is also observed in some obscure cases of enlarged spleen mentioned before.

To make the above two tests proceed as follows:—

(1) Two cc. of the blood from a prominent vein of a kala-azar case are drawn by a glass syringe and

the blood quickly centrifuged. The serum freed from the clot is introduced into a miniature test tube with a capillary pipette and then a small amount of distilled water is gently poured over the serum. A distinct *white ring* forms over the surface of the serum, in every case of kala-azar. I propose to call this the "globulin ring test" of kala-azar.

(2) The serum is collected inside miniature test tubes and then mixed with two or three parts of distilled water. A white precipitate forms in every case of kala-azar. I propose to call this the "*globulin precipitation test*" of kala-azar.

The anti-complementary properties of the above globulin-like substance.

This fact has been briefly touched upon by me in the September number of the *Indian Medical Gazette*. The following is a detailed method of showing this remarkable property of this globulin-like substance:—

One cc. of the serum of a kala-azar case is mixed with fifteen cc. of distilled water. The precipitate is collected and then washed thoroughly with distilled water. The precipitate is then dissolved in one cc. of normal saline. The following observations were then made:—

(1) Take, for instance, a hæmolytic system in which the following are the doses of the component parts:—

·15 cc. of anti-sheep amboceptor + ·5 cc. of guinea-pig's complement + ·5 cc. of sheep's corpuscles = complete hæmolysis.

(2) (a) Mix ·5 cc. of the guinea-pig's complement with ·2 cc. of the solution of the above precipitate in normal saline—incubate for half an hour.

(b) Add to this ·15 cc. of anti-sheep amboceptor + ·5 cc. of sheep's erythrocytes = no hæmolysis (I propose to call this test, "*anti-complementary globulin test*" of kala-azar).

(3) 1 cc. of the serum of the same kala-azar case + ·5 cc. of sheep's erythrocytes = complete hæmolysis (due, no doubt, to the natural complement and amboceptor frequently present in human serum).

(4) (a) Heat the serum to 55°c. for half an hour.

(b) Add ·2 cc. of the heated serum to ·5 cc. of guinea-pig complement—incubate half an hour.

(c) Add ·5 cc. of sheep's erythrocytes to (b)—incubate—complete hæmolysis.

From the above the following conclusions are made:—

1. One part of serum of a kala-azar case and two or three parts of distilled water—distinct precipitate. Such a precipitate is not obtained in any other disease except kala-azar and some rare obscure cases of enlarged spleen. Its presence is therefore of much diagnostic importance ("Globulin precipitation test").

2. Gently pour distilled water on to the top of the serum of a kala-azar case—a distinct white ring forms at the junction. This ring is not observed in any other disease except kala-azar and some rare obscure cases of enlarged spleen. Its presence is, therefore, of much diagnostic importance ("Globulin ring test").

3. The solution in normal saline of the above globulin-like substance present in the serum of kala-azar patients inhibits the action of the complement in a hæmolytic system consisting of sheep's corpuscles, anti-sheep amboceptor and guinea-pig's complement ("anti-complementary globulin test.")

4. This globulin-like substance does not inhibit the action of the natural complement normally present in the serum as long as it is not separated from the serum by the action of distilled water.

5. This globulin-like substance is probably in combination with some constituents of the serum and as long as this combination exists, it exerts no anti-complementary action. Distilled water also breaks up this combination. It is not broken up by heating the serum to 55°c.

6. We have regarded the above substance to be globulin-like in nature, but further investigations will be required to determine its chemical nature. At present it seems to differ from serum-globulin in not containing any S. or P.

7. Globulin-like substances are sometimes precipitated from the serum of cases suffering from chronic malaria, cancer of the liver, etc., by the addition of excess (15 or 20 parts) of distilled water, as has been pointed out by me in the *Indian Medical Gazette* (September, 1917). The precipitate obtained is much greater than, and must not be confounded with, what appears as a cloudiness when normal serum is diluted with excess of distilled water, due to partial precipitation of serum globulin. None of these, however, are precipitated when the serum is diluted with only *two* or *three* parts of distilled water. The properties of these globulin-like substances will form the subject of a future investigation. It cannot at present be stated whether they possess any anti-complementary properties similar to what is shown by the globulin-like protein separated from the serum of kala-azar patients. I cannot also state whether they contain any P. or S.

8. It is possible that in different diseases, globulin-like proteins, perhaps of a specific nature, are present in the serum with varying degrees of solubility in salt solution. The one present in kala-azar is characterized by being very easily precipitated by addition of small amount of distilled water to the serum. This is evidently due to the inability of NaCl in the diluted serum to hold the protein in solution.

Whether the "anti-complementary globulin test," the "globulin precipitation test" and the "globulin ring test" are absolutely pathognomonic of kala-azar can only be settled by further investigations, but, so far, they seem to be very valuable tests in the diagnosis of the disease. In some cases of very slightly enlarged spleen, the presence of these reactions led to the diagnosis of kala-azar, which was afterwards confirmed by spleen puncture. In making the ring test the serum must first be diluted ten to twenty times with normal saline.

My grateful thanks are due to Lieut.-Col. R. P. Wilson, I.M.S., for giving me every facility in carrying on my researches in the Campbell Hospital. I am also deeply indebted to Col. Sutherland, I.M.S., and his assistant, Dr. G. C. Mitter, for providing me with materials for conducting the serological portion of my work.

TUBERCULIN IN DISPENSARY PRACTICE.

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THE object of this communication is not so much to dwell on the value of tuberculin in treatment, but rather to advocate its more extended use in the practice of the many dispensaries provided throughout the country, and more especially at those situated at the headquarters of districts.

There is no question I think at the present time of the value of tuberculin properly used; yet there is a remarkable hanging back in the matter of using it. So much is made, it would appear, of the dangers of tuberculin, that doctors and patients alike shrink from employing it, or allowing it to be employed, in a very large number of cases where its benefit is lost, or it is not resorted to till too late.

I think there is no question about the very wide prevalence of tuberculosis all over India, especially in the more crowded towns, and yet, whenever one suggests to patients coming up from other places that tuberculin be used, one is met with the statement that their previous doctor was against any such treatment; and in the *Indian Medical Gazette* of December, 1914, we find a very alarming article on the dangers of tuberculin. I do not offer to criticise that article, except to say that, put in as it is, it is rather calculated to warn people off the using of tuberculin, or even to encourage some to advise their patients, so as to give them but little hope from the use of tuberculin, or even to frighten them off it entirely.

And yet, what is the position at the present day? Who will deny that non-specific drug treatment is a failure, and can often do no more than pro-

duce an amelioration of certain symptoms without influencing the cause of them at its original source. And as for sanatorium treatment, for how many is it a practicable course, and is it not absolutely true—and irrefutably true—that hygienic dietetic treatment, which alone gives such results as sanatorium treatment *without tuberculin* can claim, meets in practice with insuperable difficulties, difficulties which are based on social and economic factors which are too often beyond the control of either doctor or patient. And what is the result of sanatorium treatment with and without tuberculin? If we are to judge by the result of ridding patients of their bacilli, 20 years' experience of the hygienic dietetic cure without tuberculin gave only 15 per cent. of cases definitely rid of bacilli; whereas with tuberculin treatment superadded, fully 50 per cent. are definitely rid of bacilli. This is when we consider cases as a whole. If we consider cases separately in the first, second, and third stages, Bandelier's series of 500 cases treated with tuberculin, of which 202 were open cases of tuberculosis, gives the remarkable results shown in the following statement:—

	On total of	Stage 1.	Stage 2.	Stage 3.
Complete earning capacity on discharge.	500 cases. 69.8 %	90.4 %	80.7 %	32.8 %
Sputum changed from positive to negative.	202 open cases. 63.9 %	100 %	87.3 %	44.0 %

These results are quoted, it is true, from the results of the best workers; but they show what can be done by the use of tuberculin. If, then, with the mass of tuberculosis which we have to deal with in India we shrink from the use of tuberculin, we deny our patients the advantage of a certain very definite chance of improvement. Now in our general relation to tuberculin as practitioners and dispensary doctors, firstly we come in contact with a large number of cases where the diagnosis of tuberculosis is not and cannot be clear without the use of tuberculin. One scarcely realizes how common and frequent tubercular lesions are till one examines post-mortem results. As in animal pathology, so in human, the occurrence of tuberculosis anatomically far exceeds in extent its recognition clinically—scores of cases are treated as "fever" and let go, and eventually show up as what they really are—tuberculosis. Does not this all point to a need for the use of specific means for diagnosis? It is quite true that in this specific diagnosis tuberculin tests have only a relative value—relative only to other clinical symptoms. But although it would be unwise practice to trust to tuberculin itself indubitably to settle a diagnosis, nevertheless as