

## Some Non-Specific Serological Tests in Leprosy

ALAN MCKENZIE. *continues a pag.*

**T**HIS paper is the account of an investigation undertaken to find out what assistance might be given in the diagnosis, prognosis and treatment of leprosy by such simple blood tests as can be performed where extensive laboratory facilities do not exist. The work was done in Songea, Tanganyika Territory—a small out-station staffed by a single Medical Officer serving an area of about 14,000 square miles and with a population of approximately 100,000. The remoteness of the station forbade the use of any but the most simple examinations. 99

The patients who were the subjects of this investigation are inmates of a colony about 20 miles from the government hospital. They were visited each week by the Medical Officer (myself). The medical equipment of the colony consisted of a small brick dispensary where treatment was given twice a week, and two huts which were used as hospital wards for those requiring more constant attention. Otherwise, the patients lived in native huts under the usual conditions of native life, tilling their own fields and producing the majority of their own foodstuffs. Rough and ready treatment of the various intercurrent diseases was given by a Mission Sister assisted by native dressers; and during my weekly visits I controlled treatment and kept notes of the cases. All the specimens used in this investigation were brought in to Songea for examination and if any special examination (such as the behaviour of the patients under iodide administration) was required they were admitted to the government hospital.

The material for the examination of controls was drawn from government employees and from patients within the hospital.

The following tests were used :—

1. The Botelho Reaction.
2. Rubino's Test. (This was perhaps too ambitious, as no incubator was available, but during the period the test was employed the temperature was barely below 80° F.).
3. The Serum Formalin Reaction graded as suggested by Dye with some minor modifications.
4. The Sedimentation Reaction performed in the manner Muir had used in his investigation of the Reaction among cases of leprosy in India.

The Botelho and Rubino tests are described separately. The Sedimentation Index and the Serum Formalin Reactions are described together since it was subsequently found that more information would be acquired from their simultaneous study.

#### CLASSIFICATION OF THE CASES.

Early in this investigation, I found it impossible to apply to African natives Muir's method of classification. Accordingly on purely clinical grounds, I have worked out a system which differs from his in certain essentials. This classification creates a new group which comprises the papillary and interfollicular sub-types of his skin type and the ascending sub-type of his nerve types.

The assumption on which Muir's classification rests is apparently that anæsthesia implies *infection* of a nerve or its terminals. Clinically I have found it more satisfactory to consider anæsthesia as a result of simple *pressure* and that a clearer conception of the disease is obtained if we explain the production of anæsthesia in the three types of lesions mentioned above as being the result of fibrosis following on the resolution of a leprotic lesion or as a result of pressure from œdema within and just below the corium during the more active periods of the disease.

Muir states in LEPROSY (page 182):—“(a) The more the skin is affected the less the nerves are involved and *vice-versa*; (b) Nerve lesions are associated with a comparative paucity of the number of bacilli in the body,” but he goes on to state that “(c) consequently, the earlier lesions of leprosy are chiefly of the nerve type as there are few bacilli in the body at the outset.” This conclusion does not appear to me a logical sequence unless he is prepared to substitute for “associated with,” “caused by.”

Muir defines a skin lesion (p. 183), “as an area in which leprosy bacilli can be found” and a “nerve lesion is indicated by the area of skin in which bacilli cannot be found—but in which there is an anæsthesia.” Later, he records these conditions as not infrequently present together in the same lesion and describes the formation and contraction of fibrous tissues in the centre of a resolving bacteriologically positive skin patch. When, however, he discusses the appearance of anæsthesia in these patches, he speaks of (p. 189) “the diminution of the number of bacilli being accompanied by infection of the sensory nerve endings according to the law of relationship between skin and nerve leprosy.” Is it not more reasonable to assume that the nerve endings have

been caught in the general fibrosis and their function deranged?

In describing the nerve ascending sub-type, Muir acknowledges its close connection with the superficial sub-type of skin leprosy and gives an identical description of its histology and mode of spread. The separation of these lesions into their proper primary types appears then to rest, clinically and pathologically, on the delivery of bacilli in the tissues—always in such lesions a most difficult process.

Muir's law of relationship between skin and nerve leprosy, in conjunction with somewhat debatable indirect evidence has led him to consider infection of the main nerve trunks to be due to an ascent of the bacilli up the nerve and not as an embolic infection. Bacilli, however, have never been found in the nerve endings and the acceptance of such a scheme of infection should be delayed until every other possibility has been disproved, since it leads to such puzzling conclusions as are implied in the question: "Why the bacilli should enter these nerve sheaths more when there is a minimum of lepra bacilli in the skin and fail to enter . . . when there are far larger numbers of bacilli is difficult to say."

In skin lesions of any of these three sub-types of Muir's, I have frequently found bacilli in the periphery of the patch and anæsthesia, if present, at the centre. These two conditions have occasionally been found together (Fig. 1) and on one occasion I found bacilli and anæsthesia in the same area of several patches. More generally it was found that bacilli during treatment quickly disappeared from the periphery of the patch while anæsthesia developed at the centre.

This phenomenon was usually associated with the reappearance of pigmentation at the central and anæsthetic portion. Although with continued treatment the patch, as far as anomalies of pigmentation are concerned, may be practically invisible, the anæsthesia may or may not disappear depending, I believe, on whether the nerve endings have been completely strangled by the formation of fibrous tissue during the resolution of the lesion, or are able to recover sufficiently to function again.

A similar sequence of events may occur during an acute exacerbation of the disease during which the patch occasionally swells up presumably from local œdema since no bacilli are found, and sensation is lost, to return when the swelling has once again subsided. To my mind, the following case is not explicable on any other grounds.

P. 91. When first seen, confessed to a five years' history and presented herself for admission on account of recent swelling of some of the lesions.

On the face and body there were pale and highly raised patches whose edge was smooth and precipitous giving a keloid-like appearance. The centre of some of the patches tended to be slightly darker.

All the patches were completely anæsthetic. No bacilli could be found. There was also extensive anæsthesia on the forearms and loss of several fingers with many trophic ulcers. (Fig. 2). Three months later the patches had lost their keloid character and showed exaggerated skin folds, they were less raised above the level of the skin and their pigmentation in the centre was much more pronounced. There was now no anæsthesia to be found in any of the patches.

Two months later the lesions had not apparently changed, but small areas of anæsthesia were found in the central pigmented portion of some patches. (Figs. 2 and 3.)

If one admits that the lepra bacilli (excluding for the moment embolic methods of propagation) extend their sphere of influence slowly, and tend to confine themselves to the tissue where they have been planted, *i.e.*, bacilli planted in the corium of the skin continue to grow within the corium until a large "patch" is formed; and granted also that where the tissue is dense there is less probability of the bacilli multiplying rapidly, we can explain all the puzzles on the assumption that anæsthesia is a result of fibrosis of either a nerve ending or a nerve trunk, and that there is no need to assume, except in affections of a nerve trunk itself that there has been any direct nerve infection. A very small degree of inflammation and fibrosis in or about a nerve trunk may lead to severe trophic changes while, if anæsthesia in a skin patch is caused by fibrosis and starvation of the nerve endings from a resolving leprosy lesion we should not expect to find bacilli.

The clinical observations which first led me to doubt the value of Muir's classification are briefly summarised below.

1. Depigmented patches especially in the limbs appear in the distribution of more than one cutaneous nerve.

2. The edge is usually well defined and bacilli are generally found there if it is raised, while anæsthesia is not found except towards the centre.

3. Trophic ulcers never appear in depigmented patches.

4. The changes found over the skin distribution of visibly involved nerves are quite different; rarely do they

show any considerable depigmentation but have a dry and atrophic appearance, while in these cases the anæsthesia accurately follows the distribution of the nerve and is co-extensive with the cutaneous change.

5. The behaviour of the depigmented patch under treatment is very different from the behaviour of a pure nerve lesion. This is most strikingly seen in cases which present both lesions. Thus the local skin signs may rapidly clear up while deformities progress.

6. I have seen a large number of cases of indubitably long histories, many over ten years, who show no signs of ever having suffered from involvement of a main nerve. These show no anæsthesia except within the visible patch, there are no deformities, muscle wasting, or signs of old trophic ulcers, and palpable nerves are not thickened.

In my classification, I divide the disease into three groups :—

I. Where the deep layers of the skin are affected and bacilli are found in large numbers. This comprises all nodular cases and Muir's sub-follicular sub-type of the skin lesion. Cases where anæsthesia is also found are labelled Ia, otherwise Ib.

II. Where only the superficial layers of the skin are affected. Including the ascending nerve lesion of Muir and his papillary and inter-follicular sub-types of the skin lesion. The class is labelled IIa and IIb and IIc, according as there is anæsthesia only, bacilli only or both present in the patch, while if neither bacilli nor anæsthesia can be found in a patch but the history and appearance of the lesion justify a diagnosis of leprosy, the case is labelled simply II.

III. Here the main nerves are affected leading to regional anæsthesia, deformities and trophic ulcers.

#### CRITERIA OF CURE AND IMPROVEMENT.

*Group I.*—Improvement is recorded on the general physical condition of the patient together with the appearance of the lesions. Skin smears are frequently taken and since few of this class have become bacilli-free stress has been laid on the appearance of the bacilli, especially with regard to fragmentation or beading.

*Group II.*—Little gross physical change appears to have resulted in the majority of these patients as the result of their disease. The finer changes in the patch itself appear to be the only reliable guide to progress. The margin usually at first raised becomes level with the skin ; if it has previously contained bacilli these soon disappear and any suggestion

of œdema in the patch rapidly vanishes. Commonly though not invariably, repigmentation may occur in the centre of the patch and if this happens it is almost certain that the pigmented portion will be anæsthetic. This repigmented portion is not quite normal in appearance; the skin appears dry and atrophic and the skin folds sharp and more obvious than usual, suggesting a very fine crepe paper. If sensation is re-established in the patch the pigment tends to disappear in a peculiar manner leaving a mottled look to the old patch. This repigmentation appears to be the sequence of events in a fairly chronic case. In a more acute case with resolution the anæsthetic area is larger in extent than the area of new pigment, which is usually less deeply coloured while where the raised edge has healed quickly, a crushed paper appearance is given in contrast to the fine crepe paper of the central and more slowly resolving lesions.

*Group III.*—It is difficult to discover signs of improvement in this group, and if it be allowed that the trophic lesions, which are the signs of the disease, are due to pressure on the nerves, it will be seen that either an advancing leprosy lesion of a nerve trunk or the resolution of a similar lesion and the consequent formation and contraction of fibrous tissue will both produce an increase in the trophic lesions along the distribution of that nerve. This is well seen in certain patients who have both Group II and Group III lesions. Although there may be remarkable improvement in the skin patch, often with complete disappearance of anæsthesia, deformities of the hand and absorption of the digits may progress rapidly, while the extent of the anæsthesia in the distribution of this nerve may remain as before or extend to the full area of distribution of the nerve.

#### THE BOTELHO REACTION.

Araujo (1928) recorded the results of this test in 50 cases of leprosy and found that it was positive in only half of them. Le Cac (1930) found it positive in 72.4 of a series of leprosy cases and stated that a positive reaction was more often found in nerve than in nodular cases. Muir (1930) found the test of no practical value in making a diagnosis.

#### *Technique.*

The following method of performing the test has been used :—

To 0.6 c.c. of 24 hours old blood serum in a small test tube, the following re-agents are added, the tube being shaken after each addition.

1. Liq. ammon. fortis, 1 drop.

2. 1 per cent. nitric acid in normal saline, 3 c.c.

3. 0·5 per cent. iodine in a 1 per cent. solution of potassium iodide, 0·5 cc.

A positive reaction was recorded when a precipitate appeared which did not at once disappear on shaking.

Among 36 patients 5, or 13·8 per cent., gave a positive reaction. Three of those giving positive results were Group I cases, and the other two were both mild Group II cases. Among 18 not suffering from leprosy, there were no positive reactions.

The test appears to be of no use as a diagnostic aid, the percentage of positives being so low.

#### THE RUBINO TEST.

Rubino first described the test that bears his name in 1926, finding it positive in 66 per cent. of 18 cases, while among 713 not suffering from leprosy, positive reactions were very rare. In a second paper, in 1927, he recorded 78 per cent. of positive results among 32 leprosy patients.

Paullier and Errecart, in 1926, carried out the test among 17 cases, and five controls but found the results inconstant. Marchoux and Caro, in 1927, modified the reaction and claimed that it was then specific, obtaining among ten cases 100 per cent. of positive results as compared with 50 per cent. by the original method. Monacelli found that 12 cases in whom bacilli could be demonstrated gave positive reactions, while a pure nerve case was negative. All his 45 controls were negative.

Beltier found that with Marchoux's modification, the reaction was more sensitive; 33·3 per cent. of positive results as against 22·2 per cent.; but that among his controls the modified method gave 17·3 positive results, while none was found when using the original technique. Luz, in 1929, found positive results in less than 50 per cent. of different types of leprosy. All his control cases, tubercular and syphilitic patients were negative. Amies, in 1929, found the reaction positive in 87 out of 97 active leprosy cases, while, in 126 inactive cases, it was invariably negative. In 287 controls, there were 16 positive reactions.

The method used in this series was that of Marchoux. Well washed, defibrinated blood, of a fat-tailed sheep, was made up to the original volume with 10 per cent. formalin and left for 24 hours. It was then rewashed in saline and brought to the original volume with saline; 0·2 c.c. of this suspension were added to 1 c.c. of the 24-hour-old serum to be tested and well mixed.

Sedimentation was carried out at room temperature (from 75° F. to 80° F.) in the absence of an incubator, in small pipettes, 1 c.c. occupying a space of approximately 4 inches. Readings were taken at half-hour intervals.

The sequence of events was usually as follows:—The suspension became gradually thicker at the bottom of the pipette and clearer above until there was a distinct upper margin to the deposit. Above there was a pale brown cloud reaching nearly to the top of the column above which the serum was quite clear. Later, the cloud settled down and the whole of the supernatant fluid became clear. Readings were taken at half-hour intervals and a record made of the time taken for the deposit to acquire a sharp horizontal upper limit. The cloud never settled under 24 hours.

In one case, however, the suspension rapidly aggregated into small particles (resembling a positive macroscopic agglutination) and settled with a distinct descending upper margin. The supernatant serum was at once quite clear. This sequence of events was completed in one hour. The test with this case was repeated both in a pipette and in a small test tube along with several other sera. The sequence was constant on each occasion and with either method. This latter case I regarded as the only positive result in the series. It occurred in an old Ib case that was considered cured, and had received no treatment for a year, when small, pale patches appeared over the trunk. There were no nodular lesions and bacilli could not be found, but the patient gave a response to the iodide-sedimentation test and to the iodide-serum formalin reaction and was recommended to undergo a further course of treatment.

### *Results of the Test.*

Forty-one cases were examined and the average time taken for the upper limit of the sediment to become sharp was 99 minutes. They were grouped as follows:—

<i>Classification.</i>	<i>Number examined.</i>	<i>Average time of Sedimentation.</i>
Ia	3	9 mins.
Ib	8	108 „
IIa	21	108 „
IIb	4	96 „
III	5	90 „
Total	41	99 „



Among controls, 11 apparently normal natives and nine hospital patients, there were no positive reactions; the average time was 87 minutes.

In my hands, therefore, the reaction has proved of no use, and though in performing it at room temperature, I have not complied with the requirements of the test, yet the occurrence of one positive reaction and the absence of any real difference in the time of the reaction between normal and specific cases suggests that this omission should not vitiate the experiment.

#### THE SERUM FORMALIN AND SEDIMENTATION REACTIONS.

##### *The Serum Formalin Reaction.*

The production of a gel on the addition of commercial formalin to blood serum was first suggested as a means for the diagnosis of syphilis. It was soon shown, however, that the gel occurred in several other diseases. Fox and Mackie (1921), applied it to kala-azar and Napier (1922), modified it by the simultaneous addition of phenol and further took into account the production of opacity in the interpretation of results. He found that a full positive reaction (the formation of a gel and the production of opacity) occurred also in tuberculosis and leprosy, and in two of his cases who were suffering from malaria and showed great numbers of parasites in the blood. Baretto (1926) recorded positive reactions with the formation of Napier's opacity in malaria, syphilis, leprosy, tuberculosis, and amœbic dysentery.

Wade (1925) found that the serum formalin reaction corresponded in a general way with the globulin ratio of the serum proteids. As the ratio increased the serum formalin reaction became positive. He found high globulin ratios and positive serum formalin reactions in leprosy and tuberculous subjects and in leprosy the globulin ratio was very much increased during reactions. He further suggested using the serum formalin reaction to detect incipient leprosy.

Dye (1926) used the serum formalin reaction in trypanosomiasis, constructed a notation so that the rapidity of formation of the gel could be numerically expressed. He found that in patients suffering from trypanosomiasis the reaction value fell during treatment. He applied his modification of the test to a short series of other diseases, and found it generally high in leprosy while in secondary yaws a medium value was the rule.

Dunscombe (1927), used the method of Dye over a



The occurrence or not of opacity in the gel was also noted, but as it occurred with very great frequency, and when absent did not appear to be related to any condition, no record of this observation has been made.

Tests were made to prove the constance of the reaction. Several patients, whose condition was not changing, were examined daily for several days and the proportion of serum and formalin varied. Under these conditions, the value of the reaction did not change and it was found that a great deal of latitude could be allowed in the relative amounts of reagents without influencing the final reading.

Blood for the sedimentation test was drawn from a vein into a 5 c.c. syringe containing 1 c.c. of 5 per cent. sodium citrate solution and the syringe filled with blood up to 5 c.c. mark, well mixing the fluids.

It was carried back to the laboratory in a test tube and the examination was conducted about four hours after the collection of the specimen.

After the blood had been again well mixed it was drawn up into a glass tube 10 inches in length, marked in inches and containing within the marks approximately 3 c.c. of fluid. The lower end was sealed with a dab of plasticine, and the tube placed vertically in a rack. Readings were taken at the end of of  $2\frac{1}{2}$  and  $3\frac{1}{2}$  hours, of the distance the upper limit of the red cell mass had descended. The average of these two readings was taken as the index.

An examination of the day to day variations of this reaction showed less constancy than was seen in the serum formalin reaction, but the difference did not amount to more than 10 per cent. It was found, however, that if the estimation of the reaction was delayed for 24 hours after collecting the blood the index tended to become lower. It is, therefore, not a suitable reaction to be performed in a distant laboratory where the specimen has to be transmitted through the post.

For the sake of uniformity, the reaction was always recorded as above, but it soon became evident that by this method some of the higher reactions received values that were much less than the speed of sedimentation warranted if observed at more frequent intervals. The reaction, in my opinion, should be recorded at half-hour intervals in the case of very high reactions and the average of the figures of the first two readings multiplied by four taken as the index.

EXAMINATION OF NORMALS AND BEHAVIOUR OF THE S.I. AND S.F. REACTIONS AMONG PATIENTS SUFFERING FROM DISEASES OTHER THAN LEPROSY.

Confining myself to my own series of cases which consisted of 40 normal subjects and 116 suffering from a variety of diseases, it appears that neither of these tests shows specific significance. They have been performed among a number of normal Africans and others suffering from various diseases. On the whole, the reactions show a distinct parallel trend; a large proportion of high serum formalin reactions being associated with a high sedimentation index. There are, however, groups of cases where the reactions markedly diverge.

With regard to the sedimentation index, it appears to be a very sensitive indication of the well-being of the subject at the moment of taking. It was high in all debilitating diseases and was usually higher in proportion to the length of time sick or to the gravity of the complaint. This interpretation differs slightly from that of the originator, Fahraens, who found the reaction "most distinct when accompanied by high fever." Thus in acute febrile diseases of short duration, such as malaria or the first manifestation of relapsing fever it rose, but not so high as in subsequent attacks of the same disease or in diseases where a high temperature had been maintained over some considerable period.

For example: A hospital dresser in good health had a S.I. of 5. Six days later he had an initial attack of relapsing fever and his S.I. rose at once to 36 while a patient who presented himself for treatment after his fourth attack had an S.I. of 65. After treatment, both these cases tended to return rapidly to normal.

It was very noticeable that in contra-distinction to the behaviour of the serum formalin reaction after recovery from an acute disease the S.I. fell much more rapidly than the S.F.

How sensitive this reaction is, was well shown by an examination of 26 normal subjects all of whom were well cared for natives in the employ of the hospital. An analysis of their reactions showed that they fell into two well-marked groups and the division followed the salary of the subject. The salaries of the first group were from Sh. 10 to Sh. 12 per month while that of the second varied from Sh. 24 up to Sh. 120 per month. All were apparently healthy and had ready and immediate access to medical treatment

if unwell. The results were as follows, each group numbering 13 subjects.

		<i>Average</i> <i>S.I.</i>	<i>Greatest</i> <i>S.I.</i>	<i>Least</i> <i>S.I.</i>
Group 1 ...	...	29.0	41	11
Group 2 ...	...	8.9	19	2

The difference seems that the first group were, on account of the high local cost of meat, of necessity largely vegetarians, while those of the second could and did eat meat three or more times a week.

The interpretation of the serum formalin reaction is apparently on somewhat similar lines, as it is positive in a majority of affections in which the sedimentation index is raised. Thus, among the normals quoted above, the average value in Group 1 was 1.0 (none having a reaction of more than 2) while in Group 2 the average value was 0.32 (only three presons having positive reactions). In the complete series of 40 normals, 12 showed positive reactions and in no case was the value more than 2. In acute febrile diseases, where the reaction tends to become positive, this change does not, as a rule, take place until some days' after the commencement of the disease.

Taking again relapsing fever : among cases of an initial attack of relapsing fever whose serum formalin reaction was 0 at the febrile period of the attack, in 6 of these the reaction rose within a week to values of from 2 to 5 and in two cases in which the stay in hospital enabled the reaction to be performed two to three weeks after admission, it had fallen again to zero.

In malaria I have not found that the reaction has risen after an attack, though I have not had the same opportunity for prolonged examination, but so far I have not seen a positive serum formalin reaction in a patient of proved physical fitness (such as a member of the Police Force or K.A.R.) during the febrile period or for short periods afterwards. Similarly, during an attack of blackwater fever, a patient had a S.I. of 60 rising on the third day to 70 while the S.F.R. was never above 1. A month later his S.F.R. was 0 while his S.I. was 40.

In chronic diseases on the other hand, the two reactions show decidedly similar variations, the highest S.F. reactions, where the serum gelled and became opaque within three minutes, were given by two cases of severe and chronic non-tuberculous infection of bone, the S.I.'s being 76 and 78. Two cases of old tuberculosis gave (a) S.F. 6 ; S.I. 6 (b) S.F. 6 ; S.I. 55, while another case in which the first



FIG. 1.  
BACILLI FOUND IN EDGE OF PATCHES. ANÆSTHESIA IN DARK CENTRE AND AT UPPER PORTION OF UPPER PATCH WHERE MARGIN HAS HEALED.

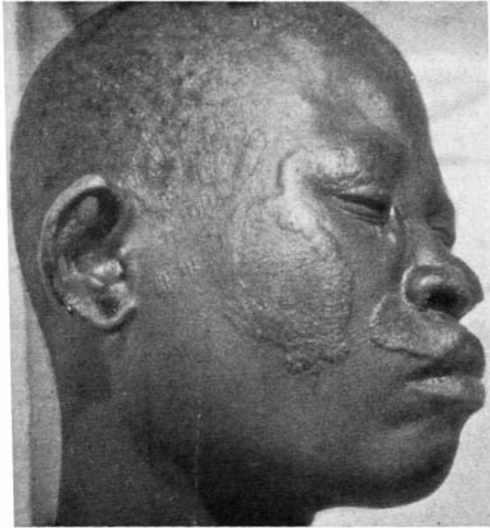


FIG. 2.  
RAISED ŒDEMATOUS-LOOKING ANÆSTHETIC PATCHES.

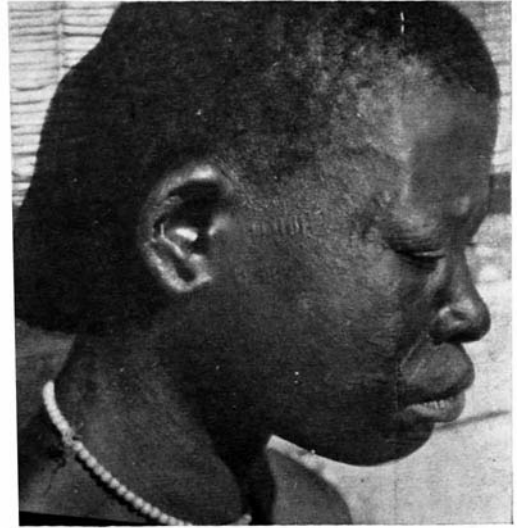


FIG. 3.  
SAME CASE THREE MONTHS LATER. ŒDEMA OF PATCHES DISAPPEARED : NO ANÆSTHESIA.



2

TREATMENT CENTRE, PERAMIHO, SONGEA.



THE OLD DISPENSARY, PERAMIHO.

symptom of ill-health was a severe hæmopotosis showed S.F.R. 3 ; S.I. 45, two days later.

An even better example is that of a patient suffering from both secondary syphilis and gonorrhœa who, under energetic treatment, gave the following results :—

<i>Date.</i>	<i>S.F.R.</i>	<i>S.I.</i>
May 6th, 1931... ..	5	66
May 14th, 1931... ..	4	55
May 25th, 1931... ..	3	50
June 11th, 1931... ..	3	47

In uncomplicated syphilis after the acute secondary symptoms have passed and also in yaws it is more usual to find that the S.F. reaction tends to be high in proportion to the S.I. Thus three consecutive cases of florid yaws were examined with the following findings :—

<i>S.F.R.</i>	<i>S.I.</i>
5	45
5	41
4	17

The following brief table gives the maximum values of the S.F. reaction and the S.I. index of the case selected, in a variety of diseases tested.

	<i>S.F.R.</i>	<i>S.I.</i>
Blackwater fever ... ..	1	60
Unspecified peripheral neuritis ... ..	1	44
Liver abscess (amœbic) ... ..	2	53
Pulmonary and glandular ... ..	6	60
Secondary syphilis ... ..	6	53
Late syphilis... ..	3	—
Florid yaws ... ..	6	60
Old yaws ... ..	1	—
Pellagra ... ..	1	46
Uncomplicated ankylost omiasis ... ..	2	53
Relapsing fever ... ..	5	—
Chronic sepsis ... ..	3	48
Chronic and nerve infection of bone ... ..	6	78
Bronchitis and Pneumonia... ..	1	69
*Trypanosomiasis ... ..	6	80

\*It is interesting to note that in a fatal case of sleeping sickness who was under treatment by Bayer 205 for six weeks, the S.I. did not vary from the initial figure of 80, while the S.F.R., which on admission was 6 + + (the serum forming a gel in less than 10 minutes), fell to 4 towards the end. This may be due to the effect of the drug itself, as it was shown by Dye that Bayer 205 added to a positive serum markedly retards the formation of the gel. (An



observation which I have confirmed, but in contrast to Dye's results have found a similar delay in other diseases). On admission, one-twentieth part of a gram of Bayer added to the serum of this patient reduced the reaction to 5. It seems reasonable to suggest, therefore, that since this drug is excreted very slowly the improvement of the reaction under treatment may be partially due to the direct effect of the drug on the blood serum.

From a consideration of these and many other cases, the following rules for interpreting the two reactions have been formulated.

Both reactions are lower in health as compared with disease. The sedimentation index is an accurate measure of the debility of the patient whatever the disease. The longer the ill-health and the greater its effect on the well-being of the subject, the higher the reaction. The serum formalin reaction is less sensitive in a majority of diseases. It is affected to a greater extent in the granulomatous diseases, *i.e.*, syphilis, yaws, leprosy (as will be shown later) and tuberculosis. It is also high in trypanosomiasis and has been found to be high in severe bone infections. Thus, in acute infections the S.I. is greater than the S.F.R., while in diseases where the S.F.R. is naturally high, if there is little debility, the S.F.R. may in proportion exceed the S.I.

*(To be continued.)*