SIMPLIFIED METHODS OF BLOOD EXAMINATION IN TUBERCULOSIS WITH EXAMPLES OF THEIR APPLICATION IN CASES.

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None of the tests about to be described can be called diagnostic for tuberculosis. All of them are measures of the general systemic state of the individual and they vary from the normal in all serious constitutional disturbances, whether connected with disease or merely with physiological stress. Tuberculosis, however, has the peculiar character of leading, in many cases, to profound constitutional disturbance without much apparent change in the outward health of the sufferer, and any method capable of bringing to light this often unsuspected deterioration in health may be of great importance. Further, while it is true that no single one of these blood-tests is diagnostic, it is also true, as the following reports will show, that, taken together, the results of the blood-sedimentation test, the Arneth Index as expressed according to the Von Bonsdorff technique, the lymphocyte-monocyte ratio and the enumeration of the total leucocytes can go far to establish a diagnosis. Apart altogether from diagnosis, however, the greatest importance of these tests lies in their value as indicators of the constitutional state of a patient at any given moment, and, when used at intervals, of the progress of a case under treatment. Nor is their application confined to tuberculosis. In all prolonged infective processes and in all chronic diseases, they are of great utility; and, requiring but little apparatus and being capable of great simplification, they are eminently suitable for use under military conditions.
Simplified Methods of Blood Examination in Tuberculosis

The Blood-sedimentation Test.

This test, first described by Fähræus (1918) (Fähræus, Hygia, 1918, No. 7, p. 369), in connection with pregnancy, was found by him to afford much information, also, in cases of tuberculosis. It depends on estimating the rate of sedimentation of the red corpuscles in a column of citrated blood. As to the biochemical and biophysical factors which underlie this test, those interested should consult the classical paper by A. Westergren (1924) on "Die Senkungsreaktion" (Westergren, A., Ergebn. d. inn. Med. u. Kinderh., Band xxvi, p. 577), in which the theories to explain increased rapidity of fall of the erythrocytes are set forth.

Here it will suffice to say that the increased rate of fall appears to go with an increased amount of fibrinogen in the plasma and, in all probability, with an increased amount of the products of cell degeneration such as are produced in caseo-necrotic processes.

In tuberculosis and in allied conditions the rate of fall of the erythrocytes, as represented by the height of the column of red cells in relation to the total height of the column of citrated blood-fluid, and in relation, also, to the time elapsed since the setting up of the test, is found to vary directly with the severity of the constitutional disturbance.

Westergren employs for the test glass tubes of standard calibre, graduated up a height of 200 millimetres; and he collects the blood from a vein into a syringe containing the appropriate amount of 3.8% solution of sodium citrate to make a mixture of one part of citrate solution to four parts of blood. This volume of citrated blood is transferred to the calibrated glass tube, and the latter set up vertically in a special type of stand provided with pressure clips to occlude the openings of the tube and retain it in the vertical position without any loss of fluid. Where, however, a large number of tests have to be performed, the operation of vein puncture takes a long time, involves the use of numerous sterile syringes, of a large number of calibrated glass tubes and a supply of expensive stands in which to stand them in position; nor is it always easy to persuade a nervous patient to submit to the puncture of a vein.

Under these circumstances, the writer has devised a simple micro-technique, applicable to a small volume of blood obtained by pricking the finger.

The simplified test is as follows:

Apparatus and Reagents Required.

Solution of 3.8% per cent sodium citrate in distilled water.
Supply of "paraffined" microscope slides prepared by dipping the slides into molten paraffin (melting at 52° C.) and allowing them to dry.
Watch glasses.
Capillary pipettes, drawn out in the flame from lengths of glass tubing in the usual way, and with rubber teats to fit.
A supply of specially prepared glass tubes, each about ten centimetres
(four inches) in length and drawn, at one end, into a narrowed neck. Each is marked, with a file cut or glass pencil mark, at a distance of five centimetres from the thick end. These pipettes are made from a special type of glass tubing, outside bore 3·5 millimetres, inside bore 1·5 millimetres, obtained from Messrs. Gallenkamp for the purpose.

A few lengths (ten inches) of rubber tubing of a size to fit the special glass tubes, above mentioned. “Bicycle valve” tubing fits well and is quite suitable.

A grease pencil.
A measuring scale graduated in millimetres.
A Bunsen burner or spirit lamp.
A glass or metal “pricker” to puncture the skin.
A length of rubber tubing for constricting the finger after puncture.
A special “rack,” made of a suitable height to contain a dozen tubes in the vertical position. The ends of this rack can easily be made from the lengths of metal strip supplied with “Meccano” toy sets. See photograph.

TECHNIQUE.

Mark off a “volume” by means of the grease pencil on the limb of a capillary pipette fitted with a rubber teat.

Puncture the skin of a finger. Constrict lightly from knuckle towards tip with the length of rubber tubing, to facilitate bleeding.
Taking the pipette by the teat, draw up one "volume" of citrate solution, a small supply of which has been poured out in a watch glass ready for use.

Admit an air bubble to mark off the "volume" of citrate and then draw up into the same pipette four successive "volumes" of blood, directly from the finger-puncture, each separated from the last by an air bubble.

Blow out the blood and citrate volumes from the pipette on to a paraffined glass slide and mix until homogeneous.

Take one of the "special" glass tubes, fitted over the narrowed end, with a length of well-fitting rubber tubing.

Holding the end of the rubber tubing between the lips, apply the thick end of the glass tube to the mixed blood and citrate on the paraffined slide and suck up a column of the mixture to the five cubic-centimetre mark.

The column of blood is approximately five centimetres in height and this column is now drawn clear of the end and the tube brought into the horizontal position so that there shall be no tendency for the blood-mixture to "pour" in either direction. The length of rubber tubing is now carefully removed and the narrowed end of the glass tube is sealed off in the Bunsen or spirit flame.

The tube, with its column of blood-mixture now held in position through the sealing of one end, is placed, open end upwards, in the special "stand," in a vertical position.

The height of the blood-column, which should be about fifty millimetres but is sure to vary a little on either side, is now read off by means of the scale and noted, as is also the exact time at which the tube was set up in the rack.

The blood-cell column gradually shortens, leaving a clear length of plasma above it. The height of the red-cell column is read at intervals, appropriate times being one hour, five hours and twenty-four hours after the setting up of the test. The height of the blood-cell column at each reading is noted and is recorded as a percentage of the total height of the column of citrated blood-fluid.

Thus, in a series of persons examined:

<table>
<thead>
<tr>
<th>Times of &quot;readings&quot;</th>
<th>Normal male aged 25</th>
<th>Tuberculous male</th>
<th>Tuberculous female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual height of blood-cell column</td>
<td>Percentage readings</td>
<td>Actual readings</td>
</tr>
<tr>
<td>At start of test</td>
<td>48 mm.</td>
<td>100</td>
<td>60 mm.</td>
</tr>
<tr>
<td>After 1 hour</td>
<td>42 mm.</td>
<td>87</td>
<td>29 mm.</td>
</tr>
<tr>
<td>After 5 hours</td>
<td>39 mm.</td>
<td>82</td>
<td>18 mm.</td>
</tr>
<tr>
<td>After 24 hours</td>
<td>24 mm.</td>
<td>50</td>
<td>16.5 mm.</td>
</tr>
</tbody>
</table>
These cases are merely taken at random from a series of observations. It is usual for the fall of red cells to be more rapid in normal females than in normal males, and the same inequality exists between otherwise comparable cases in disease. It is to be noted, too, that the sedimentation is more rapid than usual in females at the menstrual periods and that it is greatly accelerated during pregnancy. It is, in short, a better test in males than in females, so far as concerns tuberculosis, although it is quite applicable to the latter when possible sources of error have been excluded.

The rate of sedimentation in severe tuberculosis is very rapid at first, and becomes slower and slower as the column of red cells shortens and the corpuscles "ball up" and support each other.

The most striking differences between abnormal and normal bloods are seen after a period of from half to one hour. At the end of twenty-four hours the differences are less marked but are still quite definite. It is unusual for the column of red cells in "normals" to fall below half the total height of the column of fluid within twenty-four hours of the test, whereas it often falls as low as one-third or even a quarter of the total height in severe cases of tuberculosis.

The graph, reproduced below, which represents a typical observation made by the writer, serves to illustrate the order of difference to be found between tuberculous and normal persons:

**Graph I. — Red Blood-cell Sedimentation Test.**

The curves represent the height of the column of red cells expressed as a percentage of the total height of the column of citrated blood fluid.
Where large numbers of observations are being made on the same day, it may prove difficult to carry out readings at the intervals set forth in the above graph. It is now the writer's custom to make readings at one hour, five hours and twenty-four hours. Where time presses and only one reading is possible, as in cases where the preparations set up in the morning cannot be examined until the afternoon, the five-hour interval proves to be a very convenient and valuable one and has been selected as the best all-round reading in the assessment of cases.

The next method of blood examination which it is proposed to describe is Von Bonsdorff's method of expressing the "Arneth" neutrophile formula. In 1904, Arneth (Deutsch. med. Woch., 1904, No. 2, 54-56, and No. 3, 92-94) published his classical observations on the variations in the distribution of neutrophile leucocytes according to the number of nuclear divisions in each cell. His method of expressing these alterations is known to all. A much simpler, and in some ways more striking, method is that of Von Bonsdorff (Beitr. z. Klin. d. Tuberk., Supplement to Band V, Suppl. 3-5, 1912-13) which consists, not in recording a "swing" to the left, as in the Arneth formula, but in estimating the total number of nuclear lobes in 100 neutrophile leucocytes. In health, this number works out round about 250. Von Bonsdorff records counts of over 300 lobes per 100 neutrophiles, and the writer has met numerous instances of normal persons with counts as low as 225. In cases of constitutional disturbance and especially in tuberculosis, the number may fall very low. Obviously, it cannot fall below 100; but the writer has encountered counts of 110, 109, and even, on one occasion, of 102, in very severe and acute cases of pulmonary tuberculosis in the terminal stages of the disease. It is usual to find that the Von Bonsdorff count is below 200 in phthisical cases, and, while the very low figures referred to above are exceptional, it is not uncommon to meet cases with counts well below 150.

This method can only be employed effectively in blood-films which have been evenly spread and well stained. Officers of the Royal Army Medical Corps, accustomed to making blood-films for the diagnosis of malaria and trained in the use of Leishman's stain, will have no difficulty in successfully applying the Von Bonsdorff technique. Given a well-spread and well-stained film, the next thing is to have a clear understanding as to what shall be accepted as a complete nuclear division. "Young" neutrophiles tend to have band-like undivided nuclei, which may, however, show fairly deep indentations, narrowed portions suggesting impending division, or convolutions which lead to superposition of nuclear material and a false suggestion of division where none yet exists. Thus, unless the observers work on pre-arranged lines, there is room for much individual variation in the interpretation of the appearances of neutrophiles in films. Von Bonsdorff describes a method of "averaging out," but, in the course of work with post-graduate students, the writer has found the following simple rule to lead to fairly standard results:
“Always return a cell as uninuclear if you are not able to satisfy yourself that its nucleus is divided into two; always return a cell as binuclear if you are not quite certain that there are three divisions, and so on.”

A nucleus is taken to be divided if its lobes are merely united by a “thread” of nuclear material, but it is regarded as still undivided if the two larger parts are still joined together by a “ribbon.” Where there is superposition of one nuclear lobe over another, careful focussing may enable the observer to resolve the nucleus into two definite portions. If uncertain, it is better to return as “one” so as to adhere to the standard method; but nuclear superposition is rare in well-spread films. If this source of fallacy is found troublesome, it means that the film has not been well made and it is best to discard it and work with a better film.

If the above standard technique be adopted, it will be found that there is a tendency to over-emphasize the “swing to the left” in the Arneth formula and to the recording of rather low Von Bonsdorff counts. This merely requires a corresponding amplification of what is to be taken as normal; and the method has the decided advantage that it goes far to eliminate the personal equation.

The responses of tuberculous patients to the “sedimentation” and the “Von Bonsdorff” tests tend to correspond closely. The value of both lies in the insight which they give into the constitutional state of the patient at any given moment, an insight which often reveals disturbances undetected by physical examination or even by the temperature curve.

Thus, out of about 200 non-pyrexial male patients capable of graded work at Talgarth Sanatorium whose bloods were examined by my assistant,
Miss C. M. Acland, in the summer of 1927, two groups were picked out by the writer from the records as "probably unfavourable" and "probably favourable" on grounds of their "sedimentation" tests. In the "probably unfavourable" group were included 27 patients in whom the red-cell column had fallen to fifty per cent or under of the total blood-fluid column in five hours. In the "probably favourable" group were placed those whose red-cell column was not below sixty per cent of the total column in the same period.

The "Von Bonsdorff" results in these two groups are set forth in the form of "frequency curves" in Graph II.

In this graph it is seen that the curve of Von Bonsdorff counts in the "unfavourable" group, expressed as a frequency curve, reached its apex in the column "170-189." No less than sixty-eight per cent had less than 190 lobes in 100 neutrophile leucocytes. In the "favourable" group, only twelve per cent had less than 190 lobes per 100 neutrophiles and the apex of the curve fell in the "210-229" column. A curve illustrating the findings in forty-two non-tuberculous persons is introduced for comparison. It will be noticed that in this healthy group, fifty-eight per cent had Von Bonsdorff counts of over 230 lobes, whereas only twelve per cent of the "favourable" and four per cent of the "unfavourable" cases exceeded this figure.

That these observations on the blood possess considerable prognostic value is shown by the results of a "follow up" in 1929 of the patients grouped as "favourable" and "unfavourable" on blood-tests made in 1927.

<table>
<thead>
<tr>
<th>Status according to blood-finding in 1927</th>
<th>No.</th>
<th>Fit for &quot;full work&quot;</th>
<th>Fit for &quot;light work&quot;</th>
<th>Better</th>
<th>I S.Q.</th>
<th>Worse or dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Favourable&quot;</td>
<td>26</td>
<td>21</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&quot;Unfavourable&quot;</td>
<td>26</td>
<td>6</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Out of the twenty-six cases traced in the "unfavourable" group one was dead, and only six fit for full work; whereas in the "favourable" group none were dead, worse or stationary, and no less than twenty-one were fit for full work.

While the "sedimentation" and "Von Bonsdorff" tests are of great value in prognosis, and while their periodical application affords valuable information on the progress of cases under treatment, they are in no sense diagnostic of tuberculosis. They merely reflect the systemic depreciation which accompanies tuberculosis as well as other chronic diseases or trying physiological states.

A blood-test which approaches nearer to diagnostic significance is one, now to be described, which depends upon the estimation of the relationship
between the numbers of lymphocytes and monocytes as seen in well-made and well-stained blood-films.

Medlar (Amer. Rev. Tuber., 1929, vol. xx, p. 312), whose careful study of the blood in tuberculosis has thrown so much light on the question, expresses the facts underlying this test as follows:

"The mononuclear leucocyte (monocyte, S. L. C.) is the chief cell in new tubercle-formation. The lymphocyte predominates when a tuberculous lesion is healing."

Those interested in the significance of cytology in tuberculosis should study the numerous contributions to this subject by Sabin and Doan whose methods of vital staining have been of great importance in relation to some of the fundamental parts of the tuberculosis problem.¹

Apart, however, from the study of the pathogenesis of tubercles in experimentally infected animals in which vital staining is essential, a great deal can be learnt from the careful study of dried films stained by the Leishman or Giemsa method.

The method used by the writer is to count at least 100 mononuclear non-granular leucocytes and to express as a percentage of the total mononuclears, the lymphocytes, large and small, on the one hand, and the "monocytes" on the other. Thus, in a normal blood, one may expect to find from 80 to 90 per cent of lymphocytes and from 10 to 20 per cent of monocytes. The name monocyte, as here applied, refers to the large mononuclear cells with, as a rule, a horse-shoe shaped nucleus, which are sometimes called "transitional" leucocytes. They are the largest of the leucocytes normally present in the blood, the nucleus, usually but not always of characteristic horse-shoe shape, is looser in texture than the nuclei of the lymphocytes, and the cytoplasm has a peculiar ground-glass appearance and stains a fainter blue than that of either the small or the large lymphocytes. Occasionally, however, a mononuclear cell is encountered which is difficult to place, appearing larger than the average "large" lymphocyte and yet diverging from the typical monocyte type in having a rounded nucleus and a clearer cytoplasm than usual. The more one works at the interpretation of blood cytology, the less one is troubled by these uncertainties, since a habit of mind soon develops as to how to "place" a typical cell. The safest rule is the simple one, "when in doubt, call it a lymphocyte." This rule weights the scales against the monocytes so that any excess of the latter becomes all the more significant.

It should be remembered that an increase of monocytes may be masked by a still greater increase in lymphocytes and that it is only by making direct estimates of the total numbers of each type in a given volume of blood that exact truth can be attained.

¹ An important paper by these authors appears in the Journal Exper. Med., vol. xlvi, p. 627, 1927.
There is, however, a decided advantage in being able to work with dry blood-films, which can be so easily made at the bed-side and taken away for subsequent examination, and it must be added that where there is an increase of lymphocytes sufficient to mask the abnormal monocytosis, this fact in itself points to a favourable prognosis.

There are doubtless many pathological conditions besides tuberculosis in which the monocytes increase in proportion to the lymphocytes. The writer has encountered such an increase in a case of pulmonary streptothrix infection, some cases of malignant disease, and in a few cases of senility with bronchitis. In persons suspected of being tuberculous, but in whom the diagnosis has not been definitely established, the finding of a well-marked monocytosis goes far to clinch matters.

Being anxious to put the value of these various forms of blood examination to the test, the writer obtained from a medical friend at Johannesburg, a series of blood-films of native gold-mine workers for investigation. It was arranged that half of the films should be made from known cases of tuberculosis and half from healthy natives, and that the films, each with a serial number for subsequent identification, should be mixed up and sent without any further description. Thus, the writer had the task of examining the films without any knowledge of which came from the sick and which from the healthy. The idea was to ascertain how far the examination of a series of dry films could be used to distinguish the tuberculous from the non-tuberculous group.

Medlar, in the paper already referred to, has laid much stress on the significance of a polymorphonuclear leucocytosis as indicating the presence of acute tuberculosis, on the theory that a progressive tuberculous lesion, being in essentials an abscess formation, evokes a leucocytic response similar to that characteristic of septic processes in general. As the tuberculosis of African natives is usually acute, it was anticipated that an approximation to the "total leucocytes" would help. A method was, therefore, devised in which, by counting the erythrocytes with a small "counting disc" in the ocular of the microscope and the leucocytes with a larger "counting disc," the dimensions of the "field" seen with each counting disc having been ascertained with a micrometer scale, the relation of the white cells to the red cells in a given area of the film could be roughly estimated. The red cells being normally nearly a thousand times more numerous than the leucocytes, it was, of course, convenient to count a smaller number of fields, and to reduce the size of the fields when counting the erythrocytes.

The writer had ascertained, through numerous observations, that the red-cell count is not, as a rule, appreciably diminished in pulmonary tuberculosis, and so it was possible, by taking the red cells at five million per cubic millimetre of blood, to arrive at an approximation of the total leucocytes. Twenty-four dry films from South Africa were examined at Cardiff and three observations made on each, namely, the "total leucocytes,"
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the Von Bonsdorff count, and the relation of lymphocytes to monocytes. It may be said at once that the writer, selecting twelve of the films as those probably derived from the tuberculous cases, was right as to ten and wrong as to two.

Here it is only of interest to tabulate the findings under the headings subsequently ascertained from Johannesburg, of "tuberculous" and "non-tuberculous," so that the blood-picture in the former may be clearly seen and the value of examination of blood-films in tuberculosis appreciated.

The findings in the twenty-four films, according to the three criteria applied, are set forth in the following table:

<table>
<thead>
<tr>
<th>BLOOD FINDINGS IN A SERIES OF TWENTY-FOUR SOUTH AFRICAN BLOOD-FILMS.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tuberculous Cases</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>13,652</td>
</tr>
<tr>
<td>45,690</td>
</tr>
<tr>
<td>22,172</td>
</tr>
<tr>
<td>26,113</td>
</tr>
<tr>
<td>5,757</td>
</tr>
<tr>
<td>14,760</td>
</tr>
<tr>
<td>24,389</td>
</tr>
<tr>
<td>9,988</td>
</tr>
<tr>
<td>146,310</td>
</tr>
<tr>
<td>9,652</td>
</tr>
<tr>
<td>18,848</td>
</tr>
<tr>
<td>31,076</td>
</tr>
</tbody>
</table>

* Per cent. of non-granular mononuclear leucocytes.

It will be seen from the above table that, taking the three tests together, the average picture in the tuberculous blood-films was markedly abnormal. It is possible that some of the non-tuberculous cases may have been out of health in other respects. The fifth from the top has a definite leucocytosis and the sixth a very low Von Bonsdorff count. In the tuberculous series, the cases have been arranged from above downwards in order of their abnormality in respect to the relation of lymphocytes to monocytes, and it will be seen that, in this respect, all except one showed a marked increase in monocytes.

It is the writer's opinion that these simple methods of blood examination are of decided value in the assessment of progress in cases of tuberculosis, and taken together are capable of useful application in helping to establish a diagnosis.