THE MASS DETECTION OF ANÆMIA BY THE COPPER SULPHATE—BLOOD GRAVITY TECHNIQUE.

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There is a great need in the Indian Army for a rapid method of detecting anaemic men amongst large bodies of soldiers. The work of one of us (M. H.) in the Anæmia Investigation Team, General Headquarters, India, has shown that mild, easily curable, iron-deficiency anaemia is very common in recruits; severe macrocytic anaemia in Indian soldiers has been a medical problem of every monsoon campaign. The latter anaemia is usually very advanced before it is recognized, but something might be done towards its prevention by regular tests to detect the mildly anaemic men of forward units. Such tests have not hitherto been possible—it has been repeatedly shown that clinical examination is totally unreliable; mass haemoglobin estimations by the Talqvist papers are quick and easy, but very inaccurate; and it is not possible to do more than five or six estimations an hour with the Sahli haemoglobinometer. Phillips et al. (1941) showed that the haemoglobin can be calculated from the specific gravity of whole blood, which can be very quickly and accurately determined by their simple technique. "The method is based on the fact that plasma or whole blood dropped into a solution of copper sulphate of known gravity is encased in a sac of copper proteinate, and the gravity of this discrete drop is not changed for about fifteen seconds. The rise or fall of the drop during this interval shows whether it is lighter or heavier than the solution." They derived an equation on theoretical grounds relating the blood gravity and haemoglobin, but Hynes and Lehmann (1945) have produced a curve more nearly fitting the observed relation in Indian soldiers. The method described here is based on this curve.

PRINCIPLE OF THE METHOD.

A standard copper sulphate solution is chosen with a specific gravity corresponding to the particular haemoglobin level above and below which people are to be grouped. One drop of finger blood from each individual is dropped into this solution. If the drop rises the individual is classified as
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Anaemic. By this method 70 per cent of people with haemoglobin within 1 gramme of the chosen level, and the great majority of those beyond these limits, will be correctly classified. Since most individuals will have haemoglobins differing by more than 1 gramme from the chosen level, we may anticipate that about 95 per cent of the group will be correctly classified.

**Technique.**

**Preparation of Standard Solutions.**—In the field the copper sulphate solutions are best prepared from the pre-weighed salt. Any base laboratory can supply sealed ampoules containing the correct amount of finely powdered copper sulphate to make 100 ml. of a particular standard. The weight of CuSO₄.5H₂O needed is:

(desired gravity—1.001) × 159.63 grammes.

Table I shows the specific gravity corresponding to the principal haemoglobin levels, and the weight of copper sulphate needed to make 100 ml. of the corresponding solution.

<table>
<thead>
<tr>
<th>Haemoglobin grm./100 ml.</th>
<th>Whole blood Specific gravity</th>
<th>Weight of CuSO₄.5H₂O to make 100 ml. of corresponding solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.033</td>
<td>5.15 grm.</td>
</tr>
<tr>
<td>6</td>
<td>1.039</td>
<td>6.08 grm.</td>
</tr>
<tr>
<td>8</td>
<td>1.044</td>
<td>6.79 grm.</td>
</tr>
<tr>
<td>10</td>
<td>1.047</td>
<td>7.36 grm.</td>
</tr>
<tr>
<td>11</td>
<td>1.049</td>
<td>7.62 grm.</td>
</tr>
<tr>
<td>12</td>
<td>1.050</td>
<td>7.88 grm.</td>
</tr>
<tr>
<td>13</td>
<td>1.052</td>
<td>8.15 grm.</td>
</tr>
<tr>
<td>14</td>
<td>1.054</td>
<td>8.44 grm.</td>
</tr>
<tr>
<td>15</td>
<td>1.056</td>
<td>8.76 grm.</td>
</tr>
<tr>
<td>16</td>
<td>1.058</td>
<td>9.12 grm.</td>
</tr>
</tbody>
</table>

Dissolve the powder in distilled water, wash the solution into a 100 ml. volumetric flask, and make up to volume with distilled water. If distilled water is not available, only a very small error is caused by using tap-water, which even when heavily chlorinated usually contains well under 40 parts of dissolved solids per 100,000. Water which tastes very brackish or will not "lather" should not be used.

If a volumetric flask is not available, a measuring cylinder, or even a 6 oz. bottle with a 100 ml. gradation mark, may be used; for an error of 1 ml. in the volume makes an error of only about 0.2 gramme in the haemoglobin.

The solution should be stored in 6 oz. screw-capped bottles, which should be kept firmly closed when not in use. The actual test is done in the same bottles.

The solution should be discarded after 100 drops of blood have been added, for the specific gravity of a solution falls significantly after one-fortieth of its volume of blood has been added.
If very many tests are to be done the solution may conveniently be made in 500 ml. lots and used in well-stoppered gin bottles.

Performance of Test.—Prick the finger and draw a large drop of blood into a fine teated capillary pipette. Quickly, before there is time for clotting, put the pipette into the bottle with its end 2 cm. above the surface of the solution, and let a drop of blood fall. If it sinks steadily to the bottom of the solution the haemoglobin is taken to be above the level corresponding to the solution. If the haemoglobin is below this level the drop will rise as soon as it has lost the downward velocity of its entry. After fifteen seconds all drops change their nature and sink to the bottom of the bottle.

It is important that the drop should break cleanly through the surface of the solution; if it has not fallen far enough it will be tied to the surface by a “tail.” Such hanging drops need not be removed; they soon fall to the bottom.

Great care must be taken not to draw any air into the pipette with the blood, for an air-bubble included in the drop of blood will, of course, tend to make it float and lower its apparent gravity.

If pipettes are not available the blood may be dropped straight from the finger into the solution in a wide-mouthed bottle. The drop which falls from the finger is much larger than that delivered from a pipette, so 100 ml. of solution serves for only 40 tests.

The standard solution should be at the temperature of the room, otherwise the drop will be the prey of convection currents. For the same reason, the bottle must not be stood in the sun, and when it is moved it should be held by the neck.

Applications.

At the Base.—One of us (M. H.) in the Anaemia Investigation Team, General Headquarters, India, has shown that any Indian soldier with less than 14 grammes haemoglobin, and most with less than 15 grammes, will benefit from a course of ferrous sulphate. Men in training should therefore be tested with the solution corresponding to 14 grammes haemoglobin, and those found to be below this level should be given 6 grains of ferrous sulphate three times daily for three weeks and then re-tested.

On Active Service.—If during active service time cannot be found for the above procedure, the men should be tested against a solution corresponding to 11 grammes haemoglobin. All men found to be below this level should be sent into hospital for investigation and treatment.

Blood Transfusion.—Donors should be rejected if the test shows them to have less than 14 grammes haemoglobin.

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REFERENCES.