THE DETERMINATION OF BLOOD VOLUME BY THE CARBON MONOXIDE METHOD.

BY HARALD A. SALVESEN.

(From the Hospital of The Rockefeller Institute for Medical Research.)

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There is still much confusion regarding the exact value of the blood volume in human beings, though much work has been done in this field of investigation. Values for the blood volume for men are given, ranging from $\frac{1}{21}$ to $\frac{1}{8}$ of the body weight. These widely differing results have been obtained by the use of different methods.

There are one direct and several indirect methods for the determination of the blood volume.

Direct method.—Welcker (1) in 1854 was the first one to determine the blood volume by a method which still is regarded as the standard; he bled animals to death, washed out the vessels with water, and extracted the hemoglobin still remaining in the tissues by mincing the organs minus the bile and the content of the bowels, and placing them in water for several days. By comparing the hemoglobin content of the first blood and the blood washings and extracts brought together, he found the blood volumes of mammals to constitute $\frac{1}{13}$ of the body weight. The same value was obtained for human beings by Bischoff (2), who used this method on two criminals.

Welcker's method has been modified and improved by several investigators, but the general principle is the same as in 1854. The results obtained in animals with this method have differed because of incomplete washing and extraction, and the use of inexact methods for the hemoglobin determinations.

Indirect methods.—These methods must be used in experiments on living animals. There are various principles for the indirect determination of the blood volume. The best of them may be divided into two groups: (1) A known amount of an easily determinable substance, which is kept within the circulatory system for a sufficiently long time for thorough mixing, is introduced into the blood and the concentration of it deter-
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mined. (2) The blood is either (a) diluted, or (b) concentrated in various ways, and the blood volume calculated from the variation in the content of hemoglobin or corpuscles.

Group 1. (a) Carbon Monoxide Method.—The principle of this method is to administer a certain amount of carbon monoxide gas to the individual, and then to determine the degree of saturation of the blood with CO or the actual amount of CO per unit of blood. It was first used by Gréhant and Quinquaud (3), and later by Haldane and Smith (4), Oerum (5), Douglas (6), Boycott and Douglas (7), and Plesch (8). (b) The dye method of Keith, Rowntree, and Geraghty (9) is the method most widely used for the present in clinical work. A dyestuff, vital red, is injected intravenously and determined colorimetrically in the blood. The relative amounts of plasma and corpuscles are determined by the hematocrit method. (c) The antitoxin method of von Behring (10) is based upon the observation that tetanus antitoxin remains in the circulation for a long period; a known amount is injected, and the concentration then determined in the blood. (d) The acacia method of Meek and Gasser (11) has been tried only in animals so far, and the experience is not large enough to judge of its utility. Acacia is injected and determined in the blood as furfurolphloroglucine.

Group 2. (a) Dilution Methods.—The only one of these methods which has withstood criticism is based upon the observation of Cohnstein and Zuntz (12) that isotonic sodium chloride solutions are kept in the circulation for a relatively long time, and diffuse very slowly out into the tissues; this is utilized for the determination of the blood volume, the red cells being counted before and after infusion. Plesch (8) seems to have developed this method to further exactness by determining the hemoglobin instead of the cells with the help of his chromophotometer. As the variations in the cell or hemoglobin content obtained by dilution hardly exceed 10 per cent, the exactness of the method evidently depends on how accurately these constituents of the blood can be determined.

(b) Concentration Methods.—The method of Tarchanoff (13), who determined the hemoglobin before and after a steam bath and the decrease in weight through loss of water, and calculated the blood volume from these two factors, has been justified criticized and cannot be relied upon, as the water may be derived from other sources in the body than the blood. Quincke (14) transfused blood with a certain amount of red corpuscles to two anemic patients and calculated the blood volume from the increase in the red count. Lindeman (15) uses the same principle. These methods can only be used in anemia.

Of these methods only three are of practical value for physiological and clinical purposes; they are the carbon monoxide method, the infusion method of Cohnstein and Zuntz, and the vital red method. The results obtained by these methods vary. In animals, both the carbon monoxide method and the infusion method have given nearly the same values as the Welcker method; Gréhant and Quinquaud (3) found in nine dogs values from \( \frac{1}{11} \) to \( \frac{1}{13.8} \) of the body weight with the CO method,
which correspond to Welcker's own results in dogs. Douglas (6) found a close agreement between the CO method and the bleeding method in five rabbits; Boycott and Douglas (7), repeating the experiments later, found a little higher value with the CO method (2 per cent). Plesch (8), in dogs, tried subsequently the CO, the infusion, and the bleeding method, and the results were uniform. The vital red method has never been checked up by the Welcker method as far as can be seen from the literature.

In human beings the results are widely different. Bischoff's values, $\frac{1}{13}$ of the body weight, were regarded as the standard until Haldane and Smith (4), with the CO method and carmine titration, in fourteen normal men found $\frac{1}{21}$, the highest value being $\frac{1}{16}$ and the lowest $\frac{1}{30}$ (in a very fat man). Oerum (5), using the same technique, found in men $\frac{1}{19.2}$ and in women $\frac{1}{21.8}$. Plesch (8), using a gasometric method for the CO determination in four men, found the average ratio $\frac{1}{17.9}$; also in five persons, some of whom were reported to be fat, he determined the blood volume with the infusion method and found $\frac{1}{19.1}$. Bischoff's results, therefore, seem to be too high, inasmuch as the two criminals were hardly normal individuals, one at least suffering from scurvy. The method used is also open to criticism.

Douglas (16), in 1910, made a series of determinations with the CO method on himself and another subject with Haldane's technique, but waited a longer time before he took the blood sample for analysis. These results show a mean value of $\frac{1}{13.9}$ for his own and $\frac{1}{12.5}$ for the other subject, values more in accordance with those of Bischoff. He found errors in Haldane and Smith’s determinations due to incomplete mixing of the blood, as the blood sample was taken too early after the breathing of the carbon monoxide.

In 1915 Keith, Rowntree, and Geraghty (9), with their vital red method, found still higher values, the mean in normal men being $\frac{1}{11.8}$ of the weight. This method has never been controlled by the Welcker method as far as can be seen, but the authors show its reliability toward relative changes by drawing a certain amount of blood and finding a corresponding drop in the blood volume.

It may be seen from this review of the literature how uncertain is our knowledge of the blood volume in human beings. Since the carbon monoxide method in animals has given satisfactory results as compared with the standard method of Welcker, and
in human beings the results obtained by the various investigators have differed widely, it seems worth while to make further investigations in this field, especially since the technique used before has been rather difficult.

It seems certain, according to Douglas, that Haldane and Smith’s figures are too small, and so must be the figures of Oerum, as he used the same technique. There remain, therefore, only the determinations of Plesch in four persons, giving the average of $\frac{1}{17.9}$ and those of Douglas, on himself and another man, giving the values of $\frac{1}{13.9}$ and $\frac{1}{12.5}$ of the body weight.

The carbon monoxide method has been criticized by Dreyer (17) and his coworkers, who, in rabbits, used Haldane and Smith’s technique, and got so much divergence in the figures that they concluded it could not be used in its present form. They therefore determined the blood volume by injecting in rabbits’ blood a known amount of agglutinin, determined the percentage in the serum, then washed out the circulation, and determined the percentage of agglutinin in the washing. They claim the blood volume to be a function of the surface area, so that, for instance, smaller rabbits have a relatively higher blood volume than the larger ones.

The adverse criticism of the carbon monoxide method may be due to the difficulty of the technique, as the carmine titration of Haldane requires long training and a highly developed color sense. All the carbon monoxide determinations in the present paper are performed with the help of the gasometric method, described in the preceding paper, which makes the whole technique much simpler and fitted for general use.

**Blood Volume Determinations in Animals.**

The results of numerous determinations of Boycott and Douglas (7) show that rabbits’ blood constitutes from $\frac{1}{22}$ to $\frac{1}{18.1}$ of the body weight, as determined both with the carbon monoxide and the washing out method. The average of 52 rabbits with the washing out method was $\frac{1}{20.9}$, or 4.77 cc. of blood per 100
gm. of body weight, while the carbon monoxide method (with the carmine titration) gave a little higher result, \( \frac{1}{18.1} \) or 5.5 cc. per 100 gm.

**Methods.**

The arrangement used was that described by Douglas (6). It is therefore unnecessary to repeat it here.

The principle is to let the rabbit breathe into a closed system which is supplied with arrangements for removing the carbonic acid and renewing the oxygen. A measured amount of carbon monoxide is introduced into the apparatus, and 10 minutes after the entire amount is given a sample of blood is drawn from the ear vein and analyzed for carbon monoxide. At the same time a sample of the air in the chamber is taken for determination of the \( \text{O}_2, \ CO_2, \) and CO. The blood volume is calculated from the amount of carbon monoxide absorbed by the animal and the concentration of it in the blood. As in Douglas' experiments, a tube connected with a bell jar, partly immersed in a glass of water, was introduced into the respiratory chamber, and served as an indicator of the pressure in the apparatus. While the air sample was drawn, the oxygen current was cut off and the water allowed to rise in the bell jar by raising the glass in order to compensate for the negative pressure produced by the sucking out of the air.

The capacity of the apparatus (the chamber and the air in the rubber tubing, the pump, and the bell jar) was 750 cc., a little larger than that of the apparatus employed by Douglas (6), and Boycott and Douglas (7).

The carbon monoxide was prepared by heating oxalic acid and concentrated sulfuric acid, and the gas was collected over water made alkaline with sodium hydroxide, with which the gas was shaken in order to get rid of the \( \text{CO}_2 \). The gas was analyzed every 2nd day by shaking it in a Hempel pipette with cuprous chloride solution, which absorbs the carbon monoxide and \( \text{O}_2 \), and from the amount of nitrogen left the air content of the gas was calculated. The gas first evolved was discarded. The carbon monoxide content of the rest was 95 to 98 per cent.

The carbon monoxide in the blood was determined by the method described in the preceding paper, the blood, 4.5 cc. in
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all, being drawn from the ear vein without stasis and kept under paraffin oil; 2 cc. were used for each analysis. The hemoglobin was determined by the Palmer method (18). The air in the chamber was analyzed for O₂ and CO₂ in the Haldane-Henderson apparatus.

The determination of the carbon monoxide left in the chamber could not be made by gasometric methods, and the method of Haldane (19) was employed, the principle of which is to shake the carbon monoxide-containing air with blood, to estimate colorimetrically the percentage of saturation of the blood with carbon monoxide, and deduce from this value, and the percentage of O₂ present, the percentage of carbon monoxide in the air. As bloods of different species and also of different individuals show different dissociation curves for the carbon monoxide hemoglobin, as shown by Krogh (20) and Haldane (21), blood from a single sheep was used, in which the dissociation curve was previously determined (preceding paper). The dissociation curve is a hyperbola of the formula

\[
\frac{(O₂ \text{ percentage in air})}{(CO \text{ percentage in air})} \times \frac{\text{Hb CO}}{\text{Hb O}_2} = K
\]

In the blood used \( K = 179 \) at 24°.

For the determination of the dissociation curve of the blood of our sheep, 5 cc. samples of the blood were rotated in bottles of known capacity (approximately 1 liter), filled with air plus known amounts of carbon monoxide. The blood was first placed in the bottle, which was then closed by a stopper containing a three-way capillary cock. A known volume (1 to 4 cc.) of analyzed CO gas was then forced in from a micro-gas-burette, in which the volume delivered could be read over mercury to within 0.002 cc. The tubes of the cock were filled with the CO before the measured amount was admitted into the bottle, so that errors due to dead space were avoided. The bottle with the blood and gas mixture was rotated for 2 hours at 24°C. Trial showed that equilibrium was obtained in this time. Samples of 2 cc. of blood were then withdrawn and used for the determination of O₂ and CO as described in the foregoing paper. The volume of CO taken up by the 5 cc. of blood was subtracted from the volume of CO originally added, in order to estimate the amount left in the gas.
phase. The oxygen concentration remained that of atmospheric air.

The results obtained in four determinations are shown in the curve of Fig. 1. The curve is the hyperbola plotted from the formula, with 179 taken as the value of $K$; the crosses represent the results experimentally obtained.

![Fig. 1. The curve is the hyperbola plotted from the formula, with 179 taken as the value of $K$; the crosses represent the results experimentally obtained.](image)

The value of $K$ being known, small percentages of CO in air could be ascertained by shaking the latter with a known volume of the blood and determining the Hb $O_2$ and Hb CO, the calculation being per cent CO in air $= \frac{21.9 \times \text{Hb CO}}{179 \times \text{HbO}_2}$. In calculating the actual percentage of CO in the gas-sampling tube allowance was made for the amount of CO taken up by the blood with which the air was shaken. The colorimetric determination was
made by the method employed by Plesch (8), which is very simple and which gave fairly good results when used on blood with known percentages of CO. The principle is: Three samples of the same blood are saturated; No. 1 with air, No. 2 with CO, and No. 3 with the air containing the unknown percentage of CO. 0.05 cc. of each sample is diluted with 10 cc. of a 1 per cent solution of Na₂CO₃ in each of three small test-tubes of equal bore. No. 2 is added to No. 1 until the color is the same as in No. 3. If, for instance, equal color is obtained by adding 2.5 cc. of No. 2 to 5 cc. of No. 1, then the degree of saturation is $\frac{2.5}{2.5+5} = 33.3$ per cent.

Results.

In fourteen rabbits taken from the stock the blood volume was determined. In two of the female rabbits the values obtained were much higher than the average, and they later proved to be pregnant. They were placed in a separate group and the blood volume determined again, post partum. In some of the other rabbits the blood volume also was determined twice. The results are given in Tables I to V.

**TABLE I.**

*Normal Male Rabbits.*

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Date</th>
<th>Weight</th>
<th>Hemoglobin</th>
<th>Blood volume</th>
<th>Relation to body weight</th>
<th>Cc. per 100 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td>July 3</td>
<td>1,500</td>
<td>(?)*</td>
<td>70.4</td>
<td>1.191</td>
<td>5.20</td>
</tr>
<tr>
<td>89</td>
<td>8</td>
<td>1,900</td>
<td>(?)*</td>
<td>95.7</td>
<td>1.199</td>
<td>5.02</td>
</tr>
<tr>
<td>89</td>
<td>10</td>
<td>1,000</td>
<td>93.4</td>
<td>80.9</td>
<td>1.198</td>
<td>5.05</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>2,250</td>
<td>94.3</td>
<td>107.1</td>
<td>1.21</td>
<td>4.76</td>
</tr>
<tr>
<td>99</td>
<td>14</td>
<td>3,010</td>
<td>86.2</td>
<td>146.9</td>
<td>1.205</td>
<td>4.88</td>
</tr>
<tr>
<td>82</td>
<td>14</td>
<td>2,200</td>
<td>87.7</td>
<td>115.4</td>
<td>1.1906</td>
<td>5.25</td>
</tr>
<tr>
<td>98</td>
<td>15</td>
<td>2,200</td>
<td>93.4</td>
<td>111.1</td>
<td>1.198</td>
<td>5.05</td>
</tr>
</tbody>
</table>

* Determinations lost because of an incorrect hemoglobin standard.
### TABLE II.

*Normal Female Rabbits.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>July 2</td>
<td>2,300</td>
<td>(?)*</td>
<td>122.2</td>
<td>1/18.8</td>
<td>5.30</td>
</tr>
<tr>
<td>70</td>
<td>&quot;  7</td>
<td>1,250</td>
<td>83.3</td>
<td>50.8</td>
<td>1/24.6</td>
<td>4.06</td>
</tr>
<tr>
<td>88</td>
<td>&quot;  9</td>
<td>1,960</td>
<td>86.2</td>
<td>100.6</td>
<td>1/19.4</td>
<td>5.14</td>
</tr>
<tr>
<td>83</td>
<td>&quot; 11</td>
<td>2,630</td>
<td>76.9</td>
<td>129.2</td>
<td>1/20.3</td>
<td>4.93</td>
</tr>
<tr>
<td>87</td>
<td>&quot; 11</td>
<td>2,320</td>
<td>51.4</td>
<td>127.5</td>
<td>1/18.2</td>
<td>5.49</td>
</tr>
</tbody>
</table>

* Determination lost because of an incorrect standard.

### TABLE III.

*Repeated Determinations.*

*Male Rabbits.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>July 8</td>
<td>1,900</td>
<td>(?)*</td>
<td>95.7</td>
<td>1/19.9</td>
<td>5.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;  21</td>
<td>1,940</td>
<td>59.3</td>
<td>10.14</td>
<td>1/21</td>
<td>4.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;  3</td>
<td>1,500</td>
<td>(?)*</td>
<td>79.4</td>
<td>1/19.1</td>
<td>5.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;  18</td>
<td>1,540</td>
<td>75.0</td>
<td>13.87</td>
<td>1/21.1</td>
<td>4.74</td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>&quot;  10</td>
<td>2,250</td>
<td>94.3</td>
<td>18.68</td>
<td>1/21.1</td>
<td>4.76</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>&quot;  17</td>
<td>2,270</td>
<td>90.9</td>
<td>17.54</td>
<td>1/22.2</td>
<td>4.50</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>&quot;  14</td>
<td>3,010</td>
<td>86.2</td>
<td>21.08</td>
<td>1/20.5</td>
<td>4.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;  18</td>
<td>3,070</td>
<td>74.1</td>
<td>22.33</td>
<td>1/18.9</td>
<td>5.29</td>
<td></td>
</tr>
</tbody>
</table>

* Determination lost because of an incorrect standard.
TABLE IV.
Repeated Determinations.
Female Rabbits.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Date</th>
<th>Weight</th>
<th>Hemo-</th>
<th>Total oxygen capacity</th>
<th>Blood volume</th>
<th>Ratio</th>
<th>Cc. per 100 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>1919</td>
<td>1,250</td>
<td>83.3</td>
<td>7.83</td>
<td>50.8</td>
<td></td>
<td>1/24.6</td>
</tr>
<tr>
<td></td>
<td>July 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.06</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1,350</td>
<td>66.6</td>
<td>8.30</td>
<td>66.8</td>
<td></td>
<td>1/20.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.95</td>
</tr>
<tr>
<td>81</td>
<td></td>
<td>2,320</td>
<td>51.4</td>
<td>12.12</td>
<td>127.5</td>
<td></td>
<td>1/18.2</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.49</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>2,300</td>
<td>47.4</td>
<td>11.44</td>
<td>129.0</td>
<td></td>
<td>1/17.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.62</td>
</tr>
</tbody>
</table>

TABLE V.
Blood Volume in Pregnant Rabbits before and after Term.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Determination made</th>
<th>Date</th>
<th>Weight</th>
<th>Hemo-</th>
<th>Total oxygen capacity</th>
<th>Blood volume</th>
<th>Ratio</th>
<th>Cc. per 100 gm.</th>
<th>Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>Before.</td>
<td>1919</td>
<td>2,650</td>
<td>69.4</td>
<td>22.86</td>
<td>177.3</td>
<td></td>
<td>1/14.9</td>
<td>6.70 Young ones, July 15.</td>
</tr>
<tr>
<td></td>
<td>6 days post partum.</td>
<td>21</td>
<td>2,290</td>
<td>76.3</td>
<td>15.24</td>
<td>108.3</td>
<td></td>
<td>1/19.4</td>
<td>5.14</td>
</tr>
<tr>
<td>87</td>
<td>Before.</td>
<td>9</td>
<td>2,500</td>
<td>94.0</td>
<td>24.29</td>
<td>141.1</td>
<td></td>
<td>1/18.1</td>
<td>5.52</td>
</tr>
<tr>
<td></td>
<td>11 days after.</td>
<td>16</td>
<td>2,570</td>
<td>74.1</td>
<td>22.2</td>
<td>161.3</td>
<td></td>
<td>1/15.3</td>
<td>6.54 Young ones, July 20.</td>
</tr>
</tbody>
</table>

In Tables III and IV is introduced a column, "total oxygen capacity," the figures in which indicate all the oxygen with which the blood is able to combine, calculated from the blood volume and the hemoglobin percentage. In our hemoglobin standard 100 per cent equals 18.5 volumes per cent of O₂.
DISCUSSION.

The results are expressed in parts of the crude body weight, though this may vary for various causes, such as food intake, content of bowels, etc. The average value for the blood volume in seven male rabbits (eleven determinations, Tables I and III) is $\frac{1}{20.21}$ of the body weight, or 4.95 cc. per 100 gm. The average in five non-pregnant female rabbits (seven determinations, Tables II and IV) is $\frac{1}{19.99}$ of the weight, or 5.02 cc. per 100 gm. If the two determinations from Table V in rabbits post partum are added, the mean for all seven females is the same, $\frac{1}{19.99}$, or 5.0 cc. per 100 gm. The results are in accordance with those obtained by Boycott (7) and coworkers with the washing out method.

The repeated determinations in Tables III and IV show a fairly close agreement in some of the rabbits, while in Rabbits 99 and 76 there is a considerable difference. But the total oxygen capacity is nearly constant in these two rabbits. Rabbit 76 had increased in weight from 1,250 to 1,350 gm., and to get comparable values the oxygen capacity in the first determination has to be multiplied by $\frac{1,350}{1,250} = 1.08$, and this gives the value of 8.46 cc. which is very close to the value found the second time, 8.30 cc. The same phenomenon is seen in the pregnant rabbit, No. 87, of Table V. The two determinations before partus gave $\frac{1}{18.1}$ and $\frac{1}{15.3}$ of the weight, which is a large increase in the blood volume.

The hemoglobin concentration dropped, however, so that the oxygen capacity of the total blood supply of the animal remained constant, the blood merely having become diluted in the interval between determinations. About a week after partus the blood volume was again normal.

The blood volume of the rabbit, therefore, seems to be able to change normally, probably in the way that fluid passes in and out through the capillaries, a phenomenon in analogy with what has been observed in human beings in shock. Rabbits 99 (Table III), 76 (Table IV), and 87 (Table V), in which these changes in
the blood volume and constancy of the total oxygen capacity were so marked that they cannot be due to experimental error, show the smallest volume the first time, when they were unexperienced and scared when put into the apparatus, and a larger volume the second time, when they were used to the procedure. The material is too small, however, to draw any conclusion on this point, but Douglas has observed the same phenomenon of changes in the volume and constancy of oxygen capacity.

Table V shows the well known fact that pregnant animals have a larger blood volume absolutely and relatively than non-pregnant. A week post partum the blood volume is restored to the normal value.

Blood Volume Determinations in Human Beings.

The arrangement of apparatus was nearly the same as that desribed by Haldane and Smith (4), except that an ordinary Wolff bottle, filled with sticks of potassium hydroxide, was used for the absorption of the carbonic acid, as seen in Fig. 2, and the experiments lasted longer, the subjects breathing for 10 to 15 minutes after the entire amount of carbon monoxide was given. The oxygen was supplied from a cylinder as rapidly as was necessary in order to keep the amount of gas in the bag approximately constant. The estimation of the relative volume of air in the bag was facilitated by placing the bag horizontally with a scale behind it as an indicator of the degree of filling.

The apparatus was filled with carbon monoxide to the three-way stop-cock before the experiment was started. The volume of the connecting parts and the bag, filled with air to the mark, was 3,700 cc. For the calculation of the amount of carbon monoxide left the volume of the lungs must be added; this is about 3,000 cc., and the total volume of the air in which the carbon monoxide was distributed thus was 6,700 cc.

The blood, being assumed to constitute not less than \( \frac{1}{19} \) of the body weight, the amount of carbon monoxide given to the sub-

1 Potassium hydroxide was used because of the high solubility of the potassium carbonate formed during the experiment; this carbonate was washed out by rinsing the sticks with water now and then.
ject was so calculated that the saturation of the blood with carbon monoxide would not exceed 20 to 25 per cent. For instance, with the body weight 70 kilos, hemoglobin 120 per cent, blood volume at least \( \frac{70}{19} = 3.7 \) liters, and as 100 per cent hemoglobin =

18.5 volumes per cent of \( O_2 \) capacity, the capacity in this case would be 22.2 cc. per 100 cc. of blood; per 3,700 cc. it accordingly would be 821.4 cc. One, therefore, could safely give 164 cc. of carbon monoxide reduced to standard conditions (760 mm. and 0°).

The blood sample was drawn without stasis from the arm vein.
and kept under paraffin oil. The carbon monoxide was determined by the method of Van Slyke and Salvesen, 2 3 cc. being used for each determination, as the carbon monoxide content of the blood is rather small in these experiments.

The air in the bag was analyzed for oxygen, carbon dioxide, and carbon monoxide after each experiment, as in the animal experiments. In all except the last experiment the amount of carbon monoxide left was about 2 cc.: 1.47, 1.902, 2.53, 1.832, 1.96; average 1.938 cc. In the last experiment it was 4.42 cc., but in this case more oxygen was given than necessary, and the oxygen content of the bag was 33 per cent. This probably accounts for the slower absorption of the carbon monoxide, the dissociation curve of the carbon monoxide-hemoglobin being depressed when the oxygen percentage increases. If the experiments, therefore, are always performed in the same way, the amount of carbon monoxide left is constant, and if the apparatus has the same capacity as used in the present experiments, the correction which must be subtracted is 2 cc. The experiments done in this way are much simpler, the only determination which must be done being that of the carbon monoxide in the blood.

Six healthy individuals were examined, ranging in age from 23 to 37 years. The material was rather uniform as all were young people without any adipositas. The results are given in Table VI.

The average blood volume found was 3,888 cc., constituting \( \frac{1}{16.8} \) of the weight, or 5.95 cc. per 100 gm. The extremes are \( \frac{1}{14.3} \) and \( \frac{1}{19.08} \). The largest volumes were found in Nos. 1 and 6, both of whom are tall and slim, especially No. 6 who is abnormally thin, and weighs much less than would correspond with his height. In No. 1 two determinations were made and the difference found is only 7 cc. of blood.

The mean value, then, is a little larger than that of Plesch, who found \( \frac{1}{17.9} \) and smaller than that of Douglas, who found in two persons \( \frac{1}{13.9} \) and \( \frac{1}{12.5} \).

All the persons experimented on felt comfortable, and did not have any disagreeable sensations. The breathing was easy, and even a certain degree of dyspnea is not likely to interfere with the use of this method in pathological cases. Former investigators have used the carbon monoxide method in heart, kidney, and anemic cases without any difficulties.

It is hoped that the blood volume method with the easy technique for the carbon monoxide determination will be of more practical value than before.

**TABLE VI.**

**Blood Determinations in Human Beings.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Date</th>
<th>Name</th>
<th>Age</th>
<th>Weight</th>
<th>Duration</th>
<th>Hemoglobin</th>
<th>Blood volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>June 28</td>
<td>Dr. H. S.</td>
<td>30</td>
<td>68.8</td>
<td>32</td>
<td>114</td>
<td>4,504, 14.97</td>
</tr>
<tr>
<td></td>
<td>Aug. 1</td>
<td>&quot;</td>
<td>68.8</td>
<td>35</td>
<td>114</td>
<td>4,601, 14.82</td>
<td>6.74</td>
</tr>
<tr>
<td>2</td>
<td>July 29</td>
<td>Mr. A. S.</td>
<td>26</td>
<td>60.9</td>
<td>28</td>
<td>124.9</td>
<td>3,464, 17.6</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>Dr. H. A.</td>
<td>36</td>
<td>66.4</td>
<td>23</td>
<td>118</td>
<td>3,479, 19.08</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>Dr. V. S.</td>
<td>37</td>
<td>72.7</td>
<td>27</td>
<td>118</td>
<td>3,877, 18.7</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>Dr. J. T.</td>
<td>28</td>
<td>61</td>
<td>23</td>
<td>114</td>
<td>3,429, 17.7</td>
</tr>
<tr>
<td>6</td>
<td>Aug. 1</td>
<td>Mr. R. T.</td>
<td>23</td>
<td>62.7</td>
<td>22</td>
<td>116</td>
<td>4,380, 14.3</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>65.4</td>
<td></td>
<td></td>
<td>3,888, 16.8</td>
</tr>
</tbody>
</table>
Determination of Blood Volume

SUMMARY.

Determinations of the blood volume by the carbon monoxide method with the simple technique previously described for the blood analysis have been made in fourteen rabbits and six normal men. Eleven determinations in seven male rabbits show an average blood volume of \(\frac{1}{20.21}\) of the body weight, or 4.95 cc. per 100 gm. Nine determinations in five non-pregnant female rabbits show an average of \(\frac{1}{19.99}\) or 5.0 cc. per 100 gm.

In two pregnant rabbits the blood volume was largely increased, absolutely and relatively; about a week post partum it was restored to normal again.

The blood volume of rabbits may change from time to time, but the total oxygen capacity remains constant.

Seven determinations in six healthy men show an average of \(\frac{1}{16.8}\) of the body weight, or 5.95 cc. per 100 gm.

The author wishes to express his thanks to Dr. Donald D. Van Slyke on whose initiative this work was undertaken, and to Mr. Arthur H. Smith for his technical assistance during the experiments.

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THE DETERMINATION OF BLOOD VOLUME BY THE CARBON MONOXIDE METHOD
Harald A. Salvesen


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