XLVI. DETERMINATION OF CARBON MONOXIDE IN BLOOD.

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CARBON MONOXIDE in blood has been determined in several ways. Except in methods like Haldane's [1895], which is colorimetric, and Hartridge's [1912], which is spectroscopic, a combination of two chemical treatments is necessary; namely, the extraction of the CO from the blood and the subsequent determination of the quantity of the gas.

The determination of CO by means of iodine pentoxide has found a wide application, quantities as small as 0.05 cc. being estimable. The method was first applied to blood by Nicloux [1898], who extracted the CO with the vacuum-pump of Gréhant [1894]. Later on nobody seems to have used iodine pentoxide for this purpose. Plesch [1914] and Gad-Andresen [1916] liberated the CO together with the oxygen in the apparatus of Barcroft or Krogh by means of potassium ferricyanide and determined the CO by combustion.

Van Slyke and Salvesen [1919] liberated the CO together with the oxygen and determined the former gasometrically after absorption of the oxygen.

When, however, the CO content of the blood is less than 10 % of its total capacity these methods are lacking the accuracy necessary in physiological and forensic medical work. The method given by Nicloux may give better results, but he does not give any control experiments on the subject.

By combining Haldane's ferricyanide method with the use of I_2O_5 it is possible to reach a greater accuracy, if one can afford to use at least 10 cc. of blood.

1. *Determination of small quantities of CO.*

CO made in the ordinary way from formic and sulphuric acids was analysed by means of the capillary pipette described by Jordan and Schwarz [1920]. As absorbing fluids were used a solution of pyrogallic acid in strong potassium hydroxide for oxygen and carbon dioxide, and a solution of cuprous chloride in strong ammonia and ammonium chloride for carbon monoxide.

A small quantity of this analysed gas was measured in the Jordan-pipette and sucked into an evacuated flask of one litre capacity. Then outdoor air was introduced and mixed with the CO by shaking, a small quantity of water securing a thorough mixing. The resulting gas mixture was then led over I_2O_5 heated to 150°. This I_2O_5 (5 g.) was contained in a U-tube (D) of Jena glass,

being kept in position by small plugs of glass-wool or asbestos. The gas before reaching the I_2O_5 is led first through strong KOH (*A*) and then through strong sulphuric acid (*B*) to be freed from traces of SO_2 and water, which would interfere with the determination of the CO. Tube *C* is a glass spiral to retain traces of sulphuric acid. Ordinary Peligot tubes were used as wash bottles. The iodine liberated is retained in two Peligot tubes (*E* and *F*) containing 0.5 % KI solution, and afterwards titrated with 0.01 *N* thiosulphate from a micro-burette. 1 cc. of 0.1 *N* thiosulphate corresponds to 5.6 cc. of CO.

Before the apparatus is used for determinations the I_2O_5 should be subjected to a current of air devoid of reducing gases at a temperature of about 200° for several hours, until it gives off only traces of iodine at 150°; these traces should be constant in quantity in order that a reliable blank value may be obtained.

Fig. 1.

Table I indicates amounts of CO taken, as calculated from the values found with the use of the Jordan pipette and those found by titration of the iodine liberated.

Table I.

cc. CO taken	cc. CO found
0.027	0.043
0.060	0.068
0.113	0.122
0.144	0.120
0.149	0.114
0.308	0.319
0.634	0.680
1.21	1.04
1.61	1.31

In the last three determinations the gas was measured by means of a Van Slyke pipette.

2. *The extraction of the CO from the blood.*

Van Slyke and Salvesen have pointed out that for extraction of oxygen and CO from blood it is convenient to use ferricyanide together with evacuation. This principle has been applied in the following way. For the extraction a wide-mouthed bottle of 1 litre capacity is used. This bottle is closed by a rubber stopper, through which pass three tubes; two of these are bent at right angles; all three carry rubber tubes, which are closed by screw clamps (see Fig. 1). Attached to the rubber tube of the straight tube is a glass-funnel. The bottle is evacuated by the use of an ordinary suction-pump. Then a measured quantity of blood, *e.g.* 10 cc., is measured into the funnel; by opening the screw clamp below the funnel the blood is sucked into the bottle; after the blood $1\frac{1}{2}$ times its volume of distilled water are introduced in the same way and mixed with the blood by rotating; then through the funnel are added 4 cc. of fresh saturated solution of potassium ferricyanide and mixed with the contents of the flask by vigorous shaking; the flask is now placed in a bath at 40° and shaken at regular intervals during a period of about 30 minutes. There is no need to add ammonia to prevent the escape of CO_2 from the blood; on the contrary, the foaming which accompanies the liberation of CO_2 facilitates the decomposition of the CO-haemoglobin¹.

For the determination of the quantity of CO liberated after the extraction, outdoor air is introduced into the flask until atmospheric pressure is reached and the contents are thoroughly mixed; now one of the rubber tubes of one of the two bent tubes of the bottle is connected with the first Peligot tube (containing KOH) of the apparatus for CO determination, whilst the other is connected to a tube dipping into a jar filled with 2 litres of water. The last Peligot tube of the apparatus (*F*), containing potassium iodide, is connected to a suction pump, which is opened sufficiently to secure a passage of about 1 l. of gas through the apparatus in 30 minutes. When nearly all of the gas has passed, some amyl or capryl alcohol is introduced into the bottle to destroy the foam and the suction of the gas is continued until the bottle is full, when the screw clamp between the apparatus and the pump is closed and the bottle disconnected. The apparatus is now connected with another bottle containing outdoor air of which 1 or 2 l. is led through the apparatus to wash the I_2O_5 . The KI solution from the last two Peligot tubes is now titrated, allowing for the iodine which is liberated in a blank determination made by passing 2 or 3 l. of outdoor air through the apparatus.

The usefulness of the technique described was tested in the following way. Fresh horse or ox blood was saturated with CO, and the content of CO determined as described. Then known quantities of this blood were mixed with fresh blood. The CO content of the resulting mixture was again determined and compared with the CO content as calculated. As at first the results were

¹ This was found after most of the control experiments given in Table II had been accomplished; in them dilute ammonia was used.

not satisfactory, it appeared that the fresh blood itself was not always CO-free, probably on account of the air in the stables. Table II indicates the results obtained.

Table II.

	cc. of CO in 10 cc. of blood		cc. of mixed blood		cc. of CO in 10 cc. of blood	
	Fresh	Saturated	Fresh	Saturated	Calculated	Found
1	0.009	1.10	70	3	0.054	0.058
2	0	0.72	90	10	0.072	0.095
3	0.062	1.01	95	5	0.109	0.112
4	0.051	1.46	95	5	0.122	0.121
5	0	1.26	90	10	0.126	0.126
6	0.034	1.49	90	10	0.180	0.200
7	0.069	1.32	90	10	0.194	0.215
8	Not determined	1.21	10	10	0.605	0.614
9	"	1.29	10	10	0.647	0.685
10	"	1.32	10	10	0.662	0.602
11	"	1.29	0	10	1.29	1.23
12	"	1.29	0	10	1.29	1.29
13	"	1.32	0	10	1.32	1.32
14	"	1.49	0	10	1.49	1.45

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