A METHOD OF ESTIMATING THE OXYGEN AND CARBONIC ACID IN SMALL QUANTITIES OF BLOOD.

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IN the investigation of the blood-gases in small animals, or in individual organs such as the salivary glands, it is desirable to have a method which will permit of obtaining results with very small amounts of blood. The method now described was designed with the special object of fulfilling this requirement, but it may also be conveniently made use of even where large samples are available, or when it is desired to make a number of determinations of the blood-gases of a single animal. The apparatus needed is considerably simpler and the time required much shorter than with the ordinary method of extracting the oxygen and carbonic acid by a vacuum pump and subsequently analysing them.

The apparatus consists of a small glass vessel attached by tubing to a pressure-gauge of narrow bore. The vessel is so arranged that the oxygen in the sample of blood can be liberated within it by ferricyanide and the resulting increase of pressure measured by means of the gauge. From the increase of pressure the volume of the oxygen can be calculated. By similar manipulation with the use of tartaric acid in the place of ferricyanide the carbonic acid is subsequently determined.

The whole apparatus is shown in Fig. 1. Two gauges, one connected with the blood-gas vessel, and the other with a precisely similar control vessel, are fixed on a wooden stand, the front of which is painted white to form a convenient background for reading the graduations on the tubes. Each gauge is graduated on both limbs in millimetres etched on the glass, the length graduated being about 350 mm. The limbs of each gauge are connected below by a piece of india-rubber tubing of about 1·25 cm. bore, which can be compressed by a screw clamp (3cm. broad), so as to adjust the levels. The gauges are filled with water

which may be tinged with a suitable dye. The bore of the gauges is 2 to 2·5 mm. and must be even, though it need not be the same in different limbs. The evenness can be ascertained by means of a column of mercury in the usual way. One limb of each gauge is provided with

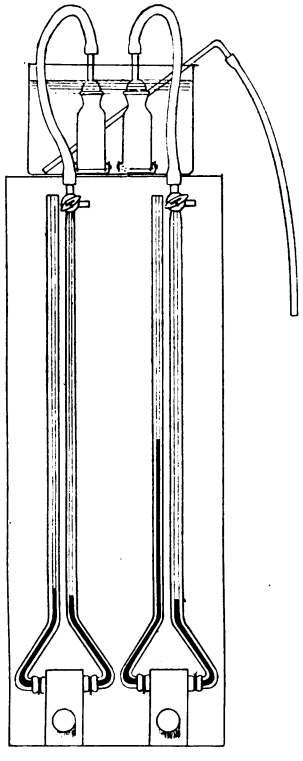

Fig. 1.

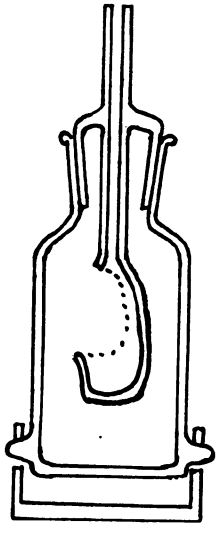

Fig. 2.

a 3-way tap close to the top. The gauges should be thoroughly cleaned before being filled, otherwise the readings will be unreliable, as shown by the fact that the water does not ascend or descend to equal distances on the two sides on compressing or relaxing the rubber tube with the taps open.

The blood-gas vessel and control vessel are of the form shown in Fig. 2. They each have a capacity of about 25 c.c. The stopper is perforated by a glass tube of narrow bore; which passes below into an open pocket capable of holding about ·3 c.c. of liquid. The pocket is so arranged that any liquid in it can easily be emptied by tilting the

vessel. To this end the orifice above the pocket should be at least 1·8 cm. in height and as broad as possible.

Before the sample of blood is collected, 1·5 c.c. of the alkaline solution (2 c.c. of strong ammonia, ·88 sp. gr., to 1000 c.c. of distilled water) used in carrying out the ferricyanide[1] method is measured with a pipette into the blood-gas vessel, and ·25 c.c. of saturated potassium ferricyanide solution is placed in the glass pocket of the stopper, which has previously been greased with resin ointment or some other adhesive lubricant.

Blood entirely deprived of its power of clotting may be collected in a pipette which delivers 1 c.c. between two marks. The pipette should be a tube of uniform bore, graduated in hundredths of a c.c. A cubic centimetre of fluid should form a column 15—20 cm. in height.

In such a tube there is no perceptible gaseous exchange except at the very surface of the column. It has already been shown that saliva and blood collected in such tubes do not lose CO_2[2], nor does a change of colour take place away from the meniscus. As the exposed portion of blood is never delivered from the pipette no error is introduced from aeration.

For the collection of coagulable blood a hypodermic syringe with special adjustments is used. This instrument is shown in Fig. 3. The syringe, which is entirely made of glass[3], should have a capacity of 1·2 to 1·5 c.c. It contains a flat glass bead, pierced with a large

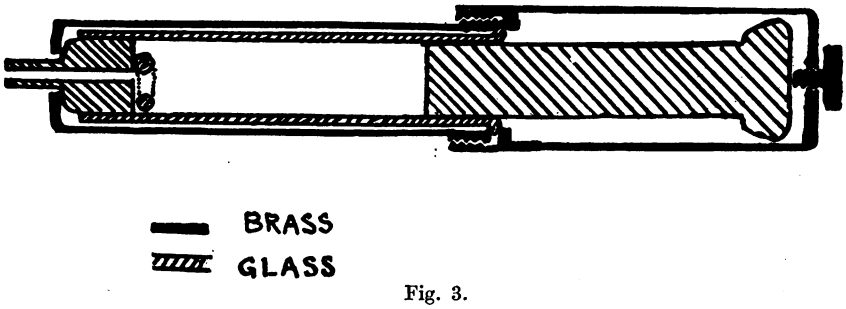

Fig. 3.

hole. The syringe is screwed into a brass case, which is so made that the distance from the shoulder of the nozzle to the end of the plunger when pulled out as far as possible is a constant quantity. The syringe

[1] This *Journal*, xxv. p. 205. 1900.

[2] This *Journal*, xxvii. p. 37. 1901.

[3] We have used an "All-glass" syringe of Burroughs and Wellcome.

has three fixed points, which will be referred to as positions I. II. and III:

Position I. when the plunger is pressed against the bead,

Position II. when the plunger is touching the back of the brass case, and

Position III. when the screw at the back of the case is screwed home and the plunger is touching the tip of the screw.

The collection of blood is carried out as follows:

(1) A 1% solution of potassium oxalate is sucked into the syringe and all the air-bubbles expelled. Oxalate is then expelled till the plunger is in position I. The syringe now contains a known quantity of oxalate.

(2) Blood is sucked in till the plunger is in position II. The blood and the oxalate are mixed by means of the bead. (The blood in the nozzle, which is quite trivial in amount, less than ·02 c.c., does not mix with the oxalate.) A mixture containing blood and oxalate in known proportions has now been obtained.

(3) The screw is screwed up till the plunger is in position III. The blood in the nozzle has now been expelled and a little of the mixture has been expelled.

(4) The mixture is expelled into the bottle, the plunger returning to position I. A known volume of a mixture of blood and oxalate of known composition, and hence a known volume of blood, has been put into the bottle.

The volume of blood delivered by the syringe is ascertained by weighing it:

(1) When empty.
(2) With nozzle just filled with water.
(3) Filled with water in position I.
(4) „ „ „ II.
(5) „ „ „ III.

The mixture is discharged cautiously into the bottom of the blood-gas vessel, care being taken that the blood lies under the ammonia solution. The blood-gas vessel is then closed and placed in the water beside the control vessel as shown in Fig. 1, care being taken not to mix the blood with the ammonia solution or spill the ferricyanide. The water is stirred two or three times by blowing air through it by means of a tube shown in Fig. 1, and the gauge is watched till the temperature of the two vessels is found to be exactly the same. In making these and other observations it is necessary first to squeeze the rubber tubing connecting

the limbs of the gauges, so as to insure that the glass above each meniscus should be wet. The two gauges are now adjusted by opening the three-way taps, so that they are both level and stand at zero. The blood-gas bottle is taken out and the blood and ammonia solution mixed without spilling any ferricyanide. When this solution is completely transparent the vessel is tilted so as to empty out the ferricyanide, and then shaken for a few minutes to liberate all the oxygen. During these manipulations it should be held either in a cloth or by the lead stand, to avoid warming the glass. It is now replaced in the water, which is stirred. When the temperature of the two vessels has again come even the gauges are adjusted so that the levels in the limbs connected with the bottles are at zero. The heights on the other limbs are read off; and if, as is usual, the temperature has risen since the original reading, so that the level is now higher in the open limb of the control tube gauge, the reading of this gauge is deducted from the reading of the other gauge.

A further correction is also necessary. If a blank experiment be made in which no ferricyanide is added, but all the other manipulations are gone through it will be found that a slight negative reading amounting to about 3·5 mm. is obtained. This is apparently due to the tension of ammonia vapour being less after the blood and ammonia solutions are mixed or to absorption of the minute amount of carbonic acid in the air within the bottle. Hence a constant determined for the ammonia solution used must be added to the reading.

The temperature of the vessel of water is now read off. As the specific gravity of mercury at 0° is 13·59 and of water at 15° ·999 it is evident that the normal barometric pressure equals that of a column of water $760 \times \frac{13{\cdot}59}{{\cdot}999} = 10340$ mm. high.

Hence the volume at 760 mm. pressure of oxygen given off equals the volume of air in the blood-gas tube and its connections multiplied by the corrected reading of the gauge in millimetres and divided by 10340. Thus if the total capacity of the blood-gas vessel and its connections to the zero of the gauge = 23·35 c.c. and that of the fluids which have been put in is 2·75 c.c. the volume of air will be 20·6 c.c. If now, the reading of the gauge = 100·0 mm. the oxygen given off will be $20{\cdot}6 \times \frac{100}{10340}$ c.c. = ·201 c.c. If the temperature were 14° this would correspond to ·191 c.c. of oxygen at 0°. If this came off 1 c.c. of blood the combined oxygen of the blood would be 19·1 %.

The capacity of the blood-gas vessel is previously ascertained by weighing it first empty and then full of water. That of the connecting tubing is determined by adjusting the gauge to zero and then raising the pressure to a certain definite amount, first with the vessel connected and afterwards with a stopper inserted into the end of the rubber tube in place of the glass tube of the vessel. The capacity of the vessel and the connecting tubing will be to that of the connecting tubing alone, as the first reduction in volume of the closed limb as read off on the gauge is to the second.

To estimate the carbonic acid the stopper of the blood-gas vessel is removed after the estimation of the oxygen and ·25 c.c. of 20 % tartaric acid solution is placed in the glass pocket; the stopper is then replaced and the gauge adjusted to zero in the same way as before. The acid is then spilt and the bottle shaken till all the CO_2 is set free, which takes a very short time. The gauge is again read off and the volume of the CO_2 calculated.

The determinations should always be made in a well-ventilated room, as the presence of an abnormally high percentage of CO_2 in the air enclosed in the bottle at the beginning of the experiment might evidently give rise to error both in the oxygen and CO_2 determinations.

A correction is necessary for any CO_2 which may be present in the solutions employed or is absorbed from the air enclosed in the bottle. This correction is obtained by previously doing a complete blank experiment, using boiled distilled water in place of blood to mix with the ammonia solution and ferricyanide. After the correction has been obtained the ammonia solution should be kept in a stoppered bottle.

A further complication is introduced by the fact that CO_2 is very soluble in water or the acid mixture of water and precipitated proteid etc. present in the bottle. To ascertain the solubility of CO_2 in this mixture as compared with its solubility in water we used a vessel of 340 c.c. capacity, provided with a mercury gauge, and immersed in water at a constant temperature. In successive experiments we filled this vessel with pure CO_2 and then forced into it 50 c.c. of boiled distilled water or 50 c.c. of blood diluted with water, ferricyanide, and acid in the proportions used for the blood-gas determinations, and after this mixture had been freed from CO_2 by shaking with air and boiling *in vacuo*. On thoroughly shaking up the liquid with the CO_2, and observing the final reading of the gauge, the relative solubilities of CO_2 in the water and in the mixture could be calculated. The mean of

several closely concordant experiments gave at 15° a coefficient of absorption of 1·001 for water, and 0·900 for the mixture. The solubility of carbonic acid in the mixture was thus 90 % of its solubility in water.

At a temperature of 13° one volume of water dissolves 1·065 volumes of CO_2 reduced to a temperature of 0°, or 1·11 volumes measured at 13°. Hence at the same temperature one volume of the blood mixture would dissolve 1·00 volume of CO_2 measured at 13°. There would therefore be no error at that temperature if in calculating the CO_2 given off by the blood the blood-gas vessel were regarded as containing no liquid. Within the ranges of temperature commonly met with the solubility of CO_2 diminishes by about 10 % for every four degrees of rise of temperature. With a bottle of about 25 c.c. capacity this would cause an error in a CO_2 determination of about 1 % for every four degrees above 13° if the vessel were regarded as containing no liquid. The simplest way of calculating the percentage of CO_2 in the blood is thus to regard the blood-gas vessel as containing no liquid, and to deduct 0·25 % from the final result for every degree C. by which the temperature of the water-bath exceeds 13°. Thus if the temperature of the bath were 17°, and the uncorrected result were 40·0 volumes of CO_2 per 100 c.c. of blood, the corrected result would be 39·6 volumes.

To test the method we have made a variety of experiments:

In the first place it was necessary to see that the gauges acted with sufficient sharpness. This was tested by the simple plan of opening both limbs and screwing up the clamp so as to raise the level of the liquid. It was found that the gauges were always practically level provided that the tube had been well cleaned with ether, nitric acid, and water before being set up. The two gauges also showed the same differences of pressure when the temperature of the bath was raised provided that the volume of the air in the vessels and connections was the same.

In order to ascertain whether any error arose from diffusion of oxygen through the ammonia solution to venous blood, we placed in the apparatus blood which had been completely deprived of its oxygen by commencing putrefaction. It was found that after the temperature had become equal in the blood-gas and control vessels the gauges kept perfectly together for a far longer time than would be needed for an analysis, hence no error arises from diffusion.

To test directly the accuracy of the determinations we took defibrinated blood, saturated with air, and determined the oxygen

capacity with the apparatus and by Haldane's method[1] with a carefully standardised hæmo-globinometer. The results were as follows:

A. *Ox-blood.*

	(1)	(2)	Mean
By hæmoglobinometer	19·60	19·80	19·70
By new apparatus	19·87	19·61	19·74

B. *Cat's blood.*

By hæmoglobinometer	12·25	12·25	12·25
By new apparatus	12·3	12·1	12·2

It is thus evident that the oxygen determinations are very accurate with saturated blood. With unsaturated blood, however, there is an evident source of slight error in the fact that the blood contains practically no dissolved oxygen when put into the apparatus, but an appreciable quantity at the end of the analysis, since at ordinary temperatures blood takes up 0·6 % of oxygen from the air. It is therefore necessary to add about that amount to the result when the blood is venous.

To test the carbonic acid determinations we employed in place of blood, 1 c.c. of a 0·200 % solution of Na_2CO_3 (which had been well heated in a platinum crucible before being weighed out). The results were as follows:

Calculated 42·1 %

Found	First series	41·6,		42·5,		42·0	Mean 42·0 %
	Second „	42·7,	42·2,	42·6,	41·9,	42·3	Mean 42·3 %

Further we have allowed the CO_2 to stay for a considerable time in the apparatus and have found that no measurable quantity is absorbed by the rubber tubing in the time taken for a determination. We have also ascertained that no appreciable change in volume is caused by the precipitation of proteids, etc. which occurs on mixing the acid with the blood and ferricyanide.

We have tested arterial blood taken directly from two cannulæ placed in the carotid and femoral arteries respectively of a dog. The former was attached to the measuring burette of Barcroft's blood-gas apparatus and 8 c.c. of blood withdrawn: the latter to a pipette containing oxalate; the former was analysed for CO_2 with the pump, the latter with the new apparatus:

CO_2 by pump	42·0 %
by new apparatus	43·5 %

[1] This *Journal*, xxvi. p. 501. 1901.

Finally, to test the consistency of the apparatus we have made three analyses of defibrinated blood from the same vessel which gave the following results:

	(1)	(2)	(3)	Mean
O	18·5	18·5	18·8	18·6
CO_2	52·4	52·7	51·2	52·1

It is thus evident that the apparatus is capable of giving sufficiently accurate results in spite of the small volume of blood used; and provided sufficient pains are taken to avoid errors due to temperature differences and to the reagents employed it is not much less accurate than the blood-pump with much larger quantities of blood.

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