DIFFERENTIAL METHOD OF BLOOD-GAS ANALYSIS.

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1. *Introductory.* In the solution of many blood-gas problems the quantity upon which the estimations depend is not the absolute amount of gas in any particular sample of blood but the difference between the gaseous contents of two samples, to wit, arterial and venous. This difference is small when the blood courses rapidly through an organ. The circulation in salivary glands and the kidney at the height of secretion furnish examples of brightly coloured venous blood. On such occasions the volume of blood passing through the organ is so great that a trivial error in the analysis of either the arterial or venous blood, causing as it does a large error in the difference between the two, produces a very serious divergence in the estimation of the oxygen used in the CO_2 given out by the organ.

By the chemical method of analysis, if the apparatus is thoroughly clean, the most probable sources of error are the following:

(1) That one or other sample of blood is not shaken sufficiently to expel the last traces of gas.

(2) That one or other sample has not acquired the temperature of the water bath before either the initial or the final reading is taken.

(3) That the blood is incompletely laked.

(4) That some ferricyanide finds its way into the blood before laking takes place.

In the last two cases too little oxygen will be given off by the defective analysis.

(5) That the solution of ammonia used in the analysis may absorb an undue amount of carbonic acid from the air.

Of these sources of error the third and fourth apply to the analyses of oxygen and depend upon the use of ferricyanide of potassium. The advantage of dispensing with this reagent would therefore be great.

The first and the second apply to analyses both of oxygen and CO_2 but more particularly to the latter, whilst the fifth source of error applies entirely to carbonic acid analyses.

The most reliable way of getting a fair comparison between arterial and venous blood is to perform four analyses, two of each. Each analysis involves four readings, so that there are in all 32 readings, any one of which may contain an error. It is best that the duplicate samples should be analysed in bottles of as nearly as possible the same size. A comparison of the pressure set up in each instrument can be made which will show whether a change in the volume of the contained gas indicates a liberation of oxygen or CO_2, or whether it is due to a passing temperature effect.

A good differential method would greatly reduce the chance of error which may be attributed to the first two causes.

The difference between the amount of oxygen in two samples of blood from the same animal may be estimated as the difference between the amounts of oxygen necessary to saturate each sample. If this principle is adopted the third and fourth sources of error disappear. Inasmuch as the acquisition of very little oxygen is usually necessary to saturate the arterial blood, the difference between the amount of oxygen taken up by the two samples is not the difference of two large quantities but is generally much greater than one of those quantities. Hence it is theoretically much more satisfactory to measure the difference between the amounts of oxygen necessary to saturate the two samples of blood than of the amounts which can be expelled from them.

The method accounts for the difference in oxygen content of the plasma as well as of the corpuscles. The fifth source of error has been specially investigated.

2. *Description of apparatus.* The apparatus consists of a manometer each limb of which can be opened to the air and is attached to a bottle.

Fig. 1 shows the apparatus from in front. *C* and *D* are the limbs of the manometer and should be made of thick glass tubing of a uniform bore of about 1 mm. Any convenient method of raising and lowering the fluid in the manometer may be adopted, in that shown each piece of glass tubing is inserted into a piece of rubber tubing. The two portions of rubber are joined by a short curved piece of glass *A*.

The rubber tubing passes underneath a screw clamp (*B*) which alters the level of the fluid.

In the manometer a solution of bile salts of S.G. 1·034 has been used.

The solution was recommended to me by Professor Brodie and, if it is made up with sufficient toluol to prevent the growth of moulds, it is an extremely satisfactory reagent for the measurement of differences of pressure in fine tubes. It has the twofold advantage of wetting the tube evenly in virtue of its power as a solvent of grease, and of exerting exactly $\frac{1}{10000}$ of the pressure of the atmosphere per millimetre. The taps at the top of the apparatus *G* and *H*, should have stoppers of the form indicated, lest the handles touch one another when they are turned, in which case there is a chance of one or other stopper being loosened.

The apparatus is seen from behind in Fig. 2.

The blood gas bottles of which *E* is one are of about 27 c.c. in capacity. They should be as nearly as possible of the same size and must be calibrated in the manner to be described later.

The bottles shown are those used by Prof. Brodie. I have to thank him for lending me the pair with which many of the determinations recorded in this article have been made.

Two forms of bottle suitable for the analysis of 5 c.c. of blood are shown in Figs. 3 and 4, both have been used in the present research. The volume of the bottle shown in Fig. 3 is about 110 c.c., that in Fig. 4, 170 c.c.

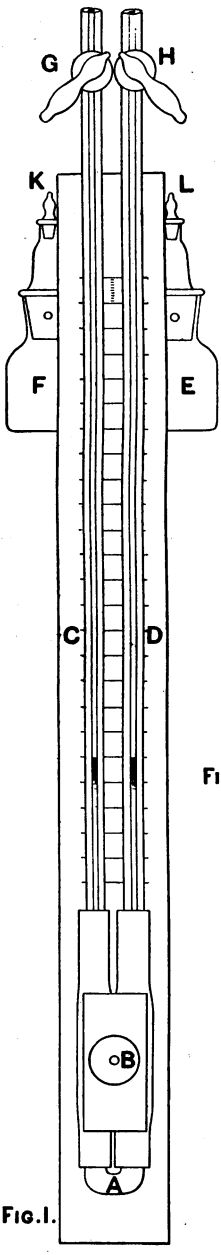

FIG. 1.

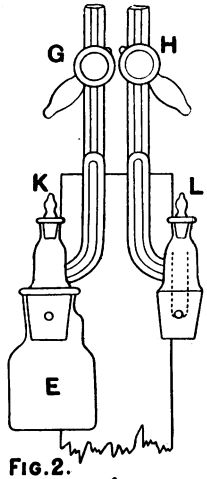

FIG. 2.

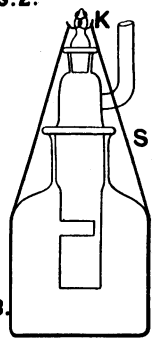

FIG. 3.

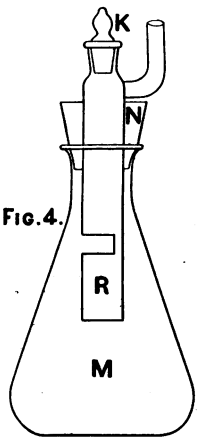

FIG. 4.

The difference between them is principally one of convenience and expense. The form shown in Fig. 4 consists of an ordinary conical flask *M* which fits into a rubber cork *N*. This form has the following advantages over that shown in Fig. 3.

(1) The conical form is better than the cylindrical one since the blood in the bottom of the flask is less likely to splash into (*R*) the reservoir for the tartaric acid, when the instrument is shaken for the purpose of saturating the blood with oxygen.

(2) This form of bottle is more easily calibrated than that shown in Fig. 3.

(3) If one flask gets broken it is easily replaced. On the other hand the rubber attachment is not so firm as the ground glass connexion. In both cases however a rubber ring *S* may be used with advantage to prevent the bottle or the small stopper *K* shifting[1].

The whole apparatus is mounted on a wooden stand and arranged so that the bottles can be immersed in a large water bath on the side of which the stand hangs.

3. *The theory of the method.* Equal quantities of arterial and venous blood are placed in the two bottles; when the temperature of the water bath has been acquired by them, or at all events when their temperature ceases to change unequally, as indicated by the movement of the fluid in the manometer, the surfaces of the bile salt solution are levelled and the instrument is shaken till the blood in each bottle is thoroughly saturated with oxygen. The amount of oxygen lost from the air on either side is not sufficient to prevent the haemoglobin taking up its full quotum. After a suitable interval of immersion of the bottles in the bath the level of the fluid surfaces in the manometer is read. The following calculation, for which I am indebted to the kindness of Sir Robert S. Ball, shows the relation between the change level and the differential absorption (or evolution) of gas.

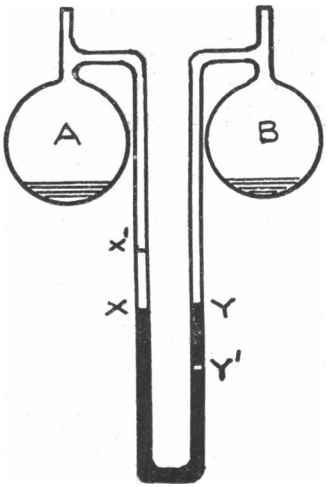

Fig. 5.

[1] Since this paper has gone to press I have had an apparatus made with conical flasks and ground stoppers which has proved satisfactory; it is calibrated with light hollow glass beads.

"The temperatures being all supposed uniform throughout, let V be the original volume of A or B at the atmospheric pressure p in millimetres of a liquid with the specific gravity 1·034.

I shall assume that in the experiment the absorption of gas on the A side is xV so that after the experiment the volume of gas on the A side if it were reduced to the original pressure p would be $V(1-x)$. But the pressure to which it is actually subjected after the experiment is $p-\rho$ if $2\rho = X'Y'$ the observed quantity. Thus after the experiment the volume on the A side as given by Boyle's law is

$$\frac{V(1-x)p}{p-\rho} \quad \text{.....(i).}$$

In like manner I suppose that after the experiment there is a change in the quantity V of gas on the B side so that the altered quantity of gas if reduced to the original pressure p would be $V(1-y)$. But after the experiment is made and the reading is being taken the pressure on that side is $p+\rho$ so that the volume is

$$\frac{V(1-y)p}{p+\rho} \quad \text{.....(ii).}$$

The difference between (ii) and (i) can be nothing else than the volume in the U tube between the levels X' and Y'. If the area of section of the tube be S in square millimetres, then since the vertical difference of X' and Y' is 2ρ we have the equation

$$\frac{V(1-y)p}{p+\rho} - \frac{V(1-x)p}{p-\rho} = 2S\rho \quad \text{.....(iii).}$$

The quantity required being the difference of the two volumes at pressure p after the experiment is $(x-y)V$ and a transformation of the equation can now be made which will give the desired quantity.

Clearing (iii) of fractions and dividing both sides by p^2 the equation assumes the form

$$V\left(x-y-\frac{2\rho}{p}+\frac{\rho x}{p}+\frac{\rho y}{p}\right) = 2S\rho\left(1-\frac{\rho^2}{p^2}\right).$$

But this equation admits of simplification for since x and y and ρ/p are small $\frac{\rho x}{p}$, $\frac{\rho y}{p}$ and $\frac{\rho^2}{p^2}$ are negligible; whence finally

$$V(x-y) = 2V\rho/p + 2S\rho.$$

Let d be the diameter of the tubing, the formula becomes

$$V(x-y) = 2\rho\left(\frac{V}{p}+0{\cdot}785d^2\right)."$$

In the case of carbonic acid analysis when the amount of gas evolved with acid is considerable p is appreciably higher than 10000 mm. of bile salt solution, the difference invading the third significant figure. Usually p is between 10200 and 10100 mm., which figure is the more correct can easily be determined at the end of the analysis. An example will show the relative importance of the quantities in the above equation.

Into bottles of 27·2 c.c. (inclusive of tubing), 2 c.c. of blood and 3 c.c. of NH_3 were placed and 1 c.c. of broken glass; therefore $V = 27{\cdot}2 - (2+3+1)$ c.c. $= 21200$ cubic millimetres, $d = 2{\cdot}20$ mm. approx.

$$V(x-y) = (2{\cdot}12 + 3{\cdot}80) = 5{\cdot}92 \text{ c.mm.}$$

This example shows that the bracket contains two quantities of the same order, the first depending upon the volume of the bottle, the second in the calibration of the tubing. The exact measurement of the volume of the bottle is of relatively little importance, a mistake of ·1 c.c. affects the answer to the extent of one part in six hundred. The calibration of the tube is all important. An error in this of ·005 c.m. would raise the right side of the equation from 5·92 to 6·09 or about 3 %. It will be of advantage therefore to arrange the sizes of the bottle and tubing so that the factor $\frac{V}{p}$ is greater relatively to $0{\cdot}785d^2$, since d^2 is not easy to measure so exactly as V considering the film of fluid which covers the inside of the tube. With tubing of a bore of one millimetre instead of 2 mm. the term $0{\cdot}785d^2$ becomes 0·785 and with bottles of the size already described, containing 1 c.c. blood, 1·5 c.c. NH_3 and ·2 c.c. glass, the equation would stand

$$V(x-y) = 2\rho\,(2{\cdot}45 + 0{\cdot}785);$$

here the second term is about one-third of the first.

With large bottles 170 c.c. in capacity the factor $\cdot 785d^2$ becomes even less important.

4. *The calibration of the apparatus.* (1) To measure the volume of the bottle. The apparatus should be set up on its wooden stand and a mark made on the glass in two places, at the same level, one on each tube. The apparatus is then taken to pieces. A little water is placed in the portion of tubing above the tap and also in the bore of the tap. Each tube with its bottle is weighed. The tube is then turned with the tap downwards and plunged into a large vessel of water up to the mark or nearly so. The bottle, which

is also under water, is put in its place and the level adjusted so that the surface of the water is now exactly at the mark. The outside of the apparatus is dried and weighed. The difference in the two weighings gives the volume to the mark. This is done for both tubes.

In general it will be found that there is some difference in the volume of the bottles, &c.; this should not be more than a few tenths of a cubic centimetre in the case of bottles of 25—30 c.c. It may be rectified by cutting a piece, of appropriate length, from a glass rod of known specific gravity. In the case of larger bottles the difference may be larger; this method of equalising the difference is unsuitable as the glass rod necessary may be so heavy as to break the bottle when shaking takes place. The bottles must be made of thin glass. The form of bottle shown in Fig. 4 is calibrated by moving one cork up or down on the glass cylinder, which should be of ample diameter. When the correct position is found it is marked on the glass. As a preliminary two glass flasks of equal size must be selected, this is not difficult. Marks should be scratched on the necks of the flasks at the position of the bottom of the rubber cork.

(2) To measure the bore of the tube. The inside of the tube should be moistened with the bile salt solution and allowed to stand in a vertical position for some minutes, the lower portion being carefully dried. The tube is then calibrated with mercury.

When these determinations have been made the volume to any point can be calculated. The apparatus is then replaced on its stand, to which it must be rigidly fixed.

5. *Some details of analysis.* Careful tests have been made of the length of time for which it is necessary to shake the apparatus in order to completely saturate the blood. This was first done on samples of 5 c.c. in bottles of 75 c.c. Samples of blood, saturated to different degrees, were put in the two bottles. After each minute of shaking the bottles were put back in the water bath, allowed to cool and a reading of the difference of level was taken. Then one tap was opened, the gas on the other side brought back to the zero, and from the pressure at constant volume the amount of oxygen absorbed was calculated. The other tap was then opened, the menisci were levelled, the taps shut and the apparatus was shaken for another minute. At the end of five minutes there ceased to be any change in level produced in the differential reading, the hæmoglobin being equally saturated on both sides, but during the sixth minute there was a little oxygen absorbed and possibly

even during the seventh. Eight minutes shaking may be regarded as ample for a sample of 5 c.c. and five minutes for a sample of 1 c.c.

The following is a sample analysis showing the detailed manipulations and the time which should be taken over each. The final difference in pressure between the oxygen in each bottle is called ΔpO_2 and of carbonic acid ΔpCO_2, and of volume ΔvO_2 or ΔvCO_2.

Quantities used—1 *c.c. blood from ærotonometers*—1·5 NH_3.

The figures in the second and third columns are mm. on the scale.

Time	Tap *G*	Tap *H*	Remarks
12.19	Open	Shut	The bottle on tube *H* (Fig. 1) is that which was handled
12.19		200	last—bottles placed in water bath.
12.22		203	
12.24		204	
12.26		205 –	
12.29		205 –	The bottles have now reached the temperature of the bath.
	Open	Open	To level before starting.
	210	210 +	
	Shut	Shut	
12.30	210	210 +	Remove from bath and shake thoroughly.
12.31—12.36			
12.38	205	211 +	
12.40	205	210 +	
12.42	205	210½	
12.44	205	210½	Analysis of oxygen complete
			$\Delta pO_2 = (110 - 105) + (110\frac{1}{2} - 110) = 5\frac{1}{2}$ mm.
12.45			Take out small stoppers *K* and *L*, Fig. 1, introduce acid.
12.50	Open	Open	
	210	210 +	
	Shut	Shut	
	210	210 +	
12.51—12.57			Spill in acid and shake.
12.59	214	204½	
1.1	214	204½	$\Delta pCO_2 = (214 - 210) + (210 - 204\frac{1}{2}) = 9\frac{1}{2}$ mm.
			Raise level of fluid in both manometers about 100 mm.
1.2		Open	Readjust level till *G* is 210.
1.3	210	40	Pressure of gas in *H* = 10160 mm. of fluid.

6. *The accuracy of the method for determinations of oxygen.* The following determinations have been made, chiefly with the view of testing the accuracy of the method for oxygen analyses. Incidentally some carbonic acid determinations were performed.

(1) Some venous blood was divided into two portions, one of these was placed in a Loewy's ærotonometer in so large a quantity that there were only a few bubbles of air in the ærotonometer, these were thoroughly shaken with the blood. The second portion was put in a similar ærotonometer and shaken with much more air. Blood with a constant oxygen content could then be delivered from either ærotonometer. The blood was analysed twice in the same apparatus by the differential method, in samples of 2 c.c. each, whilst duplicate analyses were made of each specimen (1 c.c.) by the chemical method:

	Oxygen in Blood		Difference	
	Ærotonometer A	Ærotonometer B	between A and B	ΔvO_2
(1)	19·8 %	10·6 %	9·2 %	9·2 %
(2)	19·9 %	10·6 %	9·3 %	9·7 %
Mean	19·9 %	10·6 %	9·3 %	9·5 %

(2) If determinations are made by the differential method it is possible in each case to determine how much gas one of the two samples of blood has absorbed. This is done by opening one stopper, bringing the closed side to constant volume and calculating from the observed pressure.

This procedure was followed in the cases quoted above. The oxygen absorbed by blood from A was 2·1 % and from B 11·6 % which therefore differed by 9·5 %, which agreed with the mean of the differential readings.

(3) The following determinations were made simultaneously in two different apparatus with a very different constant for d^2.

Apparatus I, volume of bottles 27·2 c.c. (less 1 c.c. glass, 1·5 c.c. NH_3 and 1 c.c. blood) $\frac{V}{p}=2{\cdot}37$, $d^2=4{\cdot}84$ sq. mm.

Apparatus II, $\frac{V}{p}=2{\cdot}49$, $d^2=1{\cdot}96$ sq. mm.

	App. I	App. II
ΔvO_2	2·16 %	2·15 %

(4) The oxygen and carbonic acid were determined in this series simultaneously. A large mass of blood from the butcher, dark in colour, was divided into two portions one of which was shaken somewhat slightly with air. The difference was determined twice in Apparatus I (constants see above).

Determination	(1)	(2)
ΔvO_2	3·08 %	3·14 %
ΔvCO_2	6·1 %	5·7 %

(5) Arterial and venous blood were withdrawn directly from the femoral vessels of a dog into which hirudin had been injected. The analysis took place simultaneously in apparatus I and apparatus III. The constants of the latter were, capacity of bottles and tubing 112·8 c.c. (less 5·58 c.c. blood, 7·5 c.c. NH_3, 1 c.c. glass) $\frac{V}{p}=9{\cdot}87$, $d^2=1{\cdot}43$ sq. mm.

The analyses were therefore on quite different scales. The results were

Apparatus	I	III
ΔvO_2	8·2 %	7·7 %

(6) Similar to (3) but with a greater difference between the samples of blood.

Apparatus	I	II
ΔvO_2	11·4 %	11·8 %
ΔvCO_2	15·4 %	15·5 %

(7) Similar to (6) and (3) but with a much smaller margin of difference between the two samples of blood used, results calculated to ·05 %.

Apparatus	I	II	III
ΔvO_2	·60 %	·55 %	·35 %
ΔvCO_2	·30 %	·9 %	·25 %

The oxygen readings in the above tests may be examined by comparing the divergence between the extreme readings of any series with actual value of ΔvO_2 as follows:

Number of readings	2	2	2	2	2	3
	(1)	(3)	(4)	(5)	(6)	(7)
Divergence	·5 %	·0 %	·1 %	·5 %	·4 %	·25 %
Mean ΔvO_2	9·5 %	2·2 %	3·1 %	8·0 %	11·6 %	·50 %

The largest divergences take place in those samples of blood in which there is greatest difference between the aeration, the error amounting to ·5 % in samples where Δ is over 5 % and being ·25 % in the samples where Δ is smallest; probably it never can be considered less than this and we may then sum up the statement of the error by saying that down to values of 2·5 % for Δ the error is not more than 10 % of Δ. If Δ be 1 % the error is $\frac{1}{4}\Delta$ and if Δ be ·5 % the error is $\frac{1}{2}\Delta$.

7. *The accuracy of the method for determination of carbonic acid.* As in all blood gas problems, the estimation of carbonic acid is much more difficult than that of oxygen. This is in part due to the larger amount of carbonic acid present whilst the actual value of Δ is usually smaller for CO_2 than for O_2; in part it is due to the greater solubility of carbonic acid in aqueous solutions and to its chemical affinity for them when they are alkaline. A systematic attempt has therefore been made to investigate the possible errors which tend to vitiate the method.

The starting point of this investigation has been an analysis of an $\frac{N}{20}$ sodium carbonate solution.

(1) This was commenced in Apparatus IV which had bottles of the shape shown in Fig. 4. The two bottles were not quite the same size, they were about 170 c.c. The value of $\cdot 785d^2 = 1 \cdot 13$.

To equalise the size of the bottles the following special method was invoked. 5 c.c. of $\frac{N}{20}$ Na_2CO_3 was placed in each bottle and 2 c.c. of saturated tartaric acid in each reservoir (R). The following values were obtained for ΔpCO_2 together with approximate values for p (at constant volume).

Observation	1	2	3	4
ΔpCO_2	2 mm.	2 mm.	3 mm.	$2\frac{1}{2}$ mm.
pCO_2	157 ,,	154 ,,	154 ,,	—

The difference of pressure, which was always in the same sense, was about $\frac{1}{60}$th of the

whole pressure and since d^2 was small compared to $\frac{V}{p}$ and V known to be about 170 c.c., it followed that one bottle was from 3–4 c.c. larger than the other.

The cork (N, Fig. 4) was therefore moved up the glass tube by an amount which was judged to decrease the volume of the bottle and its attachments to this extent. The following observations were then made. In this case p was measured more exactly than before, account being taken of the actual change of temperature.

Observation	(1)	(2)	(3)	Mean	Divergence
ΔpCO_2	1 mm.	0 mm.	1 mm.	·7 mm.	1 mm.
pCO_2	164 ,,	163 ,,	164 ,,	163·7 ,,	1 ,,

The bottles and their attachments were clearly of the same volume, with the experimental error of the apparatus. The exact volume may be calculated from the value of pCO_2. Five cubic centimetres of $\frac{N}{20}$ sodium carbonate solution would give off 2·775 c.c. of dry CO_2 at N.T.P., which may be supposed with sufficient accuracy to be evenly distributed throughout the air and the fluid in the vessel at 10° C. This under the conditions of the experiment corresponded to 2·92 c.c. of gas, the pressure set up being 163·7 mm. of bile salt solution of S.G. 1·034, the volume of the bottle would be 178 c.c.

(2) Similar determinations were made in the case of apparatus I. In this case the volume of the smaller bottle worked out to be 27·2 c.c. and the difference between the bottles ·55 c.c. The actual volume of the bottles as determined by weighing with water was 27·2 and 27·6 c.c. respectively. The bottles were equalised with glass rod.

(3) The next step was to test sodium carbonate solutions of known, but slightly different, strengths.

Two solutions were made up, one of $\frac{N}{40}$ and the other was $\frac{N}{46} \times \frac{325}{300}$. The calculated difference in the amount of gas given off at N.T.P. by 5 c.c. of each of these is ·115 c.c.

The following were five consecutive determinations:

Observation	(1)	(2)	(3)	(4)	(5)	Mean
ΔpCO_2	6 mm.	lost	6·3 mm.	6·5 mm.	6·5 mm.	6·3 mm.

This corresponded $6{\cdot}3 \times {\cdot}95$ mm. of dry gas at N.T.P. from which it appeared that

$$\Delta vCO_2 = 6{\cdot}0 \times \left(\frac{17800}{10080} + 1{\cdot}13\right) = {\cdot}113 \text{ c.c.}$$

The above series of estimations show that

(α) all the determinations were within 5 % of the mean.

(β) that the extreme variation was about 8 % of Δ.

(γ) that the observed mean value of Δ was within 2 % of the calculated value.

(4) Having established the above facts the next step was to investigate the error caused by the use of ammonia in the analysis.

For this purpose 7·5 c.c. of ammonia solution (4 c.c. of NH_3 of S.G. ·880 in 1 litre of distilled water) was placed in each flask. The fluids were analysed with tartaric acid as blood would be. The analyses were performed in a large room in which the observer was the only occupant and the ventilation was good; care was taken not to breathe into the flasks. The results of three successive observations were as follows:

Observation	(1)	(2)	(3)
ΔpCO_2	2 mm.	2 mm.	3 mm.
ΔvCO_2	·038 c.c.	·038 c.c.	·057 c.c.

In the analysis of samples of 5 c.c. of blood with this apparatus, the third of the above readings would mean an error of more than one volume per cent. whilst the two first would be ·8 vol. per cent.

Greater precautions were therefore taken to keep the ammonia solution free from excessive CO_2 in atmosphere to which it was exposed for a short time.

The burette from which the NH_3 was delivered was placed in front of an open window. Between the burette and the operator was placed a sheet of glass which was not too large to prevent the easy manipulation of the burette, but which prevented the air in the immediate vicinity of the burette from being contaminated with the breath of the operator. As the draught from the window blew towards the operator this screen was very effectual.

The following determinations were performed in the same way as the last, but with the precaution just detailed.

	(1)	(2)	(3)
Δp (CO_2)	0 mm.	0 mm.	0·5 mm.

As these observations appeared to be entirely satisfactory the screen was used in subsequent experiments.

Similar analyses were performed in Apparatus I with 1·5 c.c. of NH_3; the results were

Observation	(1)	(2)	(3)	(4)
$\Delta p(CO_2)$	0 mm.	0·5 mm.	0·5 mm.	0 mm.

This error includes any that might be introduced by rotating the bottle on the stopper.

(5) The last stage in the investigation consisted in the quantitative recovery of CO_2 from small quantities of sodium carbonate added to blood. For this purpose a solution was made by making 2 c.c. of $\frac{N}{20}$ Na_2CO_3 up to 50 c.c. with distilled water. Some of the same distilled water, which presumably contained traces of CO_2, was set aside for controls. In the first analysis apparatus IV was : the fluids put in were on one side, 7·5 c.c. of NH_3, 5 c.c. of Na_2CO_3 and 5 c.c. of blood ; on the other 7·5 c.c. NH_3, 5 c.c. of the distilled water and 5 c.c. of the same blood.

Previous experience had made it clear that a great deal of shaking was necessary for accurate CO_2 readings when blood is used: the following figures gave the levels of the manometer on each side. About half a minute's shaking with subsequent cooling was given between each.

	Initial	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10
Right side	30 mm.	26	26·5	26	26	27	27·5	28	28·5	28·5	28·5
Left side	30 mm.	37	36	34·5	34	35	35	35	35	35	35
ΔpCO_2		11	9·5	8·5	8	8	7·5	7	6·5	6·5	6·5

Correction for temperature, pressure and aqueous vapour = × ·95.

$$\Delta vCO_2 = 6{\cdot}5 \times \left(\frac{178000}{10100} + 1{\cdot}13\right) \times {\cdot}95 = 116 \text{ c.mm. or } {\cdot}116 \text{ c.c.}$$

ΔvCO_2 calculated = ·111 c.c.

Similar determinations were made with apparatus I: the bottles contained on each side ·2 c.c. broken glass, 1·5 c.c. NH_3 and 1 c.c. of blood, the right bottle in addition contained 1 c.c. of Na_2CO_3, the left 1 c.c. of distilled water. The results were as follows :

Observation	(1)	(2)	(3)	Mean
ΔpCO_2	5 mm.	3·5 mm.	4 mm.	4·2 mm.
ΔvCO_2 (N.T.P.)				·026 c.c.
ΔvCO_2 calculated				·022 c.c.

As this error is almost entirely accounted for by that incidental to the measurement of the blood in an ordinary 1 c.c. pipette, the result is as satisfactory as can be expected.

It cannot be claimed that the accuracy of the method is so great for carbonic acid as for oxygen and this disparity is accentuated by the greater precautions which are necessary in order to produce exact results. Nevertheless the method is a distinct advance on those at present in use for the comparison of the carbonic acid in small quantities of blood.

The greater part of the expense of this research has been defrayed by a grant from the Committee of the British Association for the study of the Metabolic Balance Sheet of the Individual Organs. To them my thanks are due.