I. ESTIMATION OF CARBON DIOXIDE, OXYGEN AND COMBUSTIBLE GASES BY KROGH'S METHOD OF MICRO-ANALYSIS.

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INTRODUCTION.

For the study of respiratory processes of small organisms and for many other biological purposes it is essential to have methods for analysing small air-bubbles. In 1907 two such methods were described by August Krogh [1908], viz. a method of microscopical gas-analysis, applicable to air-bubbles of 1.00–0.01 mm.³, and a method of micro-analysis for volumes between 1 and 20 mm.³.

The principle of the latter method is based upon the fact that small air-bubbles placed in a fluid behave much as semi-fluid fat drops and easily allow transport from one place to another without disunion. For analysis an air-bubble is sucked up into a capillary tube of uniform calibre about 0.25 mm. in diameter and measured. A length of 100 mm. corresponds to a volume of about 5 mm.³. Gas-absorptions are next performed one after the other in a funnel at the lower end of the capillary tube. Each absorption process being finished the air-bubble is sucked into the capillary tube again and measured anew. From the difference in length before and after absorption the absorbed quantity may be calculated. As the technique has been described by Krogh [1908, 1915] elsewhere in all details, I shall refer to these publications for further particulars.

The method is applicable to estimations of carbon dioxide and especially oxygen. Krogh has also applied the apparatus for the absorption-analysis of carbon monoxide (unpublished experiments).

In the autumn of 1916 I applied to Professor Krogh in order to obtain analyses of some gas-samples originating from gas cysts of the intestines of pigs, a pathological question I was engaged in studying. It soon turned out however that satisfactory results could not be obtained by the ordinary macro-gas-analysis, each estimation requiring samples from numerous cysts. Consequently Professor Krogh advised me to try micro-analysis of the contents of each single little cyst. As estimations of hydrogen and eventually methane were essential for the solution of the question under consideration

methods for micro-analysis of these gases had to be prepared. The results of these efforts are given below.

My best thanks are due to Professor August Krogh for his steady interest and help but for which I should not have been able to overcome the many difficulties met with during the work.

I also wish to express my sincere thanks to cand. pharm. K. L. Gad-Andresen, assistant at the laboratory, for numerous control-analyses by a Haldane-apparatus of the different gas-mixtures used for the micro-analyses.

ABSORPTION-ANALYSIS OF HYDROGEN.

It is well known that palladium, especially in colloidal form, is a good absorbing agent for hydrogen. Paal and Hartmann [1910] recommend a mixture of colloidal palladium and sodium picrate in watery solution for gas volumetric estimations of hydrogen. In this mixture palladium will act as a catalyst and rapidly transmit the hydrogen to the picric acid which is changed into 2.4.6-triaminophenol according to the formula:

1 g. picric acid consumes 834 cc. hydrogen for complete reduction.

There could be little doubt that this reagent would be well adapted to micro-analysis and the results obtained answered our expectations.

The preparation can under ordinary conditions be procured ready for use from Kalle & Co., Biebrich a. Rh. Paal and Hartmann state that Kalle's preparation even exceeds their own in absorbing power. I could not however at that time procure the reagent from Kalle and had consequently to prepare colloidal palladium myself according to the methods of Paal and Amberger [1904].

The preparation was ready for use in February 1917; it should theoretically contain about 45 % palladium, but as the palladium chloride used did not dissolve quantitatively the percentage was supposed to be about 35 % only.

The following solution of this preparation was now made according to the formula marked A in Paal and Hartmann's publication [1910, p. 249], the lower palladium percentage of the present preparation being considered:

0.66 g. colloidal palladium,
0.42 g. sodium picrate,
20.00 g. distilled water.

The solution was prepared on September 26th, 1917, and in October and November the same year was used for the analyses given below. The solution does not keep its activity for long periods; and half a year later, in May 1918, it absorbed only very slowly and anything but quantitatively. Colloidal palladium in substance also gradually loses its solubility.

Three series of analyses of different hydrogen mixtures were made by means of this palladium solution. The apparatus used had a capillary tube of uniform calibre about 0.25 mm. in diameter. The tube as well as the funnels

were filled with distilled water, slightly acidulated by means of sulphuric acid, a precaution observed once for all for the sake of carbon dioxide but of no importance for other gases. The samples to be analysed were prepared by mixing a certain quantity of hydrogen with an ample amount of atmospheric air in a 100 cc. gas-sampling-tube. Each mixture was analysed in a Haldane-apparatus to establish the exact composition. The gas was led from the sampling tube through a narrow leaden tube to the funnel, which was for safety's sake immersed in water until the air-bubble had been taken in. The bubbles analysed had a volume of 5 mm.³ on an average. The length of the bubble being measured the funnel was filled with alkaline pyrogallate solution. Oxygen has to be removed before hydrogen absorption owing to the fact that palladium catalyses oxygen and hydrogen with formation of H₂O. After the estimation of oxygen the funnel was filled with palladium mixture and hydrogen absorbed and determined.

It soon turned out that it was highly essential to maintain definite durations of the two absorption processes in order to obtain uniform results, partly to avoid loss of hydrogen by diffusion during oxygen absorption, partly to secure a complete absorption of hydrogen. A stop watch was advantageously used for this purpose and is on the whole much to be recommended to secure a uniform course of micro-analyses, where diffusion plays a part unknown in ordinary gas-analysis.

It was found in analyses of atmospheric air that 1 *minute* will suffice for a complete absorption of oxygen provided the bubble is moved up and down in the lower parts of the capillary tube twice or thrice during the process.

With regard to hydrogen absorption by the palladium mixture used it was established that 2½ *minutes* are enough to secure a complete absorption, provided the bubble is moved up and down in the lower parts of the tube *incessantly* during absorption.

Before giving the results I shall briefly mention a source of error which at the beginning caused many fallacious results. Accidentally the cause of these errors was detected when on one occasion I happened to keep the funnel under observation through the lens at the moment when pyrogallate solution was added. In the funnel was left as small a quantity of water as possible in order to exclude supersaturation when water and pyrogallate solution were mixed and the lower end of the air-bubble was consequently found very near the point where the bore widens into the funnel. At the moment when water and pyrogallate solution ran into one another I saw the end of the bubble break and the meniscus draw a little backwards into the capillary tube. This observation taken together with the result of the calculation—the oxygen percentage far too high, the hydrogen percentage far too low—cleared up the matter, a minimal part of the sample had been lost in the funnel. The phenomenon was now studied a little further and it was found that bubbles of atmospheric air never broke under conditions such that mixtures of hydrogen broke almost constantly. Furthermore it was established that air-bubbles will

break the easier the higher their percentage of hydrogen. The phenomenon is not seen when palladium mixture is added.

It is therefore essential always to keep the lower end of an air-bubble one to two millimetres within the mouth of the capillary tube when pyrogallate solution is added. As soon as this precaution was observed the phenomenon disappeared totally.

Series no. 1. 18. x. 17.

Percentage of

O₂: 11.2, 11.4, 11.0, 10.9, 11.3, 11.5, 11.2, 11.4, 11.2. Mean 11.21.

σ^* : ± 0.2 , $\sigma_m^\dagger$: ± 0.06 . Systematic error: -0.06 ± 0.06 . Macro-analysis 18. x.: 11.15 % O₂;

19. x.: 11.15 % O₂.

H₂: 46.9, 46.8, 46.9, 46.9, 47.2, 46.7, 46.8, 46.9, 46.6, 46.9. Mean 46.86.

σ : ± 0.16 , σ_m : ± 0.05 . Systematic error: $+0.81 \pm 0.05$. Macro-analysis 18. x.: 47.67 % H₂;

19. x.: 47.67 % H₂.

* σ = standard deviation. $\dagger \sigma_m$ = standard deviation of mean.

Series no. 2. 1. xi. 17.

Percentage of

O₂: 13.7, 13.6, 13.5, 13.8, 13.9, 13.6. Mean: 13.68.

σ : ± 0.15 , σ_m : ± 0.06 . Systematic error: —. Macro-analysis 1. xi. 17: —.

H₂: 35.7, 35.9, 35.9, 35.5, 35.6, 35.6. Mean: 35.70.

σ : ± 0.17 , σ_m : ± 0.07 . Systematic error: $+0.49 \pm 0.07$. Macro-analysis 1. xi. 17: 36.19 % H₂.

Series no. 3. 2. xi. 17.

Percentage of

O₂: 17.8, 17.8, 17.7, 17.8, 17.5, 17.5, 17.9, 17.8, 17.6, 18.0. Mean: 17.74.

σ : ± 0.13 , σ_m : ± 0.04 . Systematic error: $+0.13 \pm 0.04$. Macro-analysis 3. xi.: 17.87 % O₂.

H₂: 15.3, 15.3, 15.3, 15.0, 15.6, 15.4, 15.3, 15.3, 15.2, 14.9. Mean: 15.26.

σ : ± 0.2 , σ_m : ± 0.06 . Systematic error: $+0.13 \pm 0.06$. Macro-analysis 3. xi.: 15.39 % H₂.

A modified Micro-Analysis Apparatus with Combustion Funnel.

The only technical difference between this apparatus and the ordinary one as used for the hydrogen analyses is, beside the combustion funnel, the increase of the diameter of the capillary tube to about 1 mm. In the apparatus employed the capillary tube was found to be of uniform calibre, a length of 100 mm. corresponding to a volume of about 85 mm.³, about 17 times as much as the same length represents in a 0.25 mm. capillary tube.

The objects of this essential innovation were these:

1. To obtain air-bubbles so big that it might be possible to insert a platinum wire into them for combustion analysis. It had proved easy enough to construct platinum instruments small enough for air-bubbles of a volume of only 5 mm.³ but the insertion of them into such a small bubble was made impossible by the surface tension, the bubble escaping when meeting the wire.

2. To obtain better carbon dioxide analyses, the results by means of the ordinary apparatus being far too irregular, affected as they were by considerable accidental as well as systematic errors.

I soon became aware of several peculiarities which had to be considered when accurate results were to be obtained by this apparatus.

In the calculation of the results the temperature-correction is introduced simply by deducting or adding 0.1 % of a measured length for every 0.2° the temperature has changed.

A *combustion funnel* for this apparatus was constructed to render micro-analysis of combustible gases possible. For technical particulars I refer to the following chapter where the final shape of the entire arrangement for micro-analysis will be described.

Carbon Monoxide.

Two series of estimations were made, one by combustion and one by absorption. The equation for combustion of this gas is:

As the carbon dioxide formed cannot be measured with sufficient accuracy owing to diffusion, combustion has to be followed by absorption of CO_2 by potash and the CO calculated as two-thirds of the final contraction. It is consequently unnecessary to read off before the CO_2 has been absorbed. For absorption analyses acid cuprous chloride solution prepared according to the directions given by Winkler [1910] was used. As the process of absorption takes place rather slowly it is advisable to replace the reagent once during the absorption. Oxygen is also absorbed by this reagent and has therefore to be removed first by pyrogallate solution.

The gas-mixture employed was prepared by adding pure carbon monoxide to atmospheric air. The same mixture was used for both series.

Series no. 4 a. 3. v. 18.

Combustion analyses.

Capillary tube and both funnels filled with slightly acidulated distilled water. Volume of samples about 110 mm.³. Five seconds' combustion at a heat just enough to make the platinum wire visibly glowing. Absorption of CO_2 by potash. $\text{CO} = \text{two-thirds contraction} + \text{CO}_2$.

Percentage of

CO: 20.6, 20.7, 20.7, 20.5, 20.6, 20.5, 20.6, 20.7, 20.6. Mean: 20.62.

$\sigma: \pm 0.08$, $\sigma_m: \pm 0.03$.

Series no. 4 b. 3. v. 18.

Absorption analyses.

Apparatus filled with water as in series no. 4 a. Volume of samples about 71 mm.³. Oxygen absorption by pyrogallate $\frac{3}{4}$ minute, the sample being moved up and down thrice. Carbon monoxide absorption: first portion of cuprous chloride solution = 1 minute; second portion of cuprous chloride solution = $2\frac{1}{2}$ –3 minutes; the sample being kept incessantly in motion the whole time.

Percentage of

O_2 : 16.7, 16.8, 16.4, 16.6, 16.6, 16.7. Mean: 16.63.

$\sigma: \pm 0.14$, $\sigma_m: \pm 0.06$.

CO: 20.4, 20.4, 20.7, 20.5, 20.4. Mean: 20.48.

$\sigma: \pm 0.12$, $\sigma_m: \pm 0.05$.

Macro-analysis of the gas-mixture was not made, as the Haldane-apparatus was temporarily out of order.

As will be seen the CO values of the absorption estimations are slightly lower than those obtained by combustion. The latter express no doubt the exact percentage of CO, a systematic error of any importance being improbable.

Hydrogen.

One series of combustion analyses was made. The equation is:

Hydrogen is calculated as two-thirds of the contraction.

Series no. 5. 6. v. 18.

The gas-mixture was prepared by adding pure hydrogen to atmospheric air. Capillary tube and funnels filled with acidulated water. 10 seconds' combustion as in series no. 4 a. Two-thirds $\times$ contraction = H_2 .

Percentage of

H_2 : 18.4, 18.4, 18.5, 18.6, 18.5, 18.5. Mean: 18.48.

σ : ± 0.09 , σ_m : ± 0.04 . Systematic error: $+0.29 \pm 0.04$. Macro-analysis 6. v. 18: 18.77 % H_2 .

Compare further hydrogen estimations in the following chapter.

Carbon Dioxide.

The difficulty of the micro-estimation of CO_2 is due to the enormous rapidity with which this gas diffuses in most fluids, and measures which impede loss by diffusion are necessary to secure satisfactory analytical results. To this end Krogh [1908] recommends either to fill the funnel with conc. acidulated glycerol or to collect the sample in the funnel of the analysis apparatus over mercury.

I have myself spent much time seeking a satisfactory solution of this problem. As my experiences may perhaps be of some use to others working on this line and as they in my opinion clearly illustrate the essential factors to be considered in the micro-estimation of CO_2 I shall sketch the different methods tried before good results were gained.

Loss by diffusion in the absorption funnel was first considered and this part of the apparatus filled either by mercury or by conc. acidulated glycerol. Mercury gave considerably higher percentages than those obtained over water, and somewhat better results than conc. glycerol but there was still a loss in the rest of the apparatus. To prevent this the upper parts of the apparatus were filled with a saturated solution of sodium chloride, acidulated by hydrochloric acid, the absorption funnel remaining filled with mercury. This salt solution was soon replaced by a mixture of equal parts of glycerol and saturated sodium chloride solution both acidulated by hydrochloric acid. This mixture proved to be an important step forward and has ever since been used permanently in the apparatus for all gases. In the sequel it will be named "*g.s. mixture.*"

The mixture in question is thin enough for the movements in a capillary tube 1 mm. in diameter, but requires to a still higher degree than water movements of uniform speed. Augmentation of the glycerol content cannot be recommended, a mixture of 75 %, being too thick, caused accidental errors up to 1 %.

It was now justifiable to omit the filling of the funnel with mercury as being both troublesome and time-wasting. As the whole apparatus for several reasons cannot be filled throughout with mercury a systematic error bigger or smaller will always be present in CO₂ analyses.

This error being constant it will hardly pay to spend much time on diminishing it below a reasonable size which can be attained without particular arrangements.

Three series of CO₂ estimations of different mixtures were now made by this improved technique. In these and all the following series the apparatus was entirely—capillary tube as well as absorption funnel—filled with “g.s. mixt.”

<i>Series no.</i>	6	7	8
	(18. v. 18)	(21. v. 18)	(21. v. 18)
Percentage of	CO ₂	CO ₂	CO ₂
	23.8	51.3	60.8
	23.8	51.8	60.7
	23.8	51.4	60.4
	23.8	51.6	60.6
	23.7	51.5	60.5
	23.8	51.5	60.8
	23.9	51.4	60.4
	23.9	51.3	60.9
	23.8	51.4	60.6
	24.1	51.4	60.5
Mean	23.84	51.46	60.62
σ	± 0.1	± 0.15	± 0.18
σ_m	± 0.03	± 0.05	± 0.06
Systematic error	$+ 0.59 \pm 0.03$	$+ 0.95 \pm 0.05$	$+ 1.21 \pm 0.06$
Macro-analysis	24.43	52.41	61.83 % CO ₂
	(18. v. 18)	(23. v. 18)	(18. v. 18)

The accidental errors in these series are evidently of no importance as compared with the systematic ones. The latter are not proportional as might be expected to the concentrations of CO₂, the lower percentages having a relatively higher systematic error than the higher percentages.

Compare on this point the CO₂ analyses of the next chapter.

THE MODIFIED MICRO-ANALYSIS APPARATUS COMBINED WITH CLOCK-WORK AND COMBUSTION FUNNEL.

This construction represents the final shape of the apparatus I have come to during my work. The arrangement will be readily understood from Fig. 1 and annexed explanations.

Fig. 1. About $\frac{1}{4}$ nat. size.

Analysis apparatus. The apparatus is, as to the capillary tube, identical with the one described in the preceding chapter, the diameter being about 1 mm. The clock-work has necessitated a little change. The side-tube has been removed and the screw inserted in the enlarged top funnel. The screw is tightened by a very stiff mixture of pure india-rubber and vaseline melted together. The tube is filled with "g.s. mixture." This fluid undergoing no bacterial decomposition the capillary tube keeps clean for months.

When the apparatus has to be used, the screw is wound as far down as possible, the "g.s. mixture" removed from the absorption funnel by means of a pipette and replaced by a fresh solution, a small quantity of which is sucked up into the capillary tube. By following these directions one may always be sure that the gas is brought into contact with a fluid of constant composition.

Owing to the wide diameter the tube is easily cleaned without the aid of an aspirator.

Clock-work. The object of the clock-work is to secure absolutely uniform speed when the gas is sucked up into the capillary tube for measurement. All movements towards the absorption funnel have to be performed by revolving

DESCRIPTION OF FIG. 1.

A. Entire arrangement for Micro-analysis.

- a, capillary tube with water jacket and thermometer;
- b, clamp and bosshead with arrangement for stopping in vertical position;
- c, division taken as zero in measurements;
- d, screw of vulcanite with brass top;
- e, screw seen from the top with cruciform incision of the brass top;
- f, clock-work with wings for regulating the speed;
- g, metal tube fixed to the axle of the clock-work, containing a movable iron-pin to fit incisions of d;
- h, iron-pin alone;
- i, combustion funnel, carried by a clamp (compare B, a) adjustable to any angle. The conduction wires connect the platinum wire with accumulators and rheostat.

B. Stand for Micro-receivers.

- a, elastic clamp with double ball-and-socket joint for fixation of micro-receiver;
 - b and c, clips on india-rubber tubing of micro-receiver;
 - d, piece of glass tube for disconnection from mercury reservoir;
 - e, clamp holding square glass pot with excoavation for "g.s. mixture" for immersion of the absorption funnel of A, a.
- 1, position 1 of receiver, being used when the receiver has to be filled with mercury or when the last gas has to be driven out of the shorter limb for analysis;
 - 2, position 2: gas has just been sucked in, receiver clipped at both ends and levelling-tube raised again;
 - 3, position 3: gas from the longer limb is driven out through a narrow curved glass cannula into the absorption funnel, immersed in "g.s. mixture" (e). A drop of mercury regularly left in the drawn out part of the shorter limb is pushed in front of the gas so removing any foreign gas present.

the screw by hand. The most suitable speed for sucking in gas is about 100 mm. in 30 seconds. Twice the speed may very well be applied but the screw is, according to my experience, rather liable to become leaky after some time when revolved so quickly. The speed is regulated by means of a pair of wings adjustable to different angles. In order to secure the most uniform speed obtainable I have furthermore wound the clock-work before each measurement.

Coupling to the screw is effected in a few seconds and disconnecting as quickly. When the iron-pin is fixed in the top of its tube the clock-work never causes any trouble during the other manipulations of the analysis apparatus.

Combustion funnel (Fig. 1, A, i). This small instrument fixed by a clamp and a bosshead may be raised to the absorption funnel. The construction needs some explanation.

Two platinum wires, 4 cm. in length, 0.3 mm. in diameter, are flattened by a hammer at one end to an extent of about 1 mm. Incisions 1 mm. deep following the longitudinal axes of the wires are made in the middle of the flattened parts by means of a pair of scissors. The wires are insulated by platinum imbedding glass drawn out to thin threads and wound round the wires over the blowpipe. (Enamel might be tried for this purpose.) The insulation has to begin just beneath the flattened parts and must cover the wires to a length of about 3 cm. A cork, 8 mm. long, 15 mm. in diameter, is soaked in paraffin (melting point 60–70° C.) till all air is driven out of the pores. Two holes of the diameters of the insulated wires are bored near the centre of the cork 2 mm. apart from one another and the wires are inserted with the uninsulated ends first (no paraffin must touch the upper end). The flattened points have to be 15 mm. above the cork. A platinum wire 0.05 mm. in diameter is next fixed between the flattened points of the insulated wires acting as carriers of the current. Fixation is obtained by winding the wire many turns round the points. The length of the glowing-wire ought to be 5–6 mm., it may be shaped in the form of a single spiral winding for instance. Sharp curves must be avoided the wire being sure to fuse in such places. The advantage of making the wire amply long is that a broken wire may easily be welded together afterwards by simply crossing the ends till contact is obtained and increasing the current to a bright white heat. The whole arrangement allows of the replacement of a fused wire in a few minutes. The uninsulated ends of the wires on the other side of the cork are soldered to ordinary conducting wires connected to a four volt accumulator battery, a rheostat and a key.

The cork is now inserted into a piece of glass tube, 2.5 cm. long and 15 mm. in diameter. The funnel thus being formed is next filled with water (air-bubbles being avoided!) up to the glowing-wire and is now ready for use.

The rheostat may of course be of any construction at disposal, but it is essential to the task in question, that it can regulate from the slight heat of a not glowing-wire through just visible glowing to a bright white heat. A rheostat of 10 ohms fulfilled these requirements.

The heat of the glowing-wire must be regulated according to the different gases. For hydrogen the heat of a "dark" wire is sufficient, the extra heat produced at the combustion making the wire glow for a moment. For carbon monoxide a just visibly glowing-wire will do, while methane requires at least a bright red heat, the extra heat produced by the process causing the wire to glow momentarily at a bright white heat. I have often raised the temperature to a bright white heat during my methane analyses, but it is hardly essential and rather risky for the wire.

As the combustion processes under these conditions take place instantaneously it is neither necessary nor advisable to keep the wire aglow for more than five seconds. When the wire is inserted into an air-bubble and the temperature of the wire raised to a bright white heat during five seconds, the volume of the bubble will not suffer any change at all unless combustible gases are present. When the same procedure is extended to last ten seconds or more the bubble will increase slightly in volume owing to emigration of gas from the heated water in the funnel.

In estimating both hydrogen and methane the measurement after combustion of hydrogen may very well be done without removing the combustion funnel.

The course of a complete Analysis (CO_2 , O_2 , H_2 , CH_4 , N_2).

1. *Preparations.* The absorption funnel and capillary tube are filled with fresh "g.s. mixture" by means of a pipette and the screw as described before. The lower end of the funnel is inserted into the glycerol basin (Fig. 1, B, e). The clock-work is wound and connected to the screw (Fig. 1, A, d). The gas-sample is taken into the funnel and the clock-work started. When a sufficient amount of gas is sucked into the capillary tube, the clock-work is stopped, disconnected from the screw, the glycerol basin removed and the superfluous gas allowed to escape from the funnel by turning the apparatus. The lower meniscus of the sample being placed at zero by means of the restarted clock-work the first measurement is performed and noted together with the temperature of the water-jacket.

2. *Absorption of carbon dioxide.* The first measurement being finished the lower meniscus of the sample is taken down near the mouth of the funnel, "g.s. mixture" removed by a pipette and without any washing of the funnel replaced by 10 % potash. The sample is now taken down into the funnel, the upper meniscus being kept in the capillary tube but 2-3 mm. above the funnel. *It is an essential point that the fluid in the funnel must never come into contact with the "g.s. mixture" above the upper meniscus.* Absorption takes place instantaneously. As soon as the clock-work has been rewound and connected the sample is sucked back into the capillary tube together with a short column of potash below the lower meniscus stopped at zero. Second measurement. Temperature noted.

3. *Absorption of oxygen.* The second measurement being done the sample is taken down near to the funnel and the potash without any washing of the funnel

replaced by pyrogallate solution. Absorption-time about three-quarters of a minute (established beforehand for a given apparatus and pyrogallate solution by means of an oxygen estimation in atmospheric air!). The sample has to be moved up and down thrice to secure a complete absorption. The clock-work is wound anew and connected. The sucking in has to be stopped when the lower meniscus has reached a few millimetres within the capillary tube and any trace of pyrogallate is removed thoroughly from the funnel as well as the capillary tube by means of a pipette filled with distilled water. This being done the lower meniscus of the sample is taken down near the funnel and the water replaced by "g.s. mixt." The sample is next taken up to zero by means of the restarted clock-work. Third measurement. Temperature noted.

4. *Combustion of hydrogen.* The third measurement being done addition of a suitable excess of oxygen for the combustions has to take place. Either pure oxygen or atmospheric air can be applied; in the latter case it will often be necessary to remove part of the sample in order to make room for the necessary excess of oxygen (*vide* calculation below). Application of pure oxygen is advantageous as it generally allows the whole of the sample to be retained. The addition is performed in the following way. The lower meniscus of the sample is placed 1–2 mm. above the funnel, a bubble of oxygen taken into the funnel and sucked up—the screw being turned by hand—into the capillary tube below the sample but *separated* from it by a short column of "g.s. mixture." When a sufficient amount is sucked in, the apparatus is turned so that superfluous oxygen may escape from the funnel. The two air-columns are now quickly taken down into the funnel and united there. The rewound clock-work is started and the enlarged sample taken up. Fourth measurement. The difference between the fourth and third measurements represents consequently the oxygen added. The lower meniscus is next placed near the funnel and a thorough washing of the funnel—inside as well as outside—performed in order to remove any trace of glycerol. The lower 4–5 mm. of the capillary tube are equally washed by moving the screw up and down. The air-bubble may now be taken down into the absorption funnel the upper meniscus being kept 2–3 mm. within the capillary tube as emphasised before, the combustion funnel filled with water is cautiously raised, so that the glowing-wire is found in the centre of the bubble, and a current sent through the wire *without* making it glow visibly. If hydrogen be present the wire will glow up for a fraction of a second. The current is broken off by the key as soon as this has taken place and the sample is sucked up by clock-work without removing the combustion funnel. Fifth measurement. Temperature noted. Two-thirds of the contraction is hydrogen.

5. *Combustion of methane.* The fifth measurement being made the sample is taken down into the funnel again, the glowing-wire eventually centred anew in the bubble and the current *gradually* increased until the wire reaches a bright red heat. The heat should be increased cautiously as otherwise the wire may fuse owing to the extra heat produced at first by the combustion.

If methane be present the wire will for an instant glow up from a bright red to a bright white heat. The combustion is now finished, the combustion funnel removed and a small amount of potash cautiously added by means of a curved pipette to the water in the funnel so as to absorb the carbon dioxide produced by the combustion process. The clock-work is wound and started. Sixth and final measurement. Temperature noted. One-third of the contraction is methane.

6. *Nitrogen*. Calculated as residue.

Calculation. The calculation of the percentages, easily performed by a slide rule, does not require to be specially mentioned except in a case where the sample has been diminished during an analysis in order to make room for atmospheric air for combustion.

The new volume of the sample must consequently be corrected for the carbon dioxide and oxygen removed at the beginning of the analysis. The following example will explain the case.

Reading No.	Temp. ° C.	Length	Correct. length	Diff.	%
1.	13.6	110.6	110.3		
2. CO ₂ absorbed	13.8	102.4	102.0	8.3	7.5 CO ₂
3. O ₂ absorbed	13.8	101.7	101.3	0.7	0.65 O ₂
4. <i>Sample diminished to</i>	13.9	32.2	31.9		
5. Plus atmosph. air	13.9	147.3	147.0		
6. Combustion of H ₂	14.0	145.2	144.9	2.1	4.0 H ₂
7. Combustion of CH ₄ plus CO ₂ absorbed	14.1	123.7	123.3	21.6	20.7 CH ₄

$$\text{CO}_2 = \frac{100 \times 8.3}{110.3} = 7.5 \%$$

$$\text{O}_2 = \frac{100 \times 0.7}{110.3} = 0.63 \%$$

Correction of reading no. 4: $31.9 \times \frac{110.3}{101.3} = 34.7 \text{ mm.}$

$$\text{H}_2 = \frac{100 \times 2.1 \times 2}{34.7 \times 3} = 4.0 \%$$

$$\text{CH}_4 = \frac{100 \times 21.6 \times 1}{34.7 \times 3} = 20.7 \%$$

Carbon Dioxide.

The gas-mixtures for these series were prepared of pure CO₂ and atmospheric air mixed in a 100 cc. receiver. As the clamp (Fig. 1, B, e) for the glycerol basin was not ready when these analyses were made the clamp carrying the analysis apparatus had to be taken out of its bosshead down to a basin with glycerol on the table. The absorption funnel being quite filled with gas, the apparatus and the basin were raised together. The basin was not removed till the sample had been sucked up into the capillary tube. In order to avoid this troublesome and time-wasting removal of the analysis apparatus from the stand the clamp (Fig. 1, B, e) was constructed. The glycerol basin is thereby raised to the absorption funnel and fixed before the sample is taken into the funnel.

Series no.	9	10	11
	(28. v. 19)	(30. v. 19)	(18. vi. 19)
Percentage of	CO ₂	CO ₂	CO ₂
	—	21·8	—
	11·3	21·8	—
	11·0	22·0	—
	11·0	21·9	32·8
	11·4	22·0	32·9
	11·0	21·8	33·0
	11·0	21·8	33·0
	11·0	22·0	33·0
	11·2	21·9	33·0
	10·9	22·0	33·0
	11·3	21·9	32·8
	11·1	21·9	33·0
	11·2	21·9	33·0
Mean	11·12	21·90	32·95
σ	$\pm 0\cdot16$	$\pm 0\cdot08$	$\pm 0\cdot09$
σ_m	$\pm 0\cdot05$	$\pm 0\cdot02$	$\pm 0\cdot03$
Systematic error	$+ 0\cdot58 \pm 0\cdot05$	$+ 0\cdot71 \pm 0\cdot02$	?
Macro-analysis	11·70 %	22·61 %	(lost)
	(28. v. 19)	(30. v. 19)	

The *accidental errors* of these analyses have as it may be seen been reduced to such a minimum, that they may wholly be attributed to the unavoidable errors of reading. When the length of an air-bubble is about 100 mm. an error of $\pm 0\cdot1$ mm. corresponds to $\pm 0\cdot1$ %.

The *systematic errors* ought theoretically to be strictly proportional to the percentages of CO₂, found in these analyses. This proportionality is neither absolute in these series nor in the series no. 6, 7 and 8 of the preceding chapter, the tendency being that the lower percentages have a relatively higher systematic error than the higher ones. I have reason to believe that this condition will be still more pronounced at CO₂ percentages higher than the 22·6 % of series no. 10, but as such percentages are not commonly met with during biological work I have not tested the point. The training of the analyst is no doubt of great importance when dealing with a gas diffusing so rapidly and it is to be recommended that every operator should establish his individual systematic error as regards different CO₂ percentages. This is done by means of a series of micro-analyses and a macro-analysis of each CO₂-mixture. The use of the fixable glycerol-pot, mentioned above, might possibly have given more uniform errors in the present series.

The fact that the errors of these two series are considerably higher than that of the series no. 6 is no doubt wholly due to the slower movement of the clock-work, more time being left for diffusion in the funnel.

Hydrogen.

The gas-mixture used for the analyses given below was prepared by adding pure hydrogen to atmospheric air. It proved exceedingly explosive, many analyses being spoiled at the beginning. Good results were obtained, when the

current was interrupted instantaneously when the slightest trace of contraction was seen. The wire would nevertheless glow up and the combustion proceed quietly.

The oxygen percentages given are calculated by adding a third of the contraction to the values found by pyrogallate absorptions after the combustions.

A series of absorption analyses was also made, the absorbing agent being the palladium mixture mentioned at the beginning of this paper. The results proved contrary to my expectations very bad. The loss of hydrogen by diffusion during oxygen absorption was both considerable and inconstant. This method was therefore given up as inapplicable, the combustion method being far superior to it.

Series no. 12. 18. iii. 19.

Percentage of

H₂: 21.0, 21.0, 20.9, 20.8, 20.9, 20.7. Mean: 20.88.

σ : ± 0.12 , σ_m : ± 0.05 . Systematic error: $+0.12 \pm 0.05$. Macro-analysis (18. iii.): 21.0 % H₂.

O₂ (see text above): 16.6, 16.6, 16.8, 16.7, 16.8, 16.7. Mean: 16.70.

σ : ± 0.09 , σ_m : ± 0.04 . Systematic error: (-0.2 ± 0.04) . Macro-analysis (18. iii.): (16.5 % O₂ by calculation).

The accidental and systematic errors are equal.

In all ordinary circumstances, where the utmost accuracy is not wanted, one may take for granted that there is no systematic error upon analyses of hydrogen, when the percentage does not exceed about 20.

Methane.

As it proved difficult to prepare a methane pure enough for gas-analysis by chemical methods, the gas was procured from nature in the following way.

A big glass flask was nearly filled by a mixture of water and the fresh contents from the paunch of a cow. The flask was alternately saturated by CO₂ and evacuated. When the saturation was supposed to be as complete as attainable the flask was placed at 37° C. for 24 hours. A violent fermentation began after some hours, the gas produced being collected in a large receiver. The gas was freed from CO₂ by means of a Pettenkofer's absorption tube with potash. Macro-analysis of the gas so procured showed 92.9 % methane and 7.1 % nitrogen, the CO₂ formed in the combustion process being exactly equal to half of the contraction.

This concentrated methane mixture was used for the series to be given below. The plan of this series is somewhat different from that hitherto followed. It was namely thought desirable to imitate the natural conditions as closely as possible, where oxygen or atmospheric air generally has to be added to the sample. To this end small amounts of the methane mixture were measured in the analysis apparatus, whereupon atmospheric air or pure oxygen was added. Explosions were not seen in any case.

The oxygen was generated by means of a wash bottle containing oxylith on to which water was dropped. The addition of pure oxygen is most advantageous, as it allows of taking in much bigger samples than are possible when

atmospheric air is used. An oxygen-generator ought therefore always to be at hand during these combustion analyses.

The equation of combustion is:

As the contraction and the carbon dioxide formed cannot be measured separately owing to loss of CO_2 by diffusion into the water of the funnel, combustion is followed immediately by absorption of CO_2 . One-third $\times$ (contraction + carbon dioxide) = methane.

Series no. 13. 10. ii. 19.

Macro-analysis 10. ii. 19: 92.9 % methane,
7.1 „ nitrogen.

Micro-analyses:

A. A small sample is measured off and a larger amount of atmospheric air freed from CO_2 ¹ is added afterwards.

1.	92.9 % CH_4	T 13.8	11.3	corrected	11.0	equal to 10.2 mm. pure methane = 6.1 % of the mixture
	+ atmospheric air	T 13.8	167.0	„	166.7	
	Combustion + CO_2	T 13.9	136.3	„	136.0	$30.7 \times \frac{1}{3} = 10.2 \text{ mm.} = 6.1\%$
	Alkaline pyrogallate	T 14.0	125.4	„	125.0	11.0 mm. oxygen left after combustion
2.	Calculated content	12.3 mm. $\text{CH}_4 = 7.3 \%$				
	Analytical result	12.0 mm. $\text{CH}_4 = 7.2$				
3.	Calculated content	11.5 mm. $\text{CH}_4 = 6.8$				
	Analytical result	11.3 mm. $\text{CH}_4 = 6.7$				

B. A larger amount of pure oxygen is measured off and a smaller sample added afterwards.

4.	Pure oxygen	T 14.0	142.5	corrected	142.3	$12.7 = 11.8 \text{ mm. } \text{CH}_4 = 7.6 \%$
	+ 92.9 % CH_4	T 14.1	155.2	„	154.9	
	Combustion + CO_2	T 14.2	120.1	„	119.7	$35.2 \times \frac{1}{3} = 11.7 \text{ mm. } \text{CH}_4 = 7.6 \%$
5.	Calculated content	23.6 mm. $\text{CH}_4 = 14.8 \%$				
	Analytical result	23.2 mm. $\text{CH}_4 = 14.6$				
6.	Calculated content	16.2 mm. $\text{CH}_4 = 9.7$				
	Analytical result	16.0 mm. $\text{CH}_4 = 9.6$				

As it appears from the methane volumes re-calculated into per cent. the errors lie between 0 and + 0.1 %; in a single case they amount to + 0.2 %. Consequently it may be taken for granted that there is practically no systematic error in analyses of methane mixtures of 1–10 %. Mixtures containing 11–15 % may have a systematic error lying between + 0.1 and + 0.2 %.

FRACTIONAL COMBUSTION OF GAS-MIXTURES, CONTAINING BOTH HYDROGEN AND METHANE.

The fact that a heated platinum wire which is not visibly glowing causes combustion of hydrogen while on the contrary a wire of a bright red or white heat is required for combustion of methane led to the question whether it

¹ Carbon dioxide-free atmospheric air is always at hand in the potash flask.

might be possible to estimate both gases by fractional combustion. The results obtained answered our expectations.

The technique is quite the same as in ordinary combustion analyses, the only factor to be considered being, that the combustion of hydrogen takes place at the lowest possible heat by means of a "dark" wire. At the very moment the combustion occurs causing the wire to glow momentarily the current has to be broken off by a key. If the current is not interrupted instantaneously at this moment a trace of methane will also be burned.

Whether higher percentages of hydrogen can be estimated together with methane without causing a smaller or larger systematic error upon the methane percentage is a question open to future experiments. Dilution of the sample by means of an ample amount of atmospheric air will no doubt be the simplest procedure in such cases.

The methane for these analyses was obtained by means of evacuated receivers with connecting tubes and thin steel needles from the intact paunches of freshly killed cows at a slaughter-house. The gas thus collected was freed from carbon dioxide by passage through a Pettenkofer's absorbing tube filled with potash. A micro-analysis of the gas thus obtained showed a content of 52.7 % methane.

The gas-mixture employed for the series below was prepared by adding roughly estimated parts of this methane mixture, pure hydrogen, pure oxygen and pure nitrogen.

Series no. 14. 11. vi. 19.

Percentage of

H_2 : 5.3, 5.3, 5.5, 5.3, 5.6, 5.4. Mean: 5.40.

σ : 0.13, σ_m : ± 0.05 . Systematic error: $+ 0.05 \pm 0.05$. Macro-analysis 11. vi. 19: 5.45 % H_2 .

CH_4 : 9.3, 9.3, 9.3, 9.4, 9.2, 9.3. Mean: 9.30.

σ : ± 0.06 , σ_m : ± 0.03 . Systematic error: $+ 0.23 \pm 0.03$; Macro-analysis 11. vi. 19: 9.53% CH_4 , 4.12 % O_2 left after combustion.

It will be seen that the methane is left totally intact by the hydrogen combustion when the directions given are followed.

A MICRO-RECEIVER FOR SMALL GAS SAMPLES.

The quantities of gases met with in nature during biological work are often so scanty that they are lost when taken into the ordinary gas sampling tubes. Krogh has for these reasons mounted the analysis apparatus in a transport-box, serving at the same time as a stand, so that the apparatus is handy for use in nature, on board a ship or elsewhere. The small air-bubbles are then led directly into the absorption funnel of the apparatus and analysed at once. This may be practical in many cases, when only estimations of carbon dioxide and especially oxygen are wanted, but it will hardly be possible to obtain any accuracy when the combustible gases have also to be determined by the technique described in this paper. This condition led to the construction of a suitable micro-receiver for collecting and storing small gas samples during transport until analysis can take place in the laboratory.

The essential point of this simple little apparatus, making it differ from the ordinary receivers in use, is the circumstance that it has no stopcocks, the gas being forced to bubble in through a column of mercury. The sample is thus locked up between two columns of mercury. The construction and use will readily be understood from Fig. 1, *B*. The receivers are easily made by means of a blow-pipe. The dimensions are: diameter of glass tubing 6 mm.; length of longer limb 70 mm., 25 of which are drawn out for connection with

Fig. 2. Transport-box for 24 micro-receivers. The cover contributes to fix the receivers during transport
[About $\frac{1}{4}$ actual size.]

a narrow rubber tubing; length of shorter limb 50 mm., being equally drawn out. The angle between the two limbs measures 70–80°. Narrow india-rubber tubing, which is not apt to stick together, is used for connection. The rubber tubings are closed by means of small stiff clips, made of spring steel. A micro-receiver of these dimensions may hold about 1.7 cm.³ gas at ordinary pressure locked up between two mercury columns 3 cm. in height. As all contaminating air is driven out of the connecting tubing and cannula by means of mercury, even smaller air-bubbles may be taken over into the absorption funnel without loss.

A transport-box for 24 micro-receivers will be seen in Fig. 2.

As it could not be excluded beforehand that a minute diffusion might take place along the sides of the tube past the mercury, which is known not to adhere to glass like most fluids, the following control experiments were carried out.

The mercury columns serving for locking up the samples measured on an average 3 cm. in height in all three experiments. A certain pressure was established in all the receivers before they were clipped.

Experiment no. 1. Tightness test for carbon dioxide.

Micro-analysis of gas-mixture before filling of micro-receiver (series no. 10):

			21.90 % CO ₂	
„	after 15 days	(1)	21.2	„
		(2)	21.3	„

The loss has been about 0.65 % CO₂ in the course of 15 days.

A calculation of the loss per 10 % in 24 hours makes it about 0.02 %.

Experiment no. 2. Tightness test for carbon dioxide.

Micro-analysis of gas-mixture before filling of micro-receiver (series no. 11):

			32.9 % CO ₂	
„	after 4 × 24 hours	(1)	32.4	„
		(2)	32.2	„
		(3)	32.3	„

The loss has been about 0.65 % carbon dioxide in the course of 96 hours.

A calculation of the loss per 10 % in 24 hours makes it about 0.05 %.

Experiment no. 3. Tightness test for hydrogen.

Micro-analysis of gas-mixture before filling of micro-receivers *A* and *B*:

(1) 11.9, (2) 12.0, (3) 12.0 % H₂.

Micro-analysis of gas-mixture in receivers *A* and *B* after 24 hours:

A (1) 12.0, (2) 12.0 %,
B (1) 12.1, (2) 12.0 %.

No loss of hydrogen has taken place in the course of 24 hours.

Discussion of results. The issue of experiment no. 3 ensures the absolute tightness of the receivers even for hydrogen within 24 hours. The losses of carbon dioxide in 24 hours would as seen by the calculations of the results of the two first experiments be quite insignificant, the high percentages and the exceedingly long time being taken into consideration. It may also be possible that no diffusion has taken place at all, the losses being due to unnoticed impurities of the mercury.

The conclusion drawn from these preliminary experiments is, that the micro-receiver described is absolutely reliable within 24 hours. Gas samples ought consequently when the utmost accuracy attainable is wanted to be analysed before this time has elapsed.

SUMMARY.

1. The micro-analysis apparatus described by Krogh is also applicable to hydrogen and carbon monoxide estimations, a solution of colloidal palladium plus sodium picrate and an acid cuprous chloride solution serving respectively as absorbing agents.

2. A modification of this apparatus with combustion funnel is described. It is possible by means of this apparatus to estimate CO_2 and O_2 by absorption, H_2 and CH_4 by combustion in a given air-sample of about 80 mm.³—the size of a small pea. CO may be estimated either by absorption or combustion according to circumstances.

N_2 (+ inert gases) is calculated as residue.

3. Fractional combustion of hydrogen and methane appearing together is practicable by means of this technique.

4. The accidental error (standard deviation) in the present micro-analyses corresponds on an average to an error of reading of ± 0.1 mm.

5. The systematic error differs for the different gases. While being without any practical importance in estimations of O_2 , H_2 , CH_4 and probably also CO, it has to be considered in carbon dioxide analyses.

6. A micro-receiver for small gas samples is described.

REFERENCES.

- Krogh (1908). *Skand. Arch. Physiol.* **20**, 279.
— (1915). *Abderhalden's Biochem. Arbeitsmeth.* **8**, 495.
Paal and Hartmann (1910). *Ber.* **43**, 243 (compare *Abderhalden's Biochem. Arbeitsmeth.* 1915, **8**, 406).
Paal and Amberger (1904). *Ber.* **37**, 124.
Winkler (1910). Quoted by Müller in *Abderhalden's Biochem. Arbeitsmeth.* **3**, 646.