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The Use of Dixon and Gilson Respirometers in Soil and Litter Respiration Studies

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Summary

Methods used for measuring soil and litter respiration in the laboratory are outlined with special reference to manometric methods. The particular advantages of Dixon and Gilson respirometers, which are similar in theory, are discussed and the theory of the Dixon respirometer is given in detail.

In correcting the readings of these two respirometers to Normal Temperature and Pressure (N.T.P.), the bath temperature is often used, but from the theory of the instrument it is clear that the ambient temperature should be used, otherwise an error is introduced.

Introduction

Many ecologists and soil biologists are interested in measuring the metabolic activity of soil organisms, either individually or as a general soil population. Numbers of organisms are not reliable guides to activity (Stotzky 1965, p. 1550). However, it is a general biochemical property of living matter that energy-requiring reactions such as protein synthesis are linked via biochemical pathways to reactions in which energy is released, and while many micro-organisms can obtain chemical energy from anaerobic processes, aerobic organisms obtain their energy from the oxidation of food materials by molecular oxygen, carbon dioxide being evolved. One or both components of this gas exchange (respiration) can be measured to give an index of the metabolic activity of the aerobes.

General Methods

For the study of respiration of soil and litter animals, zoologists have evolved various methods, many of which involve the replacement of consumed oxygen by that generated by electrolysis of a solution, oxygen consumed being registered in a variety of ways and carbon dioxide evolved being absorbed in alkali (e.g. Helvey, 1951; Macfadyen, 1961; Phillipson, 1962). Similar types of equipment have been used in general soil studies (e.g. Greenwood and Lees, 1959; McGarity et al, 1958; Swaby and Passey, 1953; Wager and Porter, 1961; Wieringa and Mogot, 1957).

However, electrical methods often give practical difficulties and many workers use the simpler manometric methods, particularly the classical Warburg method (e.g. Bernier, 1960; Chase and Gray, 1957; Drobnik, 1960a, 1960b, 1960c; Gilmour et al, 1958; Katznelson and Rouatt, 1957; Katznelson and Stevenson, 1956; Parkinson

and Coups, 1963; Rovira, 1953; Stevenson, 1956; Webley, 1947). In this respirometer the experimental flask is attached to one arm of a manometer the other arm of which is open to the air. Small changes in bath temperature or atmospheric pressure during the experiment have a considerable effect on the readings. It is necessary to have at least one extra respirometer with only water in the flask, the readings of this respirometer (the thermobarometer) being subtracted from those of the other respirometers.

Other workers have used compensated respirometers such as that of Haldane (Lees, 1949, 1950) or of Barcroft (Stout, 1963; Johnston, 1953; Drobnik, 1958). These respirometers have the experimental flask on one side of the manometer and a compensation flask of similar dimensions on the other side. The system, being totally enclosed, is independent of changes in atmospheric pressure, and changes in bath temperature affect both flasks and cancel out.

Special flasks have been used where the conventional ones were unsuitable (Drobnik, 1958, 1960a; Parkinson and Coups, 1963; Johnston 1953; Stotzky, 1960).

However, Warburg and Barcroft respirometers have a number of practical disadvantages which make them time-consuming and tedious to use. For example, each flask and manometer must be matched, calibrated, and constants must be calculated for the volumes of substances in the flask (KOH, water, sample) in each series of measurements. The oxygen uptake is calculated from the change in height of the manometer fluid using these constants. If flasks are broken, re-matching and re-calibration are required. A direct-reading instrument which does not require calibration is therefore more convenient to use and consumes less time. This is particularly true if one wishes to examine a number of samples of different sizes and moisture contents at different temperatures. Usually in calculating the flask constants one does not know precisely the volume of soil, water, or other materials in the sample, particularly if the soil sample is fresh and intact. Webley (1947) gave a method for calculating the flask constant for oxygen when using dry soil to which a known volume of water is added. He assumed that there is no interaction of oxygen with the soil during the one hour experimental period which he used. He pointed out that the method cannot be used for carbon dioxide. Webley's method has been used by Chase and Gray (1957).

The Dixon Respirometer

During the past four years we have used Dixon respirometers (Dixon 1952, p. 6) for measuring oxygen uptake of decomposing plant material (Howard, 1967), of soil, and also of millipedes, (Bocock and Horsman in prep.).

This direct reading respirometer is very suitable for such studies and has a number of advantages over Warburg and Barcroft respirometers.

Dixon (1952) did not describe in detail the functioning of his respirometer, and the description in Umbreit et al, (1957, p. 111) contains an error which is discussed below. Although the construction of the Dixon respirometer is similar to that of the Barcroft apparatus, its theory and use are different.

In the Dixon respriometer, the pressure is kept constant and the change in gas volume is measured directly. The theory is as follows: before gas exchange occurs (Fig. 1) v is the volume in µl of gas in the reaction flask, P_o is normal atmospheric pressure (760 mm of mercury), P is the pressure in the respriometer, p is the vapour pressure of water in the reaction flask, p' is the vapour pressure of water in the burette, XO_2 represents quantity of oxygen taken up as µl of dry gas at N.T.P., α is the solubility coefficient of oxygen in the liquid in the reaction flask, v' is the initial volume of gas in the burette, v_F is the volume of liquid in the reaction flask. The reaction and compensating flasks are at bath temperature T, but the burette and manometer are at the ambient temperature T'. The tubes connecting the flasks to the manometer are of equal and constant volume and can be ignored in the following equation. As the compensation flask is there merely to isolate the apparatus from the outside atmosphere and to eliminate the effects of small changes in bath temperature, it does not enter into the calculations.

For mathematical simplicity the apparatus is assumed to be filled initially with pure oxygen, and this gas is assumed to be evolved in the reaction flask. The evolved gas displaces the manometer fluid (Fig. 2). Gas evolution takes place continuously but readings are taken at fixed times. When a reading is taken, the level of mercury in the burette is adjusted to bring the manometer fluid back to the initial level. The new volume in the burette (v'') and thus the volume change in the burette (v'' - v') are read directly. As the burette and manometer are equilibrated at T', it is this temperature at which the change in gas volume is measured, not the bath temperature T as stated by Umbreit et al (1957, p. 111). This is an important point, as the error involved may be large if the bath and ambient temperatures are greatly different, this is illustrated in Table 1.

The amount of oxygen present initially is:

$$\left(v \frac{273}{T} + v_F \alpha \right) \frac{P - p}{P_o} + v' \frac{273}{T'} \frac{P - p'}{P_o} \quad \ldots\ldots\ldots\ldots (1)$$

and that present finally is:

Table 1

Oxygen uptake of decomposing hazel leaves measured at 0°C, 5°C, 10°C

			incorrect		correct			
Barometric pressure mm Hg	bath temperature °C	$\mu l O_2/gOD/h$ uncorrected	N.T.P. factor bath temp.	$\mu l O_2/gOD/h$ at N.T.P.	ambient temperature °C	N.T.P. factor ambient temp.	$\mu l O_2/gOD/h$ at N.T.P.	error if incorrect factor used %
758	0	41.75	0.991	41.37	20.3	0.905	37.78	9.5
756	5	63.85	0.969	61.87	20.9	0.900	57.47	7.7
760	10	119.30	0.953	113.69	20.1	0.910	108.56	4.7

$$\left(v \frac{273}{T} + v_F \alpha \right) \frac{P - p}{P_o} + v'' \frac{273}{T'} \frac{P - p'}{P_o} \quad \ldots\ldots\ldots\ldots (2)$$

The difference between these is obviously XO_2 and by subtracting (1) from (2) we get:

$$XO_2 = v'' - v' \frac{273}{T'} \frac{P - p}{P_o} \quad \ldots\ldots\ldots\ldots (3)$$

Thus, the volume of oxygen evolved is measured directly and the only calculation needed is conversion of the reading to N.T.P. It is customary to adjust gas volumes to Normal Temperature and Pressure (N.T.P.). In doing so, the gas in the respirometer is assumed to be saturated with water vapour. To adjust the volume to that of dry gas at 760 mm of mercury and 0°C, the observed volume is multiplied by a figure (the N.T.P. factor) which is obtained from standard tables or nomograms.

On setting up the Dixon respirometer, the compensating flask must have the same volume as the experimental flask so that the effects of small fluctuations in bath temperature cancel out. However, as most respirometer water baths maintain the temperature to within ± 0.1 degree C, a certain amount of latitude is possible. The compensating flask is set up in the same way as the experimental slask, but with inactive material in place of the sample. For this purpose we use filter paper when dealing with leaves, small glass beads with loose soil, and tubes of glass wool with soil cores. Experience soon shows how accurately the volumes must be adjusted for adequate compensation, but calculation shows that if the flasks differ in volume by 1 cc, a temperature rise of 0.2 deg. C will result in a movement in manometer fluid position indicating a change in volume of 0.8 µl, the direction of the change depending on which flask is the larger. The magnitude of the change depends upon the difference in volume between the flasks and is independent of the size of the flasks.

The Gilson Respirometer

The Gilson differential respirometer (Umbriet et al, 1964, pp. 104-105), which is one of several recent modifications of the Dixon respirometer, has become available in Britain only during the past four years and its use is increasing. In this respirometer, instead of each manometer having a compensating flask, all the manometers are linked via a manifold to a single compensating flask. The apparatus has three valves: (a) the manifold valve, which connects the manifold to the external atmosphere. It can also be used for introducting special gas mixtures, (b) the disconnect valve, which can be used to disconnect from the manifold any manometer not required, (c) the operational valve,

which connects the two arms of each manometer above the fluid level. This valve is in the open position when setting up and equilibrating and is closed to commence readings. All of the operational valves can be operated simultaneously by a master lever.

This respirometer is fully compensated only if the total volume of compensation flask plus manifold is equal to the total volume of the experimental flasks plus connectors. The apparatus is provided with a 250 cc compensating flask designed for use with standard Warburg type respiration flasks. These are too small for many soil and litter studies and we use flasks of 70 to 100 cc volume.* With fourteen flasks having a total volume of 1400 cc and a compensating flask of only 500 cc (the largest possible size) the effect of the imbalance is merely a small rhythmic rising and falling of the manometer fluid as the bath thermostat switches on and off. In practice we find it quite easy to ignore this movement when reading the instrument. With smaller experimental flasks this effect disappears. Because the temperature control of the Gilson water bath is $\pm$ 0.03 degrees C, a small volume imbalance produces a negligible effect.

The Gilson respirometer has certain advantages over the Dixon respirometer: (a) the former is much more compact, (b) the flasks are connected to the manometer by plastic tubing and ground glass C14 connectors, the latter being mounted on stainless steel clips which fit into brackets on the bath. This makes setting up quick and easy for one operator and minimises the risks of breakage, (c) because the Gilson respirometer is available with refrigeration (working temperature -5°C to 50°C) and recording facilities, it is very useful for soil biology studies. Whereas in the Dixon respirometer the change in volume is read on a graduated tube of mercury, in the Gilson respirometer the mercury column is replaced by a piston operated by a micrometer screw with a digital readout in µl. The micrometers are calibrated to 0.2 µl and with practice a good operator can read the instrument to the nearest 1 µl. Greater accuracy is difficult to attain because of the difficulty in locating accurately the manometer fluid meniscus. No doubt this could be overcome by means of a lens. Some diffusion of gases through the plastic tubing occurs, but it only becomes a problem if the gases in the apparatus differ greatly in composition from the external atmosphere. In such cases the apparatus has to be modified.

The theory of this instrument is the same as that of the Dixon respirometer, and contrary to the statement made in the earlier Gilson handbooks, the ambient temperature not the bath temperature should be used in calculating the N.T.P. factor.

* See page 7

General Discussion

Biochemists and microbiologists have been measuring the respiration of bacterial suspensions and tissues for many years, but the application of their techniques to soil presents certain difficulties. Thus, with bacterial suspensions or tissues slices the chemical composition of the medium can be accurately controlled and one variable at a time can be altered. In such cases respiration may be measured as oxygen uptake, carbon dioxide evolution or both. and the respiratory quotient (RQ = vol CO_2 evolved/vol O_2 taken up) calculated. However, with soils, especially those in the field condition, it is not so simple. Not all of the carbon dioxide released from soils comes from respiration. Whilst oxidative decarboxylation is an important source of respiratory carbon dioxide, "straight" decarboxylations also occur, of two main types: (a) reactions catalyzed by carboxylase and co-carboxylase, (b) reactions catalyzed by the amino-acid decarboxylases. Reactions of the latter type are common in bacteria, but they are rare and probably small-scale processes among animals (Baldwin, 1952, p. 418). Furthermore, there may be inorganic reactions involving exchange of carbon dioxide in soil, and these may vary with horizon and soil type (e.g. see Russell, 1961, p. 106 et seq). Methods for determining RQ values of soils have been described (e.g. Stotzky, 1960; Rixon and Bridge 1968), but these must be regarded as suspect. With soil, measurement of oxygen uptake is obviously preferable.

The way in which the results of laboratory measurements of soil respiration are expressed depends largely on the purpose of the measurements. Results can be calculated on the basis of soil volume, dry weight or carbon content.

With soil, all laboratory respiration measurements are to some extent artificial because the soil has been removed from its normal surroundings and its aeration and other factors are altered. These effects become greater if the material is kept in the respirometer for long periods. Some of these problems are discussed by Stotzky (1965), Drobnikova and Drobnik (1965), and Domsch (1962) discusses methods and results.

With decomposing leaf litter, our experience has shown that careful handling of the sample has no appreciable effect on its respiration.

Flasks for use with Dixon and Gilson respirometers

Conventional flasks are rarely suitable for use with soil, plant material, or small animals. We use flasks like that of Parkinson and Coups (1963), but having a B40 base, or those shown in Fig. 3, which were designed in our laboratories.

In flask A the KOH is introduced into the annular well via the C14 connector at the top, an automatic pipette with a narrow plastic tube is useful for this. The bottom ground joint is a B40 cup, and the sample is placed in a base made from a B40 cone. These can be made any desired depth.

If the rate of respiration is low, the large volume of flask A can be a disadvantage. We therefore designed flask B, also using the B40 base. With our shallowest base, flask A has a volume of 135 cc and flask B 80 cc. Flask B has no alkali well, but to overcome this the sample is placed in a specimen tube and the alkali is placed in the base. Advantages in using specimen tubes are that handling of the sample is easier and fewer bases are needed.

Flask C has been used by Bocock and Horsman (in prep.) in studies on millipede respiration. Here again, the sample is placed in a specimen tube which fits into the centre well. An annular space is provided for alkali. This flask has a B29 neck and connects to the respirometer via a B29-C14 adaptor. The total volume of flask plus adaptor is 65 cc, but if necessary this can be reduced by filling the adaptor with an inert material which will not impede gas exchange (e.g. glass or plastic beads).

For this type of work it is essential for all ground glass joints to be of the highest standard if leaks are to be avoided. Serious faults in interchangeable ground glass joints are: (a) the cup or cone is not perfectly round (the joint "binds" in places when rotated); (b) the taper of one or both components is incorrect (the joint will rock about its long axis).

The large number of flasks types A and B, and bases, made for us by Gallenkamp (Widnes) Ltd., have proved entirely satisfactory. I shall be glad to supply further information to anyone interested.

With soil, litter and small animals, one does not need to shake the flasks, as is necessary when liquid media are used, so that the size of the flask is not a disadvantage.

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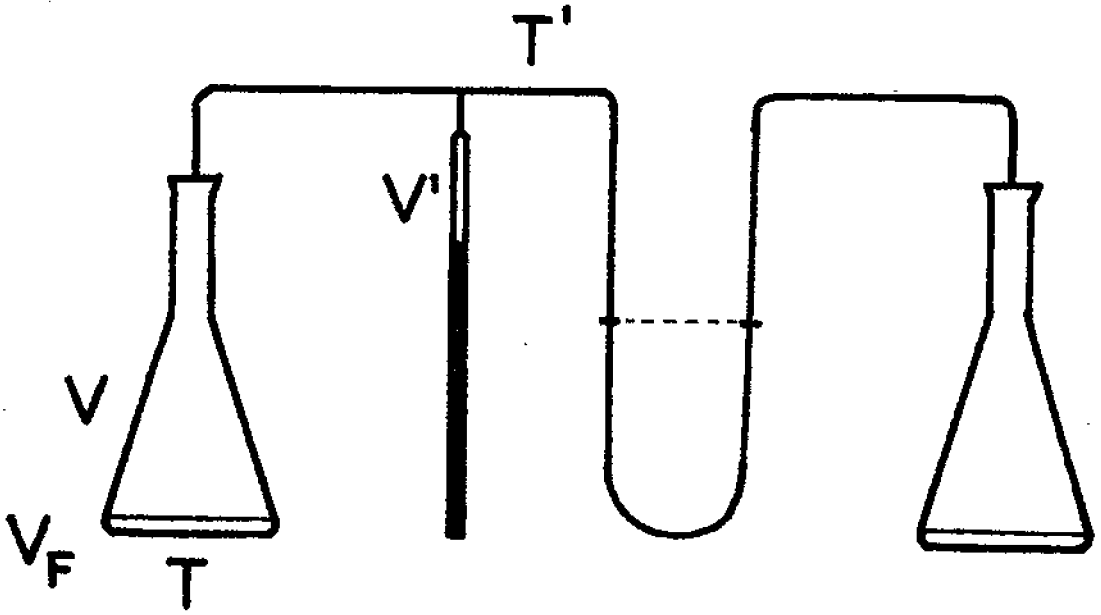


Figure 1. Conditions before gas evolution

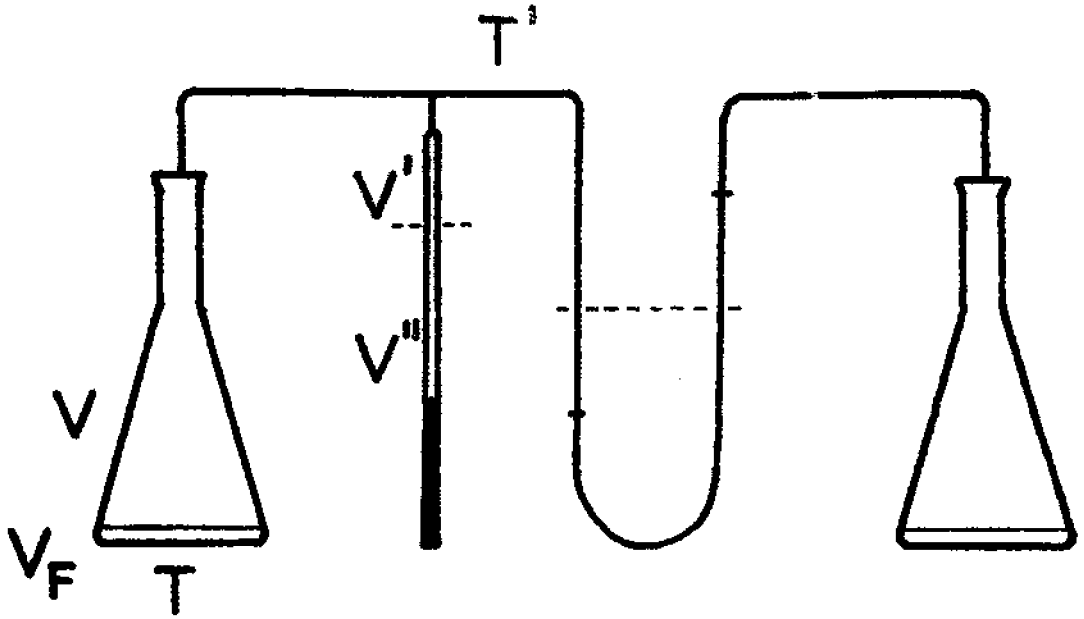
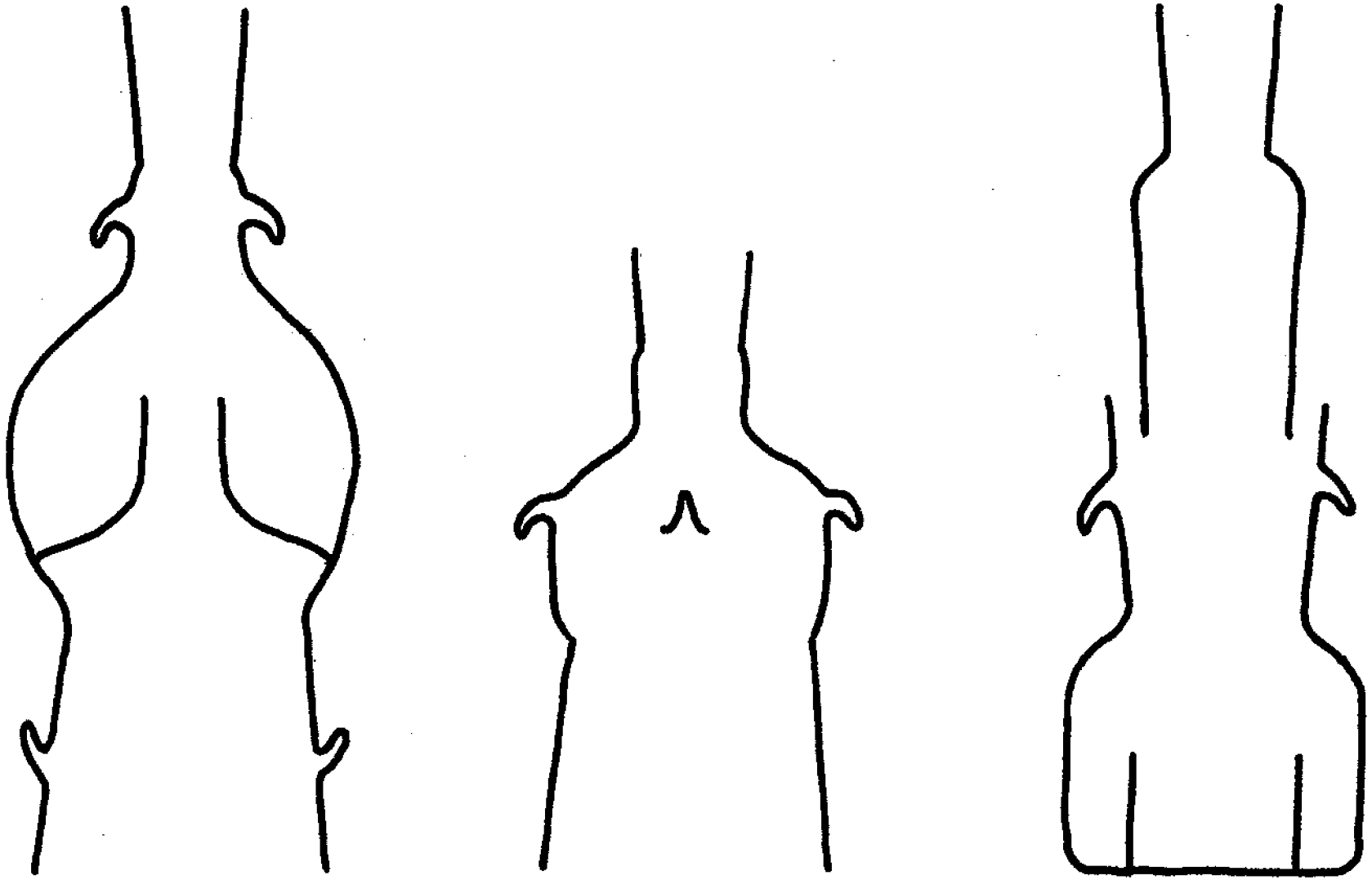


Figure 2. Conditions after gas evolution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