growth in our cultures of *C. diphtheriae*. The degree of activity was in no instance as great as that observed either with Y 47 or with the more active of its constituent factors. There is, of course, the possibility that other material having marked activity is present in these synthetic peptides at concentrations too low for chromatographic identification. The peptides P1 and P2, however, do not make up the whole of the activity for *C. diphtheriae*. S1, the slow-moving stable material, is destroyed by the action of ophio-L- α -amino-acid oxidase and hence is presumably an amino-acid. On the other hand, since according to Zeller & Maritz (1944, 1945) the presence of OH and COOH groups in amino-acids prevents attack by the oxidase, the position of S1 on chromatograms cannot be taken as indicating that it is a polycarboxylic or polyhydroxy amino-acid. Since this position is not altered after treatment with phosphatase, although inorganic phosphorus is produced, one must assume that the phosphate is liberated from some component giving no ninhydrin colour. The major part of the active material of Y 47 appears to be of a peptide or amino-acid nature, but there is some evidence for the existence of other active components as shown by the partial inactivation on osazone formation.

SUMMARY

1. The preparation is described of a concentrate from brewer's yeast which contains essential growth factors for certain strains of *Corynebacterium diphtheriae* gravis.

2. The activity of the whole preparation is associated with four of the twelve ninhydrin-positive components.

3. Most of the activity is associated with an amino-acid, S1, and a peptide, P1, while a second amino-acid, S2, and another peptide, P2, have slight activity.

4. The peptide P2 consists of serine, glycine and glutamic acid.

5. A number of synthetic glutamic acid peptides also have activity as growth factors for *C. diphtheriae*.

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A New Manometric Method for Determination of Respiratory Quotients

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Manometric determinations of the respiratory quotient (R.Q.) require measurements of oxygen uptake, carbon dioxide production and, in the case of glycolysing cells, changes in the bicarbonate concentration of the medium. Several methods, due to Dickens & Simer (1930, 1931), Dixon & Keilin (1933), and Sumerson (1939), are available. Two identical,

or practically identical, samples of the biological material are needed when these methods are used. The initial bicarbonate concentration is obtained by acidifying and killing one sample at the beginning of the experiment, while the change in bicarbonate concentration is determined by acidifying the other sample at the end of the experiment. Intermediate

values for the rate of oxygen uptake, carbon dioxide production and for the change in bicarbonate concentration during the experiment cannot be obtained. The R.Q. can therefore only be measured during one experimental period. Consequently, changes in R.Q. with time or with variations in the environmental conditions during the experiment cannot be observed. Such changes may be determined by using an adaptation of Warburg's indirect method (Laser & Rothschild, 1939), but this method is cumbersome when multiple determinations are involved.

A new apparatus with which changes in R.Q. can be measured on a single sample is described in this paper.

PRINCIPLE

The principle involves the sampling of known amounts of gas and liquid from a reaction vessel. One pair of samples is simultaneously removed from this vessel and connected with subsidiary manometers, in which their contents of gaseous CO_2 and bicarbonate are independently determined. After the initial determination at zero time (t_0), this procedure is repeated three times, the reaction in the main vessel continuing undisturbed. In this way changes in total CO_2 evolved and bicarbonate concentration of the medium are determined during three experimental periods. The corresponding changes occurring in the main vessel during the time between the taking of samples can be calculated from these data and from the readings of the manometer connected to the main vessel. As each of these readings registers the algebraic sum of the O_2 uptake and total CO_2 production, the oxygen consumption during each period can be calculated and the R.Q. determined. In the case of cell suspensions, during each experimental period the quantity of biological material in the main vessel is diminished by the amount taken out with each previous sample of the medium. This is taken into consideration in the equations which correlate the observed pressure changes with the amount of metabolizing material involved at each step. If tissue slices or whole organisms are used, the quantity of biological material remains unaltered throughout the experiment.

DESCRIPTION OF APPARATUS

Two versions of the apparatus (A and B) have been designed, differing in detail but employing the same principle.

Apparatus A. The reaction vessel is rectangular (Fig. 1, A). It is connected to a differential manometer which has a compensating vessel (Fig. 1, B) of about the same volume as the main vessel. The joint connecting the main vessel with its manometer is raised above the centre of the vessel, enabling the insertion of a turnable gas outlet (Fig. 1, C), and of

a Keilin tube (Fig. 1, D), which is hung on a short length of platinum wire fused into the inner end of the gas outlet tube. The Keilin tube can be dislodged into the main vessel at any time during the experiment by rotating the gas outlet tube. The main vessel is provided at both ends with sampling devices, on which the principle of the method is based. Both ends are identical, and only one will be described in detail. It consists of a large tap, the sampling tap (Fig. 2, C), which is joined to the main vessel in such a way that its key (Fig. 2, D) forms the vertical side of the vessel. The key contains four invaginations, the sampling cups, which are in communication with the main vessel. In the apparatus shown in Fig. 2 the cups are in two rows vertically above each other, and at such a height that the lower ones (Fig. 2, c'_1 and c'_2) are below the level of the liquid. They are therefore completely filled with the fluid medium, while the upper ones (Fig. 2, c_1 and c_2) contain the gas in the main vessel. The key is hollow; its lower end and one side of the handle are cut open and therefore filled with water from the bath, to obtain satisfactory temperature equilibration and to eliminate buoyancy.

The sampling tap (Fig. 2, C) has an upper and a lower outlet tube (Fig. 2, E and F) at the levels of the sampling cups. These outlet tubes lead to joints (Fig. 3, D) through which each is connected with a subsidiary differential manometer having its own compensating vessel (Fig. 1, G). The complete apparatus therefore consists of five differential manometers, i.e. the main manometer and two subsidiary ones on each side. The U-tube of the main manometer is in the centre, while the two subsidiary manometers on each side are so arranged that one lies inside the other. Each sampling key is provided with indicator knobs (Fig. 2, G) corresponding to the positions of the sampling cups. By rotating the key outwards from the position in which all its cups communicate with the main vessel, one pair of sampling cups can be brought into communication with the corresponding outlet tubes (Fig. 4). Together with the outlet tube, each sampling cup then becomes the experimental vessel of a differential manometer, in one of which the CO_2 content of the gas sample is measured, and in the other, the bicarbonate content of the fluid.

Determination of the gaseous carbon dioxide. The upper outlet tube contains near its lower end a roll of filter paper (Fig. 2, H) soaked with KOH which absorbs all the CO_2 in the gas sample. The change of reading in the appropriate manometer determines the amount of CO_2 absorbed. A second roll of filter paper is inserted in the same outlet tube a little higher up (Fig. 2, I). This is wetted with water to adjust the water vapour tension in the subsidiary manometer to that of the main vessel. This prevents the development of pressure changes, not due to

Fig. 1. Rear view of assembled apparatus (Type A). *A*, main reaction vessel; *B*, main compensating vessel; *C*, turnable gas outlet; *D*, Keilin tube; *E*, upper outlet tube (for CO_2 determination of gaseous sample); *F*, lower outlet tube (for bicarbonate- CO_2 determination of liquid sample); *G*, compensating vessels of subsidiary manometers; *H*, left subsidiary manometer (for determination of gaseous CO_2); *I*, left subsidiary manometer (for determination of bicarbonate CO_2); *J*, manometer board; *K*, rubber-lined clamp (partly cut away) for holding main vessel; *L*, stand; *M*, manometer tap. Length of centre manometer, base to tap, 275 mm.

CO_2 , when the gas sample from the main vessel (which is saturated with water vapour) is connected with the manometer containing KOH.

Determination of bicarbonate content of the liquid sample. The lower outlet tube contains known amounts of mercury (Fig. 2, *J*) and an organic acid (Fig. 2, *K*). When the liquid-sampling cup is connected with this outlet tube, the mercury flows into the

being superimposed on the existing readings in each manometer. From these readings the gaseous CO_2 and bicarbonate contents of the second pair of samples can be calculated. The changes in free CO_2 and bicarbonate concentration during the interval between taking the first and second samples can be calculated if the volumes of the sampling cups are known and, in the case of the gaseous samples, the

Fig. 2. Section of main vessel and sampling tap (Type A). *A*, Main reaction vessel; *B*, liquid (shown in black); *C*, barrel of sampling tap; *D*, key of sampling tap; *E*, upper outlet tube; *F*, lower outlet tube; *G*, indicator knobs; *H*, filter paper for KOH; *I*, filter paper for water; *J*, mercury; *K*, organic acid; *L*, platinum coil; c_0 , c'_0 , c_2 , c'_2 sampling cups.

sampling cup and displaces some or all of its contents which are mixed with the acid. The amount of CO_2 expelled from the bicarbonate in the sample fluid can be calculated from the change in the reading of the appropriate manometer.

After an interval for CO_2 absorption and CO_2 expulsion the sampling key is turned a second time. The first pair of sampling cups are thereby removed from their manometers and the next pair connected with them. As neither the KOH in the upper outlet tube nor the acid in the lower one are exhausted, CO_2 absorption and expulsion are repeated, new pressures

pressure in the main manometer at the time of sampling. As each sampling key contains two pairs of cups, four consecutive samples can be taken. In practice the sampling keys are turned alternately, the left one at times t_0 and t_2 , and the right one at t_1 and t_3 .

Apparatus B. This differs in one detail from apparatus A: the sampling cups are situated behind each other instead of next to each other. This is made possible by the introduction of two concentric keys in each sampling tap, the outer one forming a sleeve round the inner key. Fig. 3 shows the left

sampling tap. The inner key contains two invaginations, sampling cups c_0 , c'_0 and the outer keys two apertures constituting the sampling cups c_2 , c'_2 . At zero time the keys are in such a position that all sampling cups communicate with the main vessel. The lower invaginations (in the inner key) and the lower apertures (in the outer key) are then completely filled with liquid while the corresponding upper

APPLICATION

Technical application and sensitivity of the apparatus. The apparatus is not a microrespirometer and is therefore unsuitable for measuring small metabolic changes of the order of a few $\mu\text{l./hr.}$ Such changes are too small to give significant differences in the samples. Changes of about 200–300 $\mu\text{l./hr.}$ can be

Fig. 3. Section of main vessel and sampling tap (Type B). *A*, main reaction vessel; *B*, outer key of sampling tap; *C*, inner key of sampling tap; *D*, joints of outlet tubes for insertion of subsidiary manometers; *E*, upper outlet tube; *F*, lower outlet tube; *G*, widened portion of lower outlet tube, to provide large surface for CO_2 expulsion from bicarbonate; *H*, joint in lower outlet tube for insertion of *I* or *J*; *I*, stopper (when worked with liquids of low bicarbonate content); *J*, extension (when worked with liquids of high bicarbonate content); c_0 and c'_0 , invaginations in inner key; c_2 and c'_2 , apertures in outer key; w and w' , apertures in outer key for connecting invaginations in inner key with the outlet tubes. Length of key, 95 mm.

invaginations and apertures contain the gas in the main vessel. The outer key also has two apertures opposite the lower and upper outlet tubes (Fig. 3, w and w') through which the inner sampling cups communicate with the outlet tubes when the inner key is turned. By turning first the inner and later the outer key (Fig. 5), the inner and the outer sampling cups are successively connected with their corresponding outlet tubes. Here too the sampling is done alternately left and right.

measured at 15–30 min. intervals. These changes are large enough to be detected with accuracy in the samples, because the constants of the subsidiary manometers are small (about 0.5 for the CO_2 -absorption manometer and 0.75 for the bicarbonate- CO_2 manometer with paraffin as the manometer fluid, and 0.65 and 1.0 with Brodie solution). The sensitivity can be increased by reducing the volume of the main vessel. The size of the sampling cups partly determines the sensitivity of the apparatus,

as the volume of the cup is directly proportional to the magnitude of the change which can be measured. With this in view, the gas-sampling cups were made

to register small changes, are also large enough to hold the maximal pressures which can occur with 5% CO_2 in the gas space. On the other hand, the

Fig. 4. Schematic cross section of main vessel (Type A) at the level of the liquid (black), illustrating the sampling procedure during the course of an experiment. *CL*, barrel of left sampling tap; *DL*, key of left sampling tap; *CR*, barrel of right sampling tap; *DR*, key of right sampling tap; c'_0 and c'_1 liquid sampling cups (left); c'_2 and c'_3 liquid sampling cups (right). The arrows inside the keys indicate the direction and sequence in which the keys are turned; (a) position during temperature equilibration; (b) position from t_0 – t_1 ; (c) position from t_1 – t_2 ; (d) position from t_2 – t_3 ; (e) position from t_3 –constant pressure in h_3 . The same procedure applies automatically to the gaseous samples.

to hold about 1.2–1.5 ml. and the liquid-sampling cups 0.9–1.0 ml. Comparison of the constants shows that the subsidiary manometers for the determination of the gaseous CO_2 , while being sensitive enough

Fig. 5. Schematic cross section of left side of main vessel (Type B) illustrating the sampling procedure during the course of an experiment. *A*, barrel of sampling tap; *B*, outer key; *C*, inner key; *F*, lower outlet tube; *L*, liquid in main reaction vessel; c'_0 , liquid sampling cup in inner key; c'_2 , liquid sampling cup in outer key; w' aperture in outer key. The arrows inside the keys indicate the direction and sequence in which the keys are turned: (a) position during temperature equilibration; (b) position from t_0 – t_1 ; (c) position from t_1 – t_2 ; (d) position from t_2 – t_3 . Positions (c) and (e) (see Fig. 4) illustrating sampling on the right side are omitted.

bicarbonate- CO_2 manometers are not able to register more than about 200–300 μl . CO_2 according to the length of the manometer capillary. This is ample when liquids of low bicarbonate concentration, such as sea water, are used. For work with Ringer-

bicarbonate (about 550 μ l. CO₂/ml.) and a liquid-sampling cup of 1 ml., the manometer would be too small. This difficulty can be overcome by decreasing the amount of liquid sampled (smaller cups in the key), or by increasing the manometer constant. There are limitations to decreasing the amount of sampled Ringer-bicarbonate solution, because it is not the absolute bicarbonate content but its change which has to be determined. The constant can be increased by using a manometer fluid with high density and/or by increasing the volume of the subsidiary manometer. For this purpose an extension can be fixed on the lower outlet tube (Fig. 3, J). The manometer fluid can also be levelled after the first bicarbonate-CO₂ determination. In that case the manometer only needs to register the pressure of one sample at a time. The minimum interval between sampling depends on the magnitude of metabolism and on the time taken for the expulsion of the bicarbonate CO₂ from the acidified liquid sample. Even with high bicarbonate concentrations, this was found to be complete in about 15 min. As the samples are taken from alternate sides, the effective interval can be reduced throughout the experiment to about 7.5 min./period. The interval between sampling at t_0 and t_1 can be still further reduced if the interval between t_1 and t_2 is correspondingly prolonged.

Biological application. The apparatus is applicable to the whole range of biological material which has so far been used in macromanometric work. In addition, it enables the study of many types of material for which manometric methods have been less suitable for the following reasons: (1) the absence of CO₂ in experiments with KOH interferes with their normal metabolism; (2) experiments in the presence of CO₂ using one of the existing methods are impossible, as it is not always easy to kill one sample at the beginning of the experiment; alternatively, two identical samples of the material cannot be obtained. This group includes plants, insects and their larvae, worms, small whole organs or muscle which could be stimulated *in situ*. The apparatus can also be adapted for the simultaneous determination of O₂ uptake, CO₂ production, and N₂ fixation by absorbing CO₂ in one and CO₂ and O₂ in the other outlet tube.

Metabolism without glycolysis. Many tissues and cells show no aerobic acid production. In such cases the four lower sampling cups are unnecessary. The apparatus can therefore be used without the subsidiary manometers for the determination of bicarbonate CO₂; or a simplified apparatus can be made having only two subsidiary manometers for the determination of the gaseous CO₂.

THEORY OF THE METHOD

Nomenclature. All symbols referring to the main manometer are expressed in capital letters and those

referring to the subsidiary manometers in lower-case letters. They have the suffixes 0, 1, 2 and 3. Some of the constants have the additional suffixes O₂ or CO₂. In the case of the main manometer the suffixes refer to the time at which the readings are taken. The amounts of gas (V_g) and of fluid (V_f) in the main vessel and the constants change at each time of sampling. If V represents the total volume of the main vessel including the eight sampling cups, and if V_{g_0} and V_{f_0} , V_{g_1} and V_{f_1} , etc. represent the gas and fluid volumes at t_0 , t_1 , etc., then H_2 is the reading on the main manometer at t_2 and $K_{2:O_2}$ the O₂ constant applicable for the second time interval (t_1 - t_2) based on V_{g_2} and V_{f_2} .

The gas-sampling cups are called c_0 and c_2 on the left side and on the right side c_1 and c_3 , the appropriate manometer readings being h_0 and h_2 (left) and h_1 and h_3 (right). The corresponding liquid-sampling cups and readings are indexed with a superscript '. Thus the liquid-sampling cups are called c'_0 and c'_2 (left), c'_1 and c'_3 (right) and the readings h'_0 and h'_2 (left) and h'_1 and h'_3 (right). The volumes and constants of the subsidiary manometers also change at each time of sampling according to the volume of the sampling cup with which they become connected. As all the gaseous CO₂ which is sampled and measured in the subsidiary manometers is absorbed by KOH, the constants applicable in this case are those converting the pressure produced by a free gas and do not include the solubility factor α_{CO_2} . They are called k_0 , k_1 , k_2 and k_3 . The constants used for the determination of bicarbonate CO₂ contain the factor α_{CO_2} and are called $k'_{0:CO_2}$, $k'_{1:CO_2}$, $k'_{2:CO_2}$ and $k'_{3:CO_2}$. The suffixes in the subsidiary manometers refer to the time of sampling. Thus k'_2 and $k'_{2:CO_2}$ refer to the reading and the CO₂ constant of the subsidiary manometer with respect to the liquid removed at t_2 , but read at a convenient time during the experiment when the CO₂ expulsion from bicarbonate is complete.

In determining the gaseous CO₂ only the free CO₂ in the main vessel at the time of sampling is measured. This does not include the CO₂ which has gone into solution in the fluid in the main vessel. The free CO₂ has therefore to be converted into total CO₂ by multiplication at each step with the appropriate factor $\frac{K_{1:CO_2}}{K_1}$ or $\frac{K_{2:CO_2}}{K_2}$ or $\frac{K_{3:CO_2}}{K_3}$, where $K_{1:CO_2}$, $K_{2:CO_2}$ and $K_{3:CO_2}$ are the CO₂ constants and K_1 , K_2 and K_3 are the corresponding constants from which the factor $V_f \alpha_{CO_2}$ has been omitted. Table 1 then applies to the readings, volumes and constants of the apparatus.

Determination of gaseous carbon dioxide. Suppose that the gas in the main vessel contains no CO₂ and that at t_0 the main manometer shows no pressure difference. Connecting the sampling cup c_0 with its subsidiary manometer will then produce no pressure

Table 1. Relations between constants of the apparatus

A. Main manometer						Constants		
Time (t)	Reading	Change in reading	Gas volume	Liquid volume		K	K _{O₂}	K _{CO₂}
0	H ₀	—	V _{G0}	V _{F0}		K ₀	—	—
1	H ₁	H ₁ - H ₀ = ΔH ₁	V _{G0} - c ₀ = V _{G1}	V _{F0} - c ₀ ' = V _{F1}		K ₁	K _{1:O₂}	K _{1:CO₂}
2	H ₂	H ₂ - H ₁ = ΔH ₂	V _{G0} - (c ₀ + c ₁) = V _{G2}	V _{F0} - (c ₀ ' + c ₁ ') = V _{F2}		K ₂	K _{2:O₂}	K _{2:CO₂}
3	H ₃	H ₃ - H ₂ = ΔH ₃	V _{G0} - (c ₀ + c ₁ + c ₂) = V _{G3}	V _{F0} - (c ₀ ' + c ₁ ' + c ₂ ') = V _{F3}		K ₃	K _{3:O₂}	K _{3:CO₂}
B. Subsidiary manometer								
Sampled time (t)	Subsidiary manometer	Outlet tube	Sampling cup	Manometer reading	Constant			
0	1	t _{ul} *	c ₀	h ₀	k ₀			
0	2	t _{ll}	c ₀	h ₀	k _{0:CO₂}			
1	3	t _{ur} †	c ₁	h ₁	k ₁			
1	4	t _{lr}	c ₁	h ₁	k _{1:CO₂}			
2	1	t _{ul}	c ₂	h ₂	k ₂			
2	2	t _{ll}	c ₂	h ₂	k _{2:CO₂}			
3	3	t _{ur}	c ₃	h ₃	k ₃			
3	4	t _{lr}	c ₃	h ₃	k _{3:CO₂}			

t = outlet tube (Figs. 2 and 3, E and F); ul = upper left; ur = upper right; ll = lower left; lr = lower right.

* In apparatus B this becomes t_{ul} + w₁.

† In apparatus B this becomes t_{ur} + w_r.

change in that manometer. On the other hand, if at t₀ a pressure exists in the main manometer, the introduction of the gas volume contained in c₀ into the subsidiary manometer will produce a pressure difference depending on the pressure at which the gas is introduced and on the constant of the subsidiary manometer. The change of reading in the subsidiary manometer will be

$$\Delta_0 = H_0 \frac{K_0 c_0}{V_{G0} k_0}.$$

Similarly, on sampling gas at t₁, t₂ and t₃, the following changes of pressure in the subsidiary manometers will be produced, depending on the pressure in the main vessel at these times:

$$\Delta_j = H_j \frac{K_j c_j}{V_{Gj} k_j} \quad (j = 1, 2 \text{ or } 3).$$

Theoretically the Δ equations need two corrections to account for the solubility of gases other than CO₂ in the liquid contained in the subsidiary manometer, and the changes in this factor with variations in the relative amounts of these gases due to the O₂ uptake during the experiment. Both correction factors are small as the amount of liquid in the subsidiary manometer is small (0.4 ml.) and the gases concerned, mainly O₂, N₂, CO and H₂, have a low solubility. k₀ differs from k_{0:O₂}, k_{0:N₂}, etc. only by 0.001. Apart from the magnitude of this factor, it can safely be omitted as the CO₂ changes are calculated as differences and are unaffected by the omission of the same correction factor in all four determinations. However, because of the variations in the ratios of these gases during the experiment, this factor alters very slightly during the experiment. The Δ-values calculated from the above equations differ by less than 0.1 mm. from the theoretical figure for an O₂ uptake of 500 μl. in the main vessel. Both factors can therefore be omitted without affecting the accuracy of the result.

If the sampled gas contains CO₂, the Δ-values have to be subtracted from the corresponding readings to evaluate the amount of CO₂ present. If x_{0:CO₂} is the amount of CO₂ present in c₀,

$$x_{0:CO_2} = (h_0 - \Delta_0) k_0,$$

or

$$x_{0:CO_2} = h_0 k_0 - H_0 \frac{K_0 c_0}{V_{G0}}.$$

In this equation the term k₀, contained in Δ₀, cancels out. Similarly, the terms k₁, k₂ and k₃ contained in Δ₁, Δ₂ and Δ₃, cancel out in the subsequent equations for x_{1:CO₂}, x_{2:CO₂} and x_{3:CO₂}, which will contain the factors

$$H_j = \frac{K_j c_j}{V_{Gj}} \quad (j = 1, 2 \text{ or } 3).$$

Thus

$$x_{1:CO_2} = h_1 k_1 - H_1 \frac{K_1 c_1}{V_{G1}}.$$

The absorption of CO₂ by KOH produces a negative reading in the subsidiary manometer. To conform with the usual sign conventions the equations for x_{0:CO₂} and x_{1:CO₂} are converted into

$$x_{0:CO_2} = H_0 \frac{K_0 c_0}{V_{G0}} - h_0 k_0, \quad (1)$$

$$x_{1:CO_2} = H_1 \frac{K_1 c_1}{V_{G1}} - h_1 k_1. \quad (2)$$

x_{1:CO₂} represents the total amount of CO₂ in c₁ which includes the amount of CO₂ present in c₁ at t₀. Therefore the change of CO₂ in c₁ between t₀ and t₁ is

$$\Delta x_{1:CO_2} = x_{1:CO_2} - x_{0:CO_2} \frac{c_1}{c_0}. \quad (3)$$

On connecting c_2 with its subsidiary manometer a new pressure is superimposed on the existing reading h_0 . This does not remain as an unaltered fraction of the new reading h_2 , because part of the system in which the pressure h_0 has previously been produced, i.e. c_0 , has been removed. The fraction of h_0 included in h_2 is*

$$\frac{h_0 k_0}{k_2} \frac{t_{ui}}{t_{ui} + c_0}.$$

Similarly, on determining $x_{2:\text{CO}_2}$, h_3 will contain a fraction of the previous reading h_1 , this fraction being†

$$\frac{h_1 k_1}{k_3} \frac{t_{ur}}{t_{ur} + c_1}.$$

These factors have to be subtracted from h_2 and h_3 for the evaluation of $x_{2:\text{CO}_2}$ and $x_{3:\text{CO}_2}$:

$$x_{2:\text{CO}_2} = H_2 \frac{K_2 c_2}{V_{g_2}} - \left[h_2 - \frac{h_0 k_0}{k_2} \frac{t_{ui}}{t_{ui} + c_0} \right] k_2$$

$$\text{or } x_{2:\text{CO}_2} = H_2 \frac{K_2 c_2}{V_{g_2}} + h_0 \frac{k_0 t_{ui}}{t_{ui} + c_0} - h_2 k_2, \quad (4)$$

$$\Delta x_{2:\text{CO}_2} = x_{2:\text{CO}_2} - x_{1:\text{CO}_2} \frac{c_2}{c_1}, \quad (5)$$

$$x_{3:\text{CO}_2} = H_3 \frac{K_3 c_3}{V_{g_3}} + h_1 \frac{k_1 t_{ur}}{t_{ur} + c_1} - h_3 k_3, \quad (6)$$

$$\Delta x_{3:\text{CO}_2} = x_{3:\text{CO}_2} - x_{2:\text{CO}_2} \frac{c_3}{c_2}. \quad (7)$$

The Δ -values for CO_2 so far obtained, (3), (5), (7), are those occurring in c_1 from t_0 to t_1 , in c_2 from t_1 to t_2 , and in c_3 from t_2 to t_3 . These have to be converted into the corresponding changes/ml. of suspension, if cell suspensions are used (the amount of which changes with each time of sampling) or changes/mg. of weight or dry weight, if material such as tissue slices or whole organisms are used (the amount of which remains constant throughout the experiment). The latter equations are suffixed *a*. Thus:

ΔX_{CO_2} /ml. cell suspension

$$\Delta X_{j:\text{CO}_2} = \Delta x_{j:\text{CO}_2} \frac{V_{g_j}}{V_{f_j}} \frac{K_{j:\text{CO}_2}}{c_j} \quad (j = 1, 2 \text{ or } 3), \quad (8), (9), (10)$$

ΔX_{CO_2} /unit weight

$$\Delta X_{j:\text{CO}_2} = \frac{\Delta x_{j:\text{CO}_2}}{\text{wt.}} \frac{V_{g_j}}{c_j} \frac{K_{j:\text{CO}_2}}{K_j} \quad (j = 1, 2 \text{ or } 3). \quad (8a), (9a), (10a)$$

* In apparatus B this expression becomes

$$\frac{h_0 k_0}{k_2} \frac{t_{ui}}{t_{ui} + w_1 + c_0}.$$

† In apparatus B this expression becomes

$$\frac{h_1 k_1}{k_3} \frac{t_{ur}}{t_{ur} + w_r + c_1}.$$

Determination of bicarbonate carbon dioxide. Equations 11–14 express the bicarbonate concentrations as $\mu\text{l. CO}_2/\text{ml. fluid}$. They are calculated for the case in which the amount of mercury introduced into the subsidiary manometers on each side (Fig. 2, *J*) is greater than the volume of fluid with which the mercury changes place on sampling at t_0 and at t_1 . In this case the total volume of fluid in c'_0 and c'_1 is forced up into the lower outlet tubes of the sampling taps and remains there during the determination for the samples from c'_2 and c'_3 . This facilitates the calculation of $x_{2:B}$ and $x_{3:B}$:

$$x_{0:B} = \frac{h'_0 k'_0 c_0}{c'_0}, \quad (11)$$

$$x_{1:B} = \frac{h'_1 k'_1 c_1}{c'_1}, \quad (12)$$

$$x_{2:B} = \frac{h'_2 k'_2 c_2 - h'_0 k'_0 c_0}{c'_2}, \quad (13)$$

$$x_{3:B} = \frac{h'_3 k'_3 c_3 - h'_1 k'_1 c_1}{c'_3}. \quad (14)$$

The change in bicarbonate concentration corresponds to acid production or glycolysis (X_g):

ΔX_g /ml. cell suspension

$$\Delta X_{1:g} = x_{0:B} - x_{1:B}, \quad (15)$$

$$\Delta X_{2:g} = x_{1:B} - x_{2:B}, \quad (16)$$

$$\Delta X_{3:g} = x_{2:B} - x_{3:B}; \quad (17)$$

ΔX_g /unit weight

$$\Delta X_{1:g} = \frac{V_{f_1}}{\text{wt.}} (x_{0:B} - x_{1:B}), \quad (15a)$$

$$\Delta X_{2:g} = \frac{V_{f_2}}{\text{wt.}} (x_{1:B} - x_{2:B}), \quad (16a)$$

$$\Delta X_{3:g} = \frac{V_{f_3}}{\text{wt.}} (x_{2:B} - x_{3:B}). \quad (17a)$$

Respiratory carbon dioxide. The respiratory CO_2 (resp. CO_2) is obtained by deducting the glycolytic CO_2 from the total CO_2 for each period; thus $\Delta X_{j:\text{CO}_2}^{\text{resp.}}$ /ml. cell suspension or per unit weight:

$$\Delta X_{j:\text{CO}_2}^{\text{resp.}} = \Delta X_{j:\text{CO}_2} - X_{j:g} \quad (j = 1, 2 \text{ or } 3). \quad (18), (19), (20), (18a), (19a), (20a).$$

Determination of oxygen uptake. The O_2 uptake is calculated from the readings of the main manometer. The following equations apply:

ΔX_{O_2} /ml. cell suspension

$$\frac{\Delta H_1}{V_{f_1}} = \frac{\Delta X_{1:\text{O}_2}}{K_{1:\text{O}_2}} + \frac{\Delta X_{1:\text{CO}_2}}{K_{1:\text{CO}_2}},$$

whence

$$\Delta X_{j:\text{O}_2} = \left[\frac{\Delta H_j}{V_{f_j}} - \frac{\Delta X_{j:\text{CO}_2}}{K_{j:\text{CO}_2}} \right] K_{j:\text{O}_2} \quad (j = 1, 2 \text{ or } 3). \quad (21), (22), (23)$$

ΔX_{O_2} /unit weight

$$\frac{\Delta H_1}{\text{wt.}} = \frac{\Delta X_{1:O_2}}{K_{1:O_2}} + \frac{\Delta X_{1:CO_2}}{K_{1:CO_2}},$$

whence

$$\Delta X_{j:O_2} = \left[\frac{\Delta H_j}{\text{wt.}} - \frac{\Delta X_{j:CO_2}}{K_{j:CO_2}} \right] K_{j:O_2} \quad (j = 1, 2 \text{ or } 3).$$

(21a), (22a), (23a)

The respiratory quotient is given by the ratios in Table 2.

Table 2. Calculation of respiratory quotients

(The numbers refer to equation numbers.)

t	R.Q. with	
	Cell suspensions	Tissue slices, or whole organism
0-1	18/21	18a/21a
1-2	19/22	19a/22a
2-3	20/23	20a/23a

Table 3. Symbols to be introduced into equations 1-23

Symbol	Equation	Intro-duced for	Symbol	Equation	Intro-duced for
A	1	$\frac{K_0 c_0}{V_{\theta_0}}$	O	11	$\frac{k'_{0:CO_2}}{c'_0}$
B	2	$\frac{K_1 c_1}{V_{\theta_1}}$	P	12	$\frac{k'_{1:CO_2}}{c'_1}$
C	4	$\frac{K_2 c_2}{V_{\theta_2}}$	Q	13	$\frac{k'_{2:CO_2}}{c'_2}$
D	6	$\frac{K_3 c_3}{V_{\theta_3}}$	R	13	$\frac{k'_{0:CO_2}}{c'_3}$
E _t	4	$\frac{k_0 t_{ui}}{t_{ui} + c_0}$	S	14	$\frac{k'_{3:CO_2}}{c'_3}$
E _r	6	$\frac{k_1 t_{ur}}{t_{ur} + c_1}$	T	14	$\frac{k'_{1:CO_2}}{c'_3}$
F	3	$\frac{c_1}{c_0}$	U	21	$\frac{K_{1:O_2}}{V_{F_1}}$
G	5	$\frac{c_2}{c_1}$	V	21	$\frac{K_{1:O_2}}{K_{1:CO_2}}$
H	7	$\frac{c_3}{c_2}$	W	22	$\frac{K_{2:O_2}}{V_{F_2}}$
I	8	$\frac{V_{\theta_1} K_{1:CO_2}}{V_{F_1} c_1 K_1}$	X	22	$\frac{K_{2:O_2}}{K_{2:CO_2}}$
J	9	$\frac{V_{\theta_2} K_{2:CO_2}}{V_{F_2} c_2 K_2}$	Y	23	$\frac{K_{3:O_2}}{V_{F_3}}$
K	10	$\frac{V_{\theta_3} K_{3:CO_2}}{V_{F_3} c_3 K_3}$	Z	23	$\frac{K_{3:O_2}}{K_{3:CO_2}}$
L	8a	$\frac{V_{\theta_1} K_{1:CO_2}}{c_1 K_1}$			
M	9a	$\frac{V_{\theta_2} K_{2:CO_2}}{c_2 K_2}$			
N	10a	$\frac{V_{\theta_3} K_{3:CO_2}}{c_3 K_3}$			

Simplification of calculations. The equations contain a number of factors which are constant for a given set of experimental conditions at a given temperature. These factors can be calculated and expressed by symbols (Table 2), the introduction of which facilitates subsequent calculation. The equations can then be written down in the following way, those suffixed a referring to unit weight:

$$x_{0:CO_2} = AH_0 - h_0 k_0, \quad (I)$$

$$x_{1:CO_2} = BH_1 - h_1 k_1, \quad (II)$$

$$\Delta x_{1:CO_2} = (II) - F(I), \quad (III)$$

$$x_{2:CO_2} = CH_2 + E_1 h_0 - h_2 k_2, \quad (IV)$$

$$\Delta x_{2:CO_2} = (IV) - G(II), \quad (V)$$

$$x_{3:CO_2} = DH_3 + E_r h_1 - h_3 k_3, \quad (VI)$$

$$\Delta x_{3:CO_2} = (VI) - H(IV); \quad (VII)$$

$$\Delta X_{1:CO_2} = I(III), \quad (VIII)$$

$$\Delta X_{2:CO_2} = J(V), \quad (IX)$$

$$\Delta X_{3:CO_2} = K(VII), \quad (X)$$

$$\Delta X_{1:CO_2} = \frac{L(III)}{\text{wt.}}, \quad (VIIIa)$$

$$\Delta X_{2:CO_2} = \frac{M(V)}{\text{wt.}}, \quad (IXa)$$

$$\Delta X_{3:CO_2} = \frac{N(VII)}{\text{wt.}}; \quad (Xa)$$

$$x_{0:B} = Oh'_0, \quad (XI)$$

$$x_{1:B} = Ph'_1, \quad (XII)$$

$$x_{2:B} = Qh'_2 - Rh'_0, \quad (XIII)$$

$$x_{1:B} = Sh'_3 - Th'_1; \quad (XIV)$$

$$\Delta X_{1:B} = (XI) - (XII), \quad (XV)$$

$$\Delta X_{2:B} = (XII) - (XIII), \quad (XVI)$$

$$\Delta X_{3:B} = (XIII) - (XIV), \quad (XVII)$$

$$\Delta X_{1:B} = \frac{V_{F_1}}{\text{wt.}} (XI - XII), \quad (XVa)$$

$$\Delta X_{2:B} = \frac{V_{F_2}}{\text{wt.}} (XII - XIII), \quad (XVIa)$$

$$\Delta X_{3:B} = \frac{V_{F_3}}{\text{wt.}} (XIII - XIV); \quad (XVIIa)$$

$$\Delta X_{1:CO_2}^{\text{resp.}} = (VIII) - (XV), \quad (XVIII)$$

$$\Delta X_{2:CO_2}^{\text{resp.}} = (IX) - (XVI), \quad (XIX)$$

$$\Delta X_{3:CO_2}^{\text{resp.}} = (X) - (XVII), \quad (XX)$$

$$\Delta X_{1:CO_2}^{\text{resp.}} = (VIIIa) - (XVa), \quad (XVIIIa)$$

$$\Delta X_{2:CO_2}^{\text{resp.}} = (IXa) - (XVIa), \quad (XIXa)$$

$$\Delta X_{3:CO_2}^{\text{resp.}} = (Xa) - (XVIIa); \quad (XXa)$$

$$\Delta X_{1:O_2} = U\Delta H_1 - V \text{ (VIII),} \quad (\text{XXI})$$

$$\Delta X_{2:O_2} = W\Delta H_2 - X \text{ (IX),} \quad (\text{XXII})$$

$$\Delta X_{3:O_2} = Y\Delta H_3 - Z \text{ (X),} \quad (\text{XXIII})$$

$$\Delta X_{1:O_2} = \frac{\Delta H_1 K_{1:O_2}}{\text{wt.}} - V \text{ (VIII } a), \quad (\text{XXI } a)$$

$$\Delta X_{2:O_2} = \frac{\Delta H_2 K_{2:O_2}}{\text{wt.}} - X \text{ (IX } a), \quad (\text{XXII } a)$$

$$\Delta X_{3:O_2} = \frac{H_3 K_{3:O_2}}{\text{wt.}} - Z \text{ (X } a). \quad (\text{XXIII } a)$$

Equations I–VII and XI–XIV apply equally to cell suspensions and tissue slices or organisms. Equations VIII–XXIII and VIII *a*–XXIII *a* express the values for total CO₂ output, glycolytic CO₂, respiratory CO₂ and O₂ uptake as $\mu\text{l./ml.}$ cell suspension or $\mu\text{l./unit weight}$ for each separate period of sampling. In the case of non-glycolysing tissues, the formulae for bicarbonate-CO₂ (XI–XIV), glycolysis (XV–XVII) and for the determination of the respiratory CO₂ (XVIII–XX) do not apply. Equations VIII–X give directly the respiratory CO₂, and only equations I–X and XXI–XXIII need be calculated. Once the symbols have been calculated, the evaluation of results is obtained from equations I–XXIII. If this procedure is followed, calculation of an entire experiment may be completed in about 30 min.

MANIPULATION OF THE APPARATUS. RESULTS

The assembly and manipulation of the apparatus are simple. The wide manometer board (Fig. 1, *J*) has at its back two slides which are arranged to fit into two neighbouring brackets on the shaker bar of the tank. The manometers are not fixed to the board, but are kept in a wooden stand which is provided with slots to fit the U-shaped parts of the manometers. The main vessel is kept in a wooden block. When assembling the apparatus the vessel is not put on to the manometers as is usual, but the manometers, with the compensating vessels in position, are slid into their joints after the vessel has been fixed in a rubber-lined clamp (Fig. 1, *K*) at the back of the board. An alternative type of clamp secures the vessel round the sampling taps to allow illumination of the vessel. All compensating vessels contain water, those connected to the subsidiary manometers for the determination of gaseous CO₂ having in addition KOH on filter papers in their centre wells, to obtain the same water vapour tension as in the experimental side of the manometer. The sampling keys are greased and so inserted into the taps that all sampling cups are in open communication with the main vessel. The lower rolls of filter paper are then inserted into the upper outlet tubes and soaked with 0.2 ml. 10% KOH. The upper rolls of filter

paper are next inserted and wetted with 0.2 ml. water. (A standard size of Whatman no. 2 filter paper is being used, 15 × 75 mm., the volume of which is determined and taken into account when calculating the constants.) Redistilled mercury (3 ml.)—care must be taken that no air is trapped—and tartaric acid (0.5 ml. of 0.5 *N*) are run into each lower outlet tube. The main vessel is then filled with the amount of suspension or fluid necessary to fill and completely cover the lower sampling cups. Tissue slices or small organisms must be prevented from lodging in the lower sampling cups. This is done by placing a platinum coil in the vessel (schematically drawn in Fig. 2, *L*) or by putting the material in a small platinum-wire basket. Ringer-bicarbonate solution should be equilibrated with 5% (v/v) CO₂/O₂ before putting it into the vessel. The gas-outlet tap is introduced, and, if necessary, the Keilin tube attached. The apparatus is assembled on a solid stand (Fig. 1, *L*) which has two brackets fitting the slides of the manometer board. The vessel is secured in the clamp at the back of the manometer board and the central manometer inserted. The greased joint is worked in by gentle movement of the manometer and fixed with springs. If gassing is required or continuation of gassing with a CO₂-gas mixture, this is done in the ordinary way at this stage. Next, the outer and then the inner subsidiary manometers are slid into position in their joints and fixed. All manometers are secured in front of the manometer board by two rubber-lined clamps across the width of the board. The gassing is stopped, the gas-outlet tap closed and the manometer transferred into the bath. If necessary, gassing can be done or continued while the manometer is shaken in the bath. In this case the gas outlet (Fig. 1, *C*) is prolonged above the surface of the water by a length of rubber tubing. It is advisable to gas both sides of the main manometer in order to prevent excessive movement of the manometer fluid, which, with the narrow capillary (area 0.25 sq. mm.), may affect later readings because of the slow draining of traces of manometer fluid. Temperature equilibration is proceeded with as in any differential manometer. The subsidiary manometers do not need gassing. It is, however, important to shut all manometers simultaneously with the compensating vessel of the main vessel. This is done when the position of the fluid in the main manometer is adjusted before the reading at *t*₀. In this way small temperature differences during the experimental period do not affect the later readings of the samples.

The four subsidiary manometers should show no pressure differences up to the moment when they are connected with one of the sampling cups. The position of the sampling cups during the course of the experiment is diagrammatically shown in Fig. 4. At *t*₀ subsidiary manometers 1 and 2 (left) and the

main manometer (H_0) are read. The left sampling tap is immediately turned in order to connect the first pair of sampling cups (c_0 and c'_0) with their manometers. At t_1 subsidiary manometers 3 and 4 (right) and the main manometer (H_1) are read. The right sampling tap is then turned to connect c_1 and c'_1 with their manometers. At t_2 subsidiary manometers 1 and 2 (their pressures, h_0 and h'_0 , having in the meantime become stationary) and the main manometer (H_2) are read. The left tap is then turned once more to connect the next pair of sampling cups (c_2 and c'_2) with their manometers. At t_3 subsidiary manometers 3 and 4 (h_1 and h'_1) and the main manometer (H_3) are read and the right tap turned once more to connect the last pair of sampling cups (c_3 and c'_3) with their manometers. All readings of the main manometer (H_0 , H_1 , H_2 and H_3) have by now been taken. The remaining readings, h_2 and h'_2 (left), h_3 and h'_3 (right), are taken when the pressures in these manometers are constant.

In apparatus *B* the left inner key is turned at t_0 , the right inner key at t_1 , the left outer key at t_2 and the right key at t_3 (Fig. 5). The readings are taken in the way described for apparatus *A*.

CALIBRATION

The most accurate way of calibrating the apparatus is with mercury. In the main vessel the total volume has to be determined (including the eight sampling cups), and the separate volume of each sampling cup. The constants of the subsidiary manometers can also be obtained experimentally. It is not advisable to do so in the first instance if no previous experience of the handling of the apparatus has been gained. However, it may be useful to check the calibrated constants in the following ways.

Constants of the subsidiary manometers for the determination of gaseous carbon dioxide. The main vessel contains water and both rolls of filter paper in the subsidiary manometers on each side are wetted with water (no KOH to be included). A positive pressure is produced in the main manometer by a gas other than CO_2 . This corresponds to the reading H_0 . In this case $H_0 = H_1 = H_2 = H_3$. The left and right sampling keys are turned once.

$$k_j = \frac{H_1 K_j c_j}{V_{0j} h_j} \quad (j = 1 \text{ or } 2).$$

The manometer fluid in the subsidiary manometers is then levelled and the procedure continued.

$$k_j = \frac{H_1 K_j c_j}{V_{0j} h_j} \quad (j = 2 \text{ or } 3).$$

Constants of the bicarbonate-carbon dioxide manometers. A bicarbonate solution of known concentra-

tion is made up (approximately 50 μl . CO_2/ml .) and its concentration checked by acidifying in an ordinary manometer. The bicarbonate solution is pipetted into the main vessel and the lower outlet tubes filled with mercury and tartaric acid. After temperature equilibration, the sampling taps are turned as described. The subsidiary manometers are not levelled after the first readings left and right. If the known bicarbonate concentration/ml. is A ,

$$k'_{j:\text{CO}_2} = \frac{Ac'_j}{h'_j} \quad (j = 1 \text{ or } 2),$$

$$k'_{2:\text{CO}_2} = \frac{Ac'_2 + (h'_0 k'_{0:\text{CO}_2})}{h'_2},$$

$$k'_{3:\text{CO}_2} = \frac{Ac'_3 + (h'_1 k'_{1:\text{CO}_2})}{h'_3}.$$

Protocols

Exp. 1. This concerned the influence of light, in air, on the metabolism of *Gastrophilus intestinalis* larvae. It was done with Mr L. Levenbook as part of a study of the influence of CO on the metabolism of *Gastrophilus intestinalis* larvae. The significance of the light effect on the ratio CO_2/O_2 is not discussed here. Apparatus: type *B*; temp., 37° ; constants given in Table 4. The larvae rested on wetted filter paper.

Table 4. *Constants of apparatus used in Exp. 1*

$K_{1:\text{O}_2} = 4.4464$	$k_0 = 0.757$		
$K_{2:\text{O}_2} = 4.30$	$k_1 = 0.8303$		
$K_{3:\text{O}_2} = 4.1472$	$k_2 = 0.6441$		
	$k_3 = 0.7360$		
$A = 0.0985$	$F = 0.9387$	$L = 49.6421$	$V = 0.9831$
$B = 0.0915$	$G = 1.10$	$M = 43.8393$	$X = 0.9785$
$C = 0.10$	$H = 0.985$	$N = 44.7451$	$Z = 0.9776$
$D = 0.0948$			
$E_l = 0.4727$			
$E_r = 0.5501$			

The main vessel contained in addition to the larvae a small metal boat, filled with water. The total volume of larvae, boat and water was adjusted to 5 ml. Wet weight of four larvae, 1.9 g.

Table 5. *Manometer readings, Exp. 1*

t (min.)	Experi- mental conditions	Main manometer		Subsidiary manometer, gaseous CO_2
		$H_0 = -0.2$		$h_0 = -14.1$
0-30	Light	$H_1 = +21.2$	$\Delta H_1 = +21.4$	$h_1 = -17.5$
30-60	Dark	$H_2 = +5.2$	$\Delta H_2 = -16.0$	$h_2 = -45.6$
60-90	Light	$H_3 = +35.0$	$\Delta H_3 = +29.8$	$h_3 = -51.2$

The readings (Table 5) give the following relationships:

- I. $x_{0:\text{CO}_2} = 0.0985 \times -0.2 + 14.1 \times 0.757 = 10.66.$
- II. $x_{1:\text{CO}_2} = 0.09147 \times 21.2 + 17.5 \times 0.8303 = 16.51.$
- III. $\Delta x_{1:\text{CO}_2} = 16.51 - 0.9387 \times 10.66 = 6.51.$
- IV. $x_{2:\text{CO}_2} = 0.10 \times 5.2 + 0.4727 \times -14.1 + 45.6 \times 0.6641 = 23.24.$
- V. $\Delta x_{2:\text{CO}_2} = 23.24 - 1.10 \times 16.51 = 5.04.$
- VI. $x_{3:\text{CO}_2} = 0.09481 \times 35.0 + 0.5501 \times -17.5 + 51.2 \times 0.8303 = 31.39.$
- VII. $\Delta x_{3:\text{CO}_2} = 31.39 - 0.985 \times 23.24 = 8.54.$
- VIII a. $\Delta X_{1:\text{CO}_2} = \frac{49.6421}{1.9} \times 6.51 = 170.$
- IX a. $\Delta X_{2:\text{CO}_2} = \frac{43.8393}{1.9} \times 5.04 = 116.5.$
- X a. $\Delta X_{3:\text{CO}_2} = \frac{44.7451}{1.9} \times 8.54 = 200.3.$
- XXI a. $\Delta X_{1:\text{O}_2} = \frac{21.4}{1.9} \times 4.4464 - 0.9831 \times 170 = -117.$
- XXII a. $\Delta X_{2:\text{O}_2} = \frac{-16}{1.9} \times 4.30 - 0.9785 \times 116.5 = -150.2.$
- XXIII a. $\Delta X_{3:\text{O}_2} = \frac{29.8}{1.9} \times 4.1472 - 0.9776 \times 200.3 = -131.$

The results are given in Table 6.

Table 6. *Results of Exp. 1*

(q'_{O_2} and q'_{CO_2} represent O_2 uptake and CO_2 production/l g. larvae/hr.)

t (min.)	Experimental conditions	q'_{O_2}	q'_{CO_2}	$\frac{q'_{\text{CO}_2}}{q'_{\text{O}_2}}$
0-30	Light	234	340	1.45
30-60	Dark	300.4	233	0.775
60-90	Light	262	400.6	1.53

Exp. 2. This concerned the metabolism of 4 sheep retinæ in Ringer-bicarbonate with 0.2% glucose and 5% CO_2 /95% O_2 at 38°. Apparatus: type B; constants given in Table 7. Manometer fluids: paraffin (d , 0.788) in main manometer and in subsidiary manometers for determination of gaseous CO_2 ; α -bromonaphthalene (d , 1.4875) in subsidiary manometers for determination of bicarbonate- CO_2 . Extensions were used on the lower outlet tubes (see Fig. 3, J).

Owing to large pressure changes occurring in the subsidiary manometers for the determination of the

gaseous CO_2 at t_2 and t_3 , they were opened and levelled after the determinations of h_0 and h_1 . The expressions $E_1 h_0$ and $E_1 h_1$ in equations IV and VI, therefore, become zero and are omitted. Dry weight of retinæ, 101 mg.

Table 7. *Constants of the apparatus used in Exp. 2*

$V_{F1} = 24.0745$	$K_{1:\text{O}_2} = 3.08$	$k_0 = 0.757$	$k'_{0:\text{CO}_2} = 3.92$
$V_{F2} = 23.2613$	$K_{2:\text{O}_2} = 2.996$	$k_1 = 0.8303$	$k'_{0:\text{CO}_2} = 3.97$
$V_{F3} = 22.4792$	$K_{3:\text{O}_2} = 2.899$	$k_2 = 0.6641$	$k'_{0:\text{CO}_2} = 3.99$
		$k_3 = 0.7360$	$k'_{3:\text{CO}_2} = 4.07$
A = 0.1009	F = 0.9387	L = 43.4279	
B = 0.09488	G = 1.10	M = 38.8585	
C = 0.1026	H = 0.985	N = 37.5445	
D = 0.1029			
O = 4.2562	V = 0.7486		
P = 4.3398	X = 0.7496		
Q = 4.6820	Z = 0.7499		
R = 4.5999			
S = 4.6504			
T = 4.5361			

Table 8. *Manometer readings, Exp. 2*

t (min.)	Main manometer	Subsidiary manometers	
		Gaseous CO_2	Bicarbonate CO_2
	$H_0 = -127.4$	$h_0 = -116$	$h'_0 = +125$
0-20	$H_1 = -37.2$ $\Delta H_1 = +90.2$	$h_1 = -127$	$h'_1 = +116.6$
20-40	$H_2 = +7.8$ $\Delta H_2 = +45.0$	$h_2 = -212.5$	$h'_2 = +227.3$
40-60	$H_3 = -34.5$ $\Delta H_3 = +26.7$	$h_3 = -210.3$	$h'_3 = +216.6$

The readings (Table 8) give the following relationships:

$$\begin{aligned}
 \text{I.} \quad & x_{0:\text{CO}_2} = 0.1009 \times -128 + 116 \times 0.757 = 75. \\
 \text{II.} \quad & x_{1:\text{CO}_2} = 0.09488 \times -37.2 + 127 \times 8303 = 101.9. \\
 \text{III.} \quad & \Delta x_{1:\text{CO}_2} = 101.9 - 0.9387 \times 75 = 31.5. \\
 \text{IV.} \quad & x_{2:\text{CO}_2} = 0.1026 \times 7.8 + 212.5 \times 0.6641 = 142. \\
 \text{V.} \quad & \Delta x_{2:\text{CO}_2} = 143.3 - 1.1 \times 101.9 = 30.0. \\
 \text{VI.} \quad & x_{3:\text{CO}_2} = 0.1029 \times 34.5 + 210.3 \times 0.7360 = 158.6. \\
 \text{VII.} \quad & \Delta x_{3:\text{CO}_2} = 158.6 - 0.985 \times 142 = 18.6. \\
 \text{VIII a.} \quad & \Delta X_{1:\text{CO}_2} = \frac{43.4279}{101} \times 31.5 = 13.8. \\
 \text{IX a.} \quad & \Delta X_{2:\text{CO}_2} = \frac{38.8585}{101} \times 30.0 = 11.6. \\
 \text{X a.} \quad & \Delta X_{3:\text{CO}_2} = \frac{37.5445}{101} \times 18.6 = 6.95. \\
 \text{XI.} \quad & X_{0:B} = 125 \times 4.2562 = 532. \\
 \text{XII.} \quad & X_{1:B} = 116.6 \times 4.3388 = 506. \\
 \text{XIII.} \quad & X_{2:B} = 227.3 \times 4.6820 - 125 \times 4.5999 = 489.3. \\
 \text{XIV.} \quad & X_{3:B} = 216.6 \times 4.6504 - 116.6 \times 4.5361 = 478.3. \\
 \text{XV a.} \quad & \Delta X_{1:G} = \frac{24.0745}{101} \times (532 - 506) = 6.23. \\
 \text{XVI a.} \quad & \Delta X_{2:G} = \frac{23.26125}{101} \times (506 - 489.3) = 3.84. \\
 \text{XVII a.} \quad & \Delta X_{3:G} = \frac{22.4791}{101} \times (489.3 - 478.3) = 2.48. \\
 \text{XVIII a.} \quad & \Delta X_{1\text{CO}_2}^{\text{resp.}} = 13.8 - 6.23 = 7.57. \\
 \text{XIX a.} \quad & \Delta X_{2\text{CO}_2}^{\text{resp.}} = 11.6 - 3.84 = 7.76. \\
 \text{XX a.} \quad & \Delta X_{3\text{CO}_2}^{\text{resp.}} = 6.95 - 2.48 = 4.47. \\
 \text{XXI a.} \quad & \Delta X_{1:\text{O}_2} = \frac{90.2}{101} \times 3.08 - 0.7486 \times 13.8 = 7.57. \\
 \text{XXII a.} \quad & \Delta X_{2:\text{O}_2} = \frac{45.0}{101} \times 2.9962 - 0.7496 \times 12.0 = 7.49. \\
 \text{XXIII a.} \quad & \Delta X_{3:\text{O}_2} = \frac{26.7}{101} \times 2.899 - 0.7499 \times 6.45 = 4.44.
 \end{aligned}$$

Table 9. *Results of Exp. 2*

(Q_{O_2} , $Q_{\text{CO}_2}^{\text{resp.}}$, Q_G , metabolic quotients of O_2 , respiratory CO_2 , and glucose are in $\mu\text{l./mg. dry wt./hr.}$)

t (min.)	Q_{O_2}	$Q_{\text{CO}_2}^{\text{resp.}}$	Q_G	R.Q.
0-20	22.7	22.7	18.7	1.0
20-40	22.5	23.3	11.5	1.04
40-60	13.3	13.4	7.5	1.0

Results are given in Table 9. The figures for O_2 uptake and total CO_2 production agree closely with those obtained when using the indirect method of Warburg (1924, 1925).

SUMMARY

1. The existing manometric methods of measuring the respiratory quotient allow only one determina-

tion to be made during one experimental period. So far it has not been possible to measure changes of R.Q. with time, or after alterations of the experimental conditions during the experiment. Such changes occur on addition of substrates, inhibitors, catalysts, drugs, or on fertilization.

2. A new manometric apparatus, which allows such changes to be measured on a single sample of biological material, has been made.

3. The method involves taking samples of the liquid and gaseous phases from the main vessel. Samples are taken at the beginning of the experiment and at three convenient times during the experiment, which is unaffected by the sampling procedure. The CO_2 and bicarbonate contents of the samples are

determined in subsidiary manometers. These, together with the readings of the main manometer at the times of sampling, enable the O_2 uptake, and the production of respiratory CO_2 and acid to be independently calculated for each interval between sampling. In this way changes with time in the rates of O_2 uptake, total CO_2 production and of R.Q. can be obtained.

4. Two forms of the apparatus have been designed and are described. The theory of the method is developed and a simplified way of computing the results is given.

The instruments were made by Messrs W. G. Flaig and Sons, Waterloo Road, London, N.W. 2.

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Carotenoids, Vitamin A and 7-Dehydrosteroid in the Frog (*Rana temporaria*)

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Understanding of the biochemistry of carotenoids is rudimentary except as to the part played in the animal economy by a few of them as precursors of vitamin A. In a wider sense the animal biochemistry is complicated by species differences. Thus some animals absorb and retain so little of either the hydrocarbons or their hydroxylated derivatives as to suggest that they are not needed, whilst other species absorb relatively large quantities of different carotenoids selectively and retain them distributed over various organs. For the carotenes it could be argued that they are only absorbed as such when the capacity of enzyme systems in the gut to transform them is exceeded, and that the pigments, of no special value to the body, are slowly disposed of. Such an argument, however, neglects the regular occurrence of carotenoids in eyes, yellow bone marrow and corpora lutea and is of little value in respect to the assimilation and metabolism of hydroxylated carotenoids like lutein and zeaxanthin.

As a subject for detailed study the frog has several special advantages; carotenoids (including both β -carotene and lutein) are widely distributed in the body; the species can be studied through the life cycle, as embryos, as tadpoles and as immature and mature frogs; there is a marked seasonal cycle in both sexes and the reproductive phase entails a heavy drain on body reserves, especially in the female. Moreover, as an amphibian, the frog provides a link between work on mammals and fishes. There are, therefore, numerous factors potentially capable of correlation with data on the distribution of caro-

tenoids so as to lead to hypotheses concerning the functions of these substances.

For 10–11 weeks after using up the residual yolk the tadpole is mainly vegetarian, but when metamorphosis is complete, frogs become mainly carnivorous. Adult frogs build up large fat reserves in early summer, and from June to August the testes or ovaries enlarge considerably. The testes then shrink and during the next few months vary little in size until the spermatozoa become fully ripe; the ovaries go on developing, but after spawning in March the shrunken oviducts are orange in contrast to their earlier paleness. The corpora adiposa of Amphibia are associated with the gonads; in the frog they vary from strands of orange tissue to large cream-coloured lobes. After surgical removal of one fat body the ovary on the operated side remains small compared with the other. According to March (1937) sexually mature frogs have a body length (snout-cloaca) of about 4.8 cm. and weigh about 10 g. Applied with caution, body length is a guide to age.

FAT SOLUBLE SUBSTANCES IN THE FROG

Earlier work on carotenoids and vitamin A. Lönnberg (1929) reported the presence of a 'carotene-like pigment' in the skin (*Rana temporaria* and *R. esculenta*). Dietel (1933) noted the presence of lipochrome in skin, ovaries and liver, but thought it consisted wholly of carotene. Manunta (1934) found 'xanthophyll' and much carotene in the skin of *R. esculenta*. Rand (1935) observed vitamin A to be present in frog and toad livers with lipochrome pigments detectable in skin, liver, ovaries and eggs, oviducts, testes, kidneys,