CXXIV. THE MEASUREMENT OF TISSUE GLYCOLYSIS IN SERUM

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IN the manometric measurement of the respiration and glycolysis of animal tissues it is desirable to keep the conditions as physiological as possible. The fluid in which the tissue slices are suspended should therefore approximate as closely as possible to that which normally surrounds them in the body. Various salt solutions have been proposed, but obviously in this respect they are all inferior to serum itself. The use of serum in manometric methods, however, introduces complications, owing to "retention" effects due to its high buffering power at the physiological pH . The methods of Dickens & Šimer [1931] and Dixon & Keilin [1933] are both suitable for the measurement of the O_2 and CO_2 of respiration in any medium, including serum; Dickens & Šimer [1932] later extended their method to the measurement of the aerobic glycolysis¹ in serum; the method of Dixon & Keilin gives the glycolysis in Ringer-bicarbonate solution but has not hitherto been adapted for glycolysis in serum. The present paper describes the adaptation of the method for this purpose, making use of the later principle of Dickens & Šimer.

Principle of the method

The Dixon-Keilin method gives the respiratory exchange and the change in the amount of bicarbonate during the experimental period. In Ringer-bicarbonate solution the change of bicarbonate is equal to the glycolysis [Dixon, 1934, p. 104], but this is not the case in serum, for a part of both the acid produced and the CO_2 is buffered or "retained" by the serum proteins and consequently fails to exert its normal effect on the manometer. The true glycolysis in serum is obtained by adding a correction for the retention to the change of bicarbonate. The respiratory quantities are unaffected by the retention, as strong acid is added before taking the readings from which they are obtained.

The principle of the method is as follows [cf. Dickens & Šimer, 1932]. A "retention curve", in which the retention is plotted against the pH , is first constructed for the particular serum used. (If the serum is kept sterile, one batch, and hence one retention curve, will suffice for a large number of tissue experiments.) The retention in each experiment may then be read off from the curve if the change of pH during that experiment is known. This pH change is calculated from the changes of bicarbonate content and CO_2 pressure by applying the Henderson-Hasselbalch equation, and the necessary quantities are themselves simply obtained from the manometric readings taken in the ordinary course of the experiment. The only modification necessary in the experiments themselves is that the alkali must be introduced into the two flasks at different times and

¹ More strictly, the acid production. The term glycolysis is at present used in manometric work even when it has not been definitely shown that the acid formed is lactic acid arising from carbohydrate metabolism, and it is so used in this paper. Elliott & Schroeder [1934] suggest the term "acidogenesis".

not simultaneously as hitherto, and in consequence of this only 2 ml. of serum must be added to each flask (instead of 3 ml. as usual) in order to keep the readings within the compass of the manometer scale. (If the manometric constants have already been calculated for the larger liquid volume they must of course be recalculated.) The separate parts of the technique will now be described in detail.

Preparation of the serum

Horse serum is usually chosen for this type of work, but it is unsuitable for this method on account of the fact that it rapidly sets to a rigid gel when acidified at 40°. When this occurs in the manometer flasks the serum is no longer mixed by the shaking, and if the CO₂ evolution is not complete at the moment of gelling the remainder diffuses only slowly from the gel. Thus in estimating the bicarbonate content by the addition of acid one observes a rapid evolution of about 90 % in 3 or 4 min. which ceases abruptly at the moment of gelling and is followed by a slow output of the remaining 10 % which may take an hour or more. Besides giving misleading results, this makes it impossible to obtain the first reading *h* in the Dixon-Keilin method, for the manometric reading during the experimental period will be due partly to the action of the tissue in the right-hand flask and partly to this slow evolution of CO₂ in the left-hand flask. I have tested several samples of horse serum and obtained similar results with all: ox and pig sera behave similarly. I find, however, that rabbit and sheep sera do not give this immediate gelling with acid; although they ultimately form a gel, they remain perfectly liquid for about an hour, and as the evolution of CO₂ is complete in from 5 to 10 min. they are quite satisfactory in this respect. Sheep serum has therefore been adopted.

The serum may be obtained by allowing the blood to coagulate and then applying pressure to the clot, as described by Okamoto [1925], or it may be more convenient to obtain it by centrifuging defibrinated blood. It is not always easy to obtain a perfectly clear serum from sheep's blood by the latter method, but if the serum is allowed to stand overnight at 0° any remaining white cells aggregate and can be filtered off. It is then sterilized by passing through a Seitz filter, after which it is immunologically inactivated as described by Okamoto by heating to exactly 58° for 2 hr. and on the following day again heating to 58° for $\frac{1}{2}$ hr. It is conveniently kept in a number of sterile test-tubes containing about 10 ml. each, so that one tube can be opened for each experiment and the remainder of the stock is not infected. It may be kept in the refrigerator for months without any apparent change. Before use 1/50 of its volume of 10 % glucose is added.

Determination of retention curve

This is conveniently done in three (or, if necessary, two) manometers of the Dixon-Keilin type, by adding varying amounts of tartaric acid to the serum (in equilibrium with the O₂+CO₂ gas mixture) and noting the amounts of CO₂ evolved. These amounts are always less than the equivalents of the acid added, and the deficiency in each case gives the retention for that particular pH change. The pH values are calculated by the Henderson-Hasselbalch equation.

In each flask of manometers 1 and 2 is placed the same amount of serum as is used in the tissue experiments, together with a hanging tube ("Keilin cup") containing dried tartaric acid solution which can be dropped into the serum. By taking a different amount of tartaric acid for each flask four points on the curve are obtained. The third manometer serves for the determination of the initial conditions: in the left-hand side the total initial bicarbonate content of

the serum is obtained by the addition of excess of HCl from the side bulb, and on the right-hand side the initial CO_2 tension in the gas mixture is afterwards determined by admitting alkali to absorb the CO_2 .

The solutions added to the various manometers are as shown in the upper part of Protocol I. The purpose of adding water to the side bulbs is to keep the manometric constants the same as in the tissue experiments. For the same reason the glass rods must be placed in the centre tubes in all the flasks, even though the bottom taps are not used in most cases.

The $N/10$ tartaric acid solution is previously standardized manometrically in the usual way by adding a known amount to a bicarbonate solution and measuring the CO_2 evolved. It is difficult to measure out small volumes of the solution with sufficient accuracy and the solution is therefore weighed out into the Keilin tubes. Keilin tubes are used instead of adding the acid from the side bulbs as it is difficult to secure quantitative delivery from the latter. The tartaric solution is evaporated to dryness after weighing out in order to avoid the slight manometric changes which are observed on diluting serum in equilibrium with CO_2 [Warburg, 1925]. The usual method is to evaporate the solution in the oven, but this method is liable to lead to errors, as if the temperature of the oven is too low the evaporation from the tubes is inconveniently slow, while if it is too high some of the tartaric acid is decomposed. Appreciable decomposition appears to occur at temperatures only just above 100° and this may give rise to erratic results. The writer has obtained very much more satisfactory results by drying the tubes overnight in an evacuated desiccator over H_2SO_4 and KOH, and this method is recommended.

In order to obtain the four points on the retention curve it is necessary to dislodge the hanging tubes on the two sides of each apparatus successively and not simultaneously. This is easily arranged by having platinum hooks of different lengths on the tubes in the left- and right-hand flasks. If the hook on the right-hand side is slightly under 1 mm. in length, while that on the left-hand side is about $2\frac{1}{2}$ mm. long, it will be found that a light tap will dislodge the right-hand tube but a much stronger blow is necessary to dislodge the left-hand tube. This method has proved completely reliable, and it is in fact useful in general manometric work when it is desired to add two reagents successively without opening the manometer, for by its means two hanging tubes can be placed in the same flask and made to drop successively.

The manometers having been prepared, the determination of the retention curve proceeds as follows. The manometers are placed in the thermostat at 38° and the $\text{O}_2 + \text{CO}_2$ gas mixture is passed through the flasks at the standard rate for 10 min., exactly as described by Dixon & Keilin. The gas stream is then diverted, the outlet stoppers are closed and the manometers are shaken for a further 5 min. with the taps open and the rubber tubes still attached, after which the taps are closed and the tubes detached. The manometers are then read, shaken for a few minutes longer and read again to make sure that the readings are steady. The Keilin tubes are then dislodged in the right-hand side of each of the first two manometers and the HCl is added from the side bulb in the third. The evolution of the CO_2 is complete in a few minutes, but for safety the manometers are shaken for 20 min. before the readings are taken. The two remaining Keilin tubes are then dislodged and the alkali is introduced in the right-hand side of the third manometer. The first two manometers may be read after a further 20 min., but the third must be left for 1 hr., by which time the absorption of the CO_2 by the alkali is complete. The actual readings (in mm.) obtained in an actual determination of a curve on sheep serum are given in Protocol I.

Protocol I. *Determination of retention curve*

Manometer ...	1 14-20		2 13-64		3 14-13	
k_{CO_2} ...						
Side ...	Left	Right	Left	Right	Left	Right
In flask: 2 ml. of	Serum	Serum	Serum	Serum	Serum	Water
In side bulb: 0.3 ml. of	Water	Water	Water	Water	3 <i>N</i> HCl	Water
In bottom tap	—	—	—	—	—	20% KOH
In hanging tube: g. of <i>N</i> /10 tartaric acid solution (dried)	0.0543	0.1001	0.1971	0.2958	—	—
Time min.	Readings		Diff.	Readings		Diff.
0	100.2	100.2	0.0	107.9	108.0	— 0.1
0	Tube in			Tube in		
20	106.0	94.7	+ 11.3	125.1	90.1	+ 35.0
20	Tube in			Tube in		
40	103.0	97.8	+ 5.2	113.5	102.0	+ 11.5
80	103.0	97.8	+ 5.2	113.5	102.0	+ 11.5
<i>h</i>	6.1	11.3	—	23.5	35.1	— 59.8
x_{CO_2}	86.5	160.5	—	320.5	479	— 845
x_T	119	220	—	434	651	—
<i>R</i>	32.5	59.5	—	113.5	172	— 0
<i>B</i>	758.5	684.5	—	524.5	366	— 845
<i>C</i>	0.97	0.97	—	0.98	0.98	— 1.00
p_{CO_2}	111	116	—	128	139.5	— 105
" H_2CO_3 "	45.3	47.3	—	52.2	56.9	— 42.8
<i>pH</i>	7.324	7.280	—	7.102	6.908	— 7.396

The calculation proceeds as follows. The change of reading (*h*) due to the liberation (or absorption) of CO_2 in each flask has been written in the protocol in the column below the flask in question. Thus taking the left side of manometer 1 as an example, $h = 11.3 - 5.2 = 6.1$, and similarly with the others. On multiplying these values by the respective manometer constants (k_{CO_2}) we obtain the volume of CO_2 in $\mu\text{l.}$ (x_{CO_2}). Thus in the same example $x_{\text{CO}_2} = 6.1 \times 14.20 = 86.5$. This was liberated by 0.0543 g. of tartaric acid solution, of which 1 g. was previously found to be equivalent to 2200 $\mu\text{l.}$ of CO_2 , so that the CO_2 equivalent of this amount of tartaric acid (x_T) is $2200 \times 0.0543 = 119 \mu\text{l.}$ The retention (*R*) in this case is therefore $119 - 86.5 = 32.5 \mu\text{l.}$ of CO_2 per 2 ml. of serum.

Having found the retention, we have to calculate the corresponding *pH*, for which we require the bicarbonate content (*B*) and the CO_2 pressure (p_{CO_2}) after adding the tartaric acid. The bicarbonate content (expressed in $\mu\text{l.}$) is obviously equal to the initial bicarbonate content minus the amount of bicarbonate which has been decomposed into CO_2 . Manometer 3 shows that the initial content was 845 $\mu\text{l.}$, so that in the flask which we have chosen as example $B = 845 - 86.5 = 758.5 \mu\text{l.}$

If Warburg manometers had been used the increase in partial pressure of CO_2 would be given directly (in mm. of manometric liquid) by the readings (*h*), so that p_{CO_2} for any flask would be obtained by adding the corresponding *h* to the initial partial pressure of CO_2 , which is given by the *h* value for the right-hand side of manometer 3. But as differential manometers are used here this is only approximately true, and if great accuracy is desired the readings must be multiplied by a correction factor (*C*) in order to obtain actual CO_2 pressures. The mathematical theory has already been given by Dixon [1934, first equation on p. 26] and it will be clear from the treatment given there that *C* is equal to the first part of the expression for the manometer constant and reduces to

$$C = \cos \theta + \frac{AP_0}{2v_g}.$$

This factor, which differs only slightly from unity, must be calculated in any case in calibrating the manometers.

In the particular flask we are considering p_{CO_2} is therefore given by $105 \times 1.00 + 6.1 \times 0.97 = 111$ mm. Clerici solution. From this the amount of dissolved CO_2 ("H₂CO₃") can be obtained by multiplying by the solubility and the volume of serum (in $\mu\text{l.}$) and dividing by the P_0 value for the Clerici solution. Thus in this case "H₂CO₃" = $\frac{111}{2500} \times 0.51 \times 2000 = 45.3 \mu\text{l.}$ per 2 ml. of serum [see Dickens & Šimer, 1932]. (It will be noticed that in this experiment C can be entirely neglected without affecting the results, but under some conditions the error might not be quite negligible.)

The $p\text{H}$ can now be calculated from the equation

$$p\text{H} = 6.10 + \log \frac{B}{\text{"H}_2\text{CO}_3"},$$

so that in this case $p\text{H} = 6.10 + \log 758.5 - \log 45.3 = 7.324$. The other three points on the curve are obtained similarly, and a fifth point corresponding to

Fig. 1. Retention curve of serum.

zero retention is obtained from the initial values given by manometer 3. The retention curve can now be plotted and is shown in Fig. 1. It corresponds closely with the curve given by Dickens & Šimer, which is shown dotted. This

agreement is of course largely fortuitous, as a different serum was used, but the curves obtained with different samples of serum are as a rule very similar.

Once the retention curve has been obtained, the actual measurements of tissue glycolysis and respiration in serum involve no more trouble than when bicarbonate-Ringer solution is used.

Measurement of respiration and aerobic glycolysis in serum

The experiments with tissue are carried out precisely as described by Dixon & Keilin, except that only 2 ml. of serum are taken for each flask and that the alkali is introduced successively into the two flasks of the manometer and not simultaneously as hitherto. Only one manometer is required for a complete experiment, and the necessary readings for calculating the pH change, and hence the retention, are obtained during the ordinary course of the experiment.

The only experimental point requiring special mention is the removal of the tissue from the gelled serum for drying and weighing at the end of the experiment. This is quite easy in the case of liver slices and similar material, but with more fragile tissues such as retina it is difficult to remove the tissue quantitatively.

Fig. 2. Progress of experiment on rat retina.

The simplest method is to fill the flasks with tap water and allow them to stand for some time. The gel then redissolves and the tissue can be removed without difficulty.

In Protocol II are given the essential readings obtained in an experiment on rat retina (a strongly glycolysing tissue) and in order to show the relationship of the various quantities more clearly the whole course of the experiment is shown in Fig. 2, though it will be understood that it is not necessary to determine the whole curve in ordinary experiments.

Protocol II. *Measurement of respiration and glycolysis of rat retina in serum*

Retinae from 3 rats—3 retinae in each flask.

Inactivated sheep serum containing 0.2% glucose. $O_2 + CO_2$.

Manometer	...	...	3	
k_{O_2}	...	...	13.68	
k_{CO_2}	...	...	14.13	
Side	...	...	Left	Right
In flask: 2 ml. of			Serum	Serum
In side bulb: 0.3 ml. of			3N HCl	3N HCl
In bottom tap			KOH	KOH
Retina (mg. dry wt.)			3.9	3.9
Time min.			Readings	
0			103.2	103.7
0			HCl in	
10			71.0	135.1
20			72.0	134.3
30			72.4	133.8
90			75.5	131.0
90				HCl in
110			102.2	104.5
110			KOH in	
170			184.5	20.9
170				KOH in
250			96.9	109.6
$h_2 = -12.7 - (-0.5) = -12.2$ $x_{O_2} = -167$ $h_1 = -2.3 - (-0.5) = -1.8$ $x_{CO_2} = +147$ $h = -55.5 - (-65.5) = +10.0$ $x_B = -166$ $h_{B_1} = 65.5 - 0.5 = 65.0$ } $h_{B_2} = 55.5 - 2.3 = 53.2$ } $\Delta pH = -0.174$ $h_{C_1} = 163.6 + 2.3 = 165.9$ } $R = 70$ (from Fig. 1) $h_{C_2} = 163.6 + 12.7 = 176.3$ } $x_G = -(-166) + 70$ $= +236$ $Q_{O_2} = -28.5$ $R.Q. = 0.88$ $Q_G = +40.3$				

The respiration (x_{O_2} and x_{CO_2}) and the bicarbonate change (x_B) are obtained as usual from the readings h , h_1 and h_2 as follows [Dixon, 1934, pp. 103-4]:

$$\begin{aligned}
 x_{O_2} &= k_{O_2} \cdot h_2 \\
 x_{CO_2} &= k_{CO_2} \cdot (h_1 - h_2) \\
 x_B &= k_{CO_2} \cdot (h_1 - h) \quad [\text{alternatively } x_B = k_{CO_2} \cdot (h_{B_2} - h_{B_1})].
 \end{aligned}$$

For calculating the pH change during the experimental period four quantities are required, namely the bicarbonate contents and the CO_2 pressures at the beginning and end of the period. The respective bicarbonate contents are obviously given by the movement of the manometer on adding the HCl at these times (h_{B_1} and h_{B_2} on the diagram). The point from which h is measured is obtained as usual by extrapolation and h_{B_1} must of course be taken to the same point. The movement of the manometer on introducing the KOH gives the total CO_2 pressure at that time. As the HCl has already been added this includes

the CO_2 from the bicarbonate, which must be subtracted in order to obtain the free CO_2 at the time of addition of the HCl . Thus the partial pressure of CO_2 at the beginning of the experimental period is $C \cdot (h_{C_1} - h_{B_1})$ and at the end of the period it is $C \cdot (h_{C_2} - h_{B_2})$. On inserting these values into the Henderson-Hasselbalch equation and rearranging terms the constant factors cancel out and we obtain for the change of pH

$$\Delta \text{pH} = \log \frac{h_{B_2}}{h_{B_1}} + \log \frac{h_{C_1} - h_{B_1}}{h_{C_2} - h_{B_2}} \quad [\text{cf. Dickens \& Šimer, 1932}].$$

It should be noted that h_1 and h_2 are the actual readings of the manometer (corrected for any small initial errors), but that h , h_{B_1} , h_{B_2} , h_{C_1} and h_{C_2} are all changes of reading at the respective times. Attention must be paid to the algebraical sign of h , h_1 and h_2 ; the other quantities are of course all essentially positive. In accordance with the usual convention, we take positive values of x_{O_2} and x_{CO_2} to indicate an output of gas and a positive value of x_B to indicate an increase of bicarbonate content.

From the retention curve we can now read off the retention R corresponding to the change ΔpH , and the glycolysis is then given by $x_G = -x_B + R$ [Dixon, 1934, p. 94]. The sign of x_G is thus chosen so that a positive value indicates a production of acid (i.e. a decrease of bicarbonate content).

The Q_{O_2} and Q_G values are then obtained by dividing x_{O_2} and x_G respectively by the dry weight of tissue in the right-hand flask and correcting to 1 hr.; the r.q. is of course given by $x_{\text{CO}_2}/x_{\text{O}_2}$. The calculations for the experiment on retina are shown in the lower part of Protocol II. The results are in good agreement with the recent measurements of Elliott & Baker [1935] on rat retina in Ringer-bicarbonate solution.

Numerous blank experiments on serum containing glucose without tissue have shown that no process affecting the manometer occurs in the serum itself, the quantities h , h_1 and h_2 all being zero within a fraction of a millimetre.

Measurement of anaerobic glycolysis in serum

It is obviously not possible to determine the anaerobic glycolysis on the tissue slice which is used for the aerobic glycolysis and the respiration, and a second manometer is required for its determination. This manometer is filled with a $\text{N}_2 + \text{CO}_2$ mixture instead of the usual $\text{O}_2 + \text{CO}_2$ mixture, but otherwise the procedure and calculation are exactly the same. As there can be no O_2 absorption by the tissue h_2 will now be zero.

It is necessary to free the gas mixture completely from O_2 by passing it over hot copper, and in order to drive out all O_2 from the flasks the gas should be passed for 15 min. Actual measurements have shown that after passing the gas for 15 min. at the standard rate (200 ml. per flask per min.) less than 5 μl . of O_2 are left in each flask.

Improvements in the technique

As a result of more extensive personal experience of the Dixon-Keilin method and the investigation of certain difficulties experienced by other workers when using the method, the writer has been able to effect a considerable improvement in its accuracy and reliability.

Several workers found that the readings at the end of the experiments showed a slow drift and did not reach their final value for some hours [Elliott & Schroeder, 1934; Gemmill & Holmes, 1935]. This they attributed to a slow rate of CO_2

absorption. As such an effect causes a great waste of time, the writer attempted to discover its cause. The rate of absorption of CO_2 was first studied by filling a manometer with the 95 % N_2 + 5 % CO_2 mixture and admitting KOH to one side only. It was found that, provided that the filter-paper roll projected at least 5 mm. above the top of the central tube, the absorption of CO_2 from the flask and the tubes connected therewith was invariably absolutely complete within 1 hr., and the reading then remained unchanged for many hours. It was then thought that the alkali-soaked filter-paper might be slowly absorbing oxygen, and this was tested by repeating these experiments with the 95 % O_2 + 5 % CO_2 mixture. It was found that the effect, though present, was very small (less than 1 mm. in 3 hr.) and would be completely undetectable if KOH-papers were present on both sides as in the ordinary technique. The next possibility was that some slow reaction, not involving CO_2 , was occurring in the acidified tissue. Experiments were therefore carried out in Barcroft manometers by adding HCl to tissues in Ringer solution in the absence of CO_2 and with KOH-papers present, but it was found that the readings remained absolutely constant for many hours after adding the acid, so that this possibility also was eliminated.

These experiments appeared to show that there was no process occurring in the flasks which could produce the drifts observed on the manometer, and attention was therefore turned to the manometer tube itself. Manometric displacements were artificially produced by applying slight suction to one side of the manometer, the taps were closed so as to maintain the differences for some time and then both sides of the manometer were once more opened to the air. These experiments at once revealed the cause of the effect. With small displacements the liquid at once returned to its original zero reading, but with large displacements, such as occur in experiments with tissues, the manometer did not return to zero but still showed a reading of 2–3 mm. which gradually became less but did not reach zero for some hours. This was not due to any kind of friction effect or to incomplete wetting of the tube, for if, when the manometer was showing a reading of 2 mm., it was artificially brought to zero and released it returned to the 2 mm. reading against the action of gravity. It appears that if the liquid falls in say the left-hand tube a layer of liquid possessing a comparatively large surface is left on the walls of the tube. A surface film, perhaps mainly of bile salts, then forms on the surface of this layer, and when the liquid returns this film is compressed and exerts a surface pressure (reduction of surface tension) on that side. No such process has occurred in the right-hand side and there will therefore be a difference of surface tension between the two sides and consequently a slight negative reading, which will slowly disappear as the compressed film breaks down or redissolves.

The omission of bile salts from the Clerici solution reduced, but did not altogether eliminate, this effect, but it disappeared completely on increasing the bore of the manometer tube from about 0.8 to 2.0 mm.² (This is not permissible with ordinary Barcroft manometers, owing to the appearance of additional terms in the expression for the constant, but in this case bores up to 2.5 mm.² may be used, for these terms remain small owing to the use of a heavy liquid.) A number of tissue respiration experiments with manometers with such wide-bore tubes soon showed that the trouble was completely cleared up. All the drifts had completely disappeared, the readings were more quickly reached and remained absolutely steady and the whole method showed a great improvement in accuracy and reliability. It may be concluded that the drifts were not due to incomplete absorption of CO_2 or any such cause, but were due simply to surface tension effects in the manometer tubes. The improvement effected by

substituting wider bore tubes is so marked that the writer recommends that such tubes should always be used in this method.

Elliott & Baker [1935] found in numerous blank experiments that h_1 with their manometers was not zero, but always had a negative value of up to 2 mm., and they found it necessary to subtract this blank correction from all their values for h_1 . They state: "It is not understood why this error in h_1 should occur, or why it is always negative." This error is undoubtedly explained by the above observations. The reason why it is always negative is that the manometric liquid has necessarily been subjected to a large negative displacement for some time shortly before reading h_1 in the ordinary course of the experiment. This error does not occur in manometers with wide-bore tubes.

These observations do not invalidate any of the previous work; correct results were certainly obtained in the circumstances by applying the blank correction and by waiting until the drifts had ceased.

The Clerici solution purchased ready prepared has proved unsatisfactory in several respects, especially in the slow deposition of crystals and precipitates. It is preferable to prepare it from the pure constituents, which can now be bought fairly cheaply (from British Drug Houses). By dissolving 7 g. thallium malonate + 7 g. thallium formate in 1 ml. water a thoroughly satisfactory clear solution is obtained which undergoes no change with time, runs freely in the tubes and has a P_0 value of about 2500. Bile salts are now omitted, for the reason given above.

Instead of lanoline, the writer now uses for all the ground joints and taps the "Paraffin and Rubber Lubricant (Thick for Summer use)" supplied in tubes by British Drug Houses. Glass rods instead of mercury are always used in the KOH tubes.

Certain cases in which workers have obtained erratic results have proved on investigation to be due to their having dispensed with a flowmeter and having consequently passed insufficient amounts of gas through the flasks initially. Obviously if different amounts of CO_2 are present initially in the two flasks of the manometer considerable errors will be produced. It is absolutely necessary for accuracy that at least the specified amounts of gas should be passed through all the flasks.

Other technical details are given by Dixon [1934, pp. 96-107] and by Elliott & Schroeder [1934] who have submitted the whole method to a thorough critical examination.

SUMMARY

The method of Dixon & Keilin has been adapted to the measurement of the respiration and glycolysis of tissues in serum.

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