CCXLVII. THE MEASUREMENT OF TISSUE GLYCOLYSIS IN SERUM. II

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(Received 20 September 1937)

THE manometric method of Dixon & Keilin [1933], when used for the measurement of the metabolism of tissue slices in serum, gives directly the respiratory O_2 and CO_2 , but in order to obtain the glycolysis as well a correction for the "retention" by the serum must be applied. To evaluate the correction the retention curve of the serum must be determined, and a method of doing this has recently been worked out by Dixon [1937]. This method requires three manometers of the Dixon-Keilin type, and it gives five points on the curve.

For all practical purposes, however, it may be considered that the retention curve (which is almost a straight line) would be determined with sufficient accuracy by only three points at suitably spaced pH values, since it is merely used to determine the magnitude of a correction. In this case a considerable simplification in the determination of the retention curve becomes possible, in fact it is readily carried out in a single manometer and requires only 4 ml. of serum.

This simplification makes it possible to obtain more physiological conditions than hitherto. Owing to the greater complexity of the former method a stock of serum sufficient for a number of experiments had to be prepared, so that one retention curve would serve for the whole series of experiments. Using the present method it becomes practicable to determine the retention on a small amount of serum simultaneously with the actual experiments. It therefore becomes possible in many cases to study the metabolism of the tissue slices suspended in the animal's own fresh serum, instead of in the inactivated serum of another species. The serum, which need not then be inactivated, may be prepared while the tissue slices are being cut. The animal must of course be of such a species that the serum does not gel immediately on acidification at 38° [see Dixon, 1937]. Rabbit, rat, chicken, guinea-pig and sheep are satisfactory in this respect; horse, pig and ox cannot be used. The serum must be obtainable in sufficient amount (4 ml. for the retention curve and 4 ml. for each complete determination of tissue respiration and glycolysis). The amount obtainable from one rat is insufficient, but two moderately large rats will yield a sufficient quantity. If necessary the amount of serum used in the manometers may be reduced.

PROCEDURE

On referring to protocol I of the paper of Dixon it will be seen that manometers 1 and 2 each give two points on the curve, each point giving the conditions after adding a certain amount of tartaric acid. Manometer 3 gives the initial point, representing the conditions before adding any acid, and also supplies data for the calculation of the other points. Now if three points only are required, manometer 1 can obviously be dispensed with. Moreover, the functions of manometers 2 and 3 can be combined in a single manometer as follows:

Serum is placed in both flasks, together with hanging Keilin tubes containing suitably chosen amounts of tartaric acid solution (dried), arranged as described by Dixon. In addition, the left-hand side bulb contains excess of HCl and the right-hand bottom tap contains KOH. After filling with gas mixture the Keilin tube on the left-hand side is dislodged, the resulting reading corresponding with one of the two readings of manometer 2 of Dixon's protocol. The HCl is now added from the side bulb, liberating the CO_2 from the remainder of the bicarbonate. As excess of acid is now present all the bicarbonate is decomposed and the previous addition of the tartaric acid does not affect the final reading. Thus the manometric displacement, reckoned from the original zero, gives the initial total bicarbonate, corresponding with the first reading of manometer 3. The Keilin tube on the right-hand side is now dislodged, the resulting displacement corresponding with the second reading of manometer 2. Finally the alkali is introduced on the right-hand side and absorbs all the CO_2 . As the CO_2 which has been liberated by the tartaric acid in this flask is once more absorbed it does not affect the final reading, and the manometric displacement, reckoned as usual from the reading after the addition of the HCl, gives (when multiplied by the correction factor C) the initial CO_2 tension. The calculation from the various quantities thus determined then proceeds exactly as before.

The whole procedure and method of calculation will become clear when the protocol I of the paper of Dixon is compared with the protocol given below. In order to avoid confusion the symbol h is here used only for the displacements produced by the tartaric acid, those produced by the HCl and KOH (which give the initial conditions and correspond with the former manometer 3) being here called h_0 . For reasons of space the initial values of the various quantities, which are calculated from these latter readings, have been placed centrally in the lower part of the table, as they apply to both flasks. The initial bicarbonate (1180 $\mu\text{l.}$) is first obtained from h_0 on the left-hand side, and the other values of B are then obtained by subtracting the amounts which have been decomposed (x_{CO_2}). Similarly the initial CO_2 pressure (110 mm.) is obtained from the right-hand h_0 , and the other p_{CO_2} values obtained by adding the readings h multiplied by C . The pH values are then calculated in the usual way.

The four manometric operations need not necessarily be carried out in the order given here, which was merely chosen as giving the clearest explanation of the principles of the method. At the end of the experiment the manometer reading approaches the ends of the scale, but it will not exceed it unless the barometer is unusually high or the bicarbonate content of the serum is above the average. The protocol gives an extreme case. If it is felt that there is any danger of the fluid going beyond the scale the left-hand tap may be opened for a moment after the readings relating to the left-hand flask have been completed, so as to level the manometer approximately. After waiting for a few minutes the new zero is read and the subsequent readings are reckoned from this zero.

The retention curve given by this protocol will be found to lie somewhat more to the right than that given in Dixon's paper, owing to the higher bicarbonate content, but the two curves are parallel, showing that the retention for a given change of pH is the same.

SUMMARY

The method of Dixon for determining the retention curve of serum has been simplified so that the whole determination is carried out in a single manometer on 4 ml. of serum. This makes it possible in many cases to carry out measurements on tissue slices suspended in serum from the same animal.

PROTOCOL

Determination of retention curve of sheep serum

Manometer	7		
k_{CO_2}	14.20		
C	0.97		
Side	Left	Right	
In flask: 2 ml. of	Serum	Serum	
In side bulb: 0.3 ml. of	3 <i>N</i> HCl	Water	
In bottom tap	—	20% KOH	
In hanging tube: g. of <i>N</i> /10 tartaric acid solution (dried)	0.1025	0.2599	
Time (min.)	Readings (mm.)		Diff. (mm.)
0	101.8	102.1	— 0.3
0	Tube in		
20	95.2	108.7	— 13.5
20	HCl in		
40	60.1	143.5	— 83.4
40		Tube in	
60	76.0	128.0	— 52.0
60		KOH in	
120	4.1	200.5	— 196.4
$h =$	13.2	31.4	
$x_{\text{CO}_2} =$	187	446	
$x_{\text{T}} =$	228	577	
$R =$	41	131	
$h_0 =$	83.1	113.0	
$B =$	993	1180	
$p_{\text{CO}_2} =$	122	110	
" H_2CO_3 " =	49.8	44.9	
$p\text{H} =$	7.400	7.525	7.209

One of us (B. B.) wishes especially to express his gratitude to Sir F. G. Hopkins for his kindness in extending to him the hospitality of his Department.

REFERENCES

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