XXVIII. THE RETENTION OF ACIDS BY SERUM BASED ON THE RETENTION COEFFICIENT: ITS USE IN MANOMETRIC EXPERIMENTS

By FRANK DICKENS

*From the Research Laboratory, North of England Council of the British
Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle-upon-Tyne*

(Received 30 December 1938)

THE two-vessel method of Warburg [1924] has the unique advantage for the measurement of tissue metabolism that it permits the simultaneous and continuous reading of respiration and total acid production. Whilst serum may be used as the medium for such measurements [Warburg, 1925], and is a more physiological medium than simple saline solutions, the retention of part of the evolved lactic or carbonic acid caused by its interaction with the buffers other than bicarbonate brings with it the need for special retention corrections.

Hitherto, two procedures have been available: (1) In the original method of Warburg [1925] the retentions for lactic and carbonic acids are calculated from the data of a separate acidification experiment, and from the bicarbonate and CO_2 contents of the serum. The correction obtained applies only to experiments in which the change of CO_2 pressure is close to that found in the acidification experiment. (2) Some of the inconvenience of the former method is avoided by acidification in the actual vessels used for measurement of tissue metabolism [Warburg *et al.* 1931], all of which must therefore be provided with side-bulbs. This is not always convenient, and some loss of accuracy may result from the fact that the acidification is made with the tissue present in the vessels. With some tissues the addition of lactic acid may cause an appreciable change in metabolism. Even slight errors in reading may cause an appreciable error in the retention determination, and it is not easy to secure high accuracy in presence of respiring or glycolysing tissue. In this method also, the retention correction applies only to a pressure change close to that found in the acidification experiment. Separate retention estimations for CO_2 and lactic acid are necessary.

In the principle to be described, instead of basing the calculation of retention on the Michaelis dissociation residue, as was done by Warburg [1925], we have worked from the empirical finding that the retention, or decrease of alkali proteinate, is directly proportional to the change of $p\text{H}$. This was first shown by Van Slyke *et al.* [1923] for dialysed serum, and afterwards by Dickens & Šimer [1932] and Dixon [1937] for normal serum. Over the physiological range of $p\text{H}$ the relationship may be regarded as linear, and an essential simplification is thus introduced. It is also possible with this method to calculate the retention for any observed pressure change.

Nomenclature

The following symbols are used throughout:

H^+ = hydrogen ion concentration of the serum.

K = 1st dissociation constant of carbonic acid (for serum we take $K = 7.94 \times 10^{-7}$, $pK = 6.10$ [Hastings *et al.* 1928]).

p = partial pressure of CO_2 (mm. Brodie solution).

B = bicarbonate concentration ($\mu\text{l. per ml.}$).

β = solubility coefficient of CO_2 in serum ($\mu\text{l. CO}_2/\text{ml.}$ for a pressure of 1 atm. see below).

v_F = volume of serum in vessel (ml.).

Δu = retention of CO_2 per ml. serum per mm. change of p (where u is the hypothetical concentration of the acidic buffers other than bicarbonate, e.g. proteiates, in the serum, expressed in $\mu\text{l./ml.}$ as usual).

All pressures are reckoned in mm. Brodie fluid, of which 10,000 mm. = 1 atm. Volumes of gas, or of acids and bases considered as gases in the usual convention, are in $\mu\text{l.}$ at N.T.P. Volumes of liquids are in ml. throughout.

With these units, the solubility coefficient, β , is equal to $\alpha/10$, where α is the Bunsen absorption coefficient of the gas. For values of α for various salt solutions and for serum see Van Slyke *et al.* [1928].

The "Retention Coefficient" R

Since within the limits shown by Dickens & Šimer [1932] the retention- $p\text{H}$ curve of normal serum is linear, we may write

$$-\frac{du}{d(p\text{H})} = R \quad \text{.....(1),}$$

where R is an empirical constant which will be called the retention coefficient, denoting the retention by 1 ml. serum, expressed in $\mu\text{l.}$ per unit change of $p\text{H}$. R is a positive quantity, and its value varies between about 100 and 200 for normal sera of different species.

$$\text{Now} \quad R = -\frac{du}{d(p\text{H})} = -\frac{du}{d\text{H}^+} \frac{d\text{H}^+}{d(-\log_{10}\text{H}^+)} = 2.303 \frac{du}{d\text{H}^+} \text{H}^+ \quad \text{.....(2).}$$

It may be shown (Table I) that, to a first approximation, taking finite increases and the mean value of H^+ over the interval H_0^+ to $(\text{H}_0^+ + \Delta\text{H}^+)$

$$R = 2.303 \frac{\Delta u}{\Delta\text{H}^+} \left(\text{H}_0^+ + \frac{\Delta\text{H}^+}{2} \right) \quad \text{.....(2a),}$$

$$\text{whence} \quad \frac{\Delta\text{H}^+}{\text{H}_0^+} = \frac{2.303\Delta u}{R - \frac{2.303\Delta u}{2}} \quad \text{.....(3).}$$

Table I. Accuracy of equation (3)

From equation (1) $\Delta u/R = -\Delta p\text{H}$. From equation (2a) $\Delta u/R \sim \Delta\text{H}^+/2.303 \left(\text{H}_0^+ + \frac{\Delta\text{H}^+}{2} \right)$.

In Table I these two values of $\Delta u/R$ are compared. The difference is the error in equation 2a.

$p\text{H}$	$\text{H}^+ \times 10^8$	$\Delta\text{H} \times 10^8$	$\Delta u/R$		Difference %
			$\frac{\Delta\text{H}^+}{2.3 \left(\text{H}_0^+ + \frac{\Delta\text{H}^+}{2} \right)}$	$-\Delta p\text{H}$	
7.3979	4 (= H_0)	—	—	—	—
7.3468	4.5	0.5	0.0510	0.0511	0.2
7.3010	5	1	0.0965	0.0969	0.4
7.2596	5.5	1.5	0.1369	0.1383	1.0
7.2218	6	2	0.1737	0.1761	1.4

Acidification of serum

1. *General case.* The partial pressure of CO_2 in equilibrium with the serum increases from p_0 to $p_0 + \Delta p$. Since

$$p_0 = B_0 \text{H}_0^+ / K\beta \quad \text{and} \quad p_0 + \Delta p = (B_0 + \Delta B) (\text{H}_0^+ + \Delta\text{H}^+) / K\beta$$

therefore

$$\frac{\Delta p}{p_0} - \frac{\Delta B}{B_0} = \frac{\Delta\text{H}^+}{\text{H}_0^+} \left(1 + \frac{\Delta B}{B_0} \right) \quad \text{.....(4).}$$

Substituting from equation (3) the value of $\Delta H^+/H_0^+$

$$\frac{\Delta u}{\Delta p} = \frac{R \left(1 - \frac{p_0 \Delta B}{B_0 \Delta p} \right)}{2.303 p_0 \left(1 + \frac{\Delta B}{2B_0} + \frac{\Delta p}{2p_0} \right)} \quad \dots\dots(5).$$

Equation (5) represents the general case when p , B and u vary. Two special cases are important.

(a) *Acidification of serum by addition of lactic or other strong acid.* In a vessel of total volume v ml., containing v_F ml. serum and v_T ml. total liquid including serum, maintained at a temperature of T° absolute, let m μ l. lactic acid be added to the serum, and the resulting increase of partial pressure of CO_2 be Δp mm. Brodie.

The "vessel constant for Ringer solution" $k_{\text{CO}_2}^R$, [Warburg, 1925] is given by

$$k_{\text{CO}_2}^R = \frac{273}{10T} (v - v_T) + v_T \beta.$$

Change of bicarbonate concentration on acidification = ΔB

$$\Delta B = -\Delta p \ k_{\text{CO}_2}^R / v_F.$$

Substituting in (5) and writing the quotient with suffix L , denoting acidification by lactic acid

$$\left(\frac{\Delta u}{\Delta p} \right)_L = \frac{R}{2.303} \frac{1 + \frac{p_0 k_{\text{CO}_2}^R}{B_0 v_F}}{p_0 + \frac{\Delta p}{2} \left(1 - \frac{p_0 k_{\text{CO}_2}^R}{B_0 v_F} \right)} \quad \dots\dots(6).$$

Equation (6) shows that the retention for lactic acid is dependent on the vessel constant and volume of serum. In these respects it differs from the retention for CO_2 . The term in Δp indicates the extent of variation of the retention with CO_2 pressure-change, which was not previously calculable.

(b) *Acidification of serum by addition of carbonic acid.* Since for each equiv. of CO_2 which is combined, 1 equiv. of HCO_3^- results, accompanied by an increase of 1 equiv. in u , in this case

$$\Delta u = \Delta B.$$

Substituting in (5), and writing the quotient with suffix C , denoting retention for CO_2

$$\left(\frac{\Delta u}{\Delta p} \right)_C = \frac{R}{2.303 \left\{ p_0 + \frac{\Delta p}{2} \left(1 + \frac{p_0 \Delta u}{B_0 \Delta p} \right) \right\} + \frac{R p_0}{B_0}} \quad \dots\dots(7).$$

Equation (7) shows that the retention for CO_2 is independent of the vessel constant and the volume of serum, but depends on the particular value of Δp as is evident from the bracketed term in the denominator. This term, involving $(\Delta u/\Delta p)$ is nearly equal to $p_0 + \frac{\Delta p}{2}$ unless p_0 is large compared with B_0 , since the average value of $(\Delta u/\Delta p)$ is of the order 0.1. Consequently we may first calculate an approximate value of $(\Delta u/\Delta p)$ using the approximate relationship

$$\left(\frac{\Delta u}{\Delta p} \right)_C \sim \frac{R}{2.303 \left(p_0 + \frac{\Delta p}{2} \right) + \frac{R p_0}{B_0}} \quad \dots\dots(7a),$$

and afterwards insert this value of $(\Delta u/\Delta p)$ in the denominator of equation (7). In many cases the effect of this correction term will be almost negligible, but the calculation is very easy in practice, so that it is better to insert the correction as a routine.

Determination of the retention coefficient, R

Since R represents the retention per unit change of pH , it may be determined by any of the three methods available for determining retention [Warburg, 1925]. These are (1) acidification with a known amount of lactic acid; (2) acidification with a known amount of CO_2 and (3) dilution of the serum. The third method is complicated by the fact that since R is dependent on the concentration of non-bicarbonate buffers, the value of R will change during dilution. For this reason methods (1) and (2) are preferable.

The calculation of R may also be made by either of two methods: (a) R may be derived from the above equations (6) and (7) for $(\Delta u/\Delta p)$ or (b) R may be calculated directly from the data of the acidification experiment, using a logarithmic method. Since these calculations afford an excellent check on the accuracy of the various expressions, they will be described separately.

1a. *Calculation of R by acidification with lactic acid: derivation from $(\Delta u/\Delta p)_L$.* Let ϵ ml. of a solution of lactic acid containing $m \mu l.$ acid be added to v_F ml. serum contained in a vessel of Ringer constant $k_{CO_2}^R$, and let the observed pressure change be h mm. Brodie. Let the pressure change due to dilution of the serum thus caused be h' mm.; it should be noted that h' is negative [Warburg, 1925] and its sign must be taken algebraically. Let H be the pressure change due to acidification alone, without dilution.

Then

$$\begin{aligned} H &= h - h', \\ \Delta u &= \frac{m - Hk_{CO_2}^R}{v_F}, \\ \Delta p &= h. \end{aligned}$$

Hence $(\Delta u/\Delta p)_L$ is directly calculated from the acidification experiment. If now ϵ is a small volume, e.g. $< 5\%$ of v_F , R will remain sensibly constant during dilution. Hence from equation (6)

$$R = \frac{2.303 (m - Hk_{CO_2}^R) \left(p_0 + \frac{h}{2} - \frac{h p_0 k_{CO_2}^R}{2 v_F B_0} \right)}{h \left(v_F + \frac{p_0 k_{CO_2}^R}{B_0} \right)} \quad \dots\dots(8).$$

1b. *Calculation of R from acidification with lactic acid: logarithmic method.* In the above experiment, the initial conc. of bicarbonate = B_0 . Final conc. of bicarbonate after acidification

$$= B_1 = \frac{B_0 v_F - h k_{CO_2}^R}{v_F + \epsilon}.$$

Hence
$$\Delta pH = pH_1 - pH_0 = \log \frac{B_1}{(p_0 + h) \beta} - \log \frac{B_0}{p_0 \beta}$$

and
$$\Delta u = (m - h k_{CO_2}^R) / v_F.$$

Therefore
$$R = - \frac{\Delta u}{\Delta pH} = + \frac{m - h k_{CO_2}^R}{v_F} \bigg/ \log \frac{\left(1 + \frac{\epsilon}{v_F}\right) \left(1 + \frac{h}{p_0}\right)}{1 - \frac{h k_{CO_2}^R}{B_0 v_F}} \quad \dots\dots(9).$$

This equation eliminates the need for a dilution correction, and is the best form to use.

2a. *Calculation of R from acidification with CO_2 : logarithmic method.* In this case the acidification occurs in a special vessel of the type described by Warburg *et al.* [1931]. An outer compartment contains $NaHCO_3$ - $NaCl$ solution, into which $m \mu l.$ lactic acid are tipped, thus liberating $m \mu l.$ CO_2 . In an inner compartment of the same vessel are v_F ml. serum. Part of the CO_2 liberated from the

NaHCO_3 recombines with the buffers of the serum. The pressure change observed is h mm.

$$\begin{aligned} \text{Then} \quad \Delta u &= (m - h k_{\text{CO}_2}^R) / v_F, \\ \Delta p\text{H} &= \log \left(1 + \frac{m - h k_{\text{CO}_2}^R}{B_0 v_F} \right) \left(\frac{p_0}{p_0 + h} \right) \end{aligned} \quad \text{.....(10).}$$

Whence $R = -(\Delta u / \Delta p\text{H})$ is readily calculated.

2b. *Calculation from equation (7).* In the above experiment, Δu is given and $h = \Delta p$. Hence the quotient $(\Delta u / \Delta p)_C$ is directly obtained and from (7):

$$R = \frac{2.3 (\Delta u / \Delta p)}{1 - \frac{p_0 \Delta u^2}{B_0 \Delta p}} \left\{ p_0 + \frac{\Delta p}{2} \left(1 + \frac{p_0 \Delta u}{B_0 \Delta p} \right) \right\} \quad \text{.....(11).}$$

Vessel constants

When the values of $(\Delta u / \Delta p)_C$ and $(\Delta u / \Delta p)_L$ for the required range of Δp have been calculated by means of equations (6) and (7), it is only necessary to insert these values in equations (12) and (13) to obtain the respective vessel constants for serum [Warburg, 1925].

$$k_{\text{CO}_2}^S = k_{\text{CO}_2}^R + v_F \left(\frac{\Delta u}{\Delta p} \right)_C \quad \text{.....(12),}$$

$$\text{and} \quad k_L^S = k_{\text{CO}_2}^R + v_F \left(\frac{\Delta u}{\Delta p} \right)_L \quad \text{.....(13).}$$

The calculation of the result of a 2-vessel metabolic experiment may then be made either by the original Warburg [1925] method, or by the method of Warburg *et al.* [1931]. Average values of R.Q. for various tissues, which are required for the latter method, are given by Dickens & Šimer [1930; 1931, 2].

Retention in the Dickens-Šimer R.Q. method

In the direct method for measurement of metabolism in bicarbonate media [Dickens & Šimer, 1931; 1932; Dixon & Keilin, 1933; Dixon, 1937] the measurement of respiration is not affected by retention, unlike the same measurement by the Warburg 2-vessel method. But for the measurement of glycolysis by these methods the present principle has the advantage of greater accuracy compared with the "retention curve" estimations previously described [Dickens & Šimer, 1932; Brekke & Dixon, 1937]. $\Delta p\text{H}$ being known directly from the manometric readings [cf. Dickens & Šimer, 1932; 1933] it is only necessary to multiply this by R in order to obtain the actual retention, in $\mu\text{l.}$ per ml. serum, which occurred during the experiment. R is determined as usual by acidification by lactic acid. Only two extra vessels (with Brodie fluid in the manometers) are needed, one for acidification of serum and one for estimation of the strength of the lactic acid (cf. Table IV, vessels 2 and 4).

Dilution correction

When R is determined from the value of $(\Delta u / \Delta p)_L$ (but not by the logarithmic method) a correction h' due to the change of $p\text{H}$ of the serum on dilution with the small volume ϵ has to be applied (equation 8). The separate determination of h' is generally unnecessary, since it may be readily calculated from the value of R , as follows (the suffix D denotes dilution):

$$\Delta \text{H}_D^+ = \frac{\beta K (p_0 + h')}{B_0 \frac{v_F}{v_F + \epsilon} - \frac{h' k_{\text{CO}_2}^R}{v_F + \epsilon}} - \frac{\beta K p_0}{B_0} \quad \text{.....(14).}$$

If h' is small (it is *c.* -5 mm. for $\epsilon=0.1$ ml.) and B_0 and $k_{\text{CO}_2}^R$ are of the usual order (*c.* 500 and 1 respectively), $h'k_{\text{CO}_2}^R$ is negligible in relation to $B_0 v_F$ and since p_0 is usually *c.* 400, $(p_0 + h') \sim p_0$

$$\Delta H_D^+ = \frac{\beta K p_0}{B_0} \left(\frac{\epsilon}{v_F} \right) = H_0 + \frac{\epsilon}{v_F}$$

and

$$\Delta u = - \frac{h k_{\text{CO}_2}^R}{v_F}$$

Hence from equation (2), regarding R as constant for this slight dilution,

$$h' = - \frac{R\epsilon}{2.3 k_{\text{CO}_2}^R} \quad \dots\dots(15).$$

For the purpose of equation (15), R may be given an approximate value of 150, provided that the volume ϵ does not exceed 0.1 ml. The error in h' is then unlikely to exceed *c.* 1.5 mm. in an extreme case. If greater accuracy is desired the logarithmic method of calculation (equation 9) may be used. The dilution effect may be avoided completely by using evaporated tartaric acid solution instead of lactic acid [Warburg, 1925] and writing $\epsilon=0$ and $h'=0$ in the above equations (8) and (9), but the use of a solution is convenient.

Typical case

For an average specimen of normal serum at 37.5° the following values are typical: $R=150$, $p_0=420$, $B_0=427$, $\alpha=0.51$ [Van Slyke *et al.* 1928], or $\beta=0.051$, $p_0\beta=21.4$, $pK=6.10$ or $K=7.94 \times 10^{-7}$ [Hastings *et al.* 1928], $pH_0=7.40$ or $H_0^+=4 \times 10^{-8}$.

Table II shows values of $\Delta u/\Delta p$ for this specimen of serum. The values of $(\Delta u/\Delta p)_C$ are calculated from equation (7). Correction of the value (e.g. for $\Delta p=100$) by means of equation (7a) only changes the value from 0.122 (uncorr.)

Table II. Calculation of $(\Delta u/\Delta p)$ and ΔpH for a typical specimen of serum

For details see text. Lactic retention calc. for a vessel of total volume 13.2 ml.; for $v_F=2$ ml., $k_{\text{CO}_2}^R=1.09$; for $v_F=7$ ml., $k_{\text{CO}_2}^R=0.902$. $R=150$.

Retention for CO ₂			
Δp mm. Brodie	$(\Delta u/\Delta p)_C$ from equation (7)	Δu $=\Delta p(\Delta u/\Delta p)_C$	ΔpH $= -\Delta u/R$
0	0.135	0	0
50	0.126	6.3	-0.042
100	0.119	11.9	-0.079
150	0.112	16.8	-0.112
200	0.106	21.2	-0.141

Retention for lactic acid; $(\Delta u/\Delta p)_L$ calc. from equation (6)						
Δp mm. Brodie	For $v_F=2$			For $v_F=7$		
	$(\Delta u/\Delta p)_L$	Δu	ΔpH	$(\Delta u/\Delta p)_L$	Δu	ΔpH
0	0.240	0	0	0.175	0	0
50	0.234	11.7	-0.078	0.166	8.3	-0.055
100	0.228	22.8	-0.152	0.158	15.8	-0.105
150	0.222	33.3	-0.222	0.151	22.6	-0.151
200	0.216	43.2	-0.288	0.145	29.0	-0.193

to 0.119 (corr.), an amount which is almost negligible in practice. On the other hand the effect of the correction for various values of Δp is quite large, and this explains why with the Warburg [1925] method of calculation, the retention could only be determined for one particular experimental value of Δp .

The values of $(\Delta u/\Delta p)_L$ in Table II are calculated from equation (6) for the same specimen of serum. For the same vessel, two series of values are given to show the effect of varying v_F . The effect of Δp resembles that found for retention of CO_2 (Table II).

For a vessel of $k_{\text{CO}_2}^R$, about 1.0, an increase of p of about 100 mm. Brodie causes a decrease of the vessel constant for serum of about 5%. The effect of this in an experiment by the 2-vessel method may be to alter the metabolic quotient by an amount which may reach 10% of its value.

The change in $p\text{H}$ corresponding to any value of Δp may be readily computed from the equation: $\Delta p\text{H} = -\Delta p (\Delta u/\Delta p)/R$, in which it is important to use the correct quotient and value of Δp for CO_2 or lactic acid respectively. The values of $\Delta p\text{H}$ included in Table II show the order to be expected. This calculation is useful for two purposes; it enables one to guard against too great an acidity of the medium, and it may also be used in conjunction with Table I as a check on the error introduced by the approximation in equation (2a).

Practical details

Serum. The serum used in these experiments was obtained by allowing the blood to clot, centrifuging the supernatant serum, and inactivating by heating for two successive periods of 2 hr. at 56° .

The serum was warmed to 37° and shaken with the gas-mixture before use. It is advisable, and with larger volumes of serum essential, to pass the gas stream through the vessels containing the serum, while they are shaking in the bath at 37° . A comparison of the retention of 7 ml. serum contained in a vessel of total vol. 14 ml. gave, by previous gassing in the laboratory and then transferring to the bath $(\Delta u/\Delta p)_M = 0.131$; by gassing while shaking in the bath $(\Delta u/\Delta p)_M = 0.193$. Consequently the vessels should be provided with bored-out stoppers, and the gassing arrangement recommended by Dickens & Šimer [1933, p. 450] should be used.

Lactic acid solution. The solution, $c. M/20$, is refluxed for about 1 hr., cooled, NaCl added to make the conc. 0.9% and the strength of the solution determined manometrically. The strength remains unchanged for many weeks if the solution is kept sterile by occasionally heating in the stoppered flask in a boiling water bath.

Accurate measurement of the solution into the side-bulbs by a good, grease-free micro-pipette is essential.

The details of a complete retention estimation are given in Table III. The gas analysis is by the method described by Dickens & Šimer [1931, 1], using Clerici solution of density $c. 4$ as manometric fluid. If a Brodie thermobarometer is used, its readings must be divided by 4 before correcting the pressures in the gas analysis. Naturally the gas analysis is needed only when a new cylinder of gas is employed; correction of p_0 for various temperatures and barometric pressures may easily be made, if necessary.

In general, the acidification by lactic acid is to be preferred to the direct determination of $(\Delta u/\Delta p)_C$ as in vessel 1, Table III. Owing to the larger vessel volume necessary, the latter method is less accurate and cannot easily be applied to larger volumes of serum. An example is shown in Table III, for which the bicarbonate-R.Q. vessel of Dickens & Greville [1933] was used, and proved quite suitable; but it will be seen that the value of Δu obtained is greatly affected by a very small error in h . Consequently it is better to calculate $(\Delta u/\Delta p)_C$ from an experiment of the type shown in vessel 2. If the volume of serum is limited, 2 or 3 ml. may be used, or less with smaller vessels. The latter

Table III. *Protocol of a complete retention estimation*

Pig serum. 37.5°. O₂+CO₂ mixture passed through all vessels for 8 min. while shaking in the bath. α_{CO_2} for serum = 0.51, for Ringer solution = 0.54. Manometer fluid: Brodie solution, except No. 5 which was Clerici fluid of density 3.717. Strength of lactic acid solution: c. N/20 in 0.9% NaCl. Vessels: No. 1 bicarbonate-R.Q. type [Dickens & Greville, 1933]; Nos. 2 and 4 rectangular with side-bulb; Nos. 3 and 5 conical with side-bulb. All vessels with bored-out stoppers for gassing in bath. Barometer 758.2 mm. Hg.

Vessel no. ...	1	2	3	4	5
Exp.	CO ₂ retention	Lactic retention	Bicarbonate estimation	Strength of lactic acid	Gas analysis
Vessel vol. (ml.)	23.72	13.52	16.36	14.56	c. 20
v_T	4.10	7.10	0.70	2.1	2.3
v_F (serum)	2	7	0.5	—	—
$k_{\text{CO}_2}^R$	1.943	0.926	1.411	1.206	—
Vessel contents:					
Main pt.	2 ml. serum	7 ml. serum	0.5 ml. serum	2 ml. NaHCO ₃ -Ringer	2 ml. M/5 KMnO ₄ in N/500 H ₂ SO ₄
Outer ring	2 ml. NaHCO ₃ -Ringer	—	—	—	—
Side-bulb	0.1 ml. lactic acid	0.1 ml. lactic acid	0.2 ml. N HCl	0.1 ml. lactic acid	0.3 ml. 30% NaI, 2H ₂ O in N/500 H ₂ SO ₄
Pressure change on mixing (corrected for thermobarometer vessel):					
h	+49.5	+46.5	+155.5	+92 (Brodie)	* -81 (Clerici)
	$hk_{\text{CO}_2}^R + 96$	+43	$2hk_{\text{CO}_2}^R = B_0 = 439$	$hk_{\text{CO}_2}^R = m = 111$	$p_0 = 81 \times \frac{3.717}{13.60} \times \frac{10,000}{760}$ = 290 mm. Brodie
Dilution corr.	$h' = 0$	$h' = -5$	—	—	—
$(h - h') k_{\text{CO}_2}^R$	+96	+48	—	—	—
$(m - Hk_{\text{CO}_2}^R)/v_F = \Delta u$:	7.5	9.0	—	—	—
$\Delta u/h$	$(\Delta u/\Delta p)_C = 0.151$ for $\Delta p \sim 50$	$(\Delta u/\Delta p)_L = 0.193$ for $\Delta p \sim 45$	—	—	—
Calculation according to Warburg [1925]:					
	$(\Delta u/\Delta p)_C = 0.153$ for $\Delta p_C = 59$	$(\Delta u/\Delta p)_L = 0.192$ for $\Delta p_L = 46$	—	—	—
Calculation of R :					
(a) From equations (8) and (11)	$R = 123$	$R = 127$	—	—	—
(b) From equations (9) and (10)	$R = 123$	$R = 127$	—	—	—
Calculation of $(\Delta u/\Delta p)$, for mean value of $R = 127$, from equations (6) and (7), for above values of Δp :					
	$(\Delta u/\Delta p)_C = 0.152$	$(\Delta u/\Delta p)_L = 0.193$	—	—	—

* Corrected for pressure change of -3 mm. on mixing reagents [Dickens & Šimer, 1931, 1].

must be carefully made to provide a large surface of the serum, or equilibration with the gas mixture is slow. When using small volumes of serum care must be taken that the dilution effect is small (equation 15), and it may therefore be necessary to use evaporated tartaric acid instead of lactic acid.

Since the gas analysis will generally be done at once when the gas mixture is received, only three vessels are necessary for a complete retention estimation, viz. nos. 2, 3 and 4, Table IV. From the values of h , B_0 and m thus obtained, the retentions for lactic and carbonic acids for any desired values of Δp may at once be calculated by means of equations (9), (6) and (7). The value of R obtained

from equation (9) may be used to calculate the retention in any experiment in which the same specimen of serum is used at a value of pH within the physiological range.

Relation of retention to protein content of serum

According to Van Slyke *et al.* [1923] for dialysed horse serum the empirical relationship

$$\frac{BP}{(pH - 4.80)} = 0.68P$$

holds for the physiological range, where BP is the millimolar conc. of base bound by protein, and P the total protein in g./100 ml. Hence in our units for u

$$\Delta u = -\Delta (BP) \times 22.4,$$

and

$$R = -du/d(pH) = 0.68 \times 22.4P = 15.2P.$$

Table IV shows the relationship of R to P for 6 specimens of (undialysed) normal serum. The variation of R from $15P$ to $22P$ is rather large, not only with different species, but also with different sera of the same species. Hence it is not practicable to calculate R from the protein content of the serum, but is better to determine it manometrically for each specimen. The coincidence of the retention- pH curves for two particular specimens of horse-serum [Dickens & Šimer, 1932] and sheep-serum [Dixon, 1937] must therefore be regarded as fortuitous.

Table IV. *Value of R and total protein in various specimens of serum*

Species	...	Pig	Calf	Sheep I	Sheep II	Horse I	Horse II
Mean R^*		131	108	183	108	106	107
Total protein %		7.20	4.98	6.25	6.42	7.10	6.35
$R/(\% \text{ protein})$		18.2	21.6	22.2	16.8	15.0	16.9

* Mean of 7 estimations.

Retention by substances of known pK'

In manometric experiments made in simple NaHCO_3 -saline media, it is often necessary to consider if the retention of added substrates or other substances need be taken into account. Table V shows the retentions due to substances of various pK' , for different reactions of the medium.

Table V. *Value of R for various values of K'*

For a substance of dissociation constant K' , dissolved in conc. c' ($\mu\text{l./ml.}$) in a bicarbonate medium in equilibrium with CO_2 [Dickens & Šimer, 1932]

$$R = -\frac{d\rho}{d(pH)} c' = 2.3 \frac{H^+ K'}{(H^+ + K')^2} c'.$$

The following Table shows values of R for various values of H^+ and K' : the value of c' taken is equivalent to $M/130$ and gives $R_{\text{max}} = 100$.

pK'	R for $c' = 174$ at		
	$pH = 6.0$	$pH = 7.0$	$pH = 8.0$
4	4	0.4	0.04
5	33	4	0.4
6	100	33	4
7	33	100	33
8	4	33	100
9	0.4	4	33
10	0.04	0.4	4

Hence for a change of 0.1 pH unit the retention by $M/100$ buffer will be less than 0.5 μ l. per ml. solution, if the pK' of the buffer differs from the pH of the medium by more than 2. For other values of pK' and pH the retention can be calculated from the above equation. It should be noted that the effect of temperature and salt concentration on pK' may cause a considerable alteration in retention.

SUMMARY

The retention of acids by serum has been considered in relation to the change of pH of the serum. The "Retention Coefficient" R , representing the amount of retention per unit decrease of pH, has been used to calculate the retention of carbonic and lactic acids by serum. Methods are described for the calculation and estimation of R .

The application of this principle to the measurement of tissue metabolism in serum is described. By this means it is possible to calculate the value of the retention for any given pressure change, an advantage not possessed by other methods of determining retention.

The retention caused by the presence of substances of known dissociation constant is described.

I am grateful to Dr F. J. W. Roughton and Dr M. Dixon for useful criticism.

REFERENCES

- Brekke & Dixon (1937). *Biochem. J.* **31**, 2000.
Dickens & Šimer (1930). *Biochem. J.* **24**, 1301.
—— (1931, 1). *Biochem. J.* **25**, 973.
—— (1931, 2). *Biochem. J.* **25**, 985.
—— (1932). *Biochem. J.* **26**, 90.
—— (1933). *Abder. Handb. biol. Arb. Meth.* iv, **13**, 435.
—— & Greville (1933). *Biochem. J.* **27**, 1479.
Dixon (1937). *Biochem. J.* **31**, 924.
—— & Keilin (1933). *Biochem. J.* **27**, 86.
Hastings & Sendroy (1925). *J. biol. Chem.* **65**, 445.
—— & Van Slyke (1928). *J. biol. Chem.* **79**, 183.
Van Slyke, Sendroy, Hastings & Neill (1928). *J. biol. Chem.* **78**, 765.
—— Wu & McLean (1923). *J. biol. Chem.* **56**, 765.
Warburg (1924). *Biochem. Z.* **152**, 51.
—— (1925). *Biochem. Z.* **164**, 481.
—— Kubowitz & Christian (1931). *Biochem. Z.* **242**, 170.