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THE ACID-LABILE CO_2 IN MAMMALIAN MUSCLE AND THE *p*H OF THE MUSCLE FIBRE

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Wallace & Hastings [1942] and Wallace & Lowry [1942] have obtained relatively high values for the CO_2 content of resting mammalian muscle, which at first sight appear to be inconsistent with the theory concerning the distribution of ions in frog muscle, as presented from this laboratory [Conway & Boyle, 1939; Boyle & Conway, 1941]. With their figure of about 11 mM./kg. of total CO_2 in resting mammalian muscle, and allowing the free CO_2 to be in approximately equal concentration within and without the fibre, and the remainder of the total acid-labile CO_2 to be HCO_3^-, it follows that the ratio of HCO_3^- concentrations across the membrane would be far higher than that expected from a Donnan relation with the K concentrations. Therefore, either the mammalian muscle fibre has a very different electrolyte distribution and a different membrane permeability from that of frog muscle, or the fraction of the total CO_2 assigned by Wallace & Hastings to HCO_3^- is much too high. It was decided therefore to investigate the nature of the total available CO_2 in mammalian muscle. The solution to this problem has the further significance that it allows the *p*H value inside the muscle fibre to be determined; for whatever fraction is shown to be in truth HCO_3^- within the fibre, this, in conjunction with the free CO_2, determines the *p*H, at least within the accuracy of the *pK'* figure.

The following symbols are used in the calculations:

$[W]_s$ = g. water per kg. serum,
$[W]_m$ = g. water per kg. muscle,
$[HCO_3]_m$, $[HCO_3]_s$, $[HCO_3]_e$, $[HCO_3]_b$ = mM. HCO_3^- per kg. muscle, serum, extracellular fluid and whole blood respectively,
$[HCO_3]_{fw}$ = mM. HCO_3^- per kg. fibre water,
$[HCO_3]_{ew}$ = mM. HCO_3^- per kg. extracellular water,
$[HCO_3]_{sw}$ = mM. HCO_3^- per kg. serum water.

Similarly with the other ions, Na, Cl, K and H.

$[CO_2]_m$, $[CO_2]_s$, $[CO_2]_{fw}$ and $[CO_2]_{ew}$ are likewise mM. free CO_2 per kg. muscle, serum, fibre water and extracellular water,

$[CO_2, \text{total}]_m$ = the total acid-labile CO_2 per kg. muscle,

$[CO_2, \text{Ba sol.}]_m$ = the total Ba-soluble CO_2 in muscle.

The true H_2CO_3 is considered as included in the free CO_2 concentration.

Methods

Total acid-labile CO_2 *in muscle.* The animal was anaesthetized with ether, and then a portion of the abdominal muscle quickly excised, and introduced immediately into $N/5$ CO_2-free KOH in a weighed round-bottom centrifuge tube, the cork being removed momentarily for the purpose. 25 ml. $N/5$ KOH was used as a routine in a 50 ml. tube with about 8 g. muscle. Unless the quantity of abdominal muscle was small it was cut as rapidly as possible into approximately 2 g. portions. The whole process of removal and introduction occupied no more than 10–15 sec. from the time of sectioning the muscle.

With the leg muscle of rabbit or cat, about 10 g. were quickly excised and held over the KOH tube; quantities a few mm. thick were rapidly sectioned by sharp scissors and dropped into the alkali.

The stopper was replaced, the tube weighed, and the contents well mixed. The whole was then placed in the refrigerator for an hour, being shaken several times throughout this period.

The tube was then spun for a few minutes and 0·5 ml. volumes pipetted quickly into the outer chambers of Conway units (no. 2 size), already prepared with 0·2 ml. $N/25$ $Ba(OH)_2$ containing 5 % B.D.H. universal indicator, in the central chamber, and 0·2 ml. $2N$ H_2SO_4 in the outer chamber [Conway, 1939; O'Malley, Conway & FitzGerald, 1943].

The titrations were carried out after an hour, with $N/40$ HCl from a Conway micro-burette [Conway, 1939], to a green end-point.

The determinations were made in triplicate. The large standard units (Conway unit no. 1) were also occasionally used with 2 ml. extract, 1·3 ml. $N/40$, $Ba(OH)_2$ in the central and 0·5 ml. $2N$ H_2SO_4 in the outer chamber, with subsequent titrations of 1 ml. vol. removed from the central chambers into small tubes, using $N/10$ HCl.

Blank determinations were carried out in a similar manner, a volume of CO_2-free water corresponding to the water content of the muscle being pipetted into the alkali, and carried through the whole procedure as for muscle.

Calculation of mM. CO_2/kg. *muscle.* In the calculation it is assumed that the muscle contributes its water to the total fluid volume, the membranes being under the conditions freely permeable to all electrolytes other than protein which also escapes in a certain measure. The volume corresponding to 1 g. of muscle is thus $\frac{0{\cdot}77w+25}{w}$, where 25 ml. KOH solution is used.

For the procedure described with the no. 2 units the following formula applies:

$$\text{mM. } CO_2/\text{kg. muscle} = 0{\cdot}0625x\left(3+\frac{100}{w}\right), \tag{1}$$

where x = large divisions on burette corresponding to the CO_2 absorbed, taking the blank reading as zero absorption (each large division on the burette = 0·01 c.c.).

For the procedure with the no. 1, or standard units, the formula is

$$\text{mM. } CO_2/\text{kg. muscle} = 0{\cdot}0812x\left(3+\frac{100}{w}\right). \tag{2}$$

The Ba-*soluble fraction.* This was determined in a similar way, but prior to the CO_2 absorptions in the micro-diffusion units 5 ml. vol. of the alkali extract were pipetted into capped 15 ml. centrifuge tubes (tapered) and 1 ml. saturated $BaCl_2$ pipetted, added and mixed. These tubes were then centrifuged at about 3000 r.p.m. for at least 90 min. For abdominal muscle of rats, very young

rabbits (0·5 kg.) or guinea-pigs, there was in nearly all cases no apparent opacity left after this centrifuging and there was usually found to be extremely little on measuring with the Pulfrich Turbidimeter, the results being expressed in absolute values (using test-tubes and wedge illumination).

Total CO_2 *in blood plasma.* For this purpose the blood was collected under paraffin from a carotid cannula and centrifuged. 0·2 ml. vol. were pipetted into the outer chambers of Conway units (no. 2) with 0·2 ml. $N/10$ $Ba(OH_2)$ with 5 % B.D.H. universal indicator and 0·5 ml. $N/1$ H_2SO_4, in the outer chamber. The pipette used for the plasma was one delivering rapidly between two points. The procedure was controlled by similar deliveries of 0·2 ml. $M/50$ $KHCO_3$.

Chloride in plasma. This was carried out by a micro-diffusion procedure [Conway, 1935, 1939].

Chloride in muscle. The muscle was bubbled for an hour in 10 ml. 1·9 % Na_2SO_4 (anhydrous) per g. muscle. To 10 ml. of this extract in a 15 ml. centrifuge tube 1 ml. 10 % tungstate and 1 ml. $\frac{2}{3}N$ H_2SO_4 were added, mixed and the precipitate separated by centrifuging. 1 ml. vol. of the clear fluid were used for the analyses by the micro-diffusion procedure [Conway, 1939]. The procedure was varied by using 1·3 ml. 20 % KI instead of 1 ml. as previously described, and removing 1 ml. after 90 min. into a cell of the Spekker absorptiometer. 5 ml. water were added and mixed, and the readings controlled with $N/100$ HCl.

The initial immersion and bubbling with 1·9 % Na_2SO_4 was found advantageous for mammalian muscle, instead of the previously described method which was found useful for frog tissues, since grinding the mammalian muscle with tungstate and $\frac{2}{3}N$ H_2SO_4 gave a filtrate, from which the Cl emission in the micro-diffusion units was delayed. In the calculation it was assumed that under the conditions all the muscle water was freely available for interchanges, and that the Cl had the same concentration within as without owing to the breakdown of the membrane system. For the abdominal muscle used, with interspace value of about 25 %, the maximum error that could take place on this assumption is 5 % and the actual error would usually be less. This was considered sufficiently accurate for our purpose.

Blood in muscle. The $N/5$ KOH extracts all the blood from the muscle and probably small amounts of myohaemoglobin. The colour was compared with that of blood in $N/5$ KOH and suitably diluted.

Water in muscle. This was determined on weighed samples by heating for 12 hr. at 105° C.

Results

Rate of the alkali extraction of total CO_2 *from muscle*

In the method described for determining the total acid-labile CO_2 in muscle, an hour was allowed for the alkali extraction. It will be seen from Fig. 1 (experiments on very young rabbits, 300–500 g. wt.) that the extraction is practically complete after this time, and for the investigations to be described subsequently, the shortest extraction period possible was desirable—consistent with the removal of all or nearly all the CO_2.

The total acid-labile CO_2 *in mammalian muscle*

Table 1 gives representative data for the total CO_2 for abdominal and leg muscle (as well as the Ba-soluble fraction and the opacity of the extract with $BaCl_2$ after centrifuging 90 min. or more).

For the rat abdominal muscle the mean total CO_2 was 14·8 mM./kg. (range of 13·4–15·9). For the eight young rabbits it was 11·4 with S.D. for the single observation of 0·7 (S.D. of mean = 0·3). For seven guinea-pigs it was 10·3 with S.D. of 1·2. The mean for the leg muscle of eight rabbits was 10·6 with

S.D. of 1·2 and for the leg muscle of two cats it was 10·1 and 10·8 mM./kg. Comparing these values with those of Wallace & Hastings [1942] using a different method [Danielson & Hastings, 1939], the mean value for the leg muscle of the cat (taking their 14 control series) was 11·0 with S.D. of 1·3, agreeing very well, therefore, with the above results.

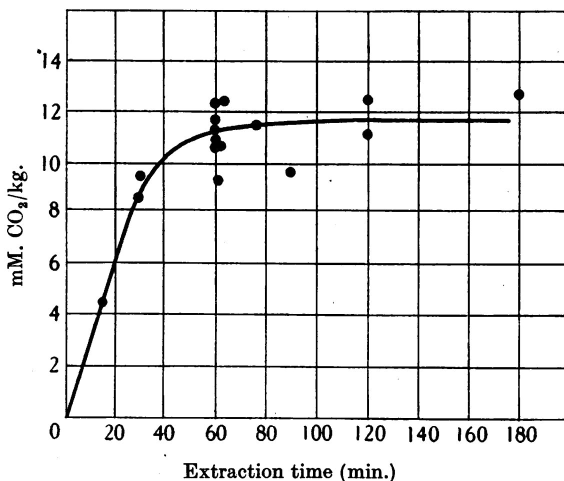

Fig. 1. Time curve of CO_2 extraction from abdominal muscles of young rabbits (about 0·5 kg.) by *N*/5 KOH. Each point in general from one rabbit; two points obtained with some rabbits.

The Ba-*soluble fraction*

The average values for the CO_2 remaining on addition of 1 vol. saturated $BaCl_2$ to 5 vol. of extract and spinning for 90 min. (occasionally much longer) at approximately 3000 r.p.m. were for the abdominal muscle of the rat, rabbit and guinea-pig, 8·0, 5·2, and 5·8 mM./kg. respectively (14·8, 11·4 and 10·3 mM./kg. being the corresponding values for the total CO_2), and for the leg muscle of rabbit and cat the value was 7·0 mM./kg. (compared with 10·6 mM./kg. for the total CO_2). Thus under the conditions of the experiment more than half the total CO_2 is not precipitated by $BaCl_2$, which is presumptive evidence that this fraction is not present in muscle as free CO_2 or HCO_3^-, or in the alkaline extract as CO_3 ion. This conclusion has been proved by two independent procedures.

(1) *Effect of addition of* $KHCO_3$ *to the muscle extracts.* To 5 ml. portions of alkaline extract of the muscle small volumes of $KHCO_3$ solution (0·1–0·2 ml.) were added and mixed before adding the saturated $BaCl_2$, corresponding volumes of CO_2-free water being added to control tubes. The results are shown in Table 2. If the $CO_3^=$ were not entirely precipitated it might be expected that higher values would be found for the analyses after centrifuging. In fact,

TABLE 1.

Animal		Total CO_2 mM./kg.	Total Ba-soluble CO_2	Turbidity of Ba extract after centrifuging, absolute units
		Abdominal muscle		
2 rats		13·4	7·4	—
2 rats		15·9	11·3	—
1 rat		—	7·9	—
3 rats		14·4	6·8	—
3 rats		15·4	6·6	0·009
	Mean	14·8	8·0	
Young rabbit		10·8	3·4	0·000
		10·7	6·6	0·000
		11·2	6·3	0·001
		12·5	6·2	0·027
		12·4	4·6	0·000
		11·4	4·2	0·010
		11·8	3·2	—
		10·7	5·9	0·000
		—	6·5	—
	Mean	11·4 ± 0·3	5·2 ± 0·4	
Guinea-pig		(8·5)	5·5	0·002
		(11·1)	6·5	0·016
		(9·4)	—	—
		(10·3)	4·9	—
		(9·5)	6·5	—
		(11·1)	6·2	—
		(12·2)	5·2	—
	Mean	10·3 ± 0·4	5·8 ± 0·3	
		Leg muscle		
Young rabbit		8·9	7·5	—
		11·2	9·1	—
		12·3	6·6	0·000
		10·7	6·4	0·000
		9·3	4·7	0·007
Grown rabbit		11·8	—	Cloudy
		9·0	7·2	—
		11·3	7·2	—
	Mean	10·6 ± 0·4	7·0 ± 0·5	
Cat		10·8	9·0	Cloudy
		10·1	7·3	,,
	Mean	10·4	8·1	

The brackets for total CO_2 for guinea-pig abdominal muscle indicate that different animals were used for determining this quantity and for the Ba-soluble fraction. The turbidities listed give absolute values over the readings for the centrifuged control without $BaCl_2$ which usually gave results not differing from water. The ± figures after the means give the S.D. of the mean values.

TABLE 2.

Animal	Muscle	Ba-soluble CO_2 in extract: Before adding $KHCO_3$ mM./kg. muscle	After adding $KHCO_3$ mM./kg. muscle	Amount of $KHCO_3$ added as mM./kg. muscle
2 rats	Abdominal	7·4	7·0	10·5
		11·3	10·2	8·6
Rabbit	Leg	7·2	6·8	4·0
		6·6	5·0	13·0
		4·3	5·2	6·0
		4·7	3·5	5·3

slightly lower values were found. All the added HCO_3^- was precipitated as $BaCO_3$, and apparently the extra bulk of the precipitate brought down a little of the Ba-soluble fraction.

The mean value of the Ba-soluble fraction without adding $KHCO_3$ was 6·9 mM./kg., and after the addition of $KHCO_3$ (corresponding to a mean value of 7·9 mM./kg. muscle) it was 6·3 mM./kg. It then seemed possible that although all the $CO_3^=$ above a certain level is precipitated, yet some $BaCO_3$ might still be held in suspension.

To test this, abdominal muscles of very young rabbits were evacuated in the cold for some hours to get rid of the major part of the total CO_2. They were then extracted in the usual manner. Evacuation in the cold was chosen rather than at room temperature, since after 90 min. or 2 hr. at room temperature the physical consistence of the muscle is altered and after adding $BaCl_2$ the extract remains markedly cloudy even with long centrifuging. Even after evacuation for some hours at 2–3° C. a certain change of a similar kind is apparent. Table 3

TABLE 3. Extract prepared from abdominal muscle—evacuated in the cold for 2½ hr.—of four young rabbits (0·3–0·5 kg. body weight)

Ba-soluble CO_2 mM./kg. muscle	$KHCO_3$ added to extract as mM./kg. muscle	Turbidity in absolute units Extract after $BaCl_2$ addition	
		Before centrifuging	After 90 min. centrifuging
2·2	0·0	0·253	0·019
2·3	1·9	0·269	0·020
3·1	3·8	0·289	0·020
3·2	5·5	0·309	0·006
2·9	7·4	0·330	0·022
3·1	9·2	0·352	0·020
2·8	10·9	0·361	0·006
2·8	12·7	0·358	0·000

shows the effect of the evacuation and of additions of $KHCO_3$ on the Ba-soluble fraction. The initial Ba-soluble fraction was 2·2 mM./kg., and after additions of $KHCO_3$ corresponding to 10·9 and 12·7 mM./kg. it was 2·8, the average of all the analyses after adding $KHCO_3$ being 2·9. This shows perhaps a slight increase after the addition but nothing comparable to the average level of 5·2 mM./kg. for the Ba-soluble fraction *in the extract of unevacuated muscle.* It may be also attributed to the fact that $BaCO_3$ is not so easily precipitated from the alkaline extract of *muscle which has been long standing.*

(2) *Turbidity studies.* The centrifuged extracts after $BaCl_2$ addition were examined, as described, in the turbidimeter (Pulfrich), the turbidities being expressed in absolute terms. For abdominal muscle the turbidity measured was often less than 0·001, such extracts appearing quite clear to ordinary observation. When compared in the turbidimeter with centrifuged control samples containing no $BaCl_2$ there was usually no measurable difference in turbidity. The question then arose as to the turbidity produced by $KHCO_3$ additions to alkaline protein solutions, with subsequent $BaCl_2$ additions. The most suitable solution for examination seemed to be the extract of evacuated abdominal muscle in which everything was otherwise similar to the analytical conditions. Several hours' evacuation of the abdominal muscle in the cold brought the total acid-labile CO_2 to about

2 mM./kg. The muscle was then extracted in the usual manner and the $KHCO_3$ added in small measured volumes (0·1–0·2 ml.) of standard solutions to 5 ml. vol. of the extract (obtained from about 17 g. muscle in 50 ml. $N/5$ KOH). After mixing, 1 ml. saturated $BaCl_2$ was added and again rapidly mixed. It may be noted that different turbidity conditions are obtained if the $KHCO_3$ additions are made *after* the $BaCl_2$ addition. There is then present a fine flocculent precipitate, whereas with the above procedure no flocculent precipitate is produced but rather a fine cloud.

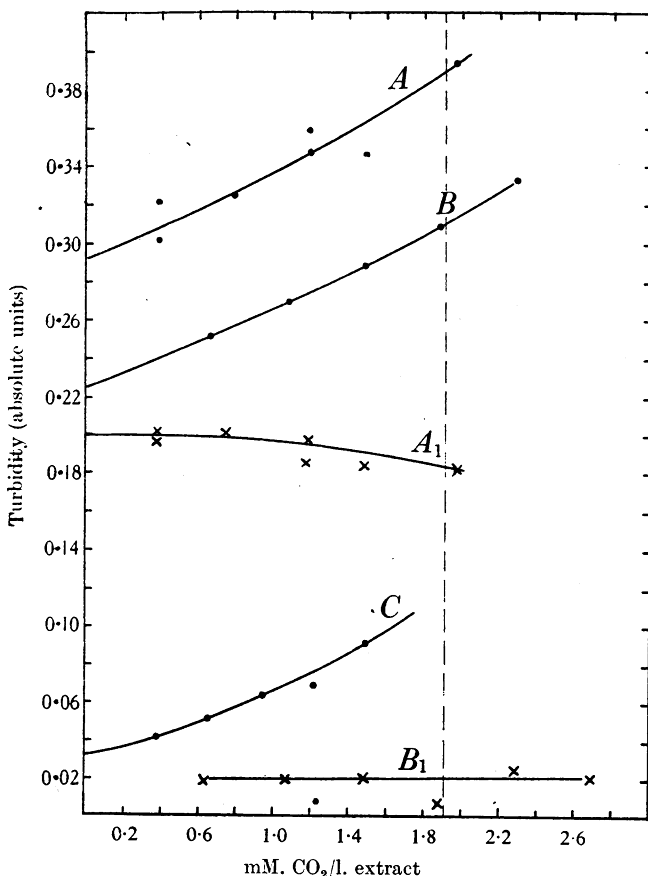

Fig. 2. Curves A and B represent turbidities of alkali extracts of evacuated abdominal muscle from young rabbits as described in text, after additions of $KHCO_3$ and then saturated. $BaCl_2$, 1 vol. to 5 vol. of extract. Curve A_1 represents the A extracts after 5 min. centrifuging. Curve B_1 represents the B extracts after 90 min. centrifuging. Curve C is for clear human serum freed of CO_2 and rendered alkaline to simulate the muscle extracts, $KHCO_3$ added and then $BaCl_2$ as for muscle. The dilution of the serum in the alkaline fluid before $BaCl_2$ addition was about 1 in 5.

Fig. 2 shows the results obtained in two such experiments (curves A and B). The remaining small amount of acid-labile CO_2 in the muscle is here not considered to be present in the extract as $CO_3^=$, but the small amount of $CO_3^=$ in the alkali itself is added on to the $KHCO_3$ additions. The abscissae give the

concentrations of carbonate present and the ordinates the turbidities in absolute values.

It will be seen that the additions cause a uniform rise in turbidity, at least up to the approximate level (dotted vertical line) of the Ba-soluble fraction of the total acid-labile CO_2 in the extracts of fresh muscle. The relation of increase in turbidity to increase in added CO_2 is nearly but not quite linear. The turbidity changes 0·10 in absolute units for a CO_2 addition corresponding to the level of the Ba-soluble fraction of fresh muscle. As already noted the turbidity of the centrifuged extracts (unevacuated muscle) after $BaCl_2$ addition is often inappreciable, averaging 0·008 for abdominal muscle, which was the muscle most examined.

Curve A_1, in Fig. 2, shows the effect on the extracts used for curve A (with $BaCl_2$ addition) of centrifuging for 5 min. The turbidity is still relatively high, but there is now a slight fall with increasing $KHCO_3$. Curve B_1 shows the effect on the B series of centrifuging for 90 min. Very little turbidity is left and there is no difference for the increasing $KHCO_3$ additions.

Curve C is for clear human serum diluted 1 in 5 with $N/5$ KOH, and which had previously been freed of CO_2 by slight acidification and exposure for an hour in micro-diffusion units. 5 ml. vol. were taken and additions of $KHCO_3$ made as above with subsequent $BaCl_2$ addition. It will be seen that the effect on the turbidity is very similar to that with extract of abdominal muscle.

The results show clearly that the comparatively large Ba-soluble fraction of the total CO_2 in the alkaline extracts cannot be present as $CO_3^=$.

The nature of the Ba-*soluble fraction*

A large fraction of the total acid-labile CO_2 in muscle is Ba-soluble in alkaline media, and this at once suggests [Henriques, 1928, 1929, 1935; Faurholt, 1924, 1925; Meldrum & Roughton, 1932, 1933; Roughton, 1935] that it may be carbamino CO_2. Now such compounds possess the characteristic property that around a pH of 7·0, when the CO_2 tension falls, they are rapidly split, yielding free CO_2.

A series of observations on the effect of exposure of strips of the abdominal muscle of guinea-pigs (numbers of which were available at the time) *in vacuo* were therefore carried out. The results of these experiments are summarized in Fig. 3 (each point being the mean of 3–6 determinations). It will be seen that both the total and Ba-soluble fraction show a rapid initial fall of 2–3 mM./kg. after which the Ba-soluble fraction falls only very slowly, and is little more than halved after full evacuation for 1 hr. at room temperature. At and after 45 min. there is no appreciable difference between the curves of the total and the Ba-soluble CO_2, so that all the free CO_2 and HCO_3^- has then disappeared from the muscle. This would seem to indicate that the greater part of the Ba-soluble fraction may not be carbamino CO_2. The mean value of the sum of the free CO_2 and HCO_3^- in the abdominal muscle of the guinea-pig is

10·3 ± 0·4 minus 5·8 ± 0·4, i.e. 4·5 ± 0·6 (see Table 1). For rabbit abdominal muscle it is 11·4 ± 0·3 minus 5·2 ± 0·3, i.e. 6·2 ± 0·4, and for the leg muscle of the rabbit it is given by 10·6 ± 0·4 minus 7·0 ± 0·5, i.e. 3·6 ± 0·6.

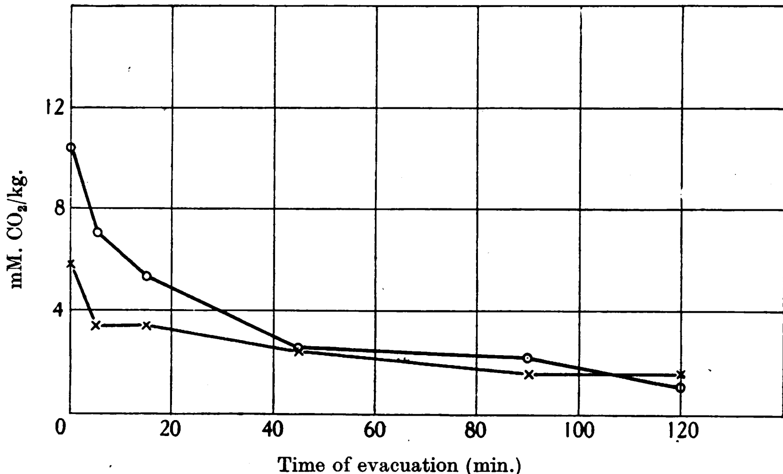

Fig. 3. Mean curves of CO_2 content of abdominal muscle of young guinea-pigs after evacuation for varying periods. Upper curve gives the total acid-labile CO_2. Lower curve gives the barium-soluble CO_2. Each point is for 3–6 analyses. Each guinea-pig was used to supply data for two time periods.

The ratio of HCO_3^- *concentration in muscle as a whole to* HCO_3^- *content of serum compared with similar ratio for chloride*

To determine this ratio for abdominal muscle we have the following data from Table 4.

The ratio $[Cl]_m/[Cl]_s$ for rabbit abdominal muscle = 26·6/99·5 = 0·267; and the ratio $[HCO_3]_m/[HCO_3]_s$ = 5·3/19·4 = 0·273. (The HCO_3^- value is obtained from Table 4 by subtracting the Ba-soluble fraction from the total acid-labile CO_2, and then a further 0·9 for the free CO_2 in solution in the total muscle water. In calculating plasma HCO_3^-, 1·0 is allowed for the free CO_2.) For the guinea-pig, assuming the same value for the free CO_2 in serum, etc.

$$[HCO_3]_m/[HCO_3]_s = 0{\cdot}186, \quad \text{and} \quad [Cl]_m/[Cl]_s = 0{\cdot}208.$$

The ratios for Cl^- and HCO_3^- are thus the same within the sampling error. If the interspace volume be calculated from the chloride analyses, assuming no chloride *inside* the fibres, there could be no intracellular bicarbonate, but such a method of interspace calculation, though giving values near to those actually found, is theoretically unsound [Conway & Boyle, 1939; Boyle & Conway, 1941]. How the true interspace volume, etc., may be calculated from chloride analyses is shown below.

TABLE 4

'Tissue'	Symbol	Value of symbol: Rabbit	Guinea-pig
Plasma	W_s (g./kg.)	924 (A)	—
	$[CO_2, \text{total}]$	20·4 (8)	—
	$[Cl]_s$	99·5 (10)	109·0 (8)
	$[K]_s$	4·9 (5)	—
	pH	7·4	—
Whole blood	W_b (g./kg.)	817 (A)	—
	$[K]_b$	45 (A)	—
	$[Cl]_b$	82 (A)	—
Muscle (abdominal)	W_m (g./kg.)	782 (5)	771 (5)
	$[CO_2, \text{total}]_m$	11·4 (8)	10·3 (7)
	$[CO_2, \text{Ba-sol.}]_m$	5·2 (9)	5·8 (6)
	$[Cl]_m$	26·6 (7)	22·7 (5)
	Blood (g./kg.)	28 (5)	29 (5)
Muscle (leg)	W_m (g./kg.)	767 (5)	767 (5)
	$[CO_2, \text{total}]_m$	10·6 (8)	—
	$[CO_2, \text{Ba-sol.}]_m$	7·0 (7)	—
	$[Cl]_m$	12·1 (6)	—
	$[K]_m$	111 (C)	—
	Blood (g./kg.)	25 (5)	24 (5)

All values as mM./kg., unless otherwise stated. The symbol $[Cl]_s$ means mM. chloride/kg. plasma and $[K]_m$ means mM. potassium/kg. muscle and similarly for the other symbols. W_s and W_m mean g. water/kg. plasma and muscle. Figures in brackets give numbers of analyses. (A) refers to Abderhalden's data [1899], and (C) to Constantino's [1911].

The pH *within the muscle fibre*

The value may be first calculated without implying a Donnan relation across the membrane.

In the Henderson-Hasselbalch equation (as given in equation (4)) $[CO_2]_{fw}$ and $[HCO_3]_{fw}$ are the concentrations of free CO_2 and HCO_3^- within the fibre (the very small value of H_2CO_3 may be neglected). A value of $pK = 6{\cdot}1$ may be assumed, as the ionic strength within the fibre will probably not differ markedly from that of blood plasma.

To calculate $[HCO_3]_{fw}$ we need to know the value of the intercellular space and this must be obtained independently of the chloride data. For the leg muscle of the rabbit we have the inulin ratio [Conway & FitzGerald, 1942] $\frac{\text{inulin/kg. muscle}}{\text{inulin/kg. plasma}} = 0{\cdot}07$. Manery & Hastings [1939] found for radioactive sodium (^{24}Na) a ratio of 0·086 with the gastrocnemius muscle of the rabbit; Hahn, Hevesey & Rebbe [1939] obtained a very similar value of 0·085.

The interchange with radioactive Na in such experiments is no doubt entirely extracellular, and it is not surprising that the inulin value should be slightly lower, as some Na may be held by fixed anions in the sarcolemma.

But, taking the sodium ratio as 0·086 for the extracellular water, this, apart from the blood in muscle, may be calculated as follows. In the calculation the

mean water content of rabbit leg muscle is considered to be 767 g./kg. muscle, and the water in serum to be 920 g./kg.

If it be supposed that in 1 kg. of leg muscle there are x kg. of extracellular fluid in addition to y kg. of blood, then

$$[\text{Na}] \text{ external to the fibres} = 0\cdot99x\,[\text{Na}]_{ew} + y\,[\text{Na}]_b = 0\cdot99x\,[\text{Na}]_{ew} + 0\cdot5y\,[\text{Na}]_s. \quad (3)$$

Here it is taken, in agreement with Wallace & Hastings [1942], that 1 kg. of extracellular fluid contains 0·99 kg. water, and that the sodium per kg. whole blood—$[\text{Na}]_b$—is approximately half that per kg. serum or $0\cdot5\,[\text{Na}]_s$.

Now $[\text{Na}]_{ew}$ may be taken as $[\text{Na}]_{sw} \times (0\cdot95/0\cdot92)$, where 0·95 is the Donnan ratio and 0·92 is the water content of the serum. Using these values as well as 0·025 found by us for the blood in the excised leg muscle of the rabbit, and the figure 0·086 above, x is found from equation (3) to be 0·075.

The extracellular fluid, apart from blood, is therefore 0·075 kg./kg. muscle. The water content of this is $0\cdot99 \times 0\cdot075 = 0\cdot074$, and the water content of 0·025 kg. blood is $0\cdot025 \times 0\cdot77 = 0\cdot019$. The total extracellular water is therefore $0\cdot074 + 0\cdot019 = 0\cdot093$; and the intracellular water

$$0\cdot767 - 0\cdot093 = 0\cdot674.$$

To determine the HCO_3^- content of the intracellular water it is necessary to assess the HCO_3^- and free CO_2 in the spaces outside the fibres and the free CO_2 within the fibres. Now

$$[HCO_3]_{sw} \text{ (mM. } HCO_3^-\text{/kg. of the serum water)} = 21\cdot0,$$

and therefore $[HCO_3]_{ew}$ (mM. HCO_3^-/kg. of extracellular water) $= 21\cdot0 \times 1\cdot05 = 22\cdot1$. Also, $[CO_2]_{sw} = 1\cdot1$, and since the solubility coefficient of CO_2 is 0·553 ml./g. serum water, 0·540 ml./g. extracellular water and 0·592 ml./g. intracellular water [as given by Wallace & Hastings, 1942] then $[CO_2]_{ew}$ (mM. free CO_2/kg. extracellular water) $= 1\cdot08$, and $[CO_2]_{fw}$ (mM. free CO_2/kg.) $= 1\cdot18$. Since the total CO_2 content of the serum is 20·4 mM./kg. (Table 4), that of whole blood may be taken as approximately 17 mM./kg. From the value of the total CO_2 content of muscle apart from the Ba-soluble fraction (that is, from $10\cdot6 - 7\cdot0 = 3\cdot6$ mM./kg.) we must subtract then the following to obtain the HCO_3^- content of the fibre water:

$0\cdot075 \times 1\cdot08$ ... free CO_2 in extracellular fluid other than blood,
$0\cdot075 \times 22\cdot1$ ... HCO_3^- in extracellular fluid other than blood,
$0\cdot025 \times 17$ ... total CO_2 in the blood in muscle,
$0\cdot674 \times 1\cdot18$ ... free CO_2 in the fibre water (or extracellular fluid) $= 2\cdot96$,

so that $3\cdot6-2\cdot96=0\cdot6$ mM. HCO_3^- is left dissolved in 0·674 kg. water, or $[HCO_3]_{fw}=0\cdot6/0\cdot674=0\cdot9$. From the Henderson-Hasselbalch equation

$$pH = pK' - \log \frac{[CO_2]_{fw}}{[HCO_3]_{fw}}, \tag{4}$$

the mean value of the intracellular $pH = 6\cdot1 - \log 1\cdot2/0\cdot9 = 6\cdot0$. No great exactness can be claimed for this figure, owing to the relative magnitude of the total interspace CO_2, and of the Ba-soluble fraction inside the muscle fibre.

Calculation of the pH *within the muscle fibre from the application of the Donnan principle and assuming a membrane permeable to* K, H, Cl *and* HCO_3 *ions*

From the Donnan relation, the ratio of the potassium activities across the membrane should be the same as the ratio of the hydrogen activities, and inversely as the chloride and bicarbonate activities. Since we are dealing with univalent electrolytes, we may without much error substitute the ratio of the concentrations. Also, to simplify the presentation, the concentration of the inorganic electrolytes in the extracellular fluid may be taken to be the same as in the plasma water, since any small differences from these values would contribute no appreciable extra accuracy to the calculations, besides being in themselves of rather uncertain value. From the Donnan relation

$$\frac{[K]_{fw}}{[K]_{sw}} = \frac{[H]_{fw}}{[H]_{sw}}. \tag{5}$$

Also,

$$[K]_{fw} = \frac{[K]_m - s \times [K]_{sw} - 0\cdot025[K]_b}{0\cdot767 - s - 0\cdot025 \times 0\cdot77}, \tag{6}$$

where $[K]_{fw}$, $[K]_m$, $[K]_{sw}$, $[K]_b$ are the mM. K/kg. of fibre water, of muscle, of serum water and of whole blood respectively; 's' is the volume of interspace water other than blood, which latter is 0·025 kg./kg. muscle (0·77 times this value, giving the water content) and 0·767 is the amount of water per kg. muscle from Table 4. Therefore, from the data of Table 4

$$[K]_{fw} = \frac{111 - s \times 5\cdot3 - 0\cdot025 \times 45}{0\cdot767 - s - 0\cdot019}$$

$$= \frac{110 - 5\cdot3s}{0\cdot748 - s}, \tag{7}$$

so that (from equation (5))

$$[H]_{fw} = \frac{110 - 5\cdot3s}{0\cdot748 - s} \times \frac{10^{-7\cdot4}}{5\cdot3}. \tag{8}$$

There remains then to calculate 's', the value of the extracellular water, apart from blood. It may be determined by using the following relation:

$$[K]_{fw} \times [Cl]_{fw} = [K]_{sw} \times [Cl]_{sw}, \tag{9}$$

when the value of $[K]_{fw}$ is inserted from equation (7), and $[Cl]_{fw}$ (as in equation (11), similar to $[K]_{fw}$) is determined as follows:

$$[Cl]_{fw} = \frac{[Cl]_m - s \times [Cl]_{sw} - 0{\cdot}025\,[Cl]_b}{0{\cdot}767 - s - 0{\cdot}025 \times 0{\cdot}77}$$

$$= \frac{12{\cdot}2 - s \times 108 - 0{\cdot}025 \times 82}{0{\cdot}748 - s}$$

$$= \frac{10{\cdot}1 - 108s}{0{\cdot}748 - s}, \qquad (10)$$

whence, from equation (9),

$$\frac{110 - 5{\cdot}3s}{0{\cdot}748 - s} \times \frac{10{\cdot}1 - 108s}{0{\cdot}738 - s} = 5{\cdot}3 \times 108, \qquad (11)$$

from which $s = 0{\cdot}071$,

which is very similar to the 0·075 value obtained above from direct analyses.

Inserting this value for 's' in equation (8)

$$[H]_{fw} = 10^{-5{\cdot}9},$$

and the pH value = 5·9, or, if we consider the pH value as the negative logarithm of the H ion activity (to correspond to equation 5 above) the value is slightly raised and becomes 6·0.

Here again no great exactness can be claimed for the result, but it is probably within ±0·1 pH, and agrees well with the previous calculation, based directly on the bicarbonate system.

Similar calculations, applied to the data for the abdominal muscle of the rabbit, give a pH within the fibres of 6·0, which is similar to that of the leg muscle.

Discussion

Ba-*soluble* CO_2 *in muscle*

The total CO_2 liberated and escaping after acidifying muscle may be termed acid-labile CO_2; as shown here, only the smaller part of this total in mammalian muscle is in the ionized HCO_3 or in the free CO_2 form, the greater part being Ba-soluble in alkaline media.

The proofs of the reality of the existence of a Ba-soluble fraction of such magnitude, and that $BaCO_3$ is not merely suspended or protected from precipitation by the proteins, have consisted in the quantitative precipitation of small amounts of CO_2 added as $KHCO_3$ to the alkaline extract before the addition of the $BaCl_2$, as well as by turbidity studies of the centrifuged samples, and turbidity effects produced by HCO_3^- additions to evacuated muscle extracts. The proof obtained from the turbidity study alone would appear conclusive for the abdominal muscles of guinea-pigs, very young rabbits and rats. The alkali extracts of leg muscles of fully grown rabbits and cats, when treated

with $BaCl_2$, show some turbidity after centrifuging, though this is no reason for supposing that such turbidity is caused by $BaCO_3$, since as with abdominal muscle this is quantitatively precipitated when small volumes of $KHCO_3$ solutions are added before the $BaCl_2$. Evidence has been presented in the paper that the greater part of this Ba-soluble fraction may be in some form other than carbamino CO_2.

The pH *inside the muscle fibre*

It will be seen that the nature of the acid-labile CO_2 in mammalian muscle as determined in these experiments altogether invalidates the *p*H calculation made by Wallace & Hastings, their calculated value of $6{\cdot}93 \pm 0{\cdot}12$ being nearly one whole unit of *p*H too high. The true value appears to be approximately 6·0, and is thus practically the same as that calculated for frog muscle, i.e. 5·9 [Boyle & Conway, 1941].

The calculations of the H-ion concentration from the study of the bicarbonate system are in agreement with a Donnan relation across the membrane for K^+, H^+, Cl^- and HCO_3^- and such a relation for K^+ and Cl^- has been demonstrated for very wide changes of these ions in frog muscle [Boyle & Conway, 1941]. Supporting evidence for mammalian muscle is given by Wilde [1943] and Darrow [1944]. It is also of interest to note that a value of 6·0 was found by Vlès [as quoted by Rous, 1925] for frozen and ground mouse tissues by various physico-chemical methods, and a figure as low as 5·6 by Rous [1925] from intravital staining of voluntary muscle in mice with the minimum disturbance of the living tissues.

The permeability of the muscle fibre to HCO_3^-

In previous communications the principle was demonstrated for frog muscle (and it has also been found for gland tissue as will be described later) that *the cell membrane in general is permeable both to cations and anions but there are size limits for these ions.* Na, Mg and Ca are excluded as ions, though they obtain entrance into cells, probably in unionized organic combination. On the other hand the muscle is not permeable to the larger anions, such as those of the phosphate esters, but is freely permeable to chloride and very probably to HCO_3^-. The permeability to HCO_3^- throws open to any particular group of cells the whole HCO_3^- buffering of the internal medium. The evidence brought forward by Wallace & Hastings [1942] and Wallace & Lowry [1942] for the impermeability of the muscle membrane may now be considered.

(*a*) Working with the leg muscles of cats they state, 'the intracellular bicarbonate remains relatively unchanged despite wide changes in the extracellular bicarbonate and it is concluded that the muscle cell is normally impermeable to the bicarbonate ion'.

With the membrane *permeable* to HCO_3^- the concentration of this ion in the fibre water is only about 1 mM./kg. Doubling the external concentration outside (other things being equal) only raises it to approximately 2 mM./kg., or from 0·7 to 1·4 mM./kg. whole muscle. The increase due to the interspace water will be at the same time about 2 mM./kg., so that altogether the total CO_2 will increase about 3 mM./kg., which corresponds almost exactly with the mean results described.

(*b*) The evidence advanced from experiments described in a subsequent paper by Wallace & Lowry [1942] is essentially similar in type. In these experiments the abdominal muscle of the rat was equilibrated *in vitro* with solutions containing very varying amounts of HCO_3^- and CO_2 with little variation in the CO_2 tension. They state, 'when the CO_2 pressure remained constant and the bicarbonate ion concentration in the equilibrium fluid was increased from 0·0 to 8·7 mM. per litre, the intracellular bicarbonate ion concentration remained nearly constant'. Seeing that they measured the extracellular fluid by means of chloride data, on the assumption that the muscle membrane was impermeable to this ion, no other result could be expected—the bicarbonate ion in the fibre so calculated should be constant no matter what the external changes—but it should be zero. What was measured as apparent bicarbonate ion was the Ba-soluble fraction of the total CO_2 and it is not surprising from the results in the present paper that this appeared to be largely independent of the external conditions. Such experiments therefore of Wallace *et al.* provide no real evidence for the impermeability of the muscle fibre membrane to HCO_3^-.

Summary

1. The total acid-labile CO_2 in muscle was determined by alkali extraction and subsequent CO_2 determination by a micro-diffusion method. It was found to be $14·8 \pm 0·6$ mM./kg. for the abdominal muscle of the rat, $10·3 \pm 0·4$ mM./kg. and $11·4 \pm 0·3$ mM./kg. for the abdominal muscle of the guinea-pig and rabbit respectively, and $10·6 \pm 0·4$ mM./kg. for the leg muscle of the rabbit.

2. The barium-soluble fraction of this total CO_2 amounted to $8·0 \pm 0·3$, $5·8 \pm 0·5$, $5·2 \pm 0·5$ and $7·0 \pm 0·5$ mM./kg. for the abdominal muscle of the rat, guinea-pig and rabbit and the leg muscle of the rabbit respectively. (The $\pm$ values give the S.D. of means.)

3. In the method for determining the barium-soluble fraction it was shown that no appreciable amount of $BaCO_3$ was held in fine suspension or protected from precipitation by proteins from the fact that HCO_3^-—as $KHCO_3$—added to the alkaline extract was precipitated quantitatively; also by turbidity studies. The latter were most satisfactory with abdominal muscle or leg muscle of rabbits of about 0·5 kg. body weight.

4. Allowing for the small amount of free carbon dioxide in the barium-insoluble fraction, it is shown that the ratio of bicarbonate concentration in muscle to that in plasma is the same as for chloride.

5. The *p*H inside the muscle fibre as determined by the Henderson-Hasselbalch equation applied to the bicarbonate system is 6·0.

6. The *p*H of the muscle fibre determined from the Donnan principle applied to a membrane permeable to K^+, H^+, Cl^- and HCO_3^- is likewise 6·0.

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