CXXXIII. THE ACID-BASE EXCHANGE IN MAMMALIAN VOLUNTARY MUSCLE.

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INTRODUCTION.

THERE are present within the muscle inorganic and organic systems which can part with their base to neutralise the acids produced during activity or in the tissue after death. There have been many investigations within recent years which deal with the acid production in muscle, the extremely important researches of Fletcher and Hopkins [1906] having been followed by those of Embden and his school [1912; 1914, 1, 2; 1921], Parnas and Laska-Mintz [1921], Meyerhof [1922] and many others and the results so obtained have been of great value in the study of the energy changes in muscle [A. V. Hill and Meyerhof, 1923]. It is necessary in the study of the heat production in muscle to obtain as much information as possible regarding the carriers of base which can part with their alkali within the zone of reaction change near the neutral point. The amount of base which can be obtained between a more alkaline and a less alkaline point by such an acid as lactic acid depends upon the absolute concentrations and the dissociation constants of the acid groups which hold the disposable base. As Meyerhof has pointed out, the exothermic changes associated with the removal of base from colloidal systems and from the amino acids are of a high order, dependent upon the heat set free in the passage from the dissociated salt form to the undissociated colloidal acid or amino acid form. The part played by the colloidal protein systems in these changes within such a reaction zone as from p_H 7.5 to p_H 6.57 will depend upon the amount of base which can be secured from such systems by lactic acid or other acids. If the muscle proteins have an acid dissociation constant of the value $10^{-5.7}$ as Meyerhof [1922] calculates from the K_b of horse serum albumin given by Pauli [1920] and the isoelectric point of serum albumin [Michaelis and Davidsohn, 1911], then undoubtedly the amount of acid which may be produced between these two points could not secure the necessary amount of base from such a system alone. Thus, even if we take the concentration of the carboxyl groups in the proteins of expressed muscle juice at so high a figure as 0.05 molar, the amount of base which could be secured between these two reaction points would only be approximately 9.5 millimols¹, because at the

¹ All concentrations referred to are per litre.

slightly alkaline reaction of p_H 7.5, the ratio of undissociated acid to colloidal anion would be $\frac{.0015}{.0485}$, and at the slightly acid reaction of p_H 6.57, the ratio would be $\frac{.011}{.039}$. Between these two points, therefore, 9.5 millimols base are obtainable for the neutralisation of an approximately equal number of millimols lactic acid, less than 1 % lactic acid being free even at p_H 6. As there may be a much greater increase in lactic acid, apart from other acid production, between these two points, it is evident that under these conditions base must be secured from some other source. As even at the more acid reaction the muscle juice is on the alkaline side of the iso-electric point of serum albumin ($2 \cdot 10^{-5}$) and therefore probably also of that of the muscle proteins, we may regard such a system as a base-carrying one. Naturally acids so weak as the amino acids (K_a 10^{-10}) and creatine cannot carry base on the acid side of neutrality. There are at least four possible base-carrying systems which might be considered, namely, bicarbonates, inorganic phosphates, the organic forerunner of the inorganic phosphates ("lactacidogen" of Embden) and the proteins. As regards the pre-formed bicarbonate the concentration even in the fresh muscle is extremely small, according to Meyerhof 0.005 molar, and in the expressed juice with which this paper deals the amount present is still less, so that attention will be directed to the remaining three systems. The concentration of the pre-formed phosphate varies from about 0.015–0.03 molar, and the lactacidogen forerunner is present also in variable but somewhat similar concentration. There are no data at present available regarding the molar concentration of the protein carboxyl groups in muscle juice.

EXPERIMENTAL.

The investigations dealt with in the following paper are concerned with the expressed juice of muscle. It was fully recognised that even with the greatest care in preparation, there will always be, in the case of expressed juice, a distinct acid production during the processes of extraction. Information of an important kind may, however, be obtained by comparing the reaction swing with the lactic and phosphoric acid increments on passing from a cooled to a warmed juice, even if the reaction of the former be distinctly more acid than that of the living tissue. In addition, it was regarded of importance to study the ultrafiltrates of the juice from frozen muscle and from the same juice after warming, in order to determine the part played by the colloidal and the non-colloidal constituents. Special attention was directed to the expressed juice of horse and dog muscle. The methods adopted for the preparation of the juice and the ultrafiltrates were as follows.

The animals were bled and, in the case of the dog, the blood was thoroughly washed out from the vessels. The muscles were then sprayed with ethyl chloride, carefully removed and frozen by being placed at once in vessels

immersed in freshly dissolved ammonium nitrate. In some cases liquid air was used, but in certain respects the after treatment of the hard frozen muscle was found to be troublesome and unsatisfactory. After freezing in the ammonium nitrate solution the muscle was rapidly minced, mixed with sand and the juice expressed in a porcelain filter-press. The juice was kept in cooled Dewar flasks until required for analysis.

The reaction of the cold juice was determined electrometrically and electrometric titration with known additions of acid or alkali carried out in order to determine the buffer value of the juice.

The pre-formed inorganic phosphate was determined by Embden's gravimetric method [1921] after removal of the protein by trichloroacetic acid. The lactic acid was determined by Meyerhof's method [1922] and the total nitrogen by Kjeldahl's method. The latter was determined simply to discover whether the concentration of the juice varied in different specimens. Another portion of the muscle juice was warmed for two hours at 40° and the reaction and buffer capacity determined in the same way and at the same temperature as in the cooled juice. The same analyses were made in the case of this warmed juice. The ultrafiltrates from the cold and warm juice were obtained in the following way. The juice was poured from the Dewar flask into a bottle, kept in melting ice, which was connected up to a gas cylinder with a tyre foot pump and with a manometer attached to a T-piece between cylinder and bottle. The exit tube from the bottle was attached to the filter-press (Walpole type) carrying two celloidin filters. Before filling the bottle with the juice the water held by the celloidin plates was driven out under pressure. About 100 cc. juice were used for filtration and the last 10–15 cc. of the first 50 cc. of the filtrate were taken for the reaction determinations and analyses. The pressure was then lowered, and the juice between the plates sucked back into the bottle. The bottle was then kept in a bath at 40° for 2 hours, and the filter-press clamping plates were also warmed. The pressure was again raised and the second portion of the ultrafiltrate from the warm juice taken for the necessary determinations.

Thus the cold and warm juice and their respective ultrafiltrates were examined and, upon the data so obtained, the study of the acid base changes produced by warming was based.

HORSE MUSCLE.

In the first case the juice alone will be considered.

Exp. 1. Analyses. The cold and the warmed juice contained 1.132 % nitrogen.

The inorganic phosphate (precipitable) and the lactic acid values in the deproteinised specimens were as follows:

	H ₃ PO ₄ (molar)	Lactic acid (molar)
Cold	.026	.026
Warmed	.041	.060

The increment in lactic acid was therefore rather more than double that in the inorganic phosphate, so that if these had a common source such as a hexose-phosphoric acid ester [see Harden, 1923], the forerunner would be of the nature of a hexose-monophosphoric acid.

Reaction determinations. 1. *Juice expressed from frozen muscle.* Reaction (18°) p_H 6.77. At this reaction the ratio of K_2HPO_4 to KH_2PO_4 (from electrometric determinations; in the sequel simply termed the ratio) corresponds to per mol phosphoric acid $\frac{455.2}{544.8}$ in millimols (mmols.).

5 cc. of this juice were taken and 0.2 cc. of 0.3N KOH added (= 12 mmols. base per litre), and the reaction was again determined at 18° when p_H was found to be 7.44. At this reaction the ratio would be $\frac{804}{196}$, an increase of 348.8 mmols. per mol phosphoric acid, and therefore the 12 mmols. actually added correspond to 0.0344M phosphoric acid. In other words, a 0.0344 molar phosphate solution of p_H 6.77 would show a reaction swing to p_H 7.44 on addition of 12 mmols. base per litre, and therefore represents the buffer value of the juice between these two reactions. On reversing the process, the addition of 12 mmols. acid to the juice at p_H 7.44 changed the reaction to the original 6.77, so that the buffer value had not altered during the period between the two determinations.

2. *The same juice after warming for 2 hours at 40° .* The reaction was now p_H 6.18 (at 18°), and it required 12 mmols. base to shift the reaction to p_H 6.72 (approximately that of the original cold juice). This corresponds to a buffer value of 0.046M phosphate. To determine the buffer capacity on the alkaline side of p_H 6.72, 18 mmols. more base were added when the reaction was found to have shifted to p_H 7.36, equivalent in buffer capacity to 0.052M phosphate.

It is evident, therefore, that the buffer capacities of the cold and warmed juice are higher than correspond to their actual phosphate contents.

Knowing the inorganic phosphate concentrations, and the reactions of the cold and warmed juice, the total base content of the phosphate systems at p_H 6.77 and at p_H 6.18 can be calculated. At p_H 6.77, the ratio in 0.026M phosphate is $\frac{.0118}{.0142}$ which therefore gives for the cold juice a total base content in its phosphate system of 37.8 mmols.

At p_H 6.18, the ratio in 0.041M phosphate is $\frac{.0068}{.0342}$, which gives for the warmed juice a total base content of 47.8 mmols. or an increase of 10 mmols. base. What is the source of this base? If the increase in phosphoric acid be regarded as derived by hydrolysis from a forerunner such as hexosemonophosphoric acid then the alkaline salt of this acid may be regarded as holding the same amount of base as phosphoric acid at the same reaction, for example, two base equivalents for each atom of phosphorus at the phenolphthalein change point, as shown in the following equation:

From the hexose so set free two molecules lactic acid may be derived, one

molecule of which, in the absence of any other source of base, would change the dibasic to the monobasic salt, giving a reaction shift of approximately p_H 9 to p_H 4¹.

As the increment in precipitable phosphate in the warmed juice is derived from a body existent in the cold juice, in all probability of the nature of a hexose-phosphate, the total molar concentration of the cold juice may be regarded as 0.041 (0.026 precipitable and 0.015 hydrolysable) and this system may possibly contain the same amount of base as a phosphate solution at the same reaction. At p_H 6.77, the cold juice considered as 0.041*M* phosphate solution should contain the maximal amount of base derivable from this source, and this value can be determined from the ratio of dibasic to monobasic at this reaction, namely $\frac{.0186}{.0224}$. At p_H 6.18 the ratio is $\frac{.0068}{.0342}$, so that, on passing from the less to the more acid, 11.8 mmols. base have been removed. The amount of lactic acid actually produced between the two reaction points was 34 mmols. (33.8 of which would secure base at p_H 6.18). This increment in lactic acid would therefore require 22 mmols. base from some other source for its neutralisation. If the original substance were the salt of a diphosphoric acid ester, then lactic acid and phosphoric acid would appear in equimolecular proportions and of the 34 mmols. lactic acid actually produced, only 15 (equal to the H_3PO_4 increment) would come from this source and 19 mmols. from some other source along with their base equivalent. In both cases the "lact-acidogen" system is unable to furnish the amount of base which must have been secured by the lactic acid produced on warming the juice, when the reaction shifted from 6.77 to 6.18. That base has actually been derived from acid groups other than the phosphoric is evident from the capacity of the warmed juice to take up more base between the reactions of the warmed and cold specimens than would be the case with a pure solution containing the same amount of phosphate. One must always bear in mind that in addition to the acid determined (lactic), other acids such as volatile fatty acids with K_a of the value 10^{-5} may be produced and these will contribute to the increase in the buffer capacity of the juice owing to their removal of base, for example, from the proteins. When alkali is added, therefore, to the warmed juice, part of this is required to satisfy the base requirements of the colloidal acid groups as well as of the phosphoric in the phosphate system.

Exp. 2. (Horse Muscle.) In this case both the juice and the ultrafiltrates will be considered.

The nitrogen percentage of the cold and warmed juice was 1.221.

Analyses	Inorganic phosphate (molar)	Lactic acid (molar)
(a) Cold juice	.0228	.0254
(b) Ultrafiltrate of cold juice	.0240	.0252
(c) Warmed juice	.0335	.0389
(d) Ultrafiltrate of warmed juice	.0340	.0386

¹ The references to this organic forerunner are based upon the studies of the phosphate mechanisms in yeast (Harden), our knowledge of the true nature of this precursor in muscle being very incomplete. Further investigation of this subject is absolutely necessary.

For 10 mmols. increase in phosphoric acid, there is therefore approximately 13 mmols. lactic acid increment, so that the process of warming has given rise approximately to an equimolecular increase in these constituents. The phosphate determinations are undoubtedly more accurate than those of lactic acid.

Reaction Determinations (Fig. 1).

(a) Juice (frozen muscle).

1. p_H (18°) 6.77, ratio = $\frac{455.2}{544.8}$.

2. + 6 mmols. base, p_H 6.98, ratio = $\frac{580.4}{419.6}$.

Buffer capacity (on alkaline side) = 0.047M phosphate.

Fig. 1 (Exp. 2). I, Cold muscle juice; Ia, Ultrafiltrate of same; II, Heated juice; IIa, Ultrafiltrate of same (Horse).

To the juice at p_H 6.98, 12 mmols. acid were added, and the reaction shifted to p_H 6.47, ratio $\frac{288.4}{711.6}$.

Buffer capacity (on acid side) = 0.041M phosphate.

(b) Ultrafiltrate of cold juice.

1. p_H (18°) 6.74, ratio $\frac{438}{562}$.

2. + 6 mmols. base p_H 7.13, ratio $\frac{662.4}{337.6}$.

Buffer capacity (alkaline side) = 0.026M phosphate.

To the juice at p_H 7.13, 12 mmols. acid were added, and the reaction shifted to p_H 6.35, ratio $\frac{232}{768}$.

Buffer capacity (acid side) = 0.027*M* phosphate.

(c) Warmed juice.

1. p_H (18°) 6.56.

2. + 6 mmols. base, 6.68.

3. + 18 „ „ 6.98.

4. + 24 „ „ 7.11.

Average buffer capacity (on alkaline side) = 0.076*M* phosphate.

(The ratios of dibasic to monobasic will now be omitted.)

(d) Ultrafiltrate of warmed juice.

1. p_H (18°) 6.63.

2. + 6 mmols. base p_H 6.86.

Buffer capacity (on alkaline side) = 0.044*M* phosphate.

To the ultrafiltrate at p_H 6.86, 12 mmols. acid were added, and reaction shifted to p_H 6.39; buffer capacity (acid side) = 0.045*M* phosphate, and on addition of 12 mmols. base to this solution, the reaction was reversed to 6.86.

The average buffer capacities and the actual phosphate concentrations may then be stated as follows:

	Buffer capacity (molar phosphate)	Phosphate (determined) (molar)
Cold juice	.0440	.0228
Ultrafiltrate	.0265	.0240
Warmed juice	.0760	.0335
Ultrafiltrate	.0445	.0340

It is evident that warming muscle juice increases the buffer capacity of the specimen just as it raises the precipitable phosphate content, but in all cases the buffer capacity is higher than the contained phosphate. There is, however, a much closer relationship between the buffer capacity and the phosphate content in the case of the ultrafiltrates than is the case with the original juice. The buffer value of the ultrafiltrate of the cold juice is 10 % higher than that of the contained phosphate, and in the filtrate of the warmed specimen 30 % higher, while the cold juice has a buffer value 93 % higher and the warmed 126 % greater than their respective phosphate contents. That is to say, on testing the buffer capacity of the juice by the addition of alkali, acid groups, other than the phosphoric, are present which soak up the added base between the reaction points determined. These acid groups which have been deprived of base by the acid production in muscle juice belong mainly to the colloidal constituents and therefore do not pass into the ultrafiltrates, although, especially in the case of the filtrate from the warmed juice, there are non-colloidal acid groups in addition to phosphoric which secure a small amount of base between the two reaction points.

The base contents of the inorganic phosphate systems of the cold and warmed juice will now be considered. The cold juice at p_H 6.77 with 0.0228*M*

H_3PO_4 had the ratio $\frac{.0103}{.0125}$ and after warming at p_{H} 6.56 with 0.0335*M* phosphate, $\frac{.0108}{.0227}$. The increase in the base content of the latter system over the former was 11.2 mmols. As in the preceding experiment, the cold juice may be regarded as originally containing 0.0335*M* phosphate (inorganic + hydrolysable) which at p_{H} 6.77 would give the ratio $\frac{.0152}{.0183}$ and for the warmed juice at p_{H} 6.56 $\frac{.0108}{.0227}$. The amount of base derivable from this system between these two reactions is then 4.4 mmols. which would neutralise slightly under that value of lactic acid. The increment, however, in lactic acid between these two points was found to be 13 mmols., so that approximately 9 mmols. acid would require to secure base from some other source, raising as a result the buffer capacity on that side above the value dependent upon the phosphate present. The colloidal acid groups deprived of, approximately, 9 mmols. base to neutralise this excess of lactic acid, which could not be neutralised by the base of the phosphate systems, would then require to take up this base equivalent in order to bring the reaction back to that of the cold juice. These values can merely be regarded as approximately true, in the first place, because lactic acid determinations are never sufficiently accurate, and, in the second place, other acids may be set free, which are not estimated, and yet which may secure base under these conditions.

In the preceding experiments there was some degree of proportionality between the phosphoric and lactic acid increments produced by heating the juice, agreeing more or less closely with the two types described by Embden, one in which the forerunner is a hexosemonophosphate, and the other, a hexosediphosphate.

In some cases, however, there may be a great departure from either of these types, in one the phosphoric may increase without the lactic acid showing even an equivalent rise, or, in the other, the lactic acid may rise without a proportional rise in the phosphoric acid.

In the former case, as will be seen from the next experiment, the amount of base which can be taken up between two reaction points does not rise so much on heating as in the preceding experiments, showing that the phosphate arises from a base-carrying source which can supply the necessary amount of base for the inorganic phosphate derived from it. An example of this kind will now be given.

Exp. 3. (Horse muscle.) The original reactions and the reaction swings on addition of base will be given in condensed tabular form, the buffer capacity being determined between the original reactions of the various specimens and the reactions obtained after the addition of the maximal amount of base.

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Analyses of these specimens.

	Inorganic phosphate H_3PO_4 (molar)	Lactic acid (molar)
Juice (cold)	.0320	.031
Ultrafiltrate	.0222	.0244
Juice (warmed)	.0590	.039
Ultrafiltrate	.0450	.029

Reactions (Fig. 2).

	Original p_H	p_H after addition of mmols. base					Buffer capacity (molar phosphate)
		3	6	9	12	18	
Juice (cold)	6.75	6.85	6.92	7.04	—	—	.0530
Ultrafiltrate of cold juice	6.75	6.97	7.23	—	—	—	.0222
Juice (warmed)	6.57	—	6.74	—	6.93	7.10	.059
Ultrafiltrate of warmed juice	6.57	—	6.85	—	7.06	7.28	.0455

Fig. 2 (Exp. 3). I, Cold muscle juice; Ia, Ultrafiltrate of same; II, Heated juice; IIa, Ultrafiltrate of same (Horse).

There is a marked disparity between the juice specimens and their ultrafiltrates as regards their phosphoric and lactic acid contents, but in both there is the same evidence of the lactic acid increment being much below that of the phosphoric acid. The ultrafiltrates of the cold and of the heated juice and of the warmed juice before filtration possess buffer capacities which are evidently due to the phosphate system present in each. Another specimen of the same heated juice which was examined had a buffer capacity of 0.060M phosphate. The buffer capacity of the cold juice is much higher than its phosphate content and this is probably due to the initial high value of the lactic acid in this juice which had already deprived colloidal carboxyl groups of their base. The very slight increase in lactic acid which occurred on warming evidently secured the necessary base from the lactacidogen which had broken down without raising the buffer value of the heated much above that of the cold juice.

It is of interest to consider the base contents of the cold and warmed juice phosphate systems. The base content of 0.032M phosphoric acid in the cold juice at p_H 6.75, can be obtained from the ratio at this reaction, namely $\frac{.014}{.018}$,

while at the reaction of the warm juice, p_H 6.57, with 0.059*M* phosphoric acid the ratio would be $\frac{.020}{.039}$ or the large increase of 33 mmols. base. If the cold juice be regarded as containing originally 0.059*M* phosphoric acid (precipitable and hydrolysable), the ratio in terms of phosphate at p_H 6.75 would be $\frac{.0261}{.0329}$ or a total base content of 85.1 mmols., while the warmed juice with the same phosphate concentration, but all in the precipitable form, would be $\frac{.020}{.039}$ at p_H 6.57, or a total base content of 79 mmols., the loss of 6.1 mmols. from this system would be accounted for by the slight rise in lactic acid which would secure its base from this source and not from the colloidal constituents so that the buffering value of the latter should not rise as a result of heating.

The other case which requires consideration is one in which lactic acid rises without an inorganic phosphate increment, and therefore the former must be derived from some other source than "lactacidogen." This condition has been observed when the muscle has been kept sterile for some hours at room temperature, and the juice then expressed compared with that obtained from the juice of frozen muscle. Under such circumstances there is a greater swing in reaction towards the acid side than in cases where there is the usual phosphate increment. Such a case will now be examined.

Exp. 4. (Horse muscle.) One portion of the muscle was frozen immediately after removal, rapidly minced and the juice expressed, while the other portion was kept for 24 hours at room temperature (14°) in a sterile vessel.

The nitrogen percentage was 1.29.

Analyses. The juice only was examined.

	Phosphate (molar)	Lactic acid
Juice from frozen muscle	.030	.012
Juice from muscle after 24 hours at 14°	.033	.032

Reaction determinations.

1. Juice from frozen muscle.

Reaction (18°) p_H 7.18, rapidly changing to 7.03 after introduction of the juice into the hydrogen electrode titration vessel, so that the effect of addition of base was really studied in a juice of the latter reaction. On adding 12 mmols. base, the reaction shifted to p_H 6.34, equivalent to a buffer capacity slightly over 0.03*M* phosphate.

2. Juice from muscle after 24 hours at 14°.

Reaction (18°) p_H 5.76. In order to test the buffer capacity of this specimen within a somewhat similar range to that in which the cold juice was examined, alkali was gradually added until the reaction was 6.18. It was then tested in the usual way for its buffer value with the following results:

Original reaction	p_H 6.18
12 mmols. base	„ 6.52
24 „ „	„ 6.78
36 „ „	„ 7.17

Buffer capacity between p_H 6.18 and 7.17 is equivalent to 0.061*M* phos-

phate. The base content of the 0.030*M* phosphate at p_H 7.03 is derivable from the ratio $\frac{.0187}{.0113}$ at this reaction, and that of the 0.033*M* phosphate at p_H 5.76 from the ratio $\frac{.0034}{.0296}$. It is evident that there is a loss instead of the usual gain, of 12.3 mmols. to neutralise part of the acids produced during the period at room temperature.

If the cold juice be taken as originally of p_H 7.18, and the total phosphate concentration (precipitable and hydrolysable) as 0.033*M*, then the ratio would be $\frac{.0227}{.0103}$, and in the juice after warming at p_H 5.76 $\frac{.0019}{.0311}$. Thus the system could have furnished 20.8 mmols. base, more than sufficient therefore to neutralise the lactic acid produced between the two reaction points. The juice at p_H 5.76 had a buffer capacity for base, however, far greater than corresponded to its phosphate content. Actually 48 mmols. base required to be added to the juice to bring it back to the reaction of the cold juice, which shows that in this case there was acid production over and above that of the 20 mmols. lactic acid.

It is evident that the changes which have taken place under those conditions are entirely different from those observed when muscle juice is rapidly warmed to 40°.

DOG MUSCLE.

Attention will now be directed briefly to some preliminary experiments on the juice expressed from the voluntary muscle of the dog. In all cases the animals were bled and the blood washed thoroughly out of the vessels.

Exp. 1. The juice from frozen muscle and the same juice after warming will alone be considered.

Analyses.

	Phosphate (molar)	Lactic acid (molar)
Cold	.014	.006
Warmed	.025	.019

The increments are roughly equimolecular.

Reaction determinations.

1. Juice (from frozen muscle).

	Original, p_H 7.14
+ 3 mmols. acid,	6.76
+ 6 " "	6.33
+ 9 " "	5.80

Buffer capacity on acid side = 0.014*M* phosphate.

2. Juice after warming.

	p_H 6.69
+ 3 mmols. base	6.86
+ 6 " "	7.01
+ 9 " "	7.16

Buffer capacity on alkaline side = 0.035*M* phosphate.

The cold juice owed therefore its buffer capacity on the acid side to the inorganic phosphate present. Unfortunately, the nitrogen percentage was not determined, and so the strength of the solution as regards colloids is not known. The warmed juice, on the other hand, shows a greater buffer capacity than its contained phosphate, as tested by the addition of alkali.

Base content of the phosphate system before and after heating.

(a) At p_H 7.14 in the cold juice with 0.014*M* phosphate, the ratio would be $\frac{.0093}{.0047} = 23.3$ mmols. base.

(b) At p_H 6.69, in the warm juice with 0.025*M* phosphate, the ratio would be $\frac{.0103}{.0147} = 35.3$ mmols. base.

The inorganic phosphate therefore shows an increase of 12 mmols. base.

(c) At p_H 7.14, in the cold juice with 0.025*M* phosphate (precipitable + hydrolysable), the ratio would be $\frac{.0167}{.0083} = 41.7$ mmols. base.

On warming the juice which in its original condition contained 41.7 mmols. base attached to the combined "lactacidogen"-phosphate system, 6.4 mmols. base are removed in the change from p_H 7.14 to 6.69. During this change 13 mmols. lactic acid have been formed, so that approximately half the base required for its neutralisation must have come from some other source than the phosphate, and therefore, on testing the base absorbing power of the warmed juice between 6.69 and 7.16, acid groups, other than phosphoric, which had been deprived of their base during the process of heating, secured the equivalent amount of base for their neutralisation and therefore raised the buffer capacity on this side.

Another example showing the changes which occur in juice on warming may be considered before the ultrafiltrates are dealt with.

Exp. 2. (Dog.)

Analyses. The nitrogen percentage of cold and warmed juice was 1.54.

	H ₃ PO ₄ (inorganic) (molar)	Lactic acid (molar)
Cold	.025	.016
Warm	.049	.032

Reactions.

(a) Cold juice p_H 6.86

+ 6 mmols. acid, p_H 6.59

+ 12 mmols. base, p_H 6.94

Buffer capacity between 6.59 and 6.94 = 0.058*M* phosphate.

It would have been better to determine the capacity up to a more alkaline point as in the succeeding case.

(b) *Warm juice.*

Reaction p_H 6.36.

+ 24 mmols. base p_H 7.02.

Buffer capacity = 0.065*M* phosphate.

The buffer capacity of the cold juice between 6.59 and 6.94 is only slightly less than that of the warmed juice between 6.36 and 7.02, and in each case is much higher than the phosphate content.

If the cold juice be regarded as containing in the form of precipitable and hydrolysable phosphate the base present in a 0.049*M* concentration at p_H 6.97, then the ratio would be $\frac{.0248}{.0242}$, and at p_H 6.36 (warmed juice) $\frac{.0115}{.0375}$, or 13.3 mmols. base have passed from this system during heating, while 16 mmols. lactic acid were produced. The greater part of the base required for neutralisation could therefore be obtained from this source without encroaching on the colloidal or other base store, hence the amount of base which can be taken up between these two reaction points in the warmed juice should not differ much from that capable of being taken up by the cold juice.

Exp. 3. (Dog.) In this case juice and ultrafiltrates were both examined.

Analyses. The nitrogen percentage of juice was 1.36.

	H ₃ PO ₄ (inorganic) (molar)	Lactic acid (molar)
Cold juice	.0214	.010
Ultrafiltrate	.0151	.015
Warmed juice	.0527	.035
Ultrafiltrate	.0341	.031

There is a marked discrepancy between the analyses of the juice specimens and their ultrafiltrates, especially affecting the phosphate values. Evidently the method employed for deproteinisation (trichloroacetic acid) removed more precipitable phosphate than passed through the celloidin plates. The juice specimens may, however, be compared with one another as may also the ultrafiltrates. The increments in phosphoric and lactic acids in the juice are approximately of the same order as in the ultrafiltrates.

Reactions (Fig. 3).

Cold juice p_H 6.20. As this reaction is much more acid than usual, in testing the buffer capacity, alkali was in the first place added until the p_H was 6.53.

(a) Cold juice p_H 6.53.

+ 12 mmols. base p_H 6.95.

Buffer capacity = 0.049*M* phosphate.

(b) Ultrafiltrate of cold juice—originally p_H 6.27, brought by addition of alkali to p_H 6.69. On the addition of 6 mmols. base the reaction shifted to p_H 7.40.

Buffer capacity = 0.016*M* phosphate.

(c) Warmed juice p_H 6.07. Alkali was added until the reaction was p_H 6.68. When 12 mmols. base were now added, the reaction shifted to 7.03 and, on a further addition of 12 mmols. to 7.41.

Buffer capacity = 0.064*M* phosphate.

(d) Ultrafiltrate of warmed juice p_H 6.18. Alkali was added until the reaction was 6.66. On addition to the solution at this reaction of 6 mmols. base, the reaction became 6.94 and on the addition of a further 6 mmols. rose to 7.30.

Buffer capacity = $0.034M$ phosphate.

The buffer capacities of the ultrafiltrate are evidently due to the inorganic phosphate present, while those of the juice specimens are much higher.

It is interesting to consider the possible source of the base necessary to neutralise the lactic acid produced in a cold juice at such an acid reaction as p_H 6.20.

1. At p_H 6.20, the cold juice, with $0.0214M$ phosphate, would have the ratio $\frac{.0036}{.0178}$ and therefore contain 25 mmols. base.

2. At p_H 6.07, in the warmed juice, with $0.0527M$ phosphate, the ratio would be $\frac{.0071}{.0456}$, and the content 59.8 mmols. base.

3. At p_H 6.20, the cold juice, with $0.0527M$ H_3PO_4 (precipitable + hydrolysable), would have the ratio $\frac{.0090}{.0437}$.

Fig. 3 (Exp. 3; Dog). I, Cold muscle juice; Ia, Ultrafiltrate of same; II, Heated juice; IIa, Ultrafiltrate of same.

Hence between p_H 6.2 and p_H 6.07 there would only be a removal of 1.9 mmols. base from the system 3.

During this very slight reaction change 25 mmols. lactic acid appeared and as only 1.9 base equivalents were obtainable from the phosphate system, and the 25 mmols. lactic acid must have secured at p_H 6.07 rather more than 24.5 mmols. base, the excess must have obtained its base from colloidal sources, as the ultrafiltrate of the heated juice shows no sign of a base-carrying system other than the inorganic phosphate. The heated juice ought therefore to have a corre-

spondingly higher buffer capacity than the cold. The rise in buffer capacity, as determined, corresponds approximately to an increase of 15 mmols. in the base-absorbing capacity of the warmed juice. The original higher value of the buffer capacity of the cold juice (over its phosphate content) is due to the preceding acid formation, which led to a reaction of p_H 6.20, having already removed base from base carrying systems other than the precipitable phosphate.

It is evident that the lactacidogen system does not contribute to the buffering value of the ultrafiltrate of the cold juice. Experiments are at present being carried out dealing with the condition of the forerunner of the precipitable phosphate in the juice.

SUMMARY.

Horse muscle juice.

1. Reaction of cold juice at 18° varies from p_H 7.18– p_H 6.77, and of heated juice from p_H 6.63 to p_H 6.18. When the juice was expressed from muscle kept at room temperature p_H was 5.76.

2. On warming the juice there is always an increase in the inorganic phosphate which is more constant than the lactic acid increment.

3. The buffer values of the juice are higher than correspond to the actual phosphate concentrations, while the actual phosphate concentrations and buffer capacities of the ultrafiltrates correspond fairly closely.

Dog muscle juice.

1. Reaction of the cold juice varies from p_H 7.14–6.20 and of the heated juice from p_H 6.69–6.07.

2. On warming the juice there is always a greater increase in the inorganic phosphate and a more constant lactic acid increment than in the horse juice.

3. The buffer values of the juice are greater than the actual phosphate concentrations, except in one case where the lactic acid content of the cold juice was extremely low. The buffer values and the phosphate concentrations of the ultrafiltrates closely correspond to one another.

GENERAL CONCLUSIONS.

1. In the expressed juice from frozen muscle there are present at least three systems which carry base in a readily disposable form, namely, the inorganic phosphates, the organic phosphate precursors and the colloidal or protein salts.

2. When lactic acid is produced as a result of heating the juice, the acid secures its base from all these systems, the buffering capacity depending especially upon the base derivable from the hydrolysed organic phosphate precursor and also from the constituents of the juice which do not pass into the ultrafiltrate.

3. If there is no inorganic phosphate increment accompanying the acid production, the buffering capacity is diminished, as the sole base-carrying systems which are then in evidence are the pre-formed inorganic phosphates and the proteins.

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