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THE HYDROGEN ION CONCENTRATION OF THE ISOLATED UTERUS.

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Introduction. The original aims of this research were (1) to determine whether the hydrogen ion concentration of minced tissue could throw light on the responses of plain muscle to changes in the hydrogen ion concentration of Ringer solutions in which it is immersed; and (2) to find whether any difference in the *c*H of the tissue, associated with relaxation and contraction, could be detected. Although the first aim has not been realised, the pursuit of it led us to experiments on the *c*H of isolated plain muscle, minced after immersion in Ringer solutions of various compositions. The results of these experiments were unexpected, and, in our opinion, of sufficient general interest to place on record. The second aim was carried out, and the results obtained are described.

Technique. The *c*H measurements were by the glass electrode method (1), the treatment of the tissue being essentially the same as described in a paper by one of us with Furusawa (2) on the hydrogen ion concentration of the muscles of the cat. The uterus of the cat was used for the greater part of this investigation, because its size is as small as can easily be adapted to the glass electrode method without undue error, and as large as may allow reasonably rapid diffusion between the interior of the tissue and the solution bathing its surface. In some experiments (as will be indicated) it was slit longitudinally to aid such diffusion.

The *c*H results found for such a minced tissue correspond to the hydrogen ion concentration of the liquid phase of the moist mince, which must consist of the fluid from the ruptured cells together with the tissue juices. Loss of carbon dioxide from these liquids cannot be prevented during the mincing process, and any difference in the rate of loss between one sample and another must be reckoned as an experimental error. It is realised that no deductions as to the absolute hydrogen ion concentration inside the living cells can be made from

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such measurements on minced tissues, but it seems probable that differences in the observed values are significant, and no method is at present known to us whereby closer indications of *c*H differences within the cell membrane can be obtained. A further point is that the tissue includes, in addition to plain muscle, variable amounts of connective tissue and a considerable relative amount of mucous membrane. We are unable to say to what extent the results we have found are due to variations in the *c*H of these structural components of the uterus, but since the muscular tissue is greatest in amount, it is probable that the *c*H of the minced tissue is determined principally by it.

The *p*H measurements of the solutions were made under liquid paraffin and at room temperature, which varied on different occasions between 15° and 18° C.

The cats were anæsthetised with ether. In the earlier experiments they were bled by cutting the carotid artery. The uterus was gently dissected, and the horns attached to threads or glass hooks at the ovaries and through the muscle near the junction. The two horns were used separately, one frequently as a control. In the earlier experiments each horn was slit longitudinally before immersion in the saline bath. About 50 c.c. of the saline solutions were contained in boiling tubes, and warmed by a water bath at 37° C. Air was led into the solution through a glass tube which ended in a hook, to which the lower end of the uterus could be attached. The upper end was attached to an isotonic lever. Variations in detail of the foregoing technique took place as experiment necessitated, and are noted below.

The solutions were made up freshly each day from British Drug Houses A.R. chemicals. The standard concentrations, in all solutions, of sodium, potassium, and calcium chloride respectively, were 0·9 p.c., 0·042 p.c., and 0·024 p.c. as in Burn and Dale's(3) solution. Sodium bicarbonate or phosphate was added in varying amounts as mentioned below.

Experimental results.

The hydrogen ion concentration of the saline solutions. As a preliminary to the measurements of the *c*H of the uterus under various conditions, some experiments were made on the hydrogen ion concentration of the salt solution of the following composition:

Table I. Salt solution.

NaCl	0·9 grm.
KCl	0·042 ,,
$CaCl_2$	0·024 ,,
$NaHCO_3$	0·05 ,,
H_2O	100·0 c.c.

This corresponds to the solution used by Burn and Dale(3) with the omission of magnesium chloride and dextrose. The results are given in the following table:

TABLE II. *p*H of salt solution.

Conditions	*p*H	
	Uterus absent	Uterus present
Freshly made up	7·55 ±0·03 (3)	—
After ¼ hour air bubbling at 37° C.	7·78 ±0·04 (7)	7·84 ±0·07 (8)
After ½ hour–2 hours' air bubbling at 37° C.	8·14 ±0·06 (8)	8·01 ±0·09 (10)
After standing 6 hours at room temperature	8·05	—

The figures given represent mean values and the mean deviations, and the numbers in brackets indicate the number of observations.

From the above figures it will be seen that such a solution becomes more alkaline either on standing in air, or on bubbling air through it, as would be expected owing to loss of carbon dioxide. No significant difference could be observed between the effect of half an hour's and two hours' bubbling, but a difference could be seen between that of a quarter of an hour and half an hour. The final *p*H attained seemed to be independent of the presence of a uterus in the solution, within the limits of experimental variation.

Calcium in saline solutions. The relatively small solubility of calcium carbonate and phosphate is of great importance in the consideration of physiological saline solutions in which the hydrogen ion concentration is varied. Calcium carbonate is much less soluble than calcium bicarbonate, and tri-calcium diphosphate much less soluble than calcium monohydrogen phosphate; consequently the solubility products of calcium carbonate and tri-calcium diphosphate respectively are the limiting factors, although the absolute amounts of CO_3'' and PO_4''' ions present in solutions at a *p*H less than 8·0 are small compared with the concentrations of HCO_3' ions and HPO_4'' ions.

It can be inferred from the constants given by Holt, Le Mer and Chown(4) and Hastings, Murray and Sendroy(5) that in the solutions used by us—*i.e.* (i) a modified Burn and Dale solution, (ii) a similar solution containing *M*/250 bicarbonate, and (iii) a solution containing *M*/250 phosphate instead of bicarbonate, as used by Evans and Underhill(6)—the products $[Ca^{\cdot}]\,[CO_3'']$ and $[Ca^{\cdot}]^3\,[PO_4'']^2$ respectively exceed the solubility products of these salts above *p*H 8·1, 7·5 and 5·7 respectively. That is, the solutions are supersaturated with respect to calcium carbonate or phosphate on the alkaline side of these figures. Solution (ii) for example, at *p*H 8·2, would have a calcium ion concen-

tration five times the equilibrium value. It was pointed out by the above workers and also by Van Dyke and Hastings(7) that similar saline solutions could remain supersaturated with respect to these substances for days. The reason for this condition of delayed equilibrium is not apparent, and it is not easily possible to determine chemically whether the excess calcium is ionised, or is combined as carbonate or phosphate, but not precipitated. Van Dyke and Hastings claim to have some indication of the state of the calcium by observing the response of a guinea-pig's uterus to constant doses of pituitary extract when placed in solutions in which all the ionic concentrations were maintained constant, with the exception of that of calcium. In this way they used the uterus as a "biological calcium electrode" and believe that the calcium in the supersaturated solutions is in an ionic form. Therefore, in view of this work, and on account of the lack of direct evidence, it may be assumed for the present that in the saline solutions under consideration the calcium is in the ionic condition, and that in spite of the absence of true ionic equilibrium its ionic concentration is constant during the course of an experiment with freshly prepared solutions, lasting only a few hours. In the case of the phosphate solutions this is only true at values of *p*H less than 7·8, at which point precipitation takes place.

Assuming that the bicarbonate solution of the composition given in Table I is in equilibrium with the CO_2 in atmospheric air (0·04 p.c.), after half an hour's air bubbling, when the *p*H has been found to be 8·1, the amount of total carbonate in the solution can be calculated to be 0·001 *M*. The amount of $NaHCO_3$ added corresponded to 0·006 *M*. Hence it follows that 83 p.c. of the carbonate has been removed during aeration, and the buffering power of the solution must be very small. This solution however is not supersaturated with regard to calcium under these conditions.

The differences of hydrogen ion concentration between the isolated uterus and its solution. If a uterus be removed from an anæsthetised cat, and placed in oxygenated saline solution of the composition described above, there is a striking and permanent difference between the hydrogen ion concentration of the muscle and that of the liquid bathing it. The muscle is invariably more acid than a relatively alkaline solution, as is shown in Table III, the difference of *p*H amounting to about 1·1. Measurements given in the table were made on the muscles, and on the solutions in contact with them at the time of their removal. The durations of immersion in the saline solution at 37° C. with air bubbling are

recorded, and the *p*H values of the muscle show no significant variation with time.

TABLE III. *p*H of cat's uterus in aerated bicarbonate buffered saline solutions at 37° C.

*p*H of uterus	*p*H of solution	Δ*p*H	Duration of immersion
7·21	(8·01)	+0·78	2–3 sec.
6·73	(8·01)	+1·28	2–3 sec.
6·89	7·72	+0·83	15 min.
6·90	7·84	+0·94	30 min.
6·94	8·13	+1·19	1 hour
6·60	7·72	+1·12	1 hour 40 min.
6·75	8·08	+1·33	2 hours
6·83	7·95	+1·12	2 hours 10 min.
6·80	7·95	+1·15	2 hours 20 min.
6·83	7·94	+1·11	2 hours 50 min.

A comparison of the values of *p*H of the muscles in contact with the above bicarbonate buffered solution (Table III) with those of muscles in contact with more acid solutions of similar composition (Table VI), indicates that the hydrogen ion concentration of the uterus is largely independent of that of the solution in which it is suspended. Muscles immersed in the acid media are indeed more acid than those in alkaline media, but the change in *p*H of the muscle is only about one-quarter of that in the corresponding solutions, and is barely outside the range of variation of individual uteri.

Similar observations on the conditions of uteri in salt solutions buffered with phosphate are recorded in the following table:

TABLE IV. *p*H of cat's uterus suspended in aerated phosphate buffered saline solutions at 37° C.

*p*H of uterus	*p*H of solution	Δ*p*H	Length of uterus	Duration of immersion	Concentration of buffer
6·99	7·32	+0·33	Contracted	15 min.	0·004 *M*
6·88	6·74	−0·14	Relaxed	15 ,,	,,
6·68	6·12	−0·56	,,	15 ,,	,,
6·79	6·12	−0·67	,,	15 ,,	,,
6·67	6·62	−0·05	Contracted	30 ,,	0·001 *M*
6·63	6·07	−0·56	Relaxed	30 ,,	,,
6·59	6·90	+0·31	Intermediate	1 hour	,,
6·34	6·90	+0·56	,,	1 ,,	,,
6·56	6·90	+0·34	,,	2½ hours	,,
6·59	6·90	+0·31	,,	2½ ,,	,,
6·62	7·05	+0·43	Relaxed	4 ,,	,,
6·82	7·03	+0·21	,,	6 ,,	,,

Here again duration of immersion in the solution appears to be without marked influence on the *p*H of the muscles. The muscle seems even more independent of the hydrogen ion concentration of the phosphate buffered solution than it is of that of the bicarbonate buffered solution.

The *p*H of the horn of a uterus taken straight from the cat, after ether anæsthesia and bleeding, was found to be 7·07 ± 0·07. The above *p*H values of the muscles after immersion are lower than this figure, indicating that acid is developed in the course of the manipulation. The stage at which this acid is formed will be discussed in a later section.

The hypothesis that the excitability of heart muscle is influenced by the difference of *p*H between the muscle and its environment (Andrus(8)) has a suggested application to unstriated muscle (Lovatt Evans(9)). We therefore examined our figures, in the hope of detecting some correlation between the degree of contraction of the uterus and the difference of hydrogen ion concentration between the muscle and the solution. If attention be confined to the cat's uterus, which tends to contract in alkaline and relax in acid media within the physiological range of *p*H, there does perhaps appear to be some such qualitative relation. The greatest differences of hydrogen ion concentration were found after immersion in bicarbonate solutions of *p*H about 8·0, in which the muscles were contracted. However, we are not inclined to regard this relation as significant but as a consequence of our happening to choose solutions which varied more widely in an alkaline sense, than in an acid sense, from the mean *p*H of the uteri. Several examples are recorded in Tables IV and VI in which a muscle is appreciably more alkaline than the solution, and although the difference of *c*H was considerable, there was no indication of contraction. Moreover, consideration of the behaviour of the guinea-pig's uterus throws further doubt on Andrus's hypothesis. This organ is commonly relaxed when in equilibrium with bicarbonate buffered solutions at a *p*H of about 8·0.

TABLE V. *p*H of the guinea-pig's uterus and of bicarbonate buffered solutions, after ½ hour immersion at 37° C.

*p*H uterus	*p*H solution	Δ*p*H
6·96	7·91	+0·95
6·76	8·27	+1·51
6·94	8·31	+1·37

Table V shows that the differences of *p*H between the modified Burn and Dale solution and the guinea-pig's uterus are of the same order as found with the cat's uterus. The uteri were taken from guinea-pigs 350 grm. in weight, and were not quite fully relaxed in the solution. Uteri from younger animals, which relax more perfectly in such circumstances, are so small that they warm up quickly in the glass electrode during measurement of their hydrogen ion concentration, and the

consequent acid formation introduces large errors. Experiments performed on them are therefore not recorded.

Comparison of conditions in bicarbonate and phosphate buffered solutions of the same pH. In a further series of experiments on cat's uteri, one horn of a uterus was placed in a bicarbonate buffered solution, the other horn in a phosphate buffered solution of initially the same pH. Concentrations of sodium, potassium and calcium chlorides were the same in all solutions. The hydrogen ion concentrations of the bicarbonate buffered solutions were adjusted by bubbling suitable mixtures of carbon dioxide and air through them throughout the experiment, and diffusion at the surface of the liquid was delayed by a one-inch layer of liquid paraffin. Air was bubbled through the phosphate buffered solutions at about the same rate. Concentrations of buffer at the pH chosen were calculated to give 0·004 *M* total carbonate and total phosphate respectively. The results are given in Table VI.

TABLE VI. pH of two horns of cat's uteri immersed in bicarbonate and phosphate buffered solutions.

Cat No.	pH of uterus	pH of bicarbonate solution	ΔpH	pH of uterus	pH of phosphate solution	ΔpH	Length of uteri	Size of uterus
1	6·66	7·44	+0·72	6·80	6·94	+0·14	Intermediate	Large
2	6·77	7·34	+0·57	6·56	7·19	+0·63	Contracted	Large
3	6·48	7·02	+0·54	6·57	6·97	+0·40	Relaxed	Medium
4	6·37	6·95	+0·58	6·40	6·95	+0·55	Intermediate	Small
5	6·67	6·41	−0·26	6·35	6·35	±0·00	Relaxed	Large
6	6·47	6·29	−0·18	6·86	6·32	−0·54	Relaxed	Large

In experiments on cats Nos. 1, 3, 5 the horn of the uterus first removed was placed in bicarbonate solution; in cats Nos. 2, 4, 6 the first horn was placed in the phosphate solution. The periods of immersion were one half hour except in cat No. 1, where the period was one and a half hours for reasons external to this experiment.

These figures lend no support to the view (Jacobs(10), Scott(11), Dale and Evans(12), Hartree and Hill(13), and others) that the cell membrane is more permeable to carbonic than to phosphoric acid, and fit in better with the finding of Stella(14), that inorganic phosphate is able to pass freely by diffusion in and out of a muscle immersed in Ringer's solution.

Development of the acid in the uterus during isolation. It has been shown above that acid is formed during the manipulation of the uterus, and that the consequent pH change is irreversible. Variations in the technique were introduced in an attempt to discover at what stage

this acid formation took place. Substitution of chloralose for ether as anæsthetic had no significant effect on the results. In experiments in which the cat was not bled previous to the removal of the uterus the acid production was frequently less, but not absent. The practice of slitting each horn of the uterus before introduction into the saline bath had no detectable effect on the final *p*H of the uterus. That some of the acid formation could take place in the animal was shown by dissecting the uterus, ligaturing one horn, removing it and plunging it in liquid air, and repeating the procedure with the other horn of the uterus after an interval of five minutes. The second horn was often more acid than the first by varying amounts up to 0·60 *p*H. That the majority of the acid formation took place previous to immersion in the saline bath was shown in experiments in which one horn of the uterus was put straight from the cat into liquid air, while the other horn was tied on to the glass hook, dipped in the saline solution at 37° C., taken out again immediately, cut off the hook, and transferred to liquid air. In one experiment the *p*H of the first horn was 7·15 and the second horn 6·73, and the time between administering the anæsthetic and placing the uterus in liquid air was 5 min. 10 sec. for the first horn, and 7 min. 58 sec. for the second horn. The time interval between the ligaturing and the liquid air immersion was 30 sec. for the first horn and 1 min. 40 sec. for the second horn. In this experiment the uterus was smaller than the average. Other experiments indicated that the acid formation was less rapid in larger organs. The experimental results were also found to be unaffected when oxygen was substituted for air bubbling.

It was therefore concluded that the acid formation took place between the time of ligaturing the uterus and the immersion in the saline bath, that it was not affected by the injury due to slitting, nor by the use of ether as an anæsthetic, and that it tended to be greater when the animal had been bled previously.

Post-mortem acid formation. Measurements were made on uteri in which post-mortem acid formation was allowed to develop to a maximum. In some cases the organ was left at room temperature overnight between two watch glasses (to prevent excessive drying), and in others it was placed in a covered boiling tube in a water bath at 37° C. for three to four hours. The results varied between 6·22 and 7·03, the mean of 16 determinations being 6·58. This value is more alkaline than that found by Furusawa and Kerridge(2) for the cardiac and gastrocnemius muscles of the cat under similar conditions. The high degree of variation may be due to the variation in buffering power of the uterus as shown

by the same workers, and is not greater than would be accounted for on that ground only. The above *p*H value would correspond to a lactic acid increase of about 1·2 millimols (or 0·11 grm. p.c.) above the resting value. This figure is of the same order as that found by Lovatt Evans(15) for the lactic acid content of the cat's uterus by analytical estimation of the lactic acid.

The influence of state of contraction or relaxation on the hydrogen ion concentration of the uterus. The possibility of acid formation associated with contraction was first investigated on cats anæsthetised with chloralose. One horn of the uterus was removed before, and the other after, injecting post-pituitary extract into the jugular vein; the results are shown in Table VII.

TABLE VII. *p*H of the cat's uterus before and after injection of post-pituitary extract.

*p*H before injection	*p*H after injection	Interval between injection and removal of second horn	Dose (international units)
7·00	6·99	5 min.	0·06
7·31	7·04	5 ,,	0·06
6·60	6·61	10 ,,	0·12
7·46	7·46	10 ,,	0·12
7·24	7·41	10 ,,	0·12
7·46	7·58	10 ,,	0·12
7·37	7·50	10 ,,	0·12

Thus no acid formation was detected in response to stimulation of the uterus of the anæsthetised cat with doses of pituitary extract sufficient to ensure full contraction for at least an hour (Knaus(16)). A similar experiment on a rabbit gave 6·72 and 6·80 as the values of *p*H for a half uterus before and after pituitary injection. If acid be formed, it may be rapidly removed by the circulation through the muscle of so adequately buffered a liquid as blood. It was determined therefore to attempt analogous experiments on the uterus suspended in lightly buffered saline solutions. Cats' uteri were suspended in about 50 c.c. of the standard saline solution buffered with 0·001 *M* phosphate, adjusted to *p*H 7·22. After some hours' immersion 0·5 c.c. of a suitable concentration of a post-pituitary extract (neutralised to *p*H 6·82) was added to one horn; after 10 minutes' contraction both the contracted and unaffected horns were removed to liquid air for *p*H determinations. The results are given in Table VIII.

Similar experiments were performed on the uteri of two young (1½ kilo) rabbits which were stimulated for 5 min. with epinine[1], and

[1] See note to Table VIII.

TABLE VIII. Comparison of the *p*H of contracted and relaxed isolated uteri.

	*p*H of relaxed uterus	*p*H of solution	*p*H of contracted uterus	*p*H of solution	Stimulus
Cat 1	6·51	7·06	6·82	7·03	Pituitary
Cat 2	6·69	7·04	6·62	7·05	Pituitary
Rabbit 1	6·68	7·16	6·82	7·32	Epinine* ($1 : 10^5$)
Rabbit 2	6·82	7·42	6·82	7·30	Epinine

* Epinine (3 : 4—dihydroxy phenyl-ethylmethylamine) is a stable substitute for adrenaline produced by Burroughs, Wellcome, Ltd. The activity of epinine is about one-tenth of that of adrenaline. The dose chosen gave about maximal contractions of the uterus.

these results are also recorded in Table VIII. In this case the solution was oxygenated and buffered with 0·004 *M* phosphate. After the relaxed muscles were removed to liquid air a sample of the solution was taken, and its *p*H is given in the table. To the rest of the solution the standard dose of epinine was added, the liquid allowed to remain with continued oxygenation for 5 min., and samples then taken for *p*H determinations gave the values 7·23 and 7·30 respectively.

It is evident that no conclusions can be drawn from the results of this method. Acid formation, if any, due to contraction, has not revealed itself either by marked acidity of the muscle or by marked acidity of the liquid in the bath which might occur if the acid were to diffuse rapidly out of the muscle. Inspection of Tables III and V, in which the states of contraction and *p*H values for uteri are given, reinforces the conclusion that no striking variation of *p*H of muscle is associated with contraction or relaxation of the muscles. The variation, if any, is masked by adventitious *p*H differences between different samples of muscle.

SUMMARY.

1. The *p*H of the minced uterus is very little affected by that of the solution in which it has been immersed. In the usual bicarbonate-buffered, aerated, saline solutions at a *p*H of about 8·0 the difference in *p*H between muscle and solution is about 1·1.

2. The effect of the *p*H of a solution on the tone of a uterus cannot be attributed to the sign or magnitude of the difference of *p*H between the muscle and the solution.

3. The variation of calcium ion concentration with *p*H of certain bicarbonate and phosphate buffered Ringer solutions is discussed.

4. Measurements on the minced tissue indicate that the hydrogen ion concentration of an isolated uterus is greater than that of the uterus *in situ*.

5. The acid formation, represented by this difference, is largely irreversible. It takes place during excision and before immersion in saline solutions.

6. After maximum post-mortem acid formation, values of *p*H of 16 samples of cats' uteri (minced) varied between 6·22 and 7·03—the mean value being 6·58.

7. Comparison of uteri before and after stimulation with drugs, both *in vivo* and *in vitro*, yielded no evidence of acid formation associated with contraction.

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