it is possible to see that apparently 4-iodine-antipyrine (I^{131}) gives acceptable results for total body water content. However, the distribution of the indicator in tissues is uneven and the water content determined for each organ differs widely from the results of SOBERMAN[1,8] with antipyrine and PRENTICE et al.[12] with tritium. The rate of disappearance of the indicator from tissues is not uniform and does not follow the rate of disappearance from plasma.

Discussion. These results sustain the early suggestion of SULLIVAN and ROSE[11] that 4-iodine-antipyrine (I^{131}) is not a suitable indicator for the measurement of total or regional water content in the rat.

FLORA et al.[13] found that this indicator reaches an equilibrium in plasma 20 min after injection, which is not altered in their observation period (2 h). Our results do not sustain this observation, being remarkable for the different rate of disappearance from plasma and tissues.

Thus far, in our experience there is no evidence that 4-iodine-antipyrine labelled with I^{131} follows the distribution of water in tissues.

Presumably this can be attributed to an early splitting of the carrier-indicator complex and the subsequent accumulation of the latter in the corporal iodine pool. However, the coincidence of the results obtained for total body water content with 4-iodine-antipyrine (I^{131}) and tritiated water cannot be explained on the grounds of their different distribution in tissue water.

Volume of distribution of 4-iodine-antipyrine (I^{131}) in tissues

	ml/100 weight (mean ± S.D.)	% of decay of radioactivity in 60 min
Whole body	67.7 ± 14.3	− 20.0
Testis	24.3 ± 3.9	− 8.6
Kidney	62.5 ± 5.2	− 1.1
Spleen	43.4 ± 5.5	+ 1.5
Liver	38.5 ± 3.8	− 1.4
Myocardium	33.5 ± 19.7	− 1.5
Lung	62.7 ± 45.3	− 0.05
Skin	105.3 ± 28.6	− 4.4

Resumen. La determinación de agua total y de agua tisular se efectuo por medio de la dilución de 4-yodo-antipirina (I^{131}) en la rata. Los resultados obtenidos muestran que la distribución regional del indicador no es similar a la del agua, aunque el volumen de agua total encontrado se aproxima a los valores medios normales. Esta distribución del indicador en los tejidos no hace aconsejable la utilización de este indicador para la medición del volumen de agua total o regional.

R. H. MEJÍA

Instituto de Fisiología, Facultad de Medicina, Universidad de Buenos Aires (Argentina), 18 September 1967.

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Electrical and Contractile Properties of Isolated Rat Atria in Buffer-Free Medium as Influenced by Changes in pH

The effects of hydrogen ion concentration on the electrical properties of the heart has been studied in Purkinje fibers[1], isolated rabbit atria[2,3] and frog ventricle[4]. In these studies the hydrogen ion concentrations were varied by alterations in the composition of the buffer systems. In view of the fact that buffers have been demonstrated to modify cardiac function[5,6] the possibility existed that the buffers themselves might have contributed to the results. In the present investigation, the effects of pH on the electrical membrane properties and developed tension of isolated rat atria were studied in buffer-free media.

Methods. Atria from male Sprague-Dawley rats were removed and suspended in a modified Krebs-Ringer bicarbonate medium with glucose as substrate[7]. The medium was aerated with 95% O_2:5% CO_2 and maintained at a pH of 7.4. Atria were stimulated at a rate of 200/min at 30 °C. The developed tension, resting potentials, magnitude and time course of action potentials and conduction time were determined as previously described[8]. The maximum rate of rise of the action potential was obtained by differentiating the output of the amplifier carrying the action potential.

The buffer-free medium was prepared by replacing the sodium bicarbonate and the potassium phosphate in Krebs-Ringer medium with equivalent concentrations of sodium and potassium chloride. This medium was aerated with 100% O_2. The pH of this medium was adjusted to the desired value (pH 3.0–12.0) by the addition of dilute NaOH or HCl. The pH was continuously monitored throughout the experimental period.

Results. Figure 1 shows the changes in atrial developed tension that occurred when the Krebs-Ringer bicarbonate medium was replaced with buffer-free medium at pH 7.4, 6.0 or 8.8. It is evident that exposure of the atria to buffer-free media resulted in a transitory positive inotropic effect that was followed by a rapid decrease in developed tension. (This is in contrast to the Krebs-Ringer bicarbonate control which showed only a small decrement in developed tension over the same period of time.) At pH 6.0 the decrement in developed tension was not significantly

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different from that of atria suspended in pH 7.4 medium. On the other hand, at pH 8.8 the decrement was significantly less. As can be seen from the Figure, the inotropic changes were reversible, for in every case the atrial developed tension returned toward the control values when the buffer-free medium was replaced with Krebs-Ringer bicarbonate.

Figures 2 and 3 summarize the electrical changes that occurred when atria were suspended in buffer-free medium

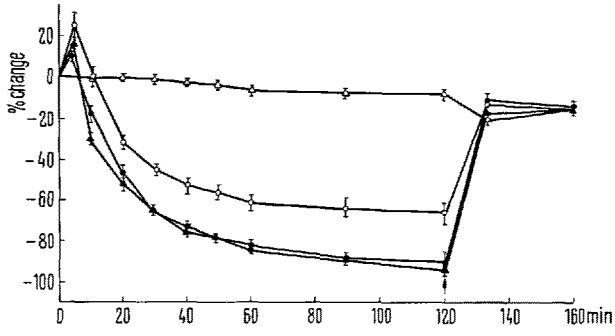

Fig. 1. Effect of pH on the developed tension of isolated rat atria suspended in buffer-free media. △—△, control in Krebs-Ringer bicarbonate medium at pH 7.4 (6 atria); ▲—▲, buffer-free medium at pH 7.4 at 0 time (6 atria); ●—●, buffer-free medium at pH 6.0 at 0 time (6 atria); o—o, buffer-free medium at pH 8.8 at 0 time (6 atria). At arrow buffer-free media was replaced with Krebs-Ringer bicarbonate. Vertical bars represent the SEM.

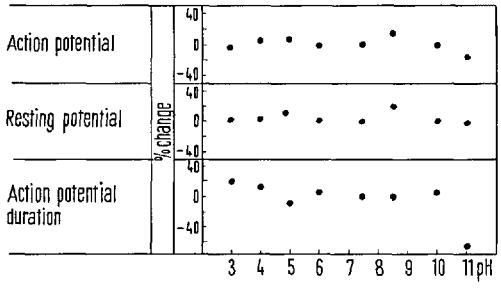

Fig. 2. Effects of pH on resting potential, action potential and action potential duration of isolated rat atria suspended in buffer-free media. Control values of these parameters were recorded after 10 min in buffer-free medium at pH 7.4 and compared with data obtained at the different pH values. Each point is the average of experiments from 6–36 atria. Determinations were made over a period of 1–10 min after the pH modification.

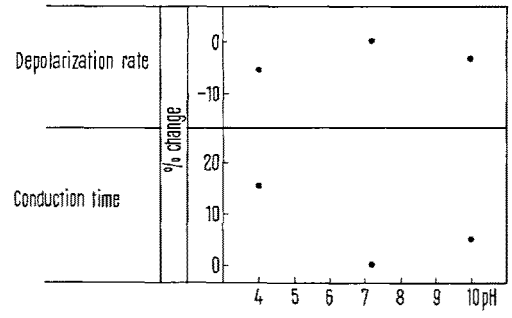

Fig. 3. Effects of pH on maximum rate of depolarization and conduction time of isolated-rat atria suspended in buffer-free media. Conditions as in Figure 2.

at acid or alkaline pH. As shown in Figure 2, the magnitude of the action potential and resting potential as well as the action potential duration showed little change over the range of pH from 4.0–10.0. At pH 3.0 the duration of the action potential was significantly increased; whereas, the magnitude of the resting and action potentials remained relatively unchanged. At pH 11.0 a significant shortening of the action potential duration was observed. This was accompanied by a small but consistent reduction of the action potential magnitude without any appreciable change in the resting potential. At the extreme range of pH, 3 and 12, the action potential disappeared within 2 min; while at pH values as low as 4.0 or as high as 10.0, essentially normal action potentials were recorded over the 10 min interval.

As shown in Figure 3, acidity (pH 4.0) reduced the maximum rate of depolarization and prolonged the conduction time. Alkalinity (pH 10.0) produced less definite changes on these 2 parameters.

All the above findings were independent of the sequence of pH changes for the results were similar whether the pH changes were from alkaline to acid or from acid to alkaline.

Discussion. The demonstration in the present study that replacement of Krebs-Ringer bicarbonate with buffer-free medium results in a rapid decrement of developed tension indicates that buffers play an important role in the regulation of cardiac contractility. From the available evidence, it would appear that it is the bicarbonate rather than the phosphate which is the more important buffer system in the medium. WHITE and SALTER[5] demonstrated a rapid decrement in the developed tension of cat papillary muscle when phosphate was substituted for bicarbonate in Krebs-Henseleit medium. BERMAN and SAUNDERS[6] demonstrated that in phosphate buffered medium (bicarbonate omitted) that glucose was relatively ineffective in restoring the contractile tension of rat ventricle strips made hypodynamic by prolonged beating in substrate-free medium. RICE and BERMAN[9] further demonstrated that in phosphate buffered medium pyruvate was more rapidly metabolized than glucose and it was proposed that in the absence of bicarbonate buffer a defect in the Embden-Meyerhof pathway occurs. Omission of glucose from the medium results in a rapid depression of atrial contractility[10], and it is possible that the rapid decrement in developed tension that occurs in buffer-free medium may in part be due to a defect in glucose metabolism[11]. The findings that the contractile decrement in alkaline medium is less than at pH 7.4 or 6.0 indicates that pH by itself independent of the presence of buffers can alter developed tension. LORKOVIĆ[4] proposed that the intracellular pH of cardiac muscle is sensitive to the pH of the environment. Thus it is possible that the better maintenance of developed tension may be related to an alteration in the intracellular pH[12]. The findings that the action potential duration of atria suspended in buffer-free medium at acid pH was prolonged while the opposite occurred at the extreme alkaline side are in agreement with other studies performed on sheep Purkinje fibers[1] and frog ventricle[4]. HECHT and HUTTER[1] demonstrated that the outward ion current of Purkinje

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fibers was greater in alkali than in acid, and this could explain the changes in action potential duration observed in this study; furthermore, the reduction of the maximum rate of depolarization as well as the increased conduction time produced at pH 3.0 may be the result of competition between H^+ and Na^+ for membrane carriers[13].

Zusammenfassung. Der Einfluss von pH-Änderungen auf die Kontraktions- und elektrischen Eigenschaften von Rattenvorhöfen wurde in pufferfreien Medien untersucht; eine Suspension von Vorhöfen in pufferfreier Krebs-Ringerlösung hatte eine zweiphasige inotrope Wirkung, (anfängliche Erregung, hierauf progressive Entspannung). Reduktion des pH von 7,4 auf 6 hatte keine bedeutende Wirkung auf die kontraktile Spannung, während eine Erhöhung des pH von 7,4 auf 8,8 das Spannungsdekrement verringerte. Saures Medium erhöhte die Dauer der Aktionspotentiale, verringerte die maximale Geschwindigkeit der Depolarisierung und verlängerte die Leitungszeit, während ein alkalisches Medium die Dauer der Aktionspotentiale verkürzte.

K.-Ch. Ko, A. L. Gimeno[14] and D. A. Berman

University of Southern California School of Medicine, Department of Pharmacology, Los Angeles (California 90033, USA), 26 June 1967.

[13] This work was supported by Research grants (No. 5T1HE-5536 and H-1999) from the National Heart Institute, U.S. Public Health Service.

[14] Established investigator from the Consejo Nacional de Investigaciones Científicay y Técnicas de la República Argentina. Present address: Segunda Catedra de Fisiología, Facultad de Medicina, Buenos Aires.

Viscosity Changes of Synovia after Application of Water Treated with Ultrasound

In our recent experiments[1,2] we demonstrated an increased permeability of water treated by ultrasound through connective tissue membranes. The effect of chemically active substances formed in sounded water on the colloids of connective tissue may be responsible for this effect. It is known that 2 principal compounds in connective tissue are present: collagen and mucopolysaccharides. Depolymerization of mucopolysaccharides determines an increase of connective tissue permeability in some experiments[3,4].

In order to prove the possibility of a similar mechanism under the conditions of our experiments, the effect of sounded water on synovia fluid has been investigated using changes in its viscosity as the measure of the degradation of hyaluronic acid.

The synovia was taken from the bovine joints and cleaned by filtration and centrifugation[4]. The relative viscosity was determined by an Ostwald capillary viscosimeter. All the measurements were made with fresh samples of synovia. After the first measurement of viscosity the synovia was diluted (5:1) with the deionized water (control), deionized water treated with ultrasound (frequency 800 kc, irradiation time 1 min, intensity 1 W/cm^2) and for comparison with hydrogen peroxide solution at a concentration of 1%. Viscosity measurements were repeated several times. The results, summarized in the Table indicate a rapid lowering of viscosity 2 min after the addition of the experimental solution. Using sounded water, the decrease of viscosity ceases within 10 min; using the hydrogen peroxide solution, the viscosity of synovia drops to the value of the viscosity of water. The decrease in viscosity under a lower concentration of hydrogen peroxide was not so evident.

Mean % of change of relative viscosity against the value of the first measurement (100%)

Min[a]	Control	Sounded water	Hydrogen peroxide
2	88.5	76.5	78.0
4	↓	74.5	76.5
6	↓	70.0	72.5
10	↓	68.0	72.0
30	↓	↓	65.5
60	↓	↓	63.0
120	↓	↓	62.5

[a] Time in min measured after adding experimental solutions.

A 0.06 mg % concentration of hydrogen peroxide as determined by polarographic method[5], was found in the water treated by ultrasound under our conditions. The decrease in viscosity when using sounded water is approximately the same as using 1.00 mg% solution of hydrogen peroxide. The presence of stabilizers in commercial hydrogen peroxide, or the additive role of free radicals in the sounded water, may be responsible for this difference.

Our results indicate that the indirect effect of ultrasound on the connective tissue may be caused by the chemical action of hydrogen peroxide[6,7] or of free radicals[8] on the mucopolysaccharides of connective tissue. These chemically active compounds can depolymerize the long chains of mucopolysaccharides, or they can only loosen the tertiary structure of polysaccharides and their hydration. Both these changes result in the decrease of anomalous viscosity of mucopolysaccharides.

Zusammenfassung. Nach Applikation von beschalltem Wasser wurde infolge physikochemischer Degradation der Polysaccharide ein Viskositätsabfall der Synovia nachgewiesen.

J. Pospíšilová

Department for Medical Physics, Medical Faculty, Purkyně University Brno (Czechoslovakia), 28 July 1967.

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