affected to the same proportion at all sarcomere lengths concerned between 1.5 and 3.0 μ. This means, if the length-tension curve is considered an index of the state of overlap between the A and I filaments (see above), that the tension delivered per unit overlap area may be varied over a substantial range by altering the degree of hydration of the fibre. This is an interesting finding because it suggests, in terms of the sliding-filament model, that the mechanical output produced by the individual active link is affected by changing the state of hydration of the cell. The nature of this effect is unclear. It may reflect a change of the interaction between the actin and myosin components due to alterations of the ionic composition[13,14] and the total ionic strength of the intracellular medium[15].

Zusammenfassung. Die Relation zwischen tetanischer Spannung und Sarkomerabstand wurde an der isolierten Semitendinosus-Faser des Frosches bei verschiedenen Graden von Hydratisierung der Faser untersucht. Es trat keine Veränderung des Länge-Spannungs-Verhältnisses auf, obwohl die Relation zwischen Länge-und Querschnittsdimensionen der Muskelfaser durch Immersion in den verschiedenen osmotischen Medien beträchtlich verschoben werden konnte. Die Ergebnisse stützen die Annahme, dass das Länge-Spannungs-Diagramm durch den Grad des Überlappens zwischen den A- und I-Filamenten und nicht durch den Querabstand zwischen den Filamenten bestimmt wird.

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[13] H. B. STEINBACH, J. cell. comp. Physiol. *24*, 291 (1944).
[14] E. BOZLER, J. gen. Physiol. *49*, 37 (1965).
[15] This study was supported by grants from the Muscular Dystrophy Association of America and from the Swedish Medical Research Council (No. B67-14x-184-03).

Distribution of 4-Iodine-Antipyrine (I^{131}) in the Rat

Since SOBERMAN et al.[1] proposed in 1948 the utilization of antipyrine for measurement of body water in mammals, wide experience has been accumulated in several species[1–8]. In some of them it has been shown that the indicator follows closely the distribution of water in the body[1,8].

In 1955 TALSO et al.[9] proposed the utilization of a derivative of antipyrine, the 4-iodine-antipyrine labelled with I^{131} for the measurement of body water in men. In this situation, the antipyrine played the role of a carrier, facilitating the penetration of the indicator (I^{131}) in the body water compartment. The volumes of distribution obtained in men[9] and sheep[10] were consistent and similar to those obtained with other chemical or radioactive indicators.

SULLIVAN and ROSE[11] suggested that an early metabolization of the carrier indicator complex may liberate the indicator (I^{131}), which will join the iodine body pool. Their suggestion was based on the finding of a lower concentration of indicator in brain than in skeletal muscle of rats.

Material and methods. In the course of studies on body composition, determination of body water content was attempted with 4-iodine-antipyrine (I^{131}) in 108 male rats from the strain bred at the Institute of Physiology.

Animals anaesthetized with sodium pentobarbital (40 mg/kg b.w.) were injected i.v. with 4-iodine-antipyrine (I^{131}), the amount injected being determined as in previous experiments in cpm[12].

Measurements were made in 9 groups of 12 animals of similar weight (mean 194.1: S.D. 15.2 g) at different time intervals, after injection: 90, 105, 120, 135, 150, 240, 300, 360 and 420 min. From the results obtained in all groups, the volume of distribution of the indicator was calculated by extrapolation to 'zero time', with the aid of the regression line logarithmic equation. In each group a sample of arterial blood was obtained at the predetermined time and immediately testis, kidney, myocardium, spleen, liver, lung and skin were extracted.

An aliquot of plasma and each organ was placed in plastic containers and their radioactivity in cpm measured in a well scintillation counter, ensuring a similar geometric efficiency for each reading. Volume of distribution in ml/100 g was calculated from the following general equations:

$$\frac{\text{cpm injected} \times 100}{\text{cpm in 1 ml of plasma} \times \text{body weight}} = \text{volume of distribution/100 g body weight}$$

$$\frac{\text{cpm in 1 g of tissue} \times 100}{\text{cpm in 1 ml of plasma}} = \text{volume of distribution/100 g tissue weight}$$

Corrections were made for plasma density to refer the results to water content in ml/100 g.

Results. From the regression line, the slope of disappearance of the indicator from plasma and tissues was calculated and expressed as % of decay of radioactivity in 60 min. The Table shows the results obtained, where

[1] R. SOBERMAN, B. N. BRODIE, B. B. LEVY, J. AXEBRO, V. HOLLANDER and M. J. STEELE, J. biol. Chem. *179*, 31 (1949).
[2] V. NOCITI, G. BRAMBILLA and I. CH. GESCON, Chir. ital. *11*, 773 (1959).
[3] V. NOCITI, G. BRAMBILLA and I. CH. GESCON, Chir. ital. *11*, 764 (1959).
[4] M. HERROLD and L. A. SAPIRSTEIN, Proc. Soc. exp. Biol. Med. *79*, 419 (1952).
[5] H. F. KRAYBILL, O. G. HANKINS and L. A. BITTE, J. appl. Physiol. *3*, 681 (1951).
[6] H. F. KRAYBILL, E. R. GOODE, R. S. B. ROBERTSON and H. S. SLOANE, J. appl. Physiol. *6*, 27 (1953).
[7] W. MEDWAY, Proc. Soc. exp. Biol. Med. *99*, 733 (1958).
[8] R. J. SOBERMAN, Proc. Soc. exp. Biol. Med. *74*, 789 (1960).
[9] P. J. TALSO, T. N. LAHR, N. SPAFFORD, G. FERENZI and H. R. O. JACKSON, J. Lab. clin. Med. *46*, 619 (1955).
[10] S. L. HANSARD and W. A. LYKE, Proc. Soc. exp. Biol. Med. *93*, 263 (1956).
[11] J. M. SULLIVAN and J. C. ROSE, J. Lab. clin. Med. *57*, 955 (1961).
[12] O. RETTORI, R. H. MEJÍA and L. A. FERNANDEZ, Acta physiol. latinoam. *14*, 221 (1964).

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.

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[13] J. H. FLORA, D. S. PHILIPS, F. ARCIDIACONO and L. A. SAPIRSTEIN, Circulation Res. *11*, 252 (1962).

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

[1] H. H. HECHT and O. HUTTER, in *Electrophysiology of the Heart* (Ed. B. TACCARDI and G. MARCHETTI, Pergamon Press, London 1965), p. 105.
[2] E. M. VAUGHAN WILLIAMS, J. Physiol. *129*, 90 (1955).
[3] E. M. VAUGHAN WILLIAMS and J. M. WHYTE, J. Physiol. *189*, 119 (1967).
[4] H. LORKOVIĆ, Circulation Res. *19*, 711 (1966).
[5] W. F. WHITE and W. T. SALTER, J. Pharmac. exp. Ther. *88*, 1 (1946).
[6] D. A. BERMAN and P. R. SAUNDERS, Circulation Res. *6*, 559 (1955).
[7] A. L. GIMENO, M. F. GIMENO, E. A. SAVINO and A. A. BEDNERS, Proc. Soc. exp. Biol. Med. *123*, 875 (1966).
[8] A. L. GIMENO, M. F. GIMENO and J. L. WEBB, Am. J. Physiol. *203*, 194 (1962).