altering the metabolic pathways leading to sprouting of the bud. Some more information is required to explain the mechanism of germination under water-deficient conditions[9].

Zusammenfassung. Das Keimen der Zuckerrohr-Achselknospen wurde als vom Knotenwasserpotential beeinflussbar beobachtet. Die Sprossentwicklung erfolgte noch bei so geringem Wasserpotential wie 1063,6 Joule/kg und selbst bei Fehlen jeglicher Wasseraufnahme. Ausgeschnittene Knospen mit einem Wasserpotential von 1063,5 bis 1823,4 Joule/kg konnten nur im Wasser gehalten austreiben, während ein Wasserpotential von 2026,0 Joule/kg tödlich war.

SUDAMA SINGH and K. K. SRIVASTAVA

Indian Institute of Sugarcane Research, Lucknow-2 (India), 29 May 1969.

[9] Thanks are due to Dr. KISHAN SINGH, Indian Institute of Sugarcane Research, for his valuable suggestions and kindly going through the manuscript.

Caffeine-Induced Contractures and Related Calcium Movements of Muscle in Hypertonic Media

Hypertonic solutions markedly reduce the mechanical responses of frog skeletal muscles to electrical stimuli while they leave the action potential normal[1–4]. The present paper describes effects of caffeine on contracture development and related ^{45}Ca movements in skeletal muscles in hypertonic media and media of normal tonicity and shows that ^{45}Ca still can be released by the action of caffeine on muscles in hypertonic media, although caffeine contracture development largely is blocked.

Methods. After excision the sartorii of the frog (*Rana pipiens*) were equilibrated for about 1 h in normal Ringer's solution before use. Normal Ringer's solution contained, in mmoles per liter of deionized water: 108 NaCl, 1.6 KCl, 1.0 $CaCl_2$, 2 *Tris* (hydroxymethyl) aminomethane-HCl buffer at pH 7.1, and 2×10^{-2} g/l of curare (D-tubocurarine chloride). Hypertonic Ringer's solution was prepared either by adding 330 m*M* sucrose to normal Ringer's or else by using 2.5 times as much NaCl and KCl as in normal Ringer's. Conventional massive stimulation[5] was used and isometric tension was recorded with a Statham ($\pm$ 4 oz) strain gauge and Sanborn amplifier and chart recorder. An atomic absorption spectrophotometer (Perkin-Elmer 303) was used for total calcium analyses[6]. ^{45}Ca movements were studied by standard techniques[6–8] and work was done at room temperature (about 23 °C). Caffeine (Eastman Organic Chemicals) was added to Ringer's solution as the free base.

Results. Exposure of muscles to the hypertonic sucrose Ringer's solution itself caused a contracture of 1–3 g (i.e. about 1–3% of maximal tetanic tention, P_0), which began within the first minute, reached a maximum within 3 min, and then started to decline after about 10 min (after 1 h the contracture tension was reduced by about half-maximum). After the completion of this work a paper by D. K. HILL[9] was published that also describes contractures of frog sartorii in hypertonic sucrose (although these are called 'changes of resting tension due to changes of the tonicity of the solution'). No such contractures were observed in the Na-K hypertonic Ringer's solution.

Table I shows that muscles in normal Ringer's solution exposed to caffeine (10 m*M*) develop a contracture tension of about 40% P_0, whereas muscles pre-soaked in either form of hypertonic Ringer's solution and then exposed to caffeine (10 m*M*) added to the hypertonic Ringer's develop much less contracture tension. This block by hypertonicity of caffeine contracture (and twitch and tetanic responses as well) appeared within 10 min after the muscles had been immersed in the hypertonic Ringer's and was almost complete by 1 h. Caffeine contractures of normal magnitude were obtained by returning a muscle to normal Ringer's solution after a soak in hypertonic Ringer's solution.

Using fresh frog sartorii, I tested for effects of hypertonicity on the total calcium content and the uptake and release of ^{45}Ca. Figures 1 and 2 demonstrate that hypertonic media (either with sucrose or extra Na and K) can cause a two- to three-fold increase in the rate of release of ^{45}Ca from frog sartorii. Control experiments show no such effect in the Achilles tendon of frog, so presumably in the whole muscle the ^{45}Ca release represents an effect on the muscle fibers per se. Furthermore, the effects of hypertonicity are shown in Figures 1 and 2 after about 120 min of washout of ^{45}Ca in normal Ringer's and thus represent an effect on essentially the slow component of ^{45}Ca release that is believed to reflect the rate of release of Ca from an intracellular locus across the surface membranes of the muscle fibers[8, 10]. This effect of hypertonicity on ^{45}Ca efflux is sustained for 1 h (Figures 1 and 2) and is somewhat better sustained in sucrose.

The addition of caffeine (10 m*M*; Figures 1 and 2) causes an increase in the rate of ^{45}Ca release in Ringer's of both normal and increased tonicity. In the latter case, the increase in ^{45}Ca release caused by caffeine appears additional to that caused by the hypertonicity itself.

Table II compares the total calcium content and the amount of ^{45}Ca uptake of frog sartorii that were exposed to ^{45}Ca for 10 min in either normal Ringer's solution (C) or in hypertonic Ringer's solution (E). Statistically there is a significant increase of 0.22 µmoles of calcium per

[1] A. L. HODGKIN and P. HOROWICZ, J. Physiol. *136*, 17P (1957).
[2] J. V. HOWARTH, J. Physiol. *144*, 167 (1958).
[3] J. TIGYI and F. SHIH-FANG, Acta Physiol. Acad. Sci. Hung. *22*, 293 (1962).
[4] P. VARGA-MANYI and J. TIGYI, Acta Physiol. Acad. Sci. Hung. *22*, 287 (1962).
[5] A. SANDOW and A. ISAACSON, J. gen. Physiol. *49*, 937 (1966).
[6] A. ISAACSON and A. SANDOW, J. gen. Physiol. *50*, 2109 (1967).
[7] C. P. BIANCHI and A. M. SHANES, J. gen. Physiol. *42*, 803 (1959).
[8] A. ISAACSON and A. SANDOW, J. Pharmac. exp. Ther. *155*, 376 (1967).
[9] D. K. HILL, J. Physiol. *199*, 637 (1968).
[10] A. M. SHANES and C. P. BIANCHI, J. gen. Physiol. *42*, 1123 (1959).

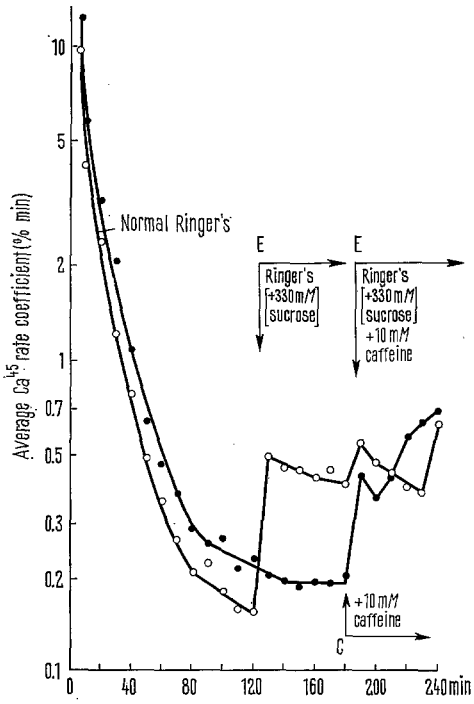

Fig. 1. The average rate coefficient of ^{45}Ca release (in % per min) from frog sartorii labeled for 3 h in ^{45}Ca 1 μc/ml, before start of washout. At 120 min, hypertonic Ringer's solution (plus 330 m*M* sucrose) replaces the normal Ringer's solution for the experimental set, E (open circles). At 180 min, caffeine (10 m*M*) is added to both the experimental (hypertonic) and control muscles. The data shown are the averages of 3 contralateral pairs of frog sartorii, i.e. 3 experimental and 3 control muscles.

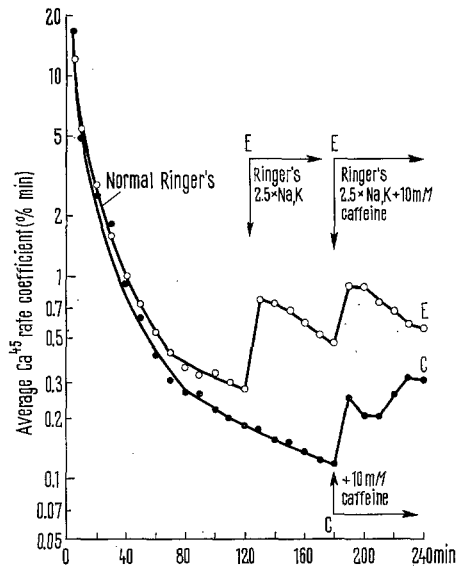

Fig. 2. The same conditions as those in Figure 1, except that the experimental muscles (E) are exposed to Ringer's solution made hypertonic with 2.5 times as much Na and K as in normal Ringer's. The data shown are the average of 2 muscles (control) and 3 muscles (experimental).

gram of muscle (wet weight) in those muscles treated with hypertonic Ringer's containing sucrose but no significant change in those treated with Na and K. Since the ^{45}Ca influx into the muscle fibers is calculated from the ^{45}Ca remaining in the muscle after the 120-min washout of the extracellular space[7], I conclude from Table II that there is no statistically significant increase in influx of ^{45}Ca in hypertonic Ringer's. But the large scatter in these data may hide a significant increase in ^{45}Ca uptake.

Discussion. The block of caffeine contracture owing to hypertonicity (Table I) agrees with reports of PODOLSKY and SUGI[11] and APRIL and REUBEN[12]. I did not observe any enhancement of the caffeine contractures, which CAPUTO[13] reported, even when the tests were made at the same concentrations of caffeine (3.9 m*M*) and the same degree of hypertonicity (two-fold Na) that he used.

Since an increased release of ^{45}Ca by caffeine was observed even in hypertonic media (Figures 1 and 2), I infer that under these conditions caffeine still is able to cause an increase in free calcium within the myofibrillar space, probably by acting on the sarcoplasmic reticulum that normally binds calcium in resting muscle[6, 8, 11, 14, 15]. The block of caffeine contracture by hypertonic media (Table I), even when the myofibrils are by inference exposed to an increased amount of free calcium, supports the conclusion of APRIL et al.[16] that an increase in ionic strength in the interior of muscle fibers is the effect of hypertonicity that causes the block in contracture tension.

Thus I infer that the major effect of hypertonicity in blocking twitch responses occurs at a late phase in excitation-contraction (E-C) coupling, i.e. after Ca^{++} has

Table I. Contracture tension (% P_0) in caffeine (10 m*M*) in hypertonic and normal Ringer's

Normal Ringer's	Hypertonic Ringer's		
	Pre-soak time in[a] hypertonic Ringer's (min)	2.5 × (Na + K)	330 m*M* sucrose
39	0	36	–
45	0	28	–
37[b]	8	12	–
	10	10	–
	10	1	–
	60	2	–
	60	–	6
	60	–	2

[a] These muscles after equilibration and recording of P_0 and twitch tension in normal Ringer's were pre-soaked in hypertonic Ringer's for the periods shown; caffeine (10 m*M*) then was added to the hypertonic Ringer's and the contractures were recorded. The control muscles were kept in Ringer's of normal tonicity throughout (except for the eventual addition of caffeine, 10 m*M*). Control values of P_0 and twitch tension were obtained before adding caffeine. [b] After a 10-min exposure to hypertonic sucrose Ringer's followed by 2-h reversal in normal Ringer's.

[11] R. J. PODOLSKY and H. SUGI, J. gen. Physiol. *50*, 2496 (1967).
[12] E. APRIL and J. P. REUBEN, Fedn. Proc. *27*, 375 (1968).
[13] C. CAPUTO, J. gen. Physiol. *50*, 129 (1966).
[14] A. WEBER and R. HERZ, J. gen. Physiol. *52*, 750 (1968).
[15] C. P. BIANCHI, J. gen. Physiol. *44*, 845 (1961).
[16] E. APRIL, P. W. BRANDT, J. P. REUBEN and H. GRUNDFEST, Nature *220*, 182 (1968).

been released from the sarcoplasmic reticulum. This does not exclude a lesser effect of hypertonicity at an earlier stage of E-C coupling, e.g. the reduction in the amount of calcium released at the surface membranes of muscle fibers reported by HOMSHER and BRIGGS[17], although not observed by CURTIS[18].

The activation of contractures by hypertonic sucrose media may result from the increase in total calcium content (0.22 μmoles/g, Table II) in frog sartorii. This is sufficient calcium to cause considerable activation of contraction[19] if it is bound to the myofibrils. The increase in the rate of ^{45}Ca release in the slow component in hypertonic Ringer's solution (Figures 1 and 2) may be an indirect effect of the change in external tonicity that should cause a rise in the internal ionic strength. Raising the ionic strength (with KCl) causes a decrease in the amount of calcium bound to sarcoplasmic reticulum preparations[20], and the same process may occur within intact fibers. An additional effect of the hypertonicity might be an increase in Ca^{++} concentration resulting from shrinkage of the fibers, although the direct effect of even removing 60% of the fiber water would not itself increase the concentration of Ca^{++} enough to cause contracture[21]. The enlargement of the elements of the sarcoplasmic reticulum shown by DYDYNSKA and WILKIE[22] to occur in hypertonic media suggests that the permeability properties of cell membrane systems also may be affected by hypertonicity. LÜTTGAU and OETLIKER[23] view the T-tubular system (TTS) as the site of action of caffeine. If they are correct, swelling of the TTS in hypertonic Ringer's may alter these hypothetical TTS sites for caffeine, thereby reducing its effectiveness in causing contracture.

Table II. Uptake of ^{45}Ca and analysis of total calcium (in μmoles Ca/g) of muscles exposed to ^{45}Ca in hypertonic or normal Ringer's for 10 min[a]

Time of washout (min)	Normal Ringer's (C)	Hypertonic Ringer's (E)	
		330 m*M* sucrose	2.5 × (Na + K)
^{45}Ca uptake			
0	0.299 ± 0.018	0.434 ± 0.056 (E-C)/C = 45.3% P[c] = 0.05	
120	0.0375 ± 0.0119	0.0641 ± 0.0267 (E-C)/C = 71.1% $0.1 < P < 0.2$	
0	0.231 ± 0.021		0.320 ± 0.017 (E-C)/C = 38.7% $P < 0.01$
120	0.0103 ± 0.0015[b]		0.0170 ± 0.0030[b] (E-C)/C = 64.4% $0.05 < P < 0.1$
Total calcium analysis			
120	2.54 ± 0.16	2.75 ± 0.18 (E-C) = + 0.22 ± 0.076 $P < 0.05$	
120	2.23 ± 0.37		2.01 ± 0.25 (E-C) = − 0.22 ± 0.13 $0.1 < P < 0.2$

[a] After exposure to ^{45}Ca, the muscles were washed out for 120 min in normal Ringer's. Calcium concentrations are given per gram of final wet weight of muscles after 120 min in normal Ringer's. Dispersions are given by the standard error. Uptake of ^{45}Ca is calculated from the ^{45}Ca space (ml/g) of the muscles (at 0 and 120 min) multiplied by the calcium concentration in the Ringer's (1 μmole/ml). Total calcium analysis is given for the same sets of muscles. [b] 5 muscles were used in general except in this instance where 4 were used. [c] *P* values are calculated from the *t*-test for paired variates.

The increase in total calcium content which occurs in Ringer's solution made hypertonic with sucrose presumably accounts for the small contractures found in this medium. No such increase was observed in hypertonic Ringer's having 2.5 times the normal concentrations of Na and K, nor was a contracture detected in this solution. A close reading of Hill's contribution[9] suggests that he found a contracture in excess Na-K Ringer's, as he most certainly did in sucrose hypertonic Ringer's solution. But the sensitivity of his tension recording was far greater than in the present work, and this may account for the difference[24,25].

Zusammenfassung. Die mit Koffein erzeugten Kontrakturen des M. sartorii von *Rana pipiens* waren meistens gehemmt, wenn die Ringer-Lösung entweder mit 330 m*M* Rohrzucker oder mit 2.5 × normaler Dosis von Na und K hypertonisiert wurde. Koffein kann die Geschwindigkeit der ^{45}Ca-Abgabe von Muskeln in hypertonischer oder normaler Ringer-Lösung erhöhen.

A. ISAACSON[26]

Division of Physiology, Institute for Muscle Disease, Inc., New York (N.Y. 10021, USA), 4 June 1969.

[17] E. HOMSHER and N. BRIGGS, Fedn. Proc. *27*, 375 (1968).
[18] B. A. CURTIS, J. gen. Physiol. *50*, 255 (1966).
[19] A. SANDOW, Pharmac. Rev. *17*, 265 (1965).
[20] A. P. CARVALHO and B. LEO, J. gen. Physiol. *50*, 1327 (1967).
[21] A. WEBER, R. HERZ and I. REISS, Proc. R. Soc. B. *160*, 489 (1964).
[22] M. DYDYNSKA and D. R. WILKIE, J. Physiol. *169*, 312 (1963).
[23] H. C. LÜTTGAU and H. OETLIKER, J. Physiol. *194*, 51 (1958).
[24] *Acknowledgments.* I thank Dr. ALEXANDER SANDOW for suggesting this study and for many discussions including those during preparation of this manuscript. I am also grateful to Dr. A. P. CARVALHO for help with the Atomic Absorption Spectrophotometer, JOHN POLK for technical assistance and to EDWIN GEFFNER for his helpful criticism of this manuscript.
[25] This investigation was supported by grants from Muscular Dystrophy Associations of America and the U.S. Public Health Service (grant No. NB-14262-04-06 to Dr. SANDOW).
[26] Present address: Department of Biology, Paterson State College, Wayne (New Jersey 07470, USA).