present experiments fairly large changes in the serum concentration of ionized calcium were only to a small degree reflected in the effluent from the perfusion system.

The difference between pH in the serum after ultrafiltration and in the blood probably reflects that serum from the more or less metabolically acidotic animals during the ultrafiltration procedure was equilibrated with a $P_{CO_2} = 40$ mm Hg.

The marked effect of pH may be explained in different ways: A pH induced increase in serum concentration in ionized calcium can hardly have been of a magnitude to cause the observed change, provided that the permeability from blood to CSF has not changed considerably.

Changes in the secretion rate of CSF may explain part of the effects observed. However, GRAZIANI et al.[15] have previously demonstrated that only part of the Ca-flux from plasma to CSF was affected by the secretion rate, and inspection of the data from the present experiments showed no tendency at all towards direct proportionality between the concentration of ionized calcium in the cisternal effluent and the rate of secretion of CSF.

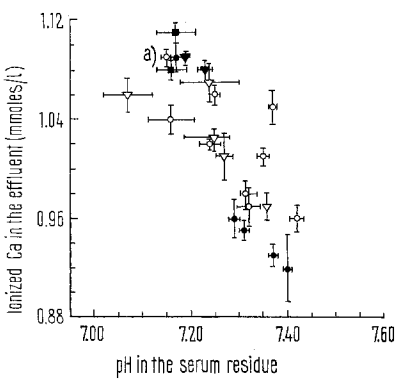

Fig. 2. The concentration of ionized calcium in the effluent as a function of the pH in the serum residue after ultrafiltration. Symbols as in Figure 1 (23 experiments on 23 rabbits).

It is known that pH affects the binding of calcium to subcellular structures, for instance in liver. Calcium is also bound to cerebral tissue[16]. An interference with this calcium binding by a decrease of pH may explain the observed increases in ionized calcium concentration in the effluent, provided that pH in the latter changed in the same direction as pH in plasma.

However, if a simple change in binding conditions took place, as a consequence of a change of pH, one would not expect this to cause the observed constant increase of ionized calcium concentration in the cisternal effluent, but rather an initial sudden change followed by an approach to a constant concentration.

Calcium has been shown to be transported between the extracellular and intracellular compartments in the brain, for instance through sodium-calcium exchange, demonstrated in cerebral cortex from cats[17]. An interference of pH with such transport-mechanisms may also explain the observed effects of pH.

The possible physiological significance of this phenomenon awaits exploration.

The results also suggest that in studies of calcium transport into and out of the CSF the pH of this fluid and blood should be rigorously controlled.

Zusammenfassung. Es wird am Kaninchen gezeigt, dass die Konzentration ionisierten Kalziums im Ausfluss eines ventriculo-cisternalen Perfusionssystems nur wenig von der Plasmakonzentration beeinflusst ist. pH-Erniedrigung im Blut erhöhte die Konzentration erheblich.

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Stimulation by Caffeine of the Calcium Efflux in Barnacle Muscle Fibers

There is evidence that the contraction of skeletal muscle caused by caffeine is associated with little or no depolarization of the fiber membrane[1–3]. Contraction is explained as being due to the release by caffeine of internally 'bound' calcium[4,5]. There also is evidence that caffeine stimulates ^{45}Ca efflux in frog and crab muscle[6,7], and ^{89}Sr efflux in barnacle muscle fibers[8]. The purpose of the following communication is to describe the kinetic results obtained by loading single barnacle fibers with radiocalcium and to show that caffeine, whether applied externally or internally, causes a rise in the Ca efflux.

Single muscle fibers were isolated by dissection from the depressor muscle bundles of specimens of *Balanus nubilus* or *B. aquila*. Fibers were cannulated in the same way as crab muscle fibers[3] and were then loaded with ^{45}Ca by means of a microinjector[9] as modified by CALDWELL and WALSTER[3]. The composition of the bathing fluid used was as follows (m*M*/l): NaCl 465, KCl 10, $CaCl_2$ 10, $MgCl_2$ 10, $NaHCO_3$ 10 and pH 7.8. Caffeine was obtained from Sigma Chemical Company.

The activity of ^{45}Ca in the wash-out specimens and the activity remaining in the fiber at the end of the experiment were assayed with plastic phosphor scintillators (Nuclear Enterprises 102A). The thickness of the wall of the phosphor cell (outer) was 3 mm. The solid 'inner' phosphor was designed so as to displace the 1.2 ml of wash-out sample, leaving only a fine film of fluid lying in

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between the inner and outer phosphor blocks. Silicone fluid and Perspex were used as optical couplers. All experiments were done between 22 and 24°C.

The loss of ^{45}Ca from fibers loaded by microinjection was consistently non-exponential, as illustrated in Figure 1. The rapid phase was found to have a half-time of 6.9 ± 0.8 min and the slow phase a half-time of 36.9 ± 4.5 min (mean $\pm$ S.E., n = 9). Departure from exponential kinetics is attributed as being due to prompt sequestration or binding of ^{45}Ca by the sarcoplasmic reticulum and mitochondria. Behavior of this type has also been reported by ASHLEY[8] who employed ^{89}Sr instead of ^{45}Ca. Taking the average rate constant for ^{45}Ca efflux as 0.7×10^{-4} sec^{-1}, the internal Ca^{2+} concentration as 2 mM (see BITTAR[10]) and the diameter of the fibers as 1 mm, the resting rate of Ca^{2+} efflux is calculated to be 3.5 pmole/cm^2 sec. This value is not very different from 1.8 pmole/cm^2 sec reported by LOWE[11] for crab muscle fibers, but at least one order of magnitude greater than that reported by HODGKIN and KEYNES[12] for squid axons.

Figure 2 shows the result of an experiment in which a 1 cm column of distilled water was injected into the fiber (4 experiments). One sees only a very slight and transitory rise in the Ca efflux, an effect attributable to the brief time it takes to complete the procedure of injecting the suspended fiber in air and not in artificial sea water.

Figure 3 shows the effect on radiocalcium efflux of external application of 10 mM caffeine. Caffeine, it will be seen, caused a prompt and substantial rise in the Ca efflux (18 experiments). Estimates of the size of the caffeine effect calculated on the basis of the change in rate coefficient for ^{45}Ca efflux led to an average value of $73.2 \pm 16.1\%$ ($n = 18$).

Additional experiments were carried out to investigate the effect of internally applied caffeine. The strongest solution possible viz. approximately 100 mM at room temperature was used, with the result shown in Figure 4 (10 experiments). The effect, which was a transitory and moderate rise in the Ca efflux, is in sharp contrast to that observed with external application of 10 mM caffeine. Estimates of the size of the effect gave an average value of 39.6 ± 19.2 ($n = 10$). This result may be related to the fact that only regional contractions were observed following the injection of caffeine.

The results of the experiments presented here show that the rate constants for ^{45}Ca efflux in barnacle muscle

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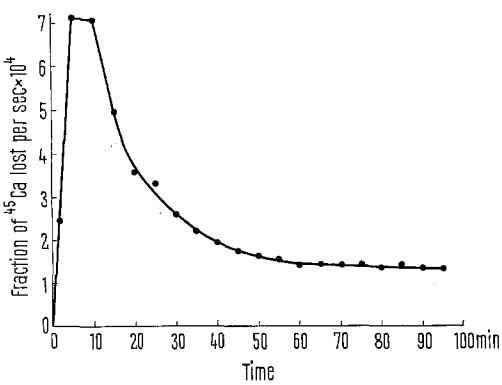

Fig. 1. The rate coefficient curve for ^{45}Ca efflux from a barnacle muscle fiber in artificial sea water.

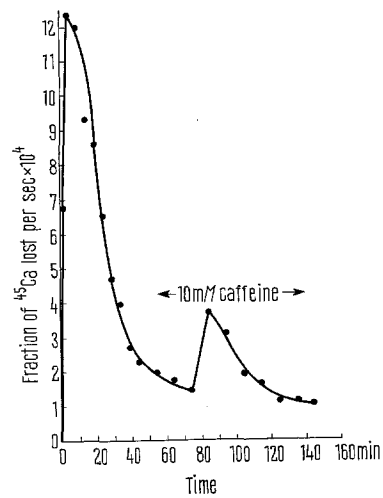

Fig. 3. The effect on the rate coefficient for ^{45}Ca efflux of external application of 10 mM caffeine.

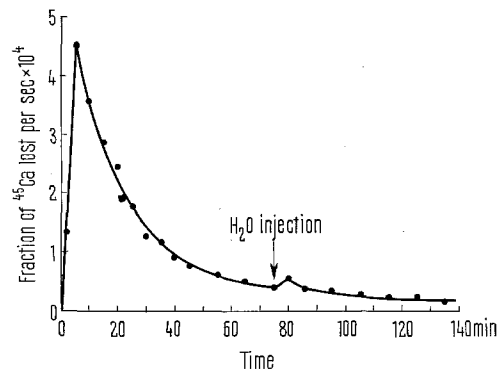

Fig. 2. The effect of injecting a 1 cm column of distilled water on the rate coefficient for ^{45}Ca efflux.

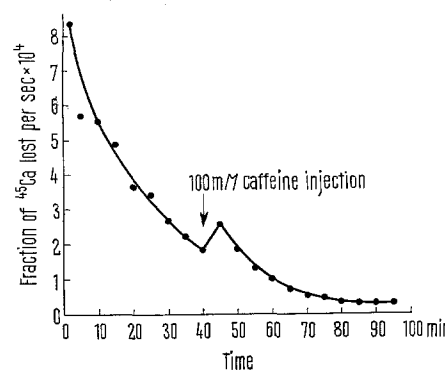

Fig. 4. The effect on the rate coefficient for ^{45}Ca efflux of internal application of a 1.5 cm column of a 100 mM caffeine solution.

fibers are not independent of time. Similar kinetics have been seen in frog[6] and crab[7] muscle fibers, and are most simply interpreted on the theroy that the bulk of the injected ^{45}Ca is fairly quickly bound or sequestered. That the large fall-off in the rate constant for Ca^{2+} efflux during the initial phase of the experiment is not the consequence of a deteriorating Ca^{2+} pump is indicated by the fact that reintroducing the microinjector into the fiber during the early part of the slow phase of Ca efflux fails to modify the behavior of ^{45}Ca emergence. On the other hand, it could be argued that the initial rate constants for ^{45}Ca efflux are high because of the contraction of these fibers following loading with radiocalcium. This would be explained as being the result of the squeezing out of ^{45}Ca mainly from the T-system.

The experiments with caffeine show that barnacle fibers are not as sensitive to the alkaloid as crab muscle fibers. It will be remembered that CALDWELL and WALSTER[3] found crab fibers to always shorten when treated internally with caffeine, even in concentrations as low as 1 m*M*. This is not true of barnacle fibers, since concentrations as high as 100 m*M* usually caused only local, weak contractions. This point is worth emphasizing in view of the conclusion by AXELSSON and THESLEFF[1] that internal application of caffeine to frog muscle does not cause a contraction. It is thus quite possible that differences in experimental results may not just be related to species differences but also to the presence of a non-uniform population of fibers within the same muscle bundle.

One explanation for the failure of caffeine in both instances to exert an effect on the radiocalcium efflux as great as that observed in crab muscle fibers[11] is as follows. Caffeine, be it applied externally or internally, mobilizes a large fraction of the 'bound' calcium, resulting in considerable dilution of the injected ^{45}Ca by the inactive calcium. Thus failure to see a fall in the rate constant for ^{45}Ca efflux could mean that the Ca^{2+} pump is not easily saturated or that the action of caffeine is actually rather marked. On this view the effect of injected caffeine is only small in comparison to that of externally applied caffeine because of a physically weaker ejection of the accumulated ^{45}Ca from the region of the T-system[13].

Zusammenfassung. Die Befunde ergeben, dass einzelne Muskelfasern der Entenmuschel durch Mikroinjektion mit ^{45}Ca aufgeladen werden können. Eine Umspülung der Muskelfasern mit einer coffeinhaltigen Lösung bewirkt ein stärkeres Ausströmen des Calciums als eine Mikroinjektion des Coffeins in die Fasern direkt.

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Modification of Hexose of a Glycoprotein Obtained from the Urine of Aminonucleoside of Puromycin Nephrotic Rats

Recently we described the isolation and purification of a glycoprotein from rat urine (MUPpg) with chemical and immunologic characterestics similar to the non-collagen-like glycoprotein of glomerular basement membrane (GBM)[1,2]. Several investigators have demonstrated alterations in the chemical composition of GBM in nephrotic animals[3–11]. Aminonucleoside puromycin (AMP) consistently produces a non-immunologic nephrotic syndrome in rats when administered either by a single large injection or by daily intraperitoneal injections[12,13]. This disease is associated with altered glomerular capillary permeability[14–16], which may occur as a result of alteration in basement membrane chemical composition[6]. The present study was designed to determine whether quantitative or qualitative alterations in MUPpg occurred in association with the development of AMP nephrosis.

Materials and methods. Two consecutive 24 h urines were collected from 50 Sprague-Dawley rats (150 g) at the beginning of the study. Urine volumes were recorded and urinary protein excretion measured. The urine samples were pooled and MUPpg, a glycoprotein similar in chemical composition but of a larger molecular weight then the non-collagenous GBM glycoprotein described by KEFALIDES[17] was isolated by ion exchange chromatography on Diethylaminoethylcellulose chromatography (DEAE) followed by Sephadex G 200 gel chromatography as previously described[1,2]. Following baseline studies, 2 groups of animals were used.

Group I. A) 15 rats in this subgroup received a single large dose (10 mg/100 g body wt.) of aminonucleoside of puromycin (AMP Sigma Biochemicals, St. Louis, Missouri). B) 15 rats received daily i.p. injections of AMP (2mg/100g body wt.) for 10 days.

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