

seems that these facts may rather support the existence of different mechanisms between them. We were much interested in linking the HS activity with the regressive effect of lentinan against transplantable tumour.

Riassunto. E dimostrato che lentinan, polisaccaride antineoplastico, è capace di aumentare la sensibilità del

topo contro istamina. La presomministrazione di lentinan invece non aumenta l'attività del fattore istamina sensibilizzante della *Bordetella pertussis*. Lentinan non induce linfocitose nel topo.

R. HOMMA and K. KURATSUKA⁷

Department of General Biologics Control,
National Institute of Health,
10-35 Kamiosaki, 2-Chome, Shinagawa-shu,
Tokio (Japan),
14 August 1972.

⁷ Acknowledgment: Thanks are due to Drs. G. CHIHARA and Y. Y. MAEDA, National Cancer Centre, Research Institute, Tokyo, for kindly supplying lentinan.

Fluxes of Magnesium and Calcium During Induced Activity of Frog Sartorius Muscle

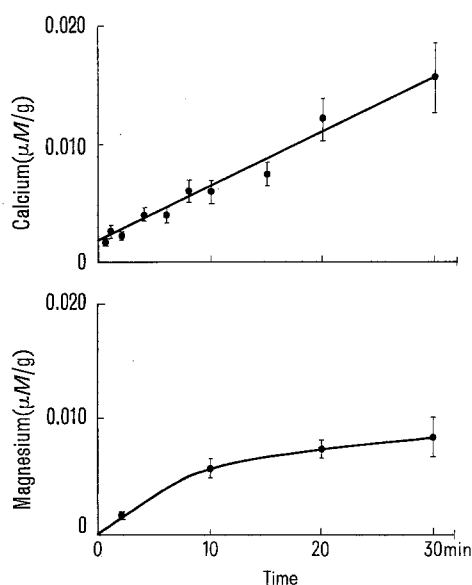
Fluxes of calcium have been extensively studied in relation to excitation-contraction coupling in muscle, in particular the increases in both influx and efflux which can be elicited in frog skeletal muscle either by caffeine or by electrical stimulation¹⁻³. Data on magnesium transport and permeability is by comparison sparse⁴⁻⁶. GILBERT⁴ found that although frog sartorius was permeable to Mg the extent of exchangeable Mg was only about one-quarter of the muscle total, and that exchange occurred in 3 phases in whole muscle, the slowest phase being interpreted as truly intracellular Mg. Elevated Mg abolishes frog muscle response to indirect stimulation without influencing resting or surface potential⁷, and stimulation in vivo has been reported to significantly increase the exchange of rat gastrocnemius Mg with that of plasma⁸. The present experiments investigated the fluxes of both Ca and Mg during electrical stimulation and caffeine contracture in excised sartorius muscles of frog.

Materials and methods. All experiments used whole sartorius muscles of male *R. temporaria*; muscles (from both winter and summer frogs) ranged in fresh weight

from 30–90 g. Mg in freshly-dissected muscles, determined by atomic absorption on nitric acid digests, was 10.08 ± 0.24 (mean, s.e., $n = 16$) $\mu\text{M/g}$ wet weight, uncorrected for muscle compartments. This value did not vary with season. For influx measurements the excised muscles were secured with one end fixed and the other attached to a strain gauge, transducer and pen recorder which monitored tension. Resting influx was measured by bathing the muscles, mounted at slightly more than skeletal length, in frog Ringer⁹ containing ^{45}Ca or ^{28}Mg at 20°C for periods from 0.5 to 30 min. At the conclusion of incubation the radioactive Ringer was quickly withdrawn from the bath and replaced by nonradioactive medium of identical composition, to allow efflux of isotope from surface and extracellular components of Ca or Mg¹⁻⁴. The efflux was continued for 90 min, and the muscles were then removed from the bath, cut free of tendon, blotted and weighed. ^{28}Mg was counted on neat extracts of muscles in 1 ml conc. HNO_3 ; ^{45}Ca was counted by liquid scintillation on aliquots of an aqueous solution of HNO_3 extract which had been evaporated to dryness. Counts were converted to concentrations by use of standards prepared from the radioactive solutions and fluxes were calculated as $\mu\text{M/g}$ wet weight.

Caffeine was incorporated in Ringer at 5 mM and invariably elicited contracture for a 10-min period of incubation at this concentration. Electrical stimulation was submaximal and direct, by square-wave pulses of 50 msec duration at frequency 0.5/sec for the last 6 min of a 10-min incubation. Both contracture and electrical stimulation experiments were limited to 10 min duration in order to reduce fatigue or damage to fibres, and the effects of these treatments were assessed by comparing the influx with that in companion muscles incubated at rest under the same conditions. The influxes were again measured after 90 min efflux had been allowed.

Results and discussion. Influxes of Ca and Mg into resting muscles are shown in the Figure. Ca influx was linear with time up to 30 min, after the bulk of the



Time-course of influx of Ca and Mg into resting frog sartorius at 20°C. Concentrations in Ringer were Ca 1.8 mM, Mg 2 mM. Each point is the mean of 6 muscles, 3 frogs. Vertical bars are standard errors.

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extracellular phases had been removed by 90 min efflux. The linearity of influx indicated that there was no significant backflux of Ca from the intracellular compartment in the course of the influx over 30 min. The curve of influx did not pass through the origin even when measured at times as short as 30 sec, indicating that influx in the first half-minute was different to the subsequent, linear process. Mg influx was of approximately the same magnitude as that of Ca, but differed markedly in its time course (Figure) although corrected for efflux and calculated in the same way as Ca influx. Although less data was available in the case of Mg the qualitative differences in influx were clearly indicated in the time-dependence; an initially high rate of influx, up to 10 min incubation, then declined gradually but steadily so that between 20 and 30 min the rate was considerably less than that of Ca. Even though based on less data the curve of Mg-influx showed a strong tendency to pass through the origin if extrapolated, so that it differed also in this respect from Ca. It can be concluded that the influx of both Ca and Mg are at least biphasic under these condi-

tions, and that the distribution of the two metals is significantly different within muscle compartments even after the surface phase and at least part of the extracellular phases of uptake have been subtracted.

The complex nature of both influxes meant that comparisons of rates of entry, over the short periods suitable for studies of stimulation and contracture, would not be valid, and therefore the influence of these treatments was assessed on the basis of total influx occurring in 10 min. The Table summarizes the results of these experiments.

Both stimulation and caffeine contracture significantly increased the influx of Ca over the resting values. The control values for Ca influx, calculated on the same rate basis as used by BIANCHI and SHANES¹, were somewhat less (0.03 as compared to $0.09 \times 10^{-12} M/cm^2$ fibre surface/sec). Neither stimulation nor caffeine contracture significantly increased the influx of Mg. It may be concluded that Mg does not use the mechanism of increased influx available to Ca during excitation, at least at the respective concentrations (2.0 mM, 1.8 mM Ca) obtaining under these conditions. The question still remains whether Mg may inhibit the influx of Ca at high extracellular concentrations of Mg relative to Ca. This would provide a mechanism of Mg blockage of muscular activity without alteration of membrane potential; such a competitive effect has been postulated for smooth muscle¹⁰.

Resumen. La representación del influjo de Ca y de Mg en músculo esquelético en reposo de rana es cualitativamente diferente; sin embargo la magnitud de dicho influjo es inicialmente similar. Estimulo mediante pulsos eléctricos o cafeína aumenta significativamente el influjo de Ca pero no el de Mg durante un período de 10 min.

J. M. O'DONNELL

Agricultural Research Council, Babraham, Cambridge (England), 3 August 1972.

Effects of electrical stimulation and of caffeine on influx of Ca and Mg in frog sartorius

	Ca ($\mu M/g$ wet weight)	Mg
Control	0.00537 ± 0.00073	0.00454 ± 0.00035
Stimulated	0.00768 ± 0.00102 (6)	0.00539 ± 0.00077 (6)
P^a	< 0.05	n.s.
Control	0.00620 ± 0.00063	0.00460 ± 0.00031
Caffeine	0.00835 ± 0.00068 (15)	0.00499 ± 0.00060 (4)
P^a	< 0.01	n.s.
	(mean, \pm S.E., No. experiments)	

^a Significance of differences between control and treated muscles, by paired-sample *t*-test. All muscles were incubated in frog-Ringer with ⁴⁵Ca or ²⁸Mg for 10 min at 20°C. Stimulation was applied for the last 6 min of incubation at frequency 0.5/sec, i.e. 108 twitches. Caffeine was present in Ringer at 5 mM. Influx was measured after the muscles had been left 90 min in nonradioactive Ringer.

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Some Functional Characteristics of the Electrorceptors (the Ampullae of Lorenzini) of Elasmobranchs

It is well known that the elasmobranchs have a well developed system of the ampullae of Lorenzini highly sensitive to the electric current¹⁻³.

The aim of this communication is to report some new functional characteristics of the electrorceptors of the elasmobranch fishes *Raja clavata* and *Trigon pastinaca*, from the Black Sea. The fish were fixed on a special platform placed in a lucite box, filled with water.

For regular respiratory movement of the fish, a plastic tube was introduced into the mouth of the fish and an adjustable flow of water led into the mouth. The ampullae of the mandibular or dorsal group were used, because of the ease of the dissection and the length of the fine nerve available⁴.

The electrical activity of the receptors was recorded from single nerve fibres or bundle of fibres connected to the receptor cell of the ampullae. The nerve impulses were amplified and displayed by conventional means. To stimulate the sensory organs, 2 small silver wire electrodes were used. All experiments were performed at 17°C. Hitherto it is only the tonic electrorceptor fibres with

spontaneous activity that were detected in the elasmobranchs^{4,5}. We, however, observed that according to the type of responses all the fibres were divided into the phasic and tonic (Figure 1; 1st and 2nd recordings). The current-threshold of the clear-cut responses were equal to 10^{-9} – 10^{-10} A/mm².

It is known that some electrorceptor fibres respond to the animal's own breathing potentials⁶. In such fibres the bursts of impulses are known to appear in time with respiration (Figure 1, 3rd recording). Along with these fibres (type 1) we also observed fibres in which the spontaneous activity was suppressed in time with respiration (type 2, Figure 2, 1st recording).

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