metrically) are not necessarily coincident[7]. Despite species differences which obtain between laboratory rodents, the hours of maximal S^{35} uptake in the cartilages determined in this study appear to precede peak mitotic activity (rats) by 2–3 h[6]. This suggests that cartilage cells cannot pursue mitosis and mucopolysaccharide synthesis concurrently. It is equally probable that DNA cannot simultaneously support its own replication and the production of RNA[8]. Evidence from mouse studies, in which chondrocytes were labeled with tritiated thymidine[9], suggests that peak DNA synthesis (some time between 02.00 and 10.00) leads in phase both peak S^{35} uptake and mitosis. A somewhat similar multiparameter diurnal pattern has been recorded in different cell fractions from liver by BARNUM and his co-workers[10]. It is doubtful whether the periodicity in S^{35} retention can be explained by diurnal variations in dietary amino acid intake; one might expect dilution of intracellular pools of radiosulfur when mice are most actively feeding. Radioglycine experiments suggest, rather, that the diurnal period in the synthesis of the protein moiety of the cartilage ground substance is maintained even when animals were starved before study[11].

Résumé. Nous avons administré du soufre radioactif par voie i.p. à des souris, chaque groupe recevant l'injection à un moment différent de la journée (intervalle de 3 h entre les injections à 2 groupes successifs). L'analyse microdensitométrique d'autoradiographies du traceur dans les cartilages de conjugaison (fémurs, tibias) 24 h après l'injection a démontré que la rétention était la plus grande chez les souris qui avaient reçu l'injection entre 15 et 18 h.

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The Initiation of Contraction by Extracellular Calcium in the Smooth Muscle of the Guinea-Pig *Taenia coli*

It is generally accepted that the spike height is related functionally to the influx of the cation which carries inward positive charge. Contraction height is also related to the concentration of Ca^{++} injected into the muscle fibre[1]. In addition, HAGIWARA et al.[2] reported that Ca^{++} carries the charge during the rising phase of the action potential in barnacle muscle. The possibility of this Ca spike has also been reported in the smooth muscle of the guinea-pig *Taenia coli*[3,4]. The present experiments were performed to investigate the relation between the Ca spike and the twitch tension.

Taeniae coli of the guinea-pig were incubated in modified Krebs solution. Electrical activities were observed by means of sucrose-gap method and tensions were measured with a mechano-electronic transducer RCA 5734. Experiments were performed at low temperature (18 °C) to block spontaneous spike discharge, for the purpose of observing contraction as a single twitch. Single spike and twitch were evoked by supramaximal external stimulation. Tetrodotoxin (5×10^{-6} g/ml) was used to suppress Na spike activities. The toxin also blocked the inhibitory potential of the preparation by external stimulation[5].

Figure 1 shows that the amplitude of both action potential and contraction height varied with Ca^{++} concentration in the solution; the changes in amplitude were almost parallel. The minimal concentration for the generation of action potential and contraction varied from 10^{-6}–10^{-5} *M*. Dissociation of electrical and mechanical activities was not observed in any solution, regardless of difference in Ca^{++} concentration.

As these spike heights were measured by means of the sucrose-gap method, they represent the summation of the spike potential of different muscle cells.

The possibility exists that each cell generated an all-or-none spike. However, judging from the duration of the action potential, synchronization might well have occurred. Moreover, it is also known that the spike height,

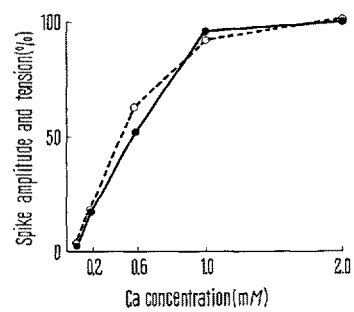

Fig. 1. The relation between spike height and twitch tension, and calcium concentration. Both spike heights and tensions were plotted against calcium concentrations as % of those observed in the solution containing 2 m*M* of calcium. Note that spike heights and contractions were parallel to each other relating to calcium concentrations. Closed circle, tension; open circle, spike amplitude.

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measured with an intracellular microelectrode, is decreased with the decrease of extracellular Ca^{++} concentration[6].

To determine whether the extracellular Ca^{++} which diffuse into the smooth muscle cell can initiate the contraction, the following experiment was performed in the K-depolarized preparation. After pretreatment of *Taenia coli* first for 30 min with a Ca^{++}-free K-Locke's solution containing 1 mM of ethylene-diamine-tetra-acetate (EDTA) and then for 10 min with a Ca^{++}-free solution without EDTA, different concentrations of Ca^{++} were added. Contractions by Ca^{++} were observed.

Figure 2 shows the relation between the initial speeds of contraction and the concentrations of Ca^{++} added. The minimal concentration of Ca^{++} required to initiate contraction varied from 10^{-6} to $10^{-5}M$ in this investigation. Initial speeds of contraction were linearly proportional to added Ca^{++} concentrations in the range from 2×10^{-5} to $1 \times 10^{-3}M$.

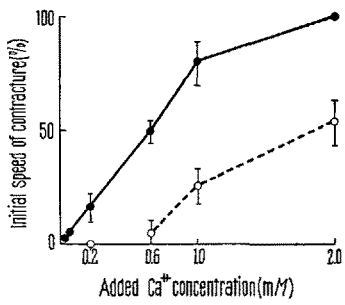

Fig. 2. The relation between initial speed of contraction produced by calcium and concentration of calcium added in the depolarized smooth muscle. This experiment was performed with isotonic measurement to observe complete relaxation of the smooth muscle. The complete relaxation was observed within 30 min of treatment with Ca-free K-Locke's solution containing 2 mM of EDTA. After an additional treatment of the preparation with Ca-free K-Locke's solution without EDTA, different concentrations of Ca were added. Initial speeds of contraction were measured and plotted against added Ca concentrations as % of the speed observed with 2 mM of Ca. Open circle, initial speeds of contraction in the presence of 1 mM manganese; closed circle, without manganese.

It is reasonable to assume that intracellular Ca^{++} in these preparations were lowered to almost identical concentrations by identical pretreatment before different concentrations of Ca^{++} were added extracellularly. If this assumption is accepted, Figure 2 shows that the initial speeds of contraction are linearly proportional to $[Ca]_0/[Ca]_i$ from 2×10^{-5} to $1 \times 10^{-3}M$. In addition, both amplitude and initial speed of contractions were strongly decreased in the presence of 1 mM of Mn^{++}, which is thought to inhibit Ca^{++} influx into the muscle cell[7].

Assuming that the action potential of the smooth muscle is 60 mV and the specific capacitance is 10 $\mu F/cm^2$, Ca influx/spike would be, roughly, more than 3×10^{-12} moles/cm^2. As it is reported that the diameter of the smooth muscle is about 4 μ and the length is about 150 μ[8], the circumferential area would be about 1.9×10^{-5} cm^2 and the volume would be about 1.9×10^{-9} cm^3. From these, it is calculated that the total Ca^{++} entering a single muscle cell is about 5.7×10^{-17} moles/spike and this Ca^{++} is also calculated to be 3×10^{-5} as a intra-cellular final concentration which may be enough to initiate the contraction of smooth muscle.

These results and calculations strongly suggest that the Ca^{++} which enter into the smooth muscle cell during the spike potential can be directly related to the contractile elements.

Further studies of the dissociation of electrical and mechanical activities should be undertaken.

Zusammenfassung. Die Ca-Wirkungen auf elektrische und mechanische Aktivität glatter Meerschweinchenmuskeln wurden untersucht. Die Resultate sprechen dafür, dass Kalziumionen, die während der Dauer des Aktionspotentials in die glatte Muskelzelle eintreten, am kontraktilen Elementarvorgang beteiligt sind.

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Metabolic Effects of Thyroxine in Infant Rats

It has been established that the thyroid hormone regulates growth, differentiation and oxidative metabolism in various animals. During the early postnatal period, thyroxine deficiency has impairing effects on the development of many organs, especially of the central nervous system, e.g. in mammals[1–4]. At about the age of 2 weeks the thyroid gland becomes important for the thermoregulation of the rat[5]. During this period, the activation of thyroid is pronounced[6,7]. Shapiro[4] found that thyroidectomy or thyroxine administration did not influence metabolic rate or body temperature in rats during the first 2 weeks of life. It was observed by Hemon[8], however, that thyroxine does not increase the oxygen consumption in rats aged 10–12 days when measured at an external temperature of 29.5 °C, but at 35 °C the increase was approximately 25%.

As the results of our pilot experiments were in conflict with the previous ones concerning the metabolic rate, the effect of thyroxine on the succinic dehydrogenase activity of liver, skeletal muscle and brain homogenates, and also

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