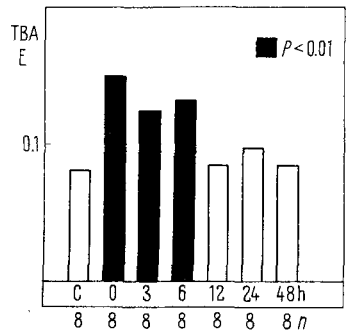


Fig. 1. TBA chromogen values in irradiated rat liver mitochondria after 180 min aerobic incubation at 37 °C.

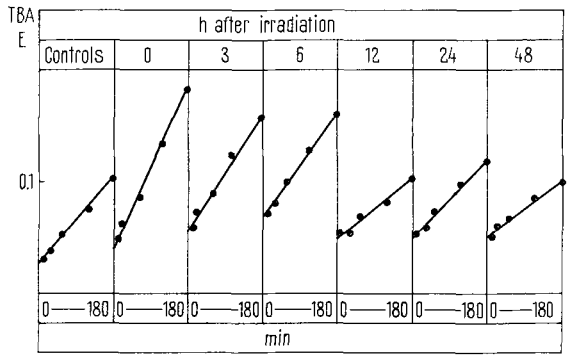


Fig. 2. The dependence of TBA chromogen production on the irradiation time in irradiated rat liver mitochondria.

incubation were higher in irradiated mitochondria, but only up to 6 h post-irradiation (Figure 2).

The higher level of TBA chromogen is not, of course, an evidence of elevated lipid peroxides after irradiation in vivo. The cell has a large number of stabilizing factors, some of which may be damaged by irradiation. The irradiation may influence these factors. In this connection it seems to be very interesting that a lipid fraction with a strong swelling effect has been isolated from the liver of irradiated rats. The results of this experiment show that although the mitochondria are transiently damaged by irradiation in vivo, they are soon reconstituted to reach pre-irradiation level according to the recovery of other morphological and functional changes[9].

*Zusammenfassung.* Der Einfluss der ionisierenden Strahlung auf die Bildung der Oxydationsprodukte von ungesättigten Fettsäuren in Lebermitochondrien ganzkörperbestrahlter Tiere wurde untersucht. Die TBS-Chromogenwerte erhöhen sich in bestrahlten Mitochondrien statistisch signifikant und sinken in 12 h nach der Bestrahlung wieder zur Norm ab.

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## Direct Action of Extracellular Ca Ions on Skeletal Muscle

It is known that the intracellular $Ca^{++}$ regulates the behaviour of the contractile structures of muscle fibre and is also involved in excitation-contraction coupling. The frog skeletal muscle in vivo contains a total of $1.4–2 \cdot 10^{-3}$ *M* Ca/kg of wet tissue. The intracellular free $Ca^{++}$ concentration is maintained at a level of about $10^{-7}M$ by the calcium binding and storing systems. In frog blood plasma the total calcium concentration was found to be about $1.8 \cdot 10^{-3}M$[1]. $CaCl_2$ is added to the physiological saline solution in a concentration between 1 and $2 \cdot 10^{-3}M$ in order to simulate the extracellular ionic conditions of cold-blooded animals.

The lowest $Ca^{++}$ concentration necessary to maintain the physiological properties of muscle membrane and end plate is about $10^{-4}M$[2,3]. By storing the muscles in Ringer's solution with 'normal' Ca content ($1–2 \cdot 10^{-3}M$), the amount of intracellularly accumulated Ca essentially increases[4–6]. This fact leads Shanes and Bianchi[5] to the conclusion that a $CaCl_2$ concentration of $1 \cdot 10^{-3}M$ corresponds better to physiological conditions than higher values.

In spite of the observed Ca influx in skeletal muscle it was found that only a very high Ca concentration in the external medium induces contractures of low tension[7]. These experiments were performed with muscles prebathed for various lengths of time in Ringer's solution with $1.8 \cdot 10^{-3}M$ $CaCl_2$. In most recent investigations, the authors found that freshly dissected muscles of *Rana esculenta* go into contracture after being dipped into Ringer's solution. The tension development depends on the Ca concentration of the external solution. The Figure shows the contracture tension at the various Ca concentrations expressed in the percentage of the maximal tension development of individual muscle in isotonic $CaCl_2$

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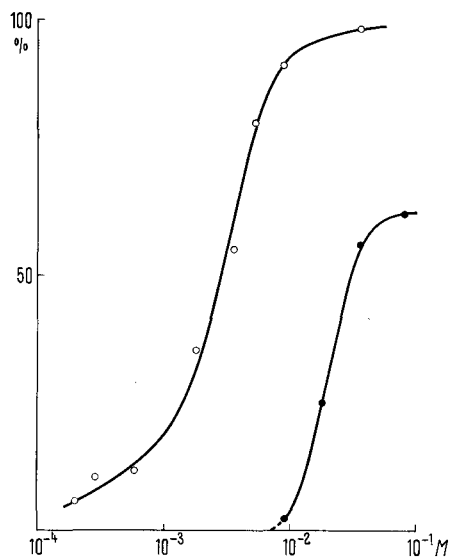
solution. The threshold lies at about $10^{-4}M$ Ca in the external solution. The authors have also found that the threshold concentration shows seasonal variations. The contractures reach the maximal values after 20–30 sec. The maximal tension is about 200 g/cm² and is of nearly the same order as that of K-contractures of whole muscle.

The Ca contractures during the rising phase of tension development are mostly superposed by several twitches. In phasic muscle, tension decreases within 1–3 min to the resting value. In tonic muscle the tension is sustained for about 20–30 min (the details will be published later).

After prebathing the muscle in Ringer's solution with $1.8 \cdot 10^{-3}M$ Ca for 5 min, the Ca concentration must be markedly higher to induce such direct 'coupling contractures'. Under these conditions the threshold value is about $10^{-2}M$. The tension development decreases considerably in absolute as well as in relative measurement (Figure).

Contracture can occur only if Ca influx is so great that the corresponding increase in the intracellular $Ca^{++}$ level cannot be prevented by Ca binding. From the decrease in contracture tension in phasic muscle after 30 sec, one may conclude that the free $Ca^{++}$ concentration in muscle fibre is diminishing in this phase. The authors' opinion is that it is not an increase in intracellular binding capacity but a decrease in membrane permeability to Ca that is responsible for this. This view is supported by investigations on Ca kinetics[4]. In these experiments it has been shown that phasic muscle takes up calcium rapidly during an initial phase lasting 30–60 sec. Thereafter the Ca influx markedly decreases until a steady level is reached several hours later. Obviously the concentration of free Ca ions is increased only during the initial phase. After Ca influx has diminished, the penetrating Ca ions are bound rapidly enough. Even a short storing of muscle in Ringer's solution with $1.8 \cdot 10^{-3}M$ $CaCl_2$ suffices to decrease the membrane permeability. The less Ca a Ringer's solution used for pre-bathing contains, the less the decrease in membrane permeability. This means that the concentration-tension curve shifts to the left with decreasing Ca concentration of the external solution in which muscle has been pre-treated.




Concentration-tension curves of $Ca^{++}$ contractures in frog sartorius. Abscissa: $CaCl_2$ concentration in log scale. Ordinate: Tension as a percentage of maximal value obtained with isotonic $CaCl_2$ solution in each individual muscle. o—o = muscles brought directly into contracture solution. ●—● = muscles pre-bathed for 5 min in Ringer's solution containing 1.8 m$M$ $CaCl_2$. Each point represents an average of 10–20 individual experiments.

If the conditions in freshly dissected muscle correspond to physiological ones, the actual extracellular $Ca^{++}$ concentration is not likely essentially to surpass $3 \cdot 10^{-4}M$; otherwise no contracture would be expected to occur at such low Ca concentration. It means that most of the Ca in the blood must be bound somehow, for example, to protein, phosphate etc. The degree of binding may also depend on pH, which can vary with the season and can reach a value of 8.

However, the authors cannot yet decide how to reconcile such a low supposed $Ca^{++}$ concentration with the requirements of heart muscle. In contrast to skeletal muscle, the intensity of heart muscle activity depends sensitively on the $Ca^{++}$ content of the surrounding fluid, though the Ca influx during a single twitch is not supposed to be sufficiently great to induce full activation[8]. Perhaps Ca in blood plasma and extracellular space is bound partially to certain organic complexes of low molecular weights, whose affinity to Ca is greater than that of skeletal muscle membrane, but smaller than that of heart muscle membrane.

After replacing $Ca^{++}$ by $Ba^{++}$ or $Sr^{++}$ solutions, contractures can also be produced which develop roughly the same tension as that of Ca contracture. It is presumed that these divalent cations act through liberation of $Ca^{++}$. $Mg^{++}$ does not induce contracture in the concentrations tested by us (up to $2 \cdot 10^{-2}M$). However, treating muscle with high Mg-Ringer's solution without Ca inhibits the Ca contracture significantly more than pre-bathing in Ringer's solution without Ca and Mg.

The authors' investigations have shown 2 important facts: (1) It is possible to obtain a contracture without membrane excitation by direct Ca influx from solutions containing a relatively small amount of Ca (threshold about $10^{-4}M$), when a freshly dissected muscle is used, as well as by micro-injection of Ca into a muscle fibre. (2) The membrane permeability to Ca in phasic frog muscle under physiological conditions seems to be relatively high. It decreases rapidly when the muscle is bathed in Ringer's solution with 'normal' $CaCl_2$ content ($1.8 \cdot 10^{-3}M$ $CaCl_2$).

*Zusammenfassung.* Frisch excidierte Sartorien von *Rana esculenta* entwickeln in Ringerlösungen Kontrakturen (ohne Erregung), deren Spannung von der Ca-Konzentration abhängt. Die wirksame Schwellenkonzention des extracellulären Ca liegt bei ca. $10^{-4}M$. Die Befunde demonstrieren sowohl die direkte Wirkung von Ca auf die mechanische Ankopplung als auch die Abnahme der Ca-Durchlässigkeit der Muskelmembran schon bei der Ca-Konzentration der 'normalen' Ringerlösung, mit der sich die Membran ins Gleichgewicht gesetzt hat.

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