

bei abgeschirmten Tieren mit aufgesetztem 10 Hz-Rechteckimpulsfeld grösstenteils kompensiert. Da die Potentialdifferenz innen/ausser auf einer unterschiedlichen Ionenverteilung beruht, muss also das elektrische Feld einen Einfluss auf das Membransystem bezüglich der Ionenpermeabilität haben.

Reversible Strukturumwandlungen an biologischen Membranen werden neuerdings lebhaft diskutiert<sup>4-7</sup>. Dabei wird allgemein angenommen, dass durch Änderung eines äusseren Parameters – einem Steuerungsprozess entsprechend – Strukturumwandlungen in den Membransystemen ausgelöst werden, die zu Funktionsänderungen an denselben führen können.

Experimentelle Arbeiten auf diesen Gebieten liegen von TASAKI<sup>8</sup> und COHEN<sup>9</sup> vor. Natürlich handelt es sich hier um äusserst verwickelte Beziehungen, die in ihrer Gesamtheit noch nicht gänzlich zu erfassen sind. Man muss aber annehmen, dass hier, besonders wegen anderen bisher von uns noch unveröffentlichten Ergebnissen, bei denen unter Einfluss elektrischer Felder Ionenverschiebungen im Blut quantitativ erfasst wurden, ein wichtiger Schlüssel zu den physiologischen Auswirkungen der luftelektischen Felder liegt.

**Summary.** The potential across the skin of *Rana esculenta* was remarkably reduced under 'Faraday conditions' in comparison to animals kept under 'normal conditions'. A pulsating electric field (10 Hz) practically abolished this effect.

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- <sup>4</sup> J. P. CHANGEUX, Proc. natn. Acad. Sci., USA 57, 335 (1967).
- <sup>5</sup> J. P. CHANGEUX und T. PODLESKI, Proc. natn. Acad. Sci., USA 59, 944 (1968).
- <sup>6</sup> R. BLUMENTHAL, J. P. CHANGEUX und R. LEFEVER, J. Membrane Biol. 2, 351 (1970).
- <sup>7</sup> T. L. HILL und Y. CHEN, Proc. natn. Acad. Sci., USA 65, 1069 (1970).
- <sup>8</sup> I. TASAKI und I. SINGER, in *Biological Membranes* (Ed. D. CHAPMAN; Academic Press London, New York 1968), p. 347.
- <sup>9</sup> L. B. COHEN, R. D. KEYNES und B. HILLE, Nature, Lond. 218, 438 (1968).

## Recovery of Isometric Twitches after Glycerol Removal

The efflux of glycerol from frog skeletal muscle leads to the dissociation of excitation-contraction (E-C) coupling caused by disconnection of transverse tubules from the surface membrane<sup>1-5</sup>. Although there are observations about the reversibility of certain morphological<sup>3, 4, 6, 7</sup> and physiological<sup>8, 9</sup> changes produced by glycerol removal, no substantial restoration of E-C coupling has been observed<sup>1, 8, 9</sup>.

This communication describes the effect of glycerol on twitch response of isolated fast frog muscle fibre. We used 110, 132, 165, 220, and 400 mM/l glycerol (R+G) prepared in ordinary Ringer solution (R). The fibre was stimulated by single pulses through 2 platinum plate electrodes (30 × 5 mm) oriented longitudinally and separated by a distance of 7 mm. The strength of stimulus was usually 2-3 times higher than threshold (about 20 V on the electrodes) and its duration was 0.2 msec. The length of fibre was adjusted to produce maximum twitch tension. It was recorded by means of RCA-5734 transducer tube. All experiments were performed in winter at 18-23°C.

Twitch tension changes, during the exposure of muscle fibre to glycerol, were similar to changes of fibre volume in R+G<sup>10, 11</sup>. The treatment with R+G led for the first 1-2 min to transient decrease, and in the case of R+400 G, even to disappearance of twitches. During the next 30 min the twitches recovered their initial value and in 165-400 mM glycerol surpassed it by 20-40% (Figure 1).

After a 0.5-2 h exposure to R+G, this solution was quickly changed for R. The procedure led to brief potentiation of twitches by 20-70% and then to their decrease and disappearance (Figure 1). In the case of 165-400 mM glycerol, isometric twitches disappeared in all fibres within 1-3 min. In the case of 110-132 mM glycerol, the complete disappearance of twitches was observed only in 30-50% of fibres.

Glycerol removal rendered the muscle fibres, for some time, very susceptible to mechanical injuries<sup>9</sup>. In many

fibres we observed the appearance of local ruptures of sarcolemma, resulting later on in necrosis. Such injured fibres made up 20, 40 and 90% of all fibres after washing out 165, 220 and 400 mM glycerol, respectively ( $n=18-40$ ).

In case of 110-220 mM glycerol removal, if there was no injury, we observed partial or complete recovery of twitches. It proceeded in a following way: in 2-30 min after disappearance of isometric twitches, a single stimulus began to elicit one or several spreading nodes of contraction, each being 100-200 microns in length. These nodes spread along the fibre without visible decrement, at the rate about 3 mm/sec. Such kind of contractile response could continue from a few minutes to 1-2 h<sup>12</sup>. At a certain experimental stage, the fibre began to respond to electrical stimulus by very weak, fast movement, followed by spreading nodes. Under isotonic conditions, this fast movement gradually increased to energetic shortening. By that time the nodes disappeared, but as a rule fibre still failed or almost failed to produce isometric twitches.

- <sup>1</sup> J. N. HOWELL, J. Physiol. 207, 515 (1969).
- <sup>2</sup> P. W. GAGE and R. S. EISENBERG, J. gen. Physiol. 53, 298 (1969).
- <sup>3</sup> S. A. KROLENKO, Cytology (Russ.) 10, 803 (1968).
- <sup>4</sup> S. A. KROLENKO, Nature, Lond. 221, 969 (1968).
- <sup>5</sup> B. EISENBERG and R. S. EISENBERG, J. Cell Biol. 39, 451 (1968).
- <sup>6</sup> S. A. KROLENKO, S. JA. ADAMJAN and N. E. SHWINKA, Cytology (Russ.) 9, 1346 (1967).
- <sup>7</sup> S. A. KROLENKO, Cytology (Russ.) 12, 861 (1970).
- <sup>8</sup> E. G. HENDERSON, J. gen. Physiol. 56, 692 (1970).
- <sup>9</sup> R. S. EISENBERG, J. N. HOWELL and P. C. VAUGHAN, J. Physiol. Lond. 215, 95 (1971).
- <sup>10</sup> C. CAPUTO, J. gen. Physiol. 52, 793 (1968).
- <sup>11</sup> S. A. KROLENKO and S. JA. ADAMJAN, Cytology (Russ.) 9, 185 (1967).
- <sup>12</sup> In a few cases the spreading nodes of contraction arise without stimulation<sup>6</sup>.

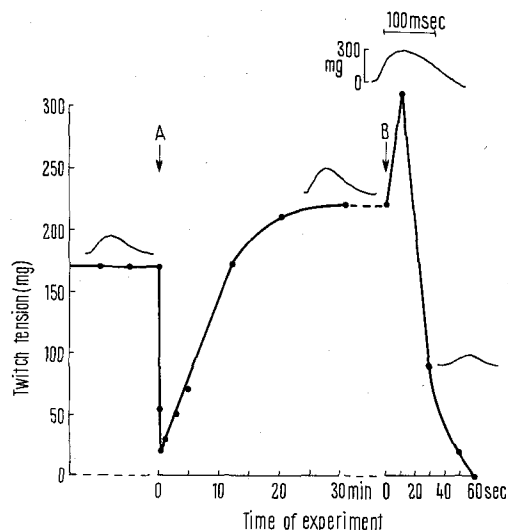


Fig. 1. Changes in twitch tension of single muscle fibre during loading with glycerol and at the beginning of its removal. The arrow A indicates the moment when R was changed for R+220 mM glycerol. The arrow B indicates the beginning of glycerol removal. Note the change in time scale from min to sec at the beginning of glycerol removal. Myograms typical for different stage of experiment are shown close to corresponding points of the curve.

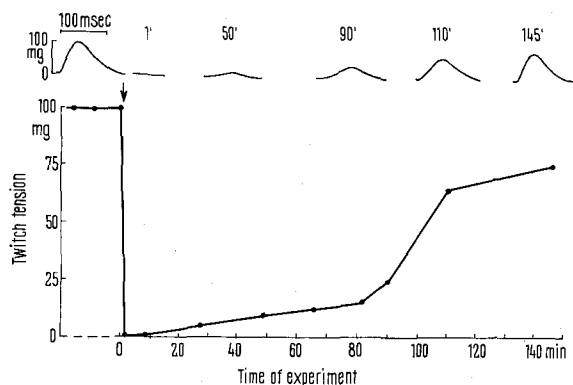


Fig. 2. Restoration of twitch tension after removal of 165 mM glycerol. The arrow indicates the moment when R+165 mM glycerol was changed for ordinary R. The figures besides the myograms denote min of experiment when these samples were recorded.

Little by little the amplitude of isometric contractions increased, usually as S-shape curve and approached the constant level which was usually equal to, or a bit less than, the initial one (Figure 2).

The time course of twitch tension recovery varied considerably in experiments with the same glycerol concentration. For example, after the removal of 165 mM glycerol, the extreme values for halftime of twitch recovery were 12 and 200 min. In spite of this variability, there is no doubt that the recovery of twitches proceeded faster and easier after 110–132 mM glycerol than after 165–220 mM. In the former case, the complete restoration was accomplished for 20–60 min and in the latter it was delayed usually for several hours. In case of 400 mM glycerol, in accordance with other evidence<sup>1,2</sup>, no restoration of isometric twitches was observed during 5–10 h of experiment, although in a few instances weak isotonic shortening appeared.

We considered the recovery of normal isometric twitches after glycerol removal as a result of restoration of normal connection between the T-tubules and surface membrane. However, the time course of twitch recovery, besides changes in the T-system, may reflect some other reversible alterations produced by glycerol efflux in muscle fibre, most probably, in its surface membrane.

**ВЫВОДЫ.** 1. Исчезновение одиночных изометрических сокращений, вызванное отмывкой 110–220 mM глицерина из изолированных мышечных волокон лягушки, носит обратимый характер. 2. На определенном этапе отмывки глицерина в ответ на одиночное электрическое раздражение возникают локальные сокращения волокна, распространяющиеся по волокну со скоростью порядка 3 мм/сек. 3. Выход глицерина резко повышает чувствительность волокна к механическим воздействиям и часто сопровождается развитием некроза.

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## The LD<sub>50</sub> Value of Tetraethyl Lead<sup>1</sup>

Ecologically speaking, lead is under attack. Among other things, it is regarded as the leading cause of childhood poisoning in the United States today<sup>2,3</sup>. Looking at the subject of lead from a different aspect, the Director-General of Research and Operations for the Canadian Food and Drug Directorate has indicated that an estimated 15 tons of lead enter the atmosphere over Los Angeles each day<sup>4</sup>. As most of this comes from the residue of burned, leaded gasoline, it seems worthwhile to closely examine the biological effects of some of the compounds involved. Tetraethyl lead was chosen as the

starting point. This paper represents a preliminary report on the toxicity of this compound.

Although the literature revealed a minimal lethal dose for the intraperitoneal route of administration<sup>5</sup> and an

<sup>1</sup> Funded in part by National Science Grant No. GI-4.

<sup>2</sup> J. GREENGARD, *Clin. Pediat.* 5, 269 (1966).

<sup>3</sup> J. R. CHRISTIAN, B. S. CELEWYCZ and S. L. ANDELMAN, *Am. J. Public Health* 54, 1245 (1964).

<sup>4</sup> A. B. MORRISON, *The Ensign* 7, 64 (1971).