# THE DEMARCATIONAL POTENTIAL DIFFERENCE AS A CHARACTERISTIC OF THE CHANGING STATE OF POLARIZATION OF SKELETAL MUSCLES AT DIFFERENT AGE PERIODS

I. I. Gokhblit and I. A. Kornienko

Laboratory of Age Physiology and Pathology (Head — Prof. I. A. Arshavskii) of the Institute of Normal and Pathological Physiology (Director — Active Member AMN SSSR V. N. Chernigovskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. V. Parin)

Translated from Byulleten' éksperimental'noi biologii i meditsiny Vol. 49, No. 2, pp. 26-31, February, 1960.

Original article submitted November 26, 1958.

It is now established [1-8] that at different stages of ontogenesis the nerves of mammals, and the skeletal muscles which they supply, are characterized by low lability, a low level of excitation, a long constant of the time of development of excitation (i.e., a long chronaxie), a long constant of accommodation ($\lambda$), a long refractory phase (absolute and relative), and a low coefficient of resistance, i.e., a low accomodational resistance to the action of an alternating stimulus. In newborn rats, on the 3rd-4th day of life, and in rabbits and cats in the intrauterine period, excitation in a nerve develops not at the cathode (in accordance with Pfluger's law of polarity), but at the anode. At early stages in the life of mammals, the nerve conductor responds to a single stimulus not by a single action current but by a diminishing discharge of impulses. These findings are evidence that at early age periods the neuromuscular apparatus is characterized by signs typifying a catelectrotonic state of the tissue. In the process of ontogenesis, and especially at critical stages in the individual development of the animal, the neuromuscular apparatus acquires signs typifying an anelectrotonic state of the tissue. This is shown by a gradual increase in the lability, level of excitation, shortening of the constant of accommodation and of the refractory phase (absolute and relative), an increase in the coefficient of resistance, and an increased ability to respond (albeit in the region of the cathode) to a single stimulus by a single action current [1-8]. These characteristic features of the main parameters of the neruomuscular apparatus suggest that their differences, depending on the age of the animal, can be explained only if it is accepted that they are due to variations in the properties of the membranes of nerve and muscle tissue.

In the present investigation an attempt has been made to demonstrate by more direct methods the possibility of an association between the polarization properties of the neuromuscular apparatus and the physiological characteristics listed above.

One direct index of the state and level of polarization of nerve and muscle tissue is the magnitude of the resting current. If, in the process of ontogenesis the change in the properties of the neuromuscular apparatus is in fact due to changes in the membrane, it is evident that this must be reflected above all in the magnitude of the demarcation potential difference. We made our starting point the membrane theory of action potentials, according to which the damaged part of the tissue is depolarized and becomes electrically negative in relation to the undamaged surface of the tissue. The magnitude of the potential difference thus created depends on the degree of polarization of the undamaged surface of the tissue.

## METHOD

Experiments were carried out on unanesthetized rabbits of different ages, starting from the first days of life. The gastrocnemius muscle was carefully dissected and a transverse incision made in its distal end. The nerve was left intact during this procedure. The core of one nonpolarizing electrode (Zn, $ZnSO_4$, NaCl), moistened with Ringer's solution, was placed in contact with the incised surface of the muscle, and the core of another electrode was applied to the middle of the undamaged surface of the muscle. The demarcation potential difference was recorded by means of a mirror galvanometer ($1 : 10^{-9}$ A), included in an ordinary potentiometric compensation circuit, at room temperature 18-20°. We made 78 observations.

## RESULTS

From the results obtained, the rabbits investigated could be divided into 3 age groups. The first group comprised rabbits aged from one to 10-12 days old (40 experiments), the second group—rabbits aged from 12 days to $1^1/_2$ months (18 experiments) and the third group—adult rabbits (20 experiments).

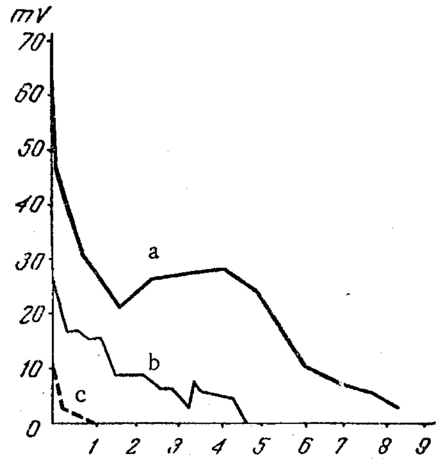


Fig. 1. Changes in the magnitude and duration of the demarcation potential difference in rabbits. a) In the third age group; b) in the second; c) in the first group (see text).

In each experiment we recorded the magnitude of the demarcation potential difference arising after infliction of the transverse incision, and the changes taking place in this magnitude as it gradually disappeared with time.

In the rabbits of the third group, the magnitude of the demarcation potential difference varied between limits of 30 to 100 mv. The mean value in the majority of experiments was 45-65 mv. In two experiments a potential difference of 25 mv was observed, and in another two experiments, one of 110-140 mv.

In the rabbits of the first age group the recorded magnitude of the demarcation potential difference varied between limits of 3 and 18.5 mv. The mean value in the majority of experiments was 6-11 mv. In 2 rabbits in the period of maturation, values of 22.5 and 33 mv were recorded.

In the rabbits of the second age group, the demarcation potential difference varied between limits of 15 and 46 mv. The mean value of the potential difference was 25-35 mv.

The length of time taken for the demarcation potential difference to disappear varied in the rabbits of the third age group between limits of 3 and $8^1/_2$ hours, and in the majority of experiments it was 6-8 hours. In rabbits under 10-12 days old (the first age group) the time taken for the demarcation potential difference to disappear varied between limits of 20 minutes and $2^1/_2$ hours. The mean time in the majority of experiments was 50-80 minutes. In only one rabbit (12 days), in which the magnitude of the demarcation potential difference was 33 mv the time taken for it to disappear was $3^1/_2$ hours. In rabbits aged from 12 days to $1$-$1^1/_2$ months (second age group), the time taken for disappearance of the demarcation potential difference varied between limits of 2 and $6^1/_2$ hours. The mean value in the majority of experiments was 3-4 hours. In one experiment the time taken for the disappearance of the potential difference was 1 hour.

In Fig. 1 are shown typical curves of the changes in magnitude and duration of the demarcation potential difference in rabbits in the various age periods.

The top curve (a) shows the dying away of the potential difference in adult rabbits. A sharp fall in the potential difference is seen during the first hour, and then for a short time the value of the potential difference is stabilized, after which it gradually tends to zero. The bottom curve (c) shows the disappearance of the demarcation potential difference in the rabbits of the first age group. This can be seen to occur within the first hour. The middle curve (b) represents the rabbits of the second age group, in which, after an initial, fairly sharp fall, the demarcation potential difference gradually tends towards zero.

Can it be considered that the difference which we found in the time taken for the demarcation potential differences to disappear may be used as an additional index of the state of muscle tissue, which is determined by age?

Dubois-Raymond first directed attention to the fact that the potential difference found in the isolated muscle of a frog between normal and injured areas fell by roughly 75% during the first 30 minutes. The progressive fall in the demarcation potential at the injured end of a nerve was subsequently studied by Engelman [11]. In order to account for this phenomenon, Gerard [12] put forward the hypothesis that at the line of demarcation from the site of injury a pseudomembrane is formed from the coagulating plasma. After the newly formed membrane has become completely polarized, the demarcation current disappears. Gerard attributed essential importance here to oxidative processes, as the factor producing and maintaining the electrical polarization of the limiting membranes. It must be pointed out that, in Gerard's hypothesis, repolarization of the regenerated membrane is looked upon as a passive process. In order to provide an explanation for the phenomenon described, we may turn to experiments which we carried out on the myocardium of the ventricle of the frog's heart.

If the anterior surface of the wall of the frog's ventricle is subjected to local cathode alteration through nonpolarizing electrodes, the following phenomenon can be observed at a suitable potential difference. The depolarization arising immediately beneath the cathode relaxes the altered area of the myocardium and excludes it from systolic activity. Immediately beneath the electrode, and in a shape corresponding strictly to it, an area of relaxed, red myocardium is formed, with the appearance of local diastole or asystole, lasting during systole of the ventricle. Alongside the electrode the state of polarization of the myocardium is increased. Closer to the periphery of the ventricle a state of depolarization is also induced perielectrotonically, which also arises beneath the electrode [2].

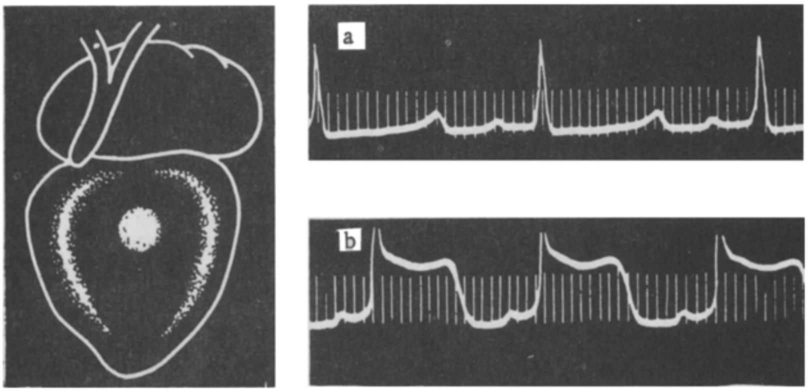


Fig. 2. Changes in the electrogram of the frog's heart. a) Before alteration; b) immediately after cathode alteration. On the left – scheme of distribution of contrast changes on the heart muscle after cathode alteration.

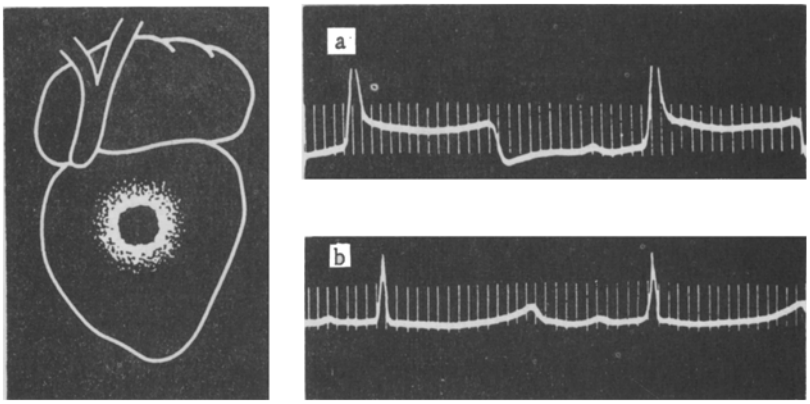


Fig. 3. Gradual disappearance of the monophasic potentials in the electrogram of a frog's heart during the formation of a necrotic zone at the site of cathode alteration (a,b). On the left — diagram of the contrast changes observed during the development of necrosis.

The asymmetry caused by local cathode alteration leads to the conversion of the biphasic action current of the ventricle into a monophasic current (Fig. 2).

If, during local cathode alteration of the ventricle, the current is increased and, in particular, its action is prolonged, then under these circumstances it can be seen how the depolarization formed beneath the electrode gradually changes into an irreversible necrosis. The relaxed, red myocardium beneath the electrode disappears and, in its place, is formed a white necrotic focus, around which appears a border of red, relaxed myocardium, not previously seen here, in apparent asystole. This depolarized border of relaxed myocardium, as it moves across to the periphery of the focus of alteration, naturally continues to maintain the monophasic character of the action potential of the ventricle of the heart.

When the border of depolarization arises at the periphery it remains for 30-60 minutes, after which it disappears completely, whereas the focus of necrosis is preserved. With the disappearance of the border of depolarization, its monophasic character is gradually lost and is replaced by the normal biphasic action potential (Fig. 3).

The gradual disappearance of the monophasic potential is none other than the expression of the gradual disappearance of the demarcation current. The disappearance of the monophasic and renewal of the biphasic action potential are evidence of the depolarization of the membrane which is regenerating at the border with the focus of necrosis. Regeneration of the membrane must be regarded as a process characterizing an active reaction which takes the form of an increase in metabolism and polarization in contiguous points of the tissue at the border with the parabiotic focus of injury.

It may be thought that the phenomena demonstrated in the ventricle of the frog's heart also take place in skeletal muscle after injury caused by a transverse incision.

The time taken for disappearance of the demarcation action potentials thus represents the time during which the area of muscle, injured by a transverse incision, passes into a state of irreversible necrosis. Differences occurring in the time taken for the demarcation potential differences to disappear are a reflection of differences in the time taken for the altered muscle tissue to pass into a state of irreversible necrosis. This length of time varies in accordance with the age of the animal. It is natural to suppose that a difference so striking as that which characterizes the duration of the change into a state of irreversible necrosis is, in all probability, an additional and frequent expression of low functional resistance or of low accommodational resistance to the alteration in early stages, and of the gradual increase in the functional resistance in the process of ontogenesis.

If the magnitude of the demarcation current is taken as an index of the degree of polarization, it must be concluded that the level of polarization of muscle tissue is very low in early stages and increases each time a change is made from one stage of individual development to the next.

A study of the resistance of the gastrocnemius muscle, with its connections to nerve centers intact, to an audiofrequency (5000 cps) or high frequency (1,000,000 cps) current enabled the coefficient of polarization to be deduced. In adult rabbits the total apparent resistance was 1500 ohms and the coefficient of polarization about 4. In rabbits aged under 8-10 days, the total apparent resistance to an audiofrequency current was about 600 ohms and the coefficient of polarization 1.5. In rabbits aged from 10 days to 1 month, the total apparent resistance was 750 ohms and the coefficient of polarization 2.

It has already been stated that, in early stages of ontogenesis, the neuromuscular apparatus is characterized by the typical state of a catelectrotonic syndrome. In the light of the results obtained, this state may be explained by a lowering of the level of polarization of the muscle and also, evidently, of the nerve tissue. The acquisition by the neuromuscular apparatus, in the process of ontogenesis, of the typical properties of an anelectrotonic syndrome may be explained by a gradual increase in the level of polarization.

In the muscles of the frog and rabbit, a relationship has been found [9, 10] between the demarcation potential and the time taken for its disappearance from subordinating influences from the nerve centers.

It may be postulated that the differences which we found in the level of polarization in accordance with the age of an animal are due to changes in the course of ontogenesis in the subordinating or trophic influences from the nerve centers on the muscle tissue. The transformation of polarized membrane structures in the muscle tissue, brought about by the influence of the nerve centers, not only leads to an increase in its lability but also makes it possible for potential energy to develop in the muscle on an ever-increasing scale, while in a state of low resistance.

## SUMMARY

Experiments were carried out on rabbits of various ages. The authors investigated the value of the demarcation potential difference in the gastrocnemius muscle and the time interval until it vanishes. In adult rabbits the average value of the demarcation potential difference was 45-65 mv. The time interval until it disappeared equalled 6-8 hours. In rabbits under 10-12 days old this average value amounted to 6-11 mv, with the average time interval until its complete disappearance equalling 50-80 minutes. In rabbits aged from 12 days to 1-1½ months these two figures were 25-35 mv and 3-4 hours.

The above data serve as additional characteristics of the changes occurring in the functional condition of the neuromuscular apparatus at various age periods.

## LITERATURE CITED

[1] I. A. Arshavskii, Uspekhi Sovremennoi Biol. 32, 1, 18 (1951).

[2] I. A. Arshavskii and O. Kurmaev, Fiziol. Zhur. SSSR 18, 4, 592 (1935).

[3] I. A. Arshavskii and V. M. Kushnarev, Problems of Experimental Biology and Medicine 1, p. 48 (Moscow, 1951) [In Russian].

[4] I. A. Arshavskii and A. A. Oganisyan, Byull. Eksptl. Biol. i Med. 13, 3-4, 79 (1942).

[5] I. A. Arshavskii and V. D. Rozanova, Fiziol. Zhur. SSSR 26, 6, 629-640 (1939).

[6] Z. M. Esipovich, The Functional Resistance of the Peripheral Nervous System at Various Age Periods. Author's abstract of candidate's dissertation (Moscow, 1954) [In Russian].

[7] A. A. Oganisyan, Fiziol. Zhur. SSSR 34, 1, 87 (1948).

[8] V. D. Rozanova, Arkh. Biol. Nauk SSSR 46, 1, 123 (1937); Fiziol. Zhur. SSSR 25, 4, 391, 403 (1938); 3, 346 (1941).

[9] M. N. Farfel', Byull. Eksptl. Biol. i Med. 24, 3, 183-189 (1947).

[10] L. G. Khrolinskii, Fiziol. Zhur. SSSR 40, 4, 472 (1954).

[11] T. W. Engelman, Arch. ges. Physiol. 15, 116 (1877).

[12] R. W. Gerard, Am. J. Physiol. 93, 498-541 (1930).