# MAGNITUDE OF THE MEMBRANE POTENTIAL OF THE SKELETAL MUSCLES OF DOGS AT DIFFERENT AGE PERIODS

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Laboratory investigations have shown that in the early stages of pre- and postnatal individual development the neuromuscular apparatus of mammals is characterized by a series of signs collectively forming the catelectrotonic syndrome. This takes the form of diminished lability, prolonged absolute and relative refractory phases, a high constant of accomodation, small demarcation potentials and impedance, absence of post-tetanic activation, and a tonic type of activity of the skeletal muscles.

During ontogenesis, and expecially starting with the period of assumption of the standing posture (in dogs, from the 16th-18th day) and still more after learning to perform locomotor acts (2.0-2.5 months), the neuromuscular apparatus acquires the signs of an anelectrotonic syndrome: the lability increases, the absolute and relative refractory phases and the constant of accommodation decrease, thedemarcation potential and the impedance are increased [1-6, 8, 12-14]. These changes in the properties of the skeletal muscles in ontogenesis must be expressed more accurately in changes in the membrane potential (MP).

At the present time changes in the MP in skeletal muscles have been studied only in rats [9, 11, 15], animals whose ontogenesis differs from the individual developement of animals such as dogs, cats, and hares.

The object of the present investigation was to assess the magnitude of the MP of the skeletal muscles in dogs at different age periods.

## EXPERIMENTAL METHOD

Investigations were conducted on dogs ranging from puppies 1 day old to adult animals, some of which were over 10 years old. The test object was the gastrocnemius muscle with its blood and nerve supply intact. The skin around the expose muscle was sutured in such a way that the muscle could be bathed in physiological saline warmed to the body temperature of the experimental animals. The solution was changed periodically.

The MP of the fibers was recorded in parts of the muscle free from nerves by means of Pyrex glass microelectrodes with a point less than $1\mu$ in diameter. To measure the MP of the muscle fibers of the puppies age between 1 day and approximatley 2 weeks, microelectrodes with a point $0.2$-$0.3\mu$ in diameter were chosen. Their resistance varied from 42 to 75 m$\Omega$. For the adult animals microelectrodes with a point up to $0.8\mu$ in diameter were used. The extreme values of the resistances of the microelectrodes used for the dogs of all ages were 12-75 m$\Omega$. The microelectrodes were filled with a 2.8 M solution of KCl. A cathode repeater was used as in the scheme described by M. M. Bongard and A. L. Byzov [7]. The MP was measured by an ocillograph with a type Sl-19 dc amplifier. In each experiment the MP of 40-50 muscle fibers were measured.

Because of the absence of data in the literature on the character of the changes in diameter and length of the muscle fibers of dogs in different age periods, the principal criterion of integrity of the fiber during insertion of the microelectrode had to be the stability of the MP in time. If the MP was unchanged immediately after puncture of the surface of the fiber by the microelectrode, its magnitude was recorded. In cases when the magitude of the MP fell continuously or became stabilized, but at a lower level, immediately after insertion of the microelectrode into the fiber, this particular value of the MP was disregarded.

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Amplitude of Membrane Potential in Dogs of Different Ages

| Age of animals | Number of experiments | Number of fibers tested | $M \pm m$ | Extreme values MP (in mV) |
|---|---|---|---|---|
| 1 day | 4 | 187 | 26±1,49 | 15—40 |
| 2—14 days | 16 | 721 | 36±2,32 | 15—70 |
| 17—22 " | 4 | 192 | 58±3,07 | 25—75 |
| 1—1½ months | 9 | 369 | 52±3,03 | 30—80 |
| 2—3 " | 3 | 149 | 76±1,46 | 40—90 |
| 6 months | 5 | 230 | 77±1,04 | 50—95 |
| adult | 4 | 188 | 82,2±0,85 | 70—100 |
| old (10-12 years) | 4 | 198 | 60,1±1,28 | 40—90 |

The results of recording the action potentials of the muscle fibers in response to a single indirect stimulus showed that in puppies during the first days of life the amplitude of the action potential is stable for several minutes (usually 5-10 min), after which the amplitude of the response falls off sharply and disappears. In adult animals the action potentials of the same fiber can be recorded for 1 h or longer. These values were taken as criteria of the stability of the MP. The MP of one fiber was usually measured for a period of 10-30 sec, i.e. long enough for this potential to be maintained at a constant level.

## EXPERIMENTAL RESULTS

Altogether 49 experiments were carried out. The results were placed into age groups, each of which was defined by its characteristic signs with respect to other electrophysiological indices. The mean amplitudes of the MP in the dogs of different age periods are shown in the table. In puppies during the first days of life the minimal amplitude of the MP, not recorded in subsequent age periods, was 15 mV.

The highest MP of the adult dogs, not recorded in previous age periods, was 100 mV. The results in the table show that the MP of the gastrocnemius muscle of the dog increases from the first days of life until the adult state. In the age periods from 17-22 days and from 2-3 months the mean amplitude of the MP rose by a greater amount than in the preceding and succeeding age periods. The first increase in MP (at the age of 17-22 days) coincided with assumption of the standing posture and with the appearance of the first locomotor acts, the second (2-3 months) with the time when the animal obtains complete mastery of locomotion in the environment and becomes completely independent of the mother. The mean MP of the muscles was lower in the dogs over 10 years old. In the muscles of the old dogs, membrane potentials of 45-50 mV characteristically were recorded alongside those of 90 mV.

The difference between the MP of the animals aged 17-22 days and 1.0-1.5 months was not significant, and this was also true of the difference between the dogs aged 2-3 and 6 months. The differences between the MP of the dogs of the other age groups and of the corresponding groups of younger animals were statistically significant.

Investigations by O. A. Martynenko [10] have shown that the concentration of sodium ions in the cytoplasm of the muscle fibers of the rat in the early stages of ontogenesis is higher, and the concentration of potassium ions is lower than in sexually mature rats. With age the increase in the MP of the muscle fiber is accompanied by an increased in the concentration of potassium ions in the cytoplasm of the muscle fiber. In old rats a decrease in the intracellular concentration of potassium was found [15].

How can the increase in the MP after the 16th day of life and in the 2nd-3rd months be explained? Laboratory investigations have shown that the maturation of the myoneural junction takes place gradually. The first, ill-defined signs showing that myoneural junctions are beginning to acquire adult properties appear on the 16th-18th day of life, and they gradually become stabilized only by the age of 3 months. This is reflected by the appearance of physiological indices which are not found before the 16th-18th day of life, such as the phase of exultation, after-hyperpolarization, post-tetanic activation, and the ability of curare to block neuromuscular transmission [1, 2, 12]. Not until the age of 2.5-3 months is the ability to develop Wedensky inhibition, a collective manifestation of the signs of the anelectrotonic syndrome, seen for the first time. It is natural to assume that the gradual increase in MP demonstrated in the present experiments, which is particularly marked at the milestones of postnatal ontogenesis, is due to maturation of the myoneural junction. The decrease in the MP in old dogs may be attributed to destruction of the myoneural junction in the involution period of ontogenesis, as is confirmed by morphological data.

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