METHODS

SIMULTANEOUS MEASUREMENT OF ELECTROMOTIVE FORCE AND CURRENT STRENGTH (QUANTITY OF ELECTRICITY) AS A METHOD OF INVESTIGATION OF BIOELECTRIC PHENOMENA

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Following the work of V. I. Chagovets (1896, 1903), the concept of biocurrents originating in a peculiar concentration element formed between injured or excited and intact or "resting" areas of tissue has persisted in modern electrophysiology.

Such a generator, like any generator in general, can be characterized either by current strength or by electromotive force (e.m.f.) – voltage in the external circuit of the generator – and their changes under various conditions. In order that certain current strength and voltage be maintained in the external circuit of the generator the generator must transform some form of energy into electric energy; in other words, energy transformation and its expenditure occur only when the generator is working: current flows in the external circuit and the difference of potential at the generator terminals tends to zero. It is for the maintenance of this difference that the expenditure of transformed energy is, in the final analysis, required. The quantity of generated electricity Q characterizes the work of the generator. This work can be calculated according to the formula: $A = ItE$, where A – work; I – current strength; t – time; E – e m f; $It = Q$ where Q – quantity of electricity.

In chemical sources of current it is the heat of the chemical reaction or osmotic work involved in equalizing ionic concentrations that is transformed. The question naturally arises as to the nature of energy which is transformed into biocurrent on excitation and injury of tissue. It may be supposed that such energy in any tissue of a living organism must be chemical, i. e., energy of metabolism, since it has been shown in a number of investigations that a connection exists between the state of metabolism in the tissue and bioelectric phenomena.

The main method used in modern electrophysiology is the measurement of e m f of tissue as a more precise index than current strength and independent of changes in the internal resistance of the generator (tissue) when the compensatory method of e m f measurement is employed.

Using ordinary nonpolarizing electrodes (Zn-$ZnSO_4$) with agar corks with considerable resistance (many thousand ohms) and completing the circuit for very brief periods with balanced measurement of e m f conditions are created under which no appreciable current flows in the generator circuit and, therefore, the generator performs no work. In order to maintain these conditions modification of the methods used in studying biocurrents is required.

In an attempt to study the relation of injury currents (e m f) to tissue metabolism (energy being transformed), and taking the hypotheses cited above as a basis, the following points were investigated in the present work: 1) relation of fall in e m f and in strength of injury current to electrode resistance; 2) relation between some aspects of tissue metabolism and the quantity of electric energy generated by the tissue.

METHODS

The experiments were performed on the sartorius muscle of autumn and winter frogs. The pelvic end of the muscle was injured by a special clamp. The muscle was placed in a paraffin bath so that the injured end was in one chamber of the bath and the intact end in the other. Each chamber was filled with Ringer solution or solution of the substance whose effect was being studied. The chambers were divided by a partition consisting of a mixture of paraffin with paraffin oil. Injury current was led off by nonpolarizing electrodes ($Zn-ZnSO_4$) with resistance of 800, 1,500, 2,200, 11,000, 50,000 ohms. Low resistance electrodes (800 ohm) were prepared according to the scheme described by A. V. Lebedinsky and A. S. Mozzhukhin [3]. In order to obtain electrodes with resistance of 1,500, 2,200, 11,000 and 50,000 ohms the original 800 ohm electrodes were connected with appropriate resistances. To measure the current strength (quantity of electricity generated), the muscle was included by means of appropriate electrodes in the circuit of a mirror galvanometer with resistance of 2,000 ohms, graduated with respect to current strength, for 6 hours. To measure the e.m.f, the muscle was included in the circuit of a balanced installation in which the same mirror galvanometer served as the "zero" instrument.

The energy generated by the muscle was calculated [3] according to the formula:

$$E=0.24\left(QU_{cp}+\frac{QU_{cp}}{5}\cdot 2\right)$$

where $Q = I_{cp}t$.

where E – energy in small calories; Q – quantity of electricity; U – e m f; I – current strength; t – time; Ucp and I cp were calculated as a mean of a number (7) of measurements during the 6 hours of experiment. The value of liberated energy was expressed in microcalories for the sake of more convenient comparison with the majority of literature data.

The influence of impairment of carbohydrate-phosphorus metabolism was studied in experiments in which the intact end of the muscle was poisoned by substances listed in the table. The concentrations indicated in the table are, according to data available in the literature(Belitser[1], Parnas, 1940 and others) the most effective in disturbing carbohydrate-phosphorus metabolism in skeletal muscle.

In most of the experiments the intact portion of the muscle was subjected to the action of the various substances throughout the whole experiment (6 hours). In some experiments the intact portion of the muscle was exposed to the action of monoiodoacetate solution for only 30 minutes; the substance was then replaced by Ringer solution or solution of vitamin B_1, which is designated in Fig. 2 as "washing".

RESULTS

Figure 1 shows changes in strength of current generated by the muscle with various resistance of the external circuit. The greatest current strength is observed when this resistance equals 3500-4200 ohms (electrode resistance 1500-2200 ohms). Measurement of resistance in the area of the muscle lying between the electrodes according to A. V. Lebedinsky's scheme [2] yields a value equal to 3000-4000 ohms. These data show good correspondence with the well-known fact that the generator performs most work (and hence the greatest transformation of energy) when the internal and external resistances of a circuit are equal. This fact was utilized in experiments in which the quantity of electric energy generated by the muscle under conditions of impaired carbohydrate-phosphorus metabolism was being studied. The results of these experiments are presented in the table and in Fig. 2. The data show that impairment of carbohydrate-phosphorus metabolism by one or other enzyme poison lowers the electric energy generated by the injuered muscle.

It follows from the table and Figs. 1 and 2 that, on the one hand, under certain conditions the maximal electric energy liberated by the injured muscle can be measured, and, on the other hand, the value of energy thus liberated can undergo a change when carbohydrate-phosphorus metabolism is disturbed. Changes in generation of electric energy are most marked when the muscle is poisoned by monoiodoacetate, fluoride and hydrazine (which interfere with the reactions connected with transfer of phosphate to the ATP-phosphocreatine system), 2,4-dinitrophenol (which interferes with conjugated oxidative phosphorylation) and veratrine (which accelerates breakdown of phosphorus compounds).

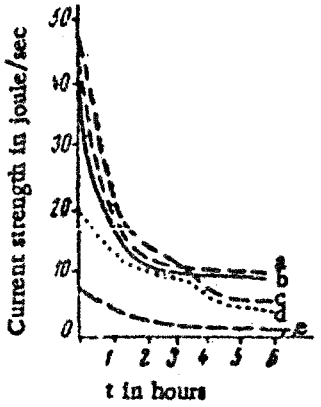

Fig. 1. Strength of current generated by the muscle with various resistance in the external circuit.
A) 4200 ohm; B) 3500 ohm; C) 2800 ohm; D) 1300 ohm; E) 52000 ohm (electrode resistance; A) 2200 ohm; B) 1500 ohm; C) 800 ohm; D) 1100 ohm; E) 50000 ohm).

It can thus be assumed that during generation of injury biocurrent energy liberated in the process of carbohydrate-phosphorus metabolism is used up.

There is a concept at present which proposes that liberation of energy in muscle tissue takes place by way of the ATP-phosphocreatine system. It is possible that this system is the direct energy source in the generation of biocurrent. It may be supposed that both glycogen breakdown and respiration area energy sources in the resynthesis of this system. The energy potential of the ATP-phosphocreatine system exceeds several times the quantity of energy required for the generation of injury current. However, formalin-fixed muscle which is, apparently, free of even traces of metabolic processes, still gives some difference of potential. But this difference of potential is not great (one half to one third of the e m f of control muscle) and the formalin-treated muscle cannot produce any significant quantities of electric energy (the total amount of electric energy generated by control muscle is 8-10 times greater); the formalin-treated muscle behaves as a discharged accumulator (element): when it is switched in by way of low resistance electrodes it shows, in the first few moments, some difference of potential and current strength but these values decrease markedly in the course of a few seconds. All these considerations not only do not refute the dependence of injury current on metabolic processes but would even appear to support this hypothesis (A. S. Mozzhukhin [4]).

Quantity of Electric Energy Liberated by Injured Muscle During 6 Hours Under Various Conditions of Impairment of Carbohydrate-Phosphorus Metabolism (all figures are average of 10 experiments)

No. of experiment	Enzyme poison or metabolite	Energy liberated in 10^{-3} microcalories per 100 mg muscle	Concentration of poison in which the intact portion of muscle was immersed
1	Control	1.0	–
2	Sodium monoiodoacetate	0.4	0.001 M
3	Sodium fluoride	0.5	0.02 M
4	Hydrazine sulfate	0.35	0.02 M
5	2,4-dinitrophenol	0.35	1:10 000
6	Sodium arsenate	1.0	0.02 M
7	Veratrine	0.4	1:10 000
8	Sodium cyanide	0.8	0.02 M
9	Phlorizin	0.8	0.01 M
10	Glyceraldehyde	0.65	0.02 M
11	Sodium pyruvate	1.1	0.02 M
12	Sodium lactate	0.95	0.02 M
13	Sodium sulfite	0.8	0.02 M
14	Formalin	0.1	20%

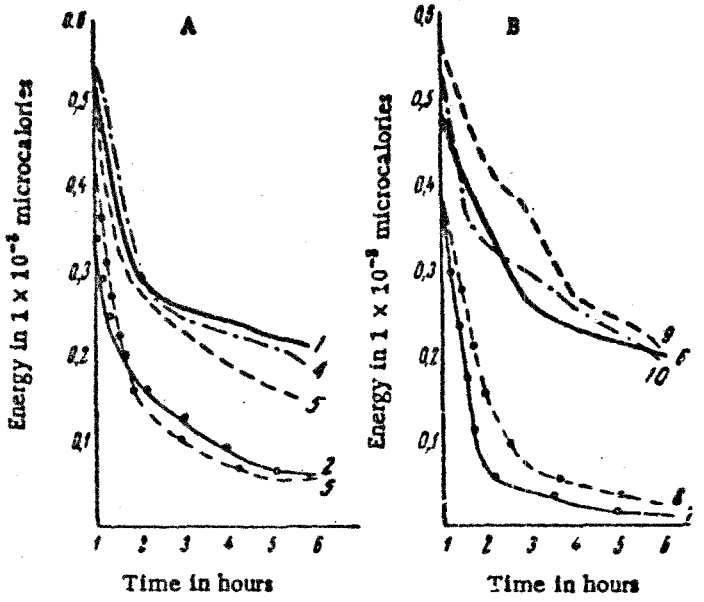

Fig. 2. The effect of poisoning isolated muscle by agents which impair carbohydrate-phosphorus metabolism on the quantity of energy generated (separate experiments with initial current strength of 1.5μ A and e m f of 50 mv).
Curves: A) 1) control, 2) monoiodoacetate poisoning with Ringer solution "washing", 3) phlorizin poisoning, 4) monoiodoacetate poisoning with vitamin B_1 solution "washing", 5) 2,4-dinitrophenol poisoning. B) 6) cyanide poisoning, 7) veratrine poisoning, 8) hydrazine sulfate poisoning, 9) lactate treatment, 10 arsenate poisoning.

It is possible that those small e m f and current values which are observed in formalin treated muscle are determined by liberation of structural energy or represent an accumulation of metabolic energy, or formalin may "fix" the differences of ionic concentrations in the muscle and the preparation represents a concentration element without continuously maintained difference of concentrations which are quickly equalized. Whatever the situation, metabolism evidently accounts for 0.9 of all the energy used in the generation of electric energy.

Simultaneous measurement of the quantity of electricity generated by muscle and of the e m f thus provides the opportunity for calculating the energy used in maintaining the potential difference between the injured and intact areas of tissue. Decrease in electric energy generated by the injured muscle when its carbohydrate-phosphorus metabolism is impaired proves that injury current and e m f are related to metabolism.

SUMMARY

Simultaneous measurement of the quantity of electricity generated by the muscle and of e m f gives the opportunity to estimate the energy which is used for maintenance of the difference of potentials between the injured and intact areas of tissue. Reduction of the value of electric energy, generated by the injured muscle, when the processes of carbohydrate-phosphorus m tabolism are disturbed proves the relationship of generation of biocurrent of the injured area to the condition of metabolism.

LITERATURE CITED

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