PHOSPHAGEN IN THE ELECTRICAL ORGAN OF TORPEDO

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(With Three Text-figures.)

INTRODUCTION.

The electrical organs of the fishes seem to have attracted remarkably little biochemical interest. But they have been the subject of intensive physiological studies at the hands of Marey, D'Arsonval, and others, a review of whose work is to be found in Richet's Dictionnaire de Physiologie. As long ago as 1666, before the Leyden jar was discovered, Redi attributed the unpleasant results of contact with the fish Torpedo to the half-moon-shaped organs which he found on either side of the head, and which he regarded as muscular. It has since been shown that the organs in question are, in fact, derived by the functional modification of muscle cells, and that between the activities of the two organs, i.e. the electrical and the muscular, there is a very considerable degree of resemblance.

In muscle, the parallel arrangement of the fibres prevents any summation of the action potentials which accompany activity, and in a frog's gastrocnemius the potential is only of the order of 0.05 volt. But in the electrical organs the cells are arranged in columns reminiscent of Volta's pile, with about 550 columns in each organ, and about 400 cells in each column, and a summation of the action potentials can occur, producing resultant potential differences of considerable magnitudes. D'Arsonval, for example, observed Torpedos of diameters of 25–35 cm, and found that the E.M.F. varied from 8 to 17 volts and the current from 1 to 7 amperes on open circuit, while on closed circuit the p.d. exceeded 300 volts.

In spite of the apparent difference between the natures of the two types of activity, there is a close resemblance between them. Lever-tracings of a single muscle twitch and galvanometric tracings of a single impulse from an electrical organ are exactly analogous, both organs can be tetanised, and both are similarly affected by drugs such as strychnine, veratrine, curare, etc., and both show the same phenomenon of latent period. In short there is so close a parallel between the two organs that one might expect to find that both make use of the same, or at any rate very similar, chemical reactions for their energy supplies. There are, indeed, a number of observations which favour such a supposition; Krukenberg (10) and Weyl (14) showed that the organ contains creatine, Schultze (13) that it contains
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creatine, while Buytendijk (a) finds that in the fresh organs of young animals there is about 0.09 per cent., and in old about 0.04 per cent. of glycogen. Very recently, in a paper not available to the present author until his work was completed, Kisch (g) confirmed the work of the earlier authors, and showed that the creatine is present as phosphagen, but did not determine the velocity constants of the hydrolysis of the compound although its physiological behaviour was well studied.

Mention might here be made also of electrical organs of the type which are believed to be derived from glandular rather than from muscular sources, as is the case, for example, with the organ of Malapterurus. This organ too, it appears, shows a close similarity to muscle, and it would be very interesting to know how its chemical mechanisms compare with those of muscle. It seems not unlikely that such knowledge might do something towards the discovery of the energy-source of glandular activity, and throw some light upon the systems whereby the actual mechanical response of muscle is brought about.

METHODS.

The first experiment was devoted to the isolation of creatine from the electrical organ, and the later work was designed to detect and identify any phosphagen which might be present, and to examine its behaviour to some extent. In all the experiments dealing with phosphagen the specimens were cooled to 0°C. before removal of the organs for analysis, but the method employed for the cooling varied in different cases and will be described later. The tissue, after removal, was dropped into an ice-cooled beaker, weighed, and then extracted with ice-cold 10 per cent. trichloracetic acid by grinding with ice-cold, acid-washed quartz sand. This operation was difficult at first on account of the tendency of the discs of which the organ is composed to separate and escape the action of the pestle by reason of their very slippery nature. This was easily overcome by first macerating the tissue with dry sand, then adding the acid a little at a time, but grinding vigorously throughout. From 5 to 10 c.c. of the acid were used for each gram of tissue. The extracts were next filtered under pressure through small Gooch crucibles, previously prepared, washed, dried, and cooled, and the filtrates were received in ice-cooled tubes. The pH was then rapidly adjusted to the desired value by the addition of 40 per cent. soda.

The method of Fiske & Subbarow (6) was employed throughout for the estimations of phosphorus (ref. accompanying paper).

The individual experiments may now be described.

EXPERIMENTS.

Exp. 1. About 180 gm. of the electrical tissue, from a fish which died soon after capture, were extracted with water and worked up by a slight modification of the method described by Hunter (8).

Fig. 1 is taken from a microphotograph of some of the crystals, which show the crystal form characteristic of creatine.
The mother liquors gave the Jaffé reaction, the Weyl-Salkowski reaction, and the ferric chloride test for creatinine, and therefore contained that substance, but the amount present was such that it might easily have been formed from creatine during the operations of extraction and concentration of the extract. The crystals themselves gave none of these reactions after being filtered off and well washed with 88 per cent alcohol, but if a solution of the crystals was previously treated for a few moments with boiling acid or alkali, powerful positive reactions were obtainable.

Exp. 2. A specimen, caught on the previous night and in excellent condition, was taken from the aquarium and transferred to a large bowl filled with small pieces of ice. The animal struggled violently when put into the ice, but soon became and remained perfectly quiet. After being left in the ice for about 20 min., one of the organs was removed as rapidly as possible, freed from its closely adherent skin, and a portion weighing 7.78 grams was extracted in the manner already described. The filtered extract was treated at once with 40 per cent. soda till the colour of a little added thymol blue showed a perceptible change, when the pH was determined by means of a capillator, and found to be 1.7. The following experiments were performed on this solution.

(a) 2 c.c. were taken, neutralised to phenolphthalein with 40 per cent. soda, and treated with 1 c.c. of Fiske & Subbarow’s calcium reagent (10 per cent. CaCl₂, saturated with Ca(OH)₂) to precipitate inorganic phosphates. The precipitate was centrifuged down after the tube containing it had been allowed to stand in ice for 10 min., the centrifugate being poured into a 50 c.c. flask, when the precipitate was washed with 4 c.c. of water and a further 1 c.c. of the calcium solution. The precipitate was again centrifuged down, the washings being added to the former centrifugate. The combined centrifugates ("creatine fraction") were then treated with
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5 c.c. of a 2-5 per cent. solution of ammonium molybdate in 5N H₂SO₄ and allowed to stand for 20 min., during which time the phosphagen was broken down, liberating free phosphate. Meanwhile, the inorganic precipitate was dissolved in a trace of dilute sulphuric acid and transferred to another 50 c.c. volumetric flask and treated with 5 c.c. of the molybdate reagent, diluted to about 40 c.c. with distilled water, and then treated with 2 c.c. of 0-25 per cent. 1-2-4-aminonaphtholsulphonic acid, a standard solution being made up at the same time, and the colours were compared after 15 min. The "creatine fraction" was treated in exactly the same manner.

The same method was employed in a number of the other estimations and is described here in detail for that reason. The results of this experiment are given in Table I.

(b) A second sample of 2 c.c. was taken in a 50 c.c. volumetric flask, treated with the colour reagents as rapidly as possible, made up to the mark, and at once trans-

ferred to the colorimeter, a standard solution being prepared in exactly the same way and at the same time. Readings were begun at once and continued at intervals till no further alteration took place. The first reading was taken 2½ min. after the addition of the molybdate reagent to the unknown. Creatine phosphate breaks down very rapidly in the presence of the molybdate ion in acid solution, and the phosphate content of the solution therefore increases, as Fig. 2 shows. The unknown was kept at a depth of 30 mm. throughout, and the readings of the standard are plotted against the time in minutes. Eggleton & Eggleton, the elaborators of this method, have shown (5) that the points on a curve plotted in this manner lie on a straight line for the first 8 min. (less in the present case, since the reaction temperature was 27°C instead of about 18°C.), so that by backward extrapolation to zero time it is possible to obtain a value for the initial phosphate content of the solution, while from the final reading one can obtain the final phosphate content, and subtracting from this the initial value, the amount of phosphate derived from the phosphagen which has broken down. The results are given in Table I.
(c) A value for the reaction-velocity constant of the hydrolysis under the conditions of the last experiment can be calculated from Fig. 2 and the value of this constant in the absence of molybdate was next determined. The remainder of the extract was heated to 28°C as quickly as possible by immersion in a hot bath, and then transferred at once to an electrically regulated incubator at the same temperature. The observed variation on the temperature of the latter was ± 0.5°C during the experiment.

1 c.c. samples of the fluid were taken at intervals, beginning at once, and the inorganic P was estimated after precipitation and washing as described for the earlier experiments. The phosphorus of the phosphagen fraction was not estimated, since the estimations of the inorganic fraction alone occupied the single observer almost completely in the earlier stages, while in the later stages the amount of P in the phosphagen fraction became too small for accurate estimation. The same flasks and pipettes were used in each estimation, and 15 min. were always allowed for the colour development. The results are given in Fig. 3, in which the amount of phosphate is plotted, in terms of mg. inorg. P per c.c. of the reaction fluid, against the time in hours.

(d) The phosphagen of the extract being now completely hydrolysed, the creatine of the solution was estimated by the method of Folin(7), after sugars had been removed by the method of van Slyke(13). To check losses involved, a solution of creatine containing the expected amount of creatine was diluted to 7 c.c., treated with the van Slyke reagents and centrifuged, and then autoclaved for 45 min. at 123-124°C with its own volume of N HCl, neutralised to litmus, and treated with picric acid and soda in the usual way, the colour produced being compared with that produced in a standard solution of creatinine. In this way it was found that 70 per cent. of the creatine was converted and recovered. 7 c.c. of the extract were then subjected to exactly the same series of operations, and the original extract was thus found to contain 0.715 mg. of creatine per c.c.
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The results of this series of experiments are given in Table I. They are expressed as mg. of P or of creatine per 100 gm. of tissue, allowance having been made for the dilution of the extract by tissue water, supposing that the water content of the organ is 91·5 per cent. as in T. ocellata, the species actually used being, however, T. marmorata.

Table I.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Method</th>
<th>I-P mg. %</th>
<th>C-P mg. %</th>
<th>(I + C) P mg. %</th>
<th>% C-P</th>
<th>Total creatine mg. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (a)</td>
<td>Calcium separation</td>
<td>53·5</td>
<td>11·0</td>
<td>64·5</td>
<td>17</td>
<td>340</td>
</tr>
<tr>
<td>2 (b)</td>
<td>Extrapolation</td>
<td>49·6</td>
<td>15·9</td>
<td>65·5</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>2 (c)</td>
<td>Reaction curve</td>
<td>52·0</td>
<td>12·4</td>
<td>64·4</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2 (d)</td>
<td>Creatine estimation</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Average for Exp. 2</td>
<td></td>
<td>51·7</td>
<td>13·1</td>
<td>64·8</td>
<td>20</td>
<td>340</td>
</tr>
</tbody>
</table>

Exp. 3. (a) The conditions of cooling in the last experiment were bad for two reasons. In the first place, the sudden exposure of the fish to cold probably resulted in sudden stimulation, and in the second place the animal was exposed to the action of a hypotonic medium during the cooling. In this animal, as the figures of the last experiment show, only 20 per cent. of the sum of the inorganic and labile fractions of the phosphorus was accounted for by phosphagen. In the next experiment, therefore, the animal was placed in a bowl of sea water which was then put into the ice-chest for an hour, and finally into a freezing mixture. In this manner it was possible to cool the animal very slowly and gently, without any sudden stimulation, while the effect of exposure to a hypotonic medium was also avoided.

When the temperature had fallen to between 5 and 4° C., respiration appeared to have ceased, and no response followed when the tail fins were gently gripped with forceps. When 3° C. was reached, most of the water was poured off, and a small portion of one organ was removed, dropped into an iced beaker, weighed, extracted, etc., in the usual fashion. 4 c.c. of the filtered extract were taken for estimation of the inorganic and phosphagen fractions of the phosphorus.

(b) The same animal was now covered with fresh sea water, when the respiration slowly returned, and with it the tail reflex. Placed in the aquarium the animal swam vigorously and seemed none the worse for its experiences, and being still in good condition next morning, it was again captured and placed in a bowl of sea water. The nerves supplying the intact organ were cautiously exposed and then stimulated electrically for half an hour with rather slow induced shocks, these being applied for 4 min. periods with 4 min. intervals. A small piece of tissue was then removed from the intact organ, dropped into an ice-cooled beaker, extracted, and the phosphate fractions were estimated in the usual way.

(c) Finally, after the fish had been allowed to rest for 20 min., another portion of the electrical organ was taken, transferred to a beaker, immersed in a bath at 45° C. and kept at that temperature for an hour, the beaker being covered to prevent evaporation as far as possible. At the end of that time, the beaker was cooled to 0° C. and the tissue extracted, etc., in the usual way.
The results of this series of experiments follow in Table II, together with the average figures for the first series, and some data from Eggleton & Eggleton (6) for the coracomandibular muscle of *Raia claviata*, a closely related species, with which they may be compared. All figures represent mg. per 100 gm. of tissue, and are corrected for the water content of the tissue.

### Table II

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Treatment</th>
<th>I-P mg. %</th>
<th>C-P mg. %</th>
<th>(1 + C) P mg. %</th>
<th>% C-P</th>
<th>Total creatine mg. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (a)</td>
<td>Cooled in ice</td>
<td>51.7</td>
<td>13.1</td>
<td>64.8</td>
<td>20</td>
<td>340</td>
</tr>
<tr>
<td>3 (a)</td>
<td>Cooled in sea water</td>
<td>25.1</td>
<td>27.4</td>
<td>62.5</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td>3 (b)</td>
<td>Stimulated 30 min.</td>
<td>84.4</td>
<td>Trace</td>
<td>84.4</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>3 (c)</td>
<td>1 hour at 45°C.</td>
<td>50</td>
<td>40</td>
<td>90</td>
<td>45</td>
<td>440</td>
</tr>
<tr>
<td>Coracomandibular of <em>Raia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION.**

From the curves of Figs. 2 and 3 the following velocity constants were calculated:

(i) at 27°C in 0.5 N acid in presence of 0.25 per cent ammonium molybdate,

\[ k = 127 \times 10^{-3}; \]

(ii) at 28°C, pH 1.7, molybdate absent, \( k = 11.4 \times 10^{-3}. \)

Fiske & Subbarow (6) have given data for the hydrolysis of the creatine phosphate of cat muscle in crude extracts under a variety of conditions from which it can be calculated that under the conditions of (ii) the value to be expected is about \( 12 \times 10^{-3} \), so that there is a good agreement. From the same data, it can be calculated that under the conditions of (i), but molybdate being absent, the constant would be expected to be \( 9.3 \times 10^{-8} \). The molybdate ion has thus produced an acceleration of about 14 times. According to Meyerhof & Lohmann (11) the acceleration factor is about 30 in pure preparations of creatine phosphate, but rather less, usually of the order of 15, in crude extracts. (All \( k \) values quoted here are expressed in terms of natural logarithms with the minute as time unit.)

The phosphagen of the extracts of the electrical organ of *Torpedo* therefore has hydrolysis constants which agree well with those of creatine phosphate; creatine has been isolated from the organ and has been identified; the general behaviour of the phosphagen is that of creatine phosphate. It is therefore concluded that the organ in question contains creatine phosphate.

It has been mentioned that in the first experiment, where only 20 per cent. of the inorganic plus labile P was accounted for by phosphagen, the animal struggled violently at first, and was also exposed to abnormal osmotic conditions. In the second case, however, both these conditions were modified and made more normal, and the percentage then went up to 60 per cent. The reduction of this to 49 per cent. by the electrical stimulation cannot be stressed, for the reduction is rather small, but it may be significant since the stimuli were applied aerobically, and the animal was respiring in a normal manner meanwhile, and so presumably kept up an adequate
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More significant is the disappearance of phosphagen when the tissue was heated to 45°C, a process which induces rigor, and complete or almost complete disappearance of phosphagen, in muscle also.

Table II shows that the amounts of P in the phosphorus fractions, and the amount of creatine also, is less than in the coracomandibular of Raia, but against this it has to be remembered that the amount of ash is less than in muscle, so that amount of P, considered as a percentage of the dry matter of the tissue, is accordingly greater than it would be in the case of muscle. In Exp. 3 (c) the amount of inorganic labile P, in mg. per cent., is much higher than in the other experiments on the same organs, but this is probably due to some extent to evaporation during the hour for which the tissue was heated. According to Weyl(19), the organ contains 18-67 per cent. of ash, of which 12-4-17-8 per cent. is P₂O₅, corresponding to 0-25 per cent. of P₂O₅ in the whole organ. The present work shows that the inorganic plus labile P corresponds to about 0-15 per cent. of the whole, reckoned as P₂O₅, that is, 60 per cent. of the total as reckoned by Weyl. Eggleton & Eggleton(4) find that in striated frog muscle the inorganic plus labile P accounts for about the same percentage of the total P, suggesting again that the distribution of the phosphorus in the various fractions may be very similar in both organs. The following figures were obtained in a rough determination of the phosphorus distribution in frog and Torpedo; under "total" is given the amount of P as mg. per cent., while the other fractions are expressed as percentages of this total value.

<table>
<thead>
<tr>
<th></th>
<th>Total P mg. %</th>
<th>Inorg. + labile P</th>
<th>Pyro. + insol. esters P</th>
<th>Sol. esters P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rana</td>
<td>128</td>
<td>65</td>
<td>29</td>
<td>6</td>
</tr>
<tr>
<td>Torpedo</td>
<td>90</td>
<td>67</td>
<td>33</td>
<td>0</td>
</tr>
</tbody>
</table>

Though only rough figures, these do show that the similarity between the muscle and the electrical organ may be very close indeed. Unfortunately it was not possible, on account of rough weather, to obtain sufficient animals to go further into the question.

On the whole, then, it seems not unreasonable to suppose that the energy liberated in activity comes from the same source in both cases, and that the factors controlling the form in which that energy appears are purely structural. Muscle tissue would thus be regarded as so adapted as to convert the energy as far as possible into the mechanical energy of contraction, electrical changes being only very slight, whereas in the electrical organ the energy appears in the electrical form, contraction being either absent or negligible. This being the case, it is possible that a careful study of the structural differences between the tissues in question would throw light on the nature of the events immediately concerned in the contraction of muscular tissues, and, at the same time, upon the manner in which the potential differences are set up in the individual cells of the electrical organ.

Finally, it is interesting to notice that the ratio of the total number of creatine molecules to the number of phosphate ions which can be obtained in the free state...
is about 1.25, so that in the partly fatigued organ there is a slight excess of creatine over phosphate. But the creatine was estimated by a method involving the use of the Jaffé reaction, whose specificity is by no means absolute, and the value obtained is probably high on that account. Further, it is well known that when the phosphagen of muscle breaks down, some of the liberated phosphate is at once esterified, and if this is also the case in the electrical organ it seems probable that in the resting organ practically all the creatine and all the phosphate are present in the form of phosphagen. Dulière has shown that this is probably the case in resting frog muscles. Obviously it is desirable to correlate, if possible, energy output with phosphagen breakdown in the electrical organ both qualitatively and quantitatively, as has been done in the case of muscle. It is important, therefore, to have some idea of the absolute amount of phosphagen present in such an organ, and this can be obtained if we suppose that practically all the creatine of the muscle is present as phosphagen in the living organ, in situ, and at rest. At present, unfortunately, no data exist from which the maximum energy production of an electrical organ can be calculated.

SUMMARY.

1. The electrical organs of *Torpedo marmorata* contain creatine phosphoric acid, which was identified by the velocity constants of its hydrolysis under controlled conditions.

2. This compound is of functional importance in the organ, being diminished in amount by activity, heat rigor, and by conditions which are unfavourable to the organism as a whole.

3. The physiological parallel between the electrical organ and muscle appears, as far as this study has been carried, to be itself paralleled by the chemical mechanisms.

4. Creatine has been isolated from the electrical organ and has been identified, in confirmation of the earlier work of other authors.

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