CXIX. BIOCHEMISTRY OF THE ELECTRIC ORGANS OF *RAIA CLAVATA*

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It was shown in a recent publication [Baldwin & Needham, 1937] that the occurrence of certain chemical reactions characteristic of muscle can readily be demonstrated in enzyme preparations derived from the electric tissue of *Torpedo marmorata*. Electric organs are also present in elasmobranch fishes of the genus *Raia*, and in these species the electric tissue occupies the place of the lateral muscles from the tip of the tail upwards, disappearing towards its base within concentric layers of muscle, from which it can easily be separated [*v.* Burdon-Sanderson & Gotch, 1888; Ewart, 1892]. In the present work the electric organs of *R. clavata* were used. Electric organs of the kind found in *Raia* are known to be weak in their effector activity as compared with those of *Torpedo*. Thus Burdon-Sanderson & Gotch [1888] remark that "the organ though small is as perfect in structure and function as that of the *Gymnotus* or *Torpedo*", and that "the maximum electromotive force of the shock, i.e. the electromotive force corresponding to 1 cm. length of organ, we have roughly estimated to be about 0.5 V. In *Torpedo* it is probably ten times as much."

Like those of *Torpedo* the electric organs of *Raia* develop from muscular rudiments, and might therefore be expected to show signs of a chemical relationship to muscle itself, even though their effector response to stimulation is relatively feeble, and it was the purpose of these experiments to investigate the enzyme systems to be found in suitable preparations obtained from the electric tissue of a member of the genus *Raia*. The majority of the experiments were carried out on the thornback ray, *R. clavata*, at the Marine Biological Laboratory, Plymouth, and I take this opportunity of expressing my thanks to the Director and Staff of the laboratory for their hospitality and for the facilities afforded for this work.

Methods

Large fishes in good physiological condition were selected and pithed. The tails were at once removed and packed in ice for at least 20 min. The electric organs were then dissected out, care being taken to remove as much as possible of the adherent connective tissue. Each fish provided on the average about 10 g. of electric tissue. The extracts used in the preliminary experiments were prepared by freezing the tissue in a mortar surrounded with freezing mixture, crushing and grinding it to a pulp which was then just allowed to thaw, and extracting with one and a half times its own weight of ice-cold distilled water. The extracts were centrifuged and stored in the ice box. In most of the experiments quoted in this paper, use was made of acetone powders. Aqueous extracts prepared in the usual manner were treated with 10 vol. of acetone at 0°, the flocculent precipitate so obtained being collected on an ordinary filter paper, washed with acetone and ether, and finally dried *in vacuo* over concentrated

sulphuric acid for 15–20 hr. Acetone powders prepared in this way contain all the enzymes involved in the reactions which it was proposed to study, and can be extracted with water and centrifuged to give active enzyme solutions.

The reagents and methods employed were almost identical with those described by Baldwin & Needham [1937] and are therefore not described in detail here.

Simple aqueous extracts of the fresh tissue gave unsatisfactory results from the first. In the presence of phosphoglyceric acid, adenylypyrophosphate and creatine, only a small synthesis of phosphagen could be demonstrated under conditions which, in the case of similar preparations from the electric tissue of *Torpedo*, lead to the formation of several tenths of a milligram of phosphagen P. Added adenylypyrophosphate rapidly disappeared and only a small fraction of the added pyro-P was transferred to creatine: part appeared in the form of inorganic phosphate, probably owing to the action of the adenylypyrophosphatase which occurs in all muscular tissues, while the remainder could not be accounted for. Most probably this last fraction must have been transferred to glycogen with the formation of hexosephosphates, but no attempt was made to discover whether this really was the case.

Adenylypyrophosphatase occurs in muscle and also in the electric organs of *Torpedo*, and if this enzyme is at all active a phosphorylation of creatine at the expense of adenylypyrophosphate can only be demonstrated with difficulty, since adenylypyrophosphate is then rapidly broken down. It has been found possible with most of the tissues hitherto examined to inactivate this enzyme more or less completely by allowing the extracts to stand for a week or more at 0° [Lehmann, 1935], or by prolonged dialysis [Lohmann, 1934]. A combination of these methods was successfully used by Baldwin & Needham [1937] in their work on *Torpedo*, but in the present case the enzyme appeared to be unusually resistant, persisting in extracts which were already a week old and had been dialysed for at least 10 hr. Table I shows the results of some preliminary experi-

Table I

Age of extract days	mg. phosphagen P found	mg. inorganic P found
3	0.029	0.284
5	0.030	0.292
8	0.039	0.163

ments in which the extract used had been dialysed for 9 hr. against 0.5 % KCl at 0°. In each case 2 ml. of the extract received 1.2 mg. phosphoglyceric acid-P, 0.127 mg. pyrophosphate-P in the form of adenylypyrophosphate, 0.6 mg. magnesium and 6 mg. creatine. The whole sample was brought to pH 7.8 by the addition of sodium bicarbonate solution, and incubated for 10 min. at 40°. The subsequent treatment was exactly the same as that described in our earlier paper. In each case only a trace of pyrophosphate-P could be found at the end of the experiment,¹ and it will be seen that, although after 8 days less pyro-P is transferred to the inorganic fraction, the proportion transferred to creatine shows no striking increase. In experiments of this kind the yield of phosphagen-P never exceeded and seldom reached 0.04 mg. under any of the conditions tried, whereas in our work on *Torpedo* yields five or six times as great were easily

¹ The term "pyrophosphate-P" refers to the easily hydrolysable P of adenylypyrophosphate throughout this paper.

obtainable under the same conditions. It might thus appear that the physiological feebleness of the electric organ of *Raia* is paralleled by a corresponding feebleness in the enzyme systems. It was difficult however to be satisfied that the adenylypyrophosphatase of these extracts had been successfully inactivated by the treatment given. It was therefore desirable to find some other way of destroying the enzyme, and I am indebted to Dr S. Ochoa for suggesting precipitation with acetone for this purpose. The employment of acetone powder preparations not only provides enzyme solutions free from adenylypyrophosphatase but has the further advantage that it allows the preparation of enzyme solutions of any desired concentration. Mammalian muscle gives on the average about 30 mg. acetone-precipitable material per g. tissue, and this is true also of the muscle of *Raia clavata* [Ochoa, 1937, personal communication]. The electric tissue however has only 12–15 mg. acetone-precipitable substances per g., and the acetone powders were therefore made up to be twice the strength of the original extracts, i.e. to contain approximately 30 mg. acetone powder per ml., by grinding with the appropriate quantity of ice-cold distilled water, centrifuging and dialysing the clear centrifugate for 10 hr. against 0.5% KCl at 0° in thin cellophane sacs. Dialysis was carried out here as a method of purifying the enzyme preparation from crystalloids rather than with a view to inactivating adenylypyrophosphatase, though it may have contributed towards this inactivation which, as experiments showed, was almost complete. The powders themselves contained appreciable quantities of phosphagen (100 mg. phosphagen P per 100 g. powder were found in one sample), as well as inorganic phosphate and other crystalloids, but these were almost completely eliminated by this preliminary dialysis, after which the preparations were stored in the ice-box till required, and generally used within 2 days. No preservative was added.

Results

[In the ensuing tables the figures refer to mg. P added to or found in 2 ml. samples of the enzyme preparation.]

Exp. 1. Phosphorylation of adenylic acid by phosphoglyceric acid.

2 ml. enzyme preparation received 1.2 mg. phosphoglyceric acid-P, an amount of adenylic acid equivalent to 0.815 mg. pyrophosphate-P, and 0.6 mg. magnesium. The sample was incubated for 2 min. at 40° and pH 7.8. No pyrophosphate-P was found in control samples, but analysis of the experimental sample showed the formation of 0.079 mg. pyrophosphate-P, corresponding to the phosphorylation of 9.7% of the adenylic acid added. Though small, this synthesis is undoubtedly significant, especially when considered in relation to the rest of the experiments.

Exp. 2. Phosphorylation of creatine by adenylypyrophosphate.

(a) 2 ml. extract received 0.636 mg. pyrophosphate-P, 30 mg. creatine and 0.6 mg. magnesium, and was analysed after incubation for 15 min. at 40° and pH 7.8 (sample I). A control sample (II) to which no adenylypyrophosphate was added showed no synthesis of phosphagen (see Table II). Here 15% of the pyrophosphate-P added was transferred to creatine, 31% was recovered unchanged, and 52% was not accounted for. Less than 2% appeared as free inorganic phosphate.

Table II

Sample	Pyro-P added	Phosphagen P found	Inorganic P found	Pyro-P left
I	0.636	0.095	0.104	0.198
II	0	0	0.095	0

(b) In an exactly similar experiment the incubation was continued for 30 min. at 40° and the results are shown in Table III. In this case 20 % of the pyrophosphate was transferred to creatine, 6 % appeared as inorganic phosphate, 25 % was unchanged and 49 % was not accounted for.

Table III

Sample	Pyro-P added	Phosphagen-P found	Inorganic P found	Pyro-P left
I	0.636	0.128	0.144	0.159
II	0	0	0.107	0

The effect of prolonging the period of incubation from 15 to 30 min. thus increased the yield of phosphagen to some extent. In neither case did the yield of inorganic phosphate-P account for any considerable proportion of the adenylypyrophosphate added, so that the adenylypyrophosphatase activity of the extract must have been very small indeed. The fact that almost exactly 50 % of the added pyro-P disappeared in both cases is a little peculiar; this may be due to concomitant phosphorylation of other substances, presumably carbohydrate.

Exp. 3. Transfer of P from phosphoglyceric acid to creatine by adenylic acid.

2 ml. of the enzyme preparation, 0.6 mg. magnesium, 6 mg. creatine, 1.2 mg. phosphoglyceric acid-P and an amount of adenylic acid equivalent to 0.16 mg. pyro-P were incubated together for 10 min. at 40° and pH 7.8 (sample I). Control samples were set up in which phosphoglyceric acid and creatine but no adenylic acid were present (II), and in which both these components were omitted (III), and on analysis the data of Table IV were obtained. Here

Table IV

Sample	Adenylic acid (as pyro-P) added	Phosphoglyceric acid-P added	Phosphagen-P found	Inorganic P found	Pyro-P found
I	0.16	1.2	0.214	0.120	0.082
II	0	1.2	0	0.097	0
III	0	0	0	0.080	0

0.214 mg. P was transferred from phosphoglyceric acid to creatine through the intermediation of adenylic acid (sample I), no transfer at all taking place in the absence of the carrier (sample II). At the same time about 50 % of the adenylic acid added was phosphorylated (sample I). Thus adenylic acid can be phosphorylated at the expense of phosphoglyceric acid, and having thus undergone phosphorylation can then hand on phosphate to creatine, adenylic acid being again formed and rephosphorylated.

Exp. 4. Formation of pyruvic acid.

In the case of muscle it is well known that adenylic acid is phosphorylated at the expense of phosphopyruvic acid, this compound being formed from phosphoglyceric acid: the products of this reaction are adenylypyrophosphate and pyruvic acid. The same must be true of the electric tissue of *Raia clavata* as the following experiment shows. A sample of 2 ml. of enzyme preparation was left for 2 hr. at room temperature (16°) with 0.6 mg. magnesium, 1.2 mg. phosphoglyceric acid-P, 0.13 mg. pyrophosphate-P and 6 mg. creatine, the pH being 7.8 as usual. At the end of this period the mixture was deproteinized and centrifuged.

5 ml. of the centrifugate were treated with 1 ml. of a 0.5% solution of 2:4:dinitrophenylhydrazine in 2*N* HCl, a yellow crystalline precipitate coming down on standing at 0°. Although the amount of precipitate was too small to allow of positive analytical identification of a recrystallized product, there can be little doubt that this was the 2:4:dinitrophenylhydrazone of pyruvic acid. The rest of the centrifugate was submitted to the nitroprusside test for pyruvic acid, the usual deep green coloration developing on standing.

Exp. 5. Components of the phosphorylating system.

This experiment provided general confirmation of the results of Exps. 1-3 above. Five samples, each of 2 ml. of the enzyme, were set up for 10 min. at 40° and pH 7.8 with 6 mg. creatine and the other additions indicated in Table V.

Table V

Sample	Phospho- glyceric acid-P added	Pyro-P added	mg. Mg added	Phosphagen- P found	Inorganic P found	Pyro-P found
I	1.2	0.127	0.6	0.182	0.170	0.038
II	1.2	0.127	0	0.145	0.102	0.0
III	0	0.127	0.6	0.011	0.086	0.039
IV	1.2	0	0.6	0	0.097	0.0
V	0	0	0.6	0	0.080	0.0

It was shown by Lohmann [1931] that the magnesium ion is a necessary activator for the production of lactic acid by the muscle enzymes. Lehmann [1935] later showed that it functions as a coenzyme in the phosphorylation processes, and its effect is shown here by the first two samples (I and II). In this case the yield of creatine phosphoric acid was a little smaller in the absence of added magnesium, but the effect of magnesium can only be satisfactorily demonstrated when the enzyme preparation has been very thoroughly freed from it, a process which evidently requires longer periods of dialysis than were used here.

The remaining figures of Table V show that (i) adenylypyrophosphate is capable of phosphorylating creatine (sample III), (ii) the phosphorylation of creatine goes further when phosphoglyceric acid is available to rephosphorylate adenylic acid (sample I), (iii) no phosphate is transferred from phosphoglyceric acid to creatine in the absence of the carrier (sample IV).

DISCUSSION

The foregoing results demonstrate quite clearly that enzyme-containing extracts of the electric organ of *R. clavata* are capable of catalysing the following reactions:

- (1) Phosphoglyceric acid → phosphopyruvic acid.
- (2) Phosphopyruvic acid + adenylic acid → adenylypyrophosphate + pyruvic acid.
- (3) Adenylypyrophosphate + creatine → adenylic acid + creatine phosphoric acid.
- (4) Adenylypyrophosphate → adenylic acid + phosphoric acid.

The enzyme concerned in reaction 4 is destroyed by acetone precipitation.

These reactions are also catalysed by extracts of a variety of muscular tissues and of the electric tissue of *Torpedo*. In the case of striated muscle at least it is now abundantly clear that these reactions are intimately bound up with the liberation of the mechanical or thermal energy produced by muscle

working anaerobically [see the review by Parnas, 1936]. It now seems highly probable that the energy of the discharge of electric organs as exemplified by those of *Torpedo* and *Raia* must be produced by chemical processes which, if not actually identical with those found in muscle, are at any rate very similar indeed.

Qualitatively then, the electric tissue of *R. clavata* closely resembles that of *Torpedo marmorata* and is very similar also to muscle. It is difficult however to make more than a very rough comparison on quantitative lines. Comparing them weight for weight of tissue we have already seen that *Torpedo* extracts can synthesize five or six times as much phosphagen as do similar extracts from *Raia* under the same conditions, and this is in fair agreement with the difference of effector activity estimated by Burdon-Sanderson & Gotch [1888]. But the proportions of physiologically active material are probably very different in the two organs, for in that of *Raia* the individual cellular elements or disks are separated by relatively dense septa of connective tissue, which must account for a considerable part of the whole bulk of the organ [cf. the description of the organ given by Garten, 1910]. The fact that this organ contains only about half as much acetone-precipitable material as does muscle points again to the presence in it of a relatively small proportion of metabolically active disk material. But the comparison cannot be pushed any further, the amount of acetone-precipitable material in the organs of *Torpedo* being at present unknown.

However, there seems to be no reason at the present time for believing that in *Raia* the disk material is much if at all metabolically inferior to that in *Torpedo*. The relative weakness of the electric organ of *Raia* is quite probably attributable to the presence in it of a relatively large proportion of connective tissue, partly because the amount of physiologically active material is correspondingly smaller in *Raia* than in *Torpedo*, and also because the interposition of comparatively thick septa of connective tissue between the individual disks in *Raia* must probably diminish the efficiency of the discharging system very considerably.

The same conclusion was reached by Ewart [1892] who says of *R. batis*, "when due allowance is made for the structural differences, there seems no escape from the conclusion that the organ of the Skate is not only physiologically as perfect as that of the *Torpedo*, but further that, taking the plate (i.e. disk) as the unit, the value per square millimetre in the Skate is equal to, if not greater than in the *Torpedo*."

It is interesting to notice in conclusion that the electric organ of the catfish, *Malapterurus electricus*, differs from those found in the rays, *Torpedo* and *Raia*, in its embryological origin [v. Garten, 1910]. It is capable of powerful discharges like those of *Torpedo*, but originates in pre-glandular and not in pre-muscular rudiments. It is therefore interesting to compare it with organs of the type found in elasmobranch fishes. Dr D. M. Needham and the author have carried out some preliminary experiments on the electric tissue of two specimens of *Malapterurus electricus* obtained through the kindness of the Director of the Aquarium of the Zoological Society of London. We used dialysed aqueous extracts of the tissue and also acetone powders prepared in the manner described earlier in this paper, but even under conditions closely resembling those used in the experiments on *Torpedo* and *Raia*, no synthesis of phosphagen, and no phosphorylation of adenylic acid could be demonstrated. A dialysed extract prepared from a fresh acetone powder was tested manometrically to see whether any lactic acid could be formed by it from added hexosediphosphate or glucose, but even in the presence of added coenzyme I, adenylypyrophosphate and magnesium, we could find no evidence of glycolysis.

Thus the non-muscular origin of the electric tissue in *Malapterurus electricus* may perhaps be associated with the absence of the glycolytic systems, which characterize muscle and electric

tissue of muscular origin, the same type of effector response being associated with different mechanisms for energy production. If the energy for the discharge in *M. electricus* originates in glycolytic processes at all, these may be of a non-phosphorylating type similar to those found in embryonic tissue [Needham & Lehmann, 1937]; the enzymes involved in this reaction sequence cannot be extracted with water. Further investigation of this problem awaits the arrival of more material.

SUMMARY

1. Experiments are described which show a close biochemical relationship between the electric organs of *Raia clavata*, muscular tissue in general, and the electric organs of *Torpedo marmorata*.

2. The relatively small physiological activity of the electric organ of *Raia* appears to be attributable to the presence in it of a large proportion of inert material in the form of connective tissue, and there is at present no reason to suppose that the material of the disk itself is any less efficient than that of *Torpedo*.

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