CHOLINESTERASE ACTIVITY OF ELECTRIC ORGAN OF *NARCINE BRASILIENSIS* (ÖLFERS)

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The characteristic structure of the electric organs of several fishes has called attention particularly by the lacking of the contractile element. *Narcine brasiliensis* (ÖLFERS) in common with many other fishes shows a very marked electric power. The biology of this tropical fish has been recently studied by AMBACHE & SAWAYA (1951) who completed the data published in 1940 by BREDER & SPRINGER and later by COX & BREDER (1943). The discharge varies between 14 and 30 volts and is generaly accompanied by muscular activity very similar to that observed in ***Torpedo occidentalis*** (COATES & COX, 1942 p. 26).

It is known that the electric organ derives from skeletal muscles from which only modified and hypertrophied end-plates have been accumulated. The muscular movements always follow the electric discharge and both are related to the amount of acetylcholine in the organ. FELDBERG & FESSARD (1942) showed that in *Torpedo* the electric organ is very rich in acetylcholine and the same has been found by AMBACHE & SAWAYA in *N. brasiliensis*. Fishes of 180 to 350 grs. have electric organs of 12 to 20 grs. each which yeld by extraction from 143 to 187, 4 gama of acetylcholine per gram of organ, which means more than 3 mgr of acetylcholine for each electric organ. Probably this enormous quantity of acetylcholine is connected with the muscular activity after each discharge. Those movements do not occur in *Electrophorus electricus*.

NACHMANNSOHN & MEYERHOF 1941 have shown that the electric organs of *Torpedo occidentalis* as *T. marmorata* contain a high amount of cholinesterase.

FELDBERG very recently (1951) called attention to the high concentration of cholinesterase in *Torpedo*, according to the data reported by MARNAY (1937) and NACHMANSOHN & LEDERER (1938), and in his opinion acetylcholine has an electrogenic effect.

Up to now no data have been reported on the concentration of cholinesterase in the electric organs of *N. brasiliensis*. This paper deals with some experiments we have performed to determine the cholinesterase content of the electric organs of that fish.

We are indebt to Mr. CALIMERIO CARVALHO, Director of the "Aquario Municipal de Santos" for many facilities given us throughout the present work, and to the Roche Products Co. for supplying the acetylcholine chlorhydrate.

Material and methods

The animals were obtained in Santos, at the Aquario Municipal, where after capture, they stayed in large and well aerated aquaria. The electric organs were dissected either in Santos or in S. Paulo. In the first case, after dissection, the organs were immediately shipped to S. Paulo and kept in the laboratory ice-box. In the second case, the animals were brought alive to S. Paulo in seawater or simply wrapped in clothes moistened with sea water. In both cases, however, care was taken in order to avoid as much as possible a too long exposure of the dissected organs to ordinary air temperature. The time elapsed between dissection in Santos and arrival in S. Paulo never exceed 3 hours, so that no sensible differences could be detected in the cholinesterase activity between the organs dissected in Santos and in S. Paulo. Electric organs kept in the ice-box can maintain their cholinesterase activity practically indefinitely.

Electric organ (E. O.) extracts were prepared as follows: pieces of tissue were cut off, thoroughly dried in clean filter-paper and then carefully weighed up to 50 mg. This amount of tissue was next transferred to a mortar containing a small quantity of ground glass (from a sterilized microscopic slide) and 1 c. c. Pantin's sea water. After grinding more Pantin's sea water was added to complete exactly 10 c. c. From this stock solution, solutions were finally prepared which, according to the case, contained the equivalent to 1.00, 0.75, 0.50 or 0.25 mg. E. O. for each 2 c. c.

The estimation of the cholinesterase activity of the extracts was carried on using the WARBURG apparatus (MARNAY l. c.; NACHMANNSOHN & LEDERER l. c.; PAGE *et alt.* 1950). In each experiment, 4 vessels *plus* a thermobarometer were used, as follows:

Vessel	*Main chamber*	*Side-arm*
Tb	2 c.c. medium[1])	0.5 c.c. medium
A	2 c.c. medium	0.5 c.c. ACh sol.
B	2 c.c. extract	0.5 c.c. medium
C	2 c.c. extract	0.5 c.c. ACh sol.
D	2 c.c. extract	0.5 c.c. ACh sol.

After attached to their respective manometers and placed in the bath (25° ± 0.05°C) the vessels were perfused with N_2/CO_2 mixtures containing 5% CO_2, shaked at 120 complete oscillations per minute, during 10 minutes. Then, the side arm stoppers and the manometers stopcocks were closed and the five manometers shaked again during 5 minutes to complete thermobarometric regulation. Finally, the stopcocks were reopened for readjustment of the level in the two braches of each manometer and closed again for the zero readings. Readings in all five manometers were then taken at five minute intervals during 15 minutes. Directly afterwards the side-arm

1) Pantin's sea water plus ground glass.

content of each vessel was dumped into the main chamber and the evolution of CO_2 followed with 15 minute interval readings.

The principle of the technique being the hydrolysis of ACh. and the subsequent reaction between one the hydrolysis products (acetic acid) and the bicarbonate of the Pantin's sea water (which causes the CO_2 evolution), the purpose of using vessel A was of course, the control of nonemzymic hydrolysis and that of vessel B the checking of occasional of CO_2 evolution by the extract alone.

Results

In a first series of experiments, 1.00 mg ACh was used against 1.00, 0.75, 0.50 and 0.25 mg E. O. The results are exposed in table I and in the fig. 1.

TABLE I

Cholinesterase activity of the electric organ of *Narcine brasiliensis* when 1 mg ACh was added to extracts equivalent to 1.00, 0.75, 0.50 and 0.25 mg E. O.

E. O. equivalent (mg)	cu.mm. CO_2 evolved after				ACh. hydrolysed in 60 min.
	15′	30′	45′	60′	
1.00	77.3	104.2	112.3	112.3	820
	45.8	80.4	111.1	115.1	840
	90.3	106.6	107.9	107.9	789
Mean	70.1	97.0	110.4	111.7	815
0.75	40.2	72.5	90.7	95.8	670
	24.3	41.5	63.8	84.3	615
	37.8	75.5	79.6	90.4	660
Mean	34.1	63.1	78.0	90.0	657
0.50	22.7	50.8	72.2	85.5	624
	20.1	40.2	55.8	70.1	512
	10.7	21.4	33.4	41.4	303
Mean	14.4	37.4	53.8	65.7	480
0.25	12.2	21.5	31.1	40.6	296
	4.1	6.8	12.4	16.5	121
	11.0	20.6	27.5	34.4	251
Mean	9.1	16.3	23.7	30.5	223

They show that, within one hour, a) 1.00 mg E. O. split about 4/5 of the substrate ; b) 0.75 mg a little less ; c) 0.50 mg about the half and finally d) 0.25 mg hydrolysed *circa* 1/4 of the substrate. The shapes of the curves in the case of 1.00 and 0.75 mg E. O., however, and the fact that proportionally 0.50 and 0.25 mg E. O. split more ACh per hour led us to the suspicion that in the first two cases some inhibitory action might have occurred as a consequence of an unappropriate excess of substrate. We therefore performed another series of experiments using a real excess of substrate.

In this second series of experiments, 2.00 mg ACh were exposed to 1.00, 0.75, 0.50 and 0.25 mg E. O. (table II and fig. 2). The results show that in presence of 2.0 mg ACh, 1.00 mg E. O. split definitely more ACh during the first 60 minutes, but this was not the case for 0.75, 0.50 and 0.25 mg E. O.. The shape of the curves in the second hour of the experiments show, in the case of 1.00 and 0.75 mg E. O. a slope similar to that observed when 1.00 mg ACh was used as substrate.

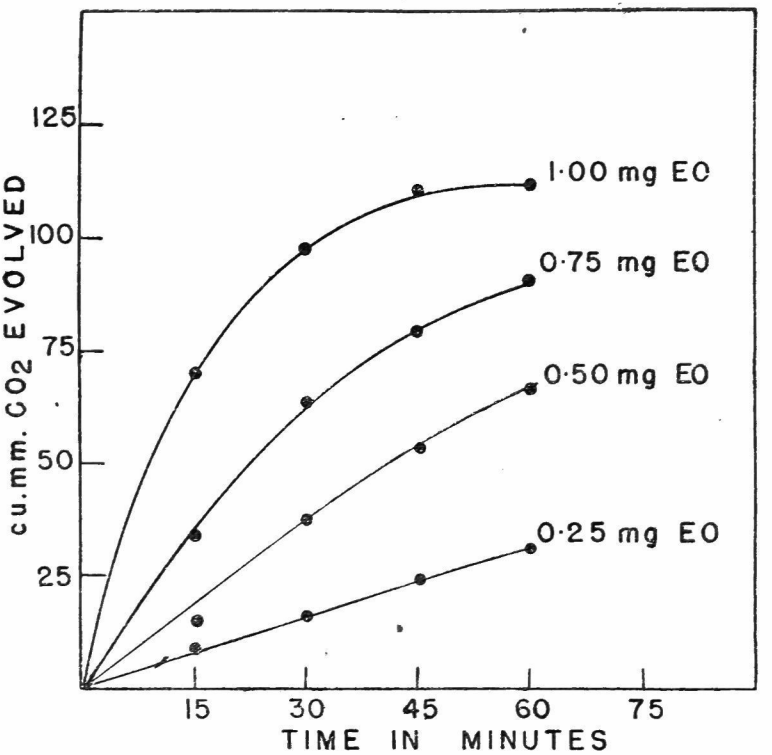

Fig. 1 : Co_2 evolution in the case of 1 mg ACh against 1.00, 0.75, 0.50 and 0.25 mg E. O.

The results here reported merely intend to furnish supplementary data to the work of AMBACHE AND SAWAYA (l. c.) on the biology of *Narcine brasiliensis*. However, they are more complete than those reported by MARNAY (l. c.) and NACHMANNSOHN & LEDERER (l. c.) for *Torpedo* in that different concentrations of enzyme and substrate were used. The data reported in tables I and II would permit a deeper analysis of the kinetics of the reaction involved, but this is out of the limits of the present work. The fact that the cholinesterase concentrations of the nervous endings in frog's striated muscle are enormous (0.795-0.404 mg ACh hydrolised by 100 mg organ in 60 minutes ; MARNAY and NACHMANNSOHN 1937, p. 40) and that the electric organs of many fishes have a great analogy with the motor end plates, led MARNAY (l. c.) to perform cholinesterase activity

determinations of extracts of electric organs (*Torpedo*). She showed that the cholinesterase activity of *Torpedo's* E. O. is about 1.000 times greater than that of the aneural part of the frog's sartorius, that is, 100 mg E. O. is able to split in 60 minutes about 150 mg ACh. These results were confirmed by NACHMANNSOHN & LEDERER. *Narcine's* electric organ exhibited a less potent cholinesterase activity (1 mg organ in 60 minutes split, against 1 mg ACh, only 0.815 mg and, against 2 mg ACh, only 1.110 mg ACh). We think, however, that MARNAY's results are probably due to the fact

TABLE II

Cholinesterase activity of the electric organ of *Narcine brasiliensis* when 2 mg ACh were added to extracts equivalent to 1.00, 0.75, 0.50 and 0.25 mg E. O.

E.O. equivalent (mg)	cu.mm. CO_2 evolved after								mg ACh hydrolysed	
	15′	30′	45′	60′	75′	90′	105′	120′	in 60′	in 120′
1.00	44.2	88.4	122.2	149.2	177.5	192.0	204.0	210.0	1.090	1.535
	49.3	97.3	137.5	160.1	192.2	195.8	197.0	198.5	1.170	1.450
	62.2	114.2	149.5	193.5	209.0	221.0	224.0	224.0	1.415	1.640
	40.5	68.8	97.2	121.5	147.2	167.4	181.8	187.0	0.912	1.365
	32.9	76.9	122.1	152.2	178.7	194.1	203.9	213.0	1.110	1.480
Mean	47.1	89.1	122.2	152.2	178.7	194.1	203.9	208.5	1.110	1.585
0.75	17.5	33.8	49.8	66.1	80.8	94.3	106.5	117.5	0.482	0.856
	27.1	55.5	73.2	96.2	116.5	137.0	153.2	165.5	0.702	1.210
	23.0	44.5	63.4	80.9	99.8	112.2	128.8	140.5	0.592	1.060
	24.6	48.0	70.0	90.8	109.0	124.5	136.3	150.0	0.665	1.095
	18.7	38.8	54.8	69.4	84.2	95.0	105.8	111.2	0.506	0.818
Mean	22.2	44.1	62.2	80.7	98.0	112.6	126.0	136.9	0.590	1.000
0.50	16.3	27.1	39.3	51.5	63.7	74.5	85.3	96.2	0.366	0.702
	9.5	18.9	31.2	40.6	48.7	56.8	65.0	71.7	0.296	0.524
	9.5	23.1	33.9	43.3	52.8	59.5	69.0	77.2	0.316	0.526
	16.1	32.4	44.2	54.8	68.4	79.0	89.7	107.3	0.400	0.783
	8.1	19.2	24.7	33.0	38.5	41.7	50.8	53.6	0.241	0.392
Mean	11.9	24.1	34.6	44.6	54.0	62.3	71.9	81.2	0.326	0.592
0.25	5.3	12.0	17.4	24.1	29.4	30.8	36.1	37.4	0.176	0.273
	2.7	6.9	9.6	13.7	16.5	19.2	20.6	24.8	0.100	0.181
	5.5	8.2	12.4	16.5	20.6	23.4	27.5	31.6	0.121	0.231
	5.5	9.6	15.1	20.6	24.7	28.8	33.0	37.1	0.151	0.271
	5.5	10.9	16.4	20.5	26.0	27.3	32.8	34.1	0.150	0.249
Mean	4.9	9.5	14.4	19.1	23.4	25.9	30.0	33.0	0.140	0.241

that she did not use E. O. extracts, but real suspensions of ground organs. It is possible then that, although she has probably used a control for CO_2 evolution by the tissue alone (this is by no means stated in her paper), some of the CO_2 evolved in MARNAY's experiments was due to fragments of tissue, which might have increased the cholinesterase activity of the

preparations. Anyway, even admitting that in the case of *Narcine* the technique employed to make the E. O. preparations did not sufficiently extract the cholinesterase from the tissue, the results clearly indicate that in

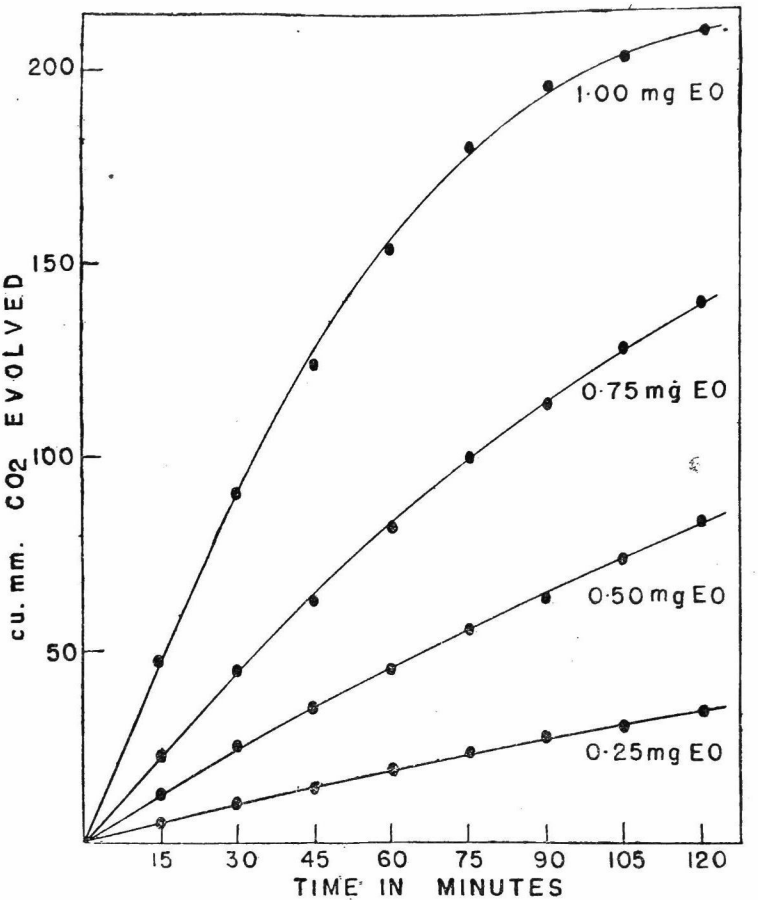

Fig. 2 Co_2 evolution in the case of 2 mg ACh against 1.00, 0.75, 050 and 0.25 mg E. O.

this ray, as in *Torpedo*, the E. O. is endowed with a very rich cholinesterase content, as compared with the frog's sartorius or muscles of rapidly moving animals, such as the lezard (MARNAY, l. c., pg. 573).

Summary

Using the manometric procedure, the cholinesterase activity of *Narcine brasiliensis* electric organ was determined, at 25°C, both extracts and ACh diluted in PANTIN's sea water. Against 1 mg. ACh, E. O. extracts equivalent to 1.00, 0.75, 0.50 and 0.25 mg organ split in 60 minutes respectively 0.815, 0.657, 0.480 and 0.223 mg ACh (mean values). Against 2 mg ACh,

the same series of extracts split in 60 minutes 1.110, 0.590, 0.326 and 0.140 ; in 120 minutes 1.585, 1.000, 0.592 and 0.241. The results indicate that in *Narcine*'s electric organ, the cholinesterase content, although high is inferior to that reported for *Torpedo*, where MARNAY found that 100 mg E. O. can split in 60 minutes about 150 mg ACh.

Sumário

Usando a técnica manométrica, determinou-se a atividade colinesterásica do órgão elétrico do Treme-treme (*Narcine brasiliensis*), a 25°C. Extratos de órgão foram obtidos em água do mar artificial segundo PANTIN e também nesse meio foi dissolvida a acetilcolina. Extratos equivalentes a 1.00, 0.75, 0.50 e 0.25 mg de órgão elétrico, quando em presença de 1 mg de ACh, hidrolisaram em 1 hora, respectivamente 0.815, 0.657, 0.480 e 0.223 mg do ester (valores médios) (tab. I, fig. 1). Os mesmos extratos, quando em presença de 2 mg de ACh, hidrolizaram em 1 hora, cerca de 1.110, 0.590, 0.326 e 0.140 mg de substância e, em 2 horas, 1.585, 1.000, 0.592 e 0.241. (tab II, fig. 2) Os resultados indicam, pois, que no órgão elétrico de *Narcine* o teôr de colinesterase, embora bem alto, é inferior ao da espécie européia proxima, *Torpedo*, onde MARNAY determinou que 100 mg de órgão elétrico podem hidrolizar em 1 hora cerca de 150 mg de ACh.

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