XXIV. ECHINOCHROME.

By ROBERT KEITH CANNAN.

From the Marine Biological Laboratories, Woods Hole and Plymouth, and the Department of Physiology and Biochemistry, University College, London.

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ECHINOCHROME is the name which was given by MacMunn [1885, 1889] to a pigment contained in the cells of the perivisceral fluid of *Strongylocentrotus lividus*, *Amphidotus cordatus*, *E. sphaera* and *E. esculentus*. On the grounds of an observed change in the spectrum under the action of strong reducing agents, MacMunn concluded that the pigment was an oxygen carrier. Griffiths [1892] confirmed this opinion but stated that the oxygen was much more firmly held than in oxyhaemoglobin, being removable only by the reducing action of the living cells. Decomposition of the pigment with boiling mineral acids was reported to yield a haemochromogen and a porphyrin. From the elaeocytes of the perivisceral fluid of the deeply coloured sea urchin, *Arbacia punctulata*, McLendon [1912] separated a pigment in a partially purified state, which he found to be spectroscopically identical with the pigment of MacMunn and the name echinochrome was, therefore, extended to it. McLendon found that the red colour of the eggs and of the test of *Arbacia* was due also to the same substance. His preparations contained no significant quantity of iron and he demonstrated that solutions of the pigment which had been submitted to a vacuum did not subsequently absorb any appreciable quantity of oxygen from the air. He consequently questioned the respiratory function of echinochrome.

During a visit to Woods Hole, the writer made the casual observation that the red colour of the aqueous extract of the elaeocytes of *Arbacia* was immediately discharged by sodium hydrosulphite and returned rapidly on shaking with air. In the absence of oxygen, the reduced substance was immediately re-oxidised by mild oxidising agents. The experiment was repeated titrimetrically and the electrode potentials observed. Substantial agreement between the titration curve traced during the reduction of the pigment with standard sodium hydrosulphite and during its re-oxidation with potassium ferricyanide, together with the rapid attainment and constancy of the electrode potentials, indicated that a reversible electrochemical system was under study. A small amount of the crude pigment was prepared and brought back to this country.

EXPERIMENTAL.

Following, in general, the method of McLendon, the echinochrome was extracted from the test of *Arbacia* with acetone. The extract was evaporated to dryness in a vacuum and the residue extracted with alcohol. Addition of ammonia to the alcoholic solution precipitated the pigment, which was then dissolved in faintly acidified water and the solution extracted with ether. This preparation was far from pure and, as its mass was small, it was, after further fractionation on similar principles, made up to a standard volume in water and this stock solution, preserved on ice, was used for the whole of the work to be reported.

The electrode studies took the usual form of determining the oxidation-reduction titration curve at a series of known $[H^+]$; the detail has been described by Cannan [1926]. Gold plated platinum electrodes were employed, the standard half cell being a saturated calomel electrode. 10 cc. of the echinochrome solution were added to 50 cc. of a buffer [Clark, 1923] of known p_H and titrated in pure nitrogen with a standard deaerated solution of the oxidising agent similarly diluted with the buffer. The p_H of each of these mixtures was assumed to be identical with that of the buffer solution diluted to the same extent with water. The latter was determined with the hydrogen electrode. The curves traced by using benzoquinone or ferricyanide as oxidising agents were identical within the experimental error. Titration of the oxidised pigment with sodium hydrosulphite was vitiated by slow persistent drifts of potential rendering accurate observations impossible. Within the accuracy of observation, however, agreement with the oxidation titration curves was obtained.

The affinity of the reduced pigment for oxygen was so great that much care was necessary to preserve solutions of it from re-oxidation by air. For this reason it was found more certain to repeat the whole titration curve at a series of $[H^+]$ than to make a series of observations of the potentials of a standard mixture of oxidised and reduced forms added to various buffer mixtures.

Analysis of the experimental curves showed that they were adequately expressed by the electrode equation

$$E_h = E_o' - 0.03006 \log \frac{[S_r]}{[S_o]}$$

where E_h is the observed potential compared with that of the normal hydrogen electrode, and $[S_r]$ and $[S_o]$ are the respective concentrations of the oxidised and reduced forms computed from the titre and the end-point determined graphically. E_o' is a constant for a given $[H^+]$. The value of the numerical factor indicates that the reversible electrode reaction is one involving two equivalents of hydrogen, thus placing echinochrome in the same group as the majority of organic oxidation-reduction systems.

Table I records the abbreviated data of a typical titration curve and indicates that the observations were within the accuracy of the experimental

technique. From the titration curves at other $[H']$ the values of E_o' have been deduced and are given in Table II. These serve to define the system over the p_H range 2 to 7.7 with some certainty.

Table I. *Titration of reduced echinochrome with M/500 potassium ferricyanide.*

$p_H=3.97$ (10 cc. echinochrome solution added to 50 cc. M/20 acid potassium phthalate). Temp. 30°. $E_o' = -0.0389$.

Ferricyanide cc.	Oxidation %	E_h (volts)		Deviation
		Found	Calc.	
0.40	2.90	-0.0852	-0.0847	-0.0005
1.00	7.25	0.0722	0.0711	-0.0011
1.60	11.60	0.0659	0.0654	-0.0005
2.20	15.94	0.0604	0.0606	+0.0002
2.80	20.29	0.0567	0.0568	+0.0001
3.40	24.63	0.0534	0.0535	+0.0001
4.60	33.34	0.0481	0.0479	-0.0002
5.80	42.03	0.0429	0.0431	+0.0002
7.00	50.72	0.0387	0.0385	-0.0002
8.20	59.41	0.0339	0.0339	—
9.40	68.11	0.0288	0.0290	+0.0002
10.60	76.81	0.0231	0.0233	+0.0002
11.80	85.51	0.0155	0.0158	+0.0003
12.40	89.84	0.0111	0.0105	-0.0006
13.00	94.19	-0.0034	-0.0026	-0.0008
13.40	97.09	+0.0053	+0.0069	-0.0016
13.80 end point				

The data may be adequately expressed by the equation

$$E_o' = E_o + 0.0601 \log [H'],$$

i.e. the value of E_o' decreases by 0.0601 v. per unit increase in p_H . The deviations from the "0.06 slope" at p_H 8.78 and 9.76 may indicate critical acid-base dissociations with ionisation constants in this region. On the other hand, they may be due to the employment of borate buffers, since experience has shown that the presence of borate sometimes leads to anomalous potentials. Neglecting, therefore, these two results, the mean value of E_o is + 0.1995.

Table II. *Relation of E_o' to p_H .*

p_H	E_o'	$0.0601 p_H$	E_o
2.21	+0.0668	-0.1328	+0.1996
3.02	+0.0176	0.1815	0.1991
3.97	-0.0389	0.2386	0.1997
4.97	-0.0992	0.2987	0.1995
6.01	-0.1614	0.3612	0.1998
6.75	-0.2056	0.4057	0.2001
7.16	-0.2308	0.4303	0.1995
7.69	-0.2632	0.4622	0.1990
8.78	-0.3220	0.5277	(0.2057)
9.76	-0.3774	0.5866	(0.2092)

Mean +0.1995

E_o from titration of fresh filtered aqueous extract of elaeocytes (Woods Hole):
6.74 -0.201 -0.405 +0.204

From a crude experiment at Woods Hole on a fresh aqueous extract of the cells of the perivisceral fluid a value of + 0.204 for E_o was obtained and

another experiment on an extract of the eggs was in close agreement. These results suggest that the pigment had been extracted in an unmodified condition and permit the conclusion that the system which has been defined above actually exists within the cells of *Arbacia*.

Attempts to demonstrate the formation of a compound of reduced echinochrome with molecular oxygen were negative. Moreover, the oxidant produced from reduced echinochrome by oxygen was identical with that formed by mild oxidising agents in the absence of oxygen. This follows from the fact that the experimental titration curves were duplicated by addition to reduced echinochrome of increasing amounts of the pigment formed by atmospheric oxygen.

An attempt was made to confirm these results on the pigment contained in *E. esculentus*. Very little pigment is, however, present and the amount extracted was insufficient for extended study. It was found, that the pigment did represent the oxidant of a reversible oxidation-reduction system but preliminary determinations of the electrode potentials suggested that these were distinctly positive to those of *Arbacia* echinochrome. Moreover, the solubilities of the two pigments differed. These qualitative observations are of interest because it would appear that *E. esculentus* holds its pigment in the partially reduced state, since the perivisceral fluid is almost colourless but rapidly turns red when removed from the animal. In *Arbacia* the echinochrome is in the oxidised state and I know of no observation of the spontaneous decolorisation of the cells *in vivo*. It would be of interest to know if the cells of *Arbacia* can effect a significant reduction of their pigment anaerobically—bacteria and yeast will slowly reduce it in the absence of oxygen—but the author has no longer access to the biological material.

The rate of oxidation of reduced echinochrome by atmospheric oxygen was too great to be measured by any simple means.

DISCUSSION.

If any respiratory rôle is to be attributed to echinochrome it is clear that it must be that of an "activator" rather than of a "carrier" of oxygen. It forms no dissociable compound with molecular oxygen. It is of interest to consider this possibility briefly in the light of the quantitative data presented since it represents a concrete case of a type of respiratory catalyst frequently postulated. At the same time it must be acknowledged that the function suggested for echinochrome is at present without physiological proof.

It is probable that within living cells there exists a dynamic equilibrium—an oxidation-reduction poise—between the inherent reducing activity of the cells and the supply of active oxygen [Cannan, Cohen and Clark, 1926]. A characteristic reduction potential may be attributed to the cell, and the state of oxidation of the echinochrome present will be conditioned by this. If the assumption be made that the pigment is the effective oxygen activator then the rate of oxygen consumption of the cell will be determined by the con-

centration of reduced pigment in equilibrium—since its oxidation by molecular oxygen is very rapid. It is improbable that the cells can, aerobically, achieve a reduction intensity (corresponding to r_H 7) sufficient to reduce a measurable fraction of the total pigment present but it is clear from the electrode equation that at reduction intensities much less than this there remains a concentration of reduced pigment which, whilst analytically insignificant, may be a sufficient molecular concentration to insure a measurable oxygen consumption.

One further implication is of interest. It is well known that fertilisation of the egg of the sea urchin is followed by an enormous increase in the oxygen consumption. J. and D. Needham [1926] failed to observe a coincident increase in the reduction potential of the egg by their method of the micro-injection of indicators, although such an increase was to be expected. Now a fall in r_H of only one unit (an increase in reduction potential of 60 millivolts) would lead to a tenfold increase in the concentration of reduced echinochrome present. The method of injection of indicators was, in the present case, incapable of observing any change in r_H less than two or three units so that it was possible for a change to have occurred corresponding to an increase of several hundredfold in the concentration of reduced pigment, and, therefore, of oxygen consumption. It will be seen that in such a hypothetical system there is wide metabolic latitude although the system is well poised. Its investigation, however, requires exact information of the rate of oxidation of reduced echinochrome and of the reduction potential of the cell under the experimental conditions.

It has frequently been recorded that in the eggs of *Arbacia* fertilisation is followed by a migration of the chromatophores to the periphery of the cell. This might be considered to be an accessory, or alternative, means for increasing the concentration of reduced pigment in effective contact with oxygen. On the other hand, though this is improbable, the supposed migration may mask an actual reduction of the echinochrome in the interior of the cell owing to an enhanced reduction intensity following fertilisation.

One final qualification must be made. We have no present means of deciding to what extent the homogeneous equilibria represented by electrode potentials are modified in a heterogeneous system such as the living cell. This is particularly cogent in the present case where the pigment exists in granules microscopically distinguishable as a separate phase. The physiological application of electrometric data must be made with caution.

SUMMARY.

1. Echinochrome has been separated from the eggs, perivisceral fluid and test of *Arbacia punctulata*. It does not form a dissociable compound with oxygen but is the oxidant of a reversible oxidation-reduction system.
2. The electrode potentials of the system have been measured over the p_H range 2.2–9.76. The normal electrode potential is + 0.1995.
3. The alleged respiratory function of echinochrome is discussed.

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