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1. The idea that the phenomenon of narcosis might be caused by an interference with oxidation is an old one. Recently it has been advocated by Verworn who tried to support it by indirect evidence.[1] Yet it is obvious that if we wish to ascertain whether or not narcosis is due to a diminution in the rate of oxidations (or, as Verworn expresses it, to an asphyxiation) there is only one way to decide the question, namely by comparing the rate of oxidations in narcotized organisms with that in non-narcotized.

If we wish to carry out such experiments we must keep in mind that organisms which are capable of muscular action cannot well be used for this purpose since we know that muscular activity can easily raise the rate of oxidation in the animal body one hundred per cent. Since the narcotics cause muscular action to cease, a narcotized animal will consume less oxygen than a free-moving animal; but it would be a mistake to state that this diminution in the consumption of oxygen was the cause of narcosis. It is the effect and not the cause of narcosis. If we wish to test the asphyxiation hypothesis of narcosis we must use organisms which possess no muscles and in which therefore this source of error does not interfere with the result. The best material for this purpose is offered in the fertilized eggs. Loeb had shown in 1895 that the eggs of the sea urchin and fish cannot develop if the oxygen is withdrawn[2] and this result has since been extended to a number of eggs of different forms. The same author showed that the addition of a small amount of KCN also inhibits segmentation. We shall see later that KCN only has this effect if it depresses the rate of oxidations in the egg below a certain level. The suppression of

[1] Verworn: *The Harvey Lectures*, 1911–12, p. 52.

[2] Loeb: *Pflüger's Archiv*, lxii, p. 249, 1895.

segmentation by lack of oxygen as well as by KCN is a reversible process.

It is also well known that anaesthetics inhibit the segmentation of the fertilized sea urchin egg reversibly. It was, therefore, an easy task to compare the rate of oxidations in equal quantities of fertilized eggs with and without the presence of narcotics. Warburg had already observed that phenyl urethane in the concentration in which it suppressed nuclear and cell division did not diminish the rate of oxidations. Only if an excessive amount of phenyl urethane was added, was a diminution in the rate of oxidations noticeable; but, as he correctly adds, the question is not whether it is at all possible to lower the rate of oxidations by adding narcotics but whether that concentration which suffices for narcosis lowers the rate of oxidations.[3]

We undertook a series of experiments on the effect of various narcotics upon the rate of oxidations in the newly fertilized eggs of *Strongylocentrotus purpuratus*. Since the method of procedure and the degree of reliability of our method have been described in previous papers no recapitulation of these points is needed.

2. *Experiments with KCN.* We determined the minimum amount of KCN necessary to suppress cell division permanently in the newly fertilized eggs of *S. purpuratus* and found that the addition of 0.7 cc. of 0.01 per cent KCN to 50 cc. of sea water was the minimum amount required. We then determined the influence of various amounts of KCN upon the rate of oxidations. Newly fertilized eggs were divided into equal lots and the amount of oxygen consumed in one hour was determined. Temperature 14°C.

TABLE I.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
1. Normal sea water	0.51	1.00
2. 50 cc. sea water + 0.7 cc. 0.01 per cent KCN	0.17	0.33
3. 50 cc. sea water + 0.9 cc. 0.01 per cent KCN	0.15	0.29

In solution 1, the eggs segmented; in solutions 2 and 3 no segmentation occurred. The eggs were transferred from solutions 2 and

[3] O. Warburg: *Zeitschr. f. physiol. Chem.*, lxvi, p. 305, 1910.

3 to normal sea water and all developed into normal larvae. The amount of KCN necessary to suppress cell division lowers the rate of oxidations to one-third of the normal amount. A repetition of the experiment confirmed the result.

TABLE II.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
1. Normal sea water	0.65	1.00
2. 50 cc. sea water + 0.4 cc. 0.01 per cent KCN	0.34	0.52
3. 50 cc. sea water + 0.7 cc. 0.01 per cent KCN	0.21	0.32
4. 50 cc. sea water + 1.0 cc. 0.01 per cent KCN	0.18	0.28

The addition of 0.4 cc. of KCN only retarded the development but did not suppress it. The addition of 0.7 cc. of KCN suppressed cell division, and the rate of oxidations was again exactly one-third of the normal.

Similar results had previously been obtained by us for the eggs of *Arbacia*.[4]

We can, therefore, state that if the rate of oxidations of the egg is lowered to one-third of the normal amount found at 14°C., the eggs cease to segment. If the prevention of segmentation by narcotics were due to the same influence we should have to expect also a lowering of the rate of oxidations to one-third of the normal rate found at 14°C.

3. We tried a number of narcotics, various alcohols, chloral hydrate, chloroform and ethyl urethane. The minimum amount required to suppress cell division permanently was ascertained for each of these narcotics. The minimal dose varies slightly for the eggs of various individuals.

a. Chloral hydrate. The minimum amount required to suppress cell division is 4.2 cc. of 0.5 per cent solution (made up in $\frac{M}{2}$ NaCl + KCl + $CaCl_2$) to 45.8 cc. of sea water. Temperature 15°C.

[4] Loeb and Wasteneys: *Biochem. Zeitschr.*, xxxvi, p. 355, 1911.

TABLE III.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
Normal sea water	0.65	1.00
2.4 cc. 0.5 per cent chloral hydrate in 50 cc. sea water	0.60	0.92
4.2 cc. 0.5 per cent chloral hydrate in 50 cc. sea water	0.57	0.88
6.0 cc. 0.5 per cent chloral hydrate in 50 cc. sea water	0.57	0.88

In the solution with 4.2 cc. and 6.0 cc. of chloral hydrate the eggs were no longer able to segment; but they segmented and developed promptly when transferred to normal sea water. In the solution with 2.4 cc. of chloral hydrate the eggs segmented. The effect of the chloral hydrate upon oxidations was practically nil. The experiment was repeated.

TABLE IV.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
Normal sea water	0.51	1.00
4.2 cc. 0.5 per cent chloral hydrate in 50 cc. sea water	0.42	0.89

Although the segmentations were completely suppressed in this solution the influence of the chloral hydrate was practically negligible. We can say with certainty: The narcotic effect of chloral hydrate upon the egg is not due to asphyxiation or a diminution in the rate of oxidations.

b. Ethyl urethane. Three cubic centimeters of a 10 per cent solution (in $\frac{M}{2}$ $NaCl + KCl + CaCl_2$) in 50 cc. of sea water are sufficient to suppress cell division.

TABLE V.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
Normal sea water	0.51	1.00
3 cc. 10 per cent ethyl urethane in 50 cc. sea water	0.46	0.98

With a few exceptions no eggs segmented in ethyl urethane, but all segmented when put back into normal sea water.

c. Chloroform. Seven cubic centimeters of 0.5 per cent $CHCl_3$ in $\frac{M}{2}$ NaCl + KCl + $CaCl_2$ were required to suppress cell division.

TABLE VI.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
Normal sea water	0.47	1.00
43 cc. sea water + 7 cc. 0.5 per cent chloroform.	0.41	0.87

No segmentation occurred in the eggs in chloroform, but they segmented and developed normally when transferred to normal sea water. The narcotic effect of chloroform is produced without any considerable lowering of the rate of oxidations. The slight lowering observed is not quite but almost within the limits of error and cannot be considered.

d. Propyl alcohol. Minimum amount necessary to suppress cell division is 3.5 cc. of 2 M propyl alcohol in 50 cc. of sea water. In order to avoid lowering of concentration of salts the 2 M solution of alcohol was made up in $\frac{M}{2}$ NaCl + KCl + $CaCl_2$.

TABLE VII.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
In normal sea water	1.00	1.00
2.0 cc. 2 M propyl alcohol in 50 cc. sea water	0.90	0.90
3.5 cc. 2 M propyl alcohol in 50 cc. sea water	0.92	0.92
5.0 cc. 2 M propyl alcohol in 50 cc. sea water	0.93	0.93

In 2 cc. of propyl alcohol a few of the eggs (less than 1 per cent) segmented. In 3.5 cc. and 5 cc. of propyl alcohol no egg segmented. A few underwent cytolysis, but as Warburg has shown, and as we were able to confirm, this does not alter the rate of oxidations in the fertilized egg. We therefore see that the narcotic effect of propyl alcohol is not accompanied by any lowering of the rate of oxidations in the egg.

e. Various other alcohols. The narcotic effect of various alcohols follows the rule that each successive alcohol of a series is two or

three times as efficient for narcosis as the previous one. The amount necessary for the suppression of segmentation in the egg of *S. purpuratus* found for the various alcohols was as follows:

	RATE OF EFFICIENCY COMPARED WITH METHYL ALCOHOL.
Methyl alcohol, 4.0 cc. 10 M in 50 cc. sea water	1
Ethyl alcohol, 5.0 cc. 4 M in 50 cc. sea water	2
Propyl alcohol, 3.5 cc. 2 M in 50 cc. sea water	6
Butyl alcohol, 3.5 cc. M in 50 cc. sea water	12

TABLE VIII.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
Normal sea water	0.87	1.00
4 cc. 10 M methyl alcohol in 50 cc. sea water	0.83	0.95
5 cc. 4 M ethyl alcohol in 50 cc. sea water	0.73	0.84
7 cc. $\frac{M}{2}$ butyl alcohol in 50 cc. sea water	0.66	0.76

No eggs segmented in the solutions containing alcohol except in the methyl alcohol in which 25 per cent of the eggs segmented. When transferred to normal sea water all the eggs segmented. The rate of oxidations in these experiments is too high to account for the narcotic effect on the basis of asphyxiation. The experiment with butyl alcohol was repeated.

TABLE IX.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	mgm.	
Normal sea water	0.84	1.00
6.5 cc. $\frac{M}{2}$ butyl alcohol in 50 cc. sea water	0.66	0.79

4. *Theoretical remarks.* The maximal lowering of the rate of oxidations found under the influence of narcotics was 20 per cent; in the majority of cases it was less than this. In the case of propyl alcohol it was less than 10 per cent; in the case of chloral hydrate it was about 10 per cent. Since the temperature coefficient for the rate of oxidations in the eggs is about 2 for 10°C.,[5] we can pro-

[5] Loeb and Wasteneys: *Biochem. Zeitschr.*, xxxvi, p. 345, 1911.

duce a lowering of the rate of oxidations of 20 per cent by lowering the temperature two or three degrees, *e.g.*, putting the eggs into sea water of 12° instead of into 15° as in these experiments. The previous experiments of Loeb have shown that the eggs of *S. purpuratus* segment not only at 12° but even at 3°C., when the rate of oxidations is less than one-half of that observed at 15°C. In addition, the experiments with KCN also show that this substance does not suppress cell division until the rate of oxidations is reduced to one-third of the normal value. From these facts we can state with certainty that the effect of narcotics upon the eggs of the sea urchin is not due to asphyxiation; a conclusion which Warburg reached also in his experiments with phenyl urethane.

Kisch[6] has recently published experiments which show that the photodynamic effect due to oxidations is raised instead of being diminished if narcotics are added to the medium.

SUMMARY.

It is shown that chloral hydrate, ethyl urethane, chloroform and various alcohols produce complete narcosis in the fertilized eggs of the sea urchin without practically lowering the rate of oxidations in the egg.

[6] Kisch: *Zeitschr. f. Biol.*, ix, p. 399, 1913.