ON CARBOHYDRATE METABOLISM IN HOMOGENISED SEA URCHIN EGGS*

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INTRODUCTION

The question of the enzymatic alterations in developing sea urchin eggs and larvae has long been the subject of intense study. Since WARBURG showed that fertilization of the sea urchin egg was connected with an increase in respiration, many attempts have been made to explain the mechanism for this increased oxidation. The question which substrate is being used has given rise to many theories. ÖHMAN[1] suggested that lipids would be the chief source of energy; he based this theory upon the low respiratory quotient of the eggs after fertilization as well as upon analyses of the lipids. HUTCHINS *et al*[2] suggested, after analysis of the ammonia liberated upon fertilization, that the increased oxidation would be due to the oxidation of protein. RUNNSTRÖM[3] found that monoiodoacetic acid did not inhibit the increased respiration occurring after fertilization and therefore concluded that glycolysis in the usual meaning of the word did not take any part in this phenomena. ÖRSTRÖM AND LINDBERG[4] and LINDBERG[5] have shown that the carbohydrate reserves decrease rapidly in eggs of certain sea urchin species after fertilization. BOREI[6] reinvestigated the question of the altered respiration in the ripening and newly fertilized egg in an intensive manner with the help of the Cartesian-diver technique, and found that the respiration consisted of two components, one which varies and one which remains constant. RUNNSTRÖM[9] and ZIELINSKI[7] analysed the alterations in the phosphate fractions during development of the newly-fertilized egg but found only small changes. LINDBERG[5] suggested in studies on homogenised eggs that carbohydrate is metabolised over hexose-6-phosphate and phosphogluconic acid.

That research on the sea urchin egg has produced so many opposite results may be explained to a large extent by the fact that different species of sea urchin often show remarkably different metabolic reactions. This may be of great help, however, to explain the significance of the different reactions.

The intention of the present investigation was to inquire if an esterification of phosphate takes place in a respiring homogenate of sea urchin eggs and which reactions can release energy for such possible esterifications.

METHODS AND MATERIALS

The material used was *Strongylocentrotus droebachiensis*, which spawns at the Swedish Westcoast from the end of February untill approximately the 20th of March.

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Respiration measurements were carried out with the Warburg apparatus at 6° C. The preparation of eggs free of jelly coating was carried out as follows: Approximately 10 ripe females were used for every experiment. The eggs were filtered through gauze and washed on the centrifuge at the lowest speed. The jelly layer was removed by treating one volume of egg suspension with one volume of 0.005 N HCl in sea water. The p_H of the solution became approximately 5. The suspension was centrifuged lightly and the hard-packed eggs, free of jelly coating, were washed three times with sea water. In case not all the jelly coating had gone into solution, the process would be repeated with somewhat stronger acid, without damage to the eggs. At the last washing the eggs were centrifuged more strongly, approximately 1000 r.p.m. for two minutes. This whole process required that the temperature be below 8° C. When the centrifuging was finished, the tube was placed in a beaker filled with ice.

For the preparation of the egg homogenate, a phosphate buffer was prepared consisting of 50 ml 3% NaCl, 50 ml 3% KCl, and 2 ml 1 M potassium phosphate buffer p_H 7.7. To 6 ml of this buffer solution at 0° C was added 8 ml of the hard-packed egg mass, by means of a pipette with a large opening. The tube was shaken several times with a vertical motion to disrupt the eggs completely. Then 0.6 ml of 0.1 M $MgCl_2$ was added, and, after shaking, also 0.6 ml 1 M NaF. In later experiments this mixture was supplemented with 1 ml of DPN[8*] containing 1 mg and 1 ml of 0.1% pyocyanine and 0.5 ml adenylic acid containing 5 mg. Of the viscous liquid thus obtained 2 ml were added to every vessel.

The Warburg vessels with additions were prepared in advance and placed in a tray containing ice. In the center cup 2 N NaOH was placed for absorption of the CO_2 formed. The incubation time for the different experiments varied because the duration of the activity of the homogenates was different. Most of the experiments were carried out so that the respiration could be measured every five minutes, and when the oxygen consumption began to show a distinct tendency to decline the incubation was stopped. The incubation periods of the experiments therefore had to vary between 20 and 40 minutes. After termination of the incubation the contents of the vessel were fixed with 10 ml of 10% trichloroacetic acid, and the filtrate made up to 50 ml volume. Aliquots of 3 ml were used for phosphate analysis according to the method of *Fiske* and *SubbaRow*. Easily-hydrolysable phosphate (designated in the tables as "ATP") was determined as the difference between the orthophosphate and the orthophosphate obtained after 10 minutes hydrolysis at 100° in 1 N H_2SO_4. Total phosphorus was determined after combustion with sulphuric acid. For the determination of alkali-labile phosphorus, 1 ml of the trichloroacetic acid filtrate was treated with 4 ml of 2 N NaOH, and was neutralised after five minutes with an equivalent amount of sulphuric acid plus an excess suitable for the phosphate determination.

The reason that none of the experiments were carried out on dialysed material is that the respiration of the homogenised eggs diminishes very rapidly; the difference between 0° and the optimum egg temperature of 6° is too small to permit inhibition of degrading reactions by cooling.

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* Abbreviations:

DPN = Diphosphopyridine nucleotide
TPN = Triphosphopyridine nucleotide
ATP = Adenosine triphosphate
HMP = Hexose monophosphate, an equilibrium mixture of glucose-6-and fructose-6-phosphate
HDP = Hexose diphosphate

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EXPERIMENTAL

RUNNSTRÖM[9] and LINDAHL[10] determined dehydrogenase activity in sea urchin egg homogenates with the aid of the methylene blue decolorisation method. The reason that they employed this method instead of the more quantitative Warburg method is that the respiration was too low to be determined in their homogenates. By first removing the jelly coating from the eggs it has become possible to obtain a homogenate the respiration of which is easily measurable in the Warburg apparatus. For this reason we have attempted to confirm their results with this method, and were able to support completely their date concerning the effect of DPN and pyocyanine. As substrate glucose was used, which, as shown in Table IV, proved to be oxidised more rapidly than any other substance tested. Possibly this effect may be particular to *Strongylocentrotus droebachiensis*.

Table II shows that homogenised sea urchin eggs are well able to make use of the energy liberated in respiration for esterification of phosphate. This esterification was markedly increased without any increase in respiration by the addition of adenylic acid. As seen from the table, a large amount of the organic phosphate formed was an easily hydrolysable phosphate ester, probably mainly ATP.

Table III shows that glucose exerted a powerful stimulation of respiration, and that this increased respiration was coupled with an esterification of phosphate. The variations in the respiration values in this table were largely caused by the different intervals during which the sea urchins were kept in the aquarium and by the varying times required for preparation of the egg homogenates. See also BOREI[6].

In Table IV, glucose, HMP, and HDP, with and without iodoacetic acid, were compared as substrates for respiration and phosphorylation. All vessels contained sodium fluoride. It may be seen that glucose and HMP were approximately equivalent as substrates for respiration, but that HDP was definitely inferior. The presence of monoiodoacetic acid (0.005 M) exerted no inhibition but rather appeared to be slightly stimulating. The formation of alkali-labile phosphate could not be detected with any substrate in the presence of monoiodoacetic acid. Since analysis of easily hydrolysable formed is unfortunately impossible in the presence of large amounts of hexose monophosphate and especially hexose diphosphate, only the values for orthophosphate are included in this experiment.

The substrate which demonstrated the best phosphorylation was glucose, but reservations must be made because of possible phosphatase action on the phospharylated substrates. Experiments were also carried out with gluconic acid and ribose as substrates, but, as has been previously demonstrated[5], these substances had no effect on respiration.

DISCUSSION

It is evident from the present investigation that the utilization of carbohydrate in the homogenised eggs of *Strongylocentrotus droebachiensis* is different from the classical metabolism in mammalian tissues in the following respects: Glucose markedly increases respiration and is not even surpassed by hexosemonophosphate. Hexose diphosphate was, at least in the fluoride-inhibited system, shown to be less rapidly oxidised than glucose. Monoiodoacetic acid did not inhibit the oxidation of glucose, HMP

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or HDP. Since 0.001 M monoiodoacetic acid almost completely inhibits the reaction:

$$\text{Phosphoglyceraldehyde} + \text{DPN} + \text{ADP} + PO_4^{-3} \rightleftarrows \text{phosphoglyceric acid} + DPNH_2 + \text{ATP}$$

this reaction may be of less importance for the respiration in the unfertilized sea urchin egg. This process is the first, after the aldolase and isomerase reactions for splitting of HDP, to require a dehydrogenation. This signifies that the aldolase reaction is of little importance on the oxidation of carbohydrate in homogenised sea urchin eggs. As further evidence against the participation of aldolase, it was shown that no alkali-labile phosphate accumulated in the presence of monoiodoacetic acid and the substates glucose, HMP, and HDP.

Despite these differences in mechanism for the most well known breakdown of carbohydrate in mammalian tissue and the one in sea urchin eggs, both mechanisms are capable of utilizing the energy obtained for the formation of energy-rich phosphate bonds. The highest ratio between the esterification of phosphate and the oxygen uptake was obtained in fully-ripened eggs at the beginning of the ripening season.

In an earlier study LINDBERG[5] suggested that carbohydrate metabolism in the unfertilized and newly-fertilized egg proceeds through the participation of Warburg's "Zwischenferment" and triphosphopyridine nucleotide with the formation of phosphogluconic acid from hexose-6-monophosphate. As has been mentioned this reaction requires TPN instead of DPN. In Table I it has been shown that DPN is necessary for the oxidation of glucose. OCHOA AND KORNBERG[13] have however proved that DPN may easily be phosphorylated to TPN by respiring liver tissue. Whether this phosphorylation reaction can take place in the sea urchin egg is not known but may be possible.

Another possibility is that a non-phosphorylating oxidation of glucose might take place. The fact that the oxidation of glucose in homogenised eggs is coupled with the esterification of phosphate makes this reaction appear unlikely. Furthermore gluconic acid is not oxidised by egg homogenates whereas phosphogluconic acid can be oxidised.

Since pyocyanine can directly oxidise reduced cozymase[14] it appears certain that the hydrogen transport system has no function in the phosphorylation by the system under investigation. In other words, despite all differences, the oxidation of glucose may produce phosphate esterification in a manner similar to the ordinary triose phosphate oxidation.

TABLE I

EXPERIMENTS SHOWING THE EFFECT OF DPN AND PYOCYANINE ON THE RESPIRATION OF HOMOGENISED SEA URCHIN EGGS

	Oxygen uptake in μM			
	H_2O	Pyocyanine	DPN	DPN + pyocyanine
Without Na F	3.72	6.50	3.18	10.20
With Na F	1.50	5.91	2.42	7.71

Pyocyanine 0.1 ml 0.1 %.
Diphosphopyridine nucleotide 0.1 ml containing 0.1 mg.
Each vessel contained 50 micromoles of glucose.

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TABLE II

EXPERIMENTS SHOWING THE EFFECT OF ADENYLIC ACID ON RESPIRATION AND PHOSPHORYLATION

		H_2O	0.1 mg DPN					1 mg DPN + 1 mg adenylic acid
			H_2O	adenylic acid				
				1 mg	2 mg	3 mg	4 mg	
Ex. 1 16 III 1948	O_2		4.1	3.5	3.5	3.1	3.1	
	P-uptake		0.0	7.8	8.2	8.2	8.2	
40 minutes' incubation	"ATP"-formed		1.4	0.2	0.4	1.2	2.1	
	Non-hydrolysable P-esters formed		1.4	7.6	7.8	7.0	6.1	
Ex. 2 13 III 1948	O_2	4.2	5.2	5.0				6.2
60 minutes' incubation	P-uptake	5.5	4.1	11.0				12.3
	"ATP"-formed	0.6	0.4	4.3				4.1
	Non-hydrolysable P-esters formed	4.9	3.7	6.7				8.2
Ex. 3 14 III 1948	O_2		4.7	5.2				
45 minutes' incubation	P-uptake		2.8	9.6				

Each vessel contained 50 micromoles of glucose, 0.1 m NaF and 3 micromoles $MgCl_2$.

TABLE III

EXPERIMENTS SHOWING THE EFFECTS OF GLUCOSE ON RESPIRATION AND PHOSPHORYLATION

		H_2O	Glucose
Ex. 1 17 III 1948	O_2	0.86	2.61
20 min incubation	P-uptake	1.5	1.9
	"ATP"-formed	—2.9	—3.1
	Non-hydrolysable P-esters formed	4.4	5.0
Ex. 2 20 III 1948	O_2	2.88	10.15
50 min incubation	P-uptake	3.7	4.3
	"ATP"-formed	1.3	—3.6
	Non-hydrolysable P-esters formed	2.4	7.9
Ex. 3 21 III 1948	O_2	1.82	5.50
30 min incubation	P-uptake	2.9	3.9
	"ATP"-formed	—0.7	—5.5
	Non-hydrolysable P-esters formed	3.6	9.4
Ex. 4 17 III 1948	O_2	2.60	4.14
65 min incubation	P-uptake	0.0	2.1
Ex. 5 18 III 1948	O_2	1.31	2.54
30 min incubation	P-uptake	0.8	3.4

Each vessel contained DPN = 0.1 mg. For other additions see text.
All values in the table are expressed in micromoles.

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TABLE IV

EXPERIMENTS SHOWING THE EFFECT OF MONOIODOACETIC ACID ON RESPIRATION AND PHOSPHORYLATION IN THE PRESENCE OF GLUCOSE, HEXOSEMONOPHOSPHATE AND HEXOSEDIPHOSPHATE IN HOMOGENISED SEA URCHIN EGGS

	Micromoles	Without iodoacetic acid				With iodoacetic acid			
		H_2O	glucose	Hmp	Hdp	H_2O	glucose	Hmp	Hdp
Ex. 1 21 III 1948 20 min incubation	O_2 P-uptake	0.7 0.6	2.5 3.4	2.4 0.7	0.8 2.2	1.1 0.4	2.8 3.0	2.3 0.2	1.6 1.2
Ex. 2 22 III 1948 30 min incubation	O_2 P-uptake		2.3 7.3	2.2 3.6	2.1 2.0		3.2 6.2	2.9 2.5	1.6 1.1
Ex. 3 23 III 1948 45 min incubation	O_2	0.6	3.5	2.5	0.7	1.4	4.2	2.7	1.5

Each vessel contained DPN, adenylic acid, and NaF in the same concentrations as in Table III.

Concentration of glucose = 100 micromoles.
,, ,, Hmp = 50 ,,
,, ,, Hdp = 50 ,,
,, ,, monoiodoacetic acid = 0.005 m.

SUMMARY

The homogenised eggs of *Strongylocentrotus droebachiensis* are able to oxidise glucose. This oxidation is markedly accelerated by the addition of diphosphopyridine nucleotide and pyocyanine.

The velocity of oxidation of glucose is at least equal to the oxidation of hexosemonophosphate, and is considerable more rapid than the oxidation of hexose diphosphate.

Monoiodoacetic acid does not inhibit the oxidation of glucose.

Upon the incubation of hexose diphosphate with homogenised eggs in the presence of monoiodoacetic acid, no formation of alkali labile phosphate could be demonstrated.

The oxidation of glucose is coupled with the esterification of phosphate.

RÉSUMÉ

Les œufs homogénéisés de *Strongylocentrotus droebachiensis* sont capables d'oxyder le glucose.

Cette oxydation est considérablement accélérée par l'addition de diphospho-pyridine-nucléotide et de pyocyanine.

La vitesse d'oxydation du glucose est au moins égale à celle de l'oxydation de l'hexose monophosphate et elle est considérablement plus rapide que l'oxydation de l'hexose diphosphate.

L'acide mono-iodacétique n'arrête pas l'oxydation du glucose.

Après avoir incubé de l'hexosediphosphate avec les œufs homogénéisés, on n'a pu démontrer aucune formation de phosphate labile en milieu alcalin.

L'oxydation du glucose est liée à l'estérification du phosphate.

ZUSAMMENFASSUNG

Die homogenisierten Eier von *Strongylocentrotus droebachiensis* können Glukose oxydieren. Diese Oxydation wird durch Zufügen von Diphosphopyridinnukleotid und Pyocyanin deutlich beschleunigt.

Die Geschwindigkeit der Glukose-oxydation ist der der Hexosemonophosphatoxydation mindestens gleich und beträchlich schneller als die der Oxydation von Hexosediphosphat.

Monojodessigsäure hemmt die Glukoseoxydation nicht.

Bei Inkubation von Hexosediphosphat mit homogenisierten Eiern, bei Anwesenheit von Monojodessigsäure, konnte keine Bildung von alkali-labilem Phosphat gezeigt werden.

Die Oxydation von Glukose ist mit der Veresterung von Phosphat verbunden.

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