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Microelectrode Studies of Oxygen Stress and Transport in Loach Eggs in Helium-Oxygen and Nitrogen-Oxygen Media

B.S. Sushko

The overall material that has by now accumulated on the /593* biological effect of helium (He), is contradictory. Alongside the viewpoint that He at normal pressure has no significant direct effect on biological systems, we have experimental confirmation that it specifically affects physiological and biochemical parameters. The authors of studies on warm-blooded animals in vivo are mainly unanimous in their conclusions, having proven during their investigations that the main factors having a biological effect are the physical properties of helium (whose density, heat capacity and thermal conductivity differ from those of nitrogen). Specifically, the body's thermal balance is disrupted [2, 4, 6 and 9]. Investigations with tissues in vitro and with protozoans have proven that helium acts directly. Thus, the replacement of N_2 by He in the respiratory cell (Warburg vessels) increases the oxygen consumption rate by different body tissues [11];

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*Numbers in the right-hand margin indicate the corresponding pages in the original.

helium in relation to other inert gases stimulates the growth and cyclosis of protozoan cytoplasm [10]. Researchers have advanced hypotheses that He and other inert gases specifically affect cell [plasma] membranes [8]. This effect correlates with the molecular weight of the inert gas and its solubility in lipids [4 and 7].

We studied the effect of helium on oxygen conditions using a model of a single cell (a loach egg) and drew our conclusions on the basis of the intracellular oxygen stress and the overall coefficient of permeability to an oxygen flow.

Research Methods

We selected a loach (Misgurnus fossilis) egg as a model for studying the oxygen conditions of a cell in helium-oxygen and nitrogen-oxygen media. We got the eggs for our experiments by first stimulating female loaches with gonadotrophin. We placed the eggs in a physiological solution containing the following:

NaCl	60 millimole/1;
KCl	1.0 ";
$CaCl_2$	0.9 ";
pH	7.4 [3].

For our investigations we used unfertilized eggs for 45 minutes to 3 hours after we got them. A perivitelline space formed in 45 minutes at 21°C in the eggs from the time that we placed them in the solution. We later stored the material in a solution balanced with atmospheric gases at 4°C.

In our experiments we placed an egg (average diameter 1.8 mm) in the centre of a thermostatically controlled, flow-type chamber on a screen with a 1 x 1 mm mesh. We selected the chamber volume (10 cm^3) and the flow rate of the incubation medium so that the medium would be completely renewed in a relatively short time interval (under 30 seconds). We set the thermostatic control at 15 and 20°C. The solutions entering the chamber were continuously saturated with a gaseous mixture of a prescribed composition by feeding the gas into saturators with microcompressors. At the same time, we prepared four solutions saturated with the following gaseous mixtures: about 21% O_2 in He (a [literally] normoxic helium-oxygen medium) and approximately 12% O_2 in He (a hypoxic helium-oxygen medium); as a control we used a normoxic nitrogen-oxygen solution (air-saturated - 21% O_2) and a solution saturated with about 12% O_2 in N_2 (a hypoxic nitrogen-oxygen medium). During our experiments an egg could be successively balanced with each of the four solutions. We recorded the oxygen stress ($\underline{P}_{O_2}$) of the solutions with a sealed Clark-type electrode in the middle of the chamber.

We determined the oxygen conditions from the $\underline{P}_{O_2}$ values inside the egg. We measured $\underline{P}_{O_2}$ by the polarographic chronoamperometric method using aged, polystyrene-coated, glass-insulated platinum microelectrodes with a tip diameter of 3 to 5 μ [1 and 5]. We measured $\underline{P}_{O_2}$ /594

1) in the central part of the egg;

2) by reading the $\underline{P}_{O_2}$ contour at one point over the egg and at five internal points [1];

3) during the change in the oxygen stress in the central part if oxygen entered or emerged from the egg.

We produced directed oxygen flows by replacing a normoxic with a hypoxic medium (discharge of O_2 from the egg) and, after balancing, again [by replacing a hypoxic] with a normoxic [medium] (entry of O_2).

Thus in all of our experiments we measured the oxygen stress intracellularly in oxygen-steady states, or in dynamic states of the transition of the egg to another steady state with rapid changes of medium.

As shown by our preliminary calculations and measurements of change, the P_{O_2} of an egg from the time t (P_t) in a transitional non-steady process is described fairly closely by the following exponential rule, which follows from the laws of diffusion [4]: $\ln\frac{P\infty}{P\infty} - \frac{P_t}{P_0} = -\frac{6}{d}p_1t$- for an incoming O_2 flow, and $\ln\frac{P_t}{P_0} - \frac{P\infty}{P\infty} = -\frac{6}{d}p_2t$

for an O_2 flow emerging from the egg. The values P_0 and P_∞ are the steady-state values of P_{O_2} at the beginning and end of the process respectively; p is the overall coefficient of the permeability of the egg to oxygen in a forward (p_1) or reverse (p_2) direction; d is the diameter of the egg.

For each recorded oxygen "input" or "output" curve we plotted a graph of the natural logarithm of the relative change in P_{O_2} in an egg (the left parts of the equations) as a function of time. We determined from the graph the value of the coefficient of the egg's permeability to oxygen.

Fig. 1 shows a block diagram of the unit on which we did our investigations.

Results of Investigations and Discussion

When we replaced the nitrogen dissolved in the medium with helium, and where the P_{O_2} of the medium was constant, the oxygen stress inside the eggs did not change. The oxygen stress and the P_{O_2} contour of the eggs proved to be dependent only on P_{O_2} and the temperature of the medium. As the P_{O_2} of the medium increased, the oxygen stress at all points inside the egg rose proportionally; as a result, the P_{O_2} gradient remained practically as before. The dependence of the oxygen stress in the central part of the eggs on the P_{O_2} of the medium appears in Fig. 2, from which we see that the relationship between the P_{O_2} of the egg [and] the P_{O_2} of the medium is close to linear. This dependence characterizes at least the media used in our investigations, with P_{O_2} ranging from 200 to 120 gPa. Because of [this] linear function, in our experiment, when the oxygen content changed in the environment (outer medium), the P_{O_2} difference between the outer, middle and central parts of the egg ($P_m - P_e = \Delta P$) remained on average at the level of 30 gPa both for the helium-oxygen and for the nitrogen-oxygen media (Table 1). We did not observe any statistical differences between ΔP at 20°C in the solutions that we tested.

In practice, ΔP is convenient for estimating the P_{O_2} gradient of a cell. Its value is determined in a steady state by two main processes: the rapidity of oxygen delivery and the rate at which the /595 oxygen is consumed by the cell. Whereas, however, the inhibition of oxygen delivery is accompanied by a rise in ΔP, a decrease in the oxygen consumption rate causes ΔP to fall in a cell. In our experiments we simulated a situation where oxygen transport and

Table 1

The Effect of Helium-Oxygen and Nitrogen-Oxygen Media on Oxygen Stress (gPa) in the Central Part of an Egg

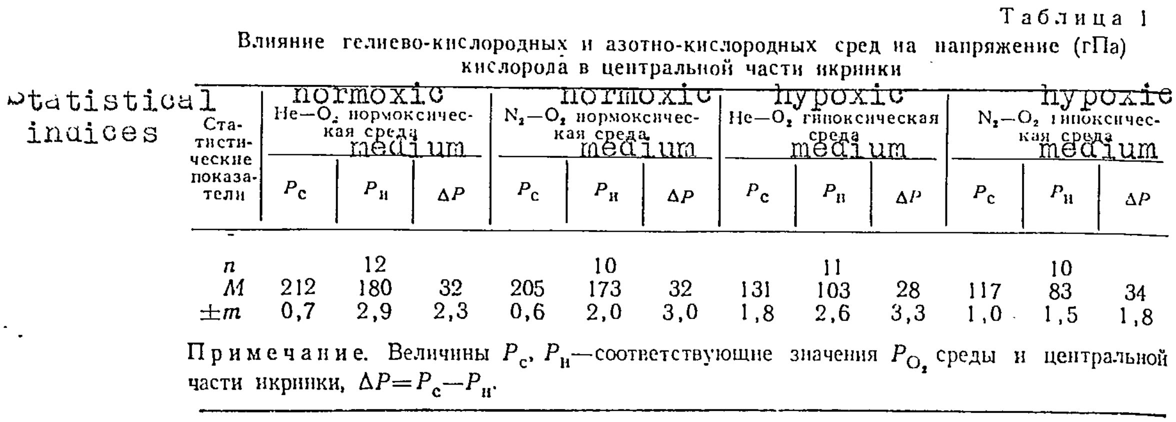

Таблица 1

Влияние гелиево-кислородных и азотно-кислородных сред на напряжение (гПа) кислорода в центральной части икринки

statistical indices Статистические показатели	normoxic He—O_2 нормоксическая среда medium			normoxic N_2—O_2 нормоксическая среда medium			hypoxic He—O_2 гипоксическая среда medium			hypoxic N_2—O_2 гипоксическая среда medium		
	$P_с$	$P_и$	ΔP	$P_с$	$P_и$	ΔP	$P_с$	$P_и$	ΔP	$P_с$	$P_и$	ΔP
n		12			10			11			10	
M	212	180	32	205	173	32	131	103	28	117	83	34
$\pm m$	0,7	2,9	2,3	0,6	2,0	3,0	1,8	2,6	3,3	1,0	1,5	1,8

Примечание. Величины $P_с$, $P_и$—соответствующие значения P_{O_2} среды и центральной части икринки, $\Delta P = P_с - P_и$.

Note. P_m and P_e are the P_{O_2} values of the medium and the central part of an egg respectively; $\Delta P = P_m - P_e$.

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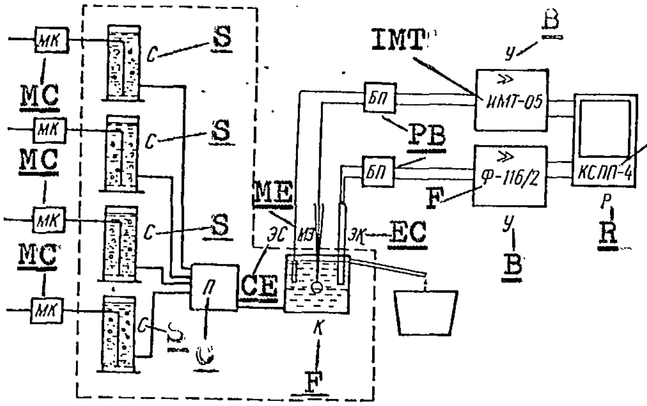
utilization by an egg were inhibited by lowering the temperature from 20 to 15°C. At the same time, the P_{O_2} difference between the environment (outer medium) and the centre of the egg decreased from 33 to 23 gPa, and the inner contour and P_{O_2} gradient of the egg changed positively (see Table 2 and Fig. 3). This drop in ΔP and the P_{O_2} gradient followed from the predominance of the inhibition of the oxygen consumption rate over the decrease in the oxygen delivery rate when the temperature fell.

If we assume that helium directly affects the oxygen transport or rate of oxygen consumption by an egg, then the constancy of ΔP may somehow be due only to the differently directed changes in oxygen transport and utilization. For a definitive conclusion, we only need data on one of these processes when exposed to a helium-oxygen medium.



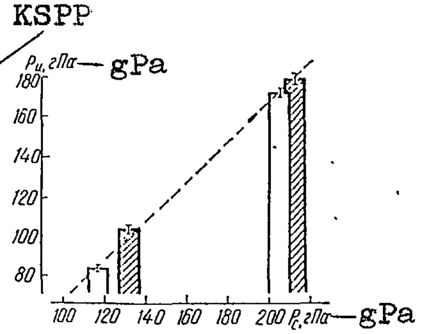


Fig. 1 — Рис. 1. Блок-схема установки.

МК — микрокомпрессор, С — сатуратор, П — переключатель растворов, К — проточная камера, ЭС — электрод сравнения, МЭ — рабочий платиновый электрод, ЭК — электрод Кларка, БП — блок поляризации электродов, У — усилитель, Р — регистратор. Штриховой линией обозначена зона термостатирования.

Fig. 2 — Рис. 2. Зависимость P_{O_2} в центральной части икринок ($P_и$) от P_{O_2} среды ($P_с$).

Светлые столбики соответствуют P_{O_2} в азотно-кислородной среде, заштрихованные — в гелиево-кислородной.

Fig. 1 - Block diagram of unit.

MC - microcompressor; S - saturator; C - solution changeover switch; F - flow-through chamber; CE - comparison electrode; ME - working platinum electrode; EC - Clark electrode; PB - electrode polarization block; B - booster; R - recorder. The broken line shows the thermostatic control zone.

Fig. 2 - P_{O_2} in central part of the eggs P (P_e) as a function of the P_{O_2} of the medium (P_m).

The blank columns correspond to P_{O_2} in a nitrogen-oxygen medium, and the shaded columns, to the corresponding value in a helium-oxygen medium.

With this purpose we did additional research on measuring the overall coefficients of the permeability of an egg to oxygen flows entering and /596 discharging. The data from this research appears in Table 3. For an incoming oxygen flow the overall coefficient of the permeability of an egg (p_{inc}) in a helium-oxygen medium proved to equal (3.9 ± 0.5 • 5.10^{-4} cm • s^{-1}*, which coincides with the mean coefficient of

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*Translator's note: This expression, including the initial parenthesis, is an exact transcription from the original Russian text, which gives no closing parenthesis.

Table 2

Overall P_{O_2} Contour of an Egg in a Normoxic Nitrogen-Oxygen Medium at 20 and 15°C

Таблица 2

Суммарный профиль P_{O_2} икринки в нормоксической азотно-кислородной среде при 20 и 15 °С

Temp. °C Температура Temp	Statistical Статистические показатели, indices	P_{O_2} в точках измерения, в гПа at measurement points in gPa						
		P_c m	P_{c1} m1	$P_{н1}$ e	$P_{н2}$ e	$P_{н3}$ e	$P_{н4}$ e	$P_{н5}$ e
20 °C	n	36	36	36	36	36	35	35
	M	205	200	198	194	189	180	174
	$\pm m$	0,4	0,6	0,5	0,6	0,9	1,1	1,0
15 °C	n	27	27	26	27	27	26	31
	M	207	202	200	198	195	189	184
	$\pm m$	0,4	0,6	0,5	0,5	0,8	1,1	0,9

Примечание. P_c—P_{O_2} среды; P_{c1}—P_{O_2} у поверхности икринки; $P_{н1}$÷$P_{н5}$—P_{O_2} в соответствующих пяти точках внутри икринки (см. рис. 2).

Note. P_m - P_{O_2} of the medium; P_{m1} - P_{O_2} at egg surface; P_{e1} to P_{e5} - P_{O_2} at corresponding five points inside egg (see Fig. 2).

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permeability of an egg to an incoming oxygen flow in a nitrogen-oxygen medium. The mean coefficients of the permeability of an egg to a discharging oxygen flow (p_{dis}) in helium-oxygen and nitrogen-oxygen media are similarly equal. The drop in the overall coefficients of permeability p_{dis} in relation to P_{inc} is in this case statistically uncertain.

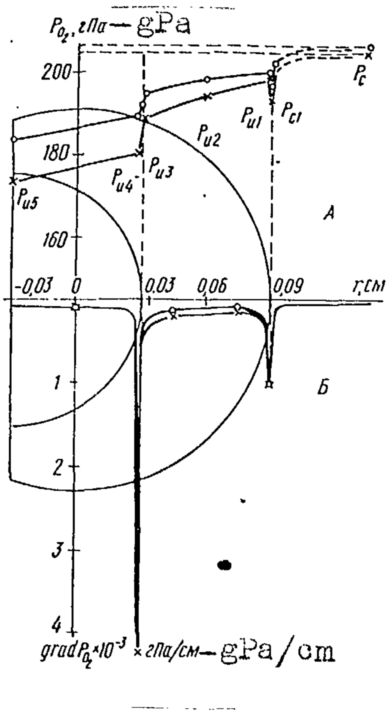

Рис. 3. Профиль (*А*) и усредненный градиент (*Б*) P_{O_2} икринки, при различных температурах.
Крестиками обозначены средние значения величин при 20 °С, кружочками — при 15 °С.

Fig. 3 - Contour (A) and averaged gradient (B) of P_{O_2} of an egg at different temperatures.

The x's show mean values at 20°C, and the circles, the corresponding values at 15°C.

Thus when we determined P_{O_2} inside an egg and the overall coefficients of permeability to oxygen in our research we could find no positive changes in these values when we replaced the nitrogen dissolved in the medium with helium. The value of P_{O_2} inside an egg proved to be dependent mainly on the rate of the processes whereby the O_2 and P_{O_2} of the medium were utilized. These data indicate that helium has no specific effect on the transport or consumption of oxygen by loach eggs under normal atmospheric pressure and where the eggs have been held for only a short time (no more than 0.5 hour) in solutions saturated with a gaseous helium-oxygen mixture.

Table 3

Values of Overall Coefficients of Permeability of an Egg to Forward ($\underline{p}_{inc}$) and Reverse ($\underline{p}_{dis}$) Oxygen Flows in Helium-Oxygen and Nitrogen-Oxygen Media

Таблица 3

Значения суммарных коэффициентов проницаемости икринки прямому ($p_{вх.}$) и обратному ($p_{вых.}$) потокам кислорода в гелиево-кислородных и азотно-кислородных средах

Statistical Статистические показатели indices	Гелиево-кислородная среда Helium-oxygen medium		Азотно-кислородная среда Nitrogen-oxygen medium	
	$p_{вх}\cdot 10^4$, см/с ent — s	$p_{вых.}\cdot 10^4$, см/с dis s	$p_{вх.}\cdot 10^4$, см/с ent	$p_{вых.}\cdot 10^4$, см/с dis s
n	8	8	8	8
M	3,9	3,5	3,9	3,45
$\pm m$	0,5	0,45	0.3	0,4

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MICROELECTRODE STUDIES OF OXYGEN STRESS AND TRANSPORT IN LOACH EGGS IN HELIUM-OXYGEN AND NITROGEN-OXYGEN MEDIA

Summary

The P_{O_2} profile in the loach (*Misgurnus fossilis*) eggs was registered by means of platinum microelectrodes by polarographic method when saturating incubational solution by helium-oxygen and nitrogen-oxygen gas mixtures under stationary conditions. Total factors of the egg penetrability to oxygen are obtained under nonstationary conditions of oxygen transport. Based on experimental data helium specific effect on oxygen transport and consumption rate in the loach eggs under conditions of their short time exposure in solutions saturated by helium-oxygen gas mixtures is supposed to be absent.

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