RESPIRATORY QUOTIENTS DURING EMBRYOGENESIS OF *RANA PIPIENS*

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(2 Figures)

Introduction

It is generally admitted that the respiratory quotient (RQ) is a relatively good indication of the type of foodstuf being burned up by an organism. In 1931, NEEDHAM, based on RQ determinations during development of several animals, suggested that a succession of energy sources occurs in ontogenesis, carbohydrates preceding proteins and proteins preceding fats. NEEDHAM's theory was at the time supported by studies performed in Annelids (FAURE-FREMIET 1924), Insects (VANEY and CONTE 1911, PIGORINI 1912), Echinoderms (WARBURG 1910, SHEARER 1922), Amphibians (BIALASCEWICS and MINCOVNA 1921, PARNAS and KRASINSKA 1921, KONOPACKI 1924) and Birds (BOHR and HASSELBACH 1900, WARBURG, NEGELEIN and POSENER 1924, KONOPACKI 1. c., NEGELEIN 1925). Back in 1931, however, NEEDHAM already recognized that "the conception of an ontogenetic succession of energy sources has to reckon with a few facts, which do not easily fit it", as, for instances, the case of the Mammalian embryo, which burns exclusively carbohydrate throughout the development. Nevertheless, in 1942, reviewing again the subject, he added new data in behalf of his view, such as the results of BOREI (1933) and VAN HERK (1933) on the sea-urchin, HOROWITZ (1940) on the Gephyrean *Urechis*, NOLF (1932) on the Trematode *Trichiurus*, of NEEDHAM (1933) on *Carcinus*, of BOEL (1935) on the grasshopper (*Melanoplus*) and, finally, of SCHLENK (1933) and AMBERSON and ARMSTRONG (1933) on two fishes (*Truta* and *Fundulus*). The list, however, of new information contrary to NEEDHAM's theory also increased, mainly as a consequence of the works of BALDWIN (1935) on the pulmonate *Limnæa*, LASER and ROTSCHILD (1939) and ÖHMAN (1940) on two sea-urchins (*Psammechinus* and *Paracentrotus*) and, finally, of RQ

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determinations performed with the Amphibian developing egg. Although he gave no comment to the results of LASER and ROTSCHILD and of ÖHMAN, NEEDHAM tried to explain what happened in *Limnæa* (that is an RQ approximately 1.0 throughout the development) by saying that in this particular case the catabolic utilisation of fat and protein is probably masked, since at least a resynthesis of fat takes place during development, as BALDWIN himself admits.

As to the Amphibian egg the present state of the question is more or less the following. In 1915, BIALASCEWICS and BLEDOWSKI, using the THUNBERG-WINTERSTEIN apparatus, reported that in *Rana temporaria* the RQs of the unfertilized and fertilized egg were respectively 0,60 and 0,61 and suggested that stored fat was consumed at the beginning of development. In 1934, BRACHET, using the MEYERHOF and SCHMIDT's technique, also reported low RQs for the early stages of development of *Rana fusca* (0,66 for the early stages of cleavage, 0.70 for the advanced blastulæ). At the beginning of gastrulation, however, the RQ suddenly rose to unity, oscillating between 0.95 and 1.00 during the rest of development. In 1946, BARTH studying the arrested egg of hybrids of *Rana pipiens* (eggs) x *Rana sylvatica* (sperm), obtained RQs below unity in the pregastrular stages, although not so low as BRACHET's. Neither were his gastrular and postgastrular RQs so close to unity. Commenting BRACHET's results, NEEDHAM (1942) suspects that in the frog's egg the succession of energy sources would only start from gastrulation on and that the same probably occurs in the fish egg (no pregastrular RQs were determined by AMBERSON and ARMSTRONG).

Now, in my opinion, BRACHET's results are to be analysed from a quite different angle. BRACHET's as well as BIALASCEWICS and LEDOWSKI's or BARTH's experiments, are by no means free of criticism on technical grounds. Thus, rather than try to find an explanation of why their results do not fit NEEDHAM's theory, I thought it preferable to repeat their work under what I consider improved experimental conditions. This paper reports RQ determinations made during the development of the common New England frog, *Rana pipiens*.

This work was suggested to me by Prof. E. J. BOELL and was done at the OSBORN ZOOLOGICAL LABORATORY of the YALE UNIVERSITY, NEW HAVEN, U.S.A. To both Prof. BOELL and the O. Z. L. my sincere thanks.

Methods

The experiments were done with batches of *Rana pipiens* eggs, during winter time. Implantations of fresh pituitary glands into one of the dorsal lymph sacs of a female provided the eggs for the artificial insemination. The eggs were stripped in a large Petri dish containing an active sperm suspension in 10% Amphibian Ringer. In order to get the very first stages of cleavage, 20 minutes after insemination, some of the eggs were already prepared for the respirometer. The rest the fertilized eggs was rinsed in 10% Ringer and then fractionated in small groups and put in finger-bowls containing fresh tap-water. No special care was taken to keep the developing eggs at a constant temperature, but they always stayed in a cool place (ca. 10°C).

In each experiment, the initial step was the removal of the egg's swelling jelly by means of a watchmaker's forceps. The naked eggs were then transferred to an Erlenmeyer containing 10% Ringer. A CO_2 free current of air was next allowed to perfuse the Erlenmeyer's liquid during 10-20 minutes in order to expell as much as possible from both eggs and solution the free or weakly bound CO_2. The eggs were then quickly pipetted from the Erlenmeyer to a small vial marked to contain exactly 1 c.c. and from the vial the desired amount of eggs plus 10% Ringer was finally introduced into the respirometer vessel. The respirometer was a BARCROFT-WARBURG apparatus. Vessels for the second method of DICKEN and SIMER (1931, I, 1933), with an approximate volume of 16 ml, were used, shaked at 120 complete oscillations per minute, at 25 $\pm$ 0.05°C.

In the experiments with the early stages (till the end of gastrula approximately), 50 eggs were employed. When later stages were used, the number of embryos introduced in one vessel decreased according to their increasing metabolic rate. After putting the eggs in the vessels, 0.3 c.c. of a 3N HCl solution were added to each of the two sidebulbs and from a microburete containing a saturated $Ba(OH)_2$ solution (carefully protected against CO_2 contamination) 0.3 c.c. were added to the center well. All these steps were taken quickly to reduce as much as possible contamination of the $Ba(OH)_2$ by the CO_2 eventually expelled from the eggs already in the vessels, thus providing fairly uniform samples for the experiments.

The stages of the eggs were determined at the beginning (after the jelly had been removed) and at the end of each experiment, using SHUMWAYS's normal tables (1941.) Besides, in all cases, a small vial filled with 10% Ringer and containing 5-10 decapsulated eggs was attached to one of the supporters of the manometers and immersed in the bath so that, during the experiment, by checking their stage under a binocular at the time of each reading, information was gained as to the stage of the eggs inside the vessels.

In each experiment, 3 vessels plus a thermobarometer were employed. At the beginning, after running the apparatus to the open air for 10 minutes and then closing the stopcocks and taking the initial readings, acid was dumped from both sidebulbs into the main chamber and center well of one of the experimental vessels, in order to determine the "initial CO_2" (as carbonate in the $Ba(OH)_2$ and as "bound CO_2" in the eggs). Records of the liberation of CO_2 in this vessel were subsequently taken every 5 minutes during 20 minutes. Readings of the oxygen uptake in the other 2 manometers were taken at half an hour intervals during one to one and a half hours. At the end, acid was tilted to the main chamber and center well of the remaining experimental vessels in order to obtain the "final CO_2", whose liberation was followed during 30 minutes with readings each 5 minutes. The CO_2 output was calculated by subtracting the initial CO_2 from the final CO_2. The technique used was thus a sort of combination of DICKEN and SIMER'S first and second methods, in that a $Ba(OH)_2$ solution was put in the center space for the CO_2 absorption, instead of putting in the outer space acidified permanganate and adding to it from one of the side bulbs acidified NaI in order to get a strongly alcaline solution. The procedure offered the following advantages: (a) Over the first method: it provided two acid deposits, one for the liberation of CO_2 from the medium

(eggs in 10% Ringer), other for the breakdown of the carbonate in the $Ba(OH)_2$ solution (in the first method, or in the method of MEYERHOF and SCHMIDT, the acid solution is first added to the alkali and a mixture of the two then thrown into the medium). (b) Over the second method : it provided the possibility of a direct and fractionated measurement of the oxygen uptake during the experiment and avoided any CO_2 accumulation.

RESULTS

Table I shows the respiratory quotients determined for the different embryonic stages of *Rana pipiens*. In computing the data for this table, results from all experimental series were taken. Those corresponding to same initial and final stages were grouped and an average RQ then calculated. In drawing the curve of fig. 1 the average RQs of table I were employed.

Between stages 2 (gray crescent) and 4-5 (4-32 cells) high RQs were obtained (maximum 1.01, minimum 0,85). From stages 4-5 to 9 (late cleavage) the RQ generally decreased to a fat level. At beginning of gastrulation a sudden rise to almost unity was observed and up to stage 12 (late gastrula) the RQ oscillated between 0.9 and 1.0 From stage 12 onwards the RQ decreased to a more or less persistent fat-protein level, except in two occasions, when it rose again to 0.9, that is, stages 19 ("heart beat") and 20. ("gill circulation"). Thus, in *Rana pipiens*, Needham's succession of energy sources in ontogenesis does not seem to take place. On the other hand, previous works on the frog's developing egg were not entirely confirmed, since high RQs were obtained with just fertilized or early cleaving eggs.

In table II, the results of two typical series of experiments are showed. The first part of the table is concerned with eggs from a same female at stages 1-4 to 14-15 ; the second part with eggs from another female at stages 16 & 17 to 20-21. The oxygen uptake is seen to increase steadily with age, as already known from previous works (BRACHET 1. c., ATLAS 1938, BARNES 1944 and MOOG 1944). The "initial CO_2" also increased. Up to stages 16 (neural tube) and 17 (tail bud), "initial CO_2" values are much higher than those for oxygen consumption or "respiratory CO_2", the ratio between them reaching 4.5 : 1. From stage 17 onwards, however, the oxygen consumption or "respiratory CO_2" values increase so fast as to equalize or even surpass those for "initial CO_2".

The fact that very early stages of cleavage exhibited high RQs led to an investigation of the RQ of the unfertilized egg, in order to find out possible metabolic changes induced by fertilization. Table III reports the results. No significative changes were detected either in the RQ or in the respiratory rate (compare tables I, II and III).

DISCUSSION

The results reported in this paper for *R. pipiens* agree with BRACHET'S for *R. fusca* only in that we both obtained low RQs for mid and late cleavages and a sudden rise to unity at the beginning of gastrulation. In *R. pipiens*, I got high RQs with the very early stages of development, whereas in BRACHET'S experiments, as a consequence of fertilization, the RQ de-

TABLE I

Respiratory Quotients during *Rana pipiens* development

Stage		Number of Experiments	Respiratory Quotients			Std. dev.
Initial	Final		Minimum	Maximum	Mean	
Gray cresc.	(2) — 2 cell (3)	4	0.95	1.16	1.01	0.05
Gray cresc.	(2) — 4 cell (4)	5	0.90	1.09	1.01	0.04
Gray cresc.	(2) — 8 cell (5)	4	0.85	0.92	0.88	0.03
Gray cresc.	(2) — 16 cell (6)	2	0.94	1.06	1.00	—
2 cell	(3) — 8 cell (5)	4	0.95	1.14	1.01	0.04
2 cell	(3) — 16 cell (6)	2	0.96	1.02	0.99	—
4 cell	(4) — 32 cell (7)	2	0.84	0.87	0.85	—
8 cell	(5) — 16 cell (6)	2	0.82	0.89	0.85	—
8 cell	(5) — 32 cell (7)	4	0.70	0.90	0.80	0.05
16 cell	(6) — Mid cleav. (8)	2	0.86	0.89	0.87	—
Mid cleav.	(8) — Mid cleav. (8)	2	0.60	0.65	0.62	—
Mid cleav.	(8) — Late cleav. (9)	6	0.66	0.85	0.75	0.03
Late cleav.	(9) — Late cleav. (9)	4	0.67	0.83	0.72	0.04
Late cleav.	(9) — Dorsal lip (10)	9	0.80	1.20	0.98	0.03
Dorsal lip	(10) — Dorsal lip (10)	6	0.79	1.13	0.90	0.05
Dorsal lip	(10) — Mid gastrula (11).	6	0.88	0.99	0.91	0.02
Mid gastr.	(11) — Mid gastrula (11).	4	0.86	0.95	0.91	0.02
Mid gastr.	(11) — Late gastr. (12)..	2	0.97	0.99	0.98	—
Late gastr.	(12) — Late gastr. (12)..	4	0.80	0.92	0.86	0.03
Late gastr.	(12) — Neural plate (13).	4	0.84	0.86	0.85	0.01
Neur. plate	(13) — Neural plate (13).	6	0.67	0.85	0.78	0.03
Neur. plate	(13) — Neural folds (14).	2	0.83	0.87	0.85	—
Neur. folds	(14) — Rotation (15)	4	0.73	0.88	0.83	0.03
Rotation	(15) — Rotation (15)	2	0.80	0.87	0.83	—
Rotation	(15) — Neural tube (16).	2	0.72	0.75	0.73	—
Neur. tube	(16) — Tail bud (17)	2	0.76	0.83	0.79	—
Tail bud	(17) — Musc. resp. (18) .	2	0.87	0.89	0.88	—
Heart beat	(19) — Heart beat (19)..	6	0.83	0.97	0.90	0.02
Gill circ.	(20) — Mouth open (21).	2	0.89	0.95	0.92	—
Operc. fold	(23) — Right operc. closed (24)	3	0.62	0.77	0.70	0.04
Right op. cl.	(24) — Right operc. closed (24)	2	0.72	0.81	0.76	—
Operc. compl.	(25) — Operc. compl. (25)	1	0.81	0.81	0.81	—

Observation : in ( ) the stage number, according to SHUMWAY.

TABLE II

Respiratory exchanges during *Rana pipiens* development

Stage	N.° of eggs.	Oxygen consumption (cu.mm./50 eggs/h.)	Initial CO2 (cu.mm.)	Respiratory CO2 (cu.mm./50 eggs/h.)	R. Q.
Gray Cresc. — 4 cell	50	5.4	9.60	5.2	0.97
2 cell — 4 cell	50	5.7	8.73	5.9	1.02
	50	5.6	8.73	5.4	0.96
Mid cleav. — late cleav.....	50	7.5	17.46	6.4	0.84
		7.8	17.46	5.8	0.74
		8.0	27.06	6.8	0.85
		7.6	27.06	5.8	0.76
Late cleav. — Dorsal lip ...	50	9.8	31.40	8.2	0.83
		10.2	31.40	8.6	0.84
Dorsal lip — Mid gastr. ...	50	12.1	45.30	10.7	0.88
		12.1	45.30	11.0	0.95
Mid gastr. — Mid. gastr. ..	50	14.1	53.20	13.1	0.94
		13.0	53.20	11.2	0.86
Late gastr. — Late gastr. ..	50	15.6	66.30	12.5	0.80
		16.6	66.30	13.8	0.84
Late gastr. — Neural plate .	50	17.1	71.20	14.5	0.85
		16.7	71.20	14.3	0.86
Neur. plate — Neur. plate..	50	17.5	86.40	14.9	0.85
		18.6	86.40	14.9	0.80
		21.1	102.90	16.9	0.80
		21.5	102.90	14.4	0.67
Neural plate — neur. folds..	50	25.4	110.80	22.2	0.87
		26.9	110.90	22.5	0.83
Neural folds — rotation	50	27.8	95.50	24.3	0.87
		27.8	95.50	25.1	0.88
Neural tube — tail bud	30	38.1	79.50	29.0	0.76
		38.4	79.50	31.8	0.83
Tail bud — musc. resp.	20	46.3	91.50	41.0	0.89
		48.3	91.50	41.8	0.87
Heart beat-heart beat.....	30	69.0	74.00	61.9	0.90
		67.5	74.00	56.3	0.83
		66.2	77.90	59.2	0.94
		68.4	77.90	65.2	0.95
		71.0	104.50	68.5	0.97
		77.0	104.50	65.8	0.85
Gill circ. — gill circ.	15	118.0	125.00	97.0	0.81
		114.0	125.00	85.0	0.74
Gill circ. — mouth open ...	15	140.0	141.00	125.0	0.89
		171.0	141.00	163.0	0.95

TABLE III

Respiratory Quotients of unfertilized eggs and early cleavage stages of *Rana pipiens*

Stage	N.° of eggs.	O2 consumption (cu.mm.)	Initial CO2 (cu.mm.)	Respiratory CO2 (cu.mm.)	R. Q.
unfertilized.....	50	10.60	13.9	11.4	1.08
	50	10.30	13.9	12.3	1.18
Gray cresc. — 4 cell	50	8.75	20.0	10.2	1.17
	50	9.91	20.0	10.6	1.07

TABLE IV

Bound CO2 in the unfertilized egg and in early cleavage stages of *Rana pipiens.*

Exper. no.	Side bulbs	Center space	Outer space	CO2 from acidific. (cu.mm.)
184	0.6 c.c. 3N HCl	0.3 c.c. sat. Ba(OH)2	1 c.c. 10% Ring.	9.99
169	0.6 c.c. 3N HCl	0.3 c.c. sat. Ba(OH)2	50 unf. eggs *plus* 10% Ringer = 1 c.c.	13.90
170	06. c.c. 3N HCl	0.3 c.c. sat. Ba(OH)2	50 fert. eggs *plus* 10% Ringer = 1 c.c.	20.00

creased from a carbohydrate level (unfertilized) to a fat level. Now, BRACHET's failure in detecting high RQs for the early stages of R. *fusca* seems to me entirely due an inadequate procedure of measuring the RQ within appropriate limits of time. In my experiments, care was taken to somemeasure the respiratory exchanges as to determine RQs corresponding to fractionated parts of cleavage, gastrulation, neurulation and so on. In many cases, the experiment startet even before the first signs of cleavages had appeared. These experiments with such early stages of development never advanced further than stage 16 (16 cells). As to other stages, an experiment, as a rule, never comprehended more than two successive stages, so that the duration of the experiments was always relatively short (one to one and a half hours, only exceptionally 2 hours). Now, BRACHET (1935) claimed that that a period of time between 12-15 hours is needed to measure the RQ of the early stages. Less than two hours, however, proved to be a long enough period for measuring both the oxygen uptake and the CO_2 output of *R. pipiens*, even in experiments starting with just fertilized eggs. Besides, MOOG (1.c.), within one to one and a half hours, was also able to measure the oxygen uptake of the same frog. On the contrary, one cannot avoid the conclusion that the long duration of BRACHET's experiments makes hard to accept his reported RQs as really corresponding to the stages they were attributed to. In fact, he started his experiments from the 2-cell stage on and extended them up to 17 hours. In determining the RQs he assigns to "cleavage", his shortest experiment lasted 11 hours. Now, *R. pipiens*, at 18°C (SHUMWAY's tables), in experiments starting at the 2-cell stage, at the end of 17 hours, would be at mid or late cleavage. At 20°C, temperature used by BRACHET, a faster development being expected, the embryos would be probably at the beginning of gastrulation. Blastulæ of *R. pipiens*. under the same conditions of those of BRACHET's *R. fusca*, that is, in experiments starting at the stage of "blastules avancées" and lasting 13-14 hours, would surely be at mid gastrula at the end of the experiment. As it is likely that BRACHET's *R. fusca* does not significantly differ from *R. pipiens* in what respects the duration of the development, it seems almost safe to say that BRACHET's RQs do not correspond the stages they were assigned to, but to much longer embryonic periods. In the case of the cleavage stages, for instances, the majority of BRACHET's RQs would rather express the respiratory quotient of the whole cleavage period up to early gastrula. Besides, an indication that BRACHET failed to obtain high RQs during the early stages of cleavage only because he used too long experiments results from his own data (1934, p. 670). In fact, the so-called "two blastomeres" stages and one of the "four blastomere" exhibited RQs above 0.7. In subsequent stages, the RQ always remained below 0.7. Thus, had Brachet's experiments initiated with two or for blastomeres not been so extended in time, he probably would have detected higher RQs for these early stages.

BIALACEWICS and BLEDOWSKI (1.c.), in spite of doing experiments of shorter duration (102 to 210 minutes), also reported low RQs for the early stages of cleavage of *R. temporaria*. They used, however, a bad procedure to detect the CO_2 output and did not care about bound CO_2 (BRACHET 1934, p. 662). Furthermore, they started their experiment 6-24 hours after fertilization, "c'est à dire, à un moment ou le development était déjà rela-

tivement avancé", as BRACHET pointed out. Finally, they observed a curious "neutral gas" eliminated by the eggs soon after they came out of the ovaries, which was never re-observed either by BRACHET (1934) or ATLAS (1. c.) or in my experiments. BRACHET considers this "neutral gas" as mere consequence of bad technique.

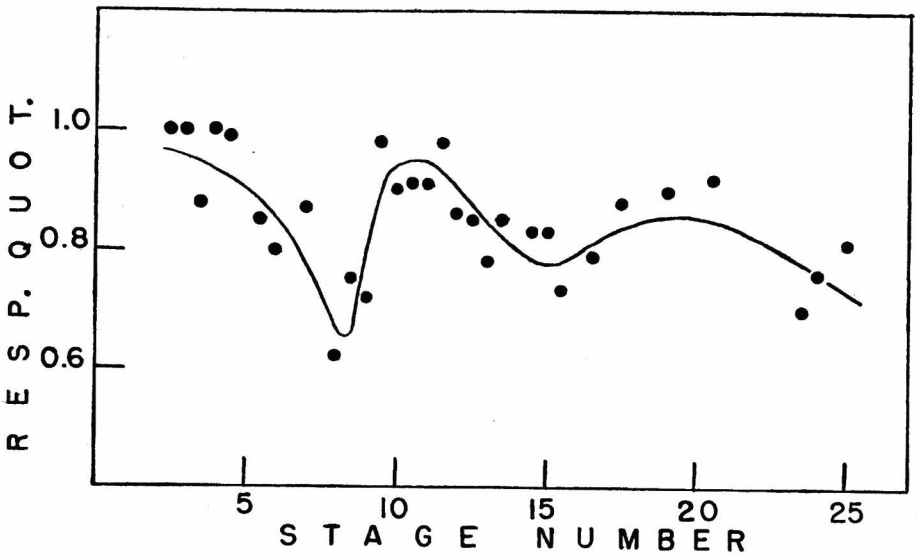

Fig. 1 Repiratory quotients during the diferent embryonic stages of *R, pipiens*

BARTH (1.c.) started his experiments from the two-cell stage on. For the pregastrular stages he reported RQs generally below 0.9 in the cases of *R. pipiens* x *R. pipiens* (N) and *R. pipiens* x *R. sylvatica* (H), although in 3 cases starting from the two-cell stage high RQs were observed (1.02 N, 1.05 H and 1.05 N). The sudden rise of the RQ to a carbohydrate level observed by BRACHET and by me at the beginning of gastrulation is not so marked in BARTH's experiments. BARTH himself recognizes that his experiments are objectionable on technical grounds. In fact, in determining the oxygen uptake and the CO_2 output, he used the so-called "three manometer method" (vessels with only one side-bulb) and this, according to him, leads to a measurement of the CO_2 production "in a manner which includes the error of oxygen consumption measurement and two errors of CO_2 measurement". Besides, as BRACHET, BARTH extended his experiments for too long periods of time. His experiments starting with two-cell stages, for instances, lasted as a rule, more than 5 hours. It is curious to remark that in the few cases where 3 hour experiments were run high RQs were obtained.

Rana pipiens unfertilized eggs exhibited RQs as high as those of the fertilized eggs undergoing the very first cleavage steps. This finding opposes BIALACEWICS and BLEDOWSKI's information that in *R. temporaria* the RQ of the unfertilized egg is as low as that of the fertilized. BACHET (1935)

also found high RQs for the unfertilized egg of *R. fusca*. The fact that in *R. pipiens* fertilization does not essentially affect the respiratory rate of the eggs confirms previous results obtained by BRACHET (1935) in *R. fusca* and STEFANELLI (1937) in *Bufo*.

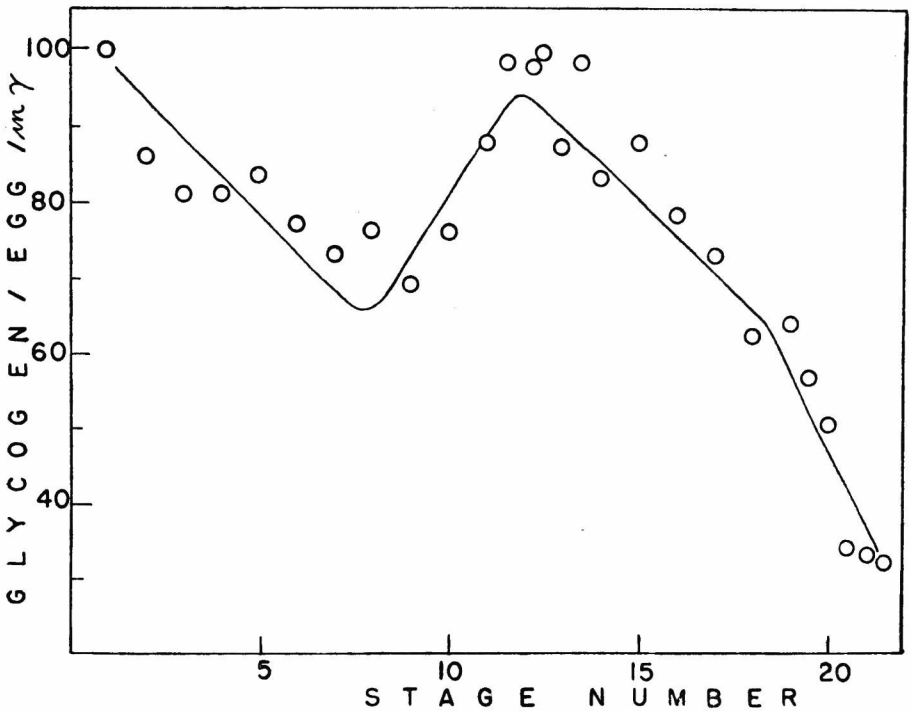

Fig. 2 The glycogen content of eggs *R. pipiens* during development. From GREGG and POMERAT's data.

As BRACHET (1934) pointed out, not much can be said about the chemical events of an organism from the simple analysis of the respiratory quotient. The RQ is the measurable expression of global oxidations in the cells and only in cases where sufficient knowledge of the oxidative intracellular reactions is available, its determination is precious in helping to understand what is going on inside the cells. For instances, a fall in the glycogen content and an increase in lactic acid formation determined concomitantly with an RQ near unity is a good indication of carbohydrate metabolism. Now, judging simply from the RQs obtained in the course of development of *R. pipiens*, one might suggest that soon after fertilization and during the very first steps of cleavage the egg possesses a carbohydrate metabolism. As cleavage proceeds, this type of metabolism gradually would change to a fat metabolism, whose maximum coincides with the period between mid and late cleavage. At the beginning of gastrulation a return to carbohydrate as energy source would take place which would persist

throughout gastrulation to be replaced towards neurulation by a fat-protein metabolism. Glycogen or lactic acid determinations made during the development of the frog's egg are, in some instances, in agreement with this interpretation of the RQs. KONOPACKI and KONOPACKA (1926), using a histochemical method, reported a loss of glycogen from the fertilized egg onwards, partially restored at gastrulation and steadily increasing again after neurulation. BRACHET and NEEDHAM (1935), although confirming the post-gastrular loss, did not find in *R. fusca* a glycogen loss before gastrulation. However, as GREGG and POMERAT (1942) pointed out, BRACHET and NEEDHAM did not report estimation of glycogen for stages suceeding fertilization and preceding gastrulation. GREGG and POMERAT, with an improved technique, obtained positive evidence that, in *R. pipiens*, even before gastrulation, the glycogen content diminishes. Starting from the just fertilized egg on and referring the stages to SHUMWAY'S tables, they showed a loss of glycogen from the fertilized egg (14% already at the grey crescent stage) to late cleavage (maximum 31%). A resynthesis occurs at early gastrula, the glycogen content reaching almost its original level at a stage just preceding the neural plate formation. Fig. 2 shows a curve drawn from GREGG and POMERAT'S data, the slope of which recall that of Fig. 1. Thus, *R. pipiens* developing egg would make large use of glycogen during early cleavage and, in preparation for gastrulation would largely resynthetize it. The lactic acid content of the developing egg of *R. temporaria* was measured by LENNERSTRAND (1934). Unfertilized and just fertilized eggs, under anaerobic conditions, showed about the same lactic acid content. Under anaerobiosis, where the lactic acid formation can be better detected, the results were the following: the unfertilized egg showed in about 20 hours a remarkable increase in lactic acid, the same being observed with the early cleavage stages and early gastrulas. The results, thus, indicate that *R. temporaria* egg makes large use of glycogen at the stages of unfertilized egg, early cleavage and gastrulation, hence, in accordance with the RQs here reported for *R. pipiens*. BRACHET (1934) reported that fertilization seems to have no effect upon the glycolysis of the egg, since unfertilized and fertilized eggs of *R. fusca* exhibited both a practically inexistent aerobic glycolysis. However, in opposition to LENNERSTRAND he observed a pronounced glycolysis in aerobisis from the gastrula stage on, which he related to the sudden rise of RQ at the beginning of gastrulation. Since, however, BRACHET (1935) found posteriorly high RQs (mean 0.99) for *R. fusca's* unfertilized eggs, no explanation is given for the fact that he could not detect lactic acid formation before fertilization.

As an attempt to correlate the RQs determined for the different stages of *R. pipiens* development with morphogenetic processes, one might say that carbohydrate burning occurs at stages charaterized by intense dynamism, such as the first cleavage steps (when cellular division meets with the difficulty of splitting thick masses of vitellus) or gastrulation (when great cell movements and important morphogenetic processes, such as preparation for neurulation, are observed). In *Amblystoma mexicanum* (BOELL, KOCH and NEEDHAM 1939), as soon as gastrulation begins, the RQ of the dorsal lip region rises to unity and almost so does that of the ventral region. To NEEDHAM (1942) and WOERDERMANN and HEATLEY (NEEDHAM 1942, p. 202) this may imply a predominant carbohydrate metabolism associated

with the release of the primary organiser. An objection against this interpretation, however, arises from the also high RQs determined for unfertilized eggs, whose metabolic activity is probably lower than that of the developing egg.

Finally, some remarks on the values expressing the respiratory exchanges in *R. pipiens*. The oxygen consumption increased steadily throughout the development. No effort, however, was made to detect that distinct inflection in the curve towards the beginning of gastrulation as reported by ATLAS (l.c.), BARNES (l.c.) and MOOG (l.c.) in *R. pipiens* and by Boell (1945) in *Amblystoma*. BRACHET (1934) found that the absolute values for oxygen consumption of some stages of *R. fusca* strongly varied from one batch to other. This was not the case in *R. pipiens*. For instances, in experiments with 5 different batches, early stages of cleavage showed a maximum oxygen consumption (in cu.mm/50 eggs/h) of 6.6 and a minimum of 3.5 (mean 5.1). BRACHET found that, in *R. fusca*, the chemically bound CO_2 in the eggs is but a weak fraction of what he calls "total respiratory CO_2", that is, the CO_2 eliminated and absorved by the alkali (also called "libre") plus the chemically bound CO_2, and that it increases only slightly throughout the development. This would indicate that the egg can at each instant of development neutralize the carbonic acid from respiration by forming an alcaline reserve. In *R. pipiens*, if one considers :

"Initial CO_2" = egg bound CO_2 + CO_2 as BaCO + CO_2 in Ringer (1)

and the fact (see table II) that the "initial CO_2" steadily increased throughout the development, the conclusion is that the egg bound CO_2 remarkably increased during the development, since in (1) the second and third terms of the second member of the equation remained practically constant.

Table IV shows that in the unfertilized egg or in the earliest stages of cleavage, the bound CO_2 is relatively small. Fertilization, however, seems to produce a slight increase in the amount of bound CO_2.

Summary

1. Experiments were made with the different embryonic stages of *Rana pipiens* in order to determine the respiratory quotient of each stage or small groups of successive stages.

2. The main purpose of the work was to find out whether or not the common New England frog shows the same fluctuation of RQ values during the development as reported by BRACHET (1934) for *R. fusca*, that is, high RQ for the unfertilized egg, low RQs from the fertilized egg up to late cleavage, a sudden rise to unity at the beginning of gastrulation up to hatching.

3. The results obtained in *R. pipiens* (table I) did not confirm the existence of low RQs soon after fertilization, although towards mid and late cleavage low values were registered. The sudden rise to unity was also observed at the beginning of gastrulation. As to the postgastrular stages, the RQs obtained were as a rule not so high as those reported by BRACHET.

4. BRACHET'S failure in detecting high RQs during early stages of cleavage is attributed to the fact that his experiments lasted too long, so that the values assigned to early stages are better representative of the whole cleavage period up to early gastrula (see discussion).

5. GREGG and POMERAT'S careful determination of the glycogen content of *R. pipiens* developmental stages and LENNERSTRAND'S measurements of the lactic acid during the development of *R. temporaria* are quoted as chemical evidence in favor of the sequence of RQ determined throughout the development of *R. pipiens*.

6. BRACHET'S sequence of RQ was not in agreement with NEEDHAM'S theory of a succession of energy sources in ontogenesis. The sequence established for *R. pipiens* in the presen work also opposes this theory.

7. A correlation between high RQs and periods of stronger dynamism (first cleavage steps, gastrulation) is suggested.

8. No significant differences were detected between the rates of oxygen consumption of the unfertilized and the fertilized eggs. The oxygen consumption increases steadily during the development, as already observed in previous works. The bound CO_2 also increases during the development, rising from a relatively small value to a high one (towards neurulation).

Sumário

1. Fizeram-se experiências com diferentes estádios embrionários de *Rana pipiens* a fim de se determinarem os quocientes respiratórios (QR) de cada estádio ou pequeno grupo de sucessivos estádios.

2. A finalidade principal da pesquisa foi verificar se a rã comum da Nova Inglaterra (Estados Unidos) mostra a mesma flutuação de valores do QR durante o desenvolvimento embrionário que a rã europea, *R. fusca*, de acôrdo com o trabalho de BRACHET (1934). Segundo êsse autor, o QR do ovo não fecundado é aproximadamente 1.0 e, como consequência da fecundação, baixa a um valor indicativo de metabolismo preponderantemente lipídico, até o início da gastrulação, quando retorna à unidade, para aí permanecer mais ou menos até a fase de vida livre.

3. Os resultados obtidos em *R. pipiens* (tabela I) não confirmam a existência de baixos QRS logo após a fecundação, embora os assinalem da metade para o fim da segmentação. O súbito retorno à unidade também foi observado no início da gastrulação. Quanto aos estádios postgastrulares, os QRS obtidos não foram, via de regra, tão altos quanto os observados por BRACHET in *R. fusca*.

4. O fato de Brachet não ter obtido QRS altos logo após a fecundação ou durante os primeiros passos da segmentação é atribuido a que, na suas experiências iniciadas com tais estádios embrionários, êle fez uso de tão longos tempos experimentais que os valores obtidos para os QRS seriam com mais propriedade referentes a todo o período de segmentação até a gastrula (veja discussão).

5. A cuidadosa determinação do teor de glicogênio nos estádios embrionários de *R. pipiens* feita por GREGG e POMERAT e as medidas de ácido lático durante a embriogênese de *R. temporaria* feitas por LENNERSTRAND são invocadas como provas químicas da sequência de QRS determinadas para *Rana pipiens*.

6. Os resultados de BRACHET (1934) não concordam com a teoria de NEEDHAM sôbre a existência na ontogênese de uma sucessão de fontes de energia (carbohidratos, depois proteinas, depois gorduras). A sequência estabelecida em *Rana pipiens* também se opõe a essa teoria, pois que, do meio para o fim da segmentação, os QRS descem a um nível lipídico e no início da gastrulação retornam ao nível que sugere a utilisação de carbohidratos.

7. Sugere-se uma correlação entre os altos QRS e os períodos de mais intenso dinamismo (primeiras segmentações, gastrulação) do desenvolvimento embrionário.

8. Não foram observadas diferenças significativas entre as taxas de consumo de oxigênio do ovo fecundado e não fecundado. O consumo de oxigênio aumenta com constância durante o desenvolvimento, como fôra observado préviamente. O "CO_2 ligado" também aumenta durante o desenvolvimento, elevando-se de um valor relativamente baixo a um alto valor (ao redor da neurulação).

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