RESPIRATION AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE DEPENDENT ENZYMES IN LETHAL AMPHIBIAN HYBRIDS

RANA NIGROMACULATA BREVIPODA ♀ × *RANA RUGOSA* ♂¹⁾

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It is well known that the development of lethal hybrid eggs produced experimentally by the interspecific crosses between anurans or between urodeles is arrested at the late blastula or early gastrula stages (Moore, 1946; Baltzer, 1952; Brachet, 1952; Zeller, 1956; Gregg, 1957). Development of the eggs of the lethal amphibian hybrid is subsequently followed by the gradual impairment in their metabolic activities when their development is arrested (Barth, 1946; Gregg, 1948; Steinert, 1951; Chen, 1953; Brachet, 1954; Chen, 1954; Gregg and Løvtrup, 1956). Based upon those findings, an attempt was made to ascertain whether or not respiration and nicotinamide adenine dinucleotide phosphate (NADP) dependent enzymes are less active in hybrid eggs than in normal eggs.

MATERIALS AND METHODS

Eggs derived from a single female frog, *Rana nigromaculata brevipoda*, were partly inseminated with *Rana rugosa* sperm and partly inseminated with sperm of the same species as the control. Both hybrid and control eggs were reared in 10 per cent amphibian Ringer's solution without phosphate and bicarbonate, at room temperature (18-23°C.).

The oxygen consumption of both normal and hybrid eggs before and during gastrulation was measured with the aid of a Warburg type manometer according to the method of Barth (1946).

The method for assaying NADP dependent enzymes was essentially the same as that described by Glock and McLean (1954). The gastrula-arrested hybrid eggs, and their control with the same developmental hours after the fertilization, were used as materials. After removing the jelly-coats with the aid of Jeweler's forceps, 400 eggs were homogenized with a glass tissue homogenizer in 5 ml. of ice-cold potassium chloride (0.15 M, pH 7.0), and the homogenates were diluted to 6 ml. After centrifugation (8000 r. p. m. for about 20 min.), the supernatants obtained were used for assaying the enzyme activity without dialysis. The dehydrogenase activity was determined spectrophotometrically by following the rates of reduction of NADP (TPN, Sigma Chem. Co.) at 340 m μ .

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RESULTS AND DISCUSSION

It has been reported that the rate of respiration of hybrid eggs showed a normal increase during early development; however, no further increase occurred when the development was arrested (Barth, 1946). Like most of the interspecific crosses between anurans, the hybrid eggs employed in the present experiment were lethal and their development was arrested at the early gastrula stage. The development of both the normal and hybrid eggs was synchronized until they reached the onset of gastrulation.

Table 1. Rate of oxygen consumption in the lethal hybrid *Rana nigromaculata brevipoda* ♀ × *Rana rugosa* ♂. (Rate—cu. mm per hour per 100 eggs)

Exptl. series	Age in hours after fertilization	Developmental stage at beginning of measurement*	Oxygen consumption		
			Control	Hybrid	H/C (%)
1	14 : 05	Early gastrula	14.6	11.5	78.8
	19 : 15	Middle gastrula	17.2	11.3	65.6
2	10 : 53	Blastula	12.7	11.8	92.9
	14 : 13	Early gastrula	14.6	12.5	85.6
	18 : 08	Middle gastrula	21.5	12.8	59.5
	20 : 33	Late gastrula	19.8	10.6	53.2

* Developmental stages were determined by reference to the chart of Shumway (1940).

Table 1 shows clearly that the oxygen consumption of the lethal hybrid was somewhat lower than that of the normal eggs during blastulation, and was greatly reduced; i.e. about 50 per cent of that of the control, at the stage when development was arrested, although the eggs were still alive. This was confirmed by twenty experiments which will be published elsewhere. The present results confirm previous results indicating the lower rate of oxygen consumption in the lethal hybrids of the combination *R. pipiens* ♀ × *R. sylvatica* ♂ (Barth, 1946), *R. esculenta* ♀ × *R. fusca* ♂ (Brachet, 1954), *Triton palmatus* ♀ × *Salamandra atra* ♂ and *T. taeniatus* ♀ × *S. atra* ♂ (Chen, 1953).

As was indicated, the activity of NADP dependent enzymes was compared between the gastrula-arrested hybrid and the control at the same developmental age. The activity of NADP dependent enzymes in the present experiment measured at pH 7.6 with glucose 6-phosphate as substrate is the summation of glucose 6-phosphate and 6-phosphogluconic acid dehydrogenase activities. A typical result illustrated graphically in Fig. 1 indicates that the rate of reduction of NADP with the supernatants of lethal hybrid egg homogenates was lower than that of the control. The lower activity of NADP dependent enzymes in the lethal hybrid eggs was noted throughout this series of experiments, although the enzyme activity was almost identical in both the hybrid and control at their blastula stage.

Subnormal glycolysis in *R. pipiens* ♀ × *R. sylvatica* ♂ hybrid embryos has been reported (Gregg, 1948). It was reported in the experiment with the lethal hybrid of

Fig. 1 Graph showing rate of reduction of NADP measured at 340 mμ in 0.05 M glycyl-glycine buffer, at pH 7.6, in the presence of 0.5 ml. of supernatant (Total nitrogen content: 365.5 μg/0.5 ml. for the control; 539.0 μg/0.5 ml. for the hybrid), 0.5 ml. of 0.1 M KCl, 0.2 ml. of NADP (1 mg/ml) and 0.125 ml. substrate (0.005 M).

1) The rate of fertilization: 97.2 % for the hybrid; 96.5 % for the control. Eggs exhibiting the normal cleavage pattern were selected. The homogenates were prepared from the material aged 20 hours 15 minutes after insemination.

2) Open circle: Hybrid. Solid circle: Control.

R. esculenta ♀ × *R. fusca* ♂ that RNA synthesis, which is low during gastrulation in the hybrid as well as in the normal eggs, does not undergo the remarkable increase which appears at neurulation in normal embryos (Steinert, 1954). Identical results indicating the lower values of the RNA and DNA contents in anuran and urodele hybrids were reported (Chen, 1954; Gregg and Løvtrup, 1956; Zeller, 1956).

As was suggested by Brachet (1965), the developmental block in the lethal hybrids, which is originated from the introduction of foreign DNA, may be the reflex of the production of abnormal messengers and of the synthesis of abnormal proteins in the cytoplasm.

The evidence that activity of the NADP dependent enzymes in the early reactions of the hexosemonophosphate shunt was depressed in the lethal hybrid suggests a subnormal synthesis of pentoses necessary for nucleic acid synthesis. In addition, the lower rate of respiration of the hybrids indicates an impairment not only of the respiratory system but also of the steps of the hexosemonophosphate shunt which needs the introduction of molecular oxygen.

SUMMARY

The rate of respiration of lethal hybrid embryos begins to diminish at the late blastula rather than before the retardation of gastrulation is appreciable. Evidence

that the activity of NADP dependent enzymes was also lower in the arrested-gastrula suggests an inhibition of the pentose cycle.

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