AGE RESISTANCE OF FROGS TO HYPOXIA COMBINED WITH TOXIC DEPRESSION OF RESPIRATORY PHOSPHORYLATION

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(Received March 28, 1958. Presented by V. V. Parin, Active Member of the AMN SSSR)

The toxic effects of 2,4-dinitrophenol (DNP), a poison which depresses the phosphorylation connected with respiration [2], are greatly enhanced in Rana temporaria by the exclusion of pulmonary respiration with the aid of curare or urethan. These experiments showed that frogs in the younger age groups (first and second years after metamorphosis), hereafter called "young frogs", are considerably more resistant than adult frogs to the combination of hypoxia, developing due to the exclusion of respiration, with toxic depression of respiratory phosphorylation. This article reports the results of experiments which we conducted in order to analyze the age difference in the resistance of Rana temporaria to this combination.

EXPERIMENTAL METHODS AND RESULTS

In the young and adult frogs, respiration was excluded by means of curare, a poison which causes peripheral paralysis of the respiratory musculature, or urethan, a narcotic which causes a central respiratory paralysis. The subcutaneous injection of DNP (in doses which would not affect the behavior of the animals) into the immobilized frogs caused the rapid death of all the adult frogs; in the young frogs, however, the poisoning developed far more slowly, and all of the animals did not die (Table 1).

The data in Table 1 deserve particular attention since the question of age resistance in cold-blooded animals is, in itself, very interesting; there is very little information on this subject in the literature [3, 4, 9].

Since there are not as yet any exact methods of determining the age of frogs, our division of the experimental frogs into 3 age groups was more or less conditional. However, the orientation indices of frogs' age which we used were the same as those used in the zoological literature [1, 8]. We allotted animals weighing 0.5-3 g, with body length of 20-35 mm (from tip of muzzle to anal orifice) to the first-year group young frogs which had undergone metamorphosis from the tadpole stage the preceding summer. The second-year group was made up of frogs weighing 5-14 g, with a body length of 40-60 mm; two-year frogs had no secondary sex characteristics. Animals weighing more than 18 g, with a body length of more than 62 mm, were regarded as adult frogs. All the third and fourth year frogs had secondary sex characteristics. The frogs used in the experiments were, for the most part, animals whose indices were most typical of their age group (for example, first-year frogs which weighed 1-1.5 g, second-year frogs weighing 6-9 g and adults weighing 20-30 g).

The experiments were conducted during the fall and winter months of the 1954-1955 and 1955-1956 seasons on 1369 animals: 695 first-year frogs (400 used as control), 232 second-year frogs (117 used as control) and 442 adult frogs (222 used as control).

There are three possible explanations of the higher resistance of first-and second-year frogs to the combination of hypoxia with toxic depression of respiratory phosphorylation: 1) young frogs are more resistant to the

TABLE 1

Age Differences in the Resistance of Frogs to Hypoxia Combined with Toxic Depression of Respiratory Phosphorylation

Time after DNP injection	Number of animals with cardiac arrest			
	urethan + DNP		curare + DNP	
	adults	young frogs	adults	young frogs
	number of animals in experiment			
	30	30	40	40
1 hour	7	2	0	0
1 hour 30 minutes	30	5	0	0
2 hours	—	11	1	0
3 hours	—	14	23	2
4 hours	—	15	33	4
5 hours	—	16	40	4
24 hours	—	21	—	14
Survived more than 2 days	0	9	0	26

Note: In all the experiments, half the minimal lethal dose of DNP was injected on a background of curare or urethan respiratory paralysis.

TABLE 2

Age Differences in Resistance of Frogs to Hypoxia of Uniform Degree Combined with Toxic Depression of Respiratory Phosphorylation (experiments with pressure chamber)

Age of Frogs	Dose of DNP in γ/g	Pressure in chamber (in mm of mercury)	Number of animals	Number that died
Adult	10	50	5	0
Second-year	10	—	5	1
Second-year	—	50	5	0
Second-year	10	50	5	4
First-year	10	—	5	0
First-year	—	50	5	0
First-year	10	50	5	5

toxic effect of DNP; 2) young frogs are more resistant to hypoxia, and also, possibly to a combination of these two factors; 3) young frogs and adult frogs are affected differently by the exclusion of respiration, so that the resultant degree of hypoxia is less in the former than in the latter. Different series of experiments were conducted to verify these different possibilities.

The resistance of frogs of different ages to the toxic action of DNP was investigated in 4 series of experiments.

We performed experiments with immersion, placing 10 first-year frogs and 10 adult frogs in glass exsiccators. Fifty ml of a 0.1% solution of DNP was poured into the smaller exsiccator containing the first-year frogs, and 1000 ml of the same solution into the larger exsiccator containing the adult frogs. The first-year and adult animals were immersed in equal depths of the solution; with the frog in its customary position, the solution came up to the shoulder girdle of the animal, and pulmonary respiration was not hindered. The toxic effect of DNP was estimated according to disturbance of the active pose (replacement of sitting position with a horizontal position, with extended extremities) and to the development of paralysis of the animal's voluntary movements. Paralysis usually developed 1-1½ minutes after the loss of the active pose.

In the immersion experiments, the active pose was disturbed sooner in the first-year frogs than in the adults (after an average of 34.6 and 47.6 minutes respectively). Since it was possible that the DNP entered the body through the skin unequally in the first-year and adult frogs due to the differences in the permeability of the skin of these two groups and in the amount of skin surface (in relation to body weight), we performed experiments in which the poison was subcutaneously administered, so that these complicating factors were eliminated.

With the administration of a uniform dose of DNP (40 γ/g) – more than the absolute lethal dose – the resistance of the frogs was estimated according to the speed with which motor paralysis developed.

Age of frogs	Number of frogs	Time paralysis set in (in minutes, extreme intervals)
First-year	10	14 – 36 (average 23.2)
Second-year	10	10 – 22 (average 17)
Adult	10	15 – 32 (average 26.5)

As the figures show, no material differences in the resistance of frogs of different ages to the toxic action of DNP were found in this experimental series (although we did get the impression that the young frogs were less resistant).

When the uniform doses of DNP which were injected were smaller than the absolute lethal dose, comparison of the resistance of the two groups (on the basis of the number of frogs which died) showed that the first-year frogs were less resistant; this dose of DNP caused the death of 8 out of the 10 first-year frogs within two days, but of only 4 of the 10 adult frogs.

When the effects of the minimal and absolute lethal doses were compared, it was found, as in the preceding experiments, that the first-year frogs were less resistant.

The reasons for the lesser resistance of these frogs will not be taken up in this paper. We only mention in passing that newborn rats and mice are also less resistant to DNP than adult animals, probably because of the imperfect state of the detoxication processes in young animals [7].

Therefore, the reason for the greater tolerance of young frogs for hypoxia combined with toxic depression of respiratory phosphorylation is not a higher resistance on their part to the toxic action of DNP.

Resistance of frogs of different ages to hypoxia. It has long been known that adult frogs can endure prolonged (about 25 days) paralysis caused by large doses of curare without ill effects [10]. We showed that if the skin is kept considerably moist constantly, young frogs can also easily endure curare paralysis for many days. Therefore, one cannot compare the resistance of frogs of different ages to hypoxia by using curare, for the hypoxia caused by curare is mild and apparently does not independently cause disturbances in the animals. Therefore, we decided to perform experiments in which the frogs were subjected to the action of low partial oxygen pressure in a pressure chamber.

Adult and first-year frogs were simultaneously placed in a pressure chamber in which the air was carefully humidifed. The greatest fall of partial oxygen pressure possible under the conditions of our method (vacuum – 36-37 mm of mercury) was created with the aid of a Komovskii pump. The animals were kept for 30 minutes in this vacuum. The air temperature in the chamber was 19°.

We present the results of 2 experiments (each with 5 adult and 5 first-year frogs):

	Number of frogs	Lost reflex of rolling over from back	Died
First-year	10	7	2
Adult	10	0	0

In the first-year frogs which survived, the reflex of rolling over when placed on their backs was restored 15 minutes after they were taken out of the pressure chamber.

The results of these experiments surprised us, since it is a well-known fact that the younger age groups of warm-blooded animals tolerate a fall of partial oxygen pressure in a pressure chamber better than adult animals.

Therefore, the greater resistance of young frogs in the subject case is not due to any greater resistance on their part to a combination of hypoxia with toxic depression of respiratory phosphorylation.

Different effect of exclusion of pulmonary rexpiration on young frogs and adult frogs. The literary data give reason to believe that exclusion of pulmonary respiration causes a lesser degree of hypoxia in young frogs than in adults: there is a greater quantity of oxygen entering through the skin in the former than in the latter, because the skin surface (in relation to body weight) is greater in the smaller animals. In order to prove the application of this theory to our experiments, we calculated (according to the well-known Benedict formula, somewhat modified by P. V. Terent'ev to facilitate calculation) the surface of the skin in frogs of different ages and the ratio of the surface to the body weight:

$$\log S = 1.0253 + 0.6667 \log p,$$

where S is the body surface in cm^2 and p is the body weight in g.

These calculations showed the ratio of the skin surface to the body weight (in cm^2/g) to be 8 in first-year frogs, 4.92 in second-year frogs and 3.6 in adults.

Therefore, after exclusion of respiration, when oxygen could only enter the frogs' blood through their skin, it is clear that the young frogs considerably less affected by hypoxia than were the adult animals. In this connection, the question arose of the resistance of frogs of different ages to a uniform degree of hypoxia combined with DNP poisoning. We obtained the answer to this question in experiments using the pressure chamber.

Resistance of frogs of different ages to a uniform degree of hypoxia combined with DNP poisoning. Frogs of the three age groups were placed simultaneously (under uniform conditions) in a pressure chamber and subjected to the action of low partial oxygen pressure, created in 3 minutes by a vacuum of about 50 mm of mercury. The animals were kept in the pressure chamber at this pressure for 25 minutes. DNP was injected subcutaneously 1 hour before the start of the vacuum (Table 2).

	Lethal doses	
	Minimal (in γ/g)	Absolute LD100 (in γ/g)
First-year	10 – 12	15 – 22
Adult	18 – 25	30 – 32

When hypoxia of uniform degree was combined with DNP poisoning, it is evident from Table 2 that the first and second-year frogs were considerably less resistant to the combination. These data are contradictory to the results of observations on warm-blooded animals of different ages [5, 6]; this is probably due to the differences in the resistance of warm and cold-blooded animals to hypoxia. The experiments in the pressure chamber confirmed the opinion expressed above that the exclusion of pulmonary respiration by means of curare or urethan creates a lesser degree of hypoxia in young frogs than in adult animals. This also explains the greater resistance of young frogs to exclusion of respiration combined with toxic depression of respiratory phosphorylation.

SUMMARY

Experiments were performed on Rana temporaria during their first and second year of life after metamorphosis. It was demonstrated that in comparison with adult frogs (aged 3 to 4 years) they possess a higher resistance to the combination of exclusion of respiration (by means of curare or urethan) with 2,4-dinitrophenol (DNP) intoxication. These age differences of resistance may apparently be explained by the fact that in younger animals the skin surface is more extensive, which provides a better oxygen supply after exclusion of the respiration. Young frogs possess a lower resistance to DNP intoxication and are less resistant than adult animals to low partial oxygen pressure, as well as to the combination of low partial oxygen pressure with DNP intoxication.

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* In Russian.

** Original Russian pagination. See C.B. Translation.