THE COMBINED EFFECT OF CYTOCHROME SYSTEM POISONS AND POISONS INTERFERING WITH RESPIRATORY PHOSPHORYLATION ON THE TOLERANCE OF MICE TO LOWERING OF ATMOSPHERIC PRESSURE

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(Received March 23, 1957. Presented by Active Member Acad. Med. Sci. USSR V. V. Zakusov)

The present investigation was prompted by our observation which showed, unexpectedly, that sodium azide and potassium cyanide increased the tolerance of animals to lowering of atmospheric pressure.

It is known that sodium azide and potassium cyanide block the cytochrome system in the tissues. In recent years a number of investigations performed on isolated tissues [7, 8, 11 etc] showed that azide could interfere with the processes of conjugated phosphorylation; this interference can be effected in concentrations insufficient to impair utilization of oxygen mediated by the cytochrome system [4].

EXPERIMENTAL METHODS

The experiments were performed in a pressure chamber (capacity 5 liters); the animals used were adult white mice weighing from 16 to 22 g. The rate of decompression was equal to 1 minute in all experiments, the time spent by the animals in the pressure chamber was 10 minutes. The experiments were thus staged under conditions of acute and brief oxygen lack. In addition to the 2-3 experimental animals the same number of control animals from the same batch were placed in the pressure chamber. Sodium azide and potassium cyanide were administered subcutaneously in the form of 0.1-0.2% solutions in doses of 15 γ per 1 g body weight. It must be noted that the potassium cyanide available was partially decomposed and therefore the minimal lethal dose (20 γ per 1 g body weight) was approximately double that given in literature references. The doses of azide used did not produce any changes in the behavior of the animals, while administration of cyanide was accompanied, in a number of experiments, by limitation of the animals' motor activity.

EXPERIMENTAL RESULTS

Results of the first series of experiments are presented in Table 1.

TABLE 1

Effect of Sodium Azide and Potassium Cyanide on the Tolerance of Mice to Lowering of Atmospheric Pressure (220-230 mm Hg)

Doses in γ per 1 g		Interval between injection of poisons and placing in pressure chamber (in minutes)	Number of animals		Number of survivals out of 5
NaN_3	KCN		Total in experiment	Survived	
–	–	–	75	11	1
15	–	10	5	0	0
		25-45	30	25	4
		90	5	0	0
–	5	5	5	0	0
		10-35	25	20	4
		45-50	5	1	0

As can be seen from Table 1, both sodium azide and potassium cyanide not only did not diminish the tolerance of mice to lowering of atmospheric pressure, but on the contrary increased this tolerance. This effect became apparent in 15-20 minutes when azide was used and continued for not more than 1½ hours. When cyanide was used the increased tolerance to hypoxia became evident within 10 minutes but proved to be more short-lived, disappearing after 45 minutes. Smaller doses of azide did not affect the animals' tolerance to lowering of atmospheric pressure.

In order to discover whether this increase in tolerance was connected with blocking of the cytochrome system or other links in tissue respiration we made use of data contained in the thesis of Z. N. Ivanova (1939). According to these data, administration of azide in the dose corresponding to approximately 60% of the lethal one led to a sharp drop in the animals' tolerance to cyanide intoxication: the mice died from 50% of the lethal dose of cyanide. Such decreased tolerance persisted up to 48 hours, whereas decreased tolerance to azide following preliminary administration of cyanide disappeared with 30-80 minutes. Z. N. Ivanova concluded that the duration of cytochrome system block elicited by these two poisons was very dissimilar.

Our experiment was performed as follows. 20 mice were given 15 γ per 1 g body weight azide and after 24 hours half the mice were checked for tolerance to lowering of atmospheric pressure and the other half for tolerance to cyanides.

It was found, as should have been expected from the data of the first series of experiments, that the tolerance to lowered atmospheric pressure was no different in animals treated with sodium azide 24 hours previously from that in the control (unpoisoned) mice: in the control group one mouse out of 10 survived decompression of 250 mm Hg, while 2 out of 10 survived in the experimental group. At the same time, the animals' tolerance to cyanide proved to be sharply reduced: of 10 control mice (which received cyanide only in the dose 10 γ/g) 9 survived, whereas of 10 experimental mice (which were given azide the previous day and cyanide on the day of the experiment) only one survived. Smaller doses of azide (10 γ/g) did not reduce the tolerance to cyanides.

Thus, 24 hours after administration of azide it still decreases tolerance to cyanides sharply but has no effect on tolerance to lowering of atmospheric pressure. The results of these experiments give grounds for distinguishing two toxic effects of azide: a more prolonged one, discovered by Z. N. Ivanova and expressed in reduction of tolerance to cyanides and a more transient one expressed in increase of animal tolerance to lowering of atmospheric pressure. Both azide effects were observed when the same doses of the substance were used.

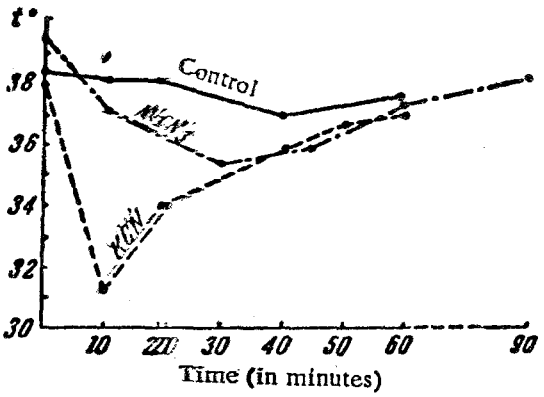
TABLE 2

Effect of Sodium Azide and Potassium Cyanide on the Tolerance of Mice to Lowering of Atmospheric Pressure (250 mm Hg) Weakened by Administration of Dinitrophenol

Substances administered	Number of animals		% survivals
	Total in experiment	Survived	
Control	30	17	57
Dinitrophenol	50	8	16
KCN and dinitrophenol	25	21	84
NaN_3 and dinitrophenol	25	24	96

It was postulated that the second azide effect, increase of tolerance to lowering of atmospheric pressure as shown by mice, could depend on lowering of body temperature under the influence of this substance. Increased tolerance of animals to hypoxia of various origins, including lowering of atmospheric pressure, associated with lowering of body temperature has been demonstrated by a number of authors [1, 2, 5, 6 etc].

To check this hypothesis, in a number of experiments the rectal temperature of mice was taken by means of a microthermometer before administration of azide and cyanide and after their administration prior to placing



Effect of sodium azide and potassium cyanide on the rectal temperature of mice.
Each curve represents mean data obtained from 5 mice.

the animals in the pressure chamber. Simultaneous temperature readings were taken from control animals. In addition, experiments were carried out on 15 mice (5 control, 5 given azide, 5 given cyanide in doses cited above) with determination of temperature at 10-minute intervals over a period of 1½ hours (see Fig).

As can be seen from the graph, both azide and cyanide elicit definite lowering of body temperature whose onset and duration correspond to the time during which the tolerance of mice to lowering of atmospheric pressure is increased by these agents. However, although administration of cyanide led to a greater lowering of temperature it was not accompanied by greater increase in tolerance to reduction of atmospheric pressure (see Table 1).

Since azide and cyanide also impair conjugated phosphorylation (by depression of respiration) it was found essential to investigate the combined effect of these poisons and dinitrophenol (DNP) – a poison that impairs conjugated phosphorylation directly. Such an investigation was all the more pertinent since it was known that DNP lowered animal tolerance to hypoxia [3, 13].

This series of experiments was carried out both in and out of the pressure chamber. Azide potentiated the effects exerted by DNP under the latter conditions: under the influence of azide the tolerance of mice to dinitrophenol intoxication was reduced. Thus, when DNP alone was given in the dose 25-30 γ/g, 16 out of 25 mice survived (64%), while when the same dose of DNP was preceded by administration of 15 γ sodium azide 10 minutes earlier, only 6 out of 25 mice survived (24%).

Different results were obtained in the pressure chamber. Sodium azide and potassium cyanide were given in the same doses as in the preceding experiments: azide was given 35 minutes prior to decompression and cyanide 20 minutes prior to decompression. DNP was given subcutaneously 30 minutes before the beginning of decompression in doses of 5, 10 and 15 γ/g in the form of 0.05-0.1% solution (with addition of sodium bicarbonate to facilitate solution). It was found that azide and cyanide abolished the DNP-elicited reduction of tolerance to lowering of atmospheric pressure. The most definite results were obtained when DNP was given in the dose 5 γ/g (Table 2). On increasing the dose of DNP to 10 γ/g the preventive effect of azide was diminished and completely eliminated when 15 γ/g DNP was given.

The difference in results obtained in and out of the pressure chamber is doubtless connected with the fact that in the former case the doses of DNP were one-fifth to one-sixth of those used in the latter case: the toxic effects of considerably smaller doses of DNP become apparent in the pressure chamber and it is these particular effects that are suppressed by azide and cyanide.

References have appeared in recent years in the literature [9, 10, 12] showing that azide suppresses the ability of DNP to activate apyrase or ATP-ase. We are not aware of a similar effect being exerted by cyanide. Therefore, antagonism in the effects of DNP and sodium azide has also been established biochemically. The question of whether this antagonism can be identified with that discovered in our experiments is still not clear and needs further investigation.

SUMMARY

Sodium azide and potassium cyanide increase the tolerance of mature white mice to reduced atmospheric pressure. This effect takes place also in those cases in which the resistance of animals is decreased by the administration of dinitrophenol.

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