THE BIOLOGICAL ACTIVITY OF CATECHOLAMINES IN RABBITS AT VARIOUS INTERVALS AFTER IRRADIATION

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In a previous report [4] we demonstrated a biphasic increase in the blood catecholamine level as well as in the aqueous humor of irradiated rabbits. There are suggestions in the literature that the blood of irradiated animals is biologically active. K. M. Larionova [3] obtained a negative inotropic effect on isolated frog heart muscle with the plasma of irradiated dogs. O. I. Komarova [1], in contrast, observed increased cardiac work with the blood of irradiated rabbits. Inasmuch as there is no uniform opinion on the biological effect of catecholamines during the development of radiation sickness we carried out experimental investigations on this problem in rabbits. A study of the problem was necessary for a correct interpretation of disturbances in hormone metabolism which are observed after exposure to ionizing radiation. We proceeded from the suppositions of Kh. S. Koshtoyants and A. A. Titaev [2,6] that the effect of the adreno-sympathetic hormones is mediated by their incorporation into the chemical dynamics of the effector organs and that the products of catecholamine metabolism take part in this process.

The experiments were carried out on 12 male rabbits which were given a total dose of 400 r on an ÉGO-2 apparatus. Acute radiation sickness developed in all the animals. All the animals survived and were observed for 3 to 4 weeks after the irradiation. Analyses of the catecholamines in each rabbit were performed before irradiation, on the day of the irradiation, on the 1st, 5th, 7th and 8th days after irradiation, and at the height of the radiation sickness (on the 12-14th day).

The biological activity of the catecholamines obtained from the aqueous humor of the anterior chamber was assayed on isolated frog heart by the method of Straub and by recording the movement of the denervated pupil of the rabbit.

Pupil diameter was measured with a Zenit-S camera. In order to film the object under study at a 1:1 ratio an oval ring and a fixed setting of the object at 0.8 m was used. The light was placed at a 30° angle from the axis of the rabbit's body at a distance of 30 cm from his eye. Photographs were taken for 30 minutes at a rate of one frame a minute after the subconjunctival injection of 0.1 ml of test material. The camera was set so that the diaphragm was at 5.6, exposure at 1/50 of a second, and film sensitivity at 65 GOST units. The negative was printed on paper containing a micrometric scale previously photographed under the same conditions. Protein precipitation and adsorption and separation of catecholamines were carried out according to previously described methods [4].

Control experiments indicated an absence of cardiac reactivity and reaction of the denervated pupil to the chemicals used. Parallel studies on quantitative changes in catecholamines were carried out by polarographic methods.

The concentration of catecholamines isolated from the anterior chamber of the normal rabbit's eye was sufficient to change the cardiac work in 30% of the trials but

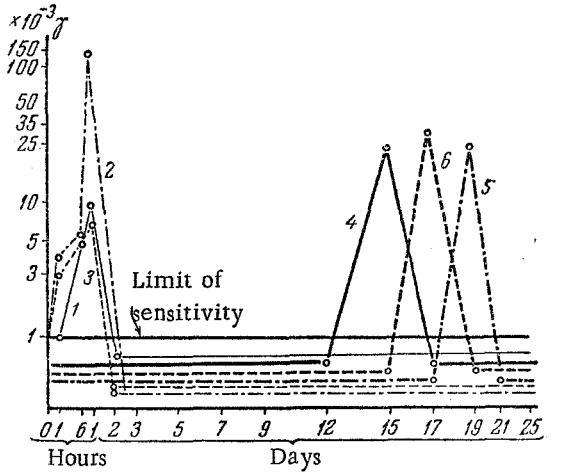

Fig. 1. Changes in the catecholamine content of the anterior chamber of the rabbit's eye following irradiation: 1-adrenaline-free; 2-free of oxidation products; 3-noradrenaline-free; 4-bound to adrenaline; 5-bound to oxidation products; 6-bound to noradrenaline.

insufficient to change the diameter of the denervated eye.

A biphasic increase in catecholamines was noted during the development of radiation sickness in the anterior chamber of the eye. The early phase occurred on the day of irradiation and the later phase at the height of the radiation sickness (Fig. 1).

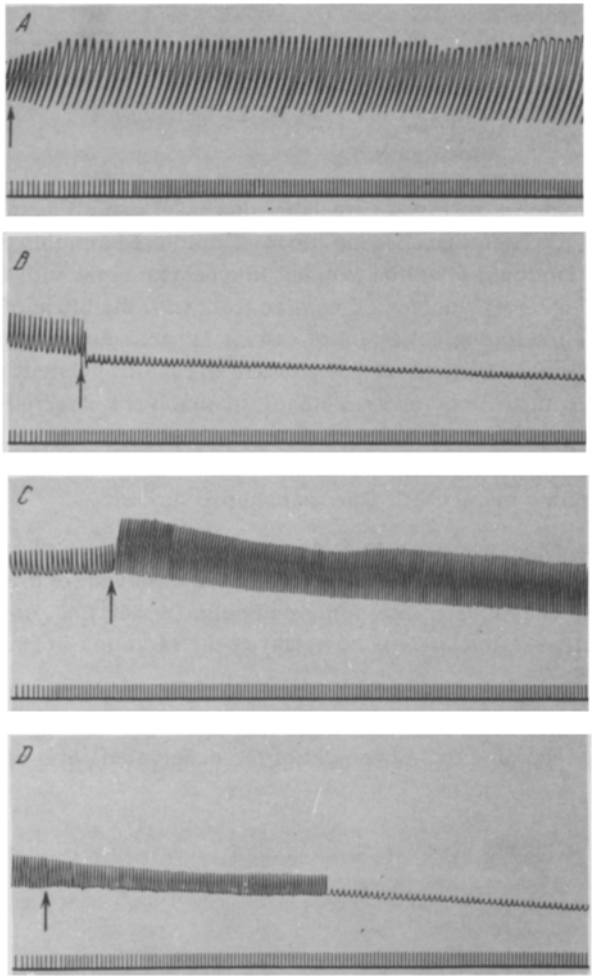

Fig. 2. Changes in work of isolated frog heart after perfusion with catecholamines obtained from anterior chamber of the eye: A) Before irradiation; B) 1 day after irradiation; C) 7 days after irradiation; D) peak of radiation sickness.

Experiments on the isolated frog heart were calibrated to evaluate the biological activity of catecholamines which developed in the anterior chamber during the course of radiation sickness. Toward this end $(2\text{-}8) \cdot 10^{-6}$ M of amine was added to the fluid obtained by canulation of the anterior chamber. After discontinuing the reaction the perfusate containing the adrenal compounds was flushed out for 10 to 15 minutes with Ringer's solution. The fluid in the cannula was

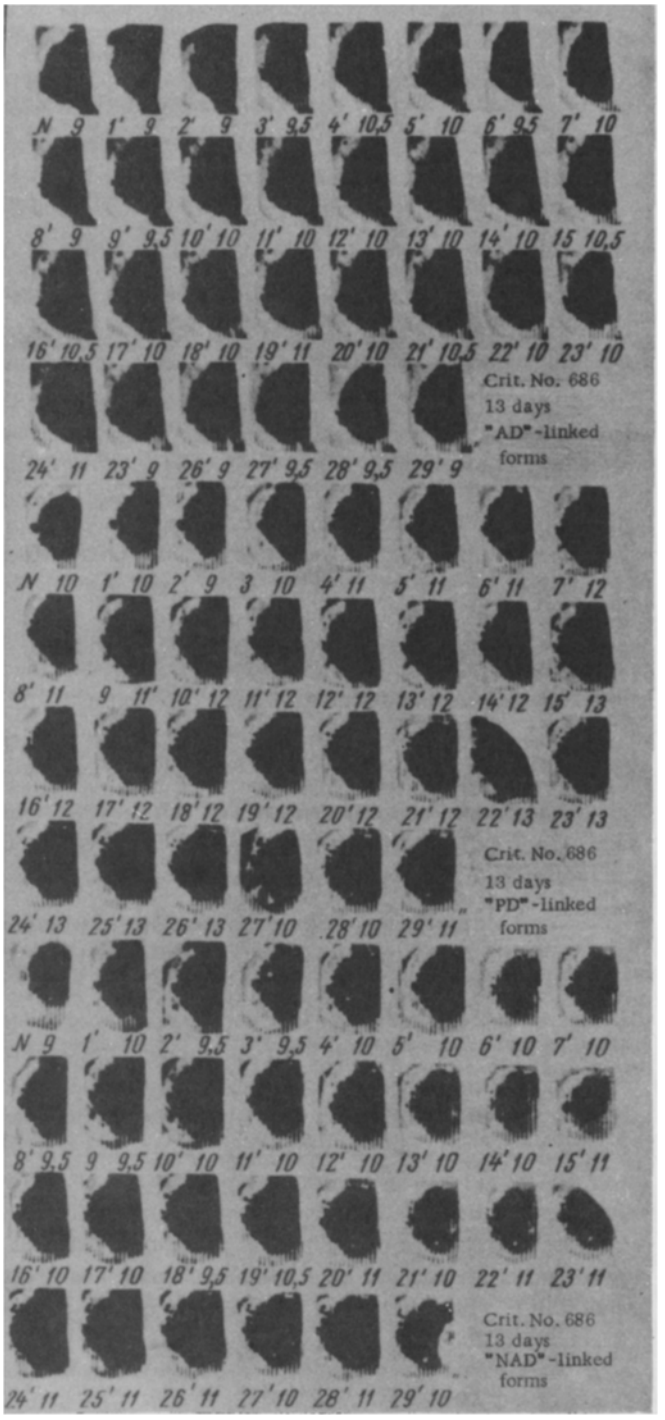

Fig. 3. Changes in the diameter of the denervated pupil of the rabbit after the injection of catecholamines isolated from the anterior chamber of the eye: numbers on the left-time after injection (in minutes); numbers on the right-pupil diameter (millimeters).

changed several times in this manner. Calculations indicated that the catecholamines isolated from the anterior chamber at different times during the radiation sickness had various effects on the isolated frog heart muscle. Thus, the perfusion of catecholamines obtained in the first and in the second phases of their increase had a negative inotropic effect in 70% of the trials and in 30% did not change the cardiac work. During the perfusion of amines obtained from the anterior chamber when there was no polarographic effect with a known quantity of catecholamines (3rd, 5th, 7th and 8th days after irradiation) an increase in cardiac effect was noted in 25% of the trials and no effect in 75%. In all probability there is a decrease in secretion of amines by the adrenal medulla to values below $1 \cdot 10^{-9}$ M (the limit of sensitivity of the polarographic method) (Fig. 2). In this connection, the data which appear at first glance to represent an artifact become fully explainable. The perfusion of small concentrations of amines of the order of $1 \cdot 10^{-8}$ to $1 \cdot 10^{-9}$ M evokes a positive inotropic effect on the isolated heart (3rd day after irradiation). The high concentration of materials ($1 \cdot 10^{-5}$ to $1 \cdot 10^{-6}$ M), on the other hand, inhibits the force of cardiac contraction (on the day of irradiation and at the height of the radiation sickness). This is verified by the data of Murav'ev [5].

In the next series of experiments, the heart was perfused with differentiated amines. Analysis of the obtained results showed clearly that perfusion of the heart with adrenaline or its "oxidation products" weakened cardiac activity to the point of complete cessation, whereas perfusion with noradrenaline increased the force of cardiac contractions.

These data can be explained by the fact that adrenaline is more toxic for the heart than noradrenaline [7] and that there exists a concentration of adrenaline which leads to a cessation of the cardiac activity. On the other hand perfusion by oxidation products also leads to a cessation of cardiac activity, for when the absolute amount of adrenaline and its oxidation products together with catecholamines exceeds by twofold the absolute concentration of noradrenaline, the dominant effect during cardiac perfusion with amines can result in a weakening of the cardiac activity.

The results of the experiments on changes in the pupil diameter after amine injection are in accord with the other data.

Calculations indicate that catecholamines evoke dilatation of the pupil in 90% of the instances (Fig. 3).

It can be concluded on the basis of the experiments described that catecholamines are biologically active during the course of radiation sickness.

SUMMARY

The biological activity of catecholamines isolated from the anterior chamber's aqueous humor at various periods of radiation sickness was assessed. For this purpose a study was made of the disturbances in the work of the isolated frog heart and the changes occurring in desympathized pupil diameters in response to catecholamine administration. On the basis of the data obtained it is shown that catecholamines are biologically active during the whole period of radiation sickness.

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