EFFECT OF ACTINOMYCIN D ON THE TOXIC ACTION OF FURFURAL WHEN ADMINISTERED REPEATEDLY

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Daily administration of furfural to albino mice was less lethal than administration on alternate days. Injections of actinomycin D abolished this effect. The oxygen tension in the muscle tissue of animals receiving furfural daily was 1.5 times higher than in control mice and mice receiving furfural on alternate days.

During repeated administration of chemicals the intensity of their toxic effect is determined not only by the dose or concentration of the agent, but also by the frequency of its administration. Under certain conditions, the more frequent administration gave a lesser effect than the less frequent, although the total dose of the compound injected was greater in the first case than in the second. This has been found, in particular, in experiments with furfural [6]. The suggestion has been made that the reason for this paradoxical effect, as with any poisonous substance, is that there is a certain optimal rhythm of action which leads to the maximal development of compensatory processes, including intracellular regeneration, and the synthesis of protein and adaptive enzymes.

If this hypothesis is true, the effect described should be abolished by blocking protein synthesis.

This problem was studied in the present investigation in which furfural was administered repeatedly and actinomycin D was given. Actinomycin D blocks DNA-dependent RNA synthesis [1, 7, 8] and is used in experiments on mammals to elucidate the mechanisms of adaptive enzyme synthesis [2]. At the same time, the oxygen tension in the tissues was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 20-25 g. Furfural was injected subcutaneously in a dose of 0.7 LD_{50} (200 mg/kg), either daily (group 1) or every other day (group 2).

Actinomycin D (Calbiochem, Los Angeles, California, 90054) was injected intraperitoneally in a dose of 70 μg/100 g body weight on the 10th day of the experiment into the animals of group 3, which received daily injection of furfural. The animals of group 4, which also received furfural daily, received two injections of actinomycin, in doses of 50 μg/100 g body weight, at intervals of 7 days on the 7th and 14th days of the experiment. Control animals (group 5) received subcutaneous injections of distilled water. The total duration of the experiment was 50 days.

The oxygen tension was determined by intravital polarography [5] in the femoral muscle. The cathode was a platinum electrode 0.2 mm in diameter. A calomel nonpolarizing electrode was used. To record the diffusion current a highly sensitive galvanometer was used, and in the course of the determination a voltage of 0.6 V was applied to the electrode. The oxygen concentration was expressed in microamperes.

Statistical analysis of the results was by the Student–Fisher method.

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TABLE 1. Effect of Actinomycin D on Toxic Action of Furfural When Administered Repeatedly

Group of animals	No. of animals	Mode of administration of furfural	Dose of actinomycin D (in μg/100 g body weight	No. of animals surviving until 50th day of expt.)	P (compared with group 1)
1	15	Daily	–	9	
2	15	On alternate days	–	4	
3	15	Daily	70.0 (single injection on 10th day of expt.)	2	0.05
4	15	"	50.0 (twice at interval of 7 days)	4	0.05
5	15	–	–	15	0,05

TABLE 2. Effect of Furfural, Administered at Different Frequencies, on Oxygen Tension in Muscles of Albino Mice

Mode of administration of furfural	Oxygen tension (in μA)	n	P (compared with control)
Control	0.009±0.001	10	–
Alternate days	0.010±0.001	10	0.1
Daily	0.014±0.0004	10	0.001

EXPERIMENTAL RESULTS

The results of the experiments with actinomycin D are shown in Table 1.

As the results in Table 1 show, after daily administration of furfural the mortality among the animals was lower than when the same dose of the compound was given on alternate days. This paradoxical effect of furfural, concerning the writers' previous observations [6], was completely abolished by actinomycin D. The survival rate was just as low in the groups of animals receiving actinomycin D in various doses against the background of daily furfural injection as in the groups receiving furfural on alternate days. In this case, adaptive changes evidently did not develop because of the blocking of protein synthesis by actinomycin D. The process of adaptation to foreign chemical substances is known to be accompanied by an increase in the content of cytochrome P450 and by intensification of protein synthesis [4]. An increase in the intensity of synthetic processes requires increased production of high-energy compounds, intensification of oxidative phosphorylation, and an increased supply of oxygen to the tissues.

Figures for the oxygen tension on the 30th day of the experiment in the tissues of the experimental and control animals are given in Table 2.

Determination of the oxygen tension in the tissues of the albino mice showed that it was about equal in the control animals and in the animals receiving furfural on alternate days. In animals receiving furfural daily, the oxygen tension in the tissues was higher than in the controls (155% of the control level), the difference being statistically significant ($P = 0.001$).

This investigation thus confirmed that an important role in the development of adaptation to the action of chemical compounds is the frequency of their administration. Daily administration of furfural led to the development of greater resistance to this poison than its administration on alternate days. This increased resistance was absent if actinomycin D was given. If the view that actinomycin suppresses protein synthesis by blocking DNA-dependent RNA synthesis [1, 2, 5, 7, 8[is accepted as valid, it can be considered that the adaptive changes leading to increased resistance to furfural when administered daily are accompanied by an increase in the intensity of synthesis of adaptive protein enzymes. This is accompanied by an increase in the oxygen tension in the tissues.

These results agree with those obtained by Sarkisov [3, 4], who showed that the effect of an external stimulus on the organism is determined not only by its strength, but also by the relationship between the rhythm of administration of the stimulus and the rhythm of intracellular regeneration. Adaptive changes under these circumstances are characterized by intensified protein synthesis, an increase in the number and size of the mitochondria, and acceleration of the cycle of intracellular regeneration [3, 4].

In the present experiments adaptation to furfural was accompanied by an increase in the oxygen tension in the tissues, possibly indicating intensification of energy-producing processes essential for the stimulation of intracellular regeneration. Abolition of adaptation to furfural by administration of actinomycin D can be regarded as confirmation of the role of protein synthesis in the development of adaptation to the action of chemical substances.

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