

THE INFLUENCE OF CONGO RED ON THE EFFECTS OF DITILINE

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In 1924 I. R. Petrov was the first to discover the ability of congo red to render curare harmless. This effect was repeatedly confirmed by other investigators [1, 2, 6 and others]. The possibility of rendering curare isolated from curare harmless was discovered in the last two investigations.

The goal of the current investigation was the clarification of the influence of congo red on the effects of bisquaternary ammonium base — ditiline (dicholine ester of succinic acid), employed in recent years in USSR as a curare-like preparation.

EXPERIMENTAL METHOD

The experimental portion of this project was begun with the performance of experiments on the abdominal rectus muscle of the frog; under the influence of ditiline contracture of the muscle was induced [4]. The isolated muscle was placed in a bath of aerated Ringer solution and 1-2 hours later, twice for each experiment, its reactivity to one or the other concentration of ditiline (1:1 — 2 mln) was checked. After repeated changes of the Ringer solution in the glass the latter was filled with a solution containing congo red $1 \cdot 10^{-6}$ — $1 \cdot 10^{-4}$, and 15-30 minutes later ditiline was added in the concentrations previously tested (a mixture of congo red and ditiline in previously stated concentrations was tested in a series of experiments. Muscle contractions were recorded on the kymograph for 3 minutes with 30 minute intervals between tests.

A total of 15 experiments with 90 tests was performed.

EXPERIMENTAL RESULTS

In experiments with preliminary treatment of the muscle with congo red two types of effects were observed; in smaller concentrations ($1 \cdot 10^{-6}$) congo red increases the contractions of the abdominal rectus muscle caused by ditiline, and in larger concentrations ($1 \cdot 10^{-4}$) suppresses these contractions (Fig. 1).

The second fact revealed itself with great clarity when mixtures of substances studied were tested.

Results obtained on the isolated muscle led on to tests of these substances on the entire organism. Experiments were carried out on 30 male frogs. The substances tested were given subcutaneously; ditiline — in doses of 5 — 10 γ per 1 g of body weight, congo red — in doses of 5 — 100 — 200 γ per 1 g body weight 15-30 minutes prior to administration of ditiline or mixed with it.

Administration of ditiline to frogs resulted in the disappearance of spontaneous movements, contractures of the forelegs, disappearance of the reflex of righting themselves when placed on the back and of the reflex to stimulation of the hindlegs. These effects of ditiline disappeared in 2-3 hours.

All the symptoms caused by ditiline could not be entirely prevented in the majority of the experiments by the administration of congo red. However, congo red did prolong somewhat the latent period preceding the onset of the effects of ditiline and in 18 of 20 experiments prevented the loss of reflexes to stimulation

of the hindlegs of the frog and decreased the intensity of the contracture of the muscles of the forelegs. Prevention of the reflex of righting themselves when placed on the back was more difficult (only in 3 out of 20 cases). Results obtained when congo red and ditiline were administered separately did not differ significantly from those obtained when a mixture of the two was given.

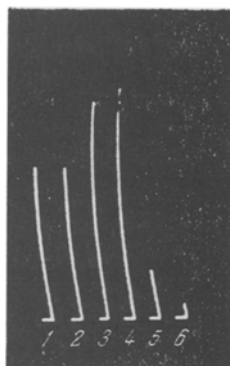


Fig. 1. Contractions of the abdominal rectus muscle of the frog under the influence of ditiline $1 \cdot 10^{-6}$ (experiment No. 13). 1 and 2) Prior to treatment with congo red; 3 and 4) after 30 minutes of treatment with congo red $1 \cdot 10^{-4}$.

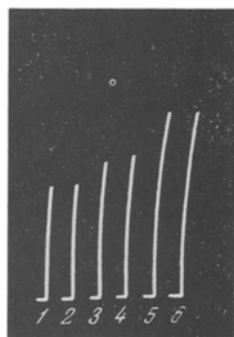


Fig. 2. Contractions of the abdominal rectus muscle of the frog under the influence of acetylcholine $1 \cdot 10^{-7}$ (experiment No. 16). 1 and 2) Prior to treatment with congo red; 3 and 4) after 15 minutes of treatment with congo red $1 \cdot 10^{-6}$; 5 and 6) after 15 minutes of treatment with congo red $1 \cdot 10^{-4}$.

In I. R. Petrov's work [3] which disclosed for the first time the antagonism between congo red and curare and in the investigations of C. J. Kensler [6], one of the last authors studying this problem, the antagonistic effect of congo red in relation to curare (more accurately "rendering it harmless") is explained by the interaction between the dye and the quaternary ammonium base. We studied the possibility of forming a compound between congo red and curarine (curare made by Shuchardt), and also ditiline with the aid of spectrographic analysis; to compare spectrums of individual substances and of their mixtures (for the opportunity to work with the spectrophotometer SF-4 and for the cooperation in the project I declare my appreciation to M. I. Kulenok). Investigations in the visible and ultraviolet fields of the spectrum did not give any grounds for believing that these substances react one with the other to form a new compound.

It is admitted that the antagonistic effect of congo red depends on the action of the dye on the muscle. The possibility of such an influence was assumed even earlier [2].

Interaction between the dye and biological structures also explains the ability of congo red to sensitize the abdominal rectus muscle of the frog to acetylcholine (Fig. 2). This sensitization, apparently, depends on inhibition of cholinesterase by the dye [6]. Apparently, the same explanation holds for the increase in the effect of ditiline (which, like acetylcholine, is a substrate of cholinesterase) which results from small concentrations of congo red.

Thus, interaction between congo red and biological structures (union with prothrombin, complement and cholinesterase) is less controversial than interaction of this dye with quaternary ammonium bases. If the latter does take place, it must lead to formation of a rather unstable compound not detectable by spectrographic analysis in the visible as well as the ultraviolet fields of the spectrum.

SUMMARY

In low concentrations ($1 \cdot 10^{-6}$) congo red intensifies the contractions of the isolated m. rectus abdominis produced by ditiline. Higher concentrations ($1 \cdot 10^{-4}$) of congo red depress these contractions. The first effect is probably connected with the depression of cholinesterase, and the second — with the action of the dye on the muscle. The formation of a congo red compound with the quaternary ammonium bases — ditiline (dicholine ester of succinic acid) and curarine (contained in curare), as suggested in pertinent literature, could not be established by means of a spectrographic analysis.

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