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THE INFLUENCE OF 1, 1, 1-TRICHLORO-2, 2-BIS (p-CHLOROPHENYL) ETHAN (DDT) UPON THE MUSCLE DISTROPHY IN RATS

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Not long ago, we were able to show that oxotremorine (the substance which activates cholinacetylase (OT) (Holmstedt and Lundgren, 1970) leads to the increase of the weight of distrophic muscles of the rat (Stern, 1971 in Press). Since we know that acetylcholin has trofic effect upon sceletal muscles (Drachman, 1967), we think that this effect of OT is probably connected with its trofic effects (Stern, 1971 in Press). Recosen (the extract of heart muscle which also activates cholinacetylase) (Bašagić, et al., 1962) had the same effect on distrophic muscle (Stern and Ristić, 1971 in Press). We have previously shown that Recosen strenghtens static tremor (ST) (Bevandić and Stern, 1964) caused by OT probably because of summarising of effects on AcH.

In another paper, we have shown that DDT causes ST which cannot be removed by atropin (Stern and Valjevac, in Press). The quantity of AcH in CNS seems to be constant (Stern and Valjevac, in Press). Some authors indicate that AcH in CNS has even decreased (Hrdina et al., 1971). Not long ago, it has been shown that DDT does not effect the activity of cholinacetylase (Ching-Chieh Yu and Booth, 1971). Tremor caused by DDT could have only been removed with mephenezin and diazepan (Stern and Valjevac, in Press) which are inhibitors of polysynaptic reflexes. We have therefore concluded that DDT causes specific form of ST at the level of medulla spinalis, and we considered it interesting to investigate the effects of DDT upon this kind of distrophy although we know that DDT doesn't increase AcH (Stern and Valjevac, in Press; Ching-Chieh and Booth, 1971) as it

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does OT or Recosen. But it is well known that DDT increases conductibility through axon (N a r a h a s h i and H a a s, 1967) and that it antagonizes tetrodotoxin (N a r a h a s h i and H a a s, 1967). Using this method, we have already shown that anabolics increase the weight of muscle mass (I g i ć et al., 1969). It should be stressed, however, that this kind of muscular disorder is not identical to the real progressive muscular distrophy (Erb's disease).

METHOD

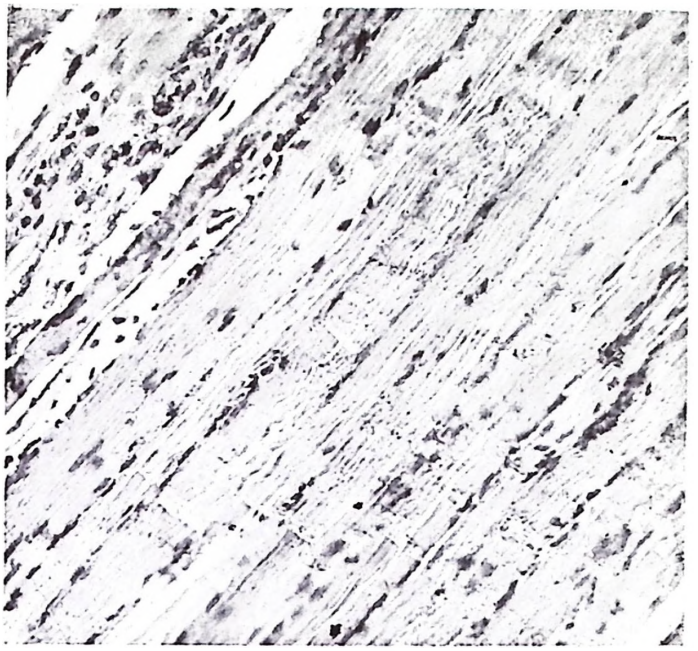
Wistar rats were used in the experiment and muscle distrophy was caused by Selye's method (S e l y e, 1965): male rats weighting 90—110 gr. had their abdominal aorta constricted just beneath renal artery. Five days later legs were exposed to ice cold water. Animals were sacrificed 14 days after operation. The wight of sceletal muscles has than been measured (medial femoral group: m. gracilis antious et posticus, m. adductor longus, magnus et brevis, m. pectineus). After measurment, it was compared with controll group and underwent histologic examination. DDT was diluted in olive oil (0,2 cc of oil per rat, and administred 25 mg/kg and 10 mg/kg per os. Controll group was administred only 0,2 cc oil per os. DDT was given from the first day of experiment i. e. after constriction of abdominal aorta until the sacrifying 15 day later. There was also one group of rats that was getting DDT for 15 days but was then left alive for another 4 days in order to see if absence of DDT administration would again lead to distrophy.

RESULTS

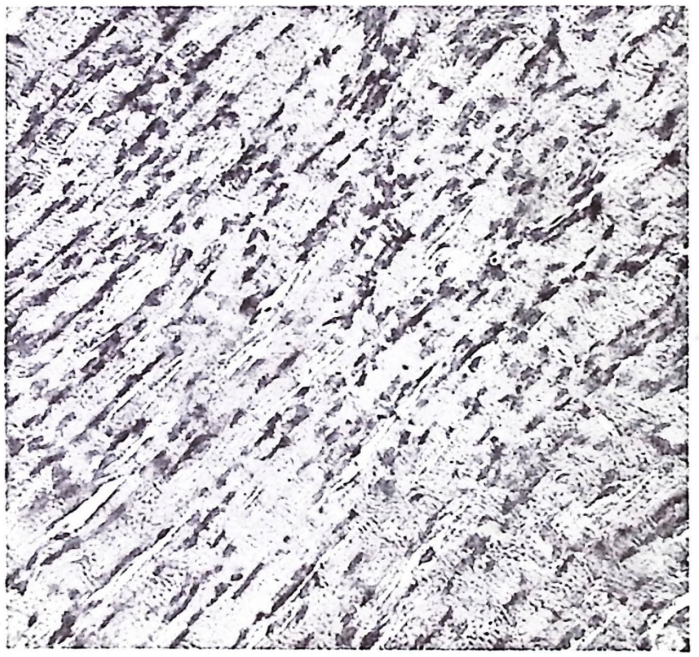
THE INFLUENCE OF DIFFERENT DOSES OF DDT UPON THE WEIGHT OF MUSCLES OF MICE WITH MUSCLE DISTROPHY

DDT Dose and way of application *	Experiment x(±SE—n)	Control x(±SE—n)	p
25 mg/kg in edible oil per os. Controll — only edible oil	X 838,57(±63,63—7)	614,37(±34,09—8)	<0,05
10 mg/kg in edible oil per os. Controll — only edible oil	* 777,50(±30,70—12)	731,10(±24,72—9)	>0,05
25 mg/kg in edible oil per os. Controll — only edible	** 906,25(±32,30—8)	876,87(±64,43—8)	>0,05

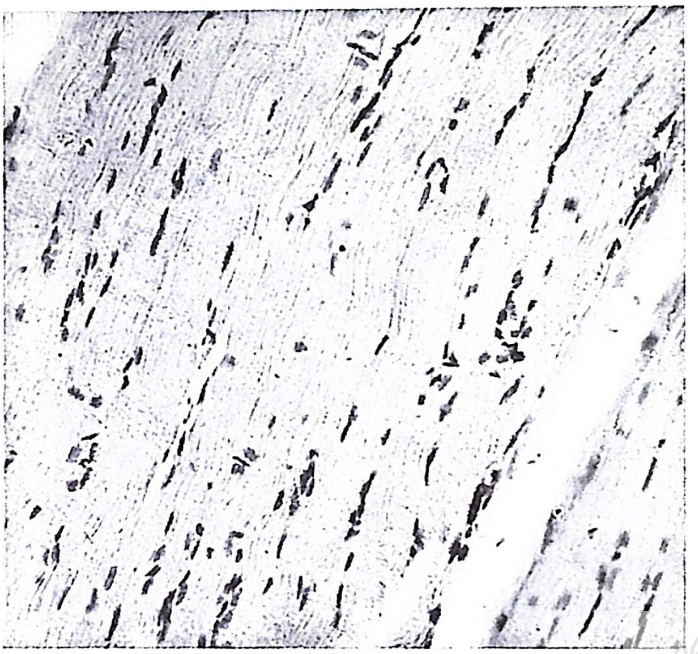
X = mean value
SE = standard error of the mean value
n = number of animals
p = probability
* = treatment with DDT was started 3 days before operation and has been continued until animals were sacrificed (15 days altogether).
** = treatment started 3 days before operation and was continued for 15 days. Treatment was then suspended for 7 days. On the 7th day animals were sacrificed.



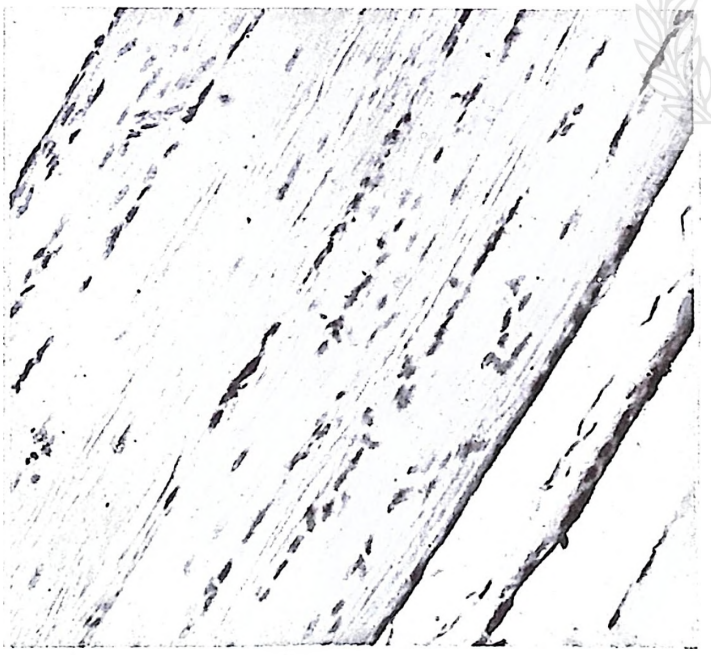
Picture 1.
Controll. Rather large muscle necrosis with resorptive reaction. Magnified 150 × Hem.—Eoz.



Picture 2.
Controll. Atrophy and degeneration of muscle fibers. Magnified 240 ×. Hem—Foz.



Picture 3.
Experiment (10 mg DDT). Muscle nearly intact. Magnified 240 ×. Hem.—Eoz.



Picture 4.
Experiment (10 mg DDT) Hipertrophy of muscle fibers. Magnified 240 ×. Hem.—Eoz.

It can be seen from the table that DDT has significantly raised the weight of distrophic muscles. We know from previous experiments that normal medial femoral group weights 900—1000 mg (Igić, et al., 1969). If DDT is suspended for 4 days after 15 days of treatment with DDT, muscles will degenerate again.

HISTOLOGIC ANALYSIS

After fixation in formalin, samples were colored with hemalaun and eozin and microscoped. We wanted to get a general impression about the condition of each sample. We also made comparisons between them and searched for atrophy, degeneration and necrotized fibers and resorptive profilerative processes in muscles. We also looked for possible hipertrophy of intact muscle tissue and for possible regeneration.

Distrophic changes (atrophy, degeneration and necrosis) were present in all samples, but various lesions were found, small ones, involving only few fibers, and very large ones. They varied from one sample to another but different lesions were sometimes seen in one sample. Distrophic changes were stronger in controll group and big lesions of muscle tissue were predominantly found in controll group. Resorptive-proliferative processes were nearly the same in experimental and in controll group. There was no significant difference in the intensity of distrophic changes in experimental subgroups which were treated with various doses of DDT.

Signs of regeneration were not found in any of the samples. Hipertrophy of smaller groups of muscle fibers was detected, in intact parts of muscles in about 50% of the samples of controll group and in experimental group which was administred 10 mg of DDT. Experimental subgroup which was getting 25 mg of DDT showed similar picture in 71%.

HISTOLOGICAL CONCLUSION

The intensity and quantity of distrophic changes in muscles, caused by Selye's method, was significantly smaller in experimental group which was given DDT than in controll group. The changes were not dependable upon the quantity of DDT. The number of necrotic foci was also smaller. One animal of the experimental group was exception and its lesions were significantly larger.

DISCUSSION

It can be seen that DDT increases the weight of distrophic muscles although it doesn't effect the activity of cholinacetylase (Ching-Chieh and Booth, 1971) and even decreases AcH in CNS (Hrdina et al., 1971). The exact mechanism of tremor caused by DDT has not yet been explained but it is very probably situated in medulla spinalis, according to us (Stern and Valjevac, in Press) and some other authors (Shankland, 1964). It has been prooved that tremor can be induced even in decapitated frog and that it can occur beneath the incision in medulla (Shankland, 1964; Henderson et al., 1970). It is well known that DDT antagonizes tetrodoxine (Narahashi and Haas, 1967). DDT will hence accelerate the conduction of impulses through axon

and spontaneous impulses from alfa motor cells will then produce stronger irritation of motor unit. This probably causes excessive draining of AcH vesicles which are located at the end of motor nerves.

DDT has probably affected distrophic muscles through α-motor neuron by increasing its function. This has then probably led to the increased release of AcH at the end of motor nerves. Underbinding of aorta, which is necessary for causing distrophia, leads to ishemia of medulla spinalis. Medulla is not entirely demaged because rats have well developed mamarian artery which supplies enough collateral (Greene Eunice .Chace, 1955) blood.

Histologic analysis showed lees demage in muscles which have received DDT than in controlls.

It is interesting that suspending of DDT cure again decreases the weight of muscles.

It this model adequatly represents Erb's disease we could conclude that it is a disease of nervous system. The similarity between Erb disease and Botulinus intoxication (Holmstedt and Lundgren, 1970; Stern, in Press) may be another proof for this. Botulinus intoxication is also characterised by decrease of AcH release at the end of motor nerves (Burgen et al., 1949), what we think, may also happen in Erb disease. Baker and Sabawala (1963) haven already shown that distrophic muscle becomes very sensitive to neostigmin. This also speks for nervous origin of Erb disease, rather than muscular.

We were even able to show that such distrophic muscles are reacting to various drugs which cause either tremor or rigor (Stern, 1971 in Press). Such reaction also show muscles intoxicated with botulinus (Stern and Valjevac, 1971). All these facts make us think a wide epidemiologic analysis should be carried out in order to see if incidence of Erb disease is related to the quantitiy of DDT in food and in examined persons.

SUMMARY

It has been shown that muscles of posterior extremities of rats with muscular distrophy caused by Selye's method regain nearly normal weight after administration of DDT.

Histologic changes (atrophy, degeneration, necrosis) in muscles treated with DDT are smaller than in controll animals.

PAVEL ŠTERN i EDITA GMAZ-NIKULIN

UTJECAJ DDT NA MIŠIČNU DISTROFIJU ŠTAKORA

KRATAK SADRŽAJ

Budući da je već poznato da DDT pojačava provodljivost kroz nerv i da izaziva statički tremor, učinjen je pokušaj da se vidi da li će DDT popraviti kliničku sliku distrofičnog mišića.

Isto tako poznato je da DDT ne povisuje količinu acetilholina u CNS, ali se ipak mogao očekivati efekt na distrofičnu muskulaturu. U jednom

prijašnjem radu mi smo postavili hipotezu da se kod mišićne distrofije (Erbovog tipa) radi o smanjenim impulsima alfa-motornih stanica medulle spinalis na okončine nerava.

Naši pokusi su pokazali da stvarno DDT povećava signifikantno težinu distrofičnih mišića štakora izazvanom metodom Selya. Osim toga, u distrofičnim mišićima štakora tretiranih DDT i histološke lezije su manje nego kod kontrolnih životinja.

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