of whether significant behavioural alteration was accomplished by the enzyme inhibitor when given alone. In addition to an exaggerated response which occurred during the first hour, a prolongation of behavioural disruption was apparent. This is revealed in Table 2 in which the average number of shocks during each 15 min period after treatment is given for one of the cholinesterase inhibitors.

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Toxicity of a nucleotoxic agent, mustine hydrochloride, and its enhancement by 5-hydroxytryptamine pretreatment

SIR,—It has been shown by numerous workers that 5-hydroxytryptamine (5-HT) possesses a marked radioprotective activity. The effects of nucleotoxic drugs show certain similarities with the effects produced by ionizing radiation. Many radioprotective agents also provide a protection against the nucleotoxic substances (Scarborough & Thomas, 1962). We have now examined the influence of 5-HT upon the toxicity of mustine hydrochloride (nitrogen mustard), a typical representative of radiomimetic poisons. For testing the specificity of the phenomenon to be described, another toxic agent, chloral hydrate, was used.

Three groups of 20 albino rats were injected intravenously with mustine hydrochloride. The first group received saline, the second 5-HT creatinine sulphate and the third chloral hydrate intraperitoneally 30 min before being given the agent. The survival was observed every 12 hr during 30 days. The results are summarised in Table 1.

TABLE 1. ENHANCEMENT OF MUSTINE HYDROCHLORIDE TOXICITY BY 5-HYDROXYTRYPTAMINE PRETREATMENT

Treatment	Pretreatment	Mortality rate after 30 days (percentage)	Mean survival time in days ± s.e.m.	"t" test (survival time)
Mustine HCl 1 mg/kg i.v.	Saline i.p.	15	26·3 ± 2·0	—
,,	5-HT creatinine sulphate 21·2 mg/kg i.p.	55	17·3 ± 2·7	$P < 0.02$
,,	Chloral hydrate 270 mg/kg i.p.	20	25·0 ± 2·3	$P > 0.05$

A significant decrease in mean survival time in the group pretreated with 5-HT was noted. The animals pretreated with chloral hydrate showed no significant alteration in survival after mustine hydrochloride. The doses of 5-HT

and chloral hydrate represent the same percentage of their LD50 and given alone had no lethal effect in preliminary tests.

The enhancement of the toxicity of mustine hydrochloride by 5-HT does not seem to be due to simple addition of toxicities since chloral hydrate (in equitoxic dose) did not alter the mean survival time. It is likely, therefore, that the described effect is a specific one.

The present finding is consistent with the experimental data of Field, Mireles & Dolendo (1962) who found that KB 95 (benzpiperylon, 4-benzyl-2-(1-methyl-piperid-4-yl)-5-phenyl-3-pyrazolone) an antagonist of 5-HT, provides a marked protection against mustine hydrochloride intoxication in mice.

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Antagonism of some spasmolytic drugs by calcium on guinea-pig isolated ileum

SIR,—We have recently pointed out that different mechanisms of action may be involved in the spasmolytic activity of the main papaverine derivatives (Santi, Ferrari & Contessa, 1963). This conclusion appears to be supported by our recent findings that changes in ionic environment may affect the *in vitro* activity of some spasmolytic drugs. The most striking effects have been observed by increasing the calcium concentration in the bath fluid.

The ileum of the guinea-pig was suspended in a 30 ml bath containing Tyrode medium at 37°; air was bubbled through the bath fluid, and the spasmolytic drugs [papaverine hydrochloride, eupaverin sulphate (1-benzyl-3-ethyl-6,7-dimethoxyisoquinoline) isoxsuprine hydrochloride] were added at concentrations, ranging from 1 to 8 μg/ml and allowed to act for 2 min before the addition of acetylcholine or histamine. Some experiments were made in anoxia, by replacing air bubbling through the bath fluid with 95% nitrogen and 5% CO_2.

Under these experimental conditions it was observed that $CaCl_2$ (300–400 μg/ml) strongly counteracted the spasmolytic activity of the drugs tested. When added after the failure of acetylcholine to stimulate the isolated gut pretreated with spasmolytic agents, $CaCl_2$ was able to restore a prolonged tonic contraction (which is abolished by atropine). This effect occurred after both isoxsuprine and eupaverin in concentrations ranging from 2 to 8 μg/ml. It was also detectable after 1–2 μg/ml papaverine but readily disappeared after increasing the papaverine concentration. Thus, $CaCl_2$ was less active against papaverine than against eupaverin and isoxsuprine. Furthermore, $CaCl_2$ was unable to remove the inhibition of the tonic phase of the acetylcholine or histamine-induced contraction caused by 2,4-dinitrophenol and by oxygen lack.

The data so far obtained indicate a clear antagonism between the excess calcium and the activity of some spasmolytic agents. Since calcium ions are believed to play a key role in muscular contraction as an excitation-contraction