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

coupling factor (Bianchi, 1961), it is tempting to assume that an interference with calcium activity may take a part in the mechanism of action of some spasmolytic drugs. On the other hand, the myolytic effect of 2,4-dinitrophenol, ascribed to impaired synthesis of high energy phosphate bonds by West, Hadden & Farah (1951), was not relieved by $CaCl_2$. This fact may account for the lower activity (compared with eupaverin and isoxsuprine) that calcium exercises against papaverine which strongly inhibits oxidative phosphorylation (Santi, Contessa & Ferrari, 1963). Thus, it seems reasonable to investigate whether papaverine may have a dual mechanism of action involving both inhibition of oxidative phosphorylation and an interference with the role of calcium, which is presumably the predominating factor in other spasmolytic agents. Finally the results with isoxsuprine, which is an isoprenaline congener, appear to be in agreement with other findings suggesting that isoprenaline may prevent the entry of calcium into the cell (Schild, 1963).

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Teratogenic activity of drugs

SIR,—Many chemical substances have the power, when administered to pregnant animals, of producing congenital malformations in the young. Methods by which new drugs possessing this action may be readily distinguished are therefore of major importance. By administering drugs initially daily throughout pregnancy and later only during the first trimester, an estimate may be made of the drugs which are most likely to exhibit teratogenic activity in man.

In the course of testing over 40 compounds by this procedure, three types of drug have emerged. Firstly, drugs which kill the mother before any effect is observed on the foetuses; secondly, drugs which kill most of the foetuses before any effect is observed on the mother; and thirdly, drugs which do not kill the mother but which produce changes within the foetuses. As previously suggested (West, 1962), an indication of a teratogenic risk may be obtained by relating foetal resorptions to the doses administered to the mother. When this relationship is made for reserpine, guanethidine and thalidomide (three drugs at one time widely used in human pregnancy), straight line graphs of quite different slopes are obtained. These are shown in Fig. 1. For this work, daily intraperitoneal doses of the drugs were given to groups of 4 rats throughout pregnancy and then to groups during the first trimester. Animals were killed on the 20th day of gestation, and foetal mortality was calculated from the number of live and dead foetuses found. For guanethidine, a horizontal line was obtained since this drug killed the mother before it was lethal to the foetuses. For reserpine, a very steep line was obtained since this drug killed all the foetuses before it was lethal