CXCIV. EFFECTS OF COLCHICINE AND RELATED SUBSTANCES ON CELL DIVISION.

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The effects of the alkaloid colchicine as a mitotic poison were first described by Lits [1934] and Dustin [1934], and the nature of its action has been further studied recently by Ludford [1936]. Amoroso [1935] has reported experiments on the inhibition of tumour growth in mice and a dog by injection of colchicine, although uniform results have not been obtained by subsequent workers. In common with a number of apparently unrelated substances, notably the cacodylates, it has been shown (Lits, Dustin, Ludford) to arrest cell division during metaphase and to maintain cells thus affected in this phase of mitosis for several hours following administration; this occurs both in tissues *in vivo* and in explanted tissue. It is, of course, most readily seen in tissues in which the mitosis rate is normally high, notably in the glands of the intestine, and it occurs as well in malignant tumours and in explants from them. It has been possible to show [Brues, 1936] in the case of the regenerating rat liver (in which the normal rate of cell division is known with reasonable accuracy) that under suitable conditions the number of mitoses seen in arrested metaphase after administration of colchicine over a given length of time is equal to the number of mitoses which would normally have occurred and gone on to completion during that time. The present study has been made in an attempt to determine the effects on mitosis of various compounds derived from colchicine, to assess the importance of the various molecular groupings of colchicine and to determine whether similar mitotic effects may be shown with colchicine derivatives which are devoid of the high toxicity of the parent alkaloid.

It has been essential to select as a test-object a tissue with a fairly high normal rate of mitosis in which the abnormal arrested metaphase can be unequivocally distinguished from the normal metaphase. Experimental tumours fulfil the first of these criteria, but owing to the frequency of abnormal mitotic figures it is often impossible to say whether a given mitosis shows the toxic effects of a mitotic poison or not. Moreover, we have observed in tumours that many cells in apparently normal later stages of division are seen after effective doses of the drug have been given and, at least in the case of certain transplanted sarcomata (probably owing to inadequate blood supply), the effect may be confined to the borders of the tumour and in some sections missed altogether.

The most satisfactory tissue for our purpose has been liver in the process of restoration following subtotal hepatectomy. In the case of the rat, the average mitosis rate during the period of rapid regeneration is nearly as great as that in most experimental tumours; and histological study is facilitated by the large size of the hepatic cells and by the highly characteristic distribution of the chromosomes under the influence of a mitotic poison. In the normal hepatic

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cell mitosis the chromosomes form a very compact group shortly after the disappearance of the nuclear membrane during prophase and remain so throughout the division: except in prophase it is impossible to distinguish and count the individual chromosomes (Pl. III, fig. 1). On the other hand, after parenteral administration of a suitable dose of colchicine, it can be seen that the chromosomes scatter widely throughout the cell shortly after disappearance of the nuclear membrane, as if they repelled one another, and it is often possible to count them (Pl. III, figs. 2 and 3). In addition, the cell in this arrested stage has the rounded border characteristic of a cell in mitosis and in most cases the cytoplasm stains much more lightly with eosin. Since we have never seen (except under the influence of a very small dosage) these abnormal figures in the same section with normal metaphases and later stages of mitosis, it is easily possible to distinguish the abnormal picture from the normal one. An appropriate dose of sodium cacodylate gives essentially the same picture in this organ.

Technique.

Using the customary aseptic precautions partial hepatectomies have been performed on a series of rats weighing 100–200 g. by a method described previously [Higgins & Anderson, 1931]. According to this method, the main and lateral lobes are removed, leaving approximately 30 % of the liver intact. This remnant has been shown [Brues *et al.* 1936] to begin increasing in size at once, whilst mitoses begin to appear in large numbers after 24 hours. We have therefore administered the substances to be tested 24–30 hours post-operatively, when mitosis is usually at its height. The animals were then killed for microscopic examination of tissues after a further interval of 6–18 hours. The drugs were given subcutaneously. Intraperitoneal administration seemed unwise since often a little ascites is seen at the time of autopsy. Microscopic examination was made on tissue fixed in 10 % formalin, sectioned in paraffin and stained with iron haematoxylin and eosin, or for purposes of rapid results on tissue smears stained with Leishman's stain, differentiated rapidly in 95 % alcohol and mounted in balsam.

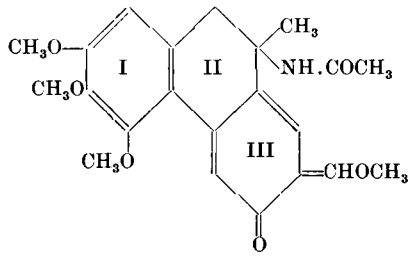
Upon administration of colchicine in aqueous solution subcutaneously, the greatest numbers of arrested mitoses were seen with a dosage of 0·1–0·2 mg. per 100 g. body weight. With doses of 0·02–0·05 mg. partial effects were seen in which the numbers of arrested mitoses were smaller, whilst many figures were seen in which some of the chromosomes had scattered, while the rest remained in a group. When large doses (0·5–10 mg.) were given, fewer figures were seen than with the smaller optimum amounts. In the case of these large doses, which usually are lethal if the experiment is prolonged to 24 hours, it was found that the number of abnormal figures seen in animals receiving the injections less than 24 hours after operation was much smaller (0–0·2 %) than in animals injected more than 24 hours after operation (1–3 %). Since these percentages are quite similar to the percentages of mitoses seen in the normally regenerating liver before and after the 24-hour interval, it seems likely that large doses of colchicine prevent cells from entering mitosis, so that the abnormal figures seen represent the cells which were already in mitosis at the time of administration. This may also be the case in a certain few rats receiving doses of 0·1 and 0·2 mg. in which only a few abnormal mitoses could be found. In no case receiving an effective dose (0·1 mg. or more) of colchicine more than 24 hours after operation did we fail to find some abnormal figures.

Since the percentage of abnormal mitoses seen is therefore not wholly dependent upon dosage, we have for purposes of assay tried to determine a

"minimum dosage" which gives partial abnormalities of mitosis, above which the typical picture of wholly scattered chromosomes is seen and below which mitoses appear normal. All dosages are expressed in mg. of substance per 100 g. body weight.

Results.

Colchicine has as its nucleus a partially hydrogenated phenanthrene ring, and Windaus [1924] has proposed the following structure for it, the only features which he regards as uncertain being the positions of the two substituents in ring III.



The colchicine employed in these experiments was obtained from the Hoffman La Roche Laboratories. Since this substance contains about 25% chloroform of crystallization, a few rats were injected with amorphous colchicine, which contains no chloroform, with entirely similar results as far as the cytological picture was concerned.

Colchicine was dissolved in 0·9% saline solution before administration. When large quantities were used, it was first dissolved in a minimum amount of alcohol, in order to facilitate solution in water. In order to control the use of alcohol in the administration of this and other substances, comparable amounts of alcohol alone have been injected into animals during hepatic regenerations, without any resulting abnormalities of mitosis being detectable.

A small series of rats were given subcutaneous injections of colchicine suspended in sesame oil, in the hope of retarding the absorption. If there is any real difference in the action of the oil solution, it appears to increase the mitotically effective dose and to decrease the minimum toxic dose, as appears in Table I.

Colchiceine [Zeisel, 1886], in which the $=CHOCH_3$ group of colchicine has been hydrolysed to $=CHOH$, was employed in saline solution. It is interesting that the minimum lethal dose of colchiceine (3 mg.) often kills within 2 hours of the time of administration, whilst the much more toxic substance colchicine, even in a dosage of 10 mg., does not kill before 8 hours after administration.

Colchicine salicylate, which is a simple molecular compound of colchicine with salicylic acid, and is in common medicinal use, appears similar to colchicine in its action and dosage, as might be expected.

Octahydrocolchicine [Windaus, 1924], which is obtained by catalytic hydrogenation of colchicine, is a derivative of *as*-octahydrophenanthrene in which the methoxymethylene group and the carbonyl group are both reduced. The specimen was prepared by Dr E. Boyland and used in saline solution.

Dimethyl- and trimethyl-colchicinic acids [Zeisel, 1888] are products of hydrolysis which contain a free amino-group in ring II and a free hydroxymethylene group in ring III. In trimethylcolchicinic acid the three methoxyl groups in ring I are preserved intact, but in dimethylcolchicinic acid one of these is hydrolysed to hydroxyl. These two compounds were used in saline solution, neutralized with $N/10$ NaOH. It will be seen that these derivatives alone have been ineffective in any sublethal dosage.

Table I.

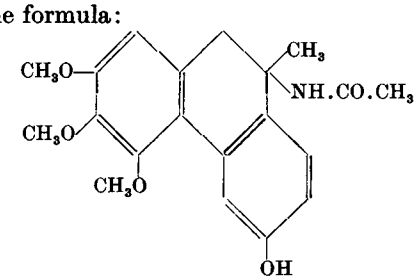
Substance	No. of animals	Minimum effective dose mg./100 g.	Optimum dose (where determined) mg./100 g.	Average lethal dose mg./100 g.
Colchicine (aqueous)	55	0·02	0·1	0·5
Colchicine in oil	5	0·1	0·2	0·2
Colchicine salicylate	5	0·05	0·1	1·0
Colchiceine	6	0·8	3·0	3·0
Octahydrocolchicine	3	3·0	10·0	10·0
N-Acetylcolchinol	6	0·9	3·0	20·0
N-Acetylcolchinol methyl ether	5	8·0	—	12·0
Colchinol methyl ether hydrochloride	4	6·0	—	20·0
Corresponding carbinol*	6	7·5	10·0	†
N-Acetyliodocolchinol	4	10·0	—	20·0
Dimethylcolchicinic acid	5	—	—	10·0
Trimethylcolchicinic acid	5	—	—	20·0

* This carbinol, which will be described elsewhere (Cohen & Cook), was obtained by the action of nitrous acid on colchinol methyl ether.

The "minimum effective dose" is the smallest dose which gives obvious abnormalities of mitosis. The "optimum dose" is the average amount which gives a maximum number of abnormal mitoses, i.e. which is effective in stopping mitosis in metaphase but which does not prevent cells from entering mitosis.

† The toxic dose of this substance is probably higher than 20 mg., but there was not sufficient material to determine this dosage.

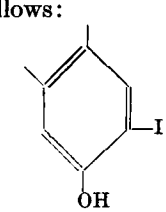
N-*Acetylcolchinol* [Windaus, 1919]. This is a more profoundly modified colchicine derivative, and on the basis of Windaus's structure for colchicine would be represented by the formula:



This was dissolved in a small amount of alcohol and made up by the addition of saline solution. The *methyl ether* [Windaus, 1919] of this compound, which was less soluble in both alcohol and water than the free phenol, was utilized in the same way. *Colchinol methyl ether hydrochloride* [Windaus, 1919] is the hydrochloride of the free base arising from hydrolytic removal of the acetyl group, and was dissolved in saline solution by neutralization with $N/10$ NaOH.

The *carbinol* obtained by the action of nitrous acid on this base was dissolved first in alcohol; with the addition of saline solution a fine colloidal suspension of the substance was obtained for injection.

N-*Acetyliodocolchinol*, an intermediate in the preparation of *N*-acetylcolchinol, in which ring III is as follows:



was injected in fine suspension.

Discussion.

Although the colchicine molecule contains a variety of molecular groupings (e.g. methoxyl, methoxymethyleneketone, acetylamino) it cannot be said from the results of this investigation that any single group is essential for the mitotic inhibiting action of colchicine. The compounds examined include colchicine derivatives in which the methoxymethylene group has been first hydrolysed to hydroxymethylene, then replaced by iodine, and finally completely eliminated, the last two stages being accompanied also by modification of the ketonic group (conversion into a phenolic hydroxyl group). Also, some of the compounds examined have had the acetylamino-group intact, others have had this group hydrolysed to the free amino-group, while in yet another case the amino-group is replaced by hydroxyl. All of these derivatives have shown activity. The inactivity of dimethyl- and trimethyl-colchinic acids is of interest. In the former case the suppression of activity might perhaps be attributed to demethylation of one of the three methoxyl groups in ring I, but trimethylcolchicinic acid appears to be anomalous, for the only modifications in the colchicine molecule are (*a*) hydrolysis of the methoxymethylene group to hydroxymethylene and (*b*) hydrolysis of the acetylamino-group to the free amino-group, whereas it has been shown that neither of these changes is necessarily accompanied by loss of activity. Possibly the inactivation of trimethylcolchicinic acid is associated with the presence of the basic grouping and also the strongly acidic hydroxymethylene group in the same molecule. The lack of specificity suggested by these results appears to warrant the examination of synthetic compounds of analogous structure, and experiments on these lines are in progress.

It will be seen that no substances are effective on mitosis in the small doses required in the case of colchicine itself, and that the toxicity roughly follows the same relative dosage with different compounds, except in the case of the colchicinic acids (which are ineffective on mitosis), and possibly in the case of the nitrogen-free carbinol, in which the lethal dose is as yet undetermined. However, the fact that the lethal effect of colchicine appears only after several hours, when the mitotic effect is beginning to wear off, suggests that the two effects may be dissociated, and this question requires further investigation.

There is obviously a wide gap between the effective and lethal doses of colchicine (and its salicylate) and those of the other substances. Some of the other differences of dosage may, however, depend upon variations in solubility and absorbability.

Summary.

A number of derivatives of colchicine have been investigated with regard to their effects as mitotic poisons. The test object was the regenerating rat liver, in which the arrested mitosis is conspicuous and is easily distinguished from the normal mitosis.

Colchicine, injected subcutaneously in aqueous or oily solutions, produces arrest in metaphase of a maximum number of mitoses only within certain limits of dosage. Larger doses appear to prevent cells from entering mitosis, whilst in smaller dosage partial effects are seen.

Colchicine, octahydrocolchicine, *N*-acetylcolchinol and four derivatives of the latter all produce similar effects to colchicine, but in considerably higher dosage. Dimethyl- and trimethyl-colchicinic acids have been ineffective in any sublethal or lethal dosage.

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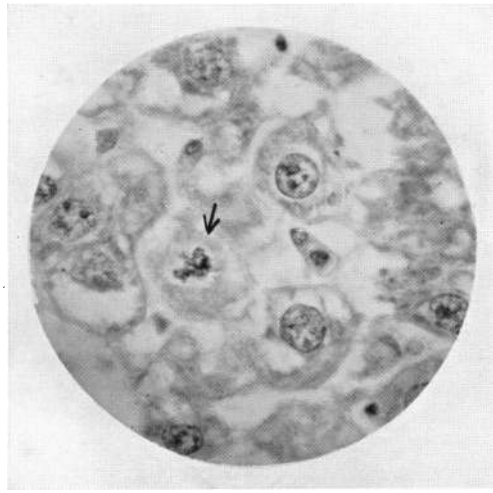

Fig. 1. Normal metaphase (indicated by arrow) in regenerating liver 30 hours after operation. ×600.

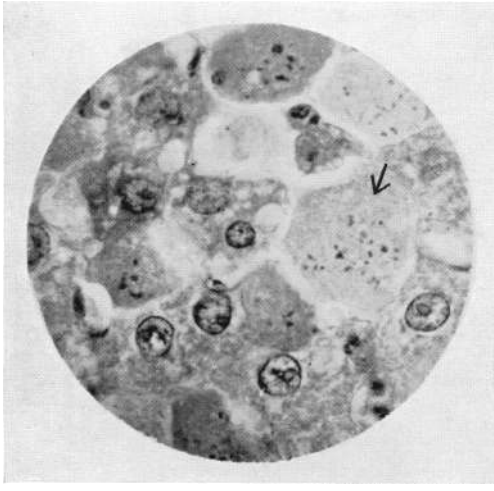

Fig. 2. Abnormal mitoses in regenerating liver 2 days after operation and 8½ hours after colchicine treatment (0·1 mg. subcutaneously). One abnormal figure is indicated by an arrow. ×600.

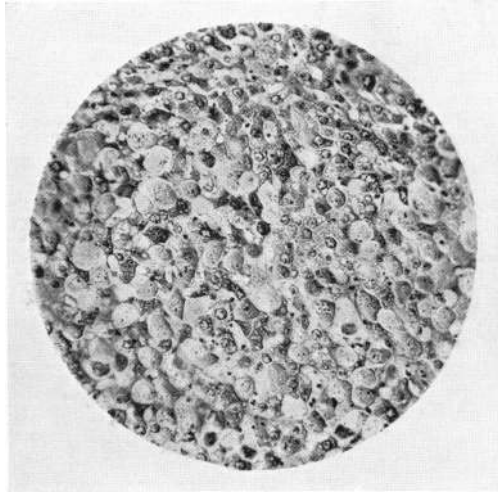

Fig. 3. Low-power view of the liver shown in Fig. 2 showing large numbers of arrested mitoses. ×95.