

The Action of Colchicine on the Intestinal Epithelium of the Cat and Dog

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SUMMARY

Varying doses of colchicine have been administered intraperitoneally to cats and dogs in order to study histologically the reactions in the epithelium of the small intestine. In normal animals of both species, approximately 1% of the total number of epithelial cells in the ileum are in mitosis at a given time. After exposure to colchicine for 5 h, the maximum number of arrested mitoses in the cat was found to be 3.77%; in the dog the maximum number rose to 17.12%. The results indicate that in the epithelial cells of the dog's small intestine, colchicine not only arrests mitosis in metaphase, but also stimulates the cells to enter mitosis.

INTRODUCTION

THE use of colchicine as a mitotic inhibitor has been widespread on the assumption that, with suitable dosage, cells that have begun to divide will be arrested in the metaphase stage of the mitotic cycle. As a result of this arrest, if an animal is killed a few hours after the administration of the drug, an accumulation of arrested metaphases will be seen, representing the number of cells that would normally have gone on to complete their division during that time. This effect is produced by interference with the formation of the spindle (Ludford, 1936; Hughes, 1950). In a recent study with the electron microscope, Inoué (1952) concluded that the action of colchicine on the spindle is 'to disorganize the orientation of the micelles in the astral rays and spindle fibers'. The assumption that any metaphase seen a few hours after colchicine administration is that of a nucleus that would normally have undergone mitosis and that has not been artificially stimulated to do so, is the basis of many biological studies. With some exceptions, its use has been confined almost entirely to experiments on mice, rats, and embryos. Little information is available regarding mitotic inhibition by this substance in larger animals. In the course of studies on the renewal and regeneration of epithelium in the small intestine of the cat (McMinn, 1954; McMinn and Mitchell, 1954), colchicine was used to arrest mitosis among the cells lining the intestinal glands (crypts of Lieberkühn). The dose employed in those studies was determined by a small series of pilot experiments, since no previous reports on the use of colchicine in the cat were available. Those experiments have been extended to include a similar series in the dog. The results of this work are now presented, since they have revealed a fundamental difference between the two species with regard to the reaction of the intestinal epithelium to colchicine.

MATERIALS AND METHODS

All experiments were performed on healthy adult cats and dogs. The animals of each species were divided into 5 groups of 4 animals in each group. One group served as normal controls, and the remaining groups received doses of colchicine (see table 1), administered intraperitoneally at 10 a.m. The animals of all groups had been starved since the previous evening, and all were killed at 3 p.m. Possible discrepancies due to diurnal mitotic variations were thus eliminated. The histological details and the method of enumerating the epithelial nuclei were the same as those previously reported (McMinn, 1954). Briefly, the mitotic counts were carried out on specimens of ileum (about 50 cm proximal to the ileo-colic junction) stained with haematoxylin and eosin. A square mask was used in one of the eyepieces of a binocular microscope. At least 2,000 nuclei were included in any single count, 4 such counts being carried out in each animal. Although mitosis occurs only in the cells lining the crypts and not in those covering the villi, the number of dividing nuclei is expressed as a percentage of the total number of epithelial nuclei in both crypts and villi. A number of duodenal specimens were also examined, but since the results were similar to those in the ileum, no further reference is made to them.

RESULTS

The results of the mitotic counts in both the normal and colchicine-treated animals are summarized in table 1, the percentages being the means of the counts in the 4 animals of each group.

TABLE 1

| | | <i>Anaphases and telophases</i> | <i>Percentages of nuclei in mitosis or arrested mitosis (means \pm S.E.)</i> | |
|-----|------------|---------------------------------|-------------------------------------------------------------------------------------------|------------------|
| cat | normal | present | 0.95 \pm 0.04 | |
| | colchicine | (mg per kg) | | |
| | | 0.1 | present | 1.27 \pm 0.30 |
| | | 0.25 | absent | 3.77 \pm 0.33 |
| | | 0.5 | absent | 1.98 \pm 0.08 |
| 1.0 | absent | 1.62 \pm 0.12 | | |
| dog | normal | present | 1.03 \pm 0.34 | |
| | colchicine | 0.05 | present | 2.51 \pm 0.25 |
| | | 0.10 | present | 2.82 \pm 0.23 |
| | | 0.15 | absent | 7.69 \pm 0.43 |
| | | 0.25 | absent | 17.12 \pm 1.00 |

From the table it can be seen that in the normal cat and dog approximately 1% of the total number of epithelial cells were in mitosis at any one time. In the cat, colchicine in a dose of 0.1 mg per kg was insufficient to cause complete arrest in metaphase of all dividing nuclei, since a considerable number of

nuclei in later phases of mitosis were still found. With larger doses, anaphases and telophases were no longer seen, and the maximum percentage of mitotic nuclei was observed with 0.25 mg per kg. Higher doses resulted in a percentage of arrested nuclei that was not significantly greater than that in the group of normal controls.

In the dog it was found necessary to give a range of smaller doses, since with 0.25 mg per kg (the highest dose given to a dog) severe toxic signs developed, such as were seen in cats receiving 1.0 mg per kg. The number of arrested nuclei found in dogs to which 0.25 mg per kg had been administered reached 17.12% of the total number of epithelial nuclei. Doses of 0.05 and 0.1 mg per kg were insufficient to cause complete metaphase arrest, while 0.15 mg per kg gave rise to nearly 8 times as many mitotic cells as in the normal group, and no later phases of the mitotic cycle were evident.

Thus the maximum percentage of dividing nuclei in the cat—3.77% after exposure of 5 h to a dose of 0.25 mg per kg, which was not the largest tolerated dose—was less than 4 times that found in the normal animal. However, in the dog, which displayed a normal figure similar to that in the cat (about 1%), the greatest number of arrested nuclei, 17.12%, was found after administering the largest tolerated dose of the series.

DISCUSSION

The results indicate the pronounced difference that exists between the cat and dog with regard to the reaction of intestinal epithelium to colchicine. The fact that the cat (of about 2 kg) seems to have a greater tolerance for colchicine than the dog (which is considerably heavier), is in keeping with the tolerance shown by the mouse, which may weigh 25 g and receive a total dose of 0.1 mg, i.e. at a rate of 4 mg per kg (Bullough, 1949), compared with the rat, for which the dose usually suggested is 1 mg per kg (Bertalanffy and Leblond, 1953; Ebling, 1954).

In the cat, doses exceeding 0.25 mg per kg do not result in a continuing increase in the number of arrested nuclei. This suggests not only that nuclei are being arrested in metaphase but also that some are being prevented from entering mitosis. Brues and Cohen (1936) and Bucher (1939) noted that doses larger than those required to give a maximal response normally caused a diminution in the number of arrested nuclei. A similar phenomenon was noted by Ebling (1954) in rat skin, and by Henry, Meyer, Weinmann, and Schour (1952) in the oral epithelium of rabbits. Bullough (1949) concluded that after about 5 h resting cells (in the ear epidermis of the mouse) were prevented from entering prophase. However, in the dog, increasing the dose increased the number of arrested nuclei. The maximum percentages found in the cat and dog are 3.77 and 17.12 respectively, after an equal period of exposure to colchicine. Since in normal animals of both species only about 1% of cells are in mitosis at a given time, it would appear that in the dog, cells are being stimulated to enter mitosis, on the assumption that the duration of the mitotic cycle is approximately the same in both species.

This apparent stimulation of mitosis by colchicine is a feature of its action that was recognized as early as 1906 by Dixon, who stated that its effect was to 'excite karyokinesis'. Paff (1939) considered that in the chick embryo colchicine could stimulate as well as inhibit cells that were rapidly dividing. In more recent experiments on tadpoles, Crişan and Mihalca (1948) were of the opinion that colchicine was stimulating cells that were about to divide. In contrast to these observations, current views on the action of colchicine have been summarized by Miszurski and Doljanski (1949), who stated: 'it is now always taken for granted that colchicine has no positive or negative effect on the rate of appearance of the mitoses before they are arrested and that its only effect is to arrest cell division that is already in progress'. Evidence in support of this is provided by the tissue culture studies on both normal and malignant cells reported by Ludford (1936), Bucher (1939), Törö and Vadász (1939), Tennant and Liebow (1940), and Hughes (1950), while in the living animal the work of Brues (1936), Buschke, Friedenwald, and Fleischmann (1943), and Bullough (1949) may be cited.

While most investigators who have used colchicine as a mitotic inhibitor have employed relatively short time intervals, others have thrown further light upon the possible stimulating action of this substance by studying the delayed effects following a single or repeated injections. Miszurski and Doljanski (1949) found an outbreak of mitotic activity in the rat's liver (where mitoses are normally uncommon) that was maximal 3 days after the injection, while Dustin (1941) distinguished between the mitoses that are seen within a few hours of injection in tissues with relatively high mitotic indices, and the 'mitoses tardives' that are found after a period of several days in various organs in which mitotic activity is normally very low.

Although the results of the present work are an indication that in the dog colchicine can stimulate the cells of intestinal epithelium to enter mitosis, it remains to be seen whether other tissues in this species exhibit a similar behaviour. The present findings have substantiated the dose used in the cat in previous reports (McMinn, 1954; McMinn and Mitchell, 1954), but have shown that it is not possible to calculate the rate of renewal of intestinal epithelium in the dog by use of the colchicine technique. They serve to emphasize the importance of carrying out studies with varying doses of colchicine, in species whose reaction to the drug is not well established, before its proposed use (for example) in the estimation of the rate of renewal of cell populations (Leblond and Walker, 1956).

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