STUDIES ON COLCHICINE DERIVATIVES

*II. Effect on Mitotic Activity of the Corneal Epithelium**

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THE present paper is an attempt to correlate the toxicity of a series of colchicine compounds with their effect on corneal mitosis. This seemed of interest because of the highly specific effect of colchicine and some of its derivatives on mitosis. The predominant effect is an arrest of mitosis in metaphase, probably due to an inhibition of spindle formation. The corneal epithelium of the rat is a test object excellently suited for studies on mitoses. A method employing mitotic counts on flat preparations of the cornea has been developed for studies on the effect of factors influencing mitotic activity.[4] The mechanism of action of colchicine,[4] of the nitrogen mustards,[6] and of thyroidectomy,[5] on mitotic activity has been analyzed by this method. For the study of the effects of colchicine and its derivatives, the corneal epithelium offers the advantage of being avascular. Colchicine and some of its derivatives have a marked effect on the capillaries,[9] one that is liable to interfere with a quantitative study of the specific effect of these compounds on mitosis in vascularized tissues. The compounds used for the toxicity studies reported in the first paper of this series[7] were used in this investigation. As relatively precise median lethal dosages (LD_{50}) were available as a result of these toxicity studies, the effects of equitoxic doses could be compared. It was our purpose to determine how great a fraction of the LD_{50} is necessary to produce a significant antimitotic effect.

MATERIAL AND METHODS

Male albino mice, Carworth Farms CF1, weighing between 15 and 35 gm., were used. The drugs were administered intraperitoneally. The methods of preparing the solutions for injection are described in the first paper.[7] Mice were sacrificed at six, twenty-four, and forty-eight hours after injection by cervical dislocation. Control mice were injected with the appropriate vehicle.

Corneal mitoses were studied on flat preparations using the method described previously for the rat.[4] Because of the smaller size of the eye of the mouse, a meridional band only 0.5 mm. wide was cut along the greatest meridian of the cornea. Counts were made of all mitoses seen on a meridional strip, 0.11 mm. in width.

RESULTS

Statistical analysis shows that an increase of mitotic figures to more than 0.7 per cent of all cells counted is statistically highly significant. Therefore, an average mitotic index of more than 0.7 per cent was accepted as indication of a minimal antimitotic dose. The average percentage of corneal epithelial cells seen in mitosis is tabulated in Table 1. The minimal effective dose for an exposure of six hours is expressed in Table 2 both in mg. per Kg. and in fractions of the LD_{50}.

DISCUSSION

In general, one can conclude from the data tabulated in Tables 1 and 2 that the less toxic

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TABLE 1

TOXICITY AND ANTIMITOTIC EFFECT OF TRIMETHYLCOLCHICINIC ACID (TMCA) DERIVATIVES

Compound		*Dose*		*Average % cells in mitosis after*		
No.	*Name*	*mg./Kg.*	*Fraction LD_{50}*	*6 hrs.*	*24 hrs.*	*48 hrs.*
1	N-acetyl TMCA methyl ether (colchicine)	7.0	2	4.6	8.2	
		5.3	1 1/2	7.7	5.7	1.8
		3.5	1	6.8	1.2	0.8
		1.77	1/2	2.9	1.3	1.8
		0.88	1/4	2.2	1.7	1.6
		0.35	1/10	1.6	1.5	0.3
		0.17	1/20	0.5	—	—
		0.07	1/50	0.2	—	—
		0.035	1/100	0.3	—	—
2	N-benzoyl TMCA methyl ether	32.0	1	2.3	0.7	—
		16.0	1/2	1.1	0.6	0.4
		8.0	1/4	0.7	0.9	0.9
		1.6	1/20	0.3	—	—
		0.64	1/50	0.4	—	—
		0.32	1/100	0.2	—	—
3	TMCA methyl ether *	92.0	2	7.0	4.7	1.6
		46.0	1	3.5	3.4	—
		23.0	1/2	3.4	3.5	—
		11.5	1/4	4.0	1.7	—
		4.6	1/10	3.6	1.5	1.4
		2.3	1/20	2.9	1.1	—
		0.92	1/50	1.4	—	—
		0.46	1/100	1.0	—	—
4	N-acetyl colchinol	56.0	1	2.9	0.9	1.2
		28.0	1/2	3.0	—	—
		14.0	1/4	0.6	—	—
		2.8	1/20	0.3	—	—
		1.12	1/50	0.4	—	—
		0.56	1/100	0.2	—	—
5	N-acetyl TMCA (colchiceine)	84.0	1	3.0	0.5	0.9
		42.0	1/2	0.4	1.6	0.5
		21.0	1/4	0.3	0.4	—
		4.2	1/20	0.3	—	—
6	TMCA	200.0	1	0.3	0.3	—
7	N-benzoyl TMCA	700.0	1	0.4	0.4	—
	Control			0.2	0.5	0.4

*d-Tartrate derivative used. The LD_{50} is calculated for the compound itself. The LD_{50} for the tartrate is 64.0 mg./Kg.

compounds are less effective on mitosis. There is one exception, that is TMCA methyl ether (compound 3, which was given in the form of its tartrate). This compound is effective at 1/100 of its LD_{50}; in other words, it has the highest therapeutic index of the compounds examined.

Data on inhibition of mitosis by compounds of this series has been reported by Brues and Cohen, who studied the effect of systemic poisoning on regenerating liver cells, and Lettré and Fernholz, who investigated the antimitotic effect in tissue cultures of chick fibroblasts. The effect of topical administration to the epithelium of the gastric mucosa of the mouse was studied by Branch. The effect on mouse sarcoma 180 and on mouse spermatogonia is reported in the first[7] and third[1] papers of this series.

The results on tissue cultures, on liver cells, and on corneal epithelium are compared in Table 3. If we designate the amount of colchicine necessary to produce an antimitotic effect as 1, the ratios of the amounts of com-

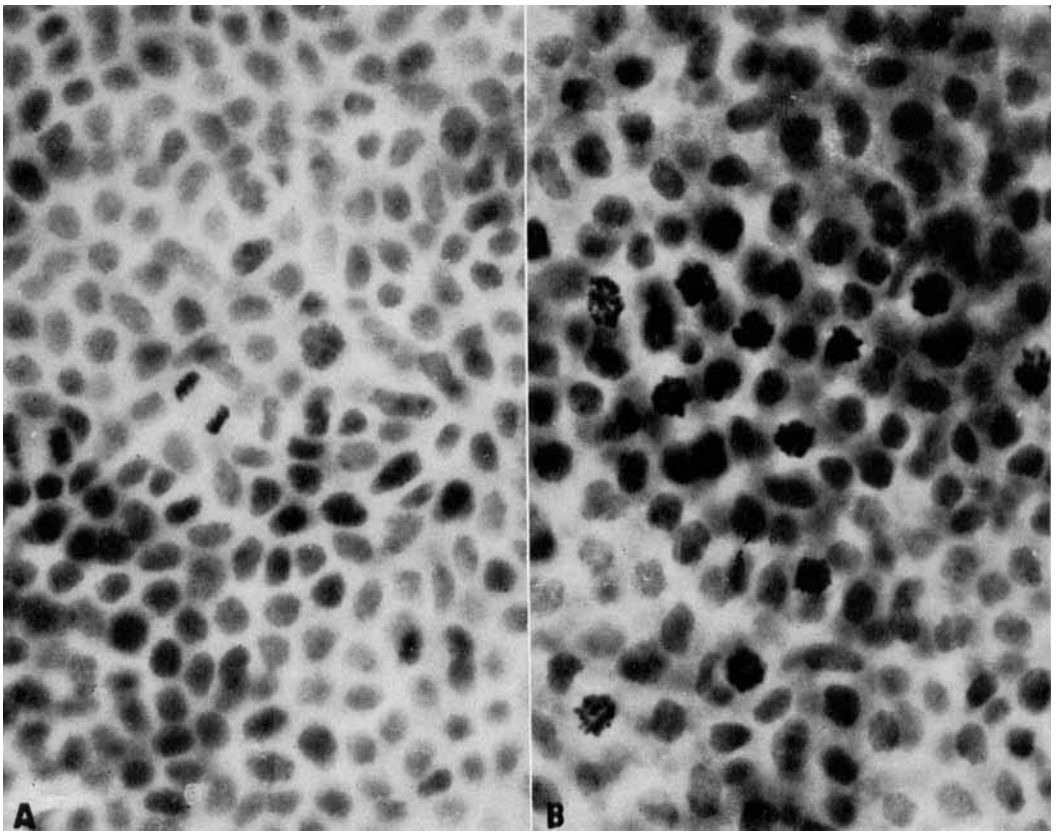

FIG. 1. *Mouse cornea. Flat preparation.*
A, *Control: Six hours after injection of normal saline.*
B, *Treated: Six hours after injection of TMCA methyl ether d-tartrate (64.0 mg./Kg.)*
(Hematoxylin. × 750.)

pounds 1, 4, and 5 are as 1:100:400 for the tissue cultures and 1:80:240 for our experiments on corneal mitoses. This is a good agreement considering the great difference in experimental conditions. The corresponding values for liver cells are 1:45:40.

Branch has chosen a different method of comparing the antimitotic effect of colchicine derivatives. Solutions of colchicine compounds are given to mice by insertion of a blunted syringe needle directly into the esophagus. After treatment, the mice are sacrificed at two-hour intervals up to sixteen hours. Mitotic counts are made on sections of the gastric mucosa. Colchicine is the most effective of the fourteen compounds examined. As in our experiments on the cornea, TMCA methyl ether *d*-tartrate is almost as effective as colchicine. The third most effective compound, according to Branch, is N-benzoyl TMCA methyl ether. This compound is not very effective in arresting corneal mitosis, but is fairly effective in arresting mitosis in mouse sarcoma 180[7] and in mouse spermatogonia.[1] This compound is less soluble in water than colchicine or TMCA methyl ether *d*-tartrate. The difference in solubility may account for the difference in antimitotic activity of N-benzoyl TMCA methyl ether by topical application to the gastric mucosa and by systemic application in the corneal epithelium.

Some differences in sensitivity of various tissues become apparent by a comparison of the results obtained in this series of papers. N-acetyl colchinol and N-benzoyl TMCA methyl ether are more active against sarcoma

TABLE 2

MINIMAL EFFECTIVE ANTIMITOTIC DOSE OF TMCA DERIVATIVES ON CORNEAL MITOSIS

Compound			*Minimal effective antimitotic dose*	
No.	*Name*	LD_{50} *mg./Kg.*	*mg./Kg.*	*Fraction* LD_{50}
1	N-acetyl TMCA methyl ether (colchicine)	3.5	0.35	1/10
2	N-benzoyl TMCA methyl ether	32.0	8.0	1/4
3	TMCA methyl ether	46.0	0.46	1/100
4	N-acetylcolchinol	56.0	28.0	1/2
5	N-acetyl TMCA (colchiceine)	84.0	84.0	1
6	TMCA	200.0	>200.0	>1
7	N-benzoyl TMCA	>700.0	>700.0	—

180 and mouse spermatogonia than against the corneal epithelium.

The mechanism of the antimitotic effect of colchicine and its derivatives seems to be similar. In corneas of mice treated with effective doses of one of the derivatives found to have an antimitotic effect (compounds 2, 3, 4, and 5) more than 90 per cent of all mitoses show the typical picture of "colchicine mitosis" (Fig. 1). Cells in "colchicine mitosis" are recognizable by their brilliantly acidophilic cytoplasm in the periphery, paler clear "mixoplasm" toward the center surrounding a dense mass of clumped chromosomes.

SUMMARY

1. A number of colchicine derivatives, the median lethal dosages (LD_{50}) of which were available, were examined for their antimitotic effect on the corneal epithelium of mice.

2. Colchicine was the most toxic of the compounds examined. In general the less toxic compounds are less effective on mitosis with the exception of TMCA methyl ether, which was given in the form of its tartrate. One hundredth of the LD_{50} had a significant effect in arresting mitosis. This indicates that TMCA methyl ether has a higher therapeutic index than the other colchicine derivatives examined.

TABLE 3

COMPARISON OF ANTIMITOTIC EFFECT ON VARIOUS TISSUES

Compound		*Minimal Effective Dose*		
No.	*Name*	*Tissue cultures* γ/cc.	*Liver mg./Kg.*	*Cornea mg./Kg.*
1	Colchicine	0.01	0.21	0.35
4	N-acetylcolchinol	1.0	9.01	28.0
5	Colchiceine	4.0	8.01	84.0
6	TMCA	inactive	inactive	inactive

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