STUDIES ON COLCHICINE DERIVATIVES

I. Toxicity in Mice and Effects on Mouse Sarcoma 180

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ALTHOUGH colchicine and its derivatives have been studied extensively, there have been relatively few reports on the comparative toxicity of these compounds.[5, 10, 12, 13] The present investigation was designed to study the relation of toxicity to chemical structure of a closely related group of colchicine derivatives. An attempt has been made to obtain relatively precise median lethal dosages (LD_{50}) in a particular strain of mice. The data thus obtained served as a basis for a comparative study of the antimitotic and other cellular and histological effects of equitoxic doses.[4, 9] By such procedures, one can differentiate more quantitatively between systemic toxicity and specific biological effects, and relate these to molecular structure.

In studies of the action of colchicine on neoplasms, two outstanding effects have been noted: (1) mitotic arrest (due to inhibition of spindle formation)[14] which is often obscured at higher concentration by necrosis, and (2) hemorrhage, which generally has been regarded as the result of injury to the proliferating capillary endothelium.[8, 14] Using these effects as criteria of activity, an attempt was made in this investigation to ascertain the minimum effective fractions of the LD_{50} of the various compounds. The compounds which would be considered more desirable from the standpoint of possible therapeutic use are those which are effective far below the LD_{50}.

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METHODS

Throughout these experiments male albino mice, Carworth Farms CF1, weighing 15 to 35 gm., were used. The mice were kept in wire-mesh cages in groups of five, and were given water and Dog Friskies ad libitum.

Trial concentration ranges, for LD_{50} determinations, were obtained from information available in the literature. Dosage levels were run at geometrically spaced intervals, each lower dose being three quarters of the preceding dose. In the event that satisfactory data could not be obtained thereby, additional concentration ranges were used. In most instances, the experiments were repeated on three occasions. In each experiment, seven to nine dosage levels were employed with five mice at each level. The diluent of choice was 0.9 per cent NaCl, 10 per cent aqueous gum acacia being used as a suspending agent for the insoluble compounds. Insoluble substances were first triturated in a small amount of the suspending agent and then diluted to correct volume. Care was exercised to keep the material in suspension prior to injection by continuous stirring and agitation. All mice received a single intraperitoneal injection. The total volume of each injection in milliliters was 1 per cent of the weight of the mouse in grams. The mice were observed and mortality recorded for a period of ten to thirteen days after an injection of the compounds.

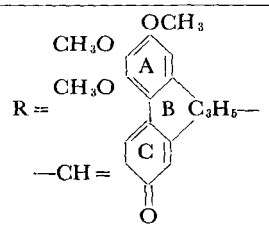
Approximate LD_{50} figures were obtained by plotting the data on log probability paper

TABLE 1

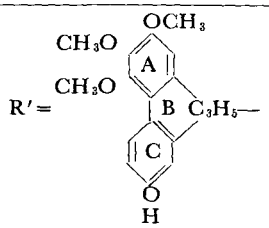
TOXICITY DATA ON COLCHICINE DERIVATIVES*

Compound		*Mol. wt.*	LD_{50} *mg./Kg.*	LD_{50} *μM./Kg.*	*Min. eff. anti-tumor dose μM./Kg.*	*Vehicle*
N-benzoyl TMCA	R{$NHCOC_6H_5$, OH	447.5	>700	>1600	—	10% Gum acacia
TMCA	R{NH_2, OH	343	200	510	—	10% Gum acacia
N-acetyl TMCA (colchiceine)	R{$NHCOCH_3$, OH	385.4	84	213	50	10% Gum acacia
N-acetyl colchinol	R′ $NHCOCH_3$	357.4	56	157	14	10% Gum acacia
TMCA methyl ether	R{NH_2, OCH_3	525.8	[46]†	120	6	Saline
N-benzoyl TMCA methyl ether	R{$NHCOC_6H_5$, OCH_3	461	32	69	17	10% Gum acacia
N-acetyl TMCA methyl ether (colchicine)	R{$NHCOCH_3$, OCH_3	399	3.5	9	4.5	Saline

*



TMCA = Trimethylcolchicinic Acid



NOTE: The structures assigned to colchicine and its derivatives are tentative and as yet indefinite. The original structure by Windaus[17] may have to be modified in the light of the evidence that the "B" ring may be seven-membered: Barton, Cook, and Loudon,[3] and previous papers. Dewar[7] has suggested that the "C" ring is also seven-membered, a point of view that has been supported by Arnstein, Tarbell, Huang, and Scott.[2]

† *d*-Tartrate derivative used. Value calculated for simple compound.

when feasible. The data was then analyzed by the method of Reed and Muench.

In the tumor studies, the transplantable tumor, sarcoma 180, was employed and implanted bilaterally into 2- to 3-month-old male Carworth Farms, CF1, mice. The tumor fragments were placed subcutaneously midway between the axillary and inguinal regions. The effect of the drugs was observed after a single injection of each compound had been administered intraperitoneally to mice bearing seven- or eight-day-old tumors. The mice were sacrified at 6 hours* after injection and the tumors excised and fixed in 10 per cent formalin or Bouin's fluid. The tumors were then sectioned at 5 microns and stained with Harris's hematoxylin and eosin. Gross observations on the amount of hemorrhage and necrosis were recorded at the time of autopsy, and these findings were supplemented by microscopic examination of sectioned tumors. Mitotic counts were performed on ten adjacent, non-necrotic, oil immersion fields for each tumor. Appropriate controls, injected with the vehicle, were similarly examined.

*Mice, injected simultaneously, were sacrificed at twenty-four and forty-eight hours for other experiments.

RESULTS AND DISCUSSION

The results are summarized in Table 1, where the least toxic compound is listed first and the remainder are listed in the order of

TABLE 2

RÉSUMÉ OF TOXICITY DATA (MG./KG.) OF COLCHICINE DERIVATIVES

Compound	*Frogs*[10, 12, 13]	*Mice*	*Rats*[5]	*Cats*[10, 12, 13]
R{$NHCOC_6H_5$, OH		>700		
R{NH_2, OH	20	200	200	>10
R{$NHCOCH_3$, OH		84	30	>12.5
R′ $NHCOCH_3$	100 (8-10°C.)	56	200	10
R{NH_2, OCH_3	<20	[46]*		5
R{$NHCOC_6H_5$, OCH_3	>20	32		<25
R{$NHCOCH_3$, OCH_3	2-4 (30-32°C.) 60-100 (8-10°C.)	3.5	5.0	0.5 to 1.0

*See Table 1.

their increasing toxicity. It may be observed that there is an approximate two hundredfold increase in order of toxicity on a molar basis from the least to the most toxic compound, all of which apparently can be attributed to fairly simple modifications (a) methylation of the hydroxy methylene substituent of the "C" ring to an enol methyl ether, and (b) acylation of the amino group in the "B" ring.

Excluding N-acetyl colchinol, the trimethylcolchicinic acid (TMCA) structure may be regarded as basic to the compounds here investigated. Acetylation of the amino group on the "B" ring (colchiceine) results in a compound somewhat more than twice as toxic. Methylation of the hydroxy methylene group of the "C" ring, to form the methyl ether yields a compound (TMCA methyl ether) about four times as toxic. Both these substitutions together (colchicine) increase the toxicity by a factor greater than fifty.

Methylation of the hydroxy methylene group to form the enol methyl ether derivative has, in all cases studied in this report, rendered the analogs more toxic. Acylation (i.e., acetylation or benzoylation) in general has also increased toxicity except in the case of N-benzoyl trimethylcolchicinic acid.

Precise data on solubilities of these compounds as related to toxicity are not available but some tentative statements may be made at this time. Acetylation induced greater toxicity than benzoylation. Formation of the enol methyl ethers from the hydroxy methylene groups has given a similar result. In these instances, greater toxicity was associated with greater solubility. This may be an oversimplified interpretation as illustrated by the relatively high toxicity of N-benzoyl trimethylcolchicinic acid methyl ether, which is not soluble in water.

In Table 2, the toxicity data noted by other investigators are compared with those obtained in this study. Colchicine clearly enough is the most toxic compound of the series with the exception of the rather anomalous results obtained on frogs (and bats[11]) at low temperatures. Furthermore, removal of the enol methyl ether group on Ring C and the acetyl group on Ring B, gives TMCA, which is less toxic than either of the aforementioned derivatives in the case of the rat and mouse. However, Führer states that TMCA is more toxic than colchiceine to cats and dogs. A benzoyl group in place of the acetyl group appears to result in a decreased

TABLE 3

MITOTIC ACTIVITY OF A SERIES OF SIX COLCHICINE DERIVATIVES

Compound	*Dose fraction LD_{50}*	*Mitotic activity control = 1*	*No. tumors studied*
Colchicine	2	6.1	8
	1	7.6	12
	1/2	3.5	7
	1/4	1.4	16
	1/10	1.4	10
	1/20	1.1	12
	1/50	.9	7
	1/100	1.0	8
TMCA methyl ether	2	4.1	8
	1	4.3	15
	1/2	3.3	11
	1/4	8.2	10
	1/10	7.1	9
	1/20	3.4	12
	1/50	1.1	8
	1/100	1.1	9
Colchiceine	2	6.9	6
	1	9.0	13
	1/2	5.9	14
	1/4	4.8	14
	1/10	1.5	6
	1/20	1.0	6
N-benzoyl TMCA methyl ether	2	3.0	6
	1	5.9	15
	1/2	4.7	15
	1/4	3.1	16
	1/10	1.8	4
	1/20	1.5	12
	1/50	1.2	10
	1/100	.9	9
N-acetyl colchinol	2	3.2	6
	1	2.4	12
	1/2	2.9	13
	1/4	4.3	13
	1/10	1.9	4
	1/20	1.8	11
	1/50	1.2	10
	1/100	1.0	8

NOTE. Activities are expressed as multiples of number of mitoses of control average.

toxicity with the one exception already mentioned.

Data summarizing the mitotic counts performed in four experiments is presented in Table 3. For each experiment, the mitotic activity was expressed as a fraction of the control taken as unity and the figure in each dosage category of the table represents an average of the figures of all of the experiments.

It can be seen from the tables that the activity of colchicine declines rapidly with decreasing concentrations, there being only a slight antimitotic effect observed below the ½ LD_{50} level (1.8 mg. per Kg.). Figs. 1 and 2 show the typical "colchicine effect" on sarcoma 180, as compared with an untreated tumor. Colchiceine decreases in activity in a similar fashion, although here the break point between activity and relative inactivity occurs below the ¼ LD_{50} concentration. On the other hand, TMCA methyl ether maintains its ability to arrest mitoses when administered in doses as low as 1/20 LD_{50}. Its antimitotic activity at this level is comparable to that of colchicine at ½ LD_{50}. N-benzoyl TMCA methyl ether and N-acetyl colchinol appear to be compounds of intermediate activity against sarcoma 180. TMCA and N-benzoyl TMCA are not listed in Table 3, since these compounds have shown no antimitotic activity even at 2 LD_{50}.

Observations on the amount of hemorrhage produced by these agents indicate that, in general, the extent of the hemorrhage parallels the degree of mitotic arrest. TMCA methyl ether is the most active of these compounds in this respect, being the only one able to produce widespread hemorrhage consistently at 1/20 LD_{50}. At dosage levels higher than ¼ LD_{50}, this compound produces hemorrhage and necrosis of such degree as to obscure its antimitotic effect. This may explain the apparently anomalous decrease in the antimitotic activity of this agent at higher dosages.

Previous investigators[1, 6, 15] have attributed any antineoplastic activity colchicine may have to its hemorrhage-producing properties more than to its antimitotic effect. As TMCA methyl ether produces both hemorrhage and mitotic arrest at low levels of toxicity, further study of this compound as a possible chemotherapeutic agent is indicated.

SUMMARY

Colchicine appears to be the most toxic member of a series of related compounds. With due reservations for varying experimental conditions, differences in the order of toxicities for individual species seem to exist.

It is clear from these experiments that toxicity and antineoplastic activity do not

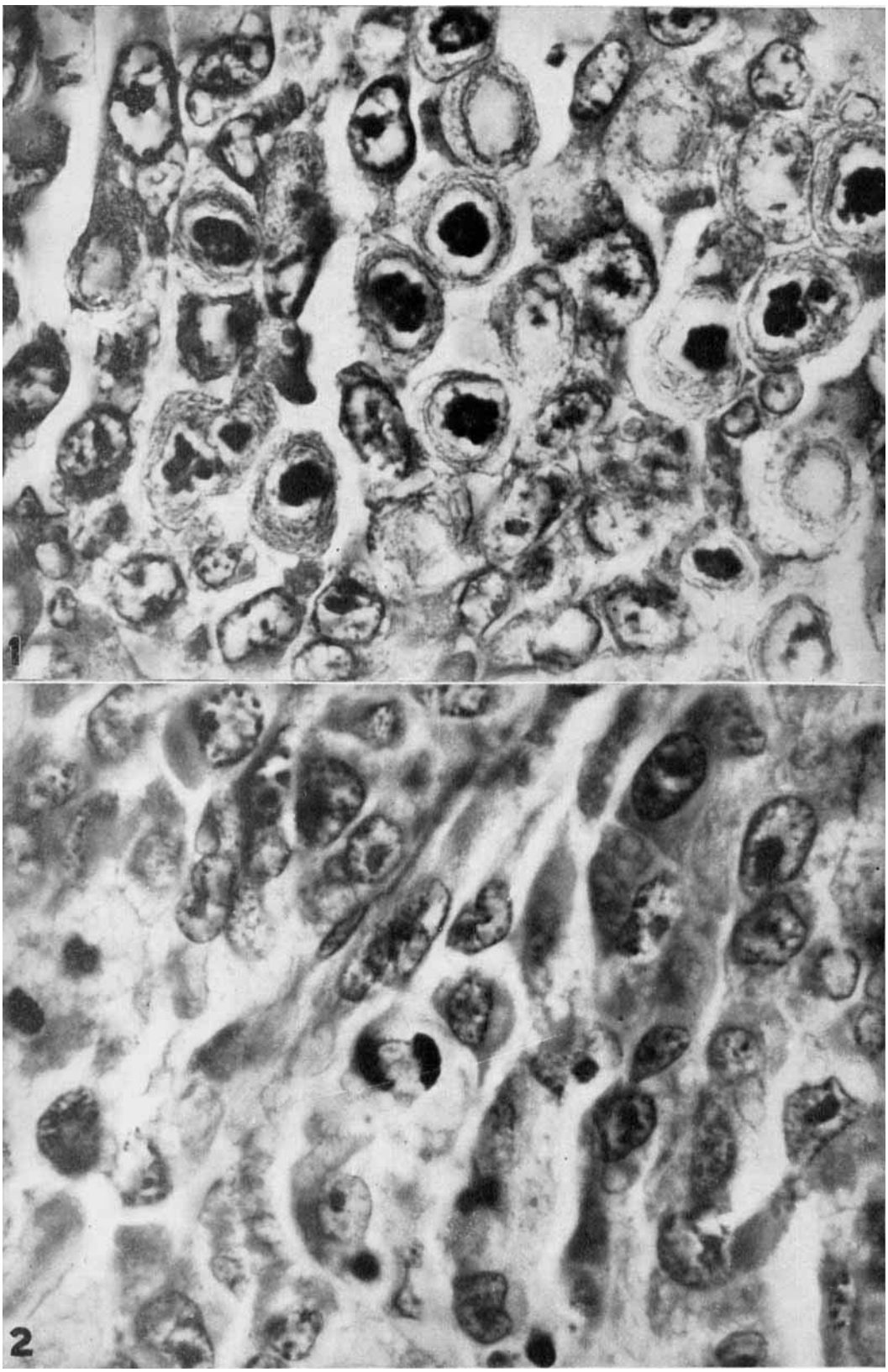


FIG. 1. *Sarcoma 180, six hours after injection of colchicine (1.75 mg./Kg.). (H. & E. ×1500.)*

FIG. 2. *Sarcoma 180, untreated. (H. & E. ×1500.)*

necessarily change in parallel manner with modification in chemical structure. In the series: colchicine, N-benzoyl TMCA methyl ether, TMCA methyl ether, N-acetyl colchinol, structural modifications were made that resulted in reduced toxicity, but that permitted retention of antineoplastic activity. The decrease in toxicity was seventeenfold (9 to 157 micromoles per kilogram) while the minimum effective antineoplastic doses varied only fourfold and ranged from 4.5 to 17 micromoles per kilogram.

REFERENCES

1. ANDERVONT, H. B.: Effect of colchicine and bacterial products on transplantable and spontaneous tumors in mice. *J. Nat. Cancer Inst.* 1: 361-366, 1940.

2. ARNSTEIN, H. R. V.; TARBELL, D. S.; HUANG, H. T., and SCOTT, G. P.: The structure of ring C of colchicine. *J. Am. Chem. Soc.* 70: 1669, 1948.

3. BARTON, N.; COOK, J. W., and LOUDON, J. D.: Colchicine and related compounds. Part V. The structure of Windaus's deaminocolchinol methyl ether. *J. Chem. Soc.* [1945]: 176-180, 1945.

4. BERGNER, A. D.: Studies on colchicine derivatives. III. Effect on mitotic activity of mouse spermatogonia. *Cancer* 3: 134-141, 1950.

5. BRUES, A. M., and COHEN, A.: The effects of colchicine and related substances on cell division. *Biochem. J.* 30: 1363-1368, 1936.

6. BRUES, A. M.; MARBLE, B. B., and JACKSON, E. B.: Effects of colchicine and radiation on growth of normal tissues and tumors. *Am. J. Cancer* 38: 159-168, 1940; addendum 38: 190, 1940.

7. DEWAR, M. J. S.: Structure of colchicine. *Nature* 155: 141-142, 1945.

8. DUSTIN, A.-P., and CHODKOWSKI, K.: Étude de la cicatrisation par la réaction colchicinique. *Compt. rend. de l'Assoc. d. anat.* 32: 170-176, 1937.

9. FLEISCHMANN, W., and ULLYOT, G. E.: Studies on colchicine derivatives. II. Effect on mitotic activity of the corneal epithelium. *Cancer* 3: 130-133, 1950.

10. FÜHNER, H.: Pharmakologische Untersuchungen über das Colchicin und seine Derivate. *Arch. f. exper. Path. u. Pharmakol.* 72: 228-238, 1913.

11. HAUSMANN, W.: Ueber den Einfluss der Temperatur auf die Inkubationszeit und Antitoxinbildung nach Versuchen an Winterschlafern. *Arch. f. d. ges. Physiol.* 113: 317-326, 1906.

12. JACOBJ, C.: Pharmakologische Untersuchung über das Colchicumgift. *Arch. f. exper. Path. u. Pharmakol.* 27: 119-157, 1890.

13. LIPPS, H.: Pharmakologische Untersuchungen in der Colchicinreihe. II. Ueber die Wirkung einiger Colchicinderivate. Der Kapillargiftmechanismus der Colchicinwirkung. *Arch. f. exper. Path. u. Pharmakol.* 85: 235-255, 1920.

14. LUDFORD, R. J.: The action of toxic substances upon the division of normal and malignant cells in vitro and in vivo. *Arch. f. exper. Zellforsch.* 18: 411-441, 1936.

15. LUDFORD, R. J.: Colchicine in the experimental chemotherapy of cancer. *J. Nat. Cancer Inst.* 6: 89-101, 1945.

16. REED, L. J., and MUENCH, H.: A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* 27: 493-497, 1938.

17. WINDAUS, A.: Untersuchungen über die Konstitution des Colchicins. *Ann. d. Chem.* 59-75, 1924.