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INTERACTION OF SOME COLCHICINE ANALOGS, VINBLASTINE AND PODOPHYLLOTOXIN WITH RAT BRAIN MICROTUBULE PROTEIN

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Abstract—The interaction of seventeen colchicine analogs, vinblastine and podophyllotoxin with rat brain microtubule protein has been sudied by measuring their ability to displace ^{3}H-colchicine. The following analogs competitively displaced ^{3}H-colchicine from rat brain microtubule protein: cholchiceinamide, *N*-desacetylthiocolchicine, demecolcine, *N*-acetyliodocolchinol, trimethylcolchicinic acid (TMCA) methyl ether, *N*-acetylcolchinol, and TMCA ethyl ether. Isocolchicine, isocolchiceinamide, iso-TMCA methyl ether, colchiceine, TMCA, *N*-benzoyl TMCA, colchicosamide, colchicoside, colchinol and colchinoic acid did not inhibit the binding of ^{3}H-colchicine to rat brain microtubule protein. A good correlation was found between the ability of a given analog to displace ^{3}H-colchicine from rat brain microtubule protein and its antimitotic or antigout properties. Podophyllotoxin and vinblastine, both potent antimitotic agents, inhibited ^{3}H-colchicine binding competitively and non-competitively respectively.

IT IS NOW widely accepted that the binding of colchicine to microtubule protein of the mitotic apparatus disrupts the organization of the mitotic spindle and thereby arrests the mitotic process.[1] While the precise mechanism of this phenomenon is unknown, several workers have shown that ^{3}H-colchicine binds to a specific protein that is believed to be a subunit of the microtubule.[2–10] This protein, which may be referred to as "microtubule protein", "colchicine binding protein" or "tubulin", has been isolated from such diverse tissues as HeLa cells,[2,3] grasshopper embryos,[4] mammalian brain,[5] neuroblastoma cell cultures,[6] Chlamydomonas flagella,[6] chick embryo brain,[7] the cilia of Tetrahymena[8] and sea urchin sperm tails.[9,10] Microtubule protein has a molecular weight of approximately 120,000 Daltons[5–7] and sediments with a sedimentation coefficient of 6S.[5,9,10] Recent work has shown that microtubule protein is composed of two non-identical monomeric subunits that have approximately the same molecular weights.[11,12]

Colchicine is well known for both its antimitotic and its antigout effects, but until recently there was no reasonable hypothesis to link these two pharmacological actions. However, in a recent review, Malawista[13] has proposed a unifying theory which suggests that the binding of colchicine to microtubule protein is responsible for both the antigout and the antimitotic activities of the drug.

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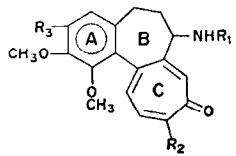
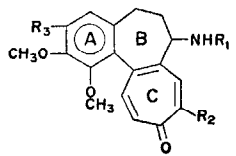
Table 1. Inhibition of colchicine binding to rat brain microtubule protein by colchicine analogs, podophyllotoxin and vinblastine



(I)



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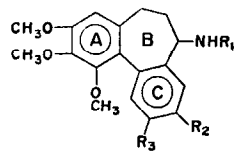

(III)

Compound	R_1	R_2	R_3	% Inhibition of ^{3}H-colchicine binding to rat brain microtubule protein*	K_I† ($M \times 10^6$)	M.E.D.‡ (mg/kg s.c.)	% Inhibition of urate-induced rat hindpaw edema§	50% Inhibition concn of TSH-stimulated ^{131}I release from mouse thyroid‖ ($M \times 10^7$)
Structure I								
Colchicine	$COCH_3$	OCH_3	OCH_3		1·4¶	1·5	87,71	2
Colchiceinamide	$COCH_3$	NH_2	OCH_3	82·1 ± 0·51	1·9	2·0	78,75	
N-desacetylthiocolchicine	H	SCH_3	OCH_3	67·1 ± 1·7	2·6		50,43	
Demecolcine	CH_3	OCH_3	OCH_3	60·2 ± 1·7	4·5		87,86	6
TMCA methyl ether	H	OCH_3	OCH_3	52·9 ± 0·9	6·6	2	78,86	
TMCA ethyl ether	H	OC_2H_5	OCH_3	38·6 ± 0·8	8·9	3	79	
Colchicosamide	$COCH_3$	HN_2	$C_6H_{11}O_7$	2·1 ± 1·5		> 920	(18)	
Colchicoside	$COCH_3$	OCH_3	$C_6H_{11}O_7$	3·2 ± 1·2			(2)	> 3000

Structure II								
Isocolchicine	$COCH_3$	OCH_3	OCH_3	2·1 ± 2·2		> 200	(7)	
Isocolchiceinamide	$COCH_3$	NH_2	OCH_3	1·0		> 500	(0)	
Iso-TMCA methyl ether	H	OCH_3	OCH_3	4·4 ± 1·2		> 1000	(16)	
Colchiceine	$COCH_3$	OH	OCH_3	6·6 ± 1·2		75	25	300
TMCA	H	OH	OCH_3	0·8 ± 1·5		600	(26)	
N-benzoyl TMCA	C_6H_5CO	OH	OCH_3	2·3 ± 2·7		> 200	(0)	
Structure III								
Colchinol	H	OH	H	6·8 ± 1·1		125	28	
N-acetylcolchinol	$COCH_3$	OH	H	51·6 ± 2·3	7·8	4	26,46,29	
N-acetyliodocolchinol	$COCH_3$	OH	I	59·3 ± 1·8	5·8	50	26	100
Colchinoic acid	$COCH_3$	COOH	H	3·7 ± 2·4		> 1000	(0)	
Other antimitotic compounds								
Podophyllotoxin				94·0 ± 0·24	0·28	2	64	3
Vinblastine				25·0 ± 1·7	197		67	6

* Incubation mixtures containing $2·5 \times 10^{-6}$ M ^{3}H-colchicine, $2·5 \times 10^{-5}$ M test compound and 100,000 *g* rat brain supernatant (1 to 1·5 mg/ml) were kept at 37° for 4 hr. Mean values ± S.E.

† Inhibition was competitive in all cases except vinblastine, where it was non-competitive.

‡ M.E.D. is the minimum effective dose in mice required to produce hemorrhage and necrosis in Sarcoma 37. Data taken from Leiter *et al.*[18-21]

§ Each value represents the mean % inhibition (eight rats) of mean control edema (eight rats) in one experiment. The dosage was 2 mg/kg (s.c.) for all drugs with the exception of TMCA where it was 10 mg/kg (s.c.). All values are significantly different from saline controls ($P < 0·05$), except those in parentheses Data taken from Zweig *et al.*[22]

‖ Data taken from Williams and Wolff.[23-24]

¶ K_m value.

In this report, we have studied the binding of seventeen colchicine analogs, podophyllotoxin and vinblastine (see Table 1) to rat brain microtubule protein by measuring their ability to inhibit the binding of ^{3}H-colchicine. Our results show the importance of ring A and the configuration of ring C in the binding of colchicine and its analogs to rat brain microtubule protein. We have also found a good correlation between the ability of the colchicine analogs, podophyllotoxin and vinblastine to inhibit ^{3}H-colchicine binding to rat brain microtubule protein and their efficacy as antimitotic and antigout agents.

MATERIALS AND METHODS

Preparation of rat brain microtubule protein. The brains of male Sprague–Dawley rats (150–200 g) were removed and homogenized in a Teflon-glass tissue grinder with 3 vol. of ice-cold 10 mM sodium phosphate buffer (pH 7·0) containing 240 mM sucrose, 5 mM $MgCl_2$ and 0·1 mM GTP. The homogenate was centrifuged at 150,000 *g* for 90 min and the resultant supernatant assayed immediately for ^{3}H-colchicine binding activity (see below). When refrigerated at 4°, the supernatant lost all ability to bind ^{3}H-colchicine within 4 days. However, the supernatant was quite stable when kept at −20° so that as much as 90 per cent of the original binding activity survived after several weeks of storage.

Colchicine binding assay. The binding of ^{3}H-colchicine to rat brain microtubule protein was measured by a modification of the filter assay developed by Weisenberg *et al.*[5] Each value represents the average of at least three determinations, which did not differ from each other by more than 5 per cent. A 200-μl aliquot of rat brain supernatant, containing 1·0 to 1·5 mg of protein, was added to 800 μl of a solution containing 10 mM sodium phosphate buffer (pH 7·0), 5 mM $MgCl_2$, 0·1 mM GTP, 240 mM sucrose and ^{3}H-colchicine. The reaction was stopped by the addition of 1 ml of an ice-cold solution containing 1×10^{-4} M colchicine. After the addition of 8 ml of cold buffer containing 10 mM sodium phosphate (pH 7·0), 5 mM $MgCl_2$ and 0·1 mM GTP, the solution was filtered under gravity through a DE 81 Whatman Chromedia filter paper (3·0 cm diameter). The filter was then washed five times with 10-ml aliquots of cold buffer containing 10 mM sodium phosphate (pH 7·0), 5 mM $MgCl_2$ and 0·1 mM GTP. The filters were then transferred to counting vials and 10 ml of scintillation fluid[14] was added and the sample counted in a Packard scintillation counter.

In those experiments which measured the per cent of inhibition of ^{3}H-colchicine binding (see Table 1), the final concentrations were ^{3}H-colchicine, $2{\cdot}5 \times 10^{-6}$ M, colchicine analogs, vinblastine and podophyllotoxin ($2{\cdot}5 \times 10^{-5}$ M). The incubation (37°) was commenced immediately after the addition of the brain supernatant and was terminated 4 hr later. For the experiments which were performed to determine the K_i values for seven of the colchicine analogs, vinblastine and podophyllotoxin (see Table 1, Figs. 2 and 3), a single concentration of each drug was chosen (5×10^{-6} M to $2{\cdot}5 \times 10^{-5}$ M for the colchicine analogs, $7{\cdot}5 \times 10^{-5}$ M for vinblastine and $7{\cdot}8 \times 10^{-5}$ M for podophyllotoxin) and incubated for 4 hr with an aliquot of rat brain supernatant and a series of different ^{3}H-colchicine concentrations (2×10^{-7} M to 2×10^{-5} M). Reciprocal plots, such as those shown in Figs. 2 and 3, were obtained for all nine drugs and the line was fitted by means of a least squares computer program.[15] The K_i values were calculated by standard procedures.

Protein assay. Protein was determined by the method of Lowry *et al.*[16]

Molecular weight determination of the colchicine complex. The molecular weight of the colchicine complex was determined by the method of Andrews[17] as modified by Wilson.[7] A 2·5 × 67 cm column of Sephadex G-100 was equilibrated with 20 mM sodium phosphate buffer (pH 6·8) containing 100 mM sodium glutamate and calibrated with the following protein standards: human γ-globulin, molecular weight 160,000; bovine plasma albumin, molecular weight 69,000; and ovalbumin, molecular weight 42,000. An aliquot of the 150,000 *g* rat brain supernatant (2·0 ml containing 14·7 mg of protein) was incubated with $2{\cdot}5 \times 10^{-6}$ M ^{3}H-colchicine for 2·5 hr at 37°, cooled to 0°, and filtered through the column. Bound ^{3}H-colchicine appeared as a single sharp peak corresponding to a molecular weight of 125,000.

Reagents. ^{3}H-Colchicine was purchased from New England Nuclear and diluted with nonradioactive colchicine to a specific activity of 0·1 Ci/m-mole. Colchicine was purchased from Fisher Scientific, while demecolcine was obtained from Pfaltz & Bauer. We are greatly indebted to the following for gifts of colchicine analogs (see Table 1) and other drugs employed in this investigation: Dr. Glenn Ullyot, Smith Kline & French Laboratories, Philadelphia (trimethylcolchicinic acid (TMCA), TMCA methyl ether, TMCA ethyl ether, *N*-acetylcolchinol, colchiceine, colchinol, colchiceinamide, isocolchicine, iso-TMCA methyl ether, isocolchiceinamide, *N*-benzoyl TMCA, colchinoic acid); Dr. Joseph Leiter, National Library of Medicine, Bethesda (colchiceine, *N*-acetyliodocolchinol, *N*-benzoyl TMCA); Dr. Harry B. Wood, National Cancer Institute, National Institutes of Health, Bethesda (demecolcine, desacetylthiocolchicine hydrochloride (DTC), TMCA, colchicoside, podophyllotoxin); Dr. André Courtin, Roussel Corp., New York (desacetylthiocolchicine hydrochloride, colchiceine, TMCA, colchicoside, colchicosamide). Vinblastine was a gift from the Eli Lilly Company, Indianapolis, Ind.

The protein standards used for the molecular weight determinations were purchased from Mann Laboratories. All other chemicals were of reagent grade.

RESULTS

When the 150,000 *g* supernatant, prepared from a rat brain homogenate, was incubated with ^{3}H-colchicine and then passed through a Sephadex G-100 column, the colchicine complex appeared as a sharp peak that corresponded to a molecular weight of 125,000 Daltons. Since this value was close to the molecular weight of microtubule protein found in other systems,[5–7] there seemed little doubt that the ^{3}H-colchicine was bound to rat brain microtubule protein. The 150,000 *g* supernatant, either freshly prepared or stored at −20°, was therefore used in all subsequent studies.

The binding of ^{3}H-colchicine to rat brain microtubule protein was dependent upon both the duration and temperature of incubation. For example, at 37°, in the presence of $2{\cdot}5 \times 10^{-6}$ M ^{3}H-colchicine, binding increased rapidly during the first hr, reaching a plateau after about 3 hr of incubation, which remained unchanged for a further 4 hr (Fig. 1). However, when the incubation was carried out at 25°, not only was the rate of binding slower than at 37° but the final level of binding was only 68 per cent of that attained at the higher temperature (Fig. 1). When the incubation temperature was lowered to 4°, the level of binding observed after 7 hr was only 5 per cent of that seen at 37° (Fig. 1).

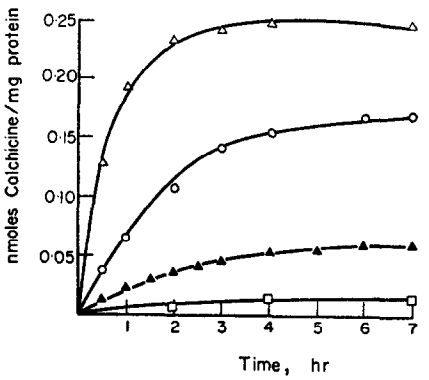

FIG. 1. Binding of ^{3}H-colchicine to rat brain microtubule protein. (△), $2{\cdot}5 \times 10^{-6}$ M ^{3}H-colchicine at 37°; (○), $2{\cdot}5 \times 10^{-6}$ M ^{3}H-colchicine at 25°; (□), $2{\cdot}5 \times 10^{-6}$ M ^{3}H-colchicine at 4°; (▲), $2{\cdot}0 \times 10^{-7}$ M ^{3}H-colchicine at 37°.

The rate of ^{3}H-colchicine binding to microtubule protein was also dependent upon the initial concentration of the drug. For example, when the ^{3}H-colchicine concentration was 2×10^{-7} M, 4 hr of incubation at 37° were required before binding reached a constant level (Fig. 1). In contrast, when the ^{3}H-colchicine concentration was 2×10^{-5} M, binding reached a level of 0·31 nmole of drug/mg of protein within 1·5 hr which did not change during a further 3·5 hr of incubation. A double reciprocal plot of ^{3}H-colchicine binding to rat brain microtubule protein after 4 hr of incubation against the initial concentration of ^{3}H-colchicine gave a striaght line relationship (Figs. 2 and 3). The K_m value for this interaction was calculated to be $1{\cdot}4 \times 10^{-6}$ M while the maximal level of binding was 0·37 nmole of ^{3}H-colchicine/mg of protein.

The inhibition of ^{3}H-colchicine binding to rat brain microtubule protein by vinblastine, podophyllotoxin and the colchicine analogs is shown in Table 1. Seven out of the seventeen colchicine analogs were quite effective in displacing ^{3}H-colchicine

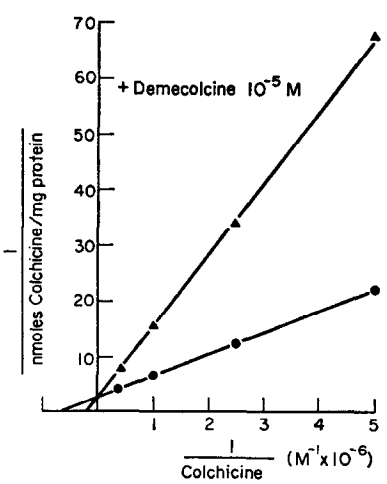

FIG. 2. Binding of ^{3}H-colchicine to rat brain microtubule protein in the presence of (▲) and absence (●) of 1×10^{-5} M demecolcine.

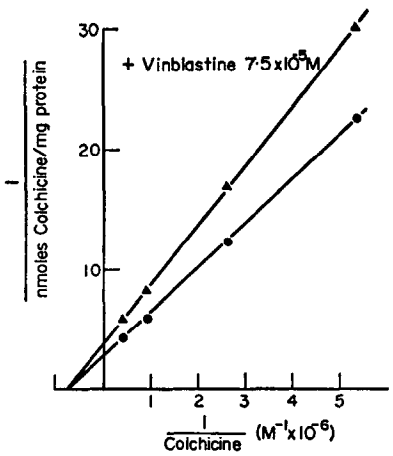

FIG. 3. Binding of ^{3}H-colchicine to rat brain microtubule protein in the presence of (▲) and absence (●) of $7{\cdot}5 \times 10^{-5}$ M vinblastine.

from microtubule protein while the remainder had little or no influence on the interaction. Podophyllotoxin also inhibited ^{3}H-colchicine binding to rat brain microtubule protein while vinblastine was somewhat less effective. However, the following anti-inflammatory drugs, several of which have been used in the treatment of gout and inflammatory processes, did not alter ^{3}H-colchicine binding to microtubule protein: acetylsalicylic acid ($2{\cdot}5 \times 10^{-5}$ M), phenylbutazone ($2{\cdot}5 \times 10^{-4}$ M), oxyphenbutazone ($2{\cdot}5 \times 10^{-5}$ M), indomethacin ($2{\cdot}5 \times 10^{-5}$ M), flufenamic acid ($2{\cdot}5 \times 10^{-5}$ M), promethazine ($2{\cdot}5 \times 10^{-5}$ M and tripellenamine ($2{\cdot}5 \times 10^{-5}$ M).

The inhibition of ^{3}H-colchicine binding to rat brain microtubule protein by seven of the colchicine analogs, vinblastine and podophyllotoxin was studied over a range of ^{3}H-colchicine concentrations (Figs. 2 and 3, Table 1). While vinblastine inhibited ^{3}H-colchicine binding in a non-competitive manner (Fig. 3), the remaining drugs were all found to be competitive inhibitors (Table 1, Fig. 2).

DISCUSSION

The binding of ^{3}H-colchicine to rat brain microtubule protein was both time and temperature dependent. Similar results have been reported by Wilson[7] for the binding of ^{3}H-colchicine to chick embryo brain microtubule protein. Wilson[7] has suggested that the binding of colchicine to microtubule protein is followed by a conformational change in the protein which results in the formation of a highly stable drug–protein complex.

The seventeen colchicine analogs can be devided into two structural categorics, namely those which can be considered as derivatives of TMCA (Table 1, structures I and II) and those which are related to colchinol (Table 1, structure III). Although colchiceine could exist in either the colchicine or the isocolchicine configuration, infrared and optical rotation measurements have led Horowitz and Ullyot[25] to suggest that this analog exists in the iso form. Since there are no physical data available for TMCA and *N*-benzoyl TMCA, these compounds have been arbitrarily assigned the iso configuration (Table 1, structure II).

The TMCA derivatives that had the iso-configuration in ring C did not dipalace ^{3}H-colchicine from rat brain microtubule protein (Table 1). Colchiceine was also a poor inhibitor of ^{3}H-colchicine binding which supports the idea that this compound exists as the iso form in solution. Demecolcine, DTC, colchiceinamide and the TMCA methyl and ethyl ethers were all good inhibitors of ^{3}H-colchicine binding (Table 1). Since inhibition was competitive (Table 1), it can be assumed that these analogs occupy the same binding site on microtubule protein as does colchicine. Although both colchicoside and colchicosamide have the same configuration as colchicine in ring C, neither of these analogs displaced ^{3}H-colchicine. Perhaps ring A of colchicine is involved in hydrophobic interactions with the microtubule protein binding site that are destroyed by the introduction of a hydrophilic sugar moiety. Alternatively, the sugar group may act as a sterile hindrance to prevent ring A from making a close contact with the binding site.

The *N*-acetyl group of colchicine does not seem to be necessary for binding, since demecolcine, DTC and the TMCA methyl and ethyl ethers all lack this moiety and yet are capable of competing with ^{3}H-colchicine for rat brain microtubule binding sites (Table 1). Nevertheless, when present, the acetyl group does appear to enhance binding (cf. colchicine and TMCA methyl ether, Table 1). The *N*-acetyl group appeared to be necessary for activity in the colchinol series (Table 1).

Podophyllotoxin, a potent antimitotic agent, competitively inhibited ^{3}H-colchicine binding to rat brain microtubule protein (Table 1). Wilson[7] has shown that when podophyllotoxin was preincubated with chick embryo brain microtubule protein the drug would inhibit the binding of ^{3}H-colchicine. However, when the same preparation was preincubated with ^{3}H-colchicine, the subsequent addition of podophyllotoxin did not alter the binding of ^{3}H-colchicine. In our experiments, both podophyllotoxin and ^{3}H-colchicine were present during the entire incubation, and under these conditions, competitive inhibition was observed. These results suggest that podophyllotoxin and ^{3}H-colchicine occupy the same binding site on rat brain microtubule protein.

Vinblastine, an antimitotic agent that disrupts a number of microtuble systems,[26–28] inhibited ^{3}H-colchicine binding to rat brain microtubule protein in a non-competitive manner (Table 1, Fig. 3). Wilson[7] has reported that vinblastine increased ^{3}H-colchicine binding to chick embryo brain microtubule protein by stabilizing the colchicine–protein complex. Our failure to observe this phenomenon may have been due to the presence of GTP in the incubation, since Wilson[7] has found that this nucleotide also stabilizes colchicine binding to microtubule protein. The non-competitive nature of the inhibition suggests that vinblastine and colchicine occupy different sites on rat brain microtubule protein. This conclusion is in agreement with previously reported data on microtubule protein from neuroblastoma cell cultures[6] and porcine brain.[29]

Since colchicine exerts its antimitotic effect by disrupting spindle microtubules,[1] it is of interest to compare the ability of the colchicine analogs to displace ^{3}H-colchicine from rat brain microtubule protein with their antimitotic activity. Leiter *et al.*[18–21] have assessed the antimitotic activity of podophyllotoxin and a number of colchicine analogs by measuring the degree of hemorrhage and necrosis found in Sarcoma 37, carried in mice, after a single subcutaneous injection of the drug. It can be seen from Table 1 that those analogs which displace ^{3}H-colchicine from rat brain microtubule protein also have the highest antitumor activity (i.e. the lowest minium effect dose values). Conversely, those analogs which are poor inhibitors of ^{3}H-colchicine binding

have weak antitumor activity (Table 1). When a ranking of the colchicine analogs and podophyllotoxin according to their ability to displace ^{3}H-colchicine from rat brain microtubule protein is compared with their ranking in the mouse Sarcoma test, a Spearman rank correlation coefficient[30] of −0·82 is obtained. This coefficient is significant at the 0·05 level and strongly suggests that the two phenomena are somehow interrelated. Although there are no comparable data available on the effect of demecolcine, DTC and colchicoside on Sarcoma 37 in mice, these analogs have been tested in other systems. For example, Schindler[31] has shown that demecolcine is as potent an antimitotic as colchicine itself. Jequier *et al.*[32] have reported that DTC is less potent than either colchicine or colchiceinamide and that colchicosamide and colchicoside are essentially devoid of antimitotic activity. These results agree quite well with the ability of these agents to prevent the binding of ^{3}H-colchicine to rat brain microtubule protein (Table 1).

Zweig *et al.*[22] have recently tested the ability of colchicine and its analogs to inhibit edema induced in the rat hindpaw by subplanter injections of sodium urate crystals. When their data are compared with those obtained in this investigation, it can be seen that the anti-inflammatory activity of the colchicine analogs correlates quite well with their ability to displace ^{3}H-colchicine from rat brain microtubule protein (Table 1). One exception is colchinol, which has about the same anti-inflammatory activity as *N*-acetylcolchinol and *N*-acetyliodocolchinol in the gout model and yet does not bind to microtubule protein as well as the two *N*-acetyl analogs. However, it is possible that the rat is capable of acetylating colchinol to give the more active form of the drug. Nevertheless, when vinblastine, podophyllotoxin and the colchicine analogs are ranked first according to their ability to displace ^{3}H-colchicine from microtubule protein and then according to their anti-inflammatory efficacy in the gout model, a Spearman rank correlation coefficient of 0·73 is obtained. This coefficient is significant at the 0·05 level and suggests that the interaction of these drugs with microtubule protein may be related to their anti-inflammatory effects. Malawista and Bensch[33] have demonstrated the colchicine-induced dissolution of microtubules present in polymorphonuclear leukocytes. Since these leukocytes are intimately involved in the inflammatory process and since colchicine inhibits leukocyte functions, such as ameboid migration and lysozomal degranulation, it seems reasonable that the leukocyte may mediate the antigout effect of colchicine.

Williams and Wolff[23,24] have recently reported that colchicine, vinblastine and podophyllotoxin blocked the TSH-stimulated release of ^{131}I from isolated mouse thyroid glands. Colchiceine and *N*-acetylcolchinol were much less effective in this system, while colchicoside was completely inactive. The ranking of these compounds according to their ability to prevent the binding of ^{3}H-colchicine to rat brain microtubule protein and to inhibit TSH-stimulated ^{131}I release from mouse thyroid is the same, with the exception of vinblastine which is more active in the thyroid system (Table 1). It is perhaps not surprising, therefore, that Williams and Wolff[23] found evidence for the binding of ^{3}H-colchicine to a soluble 6S protein which appeared to be similar to the microtubule subunit isolated from other tissues.

Recently, the inhibition by colchicine of such diverse processes as the neuronal transport of acetylcholinesterase[34] and catecholamines,[35] the glucose-stimulated release of insulin by the pancreas,[36] the nerve-stimulated release of dopamine-β-hydroxylase and norepinephrine from sympathetic nerves,[37] the acetylcholine-stimu-

lated release of catecholamines from the arenal medulla,[38] and the release of histamine from mast cells[39] and leukocytes[40] has been taken as *prima facie* evidence for the involvement of microtubules in these phenomena. However, a more recent study has raised the possibility that some of these effects may be due to pharmacological properties of colchicine that do not involve microtubules.[41] We therefore feel that a stronger case for the participation of microtubules in these processes could be made if inhibition were directly related to the ability of a given analog to bind to microtubule protein either *in vitro* or *in vivo*.

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REFERENCES

1. F. O. SCHMITT and F. E. SAMSON, *Neurosci. Res. Prog. Bull.* **6,** 129 (1968).
2. C. G. BORISY and E. W. TAYLOR, *J. Cell Biol.* **34,** 525 (1967).
3. C. G. BORISY and E. W. TAYLOR, *J. Cell Biol.* **34,** 535 (1967).
4. L. WILSON and M. FRIEDKIN, *Biochemisty, N.Y.* **6,** 3126 (1967).
5. R. C. WEISENBERG, C. G. BORISY and E. W. TAYLOR, *Biochemistry, N.Y.* **7,** 4466 (1968).
6. J. B. OLMSTED, K. CARLSON, R. KLEBE, F. RUDDLE and J. ROSENBAUM, *Proc. natn. Acad. Sci. U.S.A.* **65,** 129 (1970).
7. L. WILSON, *Biochemistry, N.Y.* **9,** 4999 (1969).
8. F. L. RENAUD, A. J. ROWE and I. R. GIBBONS, *J. Cell Biol.* **36,** 79 (1968).
9. M. L. SHELANSKI and E. W. TAYLOR, *J. Cell Biol.* **34,** 549 (1967).
10. M. L. SHELANSKI and E. W. TAYLOR, *J. Cell Biol.* **38,** 304 (1968).
11. J. BRYAN and L. WILSON, *Proc. natn. Acad. Sci. U.S.A.* **68,** 1762 (1971).
12. H. FEIT, L. SLUSAREK and M. L. SHELANSKI, *Proc. natn. Acad. Sci. U.S.A.* **68,** 2028 (1971).
13. S. E. MALAWISTA, *Arthritis Rheum.* **11,** 191 (1968).
14. G. A. BRAY, *Analyt. Biochem.* **1,** 279 (1960).
15. D. S. DAVIES, P. L. GIGON and J. R. GILLETTE, *Biochem. Pharmac.* **17,** 1865 (1968).
16. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
17. P. ANDREWS, *Biochem. J.* **91,** 222 (1964).
18. J. LEITER, J. L. HARTWELL, G. E. ULLYOT and M. J. SHEAR, *J. natn. Cancer Inst.* **13,** 1201 (1953).
19. J. LEITER, V. DOWNING, J. L. HARTWELL and M. J. SHEAR, *J. natn. Cancer Inst.* **10,** 1273 (1950).
20. J. LEITER, V. DOWNING, J. L. HARTWELL and M. J. SHEAR, *J. natn. Cancer Inst.* **13,** 379 (1952).
21. J. LEITER, J. L. HARTWELL, I. KLINE, M. V. NADKARNI and M. J. SHEAR, *J. natn. Cancer Inst.* **13,** 731 (1952).
22. M. H. ZWEIG, H. M. MALING and M. E. WEBSTER, *J. Pharmac. exp. Ther.* **182,** 344 (1972).
23. J. A. WILLIAMS and J. WOLFF, *J. Cell Biol.* **54,** 157 (1972).
24. J. A. WILLIAMS and J. WOLFF, *Proc. natn. Acad. Sci. U.S.A.* **67,** 1901 (1970).
25. R. M. HOROWITZ and G. E. ULLYOT, *J. Am. chem. Soc.* **74,** 587 (1952).
26. P. GEORGE, L. J. JOURNEY and N. M. GOLDSTEIN, *J. natn. Cancer Inst.* **35,** 355 (1965).
27. S. E. MALAWISTA, H. SATO and K. G. BENSCH, *Science, N.Y.* **160,** 770 (1968).
28. H. WIESNIEWSKI, M. L. SHELANSKI and R. D. TERRY, *J. Cell Biol.* **38,** 224 (1968).
29. R. J. OWELLEN, A. H. OWENS and D. W. DONIGAN, *Biochem. biophys. Res. Commun.* **47,** 685 (1972).
30. S. SIEGEL, in *New Parametric Statistics*, pp. 202–211. McGraw-Hill, New York (1956).
31. R. SCHINDLER, *J. Pharmac. exp. Ther.* **149,** 409 (1965).
32. R. JEQUIER, D. BRANCENI and M. PETERFALVI, *Archs int. Pharmacodyn. Ther.* **103,** 243 (1968).
33. S. E. MALAWISTA and K. G. BENSCH, *Science, N.Y.* **156,** 521 (1967).
34. K. A. C. JAMES, J. J. BRAY, I. G. MORGAN and L. AUSTIN, *Biochem. J.* **117,** 767 (1970).
35. A. DAHLSTROM, *Eur. J. Pharmac.* **5,** 111 (1968).
36. P. E. LACY, S. L. HOWELL, D. A. YOUNG and C. J. FINK, *Nature, Lond.* **219,** 1177 (1968).
37. N. G. THOA, G. F. WOOTEN, J. AXELROD and I. J. KOPIN, *Proc. natn. Acad. Sci. U.S.A.* **69,** 520 (1972).
38. A. M. POISNER and J. BERNSTEIN, *J. Pharmac. exp. Ther.* **177,** 102 (1971).
39. E. GILLESPIE, R. J. LEVINE and S. E. MALAWISTA, *J. Pharmac. exp. Thér.* **164,** 158 (1968).
40. D. A. LEVY and J. A. CARLTON, *Proc. Soc. exp. Biol. Med.* **130,** 1333 (1969).
41. J. M. TRIFARO, B. COLLIER, A. LASTOWECKA and D. STERN, *Molec. Pharmac.* **8,** 264 (1972).