The interaction of tubulin-microtubule poison complexes with anti-tubulin antisera has been investigated using radioimmunoassay. The binding of the major antiserum used in this study to tubulin does not interfere with the binding of colchicine to the tubulin or affect the decay of the colchicine-binding activity of the tubulin. Conversely, if colchicine is incubated with the tubulin, forming tubulin-colchicine complexes, the tubulin-colchicine complexes are less efficient competitors for antibody-binding sites than tubulin alone. This is the result of the formation of specific colchicine-tubulin complexes, since tubulin, incubated with lumicolchicine or isocolchicine, behaves as if the tubulin were incubated alone in the radioimmunoassay. When tubulin is incubated with other microtubule poisons, podophyllotoxin or vinblastine, the tubulin-drug complexes have diminished ability to compete with tubulin as did the tubulin-colchicine complexes. These changes observed in the binding of tubulin-microtubule poison complexes to anti-tubulin antisera in a tubulin radioimmunoassay suggest that the binding of colchicine, podophyllotoxin, or vinblastine to tubulin induces subtle conformational changes on the surface of the tubulin dimer involving antigenic determinant sites.

The highly specific interaction between an antibody and its antigenic determinant has proven to be an extremely useful tool in studying the structure and functions of many different proteins (for excellent reviews see Atassi, 1977a, 1977b, 1979). Antiseras raised against tubulin are no exception. Microtubules have been localized in cells using anti-tubulin antisera and the technique of indirect immunofluorescence (Fuller et al., 1975; Weber et al., 1975; Spooner and Holladay, 1981, for example). Anti-tubulin antisera have provided an alternative to the colchicine-binding assay for the quantitation of tubulin in cell extracts through the technique of radioimmunoassay (Morgan et al., 1977b, Bulinski et al., 1980, for example). Anti-tubulin antisera have also been used to assess structural differences and similarities between tubulins isolated from different sources (Bibring and Baxandall, 1971; Fulton et al., 1971; Morgan et al., 1978a; Hiller and Weber, 1978). Since many of the biological activities of microtubules are associated with their assembly in the cell at the appropriate time and place, it is also important to note that an antibody-antigenic determinant complex can be used to inhibit assembly of microtubules (Morgan et al., 1978b). This study examines the impact of the interaction of brain tubulin with the antisera described by Morgan et al. (1978a) on the colchicine-binding activity of the tubulin, and the effect that colchicine, podophyllotoxin, or vinblastine bound to brain tubulin has on tubulin-anti-tubulin antibody interactions. Preliminary studies of this work were presented previously (Morgan et al., 1977a, 1978c).

EXPERIMENTAL PROCEDURES

Materials—Sodium [125I]iodide was obtained from Amersham/Searle and GTP type II-S from Sigma. All other chemicals used were reagent grade. Isocolchicine was a gift from Smith Kline and French Laboratories.

Protein Purification—Brains from 14-day-old chick embryos were homogenized in 1/4 volume of 0.1 M Pipes, pH 6.8, and then centrifuged at 40,000 × g for 45 min at 4 °C. The supernatant was brought to 1 mM GTP, 0.5 mM EGTA, 1 mM MgCl₂, and 50% glycerol (v/v) and warmed to 37 °C for 30 min. Microtubules were collected by centrifugation at 40,000 × g, 30 min at 37 °C. The microtubule pellet was resuspended in 0.1 M Pipes, pH 6.8, 1 mM GTP, 1 mM MgCl₂, 0.5 mM EGTA, and 50% glycerol at 4 °C. This solution was warmed to 37 °C for 30 min, and microtubules were collected as before and stored as a microtubule pellet at −70 °C. Prior to use the pellets were resuspended in 0.1 M Pipes, pH 6.8, 1 mM MgCl₂, 1 mM EGTA, 1 mM GTP at 4 °C and centrifuged at 40,000 × g for 30 min at 4 °C to remove any insoluble material and taken through another cycle of assembly-disassembly. Microtubule protein was used at this purity or further purified to tubulin by chromatography on phosphocellulose (Himes et al., 1976).

Antibody Generation and Tubulin Radioimmunoassay—The phosphocellulose-purified tubulin was treated with 2% glutaraldehyde. After dialysis in distilled water, the tubulin was emulsified in Freund's adjuvant and injected subcutaneously into rabbits as previously described (Fuller et al., 1975; Morgan et al., 1977b, 1978a). The antisera from several bleedings of a single rabbit were pooled and used in this study. The rabbits used were rabbits 147, S-15, S-13, and 150 (Morgan et al., 1978a, 1978b).

Tubulin radioimmunoassays were performed according to previously published procedures (Morgan et al., 1977b, 1978a). For Tables I and II, the data were normalized to the amount of tubulin needed to inhibit the binding of 50% of [125I]-tubulin to the antiserum. The normalized amount of tubulin in different experiments ranged from 250 to 2300 ng of tubulin, in part due to the use of different preparations of iodinated tubulin. Standard deviations of the triplicate points for all experiments were generally ±1-3% for points between 0-20% and 80-100% and ≤±1% for points 20-80% inhibition of [125I]-tubulin bound by antiserum. Tubulin was iodinated by a modification (Morgan et al., 1978a) of the method of Wood et al. (1975) or by the method of Markwell (1979). These methods are very gentle, proceeding in buffer at pH 7-7.5. The former method is described in detail in Morgan et al. (1978a). The latter employed flint glass tubes (12 × 75 mm) in which 5 μg of Iodogen (Pierce Chemical Co.) had been adsorbed as described by Markwell (1979). After the tubes were rinsed with 0.1 M Pipes buffer, pH 6.8, an aliquot of about 25 μg of...
phosphocellulose-purified tubulin in about 25 μl was added, followed by an aliquot of [125]iodine (Amersham IMS 300) (5 mCi in about 10 μl). The reaction mixture was incubated at 4 °C for 2 min, and the reaction was terminated by removal of the mixture from the tube. The tubulin iodinated by both methods was freed of bound [125]iodine by dialysis against 0.02 M borate-buffered saline, pH 8.0, and stored for up to 2 weeks at 4 °C in 10 ml of borate-buffered saline containing 10 mg of bovine serum albumin/ml. This iodinated tubulin did not assemble into microtubules under the conditions described above, but up to 90% of the radioactive tubulin could be specifically precipitated with the anti-tubulin antisera used in this report.

Colchicine-binding Activity of Tubulin-Anti-tubulin Complexes—To assess the relationship between antibody-tubulin interactions and the colchicine-binding activity of tubulin, tubulin-antibody complexes were formed and then the colchicine-binding activity of these samples was investigated in a time-decay colchicine-binding assay. A time-decay colchicine-binding experiment yields two valuable pieces of information: the rate of decay of the labile colchicine-binding activity of the tubulin preparation and its initial colchicine-binding activity (Bamburg et al., 1973). Fig. 1 shows that the initial colchicine-binding activity of the sample was 0.6 mol of colchicine/110,000 g of protein and the half-life was about 3 h in the presence of either the immune or normal globulin fraction of the serum. The binding of this anti-tubulin antisemum (serum 147) does not interfere with the colchicine-binding site; the colchicine-binding site appears to be a distinct site independent of the antigenic determinant(s) recognized. This result contrasts with those of Aubin et al. (1976), where the colchicine-binding activity and decay were altered by anti-tubulin. The difference probably results from antibody populations raised against different sets of antigenic determinants, since they used sodium dodecyl sulfate-treated tubulin as immunogen and we used glutaraldehyde-treated tubulin.

Anti-tubulin Antibody-binding Ability of Tubulin-Colchicine Complexes—The ability of anti-tubulin antibody to bind tubulin whose colchicine-binding site was occupied was quantitatively assessed by radioimmunoassay. Tubulin (3 mg/ml = 27.3 μM) was incubated in 0.1 M Pipes, pH 6.8, and 1 mM GTP for 2 h at 37 °C and overnight at 4 °C either alone or with 10−6, 10−5, or 10−4 M colchicine, and then the ability of the tubulin-colchicine complexes to inhibit the binding of [125]tubulin to immune serum was tested by radioimmunoassay. Fig. 2 shows that tubulin, preincubated with 10−6 M colchicine, competed with [125]tubulin for antibody-binding sites as effectively as tubulin incubated without colchicine. This is to be expected since the concentration of tubulin in the initial incubation mixture was so high, most of the tubulin could not have colchicine bound to it. When the tubulin was preincubated with either 10−5 or 10−4 M colchicine, the inhibition curves were shifted, indicating a decreased ability of the tubulin-colchicine complexes to compete with [125]tubulin for antibody-binding sites. To determine whether the inhibition...
was due to a specific interaction of tubulin-colchicine complexes with the antiserum, tubulin was incubated with lumicolchicine or isocolchicine, colchicine derivatives that do not bind to tubulin (Wilson and Friedkin, 1966; Zweig and Chignell, 1973). Preincubation with $10^{-4}$ M lumicolchicine or $10^{-4}$ M isocolchicine (Fig. 3) gave results indistinguishable from controls, demonstrating that the decreased competition efficiency following preincubation with colchicine resulted from the specific formation of tubulin-colchicine complexes rather than from some nonspecific interaction between colchicine or its derivatives and tubulin. Other potential nonspecific interactions are ones between colchicine and immunoglobulins to decrease the amount of double antibody-precipitable material, an inability of colchicine-tubulin complexes to bind to the antiserum, or a colchicine-induced aggregation of the tubulin. Preincubation of rabbit antiserum (primary antiserum) with $10^{-4}$ M colchicine, in the absence of tubulin, did not alter the amount of protein precipitated with goat anti-rabbit IgG antiserum (second antiserum), indicating that there was no colchicine effect on antibody-antigen interactions under these conditions. Colchicine-induced aggregation of tubulin was investigated by incubating tubulin (2 mg/ml), prepared by 3 cycles of warm-cold polymerization, alone or with $10^{-4}$ M colchicine for 2 h at 37 °C followed by either a 1-h centrifugation at 4 °C, 120,000 × g, or an incubation overnight at 4 °C followed by the 1-h centrifugation. After the centrifugation, aliquots of the supernatant were removed, diluted, and added to the reaction mixture for the radioimmunoassay. There was no visible pellet or loss of protein from the supernatant after centrifugation from any of the tubulin samples. Furthermore, all inhibition curves generated with tubulin-colchicine complexes still required the same increased amount of protein for 50% inhibition of specific $^{125}$I-tubulin binding. The inhibition curves generated by the tubulin alone superimposed upon each other. Thus, the incubation conditions did not simply result in a drug-induced aggregation of tubulin (or denaturation of tubulin alone) which could block access to antigenic determinants. Finally, incubation of tubulin with $[^3H]$colchicine prior to a 3-h incubation with specific antiserum and then treatment with secondary antiserum resulted in specific precipitation of $[^3H]$colchicine-tubulin complexes, demonstrating that $[^3H]$colchicine-tubulin complexes do bind to anti-tubulin antiserum.

**Interaction of Tubulin-Drug Complexes with Other Anti-tubulin Antisera**—The antiserum used in the preceding experiments (antiserum 147) has been shown to recognize at least two types of antigenic determinants on chick tubulin. One type is shared with lamb or mouse tubulin; the other type is unique to chick embryo brain tubulin (Morgan et al., 1978a). To determine whether the ability to differentiate the tubulin-drug complexes from tubulin alone was unique to chick brain tubulin antiserum 147 or shared with other antisera, three additional antisera were tested, S-13 (anti-lamb brain tubulin), S-15 (anti-mouse brain tubulin), and 150 (anti-chick embryo brain tubulin) (Morgan et al., 1978a). Tubulin was incubated either alone or with $10^{-4}$ M colchicine for 2 h at 37 °C, then overnight at 4 °C, and inhibition curves were generated with the three different antisera. With all three antisera, families of inhibition curves were obtained. Table I presents the relative amount of tubulin required to inhibit the binding of $^{125}$I-tubulin by 50% for all antisera. The results are comparable to those obtained with antiserum from rabbit 147. For each antiserum tested, an increased amount of tubulin-colchicine complex is required for 50% inhibition of $^{125}$I-tubulin binding. Thus, the ability of anti-tubulin antiserum to distinguish quantitatively tubulin from tubulin-colchicine complexes seems to be shared by four different anti-tubulin antisera and may be a feature that most anti-tubulin anti-

![Fig. 3. Effect of lumicolchicine or isocolchicine on the position of the inhibition curves.](http://www.jbc.org/)

**Table I Other anti-tubulin antisera**

Chick embryo brain tubulin was purified by cycles of polymerization-depolymerization and incubated alone or with drugs as described above at 37 °C for 2 h and overnight at 4 °C. The next morning samples were diluted and used to generate inhibition curves in a tubulin RIA. Tubulin concentration during drug incubation was 3.8 mg/ml. The amount of tubulin or tubulin drug complex required for 50% inhibition of $^{125}$I-tubulin binding was determined from each inhibition curve. Then, for each antiserum, these values were normalized to the amount of tubulin incubated without any drug needed to inhibit the binding of $^{125}$I-tubulin by 50%. An example is taken from Fig. 2. The amount of tubulin alone required for 50% inhibition is 1075 ng = 1.0; tubulin plus 10^{-6} M colchicine requires 1075 ng = 1.0; tubulin plus 10^{-4} M colchicine requires 2250 ng = 2.09; tubulin plus 10^{-4} M colchicine required 4000 ng = 3.72.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Tubulin alone</th>
<th>$10^{-4}$ M colchicine</th>
<th>$10^{-4}$ M podophyllotoxin</th>
<th>$10^{-4}$ M vinblastine</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-15</td>
<td>1.0</td>
<td>2.5</td>
<td>1.39</td>
<td>2.65</td>
</tr>
<tr>
<td>S-13</td>
<td>1.0</td>
<td>2.4</td>
<td>1.0*</td>
<td>4.0</td>
</tr>
<tr>
<td>150</td>
<td>1.0</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Inhibition curve superimposes on curve generated by tubulin alone.*
serum can detect. Serum drawn early in the immunization schedule from rabbit S-13 was an exception; it did not differentiate tubulin from tubulin-colchicine (Morgan et al., 1977b).

Furthermore, either cycled or phosphocellulose-purified tubulin-drug complexes can be used to generate any of these families of curves, and 0.1 M PIPES, pH 6.8, 1 mM MgCl₂, 0.5 mM EGTA, 1 mM GTP, 5 mg/ml of bovine serum albumin can be substituted for borate-buffered saline in the RIA without effect.

**Association of the Decreased Binding of Tubulin-Colchicine Complexes with the Binding of Colchicine to the Tubulin Dimer**—The data presented above suggest that there is a colchicine-induced conformational change in the tubulin-colchicine complexes, but that the effect begins at 10⁻³ M colchicine (which is at least 10-fold greater than published values for the equilibrium constant for colchicine binding (Garland, 1978, for example)) and does not appear to be saturable. The inhibition curve continues to shift as the concentration of colchicine is increased, up to and including 10⁻¹ M colchicine. These experiments used tubulin concentrations of 2-3 mg/ml or 18-27 μM. When this concentration of tubulin is incubated with 10⁻¹ or 10⁻² M colchicine, the tubulin/drug ratio is greater than 1.0. To ensure that all available colchicine-binding sites bind colchicine, the ratio should be less than 1.0. A 10-fold excess of ligand over binding sites would probably satisfy this condition. This problem can obviously be alleviated by lowering the concentration of tubulin present in the original incubation mixture with colchicine, but then complete inhibition curves cannot be generated because of the sensitivity of the assay and dilution problems with the reactants in the radioimmunoassay. As a compromise, tubulin, at a concentration of 0.125 mg/ml or 1.1 μM was incubated alone or with 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, or 10⁻⁷ M colchicine or with 10⁻⁶ M lumicolchicine for 2 h at 37 °C and then added to the RIA. The results are shown in Fig. 4. When tubulin, at this concentration, was incubated with 10⁻⁸ or 10⁻⁹ M colchicine, its ability to inhibit the binding of ¹²⁵I-tubulin to antisem decreased. At colchicine concentrations of 10⁻⁶, 10⁻⁷, or 10⁻⁸ M, the ability to compete plateaude at the same level as those tubulin complexes formed with 10⁻⁶ M colchicine, demonstrating a saturation of the colchicine effect over 4 orders of magnitude. At 10⁻⁵ M colchicine another decrease occurred suggesting nonspecific binding or that binding of colchicine to low affinity sites on the tubulin dimer might have occurred. In another experiment the binding of ¹²⁵I-tubulin to antitubulin antisem was inhibited by 63% when 1.8 μg of tubulin-colchicine complex was added and was inhibited by only 24-27% when 1.8 μg of tubulin alone or 1.8 μg of tubulin incubated with 10⁻⁴ M lumicolchicine or 10⁻⁸, 10⁻⁹, or 10⁻¹⁰ M isocolchicine was added again showing that the effect results from the specific binding of colchicine to the tubulin dimer.

**Interactions of Tubulin-Vinblastine or Tubulin-Podophyllotoxin with Anti-tubulin Antiserum**—Two well characterized microtubule poisons are vinblastine and podophyllotoxin. The podophyllotoxin-binding site overlaps with the site to which colchicine binds. The two sites to which vinblastine binds are unique (Bryan, 1972; Bhattacharyya and Wolff, 1976). These drugs are structurally distinct from colchicine and differ in their rapid, reversible, and temperature-independent binding to tubulin. But the different binding sites are able to influence each other. Binding of vinblastine to tubulin protects the colchicine-binding activity of tubulin, to some extent, from decay (Cortese et al., 1977; Luduena, 1979). The ability of vinblastine-tubulin complexes or podophyllotoxin-tubulin complexes to compete with ¹²⁵I-tubulin for antibody-binding sites was examined to determine whether these drugs induced conformational changes that could be detected immunologically. Families of inhibition curves were generated with tubulin alone, tubulin incubated with 10⁻⁷, 10⁻⁶, or 10⁻⁵ M vinblastine, or tubulin incubated with 10⁻⁶, 10⁻⁷, or 10⁻⁸ M podophyllotoxin. The data are shown in Table II. Both tubulin-vinblastine complexes and tubulin-podophyllotoxin complexes generated a family of curves as did the tubulin-colchicine complexes. As the concentration of either drug was increased, the ability of the tubulin-drug complexes to compete with ¹²⁵I-tubulin for the antibody binding sites decreased. Thus, more tubulin-drug complexes were required to inhibit the binding of labeled tubulin by 50% as the concentration of drug was increased, as was observed with colchicine. Nonspecific interactions between either drug and immunoglobulins was ruled out when it was determined that, in the absence of tubulin, preincubation of the primary antisem with concentrations of either drug to 10⁻³ M did not interfere quantitatively with the amount of second antibody-precipitable material. The possibility of drug-induced aggregation was investigated as described above for colchicine. For the podophyllotoxin-tubulin complex, there were no visible pellets following centrifugation and no decrease in protein concentration in the supernatant, and the inhibition curves generated by incubation with podophyllotoxin were not altered by centrifugation. A drug-induced aggregation of tubulin does not appear to be interfering with the antibody binding to

**Table II**

<table>
<thead>
<tr>
<th>Drug conc</th>
<th>Normalized amount of unlabel tubulin or tubulin-drug complex required for 50% inhibition of ¹²⁵I-tubulin binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Vinblastine</td>
</tr>
<tr>
<td>None added</td>
<td>1.0</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>1.0</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>1.17</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>1.52</td>
</tr>
</tbody>
</table>

![Fig. 4. Binding of tubulin-colchicine complexes to anti-tubulin antiserum.](image-url)
Tubulin-Microtubule Poison Complexes

As was the case with colchicine, the detection of reduced ability of drug-tubulin complexes to compete in the RIA was not restricted to this chick brain tubulin antiserum (147). Table I shows that both lamb brain tubulin antiserum (S-13) and mouse brain tubulin antiserum (S-15) recognize differences between tubulin and tubulin-drug (i.e. colchicine, podophyllotoxin, or vinblastine) complexes. Thus, the ability of anti-tubulin antiserum to quantitatively distinguish tubulin from tubulin-drug complexes seems to be shared by three different anti-tubulin antisera.

**Interactions of Double Drug-Tubulin Complexes with Anti-tubulin Antiserum**—Since colchicine bound to tubulin stabilizes the decay of the vinblastine-binding sites and, conversely, vinblastine bound to tubulin stabilizes the colchicine-or podophyllotoxin-binding site (for review see Luduena, 1979), the effect of binding more than one drug to tubulin was examined. Families of inhibition curves were generated with tubulin alone and with tubulin incubated with 10^{-3} M vinblastine, with 10^{-4} M podophyllotoxin, with 10^{-3} M colchicine, or with the three double-drug combinations. The data are shown in Fig. 5. The inhibition curves generated with tubulin alone, tubulin-colchicine, tubulin-podophyllotoxin, or tubulin-vinblastine complexes do not superimpose, implying that the three-dimensional configurations of these four tubulin-drug complexes are immunologically distinguishable. While colchicine and podophyllotoxin compete for the same binding site on the tubulin-dimer, podophyllotoxin binds more rapidly (Luduena, 1979). The double drug-tubulin inhibition curve generated with (colchicine-podophyllotoxin)-tubulin complexes superimposes upon the curve generated with tubulin-podophyllotoxin complexes alone, implying that podophyllotoxin-tubulin complexes were formed first in agreement with the binding characteristics of the drugs. The curve generated with (vinblastine-colchicine)-tubulin complexes is shifted furthest from the control curve and provides evidence that the conformational changes induced by a combination of colchicine and vinblastine act synergistically to induce a greater conformational change in tubulin antigenic determinants than either drug alone. In fact, this curve and that generated with (vinblastine-podophyllotoxin)-tubulin complexes are the only inhibition curves generated that plateau before zero inhibition. The (vinblastine-colchicine)-tubulin curve plateaus at about 25% of maximum ^{125}I-tubulin bound and the (vinblastine-podophyllotoxin)-tubulin curve plateaus at about 20%, suggesting that enough of a conformational change has taken place to essentially remove or block at least one antigenic determinant in the double drug-tubulin complexes.

**DISCUSSION**

The data reported here show that the binding of the antimitotic drugs colchicine, podophyllotoxin, and vinblastine to tubulin leads to decreased binding affinity of the anti-tubulin antibodies for the complexes, results best interpreted as evi-
dence of drug-induced conformational changes in the tubulin dimer. Evidence, independent of antibody probes, that the binding of these drugs does produce conformational changes in the tubulin molecule has accumulated in recent years. Vinblastine binding to tubulin can produce paracrystalline arrays in vivo or ladder-like irregular coils in vitro (Bensch and Malawista, 1969). In the case of colchicine, Garland (1978) and Lambeir and Engelborghs (1981) have used kinetic analyses of drug binding to demonstrate a ligand-induced conformational change in tubulin. Furthermore, Luduena (1979) and Luduena and Roach (1981) have shown that colchicine, vinblastine, and podophyllotoxin inhibit the alkylation of tubulin, which is consistent with drug-induced conformational changes. Finally, Detrich et al. (1982) suggest that colchicine causes a conformational change in tubulin that affects dissociation into α and β monomers, and Andreu and Timasheff (1982) and Saltarelli and Pantaloni (1982) have shown that tubulin-colchicine complexes can assemble into non-microtubule supramolecular structures.

The ability of antibodies to detect conformational changes in proteins is well documented (Tai et al., 1980; Fluie and Fluie, 1979, for example), and the anti-tubulin antibodies used in this study are extremely well characterized (Morgan et al., 1977a, 1977b, 1978a, 1978b). It is clear that the colchicine-binding site and the antigenic determinants recognized by our antibody population are physically independent entities on the tubulin molecule, since prior formation of tubulin-antibody complexes has no effect on [3H]colchicine-binding activity or on the time-decay rate of such binding, and since [3H]colchicine-tubulin-antibody complexes are specifically precipitated by secondary antibody. However, if tubulin is first incubated with colchicine to form tubulin-colchicine complexes, its ability to compete with 251-tubulin for tubulin is first incubated with colchicine to form tubulin-colchicine complexes. The conformational change in tubulin most likely reflects a conformational change in the tubulin molecule that alters its antigenic determinants. The ability of antibodies to detect conformational changes in proteins is well documented (Tai et al., 1980; Fluie and Fluie, 1979, for example), and the anti-tubulin antibodies used in this study are extremely well characterized (Morgan et al., 1977a, 1977b, 1978a, 1978b). It is clear that the colchicine-binding site and the antigenic determinants recognized by our antibody population are physically independent entities on the tubulin molecule, since prior formation of tubulin-antibody complexes has no effect on [3H]colchicine-binding activity or on the time-decay rate of such binding, and since [3H]colchicine-tubulin-antibody complexes are specifically precipitated by secondary antibody. However, if tubulin is first incubated with colchicine to form tubulin-colchicine complexes, its ability to compete with 251-tubulin for antibody-binding sites in a tubulin RIA is decreased. The decrease in antibody-binding ability plateaus at 10^{-6} M colchicine, is constant over a 4 order of magnitude increase in colchicine concentration, and is not duplicated by the non-tubulin-binding colchicine analogs lumicolchicine and isocolchicine. The saturation of the effect at 10^{-6} M colchicine is consistent with values for the tubulin-colchicine equilibrium constant, determined from direct binding experiments, that range from 0.7 to 3 × 10^{-6} M^{-1} (Bryant and Taylor, 1967; Bryan, 1972; Wilson and Meza, 1973; Bhattacharyya and Wolf, 1974; Garland, 1975; Sherline et al., 1975). This effect of colchicine binding on the antibody-binding ability of tubulin most likely reflects a conformational change in the tubulin molecule that alters its antigenic determinants. The change could decrease the tightness of the antibody-binding constants to produce the decreased competition efficiency of tubulin-colchicine complexes. The conformational change must affect the dimer at regions some distance from the drug-binding site, since the drug- and antibody-binding sites appear to be physically independent, and must be relatively mild, since the tubulin-colchicine complexes can completely inhibit the binding of 125I-tubulin to its antisemur (albeit at decreased efficiency). Thus, the binding of colchicine to tubulin does not result in the loss or blocking of an entire set of antigenic determinants, at least within the detection capability of four different tubulin antisera.

It is not clear why we observe continued progressive shifting of the inhibition curves with progressive increases in colchicine concentration, when the experiments are done at 10–20-fold higher tubulin concentrations. Two laboratories have reported that tubulin-colchicine complexes at high protein concentrations can assemble into supramolecular structures (Andreu and Timasheff, 1982; Saltarelli and Pantaloni, 1982), raising the possibility that the formation of tetramers or higher order structures by the colchicine-tubulin dimers may be a factor. At low tubulin concentrations, where the formation of tubulin tetramers and higher order structures may be rare, the formation of tubulin dimer-colchicine complexes is uncomplicated by dimer-dimer interactions and, under such conditions, saturation of the colchicine effect is observed.

The family of inhibition curves generated by complexes of tubulin with podophyllotoxin and with vinblastine suggest that these microtubule poisons also induce conformational changes in tubulin upon binding, a result supported by chemical modification experiments (Luduena, 1979; Luduena and Roach, 1981). As with colchicine, the inhibition curves shifted when tubulin was incubated with saturating concentrations of podophyllotoxin (10^{-5} M) or vinblastine (10^{-6} M). Continued shifting of the curves at higher drug concentrations may reflect binding to low affinity sites or tubulin dimer-dimer interactions, especially for vinblastine. Even at high concentrations of vinblastine that cause tubulin aggregation, the tubulin-vinblastine complexes can completely inhibit the binding of 125I-tubulin to its antisemur. Thus, the anti-tubulin antisemur may be detecting a vinblastine-induced conformational change as well as vinblastine-induced tubulin aggregation.

The double drug binding studies further support the idea of ligand-induced conformational changes. Although colchicine and podophyllotoxin compete for the same site on the tubulin dimer, podophyllotoxin binds much more rapidly and with higher affinity (Wilson et al., 1976). The double drug-tubulin inhibition curves generated with complexes of tubulin-colchicine-podophyllotoxin complexes superimpose on the one generated with tubulin-podophyllotoxin complexes, as would be expected. The curve generated with (vinblastine-colchicine)-tubulin complexes is shifted furthest from the control curve, suggesting that the binding of both vinblastine and colchicine to tubulin induced a conformational change greater than the conformational changes produced by either drug alone. In fact, this curve plateaus before it reaches zero inhibition, at about 25% of maximum 125I-tubulin bound, suggesting that such conformational change has occurred to essentially remove one or more of the antigenic determinants from the tubulin-colchicine-vinblastine complexes. This observation is consistent with the data of Luduena and Roach (1981) that the effect of colchicine and vinblastine on tubulin was greater than that of either alone. With vinblastine-podophyllotoxin-tubulin combinations, the inhibition curve superimposes, for most of its length, with that formed by tubulin-vinblastine complexes. The affinity of podophyllotoxin for viablastine paracrystals of tubulin is significantly reduced (Wilson et al., 1976). Perhaps the reason for this reduction is a vinblastine-induced conformational change in tubulin extending to regions of the podophyllotoxin-binding site.

In summary, this study has extended the use of anti-tubulin antibodies to probe the three-dimensional configuration of tubulin by detecting drug-induced conformational changes most probably involving surface locations on the tubulin dimer. The conformational changes detected are not accompanied by the loss of entire sets of antigenic determinants and suggest that these tubulin conformation changes are relatively subtle.

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J L Morgan and B S Spooner


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