# A SENSITIVE METHOD FOR MEASURING POLYMERIZED AND DEPOLYMERIZED FORMS OF TUBULIN IN TISSUES

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## ABSTRACT

A rapid method for measuring polymerized and depolymerized forms of tubulin in tissues has been developed. The procedure consists of homogenization and centrifugation of the tissue in a microtubule-stabilizing solution and depolymerization of the precipitated microtubules; polymerized and depolymerized forms of tubulin are quantitated by a colchicine-binding assay. The validity of the technique was assessed by electron microscopy and recovery studies with labeled and unlabeled preparations of polymerized and depolymerized forms of rat brain tubulin. The sensitivity of this technique allows quantitation of tubulin in 150 $\mu$g of tissue, wet weight. The method demonstrated that both the polymerized and depolymerized forms of tubulin were present in rat liver cells, and that in the fed state 31.3 ± 0.7% of the total tubulin pool was in the polymerized form.

Microtubules have been implicated in a wide variety of cellular functions on the basis of the observations that agents that disrupt microtubules, i.e., colchicine and vinblastine, inhibit the specific function under study (19). The mechanism(s) by which microtubules participate in many of these cellular processes, however, has not been defined. Several in vitro studies have indicated that there is a dynamic equilibrium between the polymerized and depolymerized forms of tubulin (11, 19), and that microtubule formation in vitro is sensitive to physiological concentrations of magnesium and calcium (20, 21, 28) and requires the presence of nucleoside triphosphates (4). These observations raise the possibility that the relative distribution of the cellular pool of tubulin between these two forms may represent an important regulatory site for microtubule-dependent functions. To examine this possibility, a simple and rapid method has been developed for the quantitation of polymerized and depolymerized forms of tubulin in small tissue samples. This technique is based on the stabilization of both polymerized and depolymerized forms of tubulin in tissue homogenates, their separation by centrifugation, and their quantitation by a colchicine-binding assay.

## MATERIALS AND METHODS

### *Materials*

Adult Sprague-Dawley rats (300–350 g) were used throughout the study. $NaI^{125}$ and [$^3$H]colchicine (3 Ci/mmol) were obtained from New England Nuclear (Boston, Mass.). Guanosine triphosphate (GTP), colchicine, and ethylene glycol-bis($\beta$-aminoethyl ether)*N,N,N',N'*-tetraacetic acid (EGTA) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Dimethylsulfoxide (DMSO) and glycerol were products of Fisher (St. Louis, Mo.). All other reagents were analytical grade.

### *Preparation of Tubulin and Microtubules from Rat Brain*

10 g of rat brain were minced with scissors and homogenized in 10 ml of an ice-cold tubulin-depolymerizing solution (TS) containing 0.25 M sucrose, 0.5 mM $MgCl_2$, 0.5 mM GTP in 10 mM phosphate buffer, pH 6.95. After centrifugation at 100,000 *g* for 30 min at 4°C, the supernatant fraction was diluted with an equal volume of glycerol, and GTP and EGTA were added to a final concentration of 1 mM. The mixture was incubated for 60 min at 37°C and centrifuged at 100,000 *g* for 45 min at 30°C. A microtubule-rich fraction was obtained by resuspending the pellet in a microtubule-stabilizing solution (MTS) containing 50% glycerol, 5% DMSO, 0.5 mM GTP, 0.5 mM $MgCl_2$, 0.5 mM EGTA in 10 mM phosphate buffer, pH 6.95 at room temperature. Resuspension and centrifugation of the pellet in cold TS yielded a depolymerized tubulin-rich soluble fraction which was further purified by one additional polymerization-depolymerization cycle and final chromatography on a Biogel A 5-m column (Bio-Rad Laboratories, Richmond, Calif.) (9). $^{125}$I-tubulin was prepared by the iodination procedure of Hunter and Greenwood (2, 12) and purified by gel chromatography (2). Labeled microtubules were prepared by the addition of $^{125}$I-depolymerized tubulin to the original rat brain supernatant fraction and by incubation under conditions favoring microtubule formation in vitro.

### *Assay of Depolymerized and Polymerized Forms of Tubulin*

Tissue samples were immersed in MTS in a buffer/tissue ratio ranging from 13:1 to 80:1 and immediately homogenized by four strokes of a loosely fitting Teflon pestle; the homogenate was centrifuged immediately at 100,000 *g* for 45 min at room temperature. The depolymerized tubulin in the supernate fraction (SN-I) was measured by a colchicine-binding assay. The pellet (PP-I) was resuspended in ice-cold TS and centrifuged at 100,000 *g* for 30 min at 4°C. The depolymerized tubulin in the supernatant fraction (SN-II) was also assayed, and any residual colchicine-binding activity in the pellet (PP-II) was measured after resuspension in ice-cold TS.

### *Determination of Colchicine-Binding Activity*

Colchicine-binding activity was determined by a modification of the charcoal separation method previously described in this laboratory (24). Since the buffer solutions used in this study differed from the buffer reported by Sherline et al. (24), it was necessary to define the incubation conditions under which colchicine-binding sites were fully saturated. As shown in Fig. 1, saturation was reached after 30 min of incubation in TS, and required 120 min in microtubule-stabilizing solution (MTS). Therefore, the assay procedure adapted involved the incubation of duplicate 25-$\mu$l samples with 5 $\mu$l of a 65 $\mu$M [$^3$H]colchicine solution (0.2 $\mu$Ci/mmol) at 37°C for 60 min when TS was used and for 150 min when MTS was used. Charcoal concentrations between 1 and 10 mg/ml adsorbed more than 99% of the free colchicine, but were also found to adsorb some of the colchicine-binding protein in diluted samples of purified tubulin. To prevent this complication, bovine albumin was added to diluted tissue samples at a final concentration of 0.1%, and 200 $\mu$l of a 0.25% charcoal suspension was used for removal of free colchicine. Under these conditions, more than 99.5% of the free colchicine was adsorbed by charcoal without loss of colchicine-binding protein. Furthermore, these assay conditions gave a linear response over a range of 15–1,500 $\mu$g of tissue protein per 25 $\mu$l of the supernatant fractions prepared in either MTS or TS (Fig. 2).

Colchicine-binding activity was converted to tubulin equivalents by assuming a 1:1 molar ratio of colchicine binding to tubulin and a molecular weight for tubulin of 110,000 (29). The colchicine-binding activity was measured in SN-I and SN-II in order to permit us to quantify, respectively, the amount of tubulin originally present in the depolymerized and polymerized forms. The tubulin content of tissues was expressed either in terms of wet weight of the tissue or as a function of DNA (17) or total protein content (18).

### *Electron Microscopy*

Pellets either were immersed in 5% glutaraldehyde and processed for sectioning or were resuspended in MTS and examined on collodion-carbon-coated grids after 1-min exposure to 0.5% uranyl acetate.

## RESULTS

### *Preservation and Depolymerization of Microtubules*

The MTS used in this study was developed on

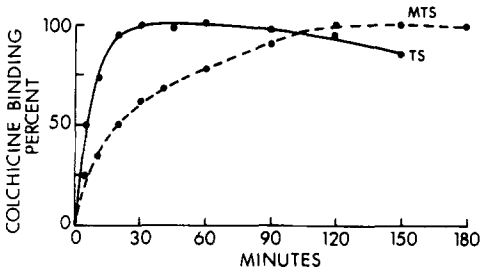


FIGURE 1 The effect of MTS and TS solutions on the rate of saturation of colchicine-binding sites. Liver tissue (0.5 g) was homogenized in 6.0 ml of either TS (4°C) or MTS (22°C), centrifuged at 4°C (TS) or 22°C (MTS), and an aliquot was assayed for colchicine-binding activity; colchicine-binding activity is expressed as a percent of maximal binding. Similar results were obtained when purified depolymerized rat brain tubulin was assayed in either solution.

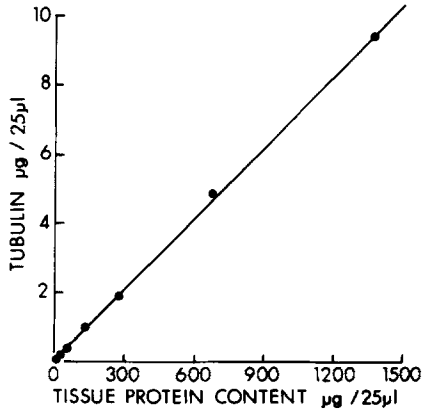


FIGURE 2 Linearity of colchicine binding over the range of tissue/extraction solution volume ratios used in the assay procedure. Liver tissue was homogenized in cold TS, centrifuged, and the supernate was diluted with additional TS. Colchicine-binding activity was determined in 25-µl aliquots, and is plotted against the corresponding total protein content of the sample. Linearity was also observed with MTS.

the basis of observations that concentrated glycerol solutions facilitate the assembly and stabilization of microtubules in vitro (6, 23). The degree to which microtubules were preserved in MTS was assessed in several ways. Brain microtubules formed in vitro were homogenized in MTS, centrifuged at 100,000 *g* for 45 min, and colchicine-binding activity was determined in the supernatant as well as in the pellet resuspended in the depolymerizing solution TS. The colchicine-binding activity measured in the soluble fraction was expressed as a percent of total colchicine-binding activity (soluble + precipitated) and was used as an index for microtubule lability. In all conditions tested, the protein released into the supernate was proportional to the corresponding release of colchicine-binding tubulin.

As shown in Table I, only 4–8% of the microtubules present in the MTS homogenizing solution underwent depolymerization during 45 min of exposure at 22° and 37°C. When the temperature was lowered to 4°C, microtubule lability was increased two- to three-fold. In the absence of glycerol and DMSO, the microtubules depolymerized almost completely at 4°C. At 22°C and at the protein concentrations used (0.1–0.5 mg/ml), 40–50% of the tubulin remained in the precipitable form, suggesting the presence of microtubules and/or tubulin aggregates in these preparations. The observation that assembled microtubules were preserved in MTS at room temperature and completely depolymerized in cold TS was further confirmed by electron microscope studies of microtubules formed in vitro (Fig. 3). Electron microscope studies of PP-I fractions prepared from rat liver homogenized in MTS also revealed the presence of long, smooth microtubules, which disappeared completely after resuspension of the pellet in cold TS. No microtubule structures were observed in the pellets obtained from livers initially homogenized in TS.

$^{125}$I-labeled microtubules were also added to 0.5 g of liver before its homogenization and centrifugation in MTS, and the percent of radioactivity which appeared in the 100,000 *g* supernatant fraction was used as an index of microtubule lability and recovery in the assay system. Similar studies were carried out by the addition of unlabeled brain microtubules to liver tissue before homogenization. The colchicine-binding assay was used to quantitate endogenous polymerized and depolymerized forms of tubulin in the liver homogenate, the amount of brain microtubules added, and the total tubulin represented by endogenous tubulin and added tubulin. Identical results were obtained with both unlabeled and $^{125}$I-labeled preparations (Table II); more than 90% of the polymerized tubulin was recovered in SN-II.

### *Preservation of Depolymerized Tubulin*

Since highly concentrated glycerol solutions not

TABLE I
*Preservation of Microtubules and Colchicine-Binding Activity*

| Homogenization solution | Temperature | Microtubule lability* | Depolymerized tubulin lability‡ |
|---|---|---|---|
| | °C | % | |
| MTS | 4° | 12–16 | <2 |
| " | 22° | 4–8 | <2 |
| " | 37° | 4–8 | 2–5 |
| MTS without glycerol and DMSO | 4° | 88–95 | 15–25 |
| | 22° | 50–60 | – |
| TS | 4° | 90–95 | 5–7 |
| " | 22° | 55–65 | – |

Values represent the range of duplicate assays of four experiments.

* Rat brain microtubules were suspended and centrifuged under the conditions indicated; microtubule lability is expressed as the percent of total tubulin which appeared in the supernatant fraction.

‡ Depolymerized tubulin lability represents the loss in colchicine-binding activity of a depolymerized tubulin-rich brain fraction, which is kept for 90 min under the conditions indicated.

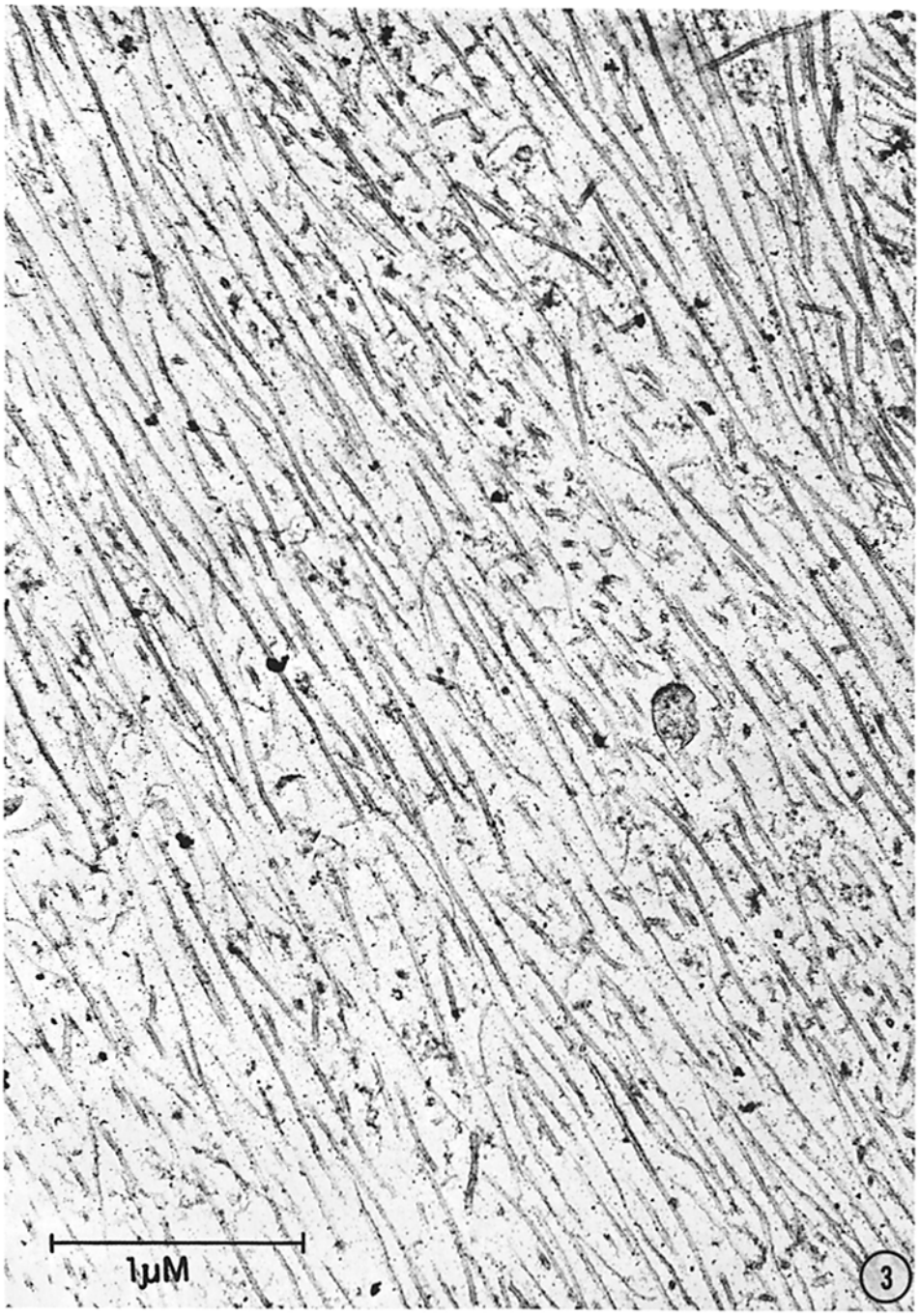


FIGURE 3 The stability of rat brain microtubules in MTS. Microtubules were formed in vitro, suspended in MTS (0.1 mg/ml, 22°C), centrifuged, and the pellet was immersed in glutaraldehyde and processed for electron microscopy. No microtubular structures were identifiable when resuspended and centrifuged in cold TS.

only stabilize microtubules but also favor their formation in vitro, it is important to demonstrate that depolymerized tubulin is also preserved under the conditions of this assay. Tubulin polymerization in vitro was prevented by keeping tubulin levels of tissue homogenates lower than 70 μg/ml –

TABLE II

*Recovery of Added Polymerized and Depolymerized Forms of Tubulin in the SN-I and SN-II Fractions of the Assay Procedure*

| Tubulin preparation | Supernatant fraction I | Supernatant fraction II |
|---|---|---|
| | % | |
| Microtubules | | |
| $^{125}$I-labeled | 5–10 | 90–95 |
| 200 μg Unlabeled | 5–10 | 90–95 |
| Depolymerized tubulin | | |
| $^{125}$I-labeled | 92–97 | 3–8 |
| 200 μg Unlabeled | 90–95 | 5–10 |

Values represent range of four experiments carried out in triplicate.

a concentration far below the levels required for microtubule formation in the test tube (10). The total tissue tubulin was measured with the colchicine-binding assay on aliquots of tissues homogenized in TS (depolymerizing buffer) at 4°C; these values were in excellent agreement (<5% variation) with total tissue tubulin levels calculated as the sum of the tubulin content of the SN-I plus SN-II fractions. Furthermore, the depolymerized tubulin was exposed to 50% glycerol at room temperature for periods shorter than 60 min, factors which should minimize microtubule formation in dilute solutions. The preservation of depolymerized tubulin, however, was assessed experimentally in three ways. (*a*) Soluble fractions of brain tissue prepared in 50% glycerol and which were demonstrated to be capable of forming microtubules in vitro at 37°C, were diluted in MTS to give a final tubulin concentration of 75 μg/ml or less. After centrifugation at room temperature, the presence of microtubules formed in vitro was examined by determining residual colchicine-binding activity in the supernatant fraction and by electron microscopy of the pellet. Neither the loss of colchicine-binding activity nor the presence of microtubules in the pellet could be demonstrated. (*b*) Unlabeled and $^{125}$I-labeled depolymerized forms of tubulin were added to MTS and homogenized with liver tissue. The appearance of radioactivity in the 100,000 *g* pellet and/or loss of colchicine-binding activity in the supernatant fraction was taken as a measure of microtubule assembly and/or adsorption of depolymerized tubulin to particulate material during the assay. As shown in Table II, neither microtubule formation nor loss of depolymerized tubulin was evident during the experimental procedure. (*c*) When the liver soluble fraction prepared in MTS was centrifuged at 100,000 *g* for 45 min at room temperature, no colchicine-binding activity was found in the precipitate, indicating that the depolymerized tubulin present in SN-I did not reassemble under these experimental conditions.

### *Stabilization of Colchicine-Binding Activity*

Since a decay in colchicine-binding activity of tissue extracts has been previously reported (29, 30), studies were carried out to assess the stability of colchicine-binding activity in the homogenizing solutions used in the present assay. The stability of depolymerized tubulin prepared from rat brain after 90 min of incubation in various media is shown in Table I. No significant decay was noted in MTS: the decay of colchicine-binding activity in TS at 4°C was minimal and significantly less than that seen with other depolymerizing solutions. The low rate of decay in TS is probably due to the presence of 0.25 M sucrose, since 1 M sucrose solutions have been reported to preserve colchicine-binding activity for at least 2 wk (8). However, 1 M sucrose cannot be used in the present assay since it would prevent effective and complete depolymerization of microtubules (23).

The stability of colchicine-binding activity was also studied in soluble liver fractions prepared in MTS or TS and incubated under conditions comparable to those used in the assay. As shown in Fig. 4, no detectable decay in colchicine-binding activity was observed in MTS even after 240 min of incubation. Since the assay procedure involves

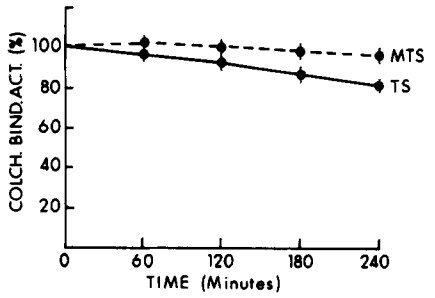


FIGURE 4 The stability of colchicine-binding activity (COLCH. BIND. ACT.) in MTS and TS. Liver tissue (0.5 g) was homogenized and centrifuged in 6.0 ml of either cold TS or MTS at room temperature. Aliquots of the supernatant fractions were incubated for varying time periods at 4°C (TS) or at 22°C (MTS) and then assayed for colchicine binding. Results are expressed as a percent of the initial colchicine-binding activity. Values represent mean ± SD of three experiments.

only 30–40 min of incubation in TS, the rate of decay observed in TS does not significantly affect the sensitivity or precision of the procedure.

### *Characterization of Colchicine-Binding Activity*

The colchicine-binding activities measured in SN-I and SN-II were further characterized by chromatography on a Biogel A 1.5-m column eluted with TS. All colchicine-binding activity in both SN-I and SN-II co-eluted with $^{125}$I-tubulin prepared from rat brain (Fig. 5). Similar elution profiles characterized the various fractions prepared from rat liver, brain, and pancreatic islet tissue.

It has recently been suggested (16, 26) that brain tubulin exists in two forms, one of which is characterized by a low colchicine-binding activity. Since the proposed method for measuring depolymerized and polymerized forms of tubulin is based on colchicine binding, it is important to demonstrate that the specific colchicine-binding activities of purified preparations of both forms of tubulin are identical. Microtubules, prepared in vitro (23) and resuspended in cold depolymerizing TS solution, exhibited a colchicine-binding activity of 4.2 ± 0.5 nmol per mg protein. After centrifugation of this suspension at 100,000 *g* for 30 min, the supernatant fraction contained >90% of the initial protein added and demonstrated a colchicine-binding activity of 4.5 ± 0.4 nmol per mg protein. Elution of the depolymerized microtubule preparation over a Biogel A 5-m column, with cold TS as eluting buffer, resulted in the distribution of the protein in two distinct peaks: the first peak appeared immediately after the void volume and contained <15% of the total protein added to the column (Fig. 6); more than 85% of the protein was recovered in the second peak which co-eluted with $^{125}$I-tubulin and on SDS-polyacrylamide gel electrophoresis formed a single band corresponding to a mol wt of 50,000–55,000 (Fig. 7). The first peak was found to consist of several proteins, one of which co-migrated with tubulin and could represent tubulin molecules initially present in 36S structures (16). Since the tubulin present in the first peak represents only 10–15% of the total protein in this fraction, it is unlikely that depolymerized microtubules eluted with cold TS contain a significant quantity of tubulin polymers, i.e., a form which does not bind colchicine. No colchicine-binding activity was detected in the first peak, whereas the protein in the second peak bound 4.0 ± 0.5 nmol of colchicine per mg protein. It may therefore be concluded that the colchicine-binding activities of tubulin derived from either the polymerized or the depolymerized form are equivalent.

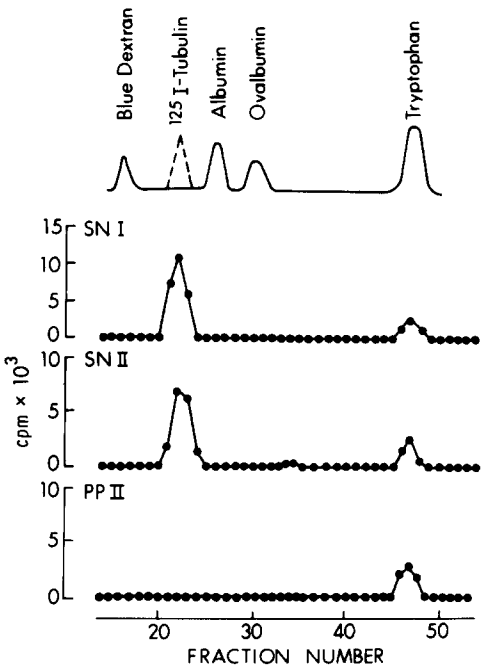


FIGURE 5 Co-elution of colchicine-binding activity in SN-I and SN-II fractions with $^{125}$I-rat brain tubulin. SN-I, SN-II, and PP-II fractions were incubated with [$^{3}$H]colchicine under conditions in which colchicine-binding sites were saturated. After adsorption of free colchicine with charcoal, aliquots were chromatographed on a Biogel A 1.5-m column, using TS as the elution buffer. The column was calibrated with $^{125}$I-tubulin, blue dextran, albumin, ovalbumin and tryptophan.

### *Polymerized and Depolymerized Forms of Tubulin in Liver Tissue*

Application of the present method to liver tissue

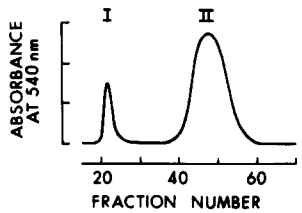


FIGURE 6 Absorbance profile (at 540 nm) of depolymerized microtubule preparations eluted on a Biogel A 5-m column with TS buffer at 4°C. Peak I appears immediately after the void volume; peak II co-elutes with $^{125}$I-rat brain tubulin.

obtained from fed rats indicated that both polymerized and depolymerized forms of tubulin are present in normal liver tissue (Table III). For determination of the degree of tubulin polymerization in vivo, tissues were homogenized in MTS, the buffer to tissue volume ratio always being kept >10 since tubulin was found to polymerize in vitro under higher concentrations. When the PP-II fraction was resuspended in TS and incubated with [$^3$H]colchicine before elution on a Biogel A 1.5-m column, no colchicine-binding protein was observed in the eluate; all the radioactivity appeared in the salt peak (Fig. 5). The total tubulin was thus determined as the sum of the SN-I and SN-II tubulin levels and represents both the depolymerized tubulin pool and the pool of cytoplasmic microtubules, known as cold labile tubulin polymers (1). Since the present experimental procedure does not allow the detection of an eventual third tubulin pool, namely membrane-bound tubulin which cannot be solubilized by cold or colchicine treatment, it remains possible that the measured total tubulin levels are underestimated. Further experiments are, however, required to assess the quantitative importance of membrane-bound tubulin as well as its eventual role in the process of depolymerization of cytoplasmic microtubules. Polymerized tubulin averaged 31.1 ± 0.7% of the total tubulin pool. As was anticipated, the polymerized tubulin content decreased significantly when liver tissue was exposed to 4°C before homogenization in MTS (Table III). The lability of liver microtubules at low temperature is comparable to that reported in other systems (1, 13). Like brain microtubules formed in vitro, liver microtubules also depolymerized up to 95% upon homogenization of the liver tissue in cold TS (Table III), provided the buffer/tissue ratio was not lower than 13:1.

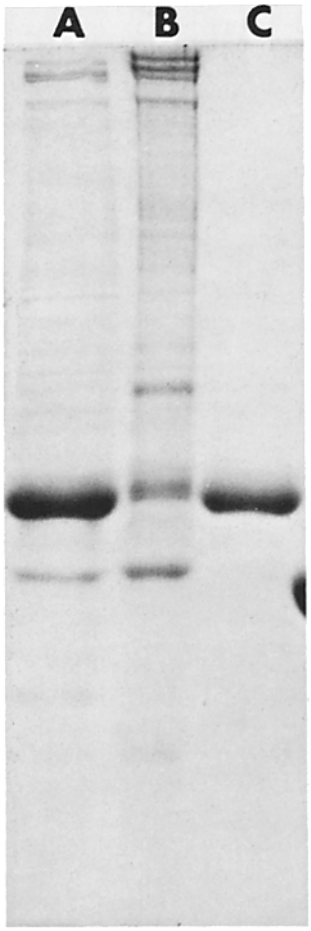


FIGURE 7 Analysis of microtubule proteins by SDS-polyacrylamide gel electrophoresis, using 10% acrylamide slabs (25). (*A*) Microtubule pellet after one assembly step. (*B*) Peak I from Biogel A 5-m column. (*C*) Peak II from Biogel A 5-m column.

## DISCUSSION

The development of a quantitative assay for measuring polymerized and depolymerized forms of tubulin requires that both forms be stabilized dur-

TABLE III
*Total and Polymerized Tubulin in Liver*

| Exp. condition | Fraction | Bound colchicine | Total tubulin content | | Polymerized tubulin | n |
|---|---|---|---|---|---|---|
| | | *pmol/mg protein* | *μg/g wet wt.* | *μg/mg DNA* | *% of total tubulin* | |
| Standard assay | SN-I | 30.1 ± 1.1 | 421.1 ± 13.8 | 176.9 ± 11.0 | 31.3 ± 0.7 | 12 |
| | SN-II | 79.0 ± 3.2 | 192.0 ± 11.2 | 79.6 ± 5.5 | | |
| Previous exposure to 4°C for 30 min | SN-I | 43.3 ± 2.1 | 576.5 ± 19.6 | – | 9.9 ± 1.5 | 4 |
| | SN-II | 25.2 ± 1.1 | 61.8 ± 6.1 | – | | |
| Initial homogenization in TS | SN-I | 43.3 ± 2.8 | 578.9 ± 22.6 | 244.0 ± 10.6 | 1.9 ± 0.2 | 5 |
| | SN-II | 8.8 ± 0.9 | 11.1 ± 0.9 | 4.7 ± 0.2 | | |

Values represent mean ± SEM. n = number of experiments.

ing the course of isolation. Although several investigators have reported the successful isolation of mitotic spindles (5, 14) and cytoplasmic microtubules (6, 15), the conditions used in these studies have not been demonstrated to maintain the stability of depolymerized tubulin. Furthermore, Forer and Zimmerman (7) have recently demonstrated that mitotic spindles isolated with hexylene glycol undergo progressive depolymerization, and hence this extraction technique is not applicable for a quantitative assay. Kane's spindle isolation medium (14) was used by Weisenberg (27) to measure changes in "particulate tubulin" during meiosis in surf clam eggs, but evidence was not provided concerning the quantitative recovery of the polymerized and depolymerized forms of tubulin. Rubin and Weiss (22) have recently demonstrated by an electron microscope morphometric technique that the microtubule extraction medium described by Filner and Behnke (6) can be used to stabilize microtubules in Chinese hamster ovary cells. However, depolymerized tubulin exhibits a significant rate of decay in solutions without glycerol or sucrose (Table I), which limits the usefulness of the procedure as an assay technique.

The MTS used in the present procedure is a modification of the medium described by Filner and Behnke and differs primarily by the addition of 0.5 mM GTP and 0.5 mM EGTA, compounds which stabilize colchicine-binding activity and microtubule structure, respectively (7, 27). The buffer to tissue volume ratio at which tissues were homogenized in MTS was always >10, since polymerization of tubulin in vitro could be demonstrated under higher concentrations. Over a range of buffer to tissue volume ratios of 10–100, the colchicine-binding assay demonstrated linear characteristics with either MTS or TS. Furthermore, these studies demonstrated that colchicine-binding activity remained stable in MTS for over 4 h and that the rate of decay in TS was extremely slow—less than 5% denaturation under the conditions of our assay. The colchicine-binding assay permits the detection of as little as 75 ng of tubulin, an amount present in 150 $\mu$g (wet weight) of tissue.

The validity of the present technique has been documented by assessing the behavior of $^{125}$I-labeled and unlabeled polymerized and depolymerized forms of rat brain tubulin under assay conditions. Specifically, it was demonstrated that microtubules were quantitatively recovered in the SN-II fraction after homogenization and centrifugation in the MTS and subsequent depolymerization in the TS. Similarly, depolymerized tubulin was quantitatively recovered in the SN-I fraction after homogenization and centrifugation in either MTS or TS. The quantitative recovery of polymerized and depolymerized forms of rat brain tubulin added to liver tissue also indicates that nonspecific adsorption of tubulin by particulate cellular fractions does not occur. The possibility that the colchicine-binding activity in SN-II is not exclusively a measure of microtubules but includes depolymerized tubulin released from previously unbroken cells is also unlikely since (*a*) rehomogenization of PP-I in MTS did not solubilize any additional colchicine-binding activity and (*b*) the specific activity of colchicine-binding activity, i.e., colchicine-binding protein per total protein, was significantly greater in SN-II than SN-I. The validity of the present experimental procedure was also suggested by electron microscope studies. Both rat brain microtubules prepared in vitro and endogenous liver cell microtubules were readily observed in PP-I fractions after homogenization and centrifugation in the MTS, and disappeared completely after exposure to cold TS.

Since the specific colchicine-binding activities of tubulin derived from the polymerized and depolymerized forms are identical, the colchicine-binding assay described appears to be a valid procedure for determining both the polymerized and depolymerized tubulin pools. It is possible that tubulin levels determined by this assay may be slightly underestimated, in that tubulin (complexes) might exist that does (do) not bind colchicine (16, 26). However, gel quantitation indicated that depolymerized microtubule preparations contained only a few high molecular weight tubulin complexes which represented <5% of the tubulin in these preparations. It should also be noted that the present procedure will not measure membrane-bound tubulin (3), if this form is not extracted by MTS or solubilized by the TS buffer.

This assay procedure indicates that tubulin is present in both polymerized and depolymerized forms in the liver cell and that in the fed state 31.3% of the total tubulin occurs in the polymerized state (Table III). The ability to measure polymerized and depolymerized forms of tubulin in small tissue samples provides a useful tool for assessing the effects of various physiological and pharmacological agents on total tubulin and the dynamic equilibrium between polymerized and depolymerized forms of tubulin. The usefulness of this procedure has been recently documented in

our studies demonstrating a parallelism between the amount of polymerized tubulin in pancreatic islets and their capability of insulin secretion (20). Such studies should help clarify the mechanism(s) by which microtubules are involved in a wide variety of cellular functions.

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