Fibroblast Tubulin†

Richard Ostlund‡ and Ira Pastan*

ABSTRACT: Tubulin purified from L cells by Sepharose 4B and DEAE-cellulose chromatography has properties similar to brain tubulin. The specific activity of colchicine binding is 1.1 mol of colchicine bound per 100,000 g of protein. From 0.07 to 0.45% of the total protein of L cells is active tubulin. Proteins differing biochemically from tubulin but coelectrophoresing with it in sodium dodecyl sulfate gels were encountered. Colchicine-binding activity of virus-transformed NRK fibroblasts measured by a filter assay using a whole cell homogenate was reduced when compared to the parent NRK cells. L cells growing on plastic or glass surfaces had five times as much colchicine-binding activity as the same cells growing in spinner culture. Apparently growth on a surface increases the tubulin content of L cells.

Tubulin, the colchicine-binding protein of microtubules, has been identified in many eukaryotic tissues (Weisenberg et al., 1968; Feit et al., 1971; Everhardt, 1969). Indeed, tubulin is the principal component of microtubules and microtubule-like structures can be assembled from solutions containing high concentrations of tubulin (Weisenberg, 1972). Microtubules appear to play an important role in maintaining cell structure and mediating a variety of cellular events including mitosis, motility, phagocytosis, and the secretion of granular products. An understanding of the properties of tubulin is essential to clarify its role in these processes.

Brain is a rich source of tubulin and most studies on the biochemistry of tubulin have employed this tissue as its source (Weisenberg et al., 1968; Eipper, 1972). We have been interested in studying the behavior of clonal lines of normal and transformed fibroblastic cells maintained in tissue culture. These cells undergo striking changes in shape, motility, growth rate, and other processes when transformed into cancer cells. Therefore, it seems important to characterize tubulin prepared from fibroblastic cells and to compare the content and properties of tubulin from normal and transformed cells.

Materials and Methods

Cells. L cells were grown on the surface of 0.5-gal glass roller bottles, 100-mm plastic dishes, or in spinner suspension culture. Eagle's medium with 10% fetal bovine serum, 50 units/ml of penicillin, 50 μg/ml of streptomycin, and 2 m*M* glutamine was used except in spinner culture where calcium was omitted and sodium phosphate increased tenfold to 10 m*M*. NRK,[1] transformed NRK, 3T3, and 3T3 cAMP^{tcs}-1 mutant cells were grown on 100-mm plastic dishes in Dulbecco-Vogt modified Eagle's medium containing 50 units/ml of penicillin, 50 μg/ml of streptomycin, and 2 m*M* glutamine. In all cases the medium was changed every other day and 24 hr before the cells were harvested. Cells were harvested at confluency.

Buffers and Extraction Methods. Before harvesting, the cells were washed four times with PBS[1] (4°) and twice with 50 m*M* sodium pyrophosphate, 2.5 m*M* $MgCl_2$, 0.1 m*M* GTP, and 0.24 *M* sucrose (pH 6.5) (buffer A) at 4°. The cells were harvested by scraping from surfaces with a rubber policeman or by centrifugation of spinner cells. The suspension was homogenized for 50 strokes with a tight-fitting Dounce homogenizer. This was left at 1° for 1 hr and then centrifuged at 16,000*g* for 30 min. Homogenizing up to 200 strokes or longer extraction times did not increase the yield. The supernatant was then chromatographed on a 0.9 × 52 cm column of Sepharose 4B equilibrated with 50 m*M* sodium pyrophosphate, 2.5 m*M* $MgCl_2$, and 0.1 m*M* GTP (pH 6.5) (buffer B). Fractions containing colchicine-binding activity were combined and applied to a 0.9 × 5 cm DEAE-cellulose column equilibrated with buffer B. The column was first eluted with 20 ml of buffer B containing 0.1 *M* NaCl and this was followed by a linear NaCl gradient consisting of 30 ml of buffer B containing 0.1 *M* NaCl and 30 ml of buffer B containing 0.5 *M* NaCl.

Filter Assay. Cells were prepared as described above up to the homogenization step. In certain experiments the cell homogenate was then centrifuged at 16,000*g* for 30 min and the cell pellet homogenized in buffer A. Then 0.17 ml of cell homogenate (or supernatant or pellet, 1–15 mg/ml) was incubated at 37° for 1 hr with a final concentration of 4 μ*M* [^{3}H]colchicine (5 Ci/mmol). The [^{3}H]colchicine was added as 13 μl of a stock solution made up in buffer B. [^{3}H]Colchicine was usually not diluted with nonradioactive colchicine. The reaction was quenched by placing the test tubes on ice. The bound colchicine was determined by placing 50-μl aliquots of the reaction mixture on 2.4-cm diameter DEAE-cellulose (DE-81) paper supported by straight pins above a plastic packing lid at 4° in a cold room. After 15–30 min the numbered filters were placed in a beaker of buffer B not containing GTP and washed eight times with that buffer, each wash lasting about 3 min. The wet filters were placed in counting vials with 15 ml of Aquasol and counted 24 hr later. The counting efficiency was 22%. All assays were done in triplicate.

Techniques. Polyacrylamide gel electrophoresis was done according to the method of Maizel using 7.5% polyacrylamide–0.1% sodium dodecyl sulfate gels (Maizel, 1969). Sucrose was determined by the methods of Dubois et al.

† From the Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received February 13, 1975.*

‡ Present address: Department of Internal Medicine, Metabolism Division, Barnes & Wohl Hospitals, St. Louis, Mo. 63110.

[1] Abbreviations used are: NRK, normal rat kidney cells; 3T3 cAMP^{tcs}-1, temperature sensitive 3T3 cells; PBS, 0.15 *M* NaCl containing 0.015 *M* sodium phosphate (pH 7.4); buffer A, 50 m*M* sodium pyrophosphate, 2.5 m*M* $MgCl_2$, 0.1 m*M* GTP, and 0.25 *M* sucrose; buffer B, 50 m*M* sodium pyrophosphate, 2.5 m*M* $MgCl_2$, and 0.1 m*M* GTP.

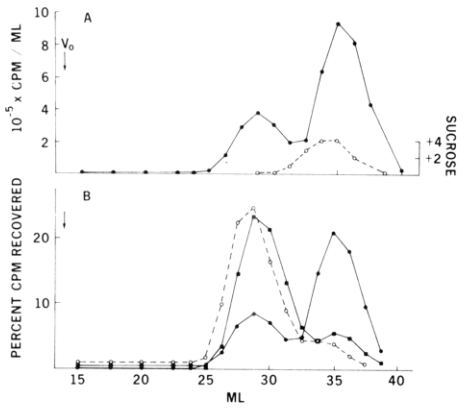

FIGURE 1: Chromatography of extracts of L cells and mouse and rat brain on Sepharose 4B. (A) An extract of 1.2 ml of L cells in buffer A was incubated with a final concentration of 4 μM [^{3}H]colchicine for 3 hr at 37° and then chromatographed on a 0.9 × 52 cm column of Sepharose 4B. The content of [^{3}H]colchicine (●—●) and the amount of sucrose in each fraction (O- - -O) were determined as described under Materials and Methods. (B) Separate experiments were done using extracts of mouse brain (■—■) and rat brain (O- - -O); the results are plotted along with those of L cells (●—●) as percent of recovered counts in each fraction. Sucrose was added to mark the included volume.

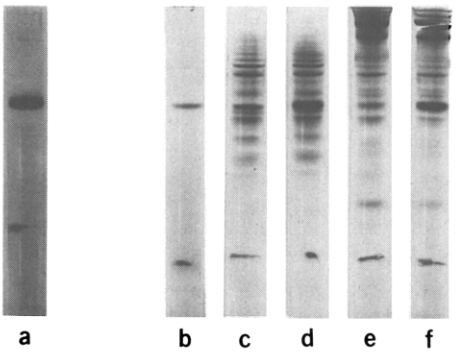

FIGURE 2: Polyacrylamide (7.5%)–sodium dodecyl sulfate (0.1%) gel electrophoresis: (a,b) fibroblast tubulin; (c) material not adhering to DEAE-cellulose; (d) gel C + fibroblast tubulin; (e) material appearing in the void volume on Sepharose 4B (see Figure 1) (f) gel e + fibroblast tubulin.

(1956), the color being rated on an arbitrary scale of 1+ to 4+. Protein was determined by the method of Lowry following trichloroacetic acid precipitation (Lowry et al., 1951).

Materials. [^{3}H]Colchicine was purchased from New England Nuclear Corp. Calf serum was purchased from Colorado Serum Co. and fetal bovine serum from Industrial Biological Laboratories. L-929 cells were obtained from Mr. C. Corey, NRK cells and their derivatives from Dr. E. Scolnick, and 3T3 and 3T3 cAMP^{tcs}-1 cells from Dr. M. Willingham, all of the National Institutes of Health.

Results

Purification of Fibroblast Tubulin. Tubulin was extracted from L cells by a modification of the pyrophosphate method described by Eipper (1972). In a typical preparation L cells were grown in two 0.5-gal roller bottles to yield 43 mg of cell protein as described under Materials and Methods. The adherent cells were washed six times with PBS, twice with buffer A, and then scraped from the surfaces of the bottles into a small amount of residual buffer. The material was homogenized 50 strokes in a Dounce homogenizer yielding 1.7 ml of homogenate and spun at 16,000*g* for 30 min yielding 1.2 ml of supernatant. This supernantant was incubated 3 hr at 37° with a final concentration of 4 μM [^{3}H]colchicine. The reaction was then quenched by placing the test tube on ice and the material was chromatographed over a 0.9 × 52 cm Sepharose 4B column at 4° as seen in Figure 1A. A peak of bound colchicine appears at 29 ml. The elution volume of this colchicine-binding material was very similar to that of tubulin extracted from mouse and rat brain (Figure 1B). In addition, the pattern of colchicine binding did not change when the extract applied to the column did not contain [^{3}H]colchicine and the column fractions were assayed for their ability to bind colchicine after chromatography on Sepharose 4B.

The fractions eluting between 26.3 and 30.0 ml were combined and applied to a 0.9 × 5 cm column of DEAE-cellulose previously equilibrated with buffer B. The column was first eluted with 20 ml of buffer B containing 0.1 *M* NaCl and then by a linear NaCl gradient from 0.1 to 0.5 *M* in the same buffer. The recovery of [^{3}H]colchicine from all column fractions was 103% of that applied. Of this, 14% did not adhere to DEAE-cellulose in the presence of 0.1 *M* NaCl. The remaining 86% appeared as a peak at 0.18 *M* NaCl and contained a nearly homogeneous single protein band when analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Figure 2a). The subunit molecular weight of this colchicine-binding protein determined in 0.1% sodium dodecyl sulfate–7.5% polyacrylamide gels prepared in 0.1 *M* sodium phosphate (pH 7.0) (Maizel, 1969) was 50,000. Trypsin, pepsin, ovalbumin, pyruvate kinase, and bovine albumin were used as standards. The specific activity of colchicine binding as calculated from the peak colchicine-binding fractions from DEAE chromatography was 1.1 mol of colchicine per 100,000 g of protein. If the tubulin dimer has a mol wt of about 100,000, this is 1.1 mol of colchicine per mol of tubulin.

The column profiles of Figure 1 and the DEAE chromatography demonstrate that the only significant colchicine-binding activity present in L-cell extracts resides in the single colchicine-binding protein. We will refer to this protein as fibroblast tubulin because of its chemical similarities to brain tubulin.

Analyzing fractions at various stages of purification of fibroblast tubulin, it became apparent that all the material in the extract with the subunit mobility of tubulin in sodium dodecyl sulfate–polyacrylamide gels did not bind colchicine and did not copurify with tubulin. For example, the gels in Figures 2b–f demonstrate that proteins which co-electrophorese with tubulin are present in the void volume of the Sepharose 4B column and in the material that did not bind to DEAE-cellulose during DEAE-cellulose chromatography. These fractions have little or no colchicine-binding activity. They make it impossible to measure active tubulin in crude extracts of L cells by polyacrylamide gel electrophoresis.

The content of tubulin in our cells can be calculated by division of the colchicine-binding capacity of a crude cell homogenate (picomoles per milligram of cell protein) by the specific colchicine-binding activity of pure fibroblast tubulin (picomoles per milligram of tubulin). By this method the tubulin content of spinner L cells in Table IV is 0.07%, and that of attached L cells is 0.45%. The tubulin content of 3T3 cells in Table V is 1.6%. These calculations assume that the colchicine-binding capacity of tubulin in crude cell extracts is the same as that of purified L cell tubulin.

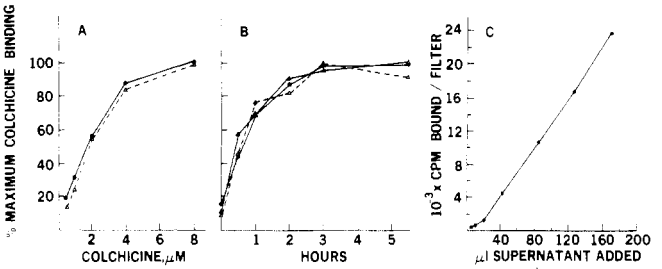

FIGURE 3: Parameters of DEAE filter assay for colchicine binding. Values are plotted for L cell buffer A supernatants (●—●), homogenates (Δ—Δ), or pellets (Δ- - -Δ) prepared as described under Materials and Methods. (A) The colchicine concentration dependence of binding was determined and the results expressed as the percent bound using the amount bound at 8 μM colchicine as 100%. (B) The time course of binding was determined by incubating aliquots of homogenate, supernatant, or pellet with [^{3}H]colchicine for 0–5.5 hr at 37°. The reaction was quenched by placing the test tube on ice. After completion of the experiment, all time points were analyzed together. (C) The dependence of colchicine binding on the concentration of tubulin was determined by adding varying volumes of buffer A supernatant to the filter assay.

Table I: Repetitive Extraction of L Cells.[a]

Cell Fraction	pmol of Colchicine Bound	
	A	B
Supernatant 1	45.2	66.5
2	56.9	
3	14.6	
4	5.8	
Pellet	11.2	121.2
	133.7	187.7

[a] A homogenate of L cells in buffer A was divided into two portions (A and B) of 0.8 ml and treated separately. Portion A was centrifuged at 16,000g for 30 min and then the pellet was repetitively extracted with 1.0 ml of buffer A and centrifuged at 16,000g for 30 min for a total of four extractions. Portion B remained at 1° for the duration of the experiment (90 min) and was then centrifuged, yielding only one extraction. Protein concentration of the initial homogenate was 2.1 mg/ml.

Filter Assay for Tubulin. As a routine assay a modification of the DEAE-cellulose filter assay of Weisenberg et al. (1968) was used as described under Materials and Methods. The assay could be performed on a whole cell homogenate, a particle-free supernatant, or a cell pellet resuspended in buffer A with reproducible results (coefficient of variation 0.06). When we examined the content of tubulin in cell homogenates, we found that the majority of colchicine-binding activity remained in the pellet after a single extraction with buffer A. However, the insoluble binding activity could be recovered by repetitive extraction (Table I). The colchicine-binding activity of the supernatant and that of the pellet seemed to be identical as judged by the following criteria. Both were saturable by micromolar concentrations of colchicine as seen in Figure 3A. Both displayed a similar time course of binding at 37° as seen in Figure 3B. In both, incubation with [^{3}H]colchicine at 4° instead of at 37° for 5.5 hr resulted in less than 15% of the total binding expected at 37°. Both activities were significantly reduced by the addition of 0.1 mM unlabeled colchicine or by boiling for 10 min (Table II). The colchicine-binding activity of homogenate fractions averaged 75.4% of the combined activities of supernatant and pellet fractions. These findings enabled us to use the colchicine-binding activity of whole cell homogenates to measure the content of active tubulin in different lines of fibroblasts.

Table II: Colchicine-Binding Activity Remaining after Boiling or Addition of 0.1 mM Unlabeled Colchicine.[a]

	Rel Act.
Homogenate	100.0
Homogenate boiled 10 min	2.8
Homogenate + 0.1 mM colchicine	2.8
Supernatant	100.0
Supernatant + 0.1 mM colchicine	1.9
Pellet	100.0
Pellet + 0.1 mM colchicine	4.9

[a] L cells were extracted and fractionated as described under Materials and Methods. Control experiments without unlabeled colchicine or boiling are taken as 100% activity.

Table III: Colchicine Binding of Total Cell Homogenate.[a]

Cell Type	pmol/mg of Protein	
	Expt 1	Expt 2
NRK	207 (1)	120 (1)
Kirsten NRK	97 (0.48)	59 (0.49)
Schmidt-Ruppin NRK	117 (0.56)	70 (0.58)
Moloney NRK	121 (0.58)	62 (0.52)

[a] Cells were homogenized in buffer A and the colchicine-binding activity of the homogenate was determined on an aliquot. NRK cells and all three transformed derivatives were analyzed together in two separate experiments. Numbers in parentheses are colchicine-binding activity relative to NRK.

In one experiment we compared the binding values obtained by the filter assay with those obtained by chromatography over a 0.9 × 5 cm DEAE-cellulose column using the technique of Eipper (1972). The filter method gave 92% of the column method. The dependence of the filter assay on the concentration of colchicine-binding material is shown in Figure 3C. Values from experiments with less than 20 μl of extract in a total of 183 μl consistently gave less than 1500 cpm per filter (after subtraction of the blank of about 500 cpm per filter) and led to results lower than expected. Therefore, assays were routinely done in the linear portion of the concentration dependence curve.

Tubulin Content of Different Fibroblast Lines. Using the filter assay, tubulin content was determined in the whole homogenate of several cell types. This measurement reflects only active colchicine-binding sites.

NRK and Transformed NRK Cells. The parent NRK cell type has previously been infected with various viruses (Benveniste and Scolnick, 1973) to yield transformed cell lines. The data in Table III demonstrate that three transformed lines of NRK cells have about half as much tubulin as the parent NRK cells. Data from two separate experiments are shown in Table III. In the second experiment the amount of tubulin was decreased in all the cells proportionately. Therefore, in making comparisons between cell types we have grown the test cells and assayed all samples in parallel. The cause of this type of variation is unknown but is not due to differences in cell density, cell feeding schedules, lots of serum, or assay conditions.

L Cells. Conditions were found which greatly altered L cell active tubulin content. L cells were grown either on glass or plastic surfaces or in spinner suspension culture

Table IV: Colchicine Binding by Cell Extracts.[a]

	pmol/mg of Protein			
	Spinner Cells		Attached Cells	
Expt No.	Supernatant	Pellet	Supernatant	Pellet
1	1.6	5.5	9.7	35.7
2	2.0	na	12.3	na
3	1.7	na	8.7	na

[a] Colchicine binding was determined in L cells grown either in spinner culture or on the surfaces of plastic petri dishes or glass roller bottles. The cells were harvested and fractionated as described under Materials and Methods. Each number represents a separate preparation; na, not assayed.

where suspension was maintained by a magnetic stirring device. L cells grown on glass or plastic surfaces were found to contain over five times as much tubulin as those grown in spinner culture (Table IV). This increase does not simply reflect the extractability of the colchicine-binding protein, since binding in the pellet was also increased in cells attached to a surface. Nor does the increase reflect differences between normal and spinner culture medium. L cells were taken from spinner culture and planted in 100-mm plastic dishes at 3,000,000 cells/dish. The cells were grown without magnetic stirring in either normal or spinner medium. Cells adhered to the plastic since stirring is essential for a suspension culture. The medium was changed every other day and the cells were grown to confluence (5.0 and 4.8 mg/dish, respectively). The rate of cell growth was identical and no morphological differences were noted. The cells were harvested and the colchicine binding of the cell homogenate determined yielding 98 pmol of colchicine/mg of protein for cells growing in normal medium and 123 pmol/mg for cells growing in spinner medium.

3T3. Willingham et al. (1973) have isolated a mutant line of 3T3 cells (3T3 cAMP^{tcs}-1) in which the cyclic AMP levels rapidly fall by over 50% when the cells are subjected to a change in temperature. Following this decline in cyclic AMP levels the flattened cells retract their processes and assume a rounded morphology. In an experiment designed to determine if rapid changes in colchicine binding take place in response to changes in cellular cyclic AMP levels, this temperature-sensitive mutant was used. Two 100-mm dishes of 3T3 cAMP^{tcs}-1 cells and two dishes of normal 3T3 cells were grown at 37° to light density as described under Materials and Methods. Then one dish of each was placed at room temperature for 30 min during which time the mutant cells underwent the expected rounding; such rounding is correlated with a decline in cellular cyclic AMP levels (Willingham et al., 1973). No changes were noted in the morphology of the control 3T3 cells. Then cells from these dishes as well as from dishes kept at 37° as controls were harvested in the usual manner after washing at 4°. Rounding up of mutant cells did not result in the loss of cells from the dish, since the total protein in the dish cooled to room temperature did not differ from that maintained at 37°. Colchicine-binding assays were then done on the cell homogenates and the results are shown in Table V. It appears that all of the cells have about the same amount of colchicine-binding activity. Therefore, rapid changes in cyclic AMP levels in the cells did not affect the amount of colchicine bound. The effect of prolonged changes in cyclic AMP levels has not been studied.

Table V: Colchicine Binding in 3T3 and Temperature Sensitive 3T3 cAMP^{tcs}-1 Cells.[a]

	Colchicine Binding (pmol/mg of Protein)
Harvested immediately	
3T3	153
3T3 cAMP^{tcs}-1	158
Harvested after 30 min at room temperature	
3T3	148
3T3 cAMP^{tcs}-1	161

[a] See Results section for experimental protocol.

Discussion

Tubulin with typical chemical properties has been isolated from mouse L cell fibroblasts. The material appears to exist as a dimer similar in molecular weight to brain tubulin based on its elution pattern on Sepharose 4B. It has a subunit molecular weight of 50,000 in sodium dodecyl sulfate–polyacrylamide gels. It binds 1.1 mol of colchicine per 100,000 g of protein, the molecular weight of the putative dimer. Porcine brain tubulin binds 1 mol of colchicine per 120,000 g (Weisenberg et al., 1968). The fibroblast protein is essentially saturated at 8 μM colchicine and displays a time course of binding similar to that of calf brain tubulin (Wilson, 1970; Owellen et al., 1972). No other protein with significant binding activity under the conditions of our assay was found.

It seems highly likely that fibroblast tubulin is a constituent of the cytoplasmic microtubules and mitotic spindle known to exist in fibroblasts. We have found microtubules to be present by electron microscopy in the cells studied in this paper. Proteins thought to be tubulin have previously been described in L cells (Feit et al., 1971; Krishan and Hsu, 1971).

We were surprised to find that some of the protein from the cell homogenate that coelectrophoresed with fibroblast tubulin in polyacrylamide gels did not bind colchicine or adhere to DEAE columns. It is not known whether these proteins represent a soluble but inactive tubulin such as that described by Kirschner et al. (1974) or whether they are merely extraneous proteins. It has been reported that in vitro (but not in vivo) phosphorylation of tubulin results in an aggregated form eluting at the void volume of Bio-Gel A 0.5 M (Eipper, 1974). A complex of in vitro phosphorylated tubulin and a soluble brain protein has been described (Murray and Froscio, 1971) which binds poorly to DEAE-Sephadex. It is possible that our co-electrophoresing proteins are similar to those modified tubulins. In any event our data suggest that it may not be valid to measure active tubulin concentrations by scanning polyacrylamide gels of crude tissue extracts.

In the following discussion we use the term "tubulin content" as a convenient notation for colchicine-binding activity, although we have not excluded the possibility that the specific activity of colchicine binding by tubulin could have changed during the manipulations described. However, we believe that this is unlikely and that the differences in colchicine binding observed are most likely to be explained by differences in the content of tubulin.

We have found that under usual extraction conditions more than half of the tubulin remains in the cell pellet, but the tubulin in the pellet can be measured by the usual filter

assay despite the insolubility of the pellet in the assay buffer. The pellet colchicine-binding activity has kinetic and saturability properties (see Figure 3) which exclude the possibility of nonspecific binding as described by Stadler and Franke (1974) in liver membranes. We believe that tubulin may exist adsorbed or polymerized in the pellet and that to measure accurately the tubulin content of a cell the whole homogenate should be used, not merely a soluble extract.

Three virus-transformed NRK cells had less tubulin per milligram of cell protein than the parent NRK (Table III), the difference being about twofold. This difference correlates with the observation that transformed cells are generally more rounded than untransformed cells and the processes of untransformed cells usually contain prominent microtubules. Since transformed cells often grow more rapidly than normal cells, the amount of time spent in different parts of the cell cyclic could produce differences in tubulin content. Indeed, in one study tubulin synthesis has been reported to be increased during the S and G_2 phases of the cell cycle (Forrest and Klevecz, 1972), although no differences were found in another study (Robbins and Shelanski, 1969).

The effect of acute changes in intracellular cyclic AMP levels on colchicine binding was investigated using a temperature-sensitive 3T3 mutant, but no differences were noted under conditions known to lower intracellular cyclic AMP (Table V).

The most significant factor we have found to affect tubulin content is the method of cell growth. L cells grown adhering to plastic dishes had at least a fivefold increase in colchicine-binding activity compared to cells grown in spinner suspension culture (Table IV). That this difference was not due to differences between normal and spinner culture medium was demonstrated by growing L cells on plastic surfaces with spinner medium (by not stirring the cells) and noting the expected increase in colchicine binding. This suggests that the physical process of attachment to the substratum may induce the accumulation of tubulin. It has been suggested that neoplastic cells may grow invasively because of deficient attachment to the substratum (Vasiliev and Gelfand, 1973). The accumulation of tubulin is one of the effects of cell attachment to the substratum. Hence, a diminished level of tubulin would be expected in transformed cells, a finding reported above.

It is of interest that the relative amount of tubulin found in cells grown under different culture conditions correlates well with the relative amount of myosin found under the same conditions (Ostlund et al., 1974). Spinner-grown L cells have less myosin and tubulin than cells grown on plastic surfaces. Further viral transformation lowers the content of both myosin and tubulin in NRK cells. These findings suggest that a common regulatory mechanism may control the level of both tubulin and myosin in cultured cells.

Tubulin prepared from brain tissue contains phosphate covalently bound to serine (Eipper, 1972). A prominent action of cyclic AMP in fibroblastic cells is to stimulate the phosphorylation of proteins. Since cyclic AMP controls the shape and other properties of fibroblastic cells, it seems possible that it does so by promoting the phosphorylation of tubulin. Using the methods described in this paper it should be possible to examine this idea.

Acknowledgment

We thank Dr. M. Willingham for his assistance with the 3T3 cAMP^{tcs}-1 cells and Ms. B. Lovelace for her assistance with cell culture.

References

Benveniste, R. E., and Scolnick, E. M. (1973), *Virology 51,* 370.

Dubois, M., Gilles, K. A., Hamilton, J. K. Rebers, P. A., and Smith, F. (1956), *Anal. Chem. 28,* 350.

Eipper, B. A. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2283.

Eipper, B. A. (1974), *J. Biol. Chem. 249,* 1398.

Everhardt, L. V., Jr. (1969), *J. Mol. Biol. 61,* 745.

Feit, H., Slusarek, L., and Shelanski, M. L. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 2028.

Forrest, G. L., and Klevecz, R. R. (1972), *J. Cell Biol. 43,* 371.

Kirschner, M. W., Williams, R. C., Weingarten, M., and Gerhart, J. C. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1159.

Krishan, A., and Hsu, D. (1971), *J. Cell Biol. 48,* 407.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.

Maizel, J. V., Jr. (1969), in Fundamental Techniques in Virology, Habel, K., and Salzman, N. P., Ed., New York, N.Y., Academic Press, p 334.

Murray, A. W., and Froscio, M. (1971), *Biochem. Biophys. Res. Commun. 44,* 1089.

Ostlund, R. E., Pastan, I., and Adelstein, R. S. (1974), *J. Biol. Chem. 249,* 3903.

Owellen, R. J., Owens, A. H., and Donigan, D. W. (1972), *Biochem. Biophys. Res. Commun. 47,* 685.

Robbins, E., and Shelanski, M. (1969), *J. Cell Biol. 43,* 371.

Stadler, J., and Franke, W. W. (1974), *J. Cell Biol. 60,* 297.

Vasiliev, J. M., and Gelfand, I. M. (1973), in Locomotion of Tissue Culture Cells, Ciba Foundation Symposium 14, New York, N.Y., Associated Scientific Publishers, p 311.

Weisenberg, R. C. (1972), *J. Mol. Biol. 61,* 745.

Weisenberg, R. C., Borisy, G. G., and Taylor, E. W. (1968), *Biochemistry 7,* 4466.

Willingham, M. C., Carchman, R. A., and Pastan, I. H. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2906.

Wilson, L. (1970), *Biochemistry 9,* 4999.