Table 1. Vinyl proton chemical shifts (ppm).

Parent compound	Acetate derivative	Spectral shift
Δ^9-THC, 6.38	6.01	+0.37
Δ^8-THC, 5.44	5.44	0.00
1, 5.72	5.80	−0.08

group in compound **1**. This acylation shift is characteristic of a primary alcohol (*6*). The integral lends further support to this assignment. We therefore conclude that the additional hydroxyl group is attached at C-11 with β-unsaturation.

The only remaining question regarding the structure of the metabolite was whether the olefinic bond has remained in the Δ^8 position or migrated to the Δ^9 position. A comparison of the NMR spectra of Δ^8-THC, Δ^9-THC, the metabolite, and their acetate derivatives showed that the double bond had not migrated (Table 1).

The large downfield shift of the vinyl proton in Δ^9-THC is attributed to deshielding caused by the anisotropy of the aromatic ring (*7*). Formation of the acetate changes the anisotropy of the aromatic ring, and the position of the vinyl proton resonance shifts upfield 0.37 ppm (*8*). In Δ^8-THC these effects are absent because of the remoteness of the vinyl proton from the aromatic ring. The resonance of the vinyl proton of the metabolite (**1**) is only slightly downfield from that of the vinyl proton of Δ^8-THC, a shift consistent with substitution at the allylic carbon. In addition, the position of the resonance of the vinyl proton in the diacetate of compound **1** has shifted only slightly relative to the resonance of the corresponding proton in compound **1**. Furthermore, the shift is opposite in nature to that observed in the Δ^9-THC system. We conclude that the vinyl proton is not at C-10 and therefore must be at C-8.

Synthesis of compound **1** from Δ^8-THC was achieved by a two-step conversion. The *trans*-Δ^8-THC was oxidized with selenium dioxide in 95 percent aqueous ethanol. Efforts to purify and characterize the major component of the oxidation were unsuccessful. Therefore, the crude product from the reaction was reduced directly with sodium borohydride in ethanol. The retention time by gas-liquid chromatography of the trimethylsilyl derivative of the resulting major product is identical to that of the TMS-derivative of the metabolite (**1**). The major product was separated by column chromatography on alumina and further purified by preparative TLC. The R_F values of the synthetic and natural metabolite are identical in several TLC systems. The mass spectra of the natural metabolite and the synthetic product are identical, as are the mass spectra of the corresponding trimethylsilyl derivatives. The NMR and infrared spectra of the natural and synthetic materials are also essentially identical.

Psychopharmacological tests indicate that the metabolite (**1**) exhibits a behavioral profile in the rat very similar to that exhibited by the Δ^8- and Δ^9-tetrahydrocannabinols with the use of the tests described by Irwin (*9*).

Burstein, Mechoulam, and co-workers have recently reported the isolation of a similar, if not identical, metabolite from rabbits injected with Δ^8-THC (*10*). The diacetate derivative of compound **1** gives a mass spectrum which is consistent with the mass spectral data for the diacetate derivative of the rabbit metabolite. These authors suggested that the additional hydroxyl group is located at the C-11 position (*10*). However, they could not exclude the possibility of the hydroxyl being located at the C-7 position.

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On the Apparent Homology of Actin and Tubulin

Abstract. *Muscle actin and ciliary A-tubulin from* Pecten irradians *have molecular weights of 46,000 and 59,000, respectively, as measured by sodium lauryl sulfate–polyacrylamide-gel electrophoresis. Tryptic and chymotryptic peptides obtained from these proteins are very dissimilar, indicating that there is little significant homology.*

Muscle actin and the microtubule structural protein tubulin (*1*) are similar in amino acid composition and in the presence of a bound nucleotide (*2–4*). These factors, coupled with the fact that both proteins apparently interact in some manner with adenosine triphosphatase proteins of motile systems, suggest that there may be some common structural basis for these similarities.

At one time, actin and tubulin were each thought to have a molecular weight of 60,000, but data based on the 3-methylhistidine content of actin has shown that the molecular weight of this protein is about 48,000 (*5*). Tubulin is devoid of 3-methylhistidine, and molecular-weight determinations by hydrodynamic methods have consistently given values near 60,000 (*2*, *4*).

Although early reports indicated a total of 1 mole of bound guanosine diphosphate (GDP) or guanosine triphosphate (GTP) per 60,000 g of tubulin (*3*), it now appears that 1 mole of nucleotide per 120,000 g is tightly bound while a second mole is apparently exchangeable with the medium (*6*). Actin was originally reported to contain 1 mole of bound adenosine diphosphate (ADP) per 57,000 g of protein, but more recent data show 1 mole per 45,000 to 47,000 g (*7*), basically in accord with the molecular-weight data discussed above.

With respect to amino acid composition, it has been pointed out that *Pecten* actin and *Tetrahymena* tubulin were no more dissimilar than actins derived from different species (*2*). However, when the apparent homology was further investigated in the same species, it was found that tubulin from the outer fibers of gill cilia of the scallop *Pecten* resembled actin from the striated adductor muscle far less than did these proteins resemble their true homologs from unrelated species (*8*). Results of fractionation of outer fiber doublets into the closely related subfiber com-

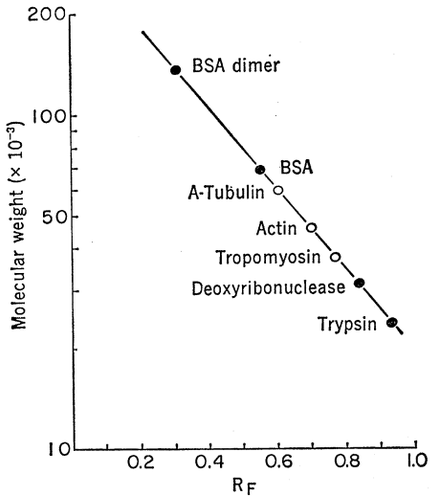

Fig. 1. Migration rate plotted against the log of molecular weight for various proteins on 5 percent polyacrylamide gels containing SLS. Solid circles are protein standards; open circles represent the resultant molecular weight at the observed migration rate for A-tubulin, actin, and tropomyosin from *Pecten*.

ponents, A- and B-tubulin, would in no way change this conclusion (*9*).

But the two proteins do resemble each other in composition far more closely than they resemble myosin, tropomyosin, or bacterial flagellin (*1*). Even though there are molecular-weight and compositional differences between actin and tubulin, these two proteins still may have some rather substantial segment of their polypeptide chains in common. Investigation of A- and B-tubulin through peptide mapping indicated that these proteins differ in only small regions of the polypeptide chain but that both differ markedly from actin (*9*); since the tubulin was derived from sea urchin flagella and the actin from molluscan muscle, no conclusion regarding local regions of similarity could be drawn.

To resolve any remaining issue of homology between tubulin and actin, I determined the minimum subunit molecular weight of these two proteins in the same species by a method not influenced by aggregation or nucleotide loss and compared tryptic or chymotryptic peptide maps of such actin and tubulin to detect homologous regions in their polypeptide chains.

Actin was prepared from the striated adductor muscle of *Pecten irradians* (*8*). A-tubulin was derived from thermal fractionation (*9*) of outer fiber doublet microtubules of *P. irradians* gill cilia (*8*). Electrophoretically, both proteins moved as single bands on urea-containing polyacrylamide gels, with actin showing only a minor tropomyosin band and A-tubulin showing some degree of higher aggregation. The contaminants in either case were no greater than 5 percent.

Molecular weights were determined at neutrality on 5 percent polyacrylamide gels containing 0.1 percent sodium lauryl sulfate (SLS) (*10*). The gels were calibrated with bovine serum albumin (BSA; molecular weight, 68,000 and 136,000), deoxyribonuclease I (molecular weight, 31,000), and trypsin (molecular weight, 23,500); bromphenol blue was used as tracking dye. The relative mobilities of these proteins, measured to the midpoint of each band, were inversely proportional to the log of their respective molecular weights.

Actin migrated at a rate corresponding to a molecular weight of 46,000 ± 3,000, while A-tubulin gave a value of 59,000 ± 4,000. The minor tropomyosin contaminant in actin moved at a rate corresponding to a molecular weight of 37,000 ± 3,000 (Fig. 1). Within experimental error, these molecular weights correlate with currently accepted molecular-weight values for actin (*5*, *7*), tubulin (*2*, *4*), and tropomyosin monomer (*11*), obtained through hydrodynamic or nucleotide-binding methods. Thus the molecular weights for actin and tubulin are about 46,000 and 60,000, respectively, regardless of the method of determination.

For comparative peptide mapping, actin and A-tubulin were reduced and alkylated (*12*), dialyzed in 0.1*M* ammonium carbonate, *p*H 8, and then digested for 24 hours at room temperature with either trypsin (treated with tosyl-L-phenylalanylchloromethane) or chymotrypsin in a protein to enzyme ratio of 100 : 1. The resulting tryptic or chymotryptic peptides were then chromatographed for 1 hour in an ascending direction with a solvent system of chloroform, methanol, and ammonium hydroxide (2 : 2 : 1, by volume) on thin-layer silica gel G plates. The chromatogram was dried in air and then subjected to electrophoresis for 45 minutes at 1000 volts, with a pyridine, acetic acid, and water (1 : 10 : 489, by volume) buffer (*13*). The plates were then dried at 100°C and developed with ninhydrin.

Composite peptide maps for tryptic and chymotryptic digests of actin and tubulin from *Pecten*, representing spots common to quadruplicate determinations, are illustrated in Fig. 2. As might be expected from size differences and content of lysine and arginine, A-tubulin (Fig. 2B) yields significantly more tryptic peptides than actin (Fig. 2A). With the exception of some small basic polar peptides to the extreme right of the maps (common to tryptic digests of most proteins) and the two pairs of acidic polar peptides directly above the origins, it would be difficult to demonstrate any appreciable degree of similarity between peptides of these two proteins.

The chymotryptic maps (see Fig. 2, C and D) are somewhat more com-

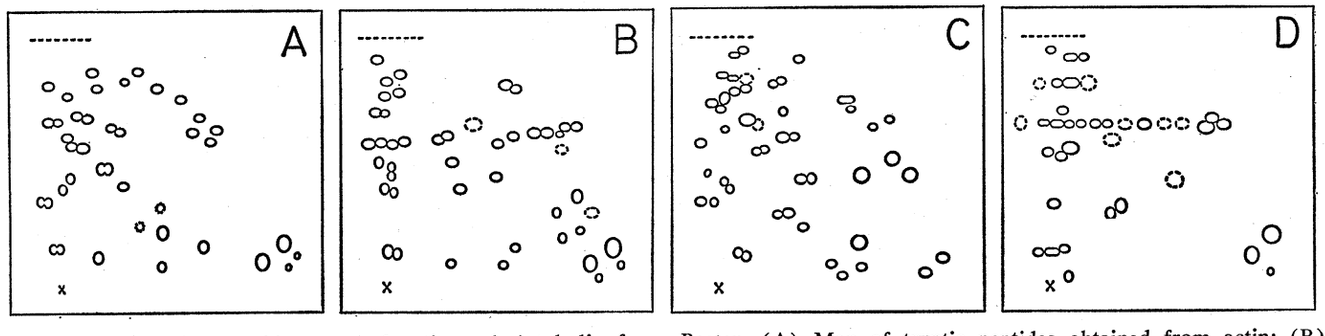

Fig. 2. Comparative peptide maps of actin and A-tubulin from *Pecten*. (A) Map of tryptic peptides obtained from actin; (B) map of tryptic peptides from A-tubulin; (C) map of chymotryptic peptides obtained from actin; and (D) map of chymotryptic peptides from A-tubulin. Chromatography was carried out in the ascending direction; X is the origin and the dotted line is the solvent front. Electrophoresis was performed with the cathode on the right.

plex because of the lesser specificity of chymotrypsin, but here also, with few exceptions, the two peptide maps are clearly dissimilar. Admittedly, in both pairs of maps the complexity is such that it would be difficult to claim noncoincidence for some of the spots, purely because there are so many of them. Therefore, the data presented here cannot completely eliminate the possibility of very restricted local homology at some points within the polypeptide chains (perhaps at the nucleotide-binding site), but the overall makeup of the two polypeptide chains is clearly different.

Thus actin and tubulin from *Pecten irradians* striated muscle and gill cilia microtubule doublets, respectively, have significantly different molecular weights that agree with values for their homologs from other species. Comparative peptide mapping, with tryptic or chymotryptic digests, shows that most of the resulting peptides from the two proteins are dissimilar. Association or interaction with an adenosine triphosphatase and the binding of molar ratios of nucleotides are the only remaining similarities between actin and tubulin. In terms of comparative mechanochemistry, a common ground for muscle contraction and ciliary beat must now be sought at the enzymatic level. In this regard, the microtubule protein of brain tissue, in a manner quite parallel to actin, significantly increases the magnesium-activated adenosine triphosphatase of myosin; and the protein complex thus formed undergoes characteristic viscosity drops on addition of ATP (*14*). It remains to be demonstrated, however, that these properties are held by other tubulin fractions of more certain purity.

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Ultrastructure of Secretory and High-Polymer Serum Immunoglobulin A of Human and Rabbit Origin

Abstract. *Electron micrographs of immunoglobulins A from human and rabbit colostrum, which were purified on tall agarose columns, revealed Y-shaped molecules (125 by 140 angstroms). The linear dimensions of the arms were 55 to 75 by 25 to 30 angstroms. A molecular model is postulated in which two immunoglobulin A monomers are superimposed on each other in a close-packed state with the secretory piece inserted in the constant region of the α-chains. High-polymer (11 or 13S) immunoglobulin A molecules (total span 100 to 110 angstroms) from human serum were composed of four arms (50 to 55 by 20 angstroms) joined at a contrast-rich center.*

Immunoglobulin A (IgA) is the predominant globulin in the secretions on mucous membranes. Secretory IgA (s-IgA) has a higher sedimentation coefficient (approximately 11*S*) than the major serum IgA component (*1*) and possesses a unique protein, referred to as the secretory piece (SP) (*1*) or transport piece (*2, 3*). This protein has been demonstrated in free form in secretions of agammaglobulinemic patients (*3*) or individuals lacking IgA (*4*). Cebra and Small (*5*) concluded that the rabbit s-IgA molecule was composed of four heavy and four light peptide chains plus one or two so-called T chains which make up the SP. In studies of human s-IgA Tomasi and Czerwinski (*6*) and Newcomb *et al.* (*7*) reached the same conclusions.

Essentially no information concerning the quaternary structure of s-IgA or high-polymer serum IgA is available. There are basically three different approaches applicable to analyses of the overall layout of protein molecules and the spatial arrangement of their subunits—hydrodynamic studies, x-ray diffraction studies, and electron microscopy. We now report on comparative studies of the size and conformation of colostrum IgA and high-polymer, serum IgA molecules by the use of electron microscopy.

Nine human and five rabbit s-IgA preparations were partially purified by centrifugation and gel filtration (*8*). The first eluted peak on Bio-Gel A-15m (Fig. 1a) contained chylomicrons by lipid staining and phase contrast microscopy; IgA was distributed throughout the second major elution peak. The ascending part of this peak contained also immunoglobulin M (IgM) and the descending part contained two, occasionally three, unidentified colostrum proteins. These contaminating proteins were detected by immunodiffusion only in fractions concentrated eight to ten times.

Only s-IgA was demonstrable within a restricted central area of the peak. Three human s-IgA preparations (3 to 5 mg of protein per milliliter) were subjected to analytical ultracentrifugation. The $s_{20,w}$ values were 11.6, 11.4, and 10.7.

A relatively weak poliovirus-neutralizing antibody activity, which coincided with the distribution of s-IgA in the A-15m fractions, was noted when IgA from human colostrum was assayed (Fig. 1a). No IgM or IgG was detected by immunodiffusion in the fractions having most antibody activity, and absorption with antiserum to IgM caused no reduction in activity.

Immunodiffusion and immunoelectrophoretic analyses of concentrated fractions from the central part of the major elution peak of A-15m curves indicated the presence of complexes of s-IgA and albumin in three preparations of human whey (Fig. 1a). Since s-IgA is rather resistant to reduction (*1, 9*), these complexes could be dissociated by mild reduction (0.1*M* 2-mercaptoethanol) and alkylation of the whey prior to gel filtration. In addition, this treatment eliminated the IgM contamination in the major s-IgA peak, the IgM subunits being recovered in the IgG elution region (Fig. 1b).

High-polymer IgA from human serum was partly purified by a combi-