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26. Supported by NIH grant R10 CA-02332, and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation. I.B.W. is a Career Scientist of the Health Research Council of the City of New York (I-190). We thank Dr. J. F. Weiss for the *E. coli* $tRNA^{Val}$ and Miss G. Carvajal for technical assistance.

25 August 1969

Microtubular Protein: Synthesis and Metabolism in Developing Brain

Abstract. *In the developing mouse brain 40 percent of the labeled soluble protein found after injection of leucine-C^{14} consists of subunits with a molecular weight of 60,000 and with other characteristics of microtubular protein. This protein has a half-life of about 4 days.*

Microtubular protein binds colchicine (*1, 2*), is composed of subunits with a molecular weight of 60,000 (*3*), and is precipitable by vinblastine (*4, 5*). Brain and axoplasm contain significant amounts of this protein (*1, 3*), and there is evidence that microtubules participate in the rapid transport of proteins from the nerve cell body down the axon (*6*).

Because of the abundance of this protein in brain and its probable importance in axoplasmic transport (*7*), we have studied its synthesis and metabolism in the brains of developing and adult mice.

Swiss albino mice, 1 or more days of age, were injected intracerebrally with 2 μc of L-leucine-1-C^{14} [30 mc/mmole, New England Nuclear (*8*)]. After injection, the mice were decapitated, and the brains were removed and homogenized in 0.01*M* phosphate buffer, *p*H 6.5, containing 0.01*M* $MgCl_2$ and 0.24*M* sucrose. Soluble and particulate components were separated by centrifugation for 1 hour at 100,000*g*.

For determination of colchicine binding activity (*3*), 1 to 2 mg of protein were incubated with 1 μc of colchicine-H^3 (2 c/mmole, New England Nuclear) and the mixture was filtered through DEAE-cellulose–impregnated filter paper (No. DE81, Whatman). For estimation of the molecular weight, the proteins were disaggregated in 0.01*M* phosphate buffer (*p*H 6) containing 0.1 percent sodium dodecyl sulfate and 0.5*M* urea, reduced with 0.15*M* β-mercaptoethanol, alkylated with 0.02*M* iodacetamide, concentrated, and subjected to electrophoresis in 0.01*M* phosphate buffer, 0.1 percent sodium dodecyl sulfate (*p*H 6) (*9, 10*). Migration in this system is proportional to molecular weight (*11*).

Results of the above-mentioned procedures were as follows. Approximately 40 percent of the labeled protein in the 100,000*g* supernatant from homogenized brains of 2- or 6-day-old mice which had received the intracerebral injection of leucine-1-C^{14} the day before was present as a single peak with a molecular weight of approximately 60,000 (Fig. 1). One milligram of the supernatant protein bound about 72,000 counts of colchicine-H^3 per minute. Upon the addition of vinblastine ($10^{-3}M$) to the supernatant a visible precipitate formed (*4*). This precipitate contained approximately 35 percent of the total soluble protein and 98 percent of the colchicine bound to protein. Electron micrographs of negatively stained preparations of the precipitate showed the characteristic morphology of microtubular crystals (*5, 12*). Gel electrophoresis showed that the precipitate contained the protein peak with molecular weight of 60,000, whereas most of the other labeled protein remained in the supernatant after addition of vinblastine (Fig. 1). After electrophoresis of the protein precipitated with vinblastine, addition of Coomassie blue revealed a deeply stained band of molecular weight 60,000; also, three faint, closely spaced, narrow bands of very high molecular weight were detected; these were not substantially labeled with leucine-C^{14}. When the initial vinblastine precipitate was dissolved by dialysis against phosphate buffer and precipitated again with vinblastine and magnesium, these contaminants were no longer detectable. The purified precipitate contained about 20 percent of the protein in the initial 100,000*g* supernatant and about 30 percent of the total incorporated leucine-C^{14}.

The microtubular protein represented 30 to 40 percent of the labeled soluble protein in mice injected with

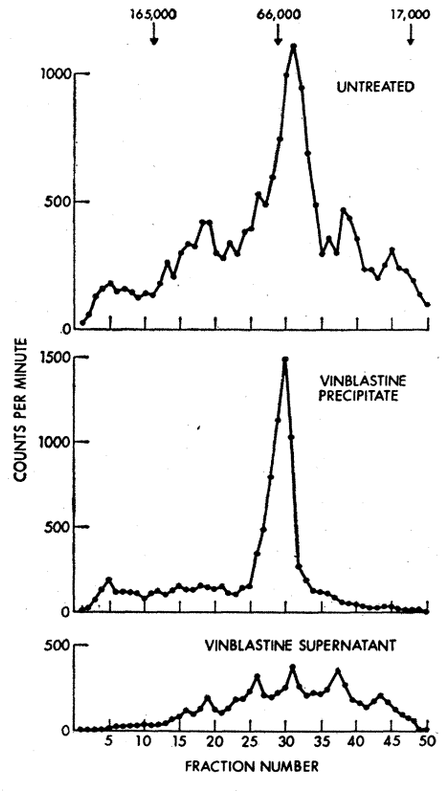

Fig. 1. Gel electrophoresis of protein in supernatant (100,000*g*) obtained from brain homogenates 24 hours after injection of leucine-C^{14}. Electrophoresis was conducted for 9 hours at 80 volts on polyacrylamide gels. The untreated supernatant (top), the protein precipitated by vinblastine (middle), and supernatant protein remaining after vinblastine precipitation (bottom) were subjected to electrophoresis simultaneously. Markers for the molecular weight estimate were a mixture of nonreduced human γ-globulin (165,000), bovine serum albumin (66,000), and hemoglobin monomers (17,000). These were subjected to electrophoresis simultaneously in a separate gel and localized by staining with Coomassie blue.

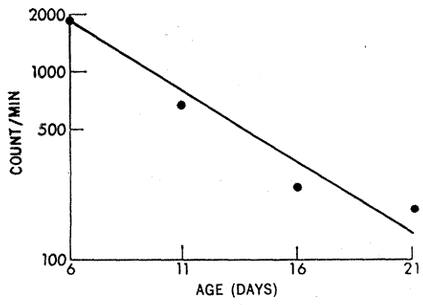

Fig. 2. Turnover of microtubule protein. Mice (5 days old) were injected intracerebrally with 2 μc of leucine-C^{14}; three were killed at each age indicated. The supernatant (100,000*g*) of the brain homogenate was isolated, 0.2 mg of the supernatant was subjected to electrophoresis, and the radioactivity (count/min) in the fractions with a molecular weight of 60,000 was determined. Since the protein content of brain increased during the period studied, corrections were made for dilution of the labeled protein, and these corrected results are shown on the ordinate.

leucine-C^{14} at 1 day, 3 days, 5 days, or 7 days after birth, and killed 1 day after injection. A peak of radioactive protein of identical molecular weight was detected in the particulate component of brain homogenates at 24 hours and at longer times after the administration of labeled amino acid. This is consistent with the finding that a substantial portion of the colchicine-binding activity in brain is in the particulate fraction (*13*). The turnover of the microtubular protein was estimated in mice injected with leucine-C^{14} at 5 days of age (Fig. 2). The half-life was approximately 4 days. In adult mice, the labeled protein with a molecular weight of 60,000 was also found 1 day after intracerebral injection of leucine-C^{14}. This peak represented only about 15 to 20 percent of the total labeled soluble protein. Other studies with embryonic axolotl brain grown in vitro and incubated for 3 hours with leucine-C^{14} upon electrophoresis of whole tissue homogenates (*14*) showed a highly labeled peak containing material with a molecular-weight of 60,000.

Because of its abundance, rapid rate of synthesis and turnover, characteristic molecular weight, colchicine-binding activity, and vinblastine precipitability, this brain protein should prove amenable to correlative studies of its metabolism and its role in brain function.

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15. Supported by grants MH-12773, K3-MH-18232, MH16816, and 5TI-MH6418 from the PHS.

10 July 1969; revised 18 August 1969

Self-Assembly of Qβ and MS2 Phage Particles: Possible Function of Initiation Complexes

Abstract. *Four kinds of particles were reconstituted with RNA and protein from the genetically unrelated bacteriophages Qβ and MS2, namely, two homologous and two heterologous, with respect to RNA and protein. However, once Qβ RNA (or MS2 RNA) reacted with a few molecules of either Qβ or MS2 protein to form a nucleoprotein complex (initiation complex), it formed a phagelike particle only with subsequent addition of the same protein.*

The small spherical RNA bacteriophages and plant viruses have been useful model systems to verify Crick and Watson's (*1*) suggestion that such small viruses would possess cubic symmetry with the shape specifications residing primarily in a single protein subunit. Self-assembly into virus-like ribonucleoprotein particles is possible in vitro (*2*). It does not appear that there is a great specificity as to the nucleic acid encapsulated in vitro. Within the cell, however, viral nucleic acid and viral protein interact with high specificity and efficiently produce infectious particles. Cellular assembly mechanisms may not involve free interaction between pools of nucleic acid and protein as in the case in vitro; alternatively there could be a highly controlled assembly coupled to mechanisms of synthesis of the component parts. We have sought experimental support for the latter possibility and have separated the process of encapsulation in vitro into an RNA-protein "initiation" stage, followed by a cooperative interaction between the coat-protein subunits. The "initiation complex," which is composed of an RNA strand and a few molecules of coat protein, provides specificity in particle formation. Similar complexes could be natural products of a coupled RNA transcription-translation system and thereby ensure efficiency and specificity in formation of mature infectious particles.

Free interaction of the four possible combinations of RNA and protein from the physically similar but genetically unrelated coliphages Qβ and MS2 (*3*) gave the expected two homologous and two heterologous phagelike particles. Electron micrographs of the phagelike particles (Fig. 1) show that both the homologous and heterologous species appeared isometric, and approximately the same dimension as authentic phages. Large and distorted particles were more frequent in reconstituted particles. In gel electrophoresis (100 minutes; 4 ma) homologous reconstituted particles migrated as the respective authentic phage (41 mm for MS2; 19 mm for Qβ), and the heterologous particles migrated as the phage from which the coat protein was derived.

In studies on repression of translation of Qβ and MS2 viral RNA it has been reported that a few molecules of phage protein can form complexes with homologous RNA but not with heterologous RNA (*4*). Contrary to these findings, we observed complex formations between homologous as well as heterologous RNA and protein. When radioactive Qβ protein was allowed to react with Qβ or MS2 RNA, complexes were readily formed, as revealed by sucrose density-gradient centrifugation. The results of a typical reaction when