gangliosides, one other point needs mentioning. In his work on the identification of the gangliosides as the substances in brains which fixed tetanus toxin, Van Heyningen[19] showed that purified gangliosides *in vitro* would in fact bind serotonin in aqueous solution. Such binding of the hormone in a reversible fashion with its tissue receptor is one of the requisites for a hormonal receptor.

Summary.—A series of gangliosides and related sphingolipids have been assayed for serotonin receptor activity by two different methods of bioassay. The most active compound was a new ganglioside called new G. Very much less activity was found in three other known gangliosides, but no activity was present in such common gangliosides as G_1, G_4, and the Tay-Sachs' ganglioside. These results indicated that the serotonin receptors are definite and specific chemical substances, possibly one or more gangliosides such as new G.

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*IN VITRO SYNTHESIS OF BRAIN PROTEIN**

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Neurons are active protein-synthesizing cells as indicated by their highly developed, ribosomal-rich endoplasmic reticulum and by results of metabolic studies on whole brain and brain slices. Cell-free systems derived from brain tissue are also active in protein synthesis.[1–6] These systems provide an *in vitro*

model for the study of control mechanisms and of products of neuronal polypeptide synthesis.

The present communication describes the characteristics of an active, reproducible brain cell-free system, and the nature of its soluble protein product.

Materials and Methods.—New Zealand white female rabbits were sacrificed by an air embolus; their brains were removed and immediately chilled in iced 0.14 *M* NaCl with 5×10^{-3} *M* $MgCl_2$ and 10^{-2} *M* Tris pH 7.4. Pia mater, blood vessels, and white matter were discarded and the remaining tissue, consisting mainly of cortical grey matter, was minced and homogenized in 4 volumes 5×10^{-3} *M* $MgCl_2$, containing 4 mg/ml bentonite, in a glass Dounce tissue homogenizer. One volume 1.5 *M* sucrose, containing 0.15 *M* KCl,[7] was then added, and the homogenate centrifuged at 2500 rpm for 10 min to remove nuclei, unbroken cells, and most of the myelin. The supernatant fluid was then centrifuged at 15,000 *g* for 10 min to remove mitochondria and nerve endings.[8] The remaining suspended material (postmitochondrial fraction) was used for the preparation of microsomes, ribosomes, and a pH 5 fraction. Microsomes were sedimented by centrifugation at 78,000 *g* for 90 min. Ribosomes were prepared by slowly adding 10% deoxycholate in 0.25 *M* sucrose to postmitochondrial fractions to a final concentration of 0.5%. They were then collected by centrifugation at 100,000 *g* for 60 min.

Ribosomal pellets were rinsed four times with 2 ml 0.25 *M* sucrose to remove deoxycholate. Microsomal and ribosomal pellets were gently homogenized in 0.25 *M* sucrose, centrifuged at 15,000 *g* for 10 min, and the material remaining suspended was frozen at −30°C. The pH 5 fractions were prepared by addition of 1 *M* acetic acid to postmicrosomal supernatant fluid to a final pH of 5.0–5.2. Precipitates were collected by centrifugation at 15,000 *g* for 40 min, dissolved in 0.1 *M* Tris pH 7.4, and frozen at −30°C. Cell fractions were used within 24 hr of preparation.

The complete system for amino acid incorporation contained in 0.7 ml, 25 μmoles Tris pH 7.4, 25 μmoles KCl, 5 μmoles $MgCl_2$, 10 μmoles glutathione, 0.5 μmoles ATP, 0.125 μmoles GTP, 25. μmoles phosphoenol pyruvate, 0.03 mg pyruvate kinase. an amino acid mixture without leucine, 5–10 μc C^{14} leucine (233 mc/mM), ribosomes or microsomes, and pH 5 protein in a ratio of 2:1 to ribosomes or microsomes. After various periods of incubation at 37°C, aliquots were removed, precipitated with 5% trichloracetic acid–2.5% tungstate (TCA-Tung),[9] washed 3 times with TCA-Tung, dissolved in 0.5 ml 1 *N* NaOH, reprecipitated with TCA-Tung, heated at 90°C for 30 min, and washed once more. Precipitates were layered on 0.45-μMillipore filters, and radioactivity was measured in a Nuclear-Chicago gas flow counter with a Micromil window with an average counting efficiency of 25%. For analytical studies, incubation mixtures were rapidly cooled in an ice bath, centrifuged at 110,000 *g* for 60 min, and the soluble fractions filtered through 1 × 30-cm columns of G 50 Sephadex (fine). Free diffusion electrophoresis was performed in a Hannig apparatus,[10] and chromatography was done on 2 gm DEAE cellulose with an exchange capacity of 0.68 meq/gm. Optical density of column effluents was monitored at 280 mμ in a Gilford recording spectrophotometer; samples were removed, placed in Bray's counting fluid,[11] and radioactivity was measured in a Packard Tri-Carb liquid scintillation counter.

Immune coprecipitation was done by incubating labeled material with known antigens and their antibodies in antibody excess at 37°C for 1 hr, keeping mixtures at 0–4° for 72–96 hr, and finally washing immune precipitates four times in ice-cold 0.85% NaCl. Precipitates were layered on Millipore filters, and radioactivity was determined as for TCA-Tung precipitates. Nonspecific antigens included ribonuclease and egg albumin. The specific antigen was an acidic protein, isolated from brain by B. W. Moore.[12, 13]

Results.—Characteristics of brain cell-free system: Incorporation of C^{14} leucine into TCA-Tung-precipitable material was highly reproducible. When complete reaction mixtures were incubated for 60 min at 37°C, 800–1000 μμmoles C^{14} leucine were incorporated per mg microsomal or ribosomal RNA. The system was active for 30 min, after which the rate of leucine incorporation was decreased. ATP and pH 5 enzymes were required for incorporation, and ribonuclease and puromycin were inhibitory (Table 1). When microsomal incubation mixtures were centrifuged at 110,000 *g* for 60 min, 10 per cent of the TCA-Tung-precipitable counts were in the soluble fraction. An additional 5 per cent were released from the particulate

TABLE 1

CHARACTERISTICS OF BRAIN CELL-FREE SYSTEM

	% Incorporation	
	Microsomal	Ribosomal
Complete (60 min)	100.0	100.0
0 time	0.5	0.3
Minus ribosomes or microsomes	1.6	1.1
Minus pH 5 fraction	47.5	3.2
Minus ATP, GTP, PEP, PK	3.2	1.8
Minus amino acids	95.0	53.5
Puromycin (50 μg)	9.6	12.7
RNase (1 μg)	3.6	1.7

TCA-Tung-precipitable counts in brain cell-free systems containing either microsomes or ribosomes, expressed as per cent incorporation of the complete system. See *Methods* for details.

fraction by 0.5 per cent deoxycholate. In ribosomal incorporation mixtures, 15 per cent of the incorporated counts were found in the soluble fraction.

Nature of Product.—To characterize the soluble protein product of the brain cell-free system, incubation mixtures were centrifuged at 110,000 *g* for 1 hr and the supernatant fluid passed through G 50 Sephadex. The external volume contained TCA-Tung-precipitable counts with less than 1 per cent free C^{14} leucine. This material was then used for electrophoresis, chromatography on DEAE cellulose, and immune coprecipitation. The bulk of TCA-Tung-precipitable material had about the same electrophoretic mobility as albumin (isoelectric point 4.9) when separated in a free-flow electrophoresis apparatus (Fig. 1). Chromatography on DEAE cellulose revealed that the radioactive material was strongly absorbed, and elution was only accomplished after washing the column with 0.1 *N* NaOH (Fig. 2). The characteristics of low isoelectric point and strong affinity for DEAE cellulose are similar to those of the protein isolated from brain by B. W. Moore.[11] This protein was found by Moore to be characteristic of brain tissue and absent in other organs. Antibody to the protein shows cross reaction with similar material isolated from several species.[14] In order to see if this unique protein accounted for part of the product of brain cell-free synthesis, immune coprecipitation was done by using

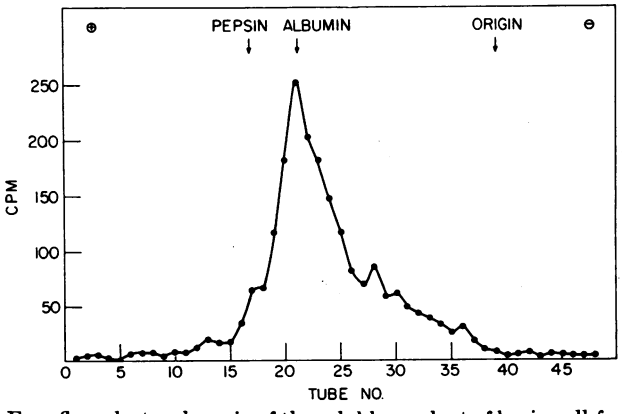

FIG. 1.—Free-flow electrophoresis of the soluble product of brain cell-free synthesis at 1580 volts, 95 ma, in 0.08 *M* Tris pH 8.7, with 0.12% EDTA. Samples were precipitated with TCA-Tung, layered on Millipore filters, and radioactivity was determined in a Nuclear-Chicago gas flow counter with a Micromil window. Arrows indicate the mobility of albumin and pepsin under similar conditions.

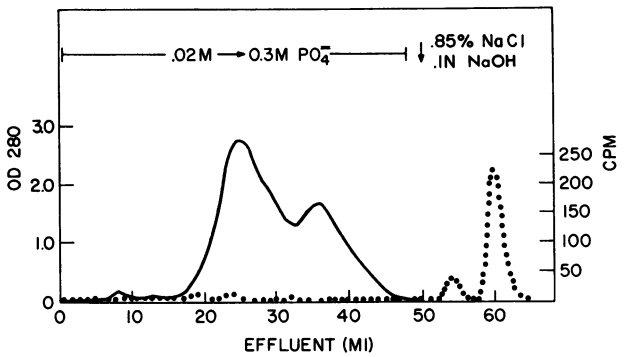

FIG. 2.—DEAE cellulose chromatography of the soluble product of brain cell-free synthesis. Optical density at 280 mμ was monitored continuously, and radioactivity of 1-ml samples determined in a liquid scintillation counter; (——), optical density at 280 mμ, (. . .), cpm.

Moore's protein and its antibody. Mixtures containing cell-free synthesized material, Moore's protein, and its antibody were incubated at 37°C for 1 hr, and kept at 0–4°C for 3–4 days. Control systems contained other antigens and antibodies in quantities that would yield more antigen-antibody precipitate nitrogen than would the brain antibrain protein mixtures. Protein synthesized in rabbit reticulocyte cell-free systems[7] and prepared in the same way as brain cell-free soluble material was also incubated with Moore's protein and its antibody. The number of counts remaining in the washed immune precipitates was compared with the total number of counts precipitable in 5 per cent TCA. Results, summarized in Table 2,

TABLE 2

IMMUNE COPRECIPITATION STUDIES ON THE SOLUBLE PRODUCT OF BRAIN CELL-FREE SYNTHESIS

	Cpm	TCA-precipitable counts, %
Ribosomal system (110,000 *g* supernate, Sephadex G 50 external volume)		
Brain—antibrain	480	15.4
RNase—anti-RNase	122	3.9
Egg albumin—antiegg albumin	66	2.1
TCA-precipitable	3120	100.0
Microsomal system (110,000 *g* supernate, Sephadex G 50 external volume)		
Brain—antibrain	197	12.7
RNase—anti-RNase	72	4.7
Egg albumin—antiegg albumin	46	3.0
TCA-precipitable	1545	100.0
Reticulocyte cell-free system (110,000 *g* supernate, Sephadex G 50 external volume)		
Brain—antibrain	44	7.1
TCA-precipitable	620	100.0

Equal amounts of soluble material synthesized in brain ribosomal, microsomal, or reticulocyte cell-free systems and separated by passage through Sephadex G 50 were precipitated either with 5% TCA or with a specific antigen and its antibody. Results are expressed both as counts per minute and as per cent of total TCA-precipitable counts present in the incubation mixtures. Total nitrogens of antigen-antibody precipitates were 75.9 μg for brain, 459.4 μg for RNA, and 134.0 μg for egg systems.

indicate that 10–15 per cent of the soluble protein formed was specifically precipitable by antibody directed against Moore's brain protein. When similar experiments were done on crude incubation mixtures, it was found that up to 21 per cent of the product was coprecipitable with the brain antibrain system (Table 3).

Discussion.—Recent studies in several laboratories indicate the importance of protein in the activity of the nervous system. An acidic protein, which may be important in nerve impulse propagation, has been isolated from squid axoplasm.[15] Proteases, when applied intracellularly to squid giant axons, cause reduction of resting potential and loss of excitability.[16, 17] Antibodies, produced in rabbits to specific areas of cat brain, cause electrical and behavioral changes when delivered to specific target areas.[18] Growth and development of nerve tissue are markedly affected, both *in vivo* and *in vitro*, by a protein isolated from either snake venom or mouse submaxillary glands.[19] This nerve growth factor promotes the growth of certain nerve cells, and may also be important in their maintenance.[20] Antibodies to nerve growth factor cause atrophy and destruction of sympathetic nerve cells.[21] The acidic protein found by Moore in brain, but not in other organs, may have a specific role in neuronal or glial functions. Recent electron microscopic observations of an anatomic relationship between ribosomal-rich endoplasmic reticulum and overlying synaptic buttons suggest that synaptic function may be associated with

TABLE 3

IMMUNE COPRECIPITATION STUDIES ON COMPLETE INCUBATION MIXTURES

	Cpm	TCA precipitable counts, %
Microsomal system (complete)		
Brain—antibrain	380	21.0
RNase—anti-RNase	165	9.2
Egg albumin—antiegg albumin	136	7.6
TCA-precipitable	1800	100.0
Ribosomal system (complete)		
Brain—antibrain	670	14.5
RNase—anti-RNase	460	9.9
Egg albumin—antiegg albumin	247	5.3
TCA-precipitable	4650	100.0

active polypeptide synthesis.[22] It has also been shown that there is a constant flow of axoplasmic protein from neuronal cell bodies.[23] This material, requiring constant synthesis, could be a major product of neuronal metabolism.[24] Schmitt has suggested that protein may function in the central nervous system as macromolecular recognition sites at synapses in a manner analogous to the specific recognition of an antigen by its antibody.[25]

Our findings indicate that cell-free systems derived from brain are among the most active in protein synthesis. A significant soluble product of this active synthetic machinery appears to be a protein unique to brain. It is characterized by a low isoelectric point, a high affinity for DEAE cellulose, and an immunologic reactivity with the antibody to a specific brain protein that has similar physical properties. These findings imply that one of the major metabolic functions of brain cells is to synthesize a specific type of protein. The active synthesis of this material further implies that it has a dynamic function in the highly specialized properties of nervous tissue.

It is hoped that *in vitro* models such as the brain cell-free system will provide the means for further correlations to be made between subcellular activities, cell metabolism, nerve impulse propagation, and synaptic function.

Summary.—Reproducible cell-free systems, active in protein synthesis, have been prepared from rabbit brain. A soluble protein product of these systems has been

characterized; it exhibits a low isoelectric point, a high affinity for DEAE cellulose, and reacts serologically with antibody to a specific brain protein.

We are grateful to Dr. B. W. Moore of Washington University for furnishing us with the brain protein and to Dr. L. Levine of Brandeis University for providing the antibody and for his advice on immune coprecipitation techniques. We especially thank Prof. Francis O. Schmitt and Dr. Peter F. Davison of the Department of Biology at the Massachusetts Institute of Technology, whose ideas are an integral part of this work. The expert assistance of Mrs. Ruth Aronson and Miss Naomi Schechter was instrumental in carrying out these studies.

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