data do not bear on the question of whether the observed heterogeneity (whatever its chemical basis) is developed at the time of synthesis, or whether this is a "postsynthesis" phenomenon similar to that described for mouse myeloma protein (*15*).

WILLIAM D. TERRY
Immunology Branch, National Cancer Institute, Bethesda, Maryland
PARKER A. SMALL, JR.*
Laboratory of Neurochemistry, National Institute of Mental Health
RALPH A. REISFELD
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases

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9. Sa is a type K γ_{2a}-protein contaminated with less than 5 percent of other immunoglobulins containing λ light chains and heavy chains other than γ_{2a}, when tested by immunodiffusion against appropriate antiserums at 200 mg/ml; Sp is a type K γ_{2b}-protein with less than 1 percent contamination when tested at 110 mg/ml; Ta is a type L γ_{2c}-protein with 5 to 10 percent contamination when tested at 60 mg/ml; Me is a type L γ_{2d}-protein without contamination when tested at 40 mg/ml. No serum proteins other than IgG were detected in any of these preparations. For example, contamination in protein Sp was estimated as follows; Sp is a type K γ_{2b}-protein, and when tested by Ouchterlony analysis at 110 mg/ml showed either no precipitation band or a very faint band with antiserum to λ-chain. This antiserum detects λ-type IgG at concentrations as low as 0.05 to 0.1 mg/ml. On the basis of the known 2:1 ratio of κ:λ, it is assumed that the λ-type contamination represents one-third of the total contamination. Therefore Sp is considered to contain about 0.3 mg of contaminating IgG in 110 mg, which is less than 1 percent. Calculations based on tests with antiserum to heavy chains yield similar estimates of contamination.
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14. Size heterogeneity would have been indicated by (i) curvature of the line when the natural logarithm of concentration was plotted against the square of the radius, and (ii) by Z-average molecular weights significantly higher than the weight-average molecular weights. For the light chain of protein Sa, averages of three determinations at 0.1, 0.2, and 0.3 mg/ml were: weight-average molecular weight 23,000 ± 800; Z-average of 20,500 ± 1,100. The averages of three determinations at 0.3, 0.6, and 0.9 mg/ml for the heavy chains were: weight-average molecular weight 49,600 ± 2,400; Z-average of 50,300 ± 1,900. These light- and heavy-chain weight averages do not differ significantly from their Z-averages. Results were based on an assumed partial specific volume of 0.71 and 0.72 respectively in a solvent of density 1.1236.
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* Present address: Department of Microbiology, University of Florida, College of Medicine, Gainesville.

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Tritiated Norepinephrine: Release from Brain Slices by Electrical Stimulation

Abstract. *Slices of rat brain and heart that had concentrated H^3-norepinephrine were superfused and electrically stimulated. Stimulation induced a marked release of H^3-norepinephrine with a threshold and a maximum repsonse. Release also occurred with increased concentrations of potassium, presumably due to neuronal depolarization. Inhibition of electrically induced release occurred with low calcium and with chlorpromazine and pentobarbital.*

Norepinephrine has been thought to be a neurotransmitter in the central nervous system, although the evidence is incomplete. Endogenous concentration and regional distribution of norepinephrine have been determined in brain (*1*). The blood-brain barrier prevents entry of administered norepinephrine into the brain from the circulation. Tritiated norepinephrine introduced into the lateral ventricle of rat brain appears to mix with endogenous norepinephrine, since its regional and subcellular distributions parallel those of the endogenous amine (*2*).

In oxygenated physiologic media, slices of tissues rich in adrenergic nerve endings concentrate H^3-norepinephrine (*3*). Norepinephrine taken up by brain slices is concentrated at nerve endings in regions rich in endogenous catecholamine, and the tissue appears to maintain histologic integrity after incubation (*4*). In addition, neurons in such preparations of brain slices maintain their resting membrane potentials and their ability to form action potentials during 2 to 3 hours of incubation (*5, 6*). Brain slices stimulated with alternating current at 1 to 10 volts have a markedly increased respiratory rate (*7, 8*). Such stimuli depolarize neurons in brain slices (*5*) and are known to elicit physiologic responses when applied to the mammalian cortex (*7*).

We used slices of rat heart and rat brain to study the effect of electrical stimulation on release of norepinephrine. Slices of heart and mid-coronal sections of brain (100 to 200 mg) were incubated for 30 minutes at 37°C in Ringer solution supplemented with tricarboxylic cycle intermediates (*3*) containing H^3-norepinephrine (25 ng and 1.25 μc per milliliter). The medium was agitated and saturated with 5 percent carbon dioxide in oxygen. After incubation, concentration of tritium in brain slices was 18 times the concentration in incubation medium; in heart slices, 6 times.

Slices were transferred to a 5-ml organ bath and bathed at 37°C with a continuous flow (2.5 ml/min) of oxygenated Krebs-Ringer medium. Serial fractions of effluent medium were collected during 2-minute intervals and assayed for total radioactivity and for H^3-norepinephrine and its metabolites (*9*). Unaltered H^3-norepinephrine accounted for about 80 percent of total radioactivity in effluent solutions; only traces of methylated catecholamine (normetanephrine) were found, and oxidized metabolites of norepinephrine accounted for most of the remaining radioactivity.

The rate of washout of H^3-norepinephrine from brain slices followed a multiphase course and was greatest during the first 10 to 15 minutes. Electrical stimuli of the order of 5 volts for 60 seconds, when applied about 20 minutes after the start of superfusion, produced striking and rapid increases in efflux of H^3-norepinephrine, with a return to base line within a few minutes (Fig. 1). Heart slices behaved similarly. At the end of these experiments, the slices had lost only a small fraction of their radioactivity.

Stimuli of 0.5 volt applied for 30 seconds did not appear to release tritium; from 1 to 4 volts, there appeared to be a direct relation between release of tritium and applied voltage. Stimuli above 4 volts, applied for 30

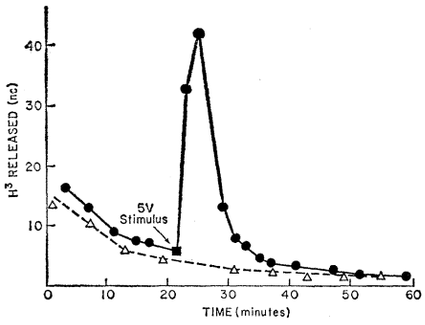

Fig. 1. Release of H^3-norepinephrine from a coronal section of rat brain, induced by a 5-volt, 60-cycle electrical stimulus delivered for 60 seconds, compared with unstimulated efflux from a similar slice. This pattern of release was typical of 18 stimulated brain slices. Closed circles, with stimulation; open triangles, without stimulation.

seconds, were almost as efficient as higher voltages (5 to 10 volts). For stimuli of 4 volts, applied for intervals of 15 to 60 seconds, there appeared to be a linear relation between duration of stimulation and release of norepinephrine. When prolonged stimulation of 10 to 12 volts was used, release of H^3-norepinephrine was apparent only during the first 2 to 4 minutes.

Stimuli of 5 volts, applied for 60 seconds, raised the temperature of the perfusion chamber to a maximum of about 37.5°C. When the bath temperature was raised from 37° to 40°C, insignificant amounts of H^3-norepinephrine were released from brain slices. Thus, effects of electrical stimulation do not appear to be due merely to heating. Efflux of C^{14} from brain slices saturated with C^{14}-urea was not altered by electrical stimulation, which indicates that enhanced diffusion did not play a role in the release of norepinephrine.

Table 1. Release of tritium from tissue slices. Data are mean differences (± standard error of mean) between base line of tritium washout and maximum release following a 5-volt, 60-cycle electrical stimulus for 60 seconds. KCl was used without electrical stimulation, and the data given represent the maximum release obtained after changing to a medium containing 33 m*M* KCl. ES, electrical stimulation.

Brain		Heart	
H^3 released (nc)	*N*	H^3 released (nc)	*N*
Electrical stimulation			
30.3 ± 2.0	18	22.4 ± 2.5	6
Ca-free medium + ES			
11.8 ± 1.0	6	4.5 ± 2.0	6
Chlorpromazine (40 μg/ml) + ES			
13.7 ± 2.2	6		
Pentobarbital (100 μg/ml) + ES			
		7.3 ± 1.0	6
*High KCl (33 m*M*)*			
24.0 ± 1.2	3	38.0	1

Effects of electrical stimulation can be reproduced by high concentrations of potassium in the perfusion medium. Marked release of tritium from brain slices was induced by Ringer media containing 16 to 66 m*M* KCl. Such media are known to depolarize nerve membranes in brain slices (*5*) and to stimulate respiration by the slices (*10*).

Absence of calcium from the medium markedly inhibited release of H^3-norepinephrine by electrical stimulation. Pentobarbital and chlorpromazine appear to inhibit release of H^3-norepinephrine from brain or heart slices by electrical stimulation (Table 1); the effect of pentobarbital on release of tritium was much greater in heart slices. These agents inhibit the increase in respiration that is observed in brain slices during electrical stimulation (*11*).

We have also found that H^3-histamine, H^3-dopamine, and some other amines which accumulate in brain slices in vitro can be released to some extent by electrical stimulation. Thus the present technique may be of some general use in investigation of the biochemistry and pharmacology of depolarization-induced release of central neurotransmitters.

ROSS J. BALDESSARINI
IRWIN J. KOPIN
National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

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Staining Tissue for Light and Electron Microscopy by Bridging Metals with Multidentate Ligands

Abstract. *"Osmium black," a pigment very useful for cytological staining in both light and electron microscopy, may be deposited selectively at the tissue-binding sites of other metal ions by bridging OsO_4 to the tissue-bound metal ion through a multidentate ligand.*

Certain osmiophilic reagents for the selective deposition of osmium black at the sites of enzymes and functional groups in tissue (*1*) have an additional property which is useful for staining tissue. These multidentate reagents have an affinity for metals bound to tissue and retain the ability to react further with the same or other unbound metal by bridging. In our studies, thiocarbohydrazide (TCH), a multidentate organic sulfur ligand, is very useful in this bridging reaction. In its simplest form, the reaction may be depicted as in Fig. 1, where M_1 is the primary and M_2 the secondary metal cation. Thiocarbohydrazide, which has been used with osmium tetroxide for the histochemical demonstration of some oxidized macromolecules (*2*), is able to

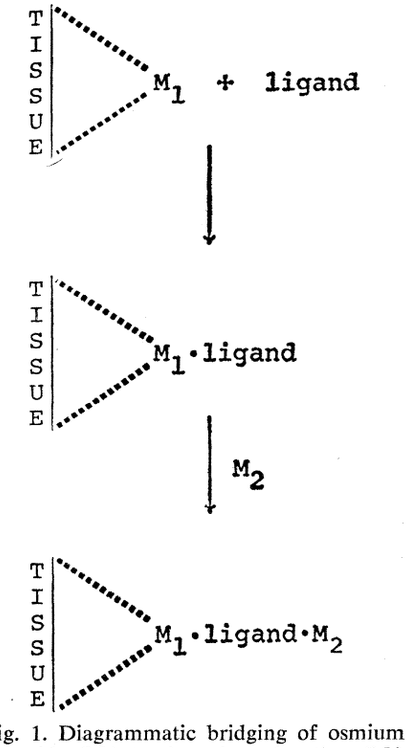

Fig. 1. Diagrammatic bridging of osmium tetroxide (M_2) to tissue-bound cation (M_1) through a multidentate ligand, TCH.