selectivity, the toxicities of this compound and two of its analogs—2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide (dioxide analog) and 2,3-dihydro-5(*N-m*-tolyl)carboxanilido-6-methyl-1,4-oxathiin (tolyl analog)—to fungi of the four taxonomic classes were determined.

A dilution series of the test compound was prepared in acetone, and 0.1 ml of such a solution was added to 10 ml of molten potato dextrose–agar in each growth tube to give concentrations of 0, 8, 32, 125, or 500 ppm; the agar was buffered at *p*H 5.3 with 0.1*M* potassium acid phthalate. For *Cytospora kunzei*, the medium described by French and Helton was used (*2*). Growth tubes were inoculated by transfer of 4-day-old mycelial discs of the test fungi to the sterile agar surface. Mycelium was allowed to grow for 2 to 14 days at room temperature, depending on the growth rate of the particular fungus. Each treatment was replicated three times.

Figure 1 shows that oxathiin and its tolyl analog have very similar patterns of fungitoxicity, which is greater in vitro than that of the dioxide analog. Being the most fungitoxic of the three, oxathiin alone was used in the remaining experiments. Figure 2 shows the lower fungitoxicity of oxathiin to Phycomycetes, Ascomycetes, and Deuteromycetes than to Basidiomycetes; the only exceptions were the deuteromycete *Verticillium albo-atrum* and the basidiomycete *Rhizoctonia solani* (isolated from aster). Both isolates of *R. solani* (one from aster, one from potato), under phase microscopy, proved to have similar dolipore septa (*3*).

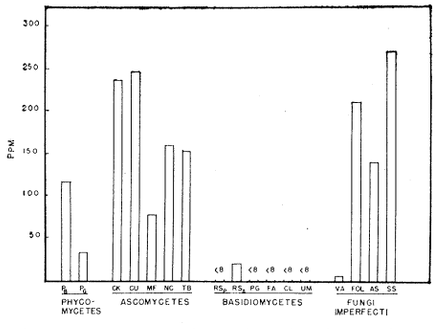

Fig. 2. Concentrations (ppm) of 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin required for 50-percent inhibition of growth of various fungi. P_B, *Pythium* sp. isolated from bean; P_G, *Pythium* sp. isolated from geranium; *CK, Cytospora kunzii; CU, Ceratocystis ulmi; MF, Monilinia fructicola; NC, Nectria coccinia; TB, Thielaviopsis basicola;* RS_B, *Rhizoctonia solani* isolated from potato; RS_A, *Rhizoctonia solani* isolated from aster; *PG, Polyporus gigantea; FA, Fomes annosus; CL, Coprinus lagopus; UM, Ustilago maydis; VA, Verticillium albo-atrum; FOL, Fusarium oxysporum* f. *lycopersici; AS, Alternaria solani;* and *SS, Stemphylium sarcinaeforme.*

Inhibition of spore germination was determined by use of spores of two other Basidiomycetes: *Uromyces phaseoli* and *Ustilago nuda* (both obligate parasites). Spores of each fungus were exposed to oxathiin at concentrations of 10, 100, 1000, and 10,000 ppm. Only 22 percent of the bean-rust spores and 52 percent of the loose-smut spores germinated on the control slides. With both fungi all concentrations of oxathiin reduced the percentage of germination to from 1 to 4 percent; a few spores germinated even in saturated solutions containing many floating crystals of oxathiin.

Thus oxathiin is fungitoxic in vitro to Basidiomycetes and may be useful for controlling plant diseases caused by them.

L. V. EDGINGTON*
G. S. WALTON
P. M. MILLER
Department of Plant Pathology and Botany, Connecticut Agricultural Experiment Station, New Haven

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* Present address: Department of Botany, University of Guelph, Guelph, Ontario.

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Electron-Microscopic Autoradiography of Rat Hypothalamus after Intraventricular H^3-Norepinephrine

Abstract. *Tritiated norepinephrine was injected into the lateral ventricles of rats, and its localization in the hypothalamus was determined by light and electron-microscopic autoradiography. Eighty percent of the autoradiographic grains were located over nerve endings and unmyelinated axons. Large, dense synaptic vesicles were present in most of the endings and axons with activity. Grains were rarely seen over myelinated axons, glia, or blood vessels.*

Parenterally administered tritiated norepinephrine (H^3-NE) has been localized in peripheral sympathetic axons of the rat pineal body by electron-microscopic autoradiography (*1*). Autoradiographic grains were located in or near unmyelinated axons containing granular ("dense-core") synaptic vesicles 400 to 500 Å in diameter. The dense vesicles have been correlated with the subcellular binding sites of peripheral norepinephrine (NE) as judged by morphological (*2, 3*), pharmacological (*4*), and fluorescent histochemical (*5*) experiments. Thus, there is good though not universal (*6*) agreement that NE-containing peripheral axons exhibit dense granular vesicles, which contain releasable NE (*4*).

We have used autoradiography to identify the structural correlate of norepinephrine binding sites within the brain. Evidence relating brain NE to the central dense granular vesicles has been mainly circumstantial. The dense granular vesicles of the central nervous system are generally 1.5 to 2.0 times as large as those described in the periphery (*7, 8*). Furthermore, the electron density of the dense "cores" is quite variable. Some investigators (*9*) have questioned the correlation between mono-amine fluorescence and the distribution of the dense vesicles in the central nervous system. Certainly, other biogenic amines present in nerve endings [obtained from density gradient fractionation of brain homogenates (*10*)] might interfere with either fluorescent or electron-microscopic histochemical methods (*11*).

Investigation of central NE binding sites is difficult because parenterally administered amine enters the brain poorly. It has been shown that tracer doses of H^3-NE injected into the lateral ventricle of the rat brain is readily taken up by the brain cells and metabolized similarly to endogenous NE (half-life of 3 to 4 hours) (*12*). The H^3-NE (but not the labeled catabolites) is found predominantly in the nerve ending fraction of brain homogenates. The labeled norepinephrine is, in addition, appropriately responsive to pharmacological agents affecting the stores of the endogenous amine. These results indicate that autoradiography following intraventricularly injected H^3-NE might provide direct evidence on the sites of uptake and binding of exogenous NE in the brain.

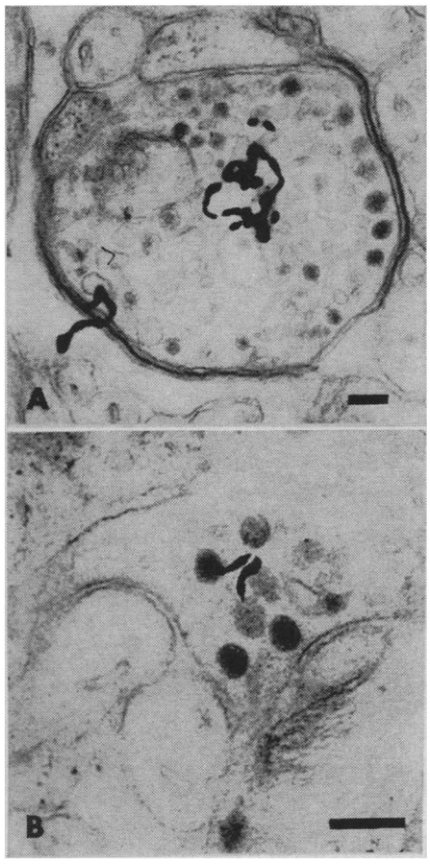

Fig. 1. Electron-microscopic autoradiographs of paraventricular hypothalamus after intraventricular injection of H^3-norepinephrine: A, unmyelinated axon; B, preterminal axon. The specimens were obtained 2 hours after the injection. The emulsion-coated thin sections were exposed for 1 to 4 weeks before a 5-minute development in Microdol-X at 15°C. Large synaptic vesicles with variable degrees of internal density are seen in both processes. The irregularly shaped silver grains are deposited on or near these large vesicles (scale, 0.25 μ).

The paraventricular hypothalamus was selected for the initial study because of the high content of endogenous NE, the presence of catecholamine fluorescent fibers (*13*), and the contiguity of this nucleus to the ependymal surface. Adult Sprague-Dawley rats were lightly anesthetized with chloral hydrate and mounted in a stereotactic headholder. Twenty microliters (20 μc) of *dl*-norepinephrine-7-H^3 (4.7 c/m mole) were injected into one lateral ventricle (*14*). The animals were killed 2 to 4 hours later, when they were fully awake and ambulatory. The brains were fixed by perfusion with buffered glutaraldehyde (*15*). Tissue blocks were then fixed with buffered osmium tetroxide (*16*), dehydrated in graded alcohols, and embedded in Maraglas-DER 732 (*17*) plastic. Pale gold thin sections and 1-μ sections were coated with Ilford L-4 emulsion (*18*), and were then exposed for 1 to 4 weeks before development and examination by electron and phase microscopy.

Examination of 1-μ sections by phase microscopy after 1 week of autoradiographic exposure reveals intense activity in the immediate paraventricular neuropil of the hypothalamus. The intensity of labeling gradually diminishes with increasing distance from the ependymal surface. Only a few grains are seen directly over ependymal cells at this time. As seen in micrographs, the majority of grains are at the peripheral borders of the large dendrites or neuronal perikarya. Grains are rarely seen within glia, myelinated axons, neurons, or blood vessels.

Examination of electron-microscopic autoradiographs confirmed the light microscopic survey. Approximately 80 percent of the grains observed in thin sections of the medial hypothalamus are located over nerve endings or unmyelinated axons. With rare exceptions, nerve endings with autoradiographic activity have identifiable dense synaptic vesicles. This type of ending constitutes less than 30 percent of the total nerve ending population in this region (*7*). Activity is sufficient to produce clusters of autoradiographic grains only in nerve endings or unmyelinated axons containing such large dense synaptic vesicles (Fig. 1). The tissue distribution of a large number of randomly observed grains is illustrated by the bar graph (Fig. 2). Apart from grains over nerve endings and axons, the only other noteworthy localization of activity is on mitochondria within dendritic processes. This is of interest in regard to the presumed mitochondrial localization of monoamine oxidase.

The observed electron density of the large synaptic vesicles seen in axonal processes containing superimposed autoradiographic grains is quite variable (Fig. 1). Although very dense vesicles can be seen in some sites which bind the H^3-NE, most of the large vesicles in labeled sites seem to be of less than maximum density. The variability of electron density within these large vesicles may relate to the duration of the fixation with osmium tetroxide (*7*). Alternatively, a difference in internal density may reflect the concentrations of bound catecholamine within vesicles. In support of the latter view, we find that activity tends

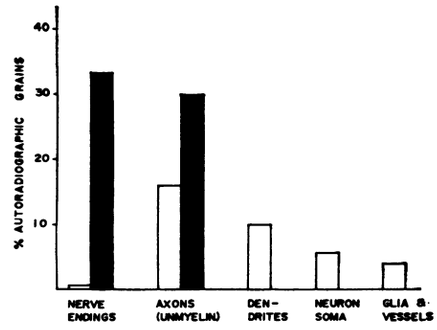

Fig. 2. The percentage distribution of 200 autoradiographic grains in paraventricular hypothalamus after intraventricular injection of H^3-norepinephrine. Values are derived from pooled electron-microscopic observations of random sections developed after exposure periods of 7 to 14 days. Solid bars indicate nerve endings and axons in which dense-core vesicles could be identified. Open bars indicate that no dense vesicles were seen. Background grains, less than 5 per 2500 μ^2.

to be associated with the less dense of the large vesicles. These vesicles, which could be regarded as being only partially filled, may be more active in taking up amine.

The relative ability of central and peripheral nerve endings to take up and concentrate norepinephrine is difficult to estimate. However, the high predilection of grains for hypothalamic endings and axons (particularly those containing dense vesicles) and the extremely low activity elsewhere indicates a selective uptake of NE by these structures. Although measurements of isotopes lost during the preparative steps for microscopy indicate good retention of labeled amine (*3*, *19*), it is possible that the unbound metabolites are lost, resulting in the relatively clean localizations seen. In fact, density gradient fractionation following ventricular injection of H^3-NE localized metabolites primarily in layers other than that of the nerve endings fraction (*12*).

We conclude that in the rat hypothalamus unmyelinated axons and nerve endings which contain dense vesicles tend to bind exogenous NE. Generalizations on the localization of endogenous brain NE should be made with caution. The caudate nucleus, which contains little endogenous NE and few dense granular vesicles (*7*), also takes up intraventricularly injected NE (*12*). Thus, sites which do not normally contain NE may nevertheless be able to bind the exogenous amine. It is of interest that the cellular distribution of autoradiographic grains in rat hypo-

thalamus following the parenteral administration of the NE precursor, 3,4-dihydroxyphenylalanine (DOPA), is very close to the distribution reported here (*20*). This suggests that exogenous NE and NE formed endogenously from DOPA finally localize in the same sites within the hypothalamus despite a widely divergent intermediate pathway. However, the structural and chemical limits of the specificity of localization require further definition.

G. K. AGHAJANIAN
F. E. BLOOM
Departments of Psychiatry and
Anatomy, Yale University,
New Haven, Connecticut

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Intravenous Injection of Thalidomide in Pregnant Rabbits

Abstract. *When administered orally to pregnant strain III rabbits, thalidomide is teratogenic to their fetuses. However, when the drug is injected intravenously to females of the same genetically controlled stock, it has no observable effect. This finding indicates that a metabolite of thalidomide formed in the intestinal tract may be the real teratogenic agent.*

Thalidomide fed to pregnant rabbits is teratogenic to their fetuses (*1–3*). It is not known whether the teratogenic agent is thalidomide itself or one or more of its metabolites, nor is the mechanism by which thalidomide produces its effects known (*4*). Hydrolysis of thalidomide before oral or intraperitoneal administration markedly reduces its teratogenic effects in strain III (*5*) and other rabbit stocks (*6*).

We have studied the effects of intravenous injection of thalidomide into pregnant rabbits because this method facilitates a rapid and direct delivery of the drug to the fetus, and thereby controls the onset of exposure and reduces the amount of drug which must be given to the doe to obtain concentrations in the blood which will be teratogenic to the fetuses. The low solubility of thalidomide prohibits its intravenous injection in large dosages. However, with the inclusion of carboxymethyl cellulose (CMC) and glucose, a supersaturated solution (1 mg of thalidomide per milliliter of solution) which is stable at 40°C for a period long enough to allow intravenous injection at a rate of 5 ml/min can be made.

One hundred milliliters of the solution was prepared by placing 0.5 g CMC (type 120, high viscosity) in a 500-ml Erlenmeyer flask which contained a test tube with circulating cold water. The flask and test tube served as a reflux condenser. Then 100 ml of distilled water, plus 8 percent to compensate for water loss during condensation, were added. The distilled water was boiled slowly for 10 minutes or until the solution became homogenous. The heat was then removed and the condenser was allowed to operate for an additional 10 minutes. Five grams of glucose and 100 mg of thalidomide (fine powder, sieve 200) were added, and the solution was heated to a slight boil until all thalidomide was dissolved (about 20 minutes). The clear solution was immediately filtered to remove any dust or fibers and was cooled to 50°C by placing it in a water bath (50°C). We injected the solution with a 30-ml syringe equipped with a two-way valve, 61 cm of polyethylene tubing (inside diameter, 0.12 cm; outside diameter, 0.16 cm), and a 22-gauge needle. The temperature of the liquid injected into the ear was about 40°C.

Satisfactory intravenous injections of 20, 50, and 150 mg of thalidomide per day to each doe were routinely made for periods varying from two to five consecutive days. Seven does of strain III produced treated litters; two of the seven produced litters at all three dosages. Each doe also produced control litters and was bred to the same male for both her control and treated litters. Controls were offspring of does which had received no intravenous injections during that pregnancy. Control and treated matings were made to avoid bias due to season or parity. All mothers were palpated for pregnancy 10 to 14

Table 1. The effects of intravenous injection of thalidomide on reproduction and on malformation of fetuses. Pregnant rabbits were iniected with varying amounts of thalidomide each day for two to five consecutive days, beginning on different days of the gestation period.

Dose given		Pregnancies per matings	Classifiable fetuses (No.)				Weighted abnormal*	Unclassified resorbed fetuses (No.)
Amount (mg/day)	Days of gestation		Total	Viable	Dead	Abnormal		
0		9/12	60	48	12	38	7	3
20	7 and 8	4/9	19	18	1	6	0	0
50	6–9	0/3						
50	6–11	0/2(1R†)						
50	8 and 9	2/5	17	15	2	12	0	1
50	9 and 10	1/2	8	6	2	7	0	0
150	6–8	1/1	7‡	0	0	0	0	1
150	6–9	0/2(1R†)						
150	6–10	4/4	19	15	4	13	1	7

* Those animals having malformations only of skull, sternum, or tail were classified as normal since this is considered to be normal strain variation (*1*). † Of the two does bred, one was palpated pregnant but she resorbed her complete litter. ‡ Taken at 21 days gestation because the doe had a broken back.