slurry sucked up by the dredge was passed through a fine mesh bag to collect any organisms present. Although several square meters of bottom were excavated to a depth of 3 m, no live shrimp were recovered. The burrow systems are evidently deeper than 3 m, and the animals are able to escape the dredge by seeking lower levels.

The dredge was used to remove the upper meter of sediment in several areas of high burrow concentration. Fresh sediment was then used to refill excavated areas. Overnight, burrows were reestablished in the same densities, and in approximately the same locations.

Polyester casts were made of burrows, using a modification of Shinn's technique (*4*), which allows the diver to mix the resin on the bottom. To remove the hardened casts the next day required one diver to steady the cast and hold the hose of the suction dredge, and another, headdown in the hole, to guide the dredge around the cast. One of the casts recovered (Fig. 2) is 2.2 m high, but the upper 30 to 40 cm of the burrow were not recovered. The burrow system is open and more than 2.5 m deep in the substrate. To the best of our knowledge this represents the deepest verified bioturbation ever recorded. In some of the cores, skeletons of recent marine foraminifera were carried down and emplaced in Pleistocene lacustrine clays.

Casts have a vertically oriented semicircular cross section, a characteristic that can also be identified in the cores. Many of the burrows were lined with eel grass, which adhered to the exterior of the casts. Several casts have a knobby exterior, similar to that of the trace fossil *Ophiomorpha nodosa* (*5*). As Seilacher pointed out (*6*), any trace fossil may have been produced by several different species of animals. Burrows of *A. serratus* have the same cross section as *Alpheus* (*4*), although their orientations differ. *Alpheus* burrows have a flat "floor" and an arched "roof"; *A. serratus* burrows have one flat and one curved "wall." Burrows of *A. serratus* have a morphology and knobby exterior similar to burrows made by *Callianassa*.

The presence of these deep burrows in the Strait of Canso is potentially of tremendous sedimentological and geochemical importance because of the profound disruption in the normal stratigraphic layering of sediments. Computer simulation of burrow complexes indicates that, in areas of average population density (nine burrows per square meter), it is virtually impossible to recover an undisturbed core. One of the cores taken in the original Atlantic Geoscience Centre study shows six burrow intersections over a total length of 1.2 m. The accuracy of age dating and stratigraphic work depends on the recognition of these burrowed areas. In areas with a high density of burrows, such work is virtually impossible.

Surface sediment south of the causeway where *A. serratus* occurs contains more water and organic carbon than otherwise similar sediment north of the causeway. The sand in the burrows also contains an unusual amount of water and exhibits different geotechnical properties from the surrounding sediment. Water content is often so high that the burrow infillings are thixotropic. Where *A. serratus* is abundant, therefore, much of the sea floor is underlain, to a depth of at least 2 m, by unstable sediment-water mixtures.

The rate of the geochemical reaction reaches a maximum value at the sediment-water interface. A burrow is an extension of this interface, and the burrows in the study areas increase the area of interface by a factor of at least 4. Sediment filling the burrows contains less clay and organic carbon than does the surface sediment it resembles, and is also associated with anomalous concentrations of lead, zinc, copper, and iron.

In the ecologically sensitive Strait of Canso, the activities of *Axius serratus* are important in burying and recycling pollutants, concentrating trace elements, and accelerating the rate of reaction at the sediment-water interface (*1*).

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Alpha-Adrenergic Receptor Identification by [^{3}H]Dihydroergocryptine Binding

Abstract. *A radioactively labeled α-adrenergic antagonist, [^{3}H]dihydroergocryptine, binds specifically to a site on rabbit uterine membranes. Binding is rapid, reaching equilibrium in less than 17 minutes at 25°C. Adrenergic agonists compete for this binding site with an order of affinities identical to the pharmacological potency order of these agents as α-adrenergic agonists (epinephrine > norepinephrine ≫ isoproterenol). The (−) stereoisomers of epinephrine and norepinephrine are 30 times more potent in competing for the site than the corresponding (+) stereoisomers. α-Adrenergic antagonists, such as phentolamine and phenoxybenzamine, potently compete for the binding sites while the β-adrenergic antagonist propranolol does not. Structural analogs of catecholamines that are devoid of α-adrenergic physiological activity do not compete for [^{3}H]dihydroergocryptine binding sites. These data suggest that α-adrenergic receptors can be directly identified and studied by [^{3}H]dihydroergocryptine binding.*

The diverse physiological effects of the endogenous catecholamines epinephrine and norepinephrine can be divided into two groups designated alpha and beta. This classification (*1*), which is based on the characteristic potency orders of catecholamines in stimulating these responses and on the selective inhibition of the responses by specific antagonists, has suggested that two distinct types of catecholamine receptors mediate the responses. Typical adrenergic responses mediated by α-adrenergic receptors are contraction of vascular, bronchial, and uterine smooth muscle. Typical β-adrenergic responses are relaxation of smooth muscle and stimulation of cardiac contractility.

Until recently adrenergic receptors, as well as other hormone receptors, were defined only in a functional sense, and their existence as physicochemical entities was inferred but not documented. The use of radioactively labeled hor-

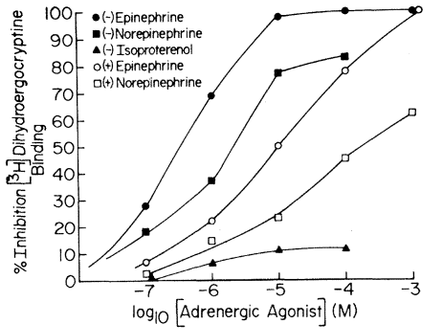

Fig. 1. Inhibition of [^{3}H]dihydroergocryptine binding to rabbit uterine membranes by adrenergic agonists. The ordinate represents the percentage of specific binding inhibited by the addition of the indicated concentrations (abscissa) of competing agents. Each value shown represents the mean of duplicate determinations from two separate experiments.

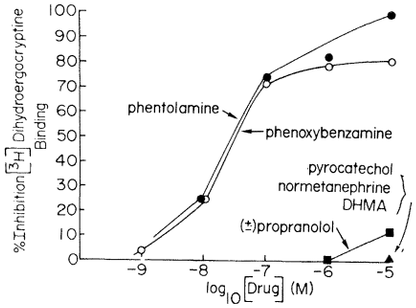

Fig. 2. Inhibition of [^{3}H]dihydroergocryptine binding to rabbit uterine membranes by adrenergic antagonists and catecholamine structural analogs. Each value shown represents the mean of duplicate determinations from two separate experiments. DHMA refers to 3,4-dihydroxymandelic acid.

mones and drugs as receptor ligands (*2*) has provided an approach for directly identifying and studying hormone receptors. Although β-adrenergic receptors have been identified by such direct binding techniques (*3*), there have been no reports of successful identification of α-adrenergic receptors by direct binding studies. Identification of α-adrenergic receptor binding sites would require that several criteria based on the known pharmacology of adrenergic agents be fulfilled.

1) Binding of the radioactive ligand should be reasonably rapid in onset as are the physiological effects of α-adrenergic agents.

2) α-Adrenergic receptor binding sites should be stereospecific, showing greater affinity for the (−) stereoisomers of α-adrenergic agonists than for the (+) stereoisomers since α-adrenergic responses exhibit such stereospecificity.

3) The order of affinities of adrenergic agonists for the binding sites should be (−)-epinephrine > (−)-norepinephrine≫ (−)-isoproterenol (*1*).

4) α-Adrenergic antagonists, such as phentolamine and phenoxybenzamine, should have high affinity for the binding sites, while β-adrenergic antagonists, such as propranolol, should not.

5) Compounds devoid of α-adrenergic activity, such as catecholamine precursors or metabolites, should not interact with the binding sites.

In our study [^{3}H]dihydroergocryptine, a potent competitive α-adrenergic antagonist, is used to identify binding sites which satisfy all of the above criteria and which therefore possess characteristics indistinguishable from those of α-adrenergic receptors.

[^{3}H]Dihydroergocryptine was prepared by New England Nuclear by catalytic reduction of ergocryptine with tritium gas using paladium as the catalyst. Under these conditions, the double bond at the 9,10 position is reduced (*4*) forming the dihydroergot alkaloid. This approach was selected for two reasons. First, catalytic reduction permits insertion of more than 1 atom of tritium per molecule of drug, thus ensuring high specific radioactivity. ([^{3}H]Dihydroergocryptine used in these experiments had specific activity of 25 c/mmole.) Second, dihydroergot alkaloids have been shown to possess even higher affinity as α-adrenergic antagonists than the native unreduced alkaloids (*5*). The labeled material was purified by thin-layer chromatography on silica gel plates and was homogeneous in a chloroform, benzene, ethanol (4:2:1) system and a chloroform, ethanol, acetic acid system (18:10:2).

Since contraction of uterine smooth muscle is an α-adrenergic response, rabbit uteri were used as a source of membranes for binding studies. New Zealand white rabbits (6 to 8 pounds; 1 pound = 1.6 kg) were killed by air embolization; the uteri were removed, stripped of fat and endometrium, minced, and homogenized for 10 to 12 seconds in an ice-cold solution (0.25*M* sucrose, 5 m*M* tris-HCl, *p*H 7.4, 1 m*M* $MgCl_2$) by means of a Tissumizer (Tekmar) at maximum speed. After filtration through one layer of cheesecloth the homogenate was centrifuged at 310*g* for 10 minutes at 4°C, and the pellet was discarded. The resulting supernatant was centrifuged at 28,000*g* for 10 minutes; the pellet was washed twice in ice-cold "incubation buffer" (50 m*M* tris-HCl, *p*H 7.55, 10 m*M* $MgCl_2$). The final pellet was resuspended in incubation buffer for use in the binding assay.

[^{3}H]Dihydroergocryptine binding was determined by incubating [^{3}H]dihydroergocryptine (9 n*M*) with rabbit uterine membranes (3 to 4 mg/ml) for 12 minutes at 25°C in 150 μl of incubation buffer. Incubations were terminated by diluting 125-μl portions with 2 ml of buffer (25°C). The diluted incubation mixtures were rapidly filtered through Whatman GFC glass fiber filters. Filters were rapidly washed with 20 ml of buffer (25°C). After drying, filters were counted in a scintillation mixture containing Triton and toluene.

"Nonspecific" binding was defined as binding not displaced by a high concentration (10 μ*M*) of phentolamine, a potent α-adrenergic antagonist which should occupy all of the α-adrenergic receptor binding sites. "Specific" or receptor binding was defined as total radioactivity bound minus nonspecific binding, and was generally 60 to 80 percent of the total radioactivity (counts per minute) bound to protein. The term [^{3}H]dihydroergocryptine binding (Figs. 1 and 2) refers to specific binding, which was equivalent to 0.13 pmole of [^{3}H]dihydroergocryptine bound per milligram of protein. A small amount of [^{3}H]dihydroergocryptine (0.5 percent of the total radioactivity filtered) was also nonspecifically adsorbed to the glass fiber filters. In separate experiments, we demonstrated that the procedures used to wash the glass fiber filters removed no specifically bound radioactivity, but only lowered nonspecific binding.

Binding of [^{3}H]dihydroergocryptine to rabbit uterine membranes was rapid, reaching equilibrium in less than 17 minutes at 25°C and remaining stable for at least 30 minutes. The binding was saturable with half-maximal saturation of the sites occurring at about 10 n*M* [^{3}H]dihydroergocryptine. The [^{3}H]dihydroergocryptine binding sites had the specificity expected of α-adrenergic receptors. Adrenergic agents competed for the binding sites (Fig. 1) in an order of affinities [(−)-epinephrine> (−)-norepinephrine ≫ (−)-isoproterenol)] identical to the order of potencies of these agents as physiological α-adrenergic agonists (*6*). The concentrations of (−)-epinephrine (0.3 μ*M*) and (−)-norepinephrine (2.0 μ*M*) which half-maximally inhibited specific binding were in the range of the concentrations of these compounds (1.6 μ*M* and 4 μ*M*) which half-maximally contract smooth muscle (*6*). Binding was stereospecific (Fig. 1), the (−) stereoisomers of epinephrine and norepinephrine being more than 30-fold more potent than the corresponding (+) stereoisomers in inhibiting binding. This agrees with the observation (*7*) that the (−) stereoisomers of α-adrenergic agonists are more potent

physiologically than the (+) isomers. The α-adrenergic antagonist, phentolamine, competed at very low concentrations for the binding causing half-maximal inhibition at 30 nM (Fig. 2) in the range of its reported (*8*) dissociation constant (8 nM) as an α-adrenergic antagonist. Higher concentrations (up to 1 mM) displaced no more binding than did 10 μM phentolamine. Phenoxybenzamine, another potent α-adrenergic antagonist, also competed at low concentrations for the binding sites causing half-maximal inhibition at 30 nM.

By contrast, (±)-propranolol, a very potent β-adrenergic antagonist, caused only a 12 percent inhibition of binding at 10 μM. In more intact preparations (aortic strips) (*9*) high concentrations of propranolol (0.1 mM to 1.0 mM) cause α-adrenergic blockade. Similarly, in this binding assay, 0.1 mM (±)-propranolol caused 50 percent inhibition of [^{3}H]dihydroergocryptine binding. Several compounds that are devoid of α-adrenergic activity did not compete for the binding sites at low concentrations. Dopamine (a catecholamine precursor) inhibited only 22 percent of the binding at 10 μM. Pyrocatechol did not inhibit binding at 10 μM. The catecholamine metabolites 3,4-dihydroxymandelic acid and normetanephrine were also ineffective in competing for the binding sites (Fig. 2).

Our data indicate that [^{3}H]dihydroergocryptine binding sites in rabbit uterine membranes satisfy the essential criteria that must be fulfilled for direct identification of α-adrenergic receptors. Binding is rapid and stereospecific, and α-adrenergic agonists and antagonists have high binding affinities that parallel their potencies in eliciting or blocking physiological α-adrenergic responses. The use of radioactively labeled α-adrenergic antagonists should be of value in the characterization of smooth muscle α-adrenergic receptors and in the study of these receptors in various physiological and pathological states.

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Uptake of Bovine Serum Albumin by Rainbow Trout from Hyperosmotic Solutions: A Model for Vaccinating Fish

Abstract. *Immersion of juvenile rainbow trout (*Salmo gairdneri*) in a solution containing either urea or sodium chloride at 1650 milliosmoles and 2 percent of bovine serum albumin (BSA) resulted in an uptake of BSA into the blood of the fish after a 3-minute exposure. Similar blood levels of BSA were also obtained by placing the fish in 1650 milliosmoles of sodium chloride for about 2 minutes, and then immersing them in 2 percent BSA solution for 3 minutes. Uptake of BSA into the fish appeared to be primarily through the lateral line system and secondarily through the gills.*

Immunization of fishes as a means of disease prevention is possible but not widely practiced because individual injection of antigen into small fish is not considered practical. Various other methods of fish immunization, including oral delivery of antigens, have been investigated (*1*) but none has been generally accepted. In a search for a practical method of fish immunization we have tested the practicability of immunization by immersion of fishes in hyperosmotic solutions containing bovine serum albumin (BSA) as an antigen.

Chemicals that we believed would aid the infusion of BSA into fish were screened by determining the threshold toxicity to 4-g trout in a 3-minute exposure. The osmotic pressure of the chemical solution was measured by the freezing point depression method on an osmometer (Advanced Instruments, Inc.), or determined by calculation. A level was selected that was hyperosmotic to the fish and that the fish could tolerate, then a 2 percent (weight to volume) BSA solution was prepared. Fish were placed in the hyperosmotic-BSA solution at 20°C for 3 minutes, after which they were transferred into flowing water at 20°C. The fish were bled about 45 minutes later and the plasma was assayed for BSA content.

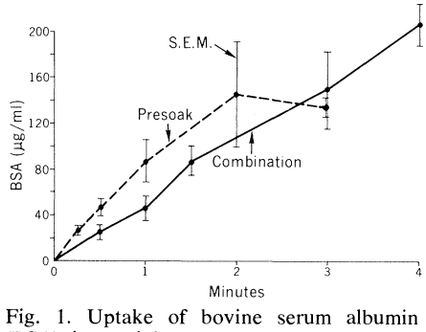

Fig. 1. Uptake of bovine serum albumin (BSA) into rainbow trout plasma after fish were placed in a solution containing 5.32 percent NaCl and 2 percent BSA (solid line) or in fish that were first immersed in a bath containing 5.32 percent NaCl followed by 3 minutes in a bath of 2 percent BSA solution (broken line). Fish were removed from the NaCl solution at various intervals and bled 45 minutes later.

The BSA in the plasma of rainbow trout (*Salmo gairdneri*) was quantified by rocket electrophoresis (*2*): the standard curve was prepared from BSA in normal rainbow trout plasma. The precipitin peaks were projected onto a screen, traced onto paper, cut out, and weighed. The assay was linear, as determined by linear regression analysis, from 10 to 250 μg of BSA per milliliter of plasma. A standard reference BSA-plasma was run in parallel with all tests. When organs were evaluated, they were placed in an equal weight of saline and sonicated to disrupt the cells. The sonicate was then tested for BSA content.

Of the chemicals tested, urea (10 percent) and NaCl (5.23 percent), each at 1650 milliosmoles, resulted in the highest plasma levels of BSA (Table 1). Because NaCl consistently gave the highest levels of plasma BSA in subsequent testing, it was tested in detail. The amount of BSA infused was dependent on the length of time the fish were held in the hypertonic NaCl-BSA solution (Fig. 1). No mortality occurred up to and including 3 minutes, but longer exposures resulted in