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.

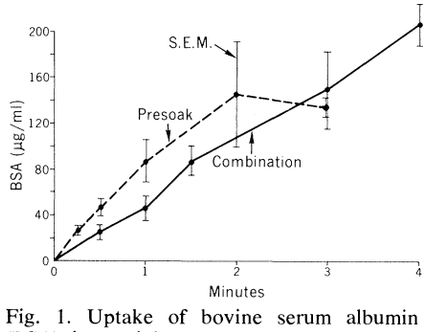

Fig. 1. Uptake of bovine serum albumin (BSA) into rainbow trout plasma after fish were placed in a solution containing 5.32 percent NaCl and 2 percent BSA (solid line) or in fish that were first immersed in a bath containing 5.32 percent NaCl followed by 3 minutes in a bath of 2 percent BSA solution (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

mortality. In other tests using a 3-minute exposure, the response of the fish to the infusion was variable: some fish showed little visible effect, whereas others lay on the bottom of the aquariums with their opercula moving rapidly for several minutes after infusion. No deaths have occurred, however, and the fish appeared normal and were feeding within 1 hour.

The route of BSA entry into fish was determined by exposing them to the hypertonic NaCl-BSA solution for 3 minutes; then samples of gills, blood, lateral line, skin mucus, stomach, and intestine were collected 0, 15, 30, 60, 120, and 180 minutes after the fish were returned to the holding aquariums. The entire gill structure was removed, mucus was scraped from the dorsal surface, and the lateral line system was dissected from each side of the fish but not from the head.

The BSA level was lowest in the plasma immediately after the fish were exposed to the NaCl-BSA solution, and then increased to a maximum of about 160 μg/ml about 60 minutes later and remained high for at least 48 hours (Fig. 2). This observation indicated that, although some BSA may have been infused directly into the blood, most was temporarily retained elsewhere in the fish and gradually entered the blood. Only traces of BSA were found in the skin mucus, stomach, and intestine, indicating that the BSA solution was not being ingested. In a separate test, the intubation of 0.5 ml of 2 percent BSA into the stomach resulted in no detectable levels of BSA in the plasma. Significant levels of BSA were found in the gill sonicate immediately after exposure, but the concentration declined rapidly during the next 2 hours.

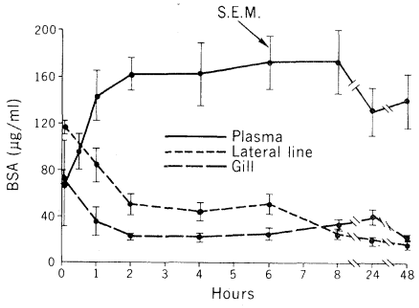

Fig. 2. Comparative uptake of bovine serum albumin (BSA) in the plasma, lateral line, and gill tissues of rainbow trout at various intervals after a 3-minute bath in a solution of 5.32 percent NaCl with 2 percent BSA.

The initial levels of BSA in the gills did not appear to be sufficient to account for the high concentration later found in the blood (Fig. 2).

The initial BSA level was significantly higher in the lateral line than in the gills, and remained higher for at least 6 hours (Fig. 2). The decrease in BSA concentration from the lateral line was correlated with the increase of BSA in the plasma. Apparently the primary route of BSA entry into fish was through the lateral line system, and the gills were a secondary route (*3*).

The lateral line of fish is a canal open to the surface, located on the sides of fish, about a third of the distance ventral to the dorsal ridge. It is continuous from the operculum to the tail and extends over the head. Although it is believed to be primarily a sensory organ (*4*), a lymph duct lies directly under the lateral line and connects to the venous system at the posterior cardinal veins (*5*). Uptake of BSA by way of the lymphatic system could possibly explain the delayed appearance of BSA in the blood and could be of immunological importance.

Hypertonic solutions have a dehydrating effect on membranes and also could possibly draw fluid from the lateral line canal, while NaCl and BSA diffuse into the canal. If this were true, the same effect should be obtained by placing the fish in a hypertonic solution first, and then in a hypotonic solution containing BSA. Figure 2 demonstrates that about the same level of BSA can be infused into fish plasma, compared with the combined NaCl-BSA solution, by first placing the fish for about 2 minutes in a hypertonic NaCl solution, and then for 3 minutes in 2 percent BSA.

The two-step infusion method has practical applications because high salt concentrations, when in combination with labile antigens, may affect the immunogenicity of some vaccines. This infusion method also may have application in delivering a variety of chemicals or chemotherapeutics. Large numbers of fish can be handled; the method is limited only by the size of the tank and by the volume of vaccine that can be prepared (*6*). Furthermore, the implication that the lateral line system takes up proteins opens a new area for evaluating the immunological systems of fish, and provides a tool for studying the phylogeny of immunity.

Table 1. Comparison of various chemicals at hyperosmotic concentrations for infusion of bovine serum albumin (BSA) into the plasma of rainbow trout.

Chemical			No. of fish infused	BSA in fish serum (mean μg/ml)	S.E.M.
Name*	Milliosmoles	Percent			
None	0		4	<10	
Urea	1650		3	129	14
NaCl	1650		6	132	25
	825		4	36	3
	412		4	19	8
$MgCl_26H_2O$	400		5	44	8
$CaCl_2$	1240		4	22	4
KCl	500		5	30	6
$NaHCO_3$	800		5	53	10
	400		5	29	4
Sucrose	1650	49	10	38	4
Polyethylene glycol†		20	5	<10	
Methanol		5	4	11	3
Dextrose	1650	30	5	14	1
	825	15	5	10	1

*All solutions of chemicals contained 2 percent BSA; fish were bled 45 minutes later. The concentration of BSA in the fish plasma was determined by electrophoresis of the plasma into a rabbit anti-BSA agarose gel (rocket electrophoresis) and measurement of the precipitin peak heights. †PEG-6000.

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6. In recent laboratory and field tests we have used the infusion method effectively to immunize fish against bacterial and viral diseases.

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