Sodium and Potassium Binding by Microsomes from Nasal Salt Gland, Harder's Gland, Kidney and Liver of Sea Gull.* (28290)

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The fundamental process of ion transport is generally recognized to be of great importance to living organisms. Although the molecular mechanism involved is not clear, biologists generally agree that transport depends on the ability of some cellular constituent to bind these ions. Recent cation binding studies have clearly demonstrated the ion exchange binding of significant amounts of Na^+, K^+, and H^+ by freshly prepared rat liver microsomes(1) and human erythrocyte ghosts (2). Only a slight preference of K^+ over Na^+ binding was noted; thus one of the basic requirements of current ion transport hypotheses is not supported by results of direct binding studies. Instead, a basically different mechanism of ion transport has been suggested by Sanui and Pace(1,3).

The nasal salt gland found in marine birds was thought to be a system particularly suitable for studying ion binding, since these glands are capable of secreting a 0.4-1.1 M solution of NaCl, a level 3-7 times greater than that found in plasma(4,5). Furthermore, electrical measurements have indicated the primary secretory mechanism to be an "active" transport of Na^+ from the blood to the gland lumen(6).

Materials and methods. Six Western gulls (*Larus occidentalis*) recently caught on the California coast and weighing from 770-1300 g each were used. The birds were killed by decapitation following intravenous administration of sodium pentobarbital. The nasal salt glands, Harder's glands, kidneys and liver were immediately removed, weighed and cooled to 0°C. The range of tissue weights from individual birds was as follows: nasal salt glands, 0.573-0.818 g; Harder's glands, 0.759-1.718 g; kidneys, 5.060-7.497 g; and liver, 18.400-22.700 g. Tissue microsome fractions were isolated in 0.25 M sucrose solution as previously described (Sanui and Pace(1)). Because of its toughness, the nasal salt gland was minced and ground in a mortar before homogenization and differential centrifugation. Aliquots of the final washed microsome materials were suspended in media containing 150 mM Na^+ or K^+ as the phosphate at pH 6.9 and allowed to equilibrate for 12 hours at 0-4°C. Due to the relatively small amount of microsome material from the nasal salt glands and Harder's glands, it was necessary to pool material from 3 birds in order to perform the experiments for these tissues. Following centrifugation for 2 hours at 132,050 *g*, the microsomes were washed twice with 0.25 M sucrose solution by resuspension and recentrifugation. Aliquots of each pellet were wet ashed in Vycor flasks using concentrated nitric and perchloric acids. The resulting white ash was dissolved in dilute HCl and Na^+ and K^+ were measured with a Perkin-Elmer model 52-C internal standard flame photometer. Total nitrogens were determined by the micro-Kjeldahl method. Separate aliquots of microsome material were used to determine Na^+ and K^+ binding, and in all cases the equilibrium pH was 6.85 $\pm$ .05.

Results. Sodium and potassium binding data for the microsomes from various tissues of the Western gull are shown in Table I below. With one exception, the binding of Na^+ or K^+ by the nasal salt gland, liver and kidney microsomes is within the range of 1.1-1.6 meq/g N. However, the binding by Harder's gland microsomes on the basis of total nitrogen is almost twice as high (2.3-2.5 meq/g N). The reason for this difference was not pursued in detail. It was noted during the experiment that the Kjeldahl nitrogen per milliliter of Harder's gland pellet was approximately half that observed in the other tissues examined, and a histological examination of

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TABLE I. Sodium and Potassium Binding by Microsomes from Various Tissues of Western Gull.

Source of microsomes		Equilibrium free Na concentration (mM)	Bound sodium (meq/g N)	Equilibrium free K concentration (mM)	Bound potassium (meq/g N)
Nasal salt gland	Pool 1	154.3	1.25	—	1.07
	" 2	150.0	1.16	143.7	1.07
Harder's gland	Pool 1	149.0	2.33	144.4	2.45
	" 2	146.3	2.52	142.6	2.48
Kidney	SG244	148.5	1.39	142.4	1.57
	SG249	151.5	1.63	143.5	1.46
	SG253	151.3	1.11	143.8	1.06
Liver	SG244	145.0	.67	143.5	1.28
	SG249	145.2	1.11	139.8	1.23
	SG253	143.0	1.23	139.3	1.16

Pool 1 = glands from birds SG224, SG229, and SG238. Pool 2 = glands from birds SG244, SG249, and SG253.

Harder's gland showed them to be mucous in nature containing large amounts of mucopolysaccharides. These two observations, coupled with the report(7) that chondroitin sulfate is capable of binding the alkali cations, suggest that the high binding seen with Harder's gland microsomes results from binding to a mucopolysaccharide low in nitrogen.

Discussion. This preliminary study demonstrates that membrane materials from various tissues of the Western gull exhibit binding of a significant amount of Na^+ and K^+ in a manner and at a level similar, for the most part, to that found with rat liver microsomes and human erythrocyte ghosts. These results provide further evidence for the apparent universality of ion exchange binding of these cations by cellular membrane materials independent of cellular function.

The similarity of the observed levels of binding under the present conditions suggest that a high binding capacity is not the reason for the high salt secreting ability of the nasal salt gland. Hokin and Hokin(8) have implicated phosphatidic acid in the secretory process of the nasal salt gland; however, the question of high specificity in cation binding still remains. Even the existence of a countercurrent mechanism in the nasal salt gland apparently cannot account for its total efficiency(5). Thus the basis for the high efficiency of the nasal salt gland seems to be linked to its unique structure(9). An electron microscopic study(10) of the nasal salt gland indicated an asymmetrical distribution of mitochondria as well as extensive infoldings of the cellular membranes, some of which appear to be continuous from the base of the cell to its apex. The significance of these epicytoplasmic spaces is not clear, but it is suggested that they may function in a manner analogous to an ion exchange column. Therefore, the highly efficient transcellular salt transport seen in the nasal salt gland could be explained in terms of the elution-type mechanism of ion transport postulated by Sanui and Pace(1,3) under the observed structural and binding characteristics of the gland.

Summary. Microsomes from the nasal salt gland, liver and kidney were capable of binding Na^+ and K^+ at a level of 1.1-1.6 meq/g N. Microsomes from Harder's gland exhibited a Na^+ and K^+ binding of 2.3-2.5 meq/g N, which possibly represents binding to a mucopolysaccharide low in nitrogen. The high efficiency of the nasal salt gland could be associated with the elution-type mechanism of ion transport.

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Properties of Poliovirus Inhibitor from Monkey Brain. (28291)

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Tissue suspensions of the central nervous system of rhesus monkeys have been reported to inhibit infectivity of all 3 types of poliovirus(1). Rapid sedimentation of the inhibitory activity, its heat lability and its virus specificity(1) suggest a possible relationship to the virus-adsorbing or receptor sites on the cell surface which have been reported for a number of viruses(2-11). Inhibition of virus by suspensions of receptor material has been shown to result from binding of virus to receptor which prevents virus from attaching to intact cells(2-11). In contrast the preliminary study of inhibitory suspension of monkey nervous tissue suggested that the antiviral action might be mediated through host cells and not by direct binding of virus because inhibition of poliovirus could be reversed by simple dilution(1). The present study of properties of the inhibitory factor, from the central nervous system tissues of monkeys, was undertaken to help determine its relationship to receptor sites, mechanism of action and possible importance in the pathogenesis of poliovirus infection.

Materials and methods. Suspensions of various organs, diluted in Medium 199, were prepared by homogenizing tissues with glass beads, glass tissue homogenizers or in an electrically driven blendor. Homogenates were stored at −25°C. Poliovirus inhibitor activity of tissue suspensions was determined by mixing equal volumes of organ homogenate and type 1, Mahoney strain poliovirus, and assaying for poliovirus plaque forming units (pfu) in monkey kidney bottle cultures (MK). A decrease of 50% or more, when compared with control plaque counts, was considered significant inhibition. The end point of antiviral activity of monkey nervous tissue homogenates occurred at a 1/20 dilution (5% suspension) of the original tissue and complete suppression of plaques occurred at 1/3 dilution (30% suspension). In the present study 10 or 20% suspensions were used.

Results. Effect of chemical and physical agents on inhibitory factor.

Organic solvents. Normal monkey brain suspension was shaken with an equal volume of ether or an equal volume of 2 parts ethanol and 1 part ether at room temperature. Aqueous phase, organic solvent phase and interphase were separated, the organic solvents allowed to evaporate at 4°C and the fractions brought back to original volume by addition of Medium 199. Assay of fractions revealed complete loss of the 80% plaque inhibiting activity of the untreated suspension. Inactivation by ether is a property shared by the inhibitory factor and previously studied receptor substance(6).

Acid. Monkey brain suspension was dialyzed overnight against 60 volumes of citric acid—disodium phosphate buffer solution at pH 2 then redialyzed against Earle's balanced salt solution at pH 7.4. This procedure resulted in loss of the 80% plaque inhibitory activity of the untreated suspension. Inactivation of inhibitory factor by acid is consistent with, but not necessarily due to the same mechanism as, the reported release of poliovirus from receptor at acid pH(9).

Trypsin. Incubation of inhibitory suspension for 1 hour at 37°C in the presence of 0.001% trypsin (twice recrystallized) resulted in loss of the 75% plaque inhibition which oc-