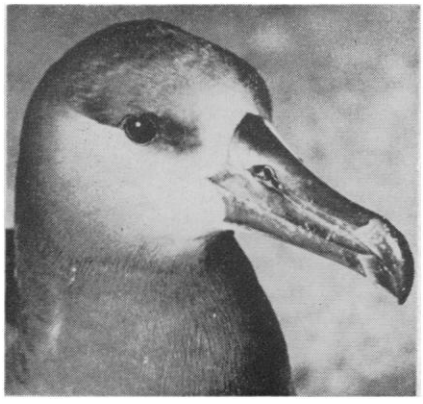
Salt Excretion by Nasal Gland of Laysan and Black-Footed Albatrosses

Abstract. Excretion of a liquid which dripped from the tip of the beak followed administration of salt loads. The Na^+ concentration in the liquid was 792 to 856 milliequivalents per liter, almost twice that in sea water. The nasal gland may thus enable these birds to meet their need for water by drinking sea water.

Recently, Schmidt-Nielsen and his coworkers have discovered that the nasal gland of two species of marine birds, the double-crested cormorant (*Phalacrocorax auritus*) and the Humboldt penguin (*Spheniscus humboldti*), excretes salt (*1, 2*). The nasal gland of birds, as these authors pointed out, has been known for a long time—in albatrosses, since 1834 (*3*)—but its function was unknown before this recent work. We have had an opportunity (*4*) to observe excretion of salt by this gland in two adult Laysan albatrosses (*Diomedea immutabilis*) and three adult black-footed albatrosses (*D. nigripes*). The two species are commonly called gooney birds. These were sent from Midway Island, where they breed. Since the sexes are indistinguishable externally, the sexes of these birds were unknown.



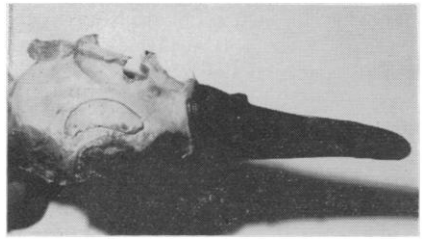


Fig. 1 (top). Head of black-footed albatross, showing tube-nostril, opening of nasal gland below nostril, and groove on beak along which nasal excretion flows to the tip. Fig. 2 (bottom). Skinned head of black-footed Albatross, showing nasal glands. The left gland has been removed to expose its bony socket. The right gland has had the capsule around it cut along the margin for differentiation in the photograph.

The birds were fed fish and given artificial sea water to drink. Voluntary drinking of this water by the birds was observed many times. A salt load was administered by feeding to each a piece of fish in which a gelatin capsule containing 0.8 g of NaCl was imbedded. Drops of the excretion appeared at the tips of the beaks of the birds, usually within 20 to 30 minutes, but occasionally in only 8 or 10 minutes. The differences may have been due to different rates of digestion of the fish. The fluid emerged from a small opening (Fig. 1) beneath the tube-nostril which is characteristic of albatrosses and other Procellariiformes. It flowed along the groove on the beak to the tip, from which it dripped or was shaken off. The drops fell at a rate of about 10 to 20 per minute during regular flow.

Samples of the nasal excretion were obtained from unrestrained birds by holding vials beneath the tips of the birds' beaks. This was tedious, for the animals often turned their heads to watch the vials, thus causing the drops to be missed. In an effort to speed up collection, the birds were restrained, but, in this case, dripping ceased within 30 to 60 seconds. Upon release of the birds, the dripping started again, within 1 to 2 minutes, usually at a greatly increased rate.

Determinations of sodium and potassium ion concentrations in the nasal excretion and blood plasma were made with a Beckman flame spectrophotometer. These data are given in Table 1. For the Laysan albatrosses, the mean value for sodium in the nasal excretion was 836 meq/lit.; for the black-footed albatrosses, 826 meq/lit. (the difference is not statistically significant). The values for the nasal excretion are like those reported by Schmidt-Nielsen and Sladen (*2*) for the penguin ($Na^+ = 726$ to 840 meq/lit.; $K^+ = 21$ to 29 meq/lit.) rather than like those for the cormorant ($Na^+ = 500$ to 600 meq/lit.; $K^+ = 5$ to 24 meq/lit.). There was no detectable change in the concentrations of these ions in the blood during excretion.

The nasal glands of these albatrosses thus act, like those of the other marine birds studied so far, to remove sodium and potassium ions from the blood. They are large and are situated in bony sockets above the eyes (Fig. 2), with ducts leading to the external openings. The similarity between the excretion in the penguin and the albatross is probably related to the fact that both are entirely marine in habitat and ingest sea water either voluntarily or accidentally. The action of this gland would enable the birds to use sea water as a source of water, in spite of its hypertonicity ($Na^+ = 420$ meq/lit.).

These albatrosses, which are large and docile, should make good subjects for studies of the action of this gland and its relationship to the water and ionic balance of marine birds. Hubert and Mable Frings have used the knowledge of the action of this gland to develop a method (*5*) for keeping these birds in captivity in apparently normal health.

Table 1. Amounts of sodium and potassium ions (meq) in the nasal excretion and blood plasma of albatrosses. Blood samples were drawn before feeding of NaCl and during excretion following feeding of NaCl. (σ_M, Standard error of the mean.)

No. of samples	Na^+ (meq/lit.) Range	Na^+ (meq/lit.) $M \pm \sigma_M$	K^+ (meq/lit.) Range	K^+ (meq/lit.) $M \pm \sigma_M$
	Nasal excretion			
7	792–856	829 ± 7.3	20 –28	24 ± 1.0
	Blood plasma before excretion			
4	162–171	167 ± 1.9	4.9– 7.0	5.7 ± 0.4
	Blood plasma during excretion			
5	159–170	164 ± 2.0	4.6– 6.0	5.4 ± 0.3

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4. These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Pennsylvania State University (NR160-464). The birds were shipped by air through the cooperation of the Bureau of Aeronautics, Department of the Navy, and naval authorities on Midway Island, to whom we wish to express our appreciation. This is paper No. 2285 in the "Journal Series" of the Pennsylvania Agricultural Experiment Station.
5. A report on this method is in preparation.

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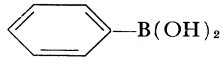
Correlation of Drug Penetration of Brain and Chemical Structure

Abstract. A study has been carried out on the permeability of the brain and a brain tumor to certain aromatic boronic acids with regard to their use in the neutron capture therapy of gliomas. The penetration of the brain by these compounds is discussed as a function of chemical substituent and benzene-aqueous partition coefficient.

The possibility of destroying differentially a group of neoplastic cells adjoining normal cells is presented by the nature of the reaction when boron-10 or lithium-6 captures a slow neutron (*1*). A large amount of energy liberated is shared between the two heavy fragments

evolved, the smaller of which is an alpha particle. In the case of the nonradioactive boron-10, the 2.5 Mev available (*2*) will propel the alpha particle only 9 μ in tissue (*3*) from the site of the reaction. In view of this short range, the lethal effect of the radiation is confined to the region of the cell containing the capturing atom of boron—hence the search for a boron-containing substance which will preferentially localize in tumor.

Since normal brain possesses a pronounced barrier mechanism not present in its tumors (*4*), the study described in this report (*5*) was motivated by an effort to learn what features a molecule should have in order to be selectively retarded in passage from blood to brain or accelerated in passage from blood to brain tumor. A series of molecules differing but little from one another in size and chemical configuration has been synthesized in order to minimize the variables. The basic moiety was phenylboronic acid:



Eight monosubstituted derivatives, most of them known compounds, were prepared for this study. These compounds were administered intraperitoneally to C3H mice bearing subcutaneously transplanted gliomas. The boron content (*6*) of 50 mg each of five tumor and five brain samples was determined at the several stated intervals after injection. Table 1 gives the average of these analyses in micrograms of boron per gram of tissue for brain and tumor at each of these stated times.

The localization factor for boron, the ratio of tumor to brain content of the element, is a measure of the relative rate of transfer between blood and the two tissues in question.

In a parallel study, approximately 10 mg of each of these nine compounds was distributed between 50 ml of a phosphate-buffered aqueous medium of *p*H 7.2 and 50 ml of benzene. The purpose of this study was to correlate the penetration of the brain by these drugs with their partition between benzene and water. Since benzene is a lipid solvent, the relative concentration of the compound in this medium could be a measure of lipid solubility. Many investigators have proposed that the penetration by drugs of the brain is mainly a function of their lipid solubility (*7*). By use of these monosubstituted phenylboronic acids, this theory could be tested. In addition, it was considered that the effect of an individual group on a molecule in enhancing or diminishing its penetrability could be determined and that this might aid in the design of effective neurotropic drugs on the one hand and tumor-seeking drugs on the other.

The compounds listed in Table 1 may be divided into three distinct groups. There are three compounds, *m*-carbamido-, *m*-carboxy-, and *p*-carboxyphenylboronic acids in the first group. All show tumor-to-brain localization factors of greater than 3, with a maximum attained 30 minutes to 1 hour following the injection. These three compounds likewise had a water-to-benzene partition coefficient greater than 50 to 60. They exhibited no effect on the central nervous

Table 1. Boron concentrations and ratios.

Time of sacrifice (min)	Concn. of B (μg/g of tissue)		Localization factor (tumor/brain)	Concn. of B (μg/ml of solvent)		Partition coefficient (aqueous/benzene)
	Tumor	Brain		Aqueous medium	Benzene	
	m-*Carbamidophenylboronic acid* (dose: 35 μg/g)					
15	12	2	6.0			
30	18	2	9.0			
60	24	4	6.0	10.9	< 0.2	> 54
120	18	6	3.0			
180	15	6	2.5			
	m-*Carboxyphenylboronic acid* (dose: 140 μg/g)					
15	57	10	5.7			
30	63	8	7.5			
60	61	10	6.1	12.1	< 0.2	> 60
120	60	13	4.6			
180	55	13	4.2			
	p-*Carboxyphenylboronic acid* (dose: 140 μg/g)					
15	44	11	4.0			
30	68	10	6.8			
60	62	8	7.7	11.4	< 0.2	> 57
120	47	9	5.2			
180	38	8	4.7			
	p-*Methoxyphenylboronic acid* (dose: 35 μg/g)					
15	29	44	0.7			
30	33	40	0.8			
60	34	38	0.9	13.1	2.5	5
120	36	34	1.1			
180	30	26	1.2			
	Phenylboronic acid (dose: 35 μg/g)					
15	34	51	0.7			
30	34	44	0.8			
60	29	30	1.0	14.4	2.3	6
120	26	29	0.9			
180	34	40	0.8			
	o-*Nitrophenylboronic acid* (dose: 35 μg/g)					
15	25	41	0.6			
30	29	41	0.7			
60	26	36	0.7	12.6	1.9	7
120	41	49	0.8			
180	43	50	0.9			
	p-*Chlorophenylboronic acid* (dose: 35 μg/g)					
15	13	69	0.2			
30	19	66	0.3			
60	33	57	0.6	6.9	5.6	1
120	32	58	0.6			
180	30	51	0.6			
	p-*Tolylboronic acid* (dose: 35 μg/g)					
15	18	53	0.3			
30	20	44	0.5			
60	30	37	0.8	8.0	5.2	2
120	31	25	1.2			
180	29	18	1.6			
	m-*Aminophenylboronic acid* (dose: 35 μg/g)					
15	25	21	1.2			
30	32	26	1.2			
60	28	23	1.2	12.4	< 0.2	> 62
120	22	17	1.3			
180	16	13	1.2			

system as judged by gross observations of the animal behavior, even in large doses.

In the second category are *o*-nitro-, *p*-methoxy-, and the unsubstituted phenylboronic acid. These three compounds had a localization factor of nearly 1, but in most cases the brain had a slightly higher boron content. These compounds produced an immediate depressant action upon the animal's spontaneous activity and responsiveness to stimuli, and soon the animals were lying flaccid and supine, unresponsive to surgical operations. The aqueous-benzene partition coefficient was from 5 to 7.

In the final category are two compounds, *p*-tolylboronic acid and *p*-chlorophenylboronic acid. At the standard dose of 35 μg per gram of mouse, the compounds were highly toxic, and the LD_{50} was approached. Coma in these animals was often accompanied by generalized twitching of the limbs. Initially, both showed a tumor-to-brain localization of 0.2 to 0.3, and thus their behavior suggests that they encounter, not a barrier slowing their penetration into brain, but an avenue facilitating it. The water-benzene partition coefficient was from 1 to 2. It is apparent that these compounds which show maximal effects on the central nervous system and greater concentration in the normal brain relative to tumor do concentrate to a greater extent in the lipid solvent, benzene, whereas those which show no obvious effect on the central nervous system and low concentration in the brain have a much higher partition coefficient in an aqueous rather than in a lipid phase.

Initially, *p*-tolylboronic acid showed a localization factor 0.3, but gradually this ratio was reversed, and after 3 hours the tumor concentration was nearly twice the brain concentration. Methyl groups attached to an aromatic nucleus are readily oxidized in vivo to a carboxyl group—for example, toluene is transformed on ingestion to benzoic acid (*8*). If this type of conversion occurs with *p*-tolylboronic acid, then *p*-carboxyphenylboronic acid would be formed and this reversal would be understandable. *p*-Chlorophenylboronic acid, on the other hand, maintained a localization factor of 0.6 even after 3 hours.

An exception to this three-category division would appear to be *m*-aminophenylboronic acid. This compound shows an effect on the central nervous system only at doses of 70 μg per gram of mouse and yet the boron localization factor is nearly 1 and the aqueous-benzene partition coefficient is greater than 60. It is conceivable that this compound might be intermediate between groups 1 and 2 or possibly that a principle other than lipid solubility is involved.

In summary, it can be stated that increased solubility in a lipid solvent is an important measure of the penetration of the brain by a drug. Of the compounds which were examined, introduction of a methyl or a chloro substituent into an aromatic nucleus definitely enhanced the penetration of a molecule into the brain, while a carboxyl or carbamido substituent markedly inhibited its entrance.

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5. My sincere thanks are due to Dr. William H. Sweet for his kind interest, great encouragement, and critical appraisal of this investigation; to Janette Robinson and Beverly Whitman for their willing cooperation in this entire study; to Dr. Adelbert Ames for valuable discussions; and to Paul Szabady for technical assistance. This work was supported by a grant from the U.S. Atomic Energy Commission under contract No. AT (30-1)-1093.
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Acute Infection of Mice with Smith Strain of Staphylococcus aureus

Abstract. Two serologically distinct variants found in a unique strain of staphylococcus produce coagulase and are phagocytized, but only one is virulent to mice. Only virulent cocci grow rapidly within leukocytes. Leukocyte destruction by the virulent strain and release of many phagocytized cocci precedes mouse death. The leukocidic agent may be delta-hemolysin.

The Smith strain of *Staphylococcus aureus* (*1*), which was isolated in 1930 by Dubos and briefly described by Smith and Dubos (*2*), is an unusual organism. We have studied in detail the mouse infections produced by this strain because it is unique. An examination of the strain showed that there were at least two cellular types in the broth culture. This differentiation was most readily made when the plasma soft agar reaction described by Finkelstein and Sulkin (*3*) demonstrated the presence of both diffuse and compact colonies in the culture. Although both types of Smith colonies produce coagulase, as determined by the tube test, only the diffuse colony in the plasma soft agar was virulent to mice. We have encountered no other staphylococcus strain with such capacity for inducing an acute infection in mice when injected by the intraperitoneal route. We have found no other strain of coagulase-positive staphylococcus which produced diffuse colonies in soft agar containing normal plasma or serum.

Smith and Dubos (*2*) stated that the strain produced pigment, was coagulase-positive, and was phage type 44A/42E. We have observed that the strain readily produced delta-hemolysin. Very rarely one may observe a colony producing beta-hemotoxin, but most colonies did not produce demonstrable alpha- or beta-hemotoxins on sheep washed-blood-cell agar plates after incubation in 10 percent carbon dioxide. We have never observed the production of staphylokinase or bacterial protease by the organism, as judged by lysis of fibrin formed around colonies on fibrinogen agar plates. The intraperitoneal median lethal dose (LD_{50}) of the diffuse-colony culture in Swiss albino mice was approximately 4×10^6 viable cells per mouse when injected as a broth suspension, but, with 0.5 ml of 5 percent hog gastric mucin, the LD_{50} was about 580 cells per mouse. Other strains of *Staphylococcus aureus* isolated from lesions of human beings and of laboratory animals showed an intraperitoneal LD_{50} of 1×10^6 viable cells in mucin, but did not consistently cause death when 1×10^9 viable cells were injected without mucin.

The presence of diffuse and compact coagulase-positive colonies in the Smith strain cultures was confirmed when the strain was sent to Finkelstein and Sulkin (*4*). The latter described their observations and noted that the Smith compact isolate was agglutinated with absorbed Group II (Cowan) antiserum and was lysed by phage type 44A. The diffuse-colony isolate, however, did not agglutinate with Groups I, II, or III absorbed antisera, nor was it lysed by type 44A phage. This suggested that the two cellular types were serologically distinct and that the compact type contained an antigen not found in the diffuse cell.

We had observed that the separation of diffuse and compact colonies of the Smith strain by the plasma soft agar also separated the mouse-virulent colonies from the mouse-avirulent. Peritoneal washings from any mouse dying from infection by the Smith strain showed only diffuse-type colonies in plasma soft agar. Each colony type, when isolated and grown through four or five broth-to-broth transfers without reisolation, showed the presence of a few cells of the other type colony. Without reisolation, a compact-colony broth culture might contain a few diffuse-type cells. Large challenge doses of the compact-type cultures produced death in an occasional mouse, but peri-