mented in various degrees by $CaCO_3$, crops out at low-tide to mid–high-tide levels on many beaches in the archipelago. The faunas from these rocks include the same species found on adjacent modern beaches. Because beach rock can form very rapidly in tropical and subtropical regions and because these rocks in the Galápagos are associated with modern sea level stands, contain modern faunas, and dip seaward, we infer that these deposits are younger than Pleistocene.

On Isla Rabida, Hertlein and Strong (*8*) reported marine mammal bone and two species of mollusks from scoriaceous talus at the base of steep cliffs above high-tide level. They concluded from the species present that the age was Pleistocene, although they listed the two mollusk species as ranging into the Recent. Our examination of the shore of Isla Rabida revealed that storm-tossed shells and bones of sea lions and birds are now being rapidly buried supratidally by debris falling from the cliffs. We conclude that these deposits at outcrop are also very young, perhaps no more than a few hundred years old.

In 1954 at Urvina Bay, Isla Isabela, the shore was suddenly uplifted 4.5 m shortly before the eruption of nearby Volcan Alcedo (*16*). The uplift exposed supratidal to subtidal bay and rocky shore communities. Now only well-skeletonized forms remain, such as calcareous algae, mollusks, echinoderms, and barnacles. Uplifted biotas may thus be quite recent and do not necessarily imply Pleistocene or older ages, as commonly assumed in the past. The relative importance and scale of localized uplift in forming the present islands have been debated by geologists (*16, 17*). These deposits and terrace deposits confirm the importance of localized uplift in shaping the islands, but they do not indicate similarity of ages.

Shallow-water, tidal, and supratidal marine fossil assemblages in the Galápagos Islands are more abundant and diverse than previously recognized. They occur in at least six geologic settings. In contrast to early paleontologic studies, our observations and conclusions (Fig. 2) corroborate independent geologic evidence (*2, 3*) that the western Galápagos Islands emerged from the sea less than 2 or 3 million years ago. The easternmost islands may be about 1 million years older on the basis of plate tectonic age estimates (*2, 3*), although these estimates do not suggest when the islands appeared above sea level. Radiometric dates on subaerial lava flows indicate that Isla Española stood above sea level at least 3 million years ago (*14*). The marine paleontological record is thus reconciled with the geologic evidence, and together they indicate that all adaptive radiation in the terrestrial biota of the Galápagos Islands occurred within the past 3 to 4 million years.

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20. This report is based on results obtained on an expedition organized by W. D. Pitt; other participants were L. Pitt and M. J. James. W. D. Pitt and M. J. James identified the fossil mollusks from Cerro Gallina and Cerro Colorado. R. Hey read a draft of the report. We thank them, F. Köster, A. DeRoi, J. DeRoi, and Ecuadorian military officials for assistance and courtesies. This is contribution 367 from the Charles Darwin Research Foundation.

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Blood-Brain Barrier: Endogenous Modulation by Adrenal-Cortical Function

Abstract. *The blood-brain barrier restricts the passage of molecules from the blood to the brain. The permeability of the barrier to iodine-125–labeled bovine serum albumin was examined in rats that had undergone adrenalectomy, adrenal demedullation, and corticosterone replacement. Adrenalectomy, but not adrenal demedullation, increased the permeability of brain tissue to the isotopically labeled macromolecule; corticosterone replacement reversed this effect. These results indicate that the blood-brain barrier may be hormonally regulated; that is, the pituitary-adrenal axis may physiologically modulate the permeability of the brain microvasculature to macromolecules.*

The blood-brain barrier (BBB) restricts the passage of polar compounds and macromolecules from the blood into the brain interstitium (*1, 2*). This barrier arises from several morphologic characteristics of the brain vasculature; namely, the tight junctions between adjacent endothelial cells and the virtual absence of fenestrations and cytoplasmic pinocytotic vesicles within endothelial cells (*1–3*). Anatomic and physiologic evidence indicates that cerebral microvascular permeability may be responsive to both neural and humoral influences (*4, 5*) and has led to speculation on the roles of these systems in maintaining homeostasis within the central nervous system through actions on the BBB (*5, 6*).

Dexamethasone and other synthetic glucocorticoids have been widely used for the clinical treatment of brain edema (*7*) and have been shown to alter the flux of water across the BBB (*8*). Furthermore, dexamethasone reduces the disruption of the BBB produced by drug-induced acute hypertension (*9*), repeated convulsive seizure activity (*10*), or hypertonic perfusion of the brain (*11*). These observations prompted our speculation that the pituitary-adrenal axis may take part in the endogenous regulation of BBB integrity and that adrenal glucocorticoids may specifically influence the permeability characteristics of the undisrupted brain microvasculature. Therefore, we investigated the effects of adrenalectomy, selective adrenal demedullation, and corticosterone replacement on the permeability of the BBB to ^{125}I-labeled bovine serum albumin (BSA; 69,000 molecular weight) in conscious, freely moving rats. We now report that total adrenalectomy, but not selective adrenal demedullation, significantly increases the permeability of ^{125}I-labeled BSA into the brain and that corticoster-

one replacement in adrenalectomized rats restores normal BBB function.

Male Sprague-Dawley rats (200 to 250 g in body weight; Zivic Miller) underwent bilateral adrenalectomy, bilateral adrenal demedullation, or sham operation. All rats were provided with both 0.9 percent saline drinking solution and tap water. At least 2 weeks after surgery, mean arterial pressure was measured by a catheter implanted in the tail artery (*12*). Freshly prepared ^{125}I-labeled BSA (10 to 20 μCi in 200 μl) (*13*) was administered through an external jugular catheter (*12*); 20 minutes later, a venous blood sample (0.5 ml) was removed. Rats were then immediately anesthetized with pentobarbital (25 mg per kilogram, intravenously) and perfused in situ with approximately 160 ml of 0.9 percent saline. Brains were removed and dissected into five regions, including the cerebellum, brainstem, rostral forebrain, ventral forebrain, and dorsal forebrain (*14*).

As verification that label remaining in the vascular lumen after perfusion did not contribute substantially to measurements of cerebrovascular permeability to ^{125}I-labeled BSA, in some experiments rats were also given freshly prepared ^{51}Cr-labeled erythrocytes (*15*). Unlike ^{125}I-labeled BSA, ^{51}Cr-labeled erythrocytes do not leave the vascular lumen; consequently, measurement of ^{51}Cr in brain tissue samples provides a direct index of perfusion adequacy. In all instances, amounts of ^{51}Cr in brain tissue were negligible (not significantly different from inset rument background radiation), indicating that perfusions in these experiments reliably cleared the vascular lumen and that intraluminal ^{125}I-labeled BSA did not contribute significantly to measurements of cerebrovascular permeability.

Adrenalectomy produced significant increases in the permeability of the BBB to ^{125}I-labeled BSA, with the magnitude of the increases ranging from 27 percent in the brainstem to 49 percent in the dorsal forebrain (Fig. 1a). In addition, mean arterial pressure and concentrations of corticosterone in plasma of adrenalectomized rats were significantly reduced (*16*) (Table 1). However, concentrations of electrolytes in plasma did not differ significantly between adrenalectomized rats and those that underwent sham operation, indicating compensation for the loss of adrenal mineralocorticoids (*17*). Increases in ^{125}I-labeled BSA measurements induced by adrenalectomy were not accompanied by changes in ^{51}Cr measurements in brain tissue after perfusion of animals that had received ^{51}Cr-labeled erythrocytes, indicating that apparent differences in permeability were not an artifact of altered perfusions of brains in the two groups.

In contrast to total adrenalectomy, selective adrenal demedullation, wherein adrenal cortical function remained intact, did not alter BBB permeability to ^{125}I-labeled BSA (Fig. 1b). Concentrations of corticosterone and electrolytes in plasma as well as mean arterial pressures also did not differ significantly between rats that had undergone sham operation or adrenal demedullation (Table 1), indicating normal adrenal-cortical

Table 1. Mean arterial pressure and concentrations of corticosterone in plasma of rats subjected to sham operations, adrenalectomy, adrenal demedullation, or adrenalectomy with corticosterone replacement.

Treatment	*n*	Mean arterial pressure (mmHg)	Corticosterone concentration (μg/dl)
Sham adrenalectomy	12	99.5 ± 2.3	13.9 ± 1.4
Adrenalectomy	12	73.4 ± 2.2*	0.5 ± 0.1*
Sham demedullation	16	109.5 ± 2.0	9.2 ± 1.7
Adrenal demedullation	16	108.5 ± 1.4	6.4 ± 1.1
Sham adrenalectomy	8	98.5 ± 3.0	10.9 ± 1.0
Adrenalectomy	7	75.0 ± 3.9*	0.6 ± 0.2*
Corticosterone replacement	7	91.4 ± 2.9	13.3 ± 1.5

*$P < 0.05$ compared to sham adrenalectomy.

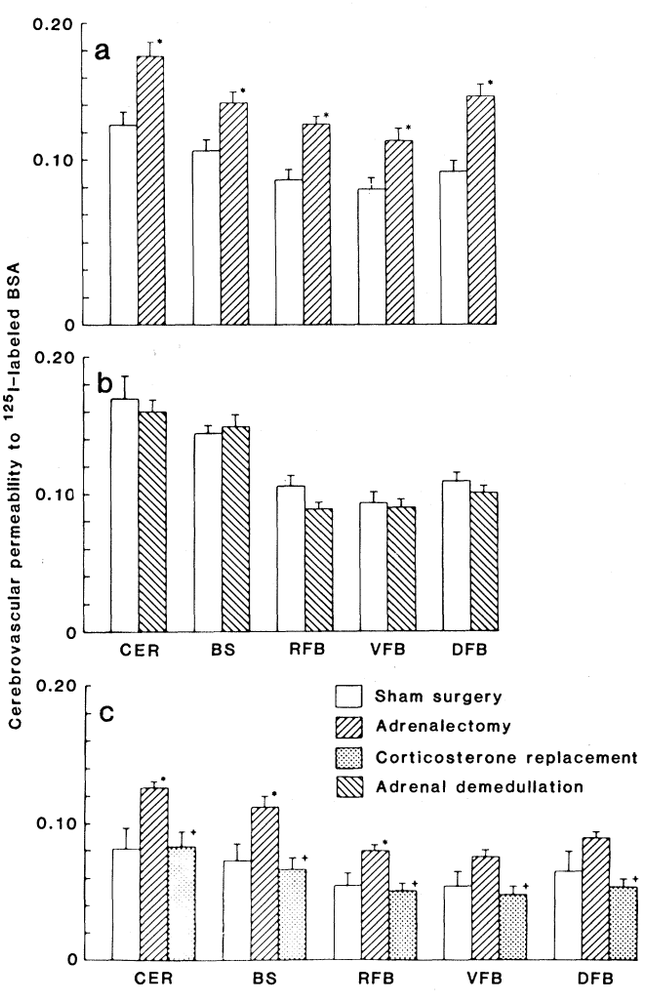

Fig. 1. Effects of (a) bilateral adrenalectomy, (b) bilateral adrenal demedullation, and (c) bilateral adrenalectomy with corticosterone replacement on the passage of intravenously administered ^{125}I-labeled BSA into brains of rats. In corticosterone replacement experiments, approximately 10 days after adrenalectomy or sham surgery, rats were given corticosterone (0.5 mg per kilogram subcutaneously in 5 percent ethanol) or vehicle twice daily for 4 days. On the following day, rats were given a final injection of corticosterone (0.5 mg per kilogram subcutaneously) or vehicle 1 hour before injection of ^{125}I-labeled BSA. Concentrations of label in blood did not differ significantly among groups within an experiment. Data were analyzed by two-way analysis of variance (ANOVA) with repeated measures (a and b) or one-way ANOVA (c). Significant differences between means ($P < 0.05$) were determined by the Newman-Keuls test (*34*). Each bar represents the amount of label (in counts per minute) per milligram of brain tissue divided by that per milligram of blood times 100 (mean ± standard error of the mean). Populations of experimental groups are listed in Table 1. Abbreviations: CER, cerebellum; BS, brainstem; RFB, rostral forebrain; VFB, ventral forebrain; and DFB, dorsal forebrain. *$P < 0.05$ compared to sham adrenalectomy values. †$P < 0.05$ compared to adrenalectomy values.

function. These results (Fig. 1, a and b) indicate that the absence of a factor or factors associated with the adrenal cortex rather than with the adrenal medulla were responsible for altered BBB permeability associated with bilateral adrenalectomy. Accordingly, administration of physiologic doses of corticosterone to adrenalectomized rats reversed the adrenalectomy-induced increases in the passage of ^{125}I-labeled BSA into the brain (Fig. 1c) (*18*) as well as the decreased mean arterial pressure associated with adrenalectomy (Table 1).

These data show that the pituitary-adrenal axis influences macromolecular permeability of the brain in conscious rats and specifically indicate that adrenal glucocorticoids may mediate altered BBB function. Moreover, although the pharmacologic administration of adrenergic agonists (for example, epinephrine, norepinephrine, or amphetamines) may increase the extravasation of ^{125}I-labeled BSA into the brain (*19*) (apparently a result of acute increases in mean arterial pressure), the physiologic loss of circulating epinephrine after adrenalectomy or adrenal demedullation does not significantly affect cerebrovascular permeability to ^{125}I-labeled BSA (Fig. 1b).

The sites or mechanisms by which manipulation of the pituitary-adrenal axis influences BBB permeability to ^{125}I-labeled BSA are not clearly established by these data. Intracisternal injections of adrenocorticotropic hormone (ACTH) and other melanotropic peptides produce dose-dependent increases in the appearance of ^{125}I-labeled albumin in the cerebrospinal fluid of rabbits (*20*). Indeed, in our experiments, concentrations of ACTH would be elevated by adrenalectomy because of the loss of adrenal glucocorticoid feedback, and the increased permeability of ^{125}I-labeled BSA may be a consequence of elevated amounts of ACTH. Alternatively, changes in permeability associated with adrenal manipulation may have been caused by changes in systemic arterial pressure (Table 1). However, brain perfusion is autoregulated between 60 and 140 mmHg (*21*), and no predictable correlation between decreases in systemic arterial pressure and permeability of the BBB has been shown (*22*). In our experiments, we were also unable to detect significant correlations between mean arterial pressure and cerebrovascular permeability within treatment groups, indicating that adrenal effects on BBB permeability are unlikely to be mediated by arterial pressure.

Passage of ^{125}I-labeled BSA from the plasma into the brain may occur at several morphologically defined sites: (i) the choroid plexus, (ii) the circumventricular organs, and (iii) the brain microvessels forming the BBB. The capillaries of the choroid plexus and the circumventricular organs are porous and have fenestrated epithelia that are readily permeable to blood-borne macromolecules; however, the apical bands of tight junctions joining the epithelial cells lining the ventricles in these locations restrict the passage of macromolecules into the cerebrospinal fluid and through the rest of the brain (*1, 23*). Because the surface area of the capillaries making up the BBB is at least 5000 times greater than the surface area of those of the circumventricular organs (*24*), the quantitatively important entry site of ^{125}I-labeled BSA into the brain appears to reside at the BBB.

Ultrastructure studies indicate that transendothelial vesicular transport (pinocytosis) is a primary cellular mechanism by which plasma protein constituents and macromolecules pass through the cerebrovascular endothelium (*25*). Under a variety of experimental conditions associated with increased BBB permeability to macromolecules—including acute hypertension (*21, 26*), ischemia (*27*), compression injury (*28*), seizures (*19, 29*), administration of histamine (*30*), and the injection of cyclic nucleotides (*31, 32*)—enhanced vesicular transport of the macromolecule horseradish peroxidase (40,000 molecular weight) has been visualized. Collectively, these data indicate that increased pinocytotic activity is an important mechanism for macromolecule entry into the central nervous system during BBB dysfunction. Therefore, glucocorticoids may directly or indirectly participate in the cellular mechanisms (*9, 31*) by which cerebrovascular vesicular transport is normally suppressed. Consequently, under conditions of reduced glucocorticoid availability (adrenalectomy), increased vesicle-mediated entry of ^{125}I-labeled BSA may occur. These actions of glucocorticoids may involve membrane stabilization (*33*) or interaction with other substances proposed to take part in the regulation of cerebrovascular permeability (*5, 6, 30 31*). Definition of the mechanisms regulating permeability and the interplay between these proposed regulators awaits further study.

In conclusion, disruptions in adrenalcortical state alter cerebrovascular permeability to ^{125}I-labeled BSA. While the magnitude of the changes is small, these observations reveal that the BBB is a dynamic interface between the peripheral and central nervous system and that the entry of macromolecules into the central nervous system may be responsive to physiologic influences such as circulating glucocorticoids. This observation challenges commonly held conceptions of the BBB and indicates that the pituitary-adrenal axis may function as a physiologic regulator of BBB permeability. As such, the pituitary-adrenal axis may regulate the entry of macromolecules and polar compounds into the central nervous system and thereby indirectly alter the central actions of diffusion-limited drugs and humoral substances.

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14. The cerebellum was removed, and a transverse

section was made at the level of the cerebral peduncles to separate the brainstem. A transverse section at the level of the optic chiasm passed through the anterior commissure and separated the forebrain rostral to this transection. The remaining tissue was divided by a horizontal section 1 mm above the anterior commissure. Tissue ventral to this cut, which included the hypothalamus and amygdala, formed the ventral forebrain, and tissue dorsal to this section, which included the hippocampus and most of the neocortex, formed the dorsal forebrain.

15. A washed suspension of rat red blood cells in Hanks buffered salt solution (HBSS) was incubated with approximately 0.75 mCi of ^{51}Cr for 1 hour at 37°C. Cells were washed repeatedly in HBSS and finally diluted with HBSS to form the injected material.
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18. The difference in the ratios of amount of label in brain tissue and in blood between Fig. 1c and Fig. 1, a and b, resulted from administration of 20 μCi of ^{125}I-labeled BSA (Fig. 1c) rather than 10 μCi (Fig. 1, a and b), which caused greater concentrations of label in the blood and increased the denominator of the ratio. Despite these differences between experiments, concentrations of ^{125}I-labeled BSA in blood did not differ between groups within an experiment.
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Receptor-Mediated Transport of Insulin Across Endothelial Cells

Abstract. *Hormones such as insulin are transported from the interior to the exterior of blood vessels. Whether endothelial cells, which line the inner walls of blood vessels have a role in this transport of hormones is not clear, but it is known that endothelial cells can internalize and release insulin rapidly with little degradation. The transport of iodine-125–labeled insulin was measured directly through the use of dual chambers separated by a horizontal monolayer of cultured bovine aortic endothelial cells. In this setting, endothelial cells took up and released the labeled insulin, thereby transporting it across the cells. The transport of insulin across the endothelial cells was temperature sensitive and was inhibited by unlabeled insulin and by antibody to insulin receptor in proportion to the ability of these substances to inhibit insulin binding to its receptor. More than 80 percent of the transported insulin was intact. These data suggest that insulin is rapidly transported across endothelial cells by a receptor-mediated process.*

The mechanism by which macromolecules such as polypeptide hormones are transported across nonfenestrated capillaries is not well understood. Endothelial cells probably have an important role in this process since they are connected by tight junctions and thus form a major barrier for the rapid diffusion of hormones to their target cells (*1–3*). The rapid onset of action after secretion of hormones is thought to be important for regulation of the metabolic homeostasis. Hence the control of hormonal transport across the vascular barrier may be a rate-limiting and regulating step for the mediation of hormonal actions in many tissues (*1–3*). To traverse the endothelial cell barrier, polypeptide hormones could diffuse between the endothelial cells or be transported through the cells. We and others have been studying the processing of insulin by endothelial cells (*4–7*). Insulin, like other polypeptide hormones, has a rapid onset of action and must therefore cross the endothelial cell barrier quickly in order to react with target muscle and adipose tissues.

We showed earlier that endothelial cells can internalize insulin rapidly (*4*). The internalized insulin is released from the cells in minutes with little degradation (*4*). We have now directly measured the transport of ^{125}I-labeled insulin across the endothelial cell barrier.

To determine whether insulin can be transported across endothelial cells, we used dual-chambered vessels, the upper chambers of which were prepared by attaching dialysis membranes to plastic cylinders (1 × 2 cm). The membrane used had a nominal cutoff of molecular weight 50,000, and within 2 hours, 44 ± 6 percent [mean ± standard error of the mean (S.E.M.)] of the ^{125}I-labeled insulin diffused from the upper to the lower chamber in the absence of cells. The culture apparatus was sterilized by exposure to ultraviolet light for 48 hours, and the cylinders were subsequently attached to the covers of 60 m*M* culture dishes—that is, to the lower chambers—with sterile vacuum grease. The membrane was coated with human fibronectin, and bovine aortic endothelial cells harvested from calf aorta (*4*) were densely seeded and allowed to form a confluent monolayer for 48 hours. The lower chambers contained Dulbecco's minimum essential medium with 10 percent calf serum and were in communication with the upper chambers, which contained the same growth medium.

For the transport studies, the medium was replaced with 0.1*M* Hepes binding buffer at *p*H 7.8 (*4*). Insulin labeled with ^{125}I, inulin labeled with ^{14}C, and other molecules were placed in the upper chamber, and the system was incubated in a shaker water bath set at 37°C. At various times, 1.0-ml samples were taken from the bottom chamber for measurement of radioactivity. The volume of the lower chamber was maintained at 9.0 ml with addition of fresh buffer.

When ^{125}I-labeled insulin (1 ng/ml) was added alone, 15 ± 2 percent of it was transported across the cellular and membrane barrier after 2 hours at 37°C (Fig. 1A). The specific transport rate for labeled insulin, calculated after subtracting the amount of labeled insulin transported in the presence of $10^{-6}M$ unlabeled insulin, was linear for the 2 hours. Addition of unlabeled insulin ($1.7 \times 10^{-9}M$ to $1.7 \times 10^{-6}M$) with the labeled insulin resulted in a progressive decrease in the amount of labeled insulin transported, indicating that the transport is a saturable process. Insulin concentrations of 10^{-9}, 10^{-8}, and $10^{-6}M$ inhibited the transport of labeled insulin by 43 ± 2, 44 ± 14, and 67 ± 7 percent, respectively (Fig. 1A). This correlated well with the ability of the unlabeled insulin to compete with labeled insulin for binding to the receptors on endotheli-