probably be an even greater difference with cells of comparable size. Leserman *et al.* (*7*) expressed reservations concerning the possible efficacy of antibody targeting for intracellular delivery of vesicle contents. While such arguments should be carefully considered, the data from our system suggest that targeted lipid vesicles may be effective in promoting the intracellular delivery of their contents under appropriate conditions. The purity of the antibody, the number of antibody molecules per vesicle, the ratio of internal volume per lipid, the number of vesicles per cell, and the retention of vesicle contents all have important roles in the efficacy of delivery.

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Adrenocorticotropic Hormone May Be Transported Directly from the Pituitary to the Brain

Abstract. *Experiments were designed to test the hypothesis that pituitary hormones may be delivered directly to the brain. Concentrations of adrenocorticotropic hormone (ACTH) in the plasma were determined in blood samples obtained simultaneously from the carotid artery, the sagittal sinus, and the jugular vein of three awake sheep. Seizures were induced electrically to stimulate ACTH secretion, and at precise intervals thereafter several simultaneous comparisons were made in each animal. In many of the post-seizure comparisons, the ACTH plasma concentrations within the sagittal sinus exceeded those within the carotid artery as well as those within the jugular vein, indicating that this hormone was released from the pituitary and carried directly through capillary beds of brain to the venous blood within the sagittal sinus. The experiment was repeated in one hypophysectomized sheep and, in this animal, ACTH concentration in the plasma was reduced, but that in the sagittal sinus still was elevated after the seizure, an indication that some ACTH (or ACTH-like material) was released from the brain itself.*

Pituitary secretions have long been thought to enter the systemic circulation directly via the cavernous sinus (*1*), but several studies have suggested that some pituitary hormones may be carried directly to the brain (*2–4*). We now report the results of experiments designed to test this hypothesis.

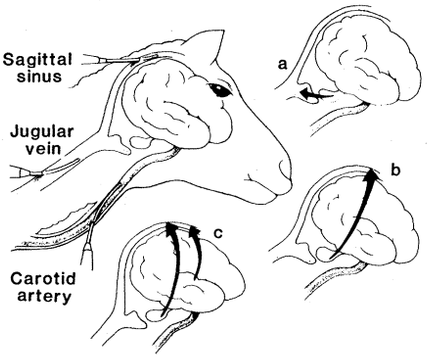

Fig. 1. The experimental model is illustrated. If a pituitary hormone is released into the cavernous sinus, jugular vein concentrations should exceed carotid artery concentrations (a). If a pituitary hormone is carried to the brain and on into the sagittal sinus, the sagittal sinus concentration should exceed the carotid artery concentration (b). Like many other hormones, ACTH is produced by both the brain and the pituitary, and the ACTH found in sagittal sinus venous blood may have come from either source (c). Since ACTH concentrations were diminished by hypophysectomy, most of the ACTH found in high concentration within the sagittal sinus must have come from the pituitary. Yet the persistence of ACTH in sagittal sinus samples after hypophysectomy supports other evidence (*9*) that this hormone is produced and released by the brain as well as the pituitary.

Blood samples were obtained at precisely timed intervals simultaneously from the carotid artery, the sagittal sinus, and the jugular vein of individual sheep; awake and unrestrained animals were used. For each sample, the concentration of adrenocorticotropic hormone (ACTH) was determined by radioimmunoassay. Electrically induced convulsions were used to stimulate ACTH secretion, and during many comparisons the ACTH plasma concentration in the sagittal sinus exceeded that within the carotid artery.

For these experiments three Suffolk sheep were anesthetized and in each animal we placed a single common carotid artery into a subcutaneous loop 10 cm long. After the carotid artery loops were well healed, the animals were anesthetized again; at craniotomy a Silastic catheter (0.16 cm in diameter) was positioned within the sagittal sinus. The catheter was passed forward into the sagittal sinus a distance of 1.0 cm from an insertion point just anterior to the entrance of the vein of Galen (*5*). The catheter was secured to the dura, the scalp, and the wool over the back of the animal, and the craniotomy wound was closed. The next day an indwelling catheter was placed, while the sheep was given a local anesthetic, into the previously prepared ca-

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rotid artery loop and into the jugular vein (Fig. 1). All catheters were kept open with dilute heparin infusions.

Subsequently, each of the three animals, treated as described above, was placed in a protective stanchion and, while the animal was awake, a grand mal seizure was induced in each by the application of 110 V between temporal scalp electrodes for 3 seconds (*6*). The seizures typically were 45 to 60 seconds in duration and included a 15-second tonic phase followed by clonic movement for 30 to 45 seconds. All animals were awake and standing at 90 seconds.

At three intervals prior to the seizure, samples were obtained simultaneously from all of the catheters. Aspiration from the jugular vein catheter and the carotid artery catheter was synchronized with the rate of aspiration from the sagittal sinus, and in all animals 8 to 12 ml of blood could by aspirated from each site in less than 1 minute, generally in 20 to 30 seconds. Post-seizure simultaneous samples from the three sites were obtained 1, 2, 5, 10, and 15 minutes after the 3-second electrical stimulus. All samples were aspirated into plastic syringes and injected into heparinized plastic tubes, which were then immediately placed on ice. The plasma was separated in a centrifuge at 4°C and stored in 1-ml portions in plastic tubes at −20°C.

In another Suffolk sheep, total hypophysectomy was performed (*7*); immediately afterward, a carotid artery loop was established and the sagittal sinus was catheterized. On the next day, additional catheters were placed in the carotid artery loop and the jugular vein. The seizure experiment was repeated, and simultaneous plasma samples from the three sites were obtained by the same methods at the same intervals. Subsequent autopsy revealed that the pituitary had been completely removed.

In samples from each of these animals radiommunoassays were performed to determine the plasma ACTH concentrations (*8*). The antibody to ACTH cross-reacts with ACTH 1-39; it has less affinity for the smaller ACTH fragments.

As shown in Table 1, a rapid increase in plasma ACTH concentrations followed the seizures, and in all four of the animals there were several instances when the sagittal sinus concentration exceeded the simultaneous carotid artery concentration. In 10 of the 15 post-seizure comparisons made on nonhypophysectomized animals, sagittal sinus concentrations were substantially higher than simultaneous carotid artery concentrations. In only one comparison did jugular vein concentration exceed that in the sagittal sinus concentration. Hypophysectomy resulted in lower ACTH plasma levels, but in both the pre-seizure and the post-seizure samples, sagittal sinus concentrations remained higher than simultaneous carotid artery and jugular vein concentrations.

These experiments were based on the premise that the simultaneous determination of hormone concentrations at the three sampling sites would allow an understanding of the direction that hormone-enriched blood flows from the pituitary. While differences in concentration of hormone between carotid artery and jugular vein indicate that hormones are being released from the pituitary, those differences alone do not tell the direction of hormone transport from the pituitary. Sampling from sites above and below the pituitary (*2*) permits the direction of secretion to be determined. If a pituitary hormone is released downward into the cavernous sinus the concentration of the hormone within the jugular vein will exceed the simultaneous concentration within both the carotid artery and the sagittal sinus (Fig. 1A). But if a pituitary hormone is added to blood flowing upward toward the sagittal sinus, the concentration of a pituitary hormone within the sagittal sinus will exceed the simultaneous concentration within the carotid artery (Fig. 1B). Since many pituitary hormones, including ACTH, are produced by the brain (*9*, *10*), higher sagittal sinus hormone concentrations could follow either pituitary release or brain release (Fig. 1C). Only by repeating the experiment after hypophysectomy could the source of sagittal sinus hormones be determined.

In these experiments the hormone concentration within the carotid artery at each given moment must serve as the baseline control. The normal fluctuations in circulating hormone concentration make this a changing baseline; it rises during periods of hormone secretion, more so for a hormone with a lengthy intravascular half-life. While a high baseline within the carotid artery makes quantitative determinations of hormone release from the pituitary more difficult, a low carotid baseline facilitates such determinations. Simultaneous comparisons made immediately after a hormone is released and before it has recirculated into the carotid artery offer the best opportunity to understand the direction of pituitary secretion.

It was the need to precisely time the stimulation of hormone release from the pituitary that led to the use of electrically induced convulsions. While most other methods of stimulation require several minutes (*11*), the convulsion trigger can be delivered in 3 seconds. Sampling can be performed at precise intervals thereafter, and the abrupt release of hormones

Table 1. The plasma concentrations (in picograms per milliliter) of ACTH at the three sampling sites: sagittal sinus (SS), carotid artery (CA), and jugular vein (JV) in the four sheep. Simultaneous samples were obtained from all three sites at 1-minute intervals before seizures and at several precisely timed intervals after seizure. In the nonhypophysectomized animals, 10 of the 15 ACTH concentrations in the sagittal sinus after seizure exceeded simultaneous carotid artery ACTH concentrations by 33 percent or more (arrows). In only one instance, the 15-minute comparison in the sheep Lizzie, did the concentration in the jugular vein exceed that in the sagittal sinus (asterisk). Although hypophysectomy reduced the amount of circulating ACTH, nevertheless significant elevations were noted in the sagittal sinus samples after seizure.

Animal	Plasma ACTH (pg/ml)							
	Before seizure (minutes)			After seizure (minutes)				
	3	2	1	1	2	5	10	15
Normal sheep								
Lizzie								
SS	283	583↑	583	1956↑	1956	2443	2718	2925
CA	366	283↑	466	1041↑	1583	2900	2583	2400
JV	166	208	583	1133	1466	1980	2550	3750*
Sultan								
SS	314	253	495	2062↑	2343	2343↑	2343↑	1875↑
CA	455	455	491	820↑	2200	1358↑	1171↑	1031↑
JV	351	431	316	632	1125	1101	1593	1781
Bathsheba								
SS	921↑	1096↑	1008↑	1821↑	5900↑	9230↑	10120↑	8730↑
CA	307↑	307↑	307↑	526↑	1490↑	1578↑	2070↑	3201↑
JV	324	295	473	1052	1678	2100	3000	2410
Hypophysectomized sheep								
Anastasia								
SS		46↑	59↑	187↑	216↑	326↑	358↑	301↑
CA		< 10↑	< 10↑	18↑	23↑	16↑	< 10↑	< 10↑
JV		< 10	< 10	< 10	< 10	< 10	< 10	< 10

that ensues allows the comparisons to be made before high hormone concentrations within the carotid artery limit the validity of the method. Since anesthesia modifies both seizure activity and pituitary function, these studies were performed in awake animals.

The high baseline pre-seizure ACTH concentrations of each of the nonhypophysectomized animals provide indirect evidence of stress from the experimental conditions alone. In one animal, Bathsheba, sagittal sinus ACTH concentrations were much higher than carotid artery concentrations prior to the seizure, suggesting that ACTH was carried from the pituitary directly to the brain prior to the convulsion. However, in each animal the convulsion caused further elevations of plasma ACTH.

The elevation of plasma ACTH concentrations noted in sagittal sinus venous blood in all of these animals indicates that this hormone was added to blood at some intracranial site. Venous blood from the frontal lobes, the parietal lobes, and the diencephalon (via the vein of Galen) converges in the sagittal sinus near the spot where our catheter tip was positioned. The high venous ACTH concentrations at this site indicate that hormone-enriched blood flowed through the capillary beds of these regions of the brain. ACTH is produced not only by the pars intermedia and the adenohypophysis, but by the brain itself (*9*), and this particular hormone may have come into sagittal sinus venous blood from either the brain or the pituitary. Since plasma ACTH was markedly reduced after hypophysectomy, most of the ACTH found within the sagittal sinus must have come from the pituitary.

The presence of substantial concentrations of ACTH in the post-seizure sagittal sinus samples after hypophysectomy verifies that ACTH (or ACTH-like material) was released from the brain as well as the pituitary by this stimulus. This demonstrates that the brain has access to two kinds of ACTH: *brain* ACTH produced locally within the brain itself (*9*), and *pituitary* ACTH carried directly to the brain from the pituitary. These may be identical molecules, but they also could by similar molecules recognized by the same antibody.

In a study of four other sheep we have confirmed the reports of others (*12*) by noting that the blood brain barrier becomes permeable to Patent Blue dye injected intravenously immediately before an electrically induced seizure. Presumably ACTH released from the pituitary by this unusual stimulas would pass into the brain. Brain ACTH carried to the sagittal sinus more certainly must have passed through a transiently open blood brain barrier.

Two vascular routes have been described (*3*) that have the potential to carry hormone-enriched pituitary blood directly to the brain. Which of these routes conveys ACTH to the brain cannot be established by this kind of study, yet these pilot physiological studies have demonstrated that, under certain circumstances, vascular mechanisms may transport a hormone from the pituitary directly to the brain. This may be true for many more of the hormones that are found commonly in the brain (*10*) and the pituitary.

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7. Hypophysectomy was performed via the transpharyngeal route and included the removal of the median eminence, the entire neurohypophysis, and the entire adenohypophysis. The animal received 10 mg of dexamethasone immediately after hypophysectomy.
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Deficient Natural Killer Cell Activity in X-Linked Lymphoproliferative Syndrome

Abstract. *The activity of natural killer cells was found to be deficient in 10 of 12 males with X-linked lymphoproliferative syndrome, a life-threatening proliferation of lymphocytes after infection by Epstein-Barr virus. The activity levels of natural killer cells from affected males were increased after treatment with interferon in vitro, but normal levels of killing were not obtained. Deficient activity of killer cells in individuals with immunodeficiency and chronic infection by Epstein-Barr virus may contribute to the development of lymphoproliferative disorders.*

In 1975 it was found that normal spleen cells of some strains of mice are selectively cytotoxic to neoplastic transformed cells in vitro (*1*). These cells were designated natural killer (NK) cells. Subsequent studies demonstrated increased activity of NK cells in the athymic nude mouse (*2*) and deficient activity in the beige mouse (*3*). The nude mouse is relatively resistant to spontaneous leukemias and lymphomas, while the beige mouse is highly susceptible to viral and chemically induced transplantable leukemias (*4*). A role for NK cells in recovery from certain viral infections was suggested by Welsh *et al.* (*5*), and recent evidence supports this concept in murine cytomegalovirus infections (*6*). It appears that NK activity is regulated by interferon (*7, 8*). Type I leukocyte or fibroblast interferon augments NK cell activity against tumor cells and virus-infected target cells.

Natural killer cells in man are Fc receptor-bearing lymphocytes with a low affinity for binding sheep erythrocyte rosettes. They effect spontaneous lysis of malignant and virus-infected target cells (*1*). Little is known about the role of the NK-interferon system during infection and malignancy in man. The X-linked lymphoproliferative (XLP) syndrome is characterized by immunodeficiency to Epstein-Barr virus (EBV) [a

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