such reactivity. The performance of this new polymer in our study warrants a thorough investigation of its applicability as a prosthetic material in diverse circumstances.

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Control of Aldosterone Secretion by the Pituitary Gland

Abstract. *In the rat, the pituitary gland is essential for the stimulation of aldosterone secretion by sodium depletion. Hypophysectomy abolishes the response to sodium depletion, whereas whole pituitary gland injections partially restore it. The response cannot be restored by injections of either adrenocorticotropin or growth hormone, nor by adrenocorticotropin plus thyroxin. The pituitary gland must secrete a hormone or possibly several hormones which are necessary for the adrenal gland to respond to sodium depletion.*

Sodium depletion is a potent stimulus of aldosterone secretion. In dog and man, the renin-angiotensin system plays an important role in this response (*1*). The evidence in sheep (*2*) and rats is not so clear (*3–5*).

The role of the pituitary in the regulation of aldosterone secretion has not been fully defined. Injections of adrenocorticotropin (ACTH) into normal dogs or those depleted of sodium (*6*) or into man (*7–9*) markedly stimulate aldosterone secretion initially. In man, with continued ACTH treatment, the secretion rate returns toward control values and even below in rare instances. Dogs (*10*) and rats (*11*) that have been hypophysectomized and humans (*12*) with hypopituitarism show a diminished response of aldosterone secretion to sodium depletion. This has usually been attributed to ACTH deficiency. However, by simultaneous infusion of angiotensin II and ACTH into hypophysectomized, nephrectomized dogs, Mulrow and Ganong (*13*) were not able to stimulate aldosterone secretion to the extent observed in sodium-depleted dogs. Hypophysectomy produced a greater reduction in aldosterone secretion in dogs with secondary hyperaldosteronism than nephrectomy did (*14*). This marked effect was attributed to the absence of ACTH. Nevertheless, this effect of hypophysectomy occurred in conscious, trained dogs with low rates of corticosterone secretion, an indication of low ACTH activity before hypophysectomy.

We studied the role of the pituitary in the response of aldosterone secretion to sodium depletion. Our results indicate that, in the rat, the pituitary is essential for the response and that the pituitary factor is not ACTH.

Male Sprague-Dawley rats (150 to 200 g), either intact or hypophysectomized, were obtained from the Charles River Breeding Laboratory. Upon arrival in the laboratory, the rats were fed for 9 to 14 days either a diet low in sodium or a diet considered to be normal in sodium (*15*). At the end of the experimental period, plasma was collected from the left lumboadrenal vein and was assayed for aldosterone by the double-isotope derivative method (*16*). In most cases, plasma from the lumboadrenal vein was also analyzed for corticosterone by the acid fluorescence method (*17*). The rate of corticosterone secretion and the adrenal gland weight were taken as indexes of ACTH activity. The sodium and potassium concentrations in arterial plasma were also measured.

The results from hypophysectomized rats fed either the normal or low-sodium diets were compared with those from similarly fed intact rats or hypophysectomized rats that had received an intramuscular injection of a whole rat-pituitary gland each day. The pituitary glands, obtained fresh from rats decapitated for other experiments, were frozen until used.

The effect of injections of ACTH on aldosterone secretion was also determined in hypophysectomized rats fed the two diets. In other experiments, the response of sodium-depleted, hypophysectomized rats to treatment with ACTH and thyroxine or to injections of growth hormone was studied. All injections were made each morning of the experimental period, including the day on which the experiment was terminated. The doses of hormones administered were: ACTH, 8 units; thyroxine, 20 μg; and growth hormone, 1 mg.

Acthar gel (*18*) was injected subcutaneously. Thyroxine (*18*), dissolved in distilled water which had been adjusted to *p*H 9.5 with 0.1*N* NAOH, was injected intraperitoneally. The amount of sodium administered with each injection was less than 0.0001 meq. Growth hormone (NIH-GH-B12, 0.97 U.S.P. unit/mg) was also dissolved in distilled water adjusted to *p*H 9.5 as above, but it was injected subcutaneously. Between 2.0 and 3.0 ml of blood from the lumboadrenal vein were collected from each rat. Collection of these samples was begun 0.5 to 5.0 hours after the final injection of the test substance. Those rats receiving the combined therapy of ACTH and thyroxine were given 50 mμ of ACTH intravenously just prior to collection of samples, instead of a final subcutaneous injection of ACTH on the morning of the adrenal vein cannulation.

In hypophysectomized rats, there was no stimulation of aldosterone secretion after sodium depletion (Tables 1 and 2), even though the width of the zona glomerulosa increased. When whole rat pituitary glands were administered to hypophysectomized rats that had been fed a low-sodium diet, there was a marked stimulation of aldosterone secretion. In the sodium-depleted group, this secretion was about nine times that found in the untreated, hypophysectomized rats, but there was only a

threefold stimulation if the rats were fed the normal sodium diet. This stimulation of aldosterone secretion in the hypophysectomized sodium-depleted group occurred despite atrophy of the adrenal glands and despite the corticosterone secretion rates which were no different from those found in simple, hypophysectomized rats, an indication of little or no ACTH activity in the injected pituitary glands.

In hypophysectomized rats on a normal or sodium-deficient diet, ACTH treatment stimulated aldosterone secretion only to the extent found in intact rats on a sodium-replete diet, but not to the extent found in sodium-depleted intact or pituitary-treated hypophysectomized rats. Yet, in rats treated with ACTH, adrenal glands were hypertrophied, and rates of corticosterone secretion were markedly elevated as compared with those in untreated hypophysectomized rats. Addition of thyroxine to the ACTH treatment did not change these results (Table 2).

Two other experiments (not shown in Tables 1 and 2) were performed to evaluate the role of ACTH. This hormone was administered to two intact rats during the 9 days on which they were fed the low-sodium diet. The rates of aldosterone secretion of these rats were 17 and 24 ng/min, respectively; they were no different from those found in intact, sodium-depleted rats not receiving ACTH. Moreover, three rats fed a low-sodium diet for 9 days were then hypophysectomized and fed the low-sodium diet for 7 more days. The ACTH was administered during the last 7 days. Despite the prolonged sodium depletion and ACTH administration, the rates of aldosterone secretion were only in the range found in intact rats on a sodium-replete diet.

Growth hormone (1 mg/day per rat) had only a slight effect on aldosterone secretion. The growth rate of these rats was slightly greater than that of rats receiving pituitary gland injections (34 ± 1 as compared to 20 ± 1 g for the 9-day period). In addition, three hypophysectomized sodium-depleted rats received a smaller dose of growth hormone (100 μg/day for each rat) with no effect on aldosterone secretion.

The rats receiving the pituitary gland injections were examined for other signs of endocrine activity of the pituitary glands. Histological examination revealed atrophied thyroid glands, an indication of little or no thyrotropin activity. The slight increase in body weight suggested some growth hormone activity. Testicular growth was normal; this fact indicated the presence of pituitary gonadotropins. The injections of the pituitary gland did not change concentrations of sodium or potassium in the plasma.

Table 1. Effects of hypophysectomy and treatment by pituitary gland injections on aldosterone secretion during sodium depletion. Means ± S.E. are shown. Numbers in parentheses indicate numbers of rats analyzed.

Diet	Secretion (ng/min)		Adrenal weight (mg/100 g of body weight)	Width of zona glomerulosa (μ)
	Aldosterone	Corticosterone		
	Normal rats			
Normal	4.0 ± 0.5 (20)	1036 ± 90 (11)	17.5 ± 0.7 (10)	44.5 ± 2.7 (10)
Low Na	18.9 ± 1.8 (15)	911 ± 81 (13)	19.2 ± 0.9 (14)	91.8 ± 4.6 (10)
	Hypophysectomized rats			
Normal	0.9 ± 0.3 (10)	< 50 (4)	7.6 ± 0.7 (9)	75.8 ± 4.4 (7)
Low Na	1.4 ± 0.5 (11)	< 50 (7)	8.7 ± 0.4 (15)	100.4 ± 4.5 (7)
	Pituitary-treated hypophysectomized rats			
Normal	2.2 ± 0.6 (3)	< 50 (3)	9.4 ± 0.8 (4)	64.8 ± 3.7 (3)
Low Na	9.9 ± 1.4 (11)	< 50 (10)	11.4 ± 0.3 (11)	110.7 ± 2.3 (6)

Our studies confirm previous reports that the pituitary gland is essential for the aldosterone response to sodium depletion in the rat (*4, 11*). Studies on the dog (*10*) and man (*12*) show that the pituitary gland is also important in the response to sodium depletion.

The necessary factor in the pituitary does not appear to be ACTH. Prolonged administration of ACTH to hypophysectomized rats fed a low-sodium diet did not restore the aldosterone response to sodium depletion, despite a marked increase in adrenal weight and corticosterone secretion. It is possible that long-term administration of ACTH first stimulated and then depressed aldosterone secretion, as it does in man on a normal sodium diet (*8*). In man receiving ACTH while on a low-sodium diet, aldosterone secretion is first stimulated but then falls toward the high base line of sodium depletion (*7–9*). In two intact rats fed a low-sodium diet, administration of ACTH during the entire period of low-sodium diet did not inhibit the aldosterone reponse to sodium depletion. Furthermore, ACTH administration cannot maintain the increased secretion of aldosterone established by sodium depletion before hypophysectomy.

Finally, the pituitary gland injections in hypophysectomized rats on a low-sodium diet stimulated aldosterone secretion and yet had no ACTH activity, as the low corticosterone secretion rate, the small adrenal size, and the morphology indicated. Conceivably, a small amount of ACTH activity was present in the injected pituitary glands, but its effect on aldosterone secretion was of longer duration than that on corticosterone secretion, adrenal weight, and histology. This seems unlikely for the reasons already cited and because of the fact that, in the dog, the effect of acute administration of ACTH upon secretion of 17-hydroxycorticoid and aldosterone is of the same duration (*6*).

The pituitary factor did not appear to be either growth hormone, thyrotropin, or at least that action of thyrotropin which stimulates thyroxine secretion. Possibly, the known pituitary

Table 2. Effects of anterior pituitary hormones and thyroxine on aldosterone secretion in hypophysectomized rats. Means ± S.E. are shown; numbers in parentheses indicate number of rats analyzed.

Diet	Secretion (ng/min)		Adrenal weight (mg/100 g of body weight)
	Aldosterone	Corticosterone	
	ACTH		
Normal	5.4 ± 2.0 (5)	641 ± 190 (3)	26.4 ± 0.8 (5)
Low Na	4.3 ± 1.0 (5)	475 ± 277 (4)	27.8 ± 1.3 (9)
	ACTH and thyroxine		
Low Na	3.2 ± 1.0 (5)	344 ± 106 (4)	21.7 ± 2.0 (6)
	Growth hormone		
Low Na	2.6 ± 1.0 (3)	< 50 (2)	11.0 ± 0.8 (5)

peptides, either alone or in combination, are necessary for the adrenal response to sodium depletion. The results of pituitary gland injections suggest that ACTH and possibly thyrotropin are not necessary for a significant response to sodium depletion.

The pituitary factor is not the primary stimulator of aldosterone secretion, since sodium depletion is also required for a marked effect of the injections of pituitary glands. Furthermore, the width of the zona glomerulosa, the site of aldosterone biosynthesis, is increased in sodium-depleted hypophysectomized rats not receiving pituitary gland injections.

The pituitary gland injections did not stimulate the secretion of aldosterone in sodium-depleted hypophysectomized rats to the same level as in sodium-depleted intact rats. This may indicate that there was an inadequate dose of the pituitary gland injected or that labile pituitary factors are also necessary for the maximum response.

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"Intrinsic" Immunological Tolerance in Allophenic Mice

Abstract. *Mice experimentally derived from pairs of conjoined, undifferentiated, cleavage-stage embryos of different histocompatibility genotypes can retain cells of each strain, which still produce their characteristic antigenic products. The animals are permanently tolerant of cells of both original types, remain free of runt disease, and display a normal and specific immune response to introduction of a foreign antigen. Absence of autoimmunity in development of ordinary animals is explainable by the "intrinsic" kind of tolerance found here.*

The immune rejection of antigenically foreign cells which ordinarily follows their introduction into an organism can be circumvented in at least two ways: by introducing the graft prenatally or early postnatally before immunological maturity (*1*), or by depressing the adult host's immune system [for example, by irradiation (*2*)] before introducing the antigen. Acceptance of grafts in both cases illustrates the phenomenon of acquired tolerance (*1*).

The earliest developmental origins of the immune system are still largely obscure, despite interesting recent investigations of the problem. It is therefore difficult to assess the precise immune status of embryos at any of the stages involved in previous prenatal inductions of tolerance, whether by way of natural vascular anastomoses, transplantation, or parabiosis (*1*, *3*). In no instance is it certain that the host completely lacked primordial cells of the immune system at the time the foreign component was first presented.

The *allophenic* mice experimentally produced by Mintz (*4*) are the first animals in which homologous cell association within the embryo unquestionably predates any immunological differentiation. These single individuals are each of multi-embryo origin. Their precocious genotypic multiplicity is established during the cleavage period, when blastomeres have been demonstrated still to be developmentally entirely labile (*4*). As a consequence, *any* tissue can ultimately consist of different genotypes of cells, related as coevals rather than as host and donor.

The animals are formed by first assembling all the blastomeres from two (or more) genetically distinctive embryos into one composite group in vitro; this is later transferred surgically to the uterus of a pseudopregnant female. Here regulation from double to single embryo size occurs during implantation. Normal development to birth frequently follows and approximately 500 healthy adults, comprising many pairs of genotypes, have been obtained (*4*, *5*). Mice arising in this manner are called *allophenic* because of the orderly coexistence in them of cells with different phenotypes ascribable to known allelic genotypic differences. The two cell populations in a tissue strikingly and invariably occupy nonrandom positions with respect to each other and therefore form consistent patterns. From analyses of such distributions it has been possible to deduce complete clonal ontogenies of several kinds of cells, including melanocytes (*5*, *6*).

Many of the animals are derived from two genetic sources differing at one or more histocompatibility loci, including the *histocompatibility-2(H-2)*-locus which exerts the strongest influence over graft acceptance. Mintz and Palm (*7*) have in fact identified in some allophenics the simultaneous presence of $H\text{-}2^k$ and $H\text{-}2^b$ erythrocytes, by agglutination and absorption methods. In the present study, grafting procedures were used to extend to skin the tests for production of diverse antigens, and also to examine for tolerance and immunity. Three kinds of experiments were conducted: (i) Some allophenic mice were grafted with skin from the original (or "parental") strains, to ascertain whether tolerance existed (Table 1). (ii) Some of the tolerant animals were challenged with skin whose histocompatibility genotype differed from either of the constituent strains, to observe whether such recipients were capable of a normal immune response. (iii) Some allophenics served as donors of skin grafts to the "parental" strains, as a direct measure of formation of the respective antigenic products (Table 2).

In certain of the strain pairs, coat color markers are also included along with *H-2* differences. The four inbred strains involved were C3Hf [genotypically $H\text{-}2^kH\text{-}2^k$ and *AA* (agouti)]; C57BL/6 [$H\text{-}2^bH\text{-}2^b$ and *aa* (*non-agouti*, or in this case black)]; CBA-*T6T6* ($H\text{-}2^kH\text{-}2^k$ and *AA*; also homozygous for the *T6* translocation); and BALB/c [$H\text{-}2^dH\text{-}2^d$ and *cc* (*albino*, which masks other color factors, in contrast to *colored*, or *CC*, in the three preceding strains)]. *AA* mice all have some black hairs and *aa* have