ments demonstrated that increasing concentrations of Na^+ or K^+ between 10 and 120 mmole/liter caused no further enhancement of the extra adenosine triphosphatase, although calcium uptake was increased.

In an earlier study of skeletal muscle microsomes, Martonosi and Feretos failed to observe differences in the adenosine triphosphatase activity in the presence of Na^+ alone, of K^+ alone, or in mixtures of Na^+ and K^+ at constant ionic strength (*11*). They presented no data to support their statement, however, and the possibility exists that the relatively small increase in the enzyme's activity seen in most mixtures of Na^+ and K^+ could have been overlooked. In the case of cardiac microsomes, several investigators have noted a (Na^+ and K^+)-activated adenosine triphosphatase enzyme system (*12–15*). This activity has been most striking in aged cardiac microsomes (*12*), and in cardiac microsomes prepared in the presence of agents such as deoxycholate (*13*) or sodium iodide (*14*).

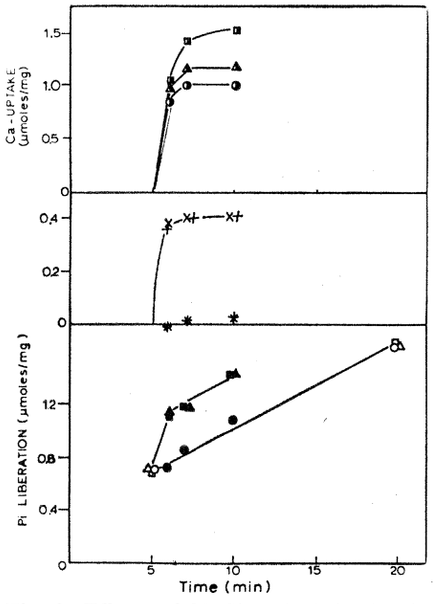

Fig. 2. Effects of NaCl and KCl on calcium uptake (top), the extra adenosine triphosphatase activity (middle), and the basic and calcium-activated activities (bottom) of skeletal muscle microsomes. (Bottom) The liberation of P_i by 0.05 mg of microsomes per milliliter of solution containing in final concentration 4.0 m*M* MgATP, 2.5 m*M* tris oxalate, and 10 m*M* histidine (*p*H 7.0) was examined in the absence (open symbols) and presence (closed symbols) of 0.10 m*M* $CaCl_2$. Samples were taken after a 5-minute period of incubation. Reactions were carried out in the absence of added alkali-metal salt (○, ●), in 0.12*M* KCl (□, ■), and in 0.12*M* NaCl (△, ▲). $CaCl_2$ was added at 5 minutes. (Middle) Extra adenosine triphosphatase activity in the absence of alkali-metal salt (*), in 0.12*M* KCl (×), and in 0.12*M* NaCl (+), calculated by subtracting the P_i liberated in the absence of added $CaCl_2$ from that liberated after the addition of $CaCl_2$ (data from lower panel). (Upper) The uptakes of Ca^{45} in the absence of alkali-metal salts (half-shaded circles), in 0.12*M* KCl (half-shaded squares), and in 0.12*M* NaCl (half-shaded triangles), determined concurrently with the measurements of adenosine triphosphatase activity. The ratios of micromoles of P_i liberated (extra adenosine triphosphatase) per micromole Ca^{++} taken up were: in no salt, 0; in KCl, 2.60; and in NaCl, 3.45. These ratios were calculated from the samples taken at 10 minutes, at which time calcium uptake had ended.

Our findings also demonstrate that the stoichiometry between calcium uptake and the calcium-activated adenosine triphosphatase of skeletal muscle microsomes can be significantly modified by low concentrations of Na^+ and K^+. This stoichiometry has been reported previously to be altered in the presence of high concentrations of Ca^{++} (*11*) or low concentrations of adenosine triphosphate (*16*). The sequestration of calcium in the absence of any discernible stimulation of the basic adenosine triphosphatase was unexpected. Although no extra adenosine triphosphatase could be detected under these conditions, significant calcium uptake was observed. It thus appears that the basic adenosine triphosphatase can, in the absence of Na^+ and K^+, provide for calcium sequestration.

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Allergic Adenohypophysitis: New Experimental Disease of the Pituitary Gland

Abstract. *The pituitary gland has been added to the roster of organs in which inflammatory disease, probably autoimmune in origin, can be induced by injection of the corresponding tissue plus adjuvants.*

Inflammatory, autoimmune diseases of several organs have been produced by injections of tissues from the corresponding organs (*1*). Organ-specific antigens occur in the pituitary gland, and they are capable of eliciting production of autoantibodies; nevertheless, no pathological lesions in the pituitary have been described (*2*). Our work concerns the experimental production, apparently for the first time, of a disease of the anterior lobe of the pituitary

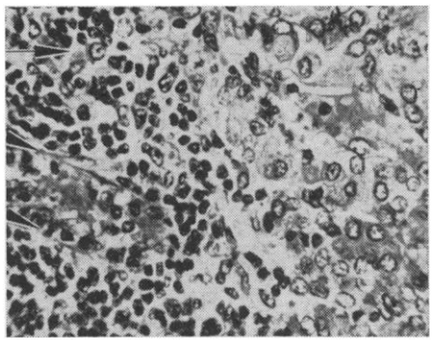

Fig. 1. Allergic adenohypophysitis. The left half reveals a dense infiltrate of lymphocytes and monocytes with darkly stained nuclei and little visible cytoplasm. The inflammatory cells surround a vein that is cut longitudinally. Similar cells are present in the vein's lumen and walls (position of wall indicated by two arrow-heads at the extreme left). Pituitary parenchymal cells, isolated in the infiltrate, are undergoing lysis (arrow). The right half consists of relatively intact parenchymal cells (larger, lighter stained nuclei, relatively abundant cytoplasm). This area is infiltrated by the advancing margin of inflammatory cells (hematoxylin and eosin, × 313).

(adenohypophysis); this disease is probably of autoimmune origin.

The disease was produced in young adult male and female Lewis rats and in first-generation hybrid rats of a cross of Lewis and BN strains by a single intracutaneous injection of pituitary tissue and an immunologic adjuvant into a right hind footpad. The tissue was prepared as homogenate (80 percent, weight to volume) in saline. It was emulsified in an equal volume of Freund's complete adjuvant (*3*). A dose of 0.05 ml of the emulsion contained 20 mg (wet weight) of tissue. The injected foot became swollen, but there were no other outward signs of disease. Nevertheless, microscopic examination of pituitaries taken 13 to 20 days after injection revealed adenohypophysitis in many of the rats.

The anterior lobe had focal aggregates and diffuse infiltration of mononuclear inflammatory cells (lymphocytes, monocytes, occasionally epithelioid cells) (Fig. 1). Frequently, these cells surrounded vessels and filled their lumens. On occasion, there was predilection for the subcapsular region. In severe instances, every part of the lobe was involved. In areas of severe inflammation, parenchymal cells exhibited degenerative (cytolytic) changes. A few posterior and intermediate lobes had minimal inflammation.

In three experiments, the influences of variations in the tissue, adjuvants, and experimental procedures were explored. Adenohypophysitis was produced with homogenates of whole pituitary glands or of anterior lobes from which most of the posterior and intermediate lobes had been removed. Both isologous tissue, derived from the same rat strain, and homologous tissue, from other rat strains, were effective. Heating the emulsion of pituitary tissue and adjuvant to 60°C for 1 hour diminished its efficacy. Addition of a second immunologic adjuvant, pertussis vaccine, produced a modest increase in the incidence of adenohypophysitis (Table 1), but this increase was not accompanied by an increase in the severity of the lesions.

The vaccine was injected on the dorsum of the foot that had received the pituitary emulsion, a procedure known to enhance autoimmune disease of the nervous system and adrenal glands (see *4*). Injection of an emulsion of pituitary tissue and adjuvant and pertussis vaccine into both hind feet was no more effective than was their injection into one foot. Adrenalectomy, another precedure that increases the severity of some autoimmune diseases (*5*), did not improve the results (Table 1) and was responsible for a number of deaths. Preliminary results indicate that adenohypophysitis can be induced in pregnant rats and may be increased in severity in postpartum animals.

No lesions were detected in thyroid or pancreas of rats with adenohypophysitis, and there were only a few minor foci of inflammatory cells in some adrenal glands and spinal cords. Conversely, the pituitary had no lesions in rats that were immunized with adrenal, pancreas, or spinal cord tissue and adjuvants.

These results lend significant support to the suggestion that certain human cases of adenohypophysitis are caused by an autoimmune reaction (*6*). The allergic nature of the experimental disease is suggested by the manner of its production and by the histologic character and specific tissue localization of the lesions, and is supported by the known antigenicity of pituitary tissue (*2*). Nevertheless, further experiments are required to prove an immunologic origin by passive transfer with lymphoid cells or blood serum, as well as to isolate the responsible antigen and elucidate the role, if any, of antigenic pituitary hormones.

Table 1. Effect of adrenalectomy and pertussis vaccine on adenohypophysitis in rats. Composite results of three experiments. Under hypophysitis incidence, the numerator is the number of rats with adenohypophysitis. The denominator is the total number of rats. Vaccine that contained 200 billion organisms per milliliter was injected in dorsum of foot immediately after an emulsion of pituitary and adjuvant were injected in footpad. Adrenalectomies were performed 4 or 5 days before challenge; animals receiving surgery were maintained on saline as the sole fluid. Adrenalectomized animals that died are not included in the results.

Pertussis vaccine	Hypophysitis incidence
No surgery	
None	6/14
0.05–0.1 ml	15/20
Adrenalectomized	
None	5/9
0.05–0.1 ml	5/11

Triplett has reported that a frog will reject a graft of its own pituitary if the animal and its pituitary were maintained apart from one another during the period of immunologic immaturity. The interpretation of this fascinating experiment depends, in part, on Triplett's assumption that pituitary antigens "normally make good physical contact with the primordial immune system during development" (*7*). If adenohypophysitis is an autoimmune disease, it may be a useful tool for testing this assumption with experiments on neonatal tolerance to pituitary antigens.

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