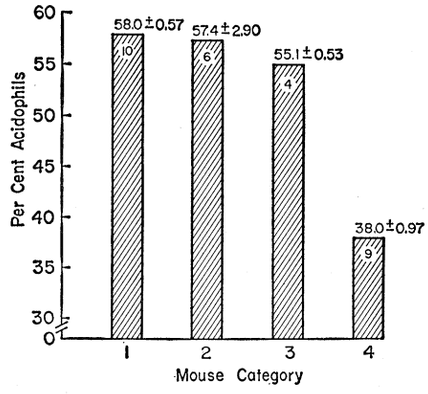

Fig. 1. Percentages of acidophils in pituitaries of female mice subjected to male and female mouse pheromones. (1) colony-housed females; (2) females exposed to restrained males for a single night; (3) females exposed to restrained males for two consecutive nights; (4) females exposed to released males on the third night after exposure to restrained males for two nights. Means ± standard deviations are shown.

stained with eosin methyl blue by a modification of the Mann-Lillie method (*14*). Pituitary sections were evaluated microscopically, and acidophil percentages were determined by the method of Johnson and Avery (*15*).

Quantitative determinations of pituitary acidophils were obtained from 29 female mice. Ten of these were removed directly from colony housing and therefore assumed to be in anestrus. Six were exposed to restrained males for one night, and four were similarly exposed for two consecutive nights. Nine were permitted access to released males on the third night after exposure to restrained males on each of the two proceeding nights. Exposure to male mice was expected to initiate FSH secretion by the pituitary gland with continued exposure sustaining this secretion until it culminated in the attainment of estrus and mating.

Acidophils constituted 58.0 ± 0.57 percent of all pituitary cells counted after colony housing for 10 days (Fig. 1). Subsequent exposure to restrained males for one and two nights resulted in respective acidophil values of 57.4 ± 2.90 and 55.1 ± 0.53 percent. Since neither value represented a statistically significant change, it cannot be stated, with certainty, that these data indicate a trend toward increased degranulation with a second night's exposure to male pheromone. Exposure for a third night to released males, however, resulted in marked degranulation with a significant decline to 38.0 ± 0.97 in pituitary acidophils ($t = 32.9$, d.f. = 11, $P < .005$). Copulation plugs were subsequently observed in 45 percent (4 of 9) of the females so exposed. Pituitary acidophil percentage was essentially equal in plugged and nonplugged females (37.6 percent for four plugged and 38.2 percent for five nonplugged), suggesting that the marked acidophil degranulation at this time probably resulted from three nights' exposure to male pheromone and may not have been related to the mating act itself.

Since vaginal smears were not examined for spermatozoa and the presence of copulation plugs represented the sole criterion of mating, the possibility that all females were bred cannot be ruled out, although it seems unlikely.

Declining concentrations of pituitary acidophils are believed to result from the discharge of acidophilic secretory granules. Desclin (*16*) interpreted increased degranulation as representing increased prolactin (luteotrophin) secretion. Conversely, higher concentrations of acidophils would represent a reduced rate of degranulation and luteotrophin secretion. In this context, the maximum acidophil concentration (58.0 percent) resulting from colony housing would be indicative of a minimum rate of luteotrophin secretion. This indicates that the state of anestrus which develops under these conditions is not attributable to continuous luteotrophin secretion and indeed supports the view that it results from failure of FSH secretion. Anestrus therefore appears to represent a state in which the suppression of secretion of both FSH and LTH is at a maximum, and FSH-LTH reciprocity does not exist.

It thus appears that male pheromone initially triggers the secretion of FSH by the female pituitary, with LTH being subsequently secreted at its assigned time in the sequence of cyclic ovarian events which are thereby initiated. Conversely, female pheromone suppresses FSH secretion which prevents initiation of these sequential events and LTH is not secreted during the ensuing period of anestrus.

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White Blood Cell Cultures in Genetic Studies on the Human Mucopolysaccharidoses

Abstract. *Cultures of white cells derived from peripheral blood of individuals homozygous and heterozygous for the inherited mucopolysaccharidoses revealed a distinct intracellular metachromatic staining with toluidine blue O. These short-term cultures circumvent the technical problems of skin fibroblast cultures and provide a simple screening procedure to detect the heterozygous state for the mucopolysaccharidoses, as well as offering an opportunity to study the heterozygous state of various inherited storage diseases.*

It has become increasingly evident that, under appropriate conditions, tissue cultures of human cells provide an opportunity to investigate the biochemical phenotype of a variety of diseases of man (*1*). The mucopolysaccharidoses represent a group of inherited diseases characterized by an abnormal accumulation of mucopolysaccharides in various organs (*2*). The observations of Mittwoch (*3*) and others (*4*), that the white blood cells of patients with the mucopolysaccharidoses contain metachromatic inclusions, suggested that cultures of the peripheral white blood cells might provide a convenient cell type. The failure of several investigators (*3, 5*) to identify metachromatic inclusions in white cells

in smears of peripheral blood of individuals heterozygous for the genetic mucopolysaccharidoses did not exclude the possiblity that cultured white blood cells might reveal a morphological abnormality.

Samples of venous blood were obtained from seven affected individuals (four with the X-linked recessive mucopolysaccharidosis known as Hunter's syndrome; two with the autosomal recessive form of the disease, Hurler's syndrome; and one with Sanfilippo's syndrome, a rare autosomally inherited mucopolysaccharidosis), in addition to some of their parents and 30 normal unrelated individuals (ages 6 months to 60 years). When samples of fresh peripheral venous blood were stained with toluidine blue O (*6*), 5 to 10 percent of the lymphocytes from all affected individuals showed metachromatic inclusions as previously described by other workers (*3*); control blood samples showed no metachromasia. White blood cell cultures were established by means of a modification of the Fedorko method (*7*) which makes use of a dextran separatory procedure (*8*). The culture medium was Eagle's minimum essential medium (*9*) containing 20 percent pooled AB serums and 5 percent beef embryo extract ultrafiltrate (*10*). Coverslips were removed from the cultures at 3, 7, and 14 days, fixed, and the cells thereon stained for metachromasia with toluidine blue O (*6*). Stained slides were scored as positive or negative for metachromasia. All the cultures studied showed a pleomorphic cell population. No difference in cell types or activity could be noted in any living or stained cultures. No cellular metachromasia was seen in cultures derived from the 30 normal individuals who were presumed to be noncarriers or from cultures established from four fathers of the children affected with the X-linked recessive form of the disease. White blood cell cultures from the seven known homozygous and eight heterozygous individuals showed marked cellular metachromasia (Fig. 1). After the cells had been in culture 3 days the metachromasia appeared as fine granules dispersed throughout the cytoplasm. At 7 days, the metachromasia was maximum and localized to the juxtanuclear area as a dense zone of pink-staining material. A marked increase in the number of metachromatic cells occurred during this interval. Although the intracellular localization of the metachromasia persisted for 14 days, a decrease in the number of positive cells was noted. Cultures of white blood cells from affected individuals still contained positive cells at 14 days, while cultures from seven of the eight heterozygotes were essentially ametachromatic.

Table 1. Metachromasia of white blood cell cultures from families with the genetic mucopolysaccharidoses and from normal, noncarrier individuals.

Subjects	Number studied	Cultures positive for metachromasia (%)
Hunter: X-linked recessive		
Propositi	4	100
Mothers	4	100
Fathers	4	0
Hurler: autosomal recessive		
Propositi	2	100
Mothers	2	100
Fathers	2	100
Sanfilippo: autosomal recessive		
Propositus	1	100
Normal, noncarriers	30	0

The successful use of white blood cell cultures appears to offer a technically simple diagnostic method to identify individuals heterozygous for the inherited mucopolysaccharidoses. In contrast to cultures of skin fibroblasts, in which metachromasia is not apparent for 5 to 8 weeks (*11*), metachromasia is evident in the cultures of white blood cells at 3 days, and definitive results are obtained in 7 days. The localization of the cellular metachromasia in the cultured white blood cells, as a fine granular accumulation of pink-staining material confined to the juxtanuclear area, is different from the diffuse cytoplasmic metachromasia noted in cultured fibroblasts (*6*) and the vesicular metachromatic inclusions described in the peripheral lymphocytes (*4*). The gradual loss of cellular metachromasia suggests that senescence, previously described in the skin fibroblast (*12*), also occurs in cultures of white blood cells. At 14 days, no metachromasia was observed in seven of the eight cultures from the known heterozygotes, while cells from homozygotes remained metachromatic. This differential loss of metachromasia is compatible with the difference in gene-dosage in the two genotypes. Previous studies on the inherited mucopolysaccharidoses made with cultured skin fibroblasts have not distinguished between individuals heterozygous and homozygous for the abnormal gene (*12*). The more rapid

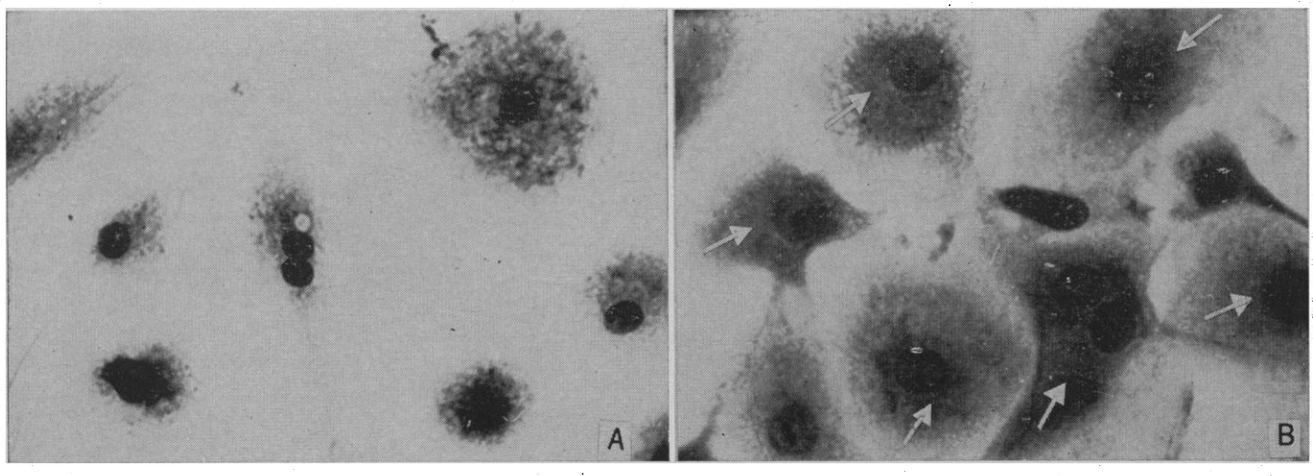

Fig. 1. Seven-day cultures of white blood cells. Preparations fixed in methanol and stained with toluidine blue O. (A) From a normal individual; (B) from an individual with Hurler's syndrome. Arrows indicate areas of metachromasia (× 1100).

appearance of ametachromatic cells in cultured white cells from heterozygous carriers, compared to the more gradual disappearance of metachromasia in cells derived from the homozygous individuals, suggests that this temporal relation may prove to be a convenient way of distinguishing heterozygous from homozygous individuals. Quantitative studies on a larger number of families will be needed to substantiate these preliminary findings.

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Potassium, Corticosterone, and Adrenocorticotropic Hormone Release in vitro

Abstract. *Incubation of rat adenohypophyses in a high concentration of potassium increases adrenocorticotropic hormone release. This increased release is suppressed by the addition of corticosterone to the incubating medium. Our findings are consistent with a process of "stimulus-secretion coupling" proposed for other glands and suggest that corticosterone may operate directly on the adenohypophysial cell membrane to inhibit releasing mechanisms.*

Douglas and co-workers (*1*) have proposed that the release of catecholamines from the adrenal medulla and of vasopressin from the neurohypophysis is initiated by a depolarization of the cell membrane. This depolarization alters the permeability of the cell membrane allowing Ca^{++} to enter the cells. The increased concentration of intracellular Ca^{++} is then thought to activate a process leading to hormone release. The process has been called "stimulus-secretion coupling" (*1*). We have now obtained evidence to suggest that the release of adrenocorticotropic hormone (ACTH) from the adenohypophysis may involve mechanisms similar to those postulated for the adrenal medulla and the neurohypophysis.

Adenohypophyses from rats adrenalectomized 1 month previously were bisected in the midsagittal plane. Pooled batches of half glands were processed immediately for ACTH (*2*). The other halves were incubated at 37°C in (i) Krebs-Ringer bicarbonate with glucose (KRB) (*3*), (ii) KRB containing a fivefold increase in K^+ (28 m*M*), isotonicity being maintained by alteration of the molarity of the NaCl, or (iii) KRB containing corticosterone (1 μg/ml). Solutions were gassed with 95 percent O_2 and 5 percent CO_2. Incubating media were assayed for adrenocorticotropic hormone content; the latter is expressed as a percentage of the content of the nonincubated halves. Parentheses enclose the 95 percent confidence limits (Table 1). All incubations were preceded by a 30-minute incubation in KRB; this medium was discarded. Exposure to a fivefold increase in K^+ significantly increased adrenocorticotropic hormone release.

Table 1. Effect of a high concentration of K^+ on adrenocorticotropic hormone release in vitro. Results are expressed as adrenocorticotropic hormone content of incubating medium (KRB, High K^+-KRB), as a percentage of nonincubated pituitary halves (parentheses enclose 95 percent confidence limits). The medium was drawn off and replaced with fresh medium at 10, 30, and 90 minutes.

Incubation time (min)	ACTH release	
	KRB (%)	High K^+–KRB (%)
1–10	0.45(0.27–0.73)	2.92(2.37–3.58)
10–30	0.41(0.25–0.68)	4.04(3.33–4.91)
30–90	0.51(0.32–0.81)	7.41(5.92–9.27)

We then investigated the effect of corticosterone on this augmented release of ACTH. The addition of corticosterone to the incubating medium significantly reduced the augmented release of ACTH from 13.40 (9.76 to 18.38) percent to 5.23 (4.01 to 6.83) percent, with a 90-minute incubation.

This effect of the high concentration of K^+ is not unique to the release of ACTH, since increased release of thyroid-stimulating hormone and luteinizing hormone have been reported under similar conditions (*4*, *5*). Indeed, Vale and Guillemin (*4*) have stated that increased concentrations of K^+ stimulate the release of ACTH in vitro. However they presented no data to support this statement.

We tentatively assume that this increased release of ACTH is secondary to a decrease in transmembrane potential. Such an effect could, however, have been due simply to a swelling of cells, such as occurs in muscle under similar conditions (*7*). But we found no significant change in weight when adenohypophyses were incubated in KRB containing a fivefold increase in the concentration of K^+.

In this system, high physiological concentrations of corticosterone depress the release of ACTH induced by high concentrations of K^+. In the intact animal, high concentrations of circulating corticosterone suppress ACTH secretion by way of a negative feedback system (*6*). It has not been firmly established where the sensing elements of the feedback system are located. These experiments strongly suggest that corticosterone may operate directly on releasing mechanisms in the adenohypophysial cell membrane.

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