indicates the strong emphasis on religion in Olmec culture, and the durability of Olmec trade relations with other areas (*16*).

DAVID C. GROVE

Department of Anthropology, State University of New York, Binghamton 13901

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5. See, for example, D. C. Grove, *Amer. Antiquity* **33**, 486 (1968).
6. See, for example, M. D. Coe, in *Handbook of Middle American Indians*, R. Wauchope, Ed. (Univ. of Texas Press, Austin, 1965), vol. 3, article 29; or M. D. Coe, *The Jaguar's Children* (Museum of Primitive Art, New York, 1965).
7. It is unfortunate that most Olmec artifacts in Mexico's central highlands are found by "treasure hunters" seeking to satisfy the demands of art collectors. Most Olmec sites known in the highlands are already looted. The possibility exists that Olmec sites, with architecture, have simply not yet been found in the central highlands.
8. The cave was reported to me and to the Instituto Nacional de Antropología e Historia by Sr. Juan DuBernard, who learned of it from one of his employees, and who conducted a preliminary visit to the cave. Permission to investigate the site was granted to D.C.G. by the Instituto.
9. The designation north and south grottoes is used to clarify the location of the various paintings.
10. M. W. Stirling, *Bull. Bur. Amer. Ethnol.* **157**, 8, 19–20 (1955).
11. ———, *ibid.*, pp. 20–21.
12. Other pre-Hispanic textiles have been found in this region. See, for example, I. W. Johnson, *Rev. Mex. Estud. Antropol.* **21**, 149 (1967).
13. More detailed descriptions and interpretations of the paintings are in preparation.
14. For example, M. Covarrubias, *Indian Art of Mexico and Central America* (Knopf, New York, 1957), pp. 67, 110.
15. The trade route hypothesis has been presented by M. D. Coe [*The Jaguar's Children* (Museum of Primitive Art, New York, 1965), pp. 122–123] and by D. C. Grove [in *Dumbarton Oaks Conference on the Olmec* (Dumbarton Oaks Research Library and Collection, Washington, D.C., 1968), pp. 179–185]. Jade sources are reported for several areas of Guerrero; see for example, A. Caso, in *Handbook of Middle American Indians*, R. Wauchope, Ed. (Univ. of Texas Press, Austin, 1965), vol. 3, p. 896.
16. Olmec presence in the central highlands and Guerrero has previously been attributed simply to the diffusion of the Olmec religious cults, or to the spread of Olmec religion by actual missionaries. At the present time, the data still best support the trade route theory, although trade may have served as a vehicle for the diffusion of religion. However, I see little data showing a widespread acceptance of Olmec religion in regions peripheral to Olmec trade routes.

3 February 1969

Pheromone-Induced Changes in the Acidophil Concentration of Mouse Pituitary Glands

Abstract. *Pituitaries of female mice in anestrus resulting from colony housing were characterized by a 58.0-percent acidophil content. Subsequent exposure to restrained male mice for one and two nights failed to evoke significant acidophilic degranulation and resulted in pituitary acidophil values of 57.4 and 55.1 percent respectively. Exposure to released males on the third night produced marked acidophilic degranulation resulting in a significant decline in pituitary acidophils to 38.0 percent. These findings support the view that female pheromone suppresses and male pheromone favors the secretion of follicle-stimulating hormone and indicate that luteotrophic hormone is secreted at its assigned time in the sequence of cyclic ovarian events initiated by the secretion of follicle-stimulating hormone.*

The term pheromone, originally proposed to describe chemical substances used for animal communication (*1*), has come to connote substances which are passed to the exterior by an animal to be received by and to evoke one or more specific responses in others of the same species (*2*).

Pheromones produce marked effects on the mouse estrous cycle. One pheromone, secreted by grouped females, results in pseudopregnancy when female mice are housed in groups of four (*3*). This effect is intensified and produces overt anestrus when groups of 30 females are housed together (*4*). Another pheromone, secreted by male mice, releases nonpregnant females from cyclic inhibition and stimulates the attainment of estrus. In addition, it blocks implantation in recently bred female mice and again stimulates the attainment of estrus (*5*). Pairing of previously isolated nonbred females with males results in a peak incidence of mating on the third night thereafter with maximum mating occurring with females previously housed in groups (*6*, *7*). Physical contact between the sexes is not a requirement for this male-induced estrus since confinement of the male in a wire basket in the presence of females continues to evoke a maximum incidence of estrus on the third night (*6*).

The female pheromone may act to block the secretion of follicle-stimulating hormone (FSH), whereas the male pheromone acts to stimulate such secretion (*8*). Blockage of implantation, however, has been attributed to failure of luteotrophic hormone (LTH) secretion (*9*), and pregnancy has been maintained by injections of luteotrophin (prolactin) on the first, second or third day after mating (*10*). It has been suggested that a reciprocal relation may exist between the secretion of FSH and LTH (*11*, *12*) and that stimulation of gonadotrophin secretion may in itself inhibit release of LTH (*11*, *13*).

Numerous studies have indicated that hormone elaboration by the pituitary gland may vary quantitatively with numerical changes in the concentration of specific pituitary cell types in response to physiological or pathological stimuli. The concept of FSH-LTH reciprocity, if true, would call for marked changes in the secretion of luteotrophin in response to male and female pheromones. We studied these changes by comparing the concentration of pituitary acidophils in grouped female mice with that in females exposed to restrained male mice for the first and second nights and to released males on the third night after removal from colony housing.

Randomly bred Swiss mice approximately 10 weeks of age were housed in the central animal facility (24°C and 55 percent relative humidity). They were given free access to food and water and kept on 12-hour nonreversed periods of alternating light and dark.

Females were housed in colony cages in groups of 25 for 10 days. At that time, ten were selected at random, and their pituitaries were collected. The remaining females were then distributed in groups of three to cages containing one male restrained in a wire basket. After the first night, one female was removed from each cage, and her pituitary gland was collected. Pituitaries were collected from a second female in each cage after the second night's residence. The wire basket was then removed, and males were released to the females on the third night. These females were examined the following day for copulation plugs, and their pituitary glands were removed.

Mice were routinely killed between 12 noon and 1 p.m. Pituitaries were removed immediately, fixed in Helly's solution for 90 minutes, embedded in Paraplast, cut into 3-μ sections, and

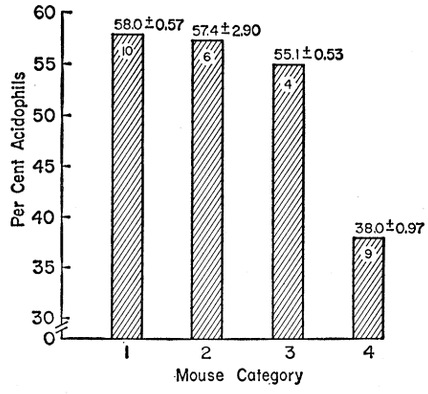

Fig. 1. Percentages of acidophils in pituitaries of female mice subjected to male and female mouse pheromones. (1) colony-housed females; (2) females exposed to restrained males for a single night; (3) females exposed to restrained males for two consecutive nights; (4) females exposed to released males 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.

THOMAS L. AVERY
St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38101

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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