tubes of germinating zoospores for the avocado roots was evident in the case of spores settling on the bottom of the petri dish at distances of up to 2 to 3 mm from the root. The germ tubes were uniformly directed toward the avocado root pieces.

3) Shortly after spore germination occurred, invasion of the root took place through unwounded tissue, and within 24 hours a brown lesion was visible in the region of elongation, identical in appearance to lesions observed in the case of infection of intact plants.

4) Evidence was obtained that the attractive substance is specific to the susceptible living avocado root, since roots killed by boiling or by propylene oxide did not attract zoospores. No chemotaxy of zoospores occurred toward actively growing roots of several other types of plants (tomato, tobacco, mandarin orange) (Table 1). There was also evidence of decreased attraction in the case of avocado varieties with some resistance to *Phytophthora cinnamomi*. Some roots of other plants (macadamia nut, sweet orange, pea) exhibited attraction for the zoospores, but this was primarily to root tips and cut ends of roots rather than to the region of elongation. As further evidence of specificity, zoospores of *Phytophthora citrophthora*, a citrus pathogen, were not attracted to avocado roots, but were attracted to citrus roots (*7*).

5) Zoospores of *P. cinnamomi* and their germ tubes showed chemotactic and chemotropic activity for aqueous extracts of susceptible avocado roots taken up on filter-paper disks. The nature of the substance is under investigation.

These results obviously have interesting implications with respect to resistance and susceptibility of plant roots to pathogens, as well as to various basic aspects of mechanisms of invasions and pathogenicity (*8*).

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References and Notes

1. W. Brown, *Botan. Rev.* **2**, 236 (1936).
2. C. J. Brokaw, *Nature* **179**, 525 (1957); L. Machlis, *Physiol. Plantarum* **11**, 845 (1958); W. Pfeffer, *Unter Botan. Inst. Tubingen* **1**, 363 (1884).
3. P. M. Goode, *Trans. Brit. Mycol. Soc.* **39**, 367 (1956).
4. N. T. Flentje, in *Plant Pathology: Problems and Progress 1908–1958* (Univ. of Wisconsin Press, Madison, 1959), p. 76.
5. G. A. Zentmyer, *Phytopathology* **50**, 660 (1960).
6. ———, *ibid.* **49**, 556 (1959).
7. The culture of *P. citrophthora* was provided by L. J. Klotz of this department.
8. This report is paper No. 1246 from the Citrus Experiment Station, University of California, Riverside.

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Patterns of Corticosteroid and Pepsinogen Change Related to Emotional Stress in the Monkey

Abstract. In association with conditioned avoidance sessions of 72 hours' duration, monkeys showed a response pattern characterized by increased levels of 17-hydroxycorticosteroids and decreased levels of pepsinogen during the stress period, with a marked and prolonged elevation of pepsinogen levels occurring during the recovery period.

Previous reports from this laboratory have described long-term studies of the effects of behavioral conditioning procedures upon the pituitary-adrenal cortical system in the monkey (*1*, *2*). A major objective in the extension of this work has been the measurement of additional endocrine or visceral functions so that centrally integrated patterns of visceral activity might be brought under investigation. In recent studies concerned primarily with the effects upon adrenal cortical activity of repeated conditioned emotional stress in the monkey, a high incidence of gastric or duodenal ulceration was observed (*3*). These observations prompted us to include, along with determinations of pituitary-adrenal cortical activity, the measurement of pepsinogen levels as a means of indirectly evaluating gastric function in animals under stress. The present report, then, describes a preliminary effort that was carried on to compare plasma 17-hydroxycorticosteroid (17-OH-CS) and pepsinogen responses during and following periods of sustained emotional stress associated with avoidance behavior in the monkey.

Four adult rhesus monkeys, two of each sex, were placed in an experimental chair-type restraining apparatus and allowed several days for adaptation according to previous studies (*4*).

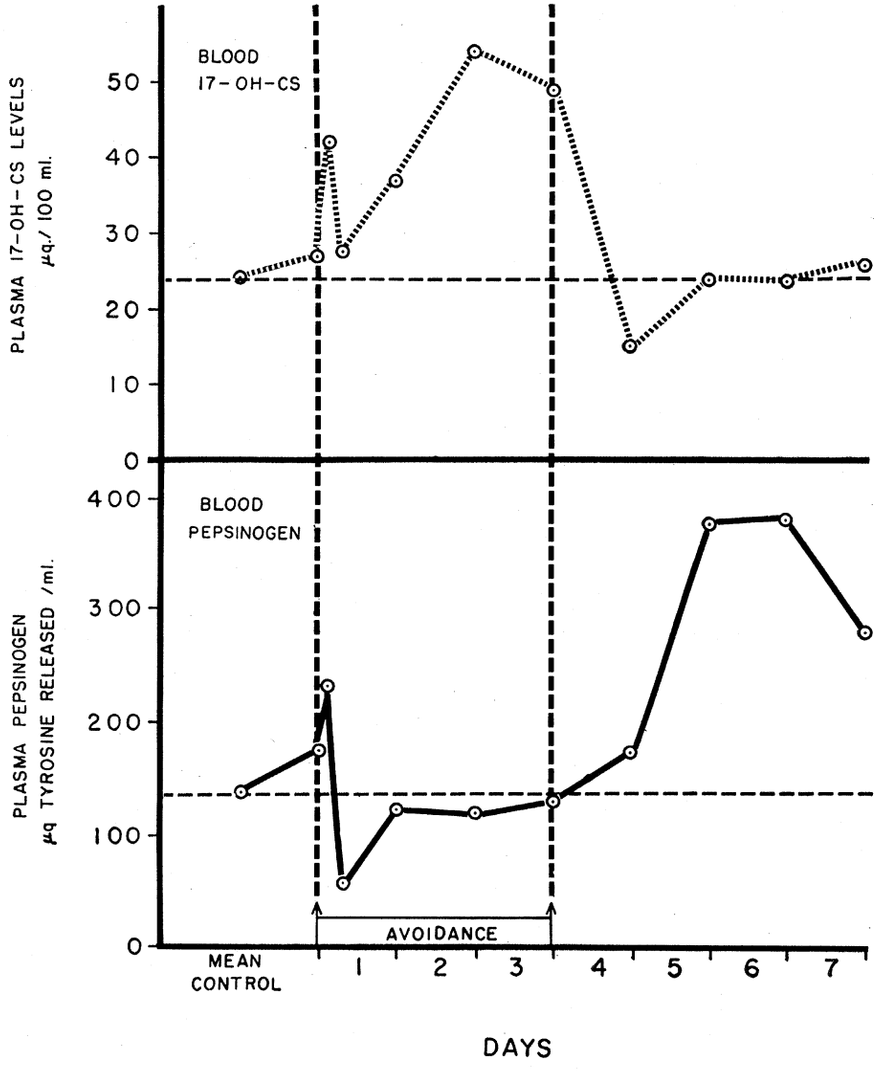

Fig. 1. Mean blood levels of 17-OH-CS and pepsinogen during 72-hour continuous avoidance sessions.

The animals were trained on a Sidman avoidance procedure, in which they must press a hand lever at a moderate, steady rate in order to avoid an electric shock to the feet which, in the absence of lever-pressing, would be delivered automatically every 20 seconds. Even though monkeys learn this procedure well and receive very few shocks, such conditioning sessions are associated with substantial emotional disturbances as evidenced by plasma 17-OH-CS elevations (*2*).

After completion of avoidance training all animals were allowed to sit quietly for a period of 10 days, during the latter part of which control biochemical measurements were made. Then they were subjected to a continuous, 72-hour avoidance session under the conditions described above. In all animals measurements of plasma 17-OH-CS (*5*) and plasma pepsinogen (*6*) were made at 9 A.M. during the final 3 days of the control period, the avoidance period, and a 5-day recovery period. In addition, blood samples were taken 2 and 6 hours after the start of the avoidance session. In one pair of monkeys, 24-hour uropepsinogen excretion (*6*) was followed and in the other pair 24-hour urinary 17-OH-CS excretion (*7*) was measured during the same periods as the plasma measurements.

The mean results of plasma pepsinogen and 17-OH-CS for the four monkeys are shown in Fig. 1. On the first day there is an initial moderate 17-OH-CS elevation, followed by a brief decline, but then a gradual increase in 9 A.M. values during the remainder of the 72-hour avoidance period. In the recovery period there is a rebound depression on the first day, followed by stabilization at pre-experimental baseline levels.

Plasma pepsinogen levels during the first two hours also showed a brief rise, but during the remainder of the 72-hour avoidance period remained below pre-experimental baseline levels. Perhaps the most striking feature of these experiments, however, is the marked, prolonged elevation in plasma pepsinogen levels which develops rather slowly during the recovery period. It is really not until the second morning after the conclusion of the avoidance period that this change becomes fully evident, and levels are still appreciably above the pre-experimental baseline 5 days after the avoidance period.

The associated changes in urinary 17-OH-CS, pepsinogen, and water excretion are summarized in Fig. 2. The urinary findings generally support the blood measurements, indicating a 17-OH-CS elevation and a pepsinogen depression during avoidance, with a prolonged pepsinogen elevation in the aftermath of avoidance. It is also of interest that urine volume tends to fluctuate substantially and in a rather smooth pattern similar to that of pepsinogen excretion although fluid intake was rigidly maintained constant in these animals.

While considerable controversy still exists over the significance of pepsinogen measurements, the fact that both plasma and urinary levels changed substantially and in the same direction in the present experiments would seem to support an interpretation of increased gastric release of pepsin. Other related studies in our laboratory also furnish some evidence in support of this interpretation (*8*). It should be emphasized, however, that firm conclusions regarding gastric changes can be made only with the direct measurement of the gastric secretory rate of pepsin.

The problem of the central regulatory mechanisms underlying the pepsinogen elevation following avoidance is a provocative one, but cannot be settled by our present data. These findings furnished little suggestion that the pepsinogen response could be dependent upon the influence of adrenal cortical hormones, since the two systems have quite different temporal patterns. The prolonged duration of the pepsinogen response also would seem to militate against an explanation based upon an underlying increase in parasympathetic activity. Additional hypotheses which might merit experimental consideration include other hormonal influences, possibly such as growth hormone, which has been shown to elicit marked increases in the secretory capacity of pepsinogen (*9*).

A word of caution may be in order against broad generalizations about gastric responses to other types of stress from this study of only a single type of stressful situation. It is also possible that modified or cumulative effects may develop with many repetitions of conditioning sessions in the same animal. It should be pointed out that the factor of sleep deprivation is an added variable in these experiments and must eventually come under separate evaluation.

In any event, the present data call attention to perhaps two general principles which may be useful in future stress research.

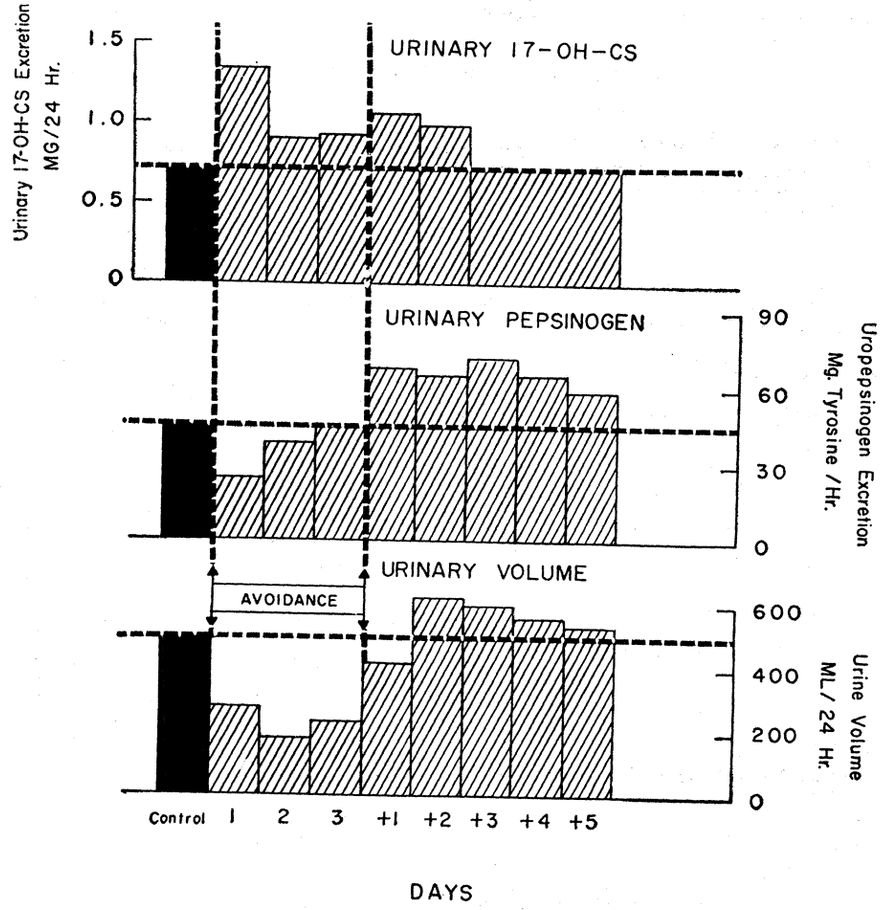

Fig. 2. Urinary volume, 17-OH-CS, and pepsinogen changes associated with 72-hour continuous avoidance sessions.

First, it appears that stressful situations must be considered in their full temporal aspects, and that increased concern with observations during a rather long recovery phase may be particularly profitable. It seems likely that, during the stress aftermath, predominance of regulatory changes associated generally with anabolic events promoting restoration and repair would be appropriate. The possibly critical nature of the temporal patterning of stress versus rest periods in the determination of visceral disorders is also suggested by these data, which indicate that rest periods must be of sufficient duration for the full development of this delayed and prolonged gastric aftereffect.

Secondly, although only two visceral systems were studied, the suggestion is raised that the over-all visceral response to stress may include a much greater variety of regulatory changes than is generally suspected. It appears an important goal in stress research, therefore, to evaluate function in many regulatory systems concurrently so as to test the hypothesis that the responses of these systems are integrated into characteristic and purposeful patterns appropriate to the actual or expected adaptive metabolic needs of the organism (*10*).

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References and Notes

1. J. W. Mason, J. V. Brady, M. Sidman, *Endocrinology* **60**, 741 (1957).
2. J. W. Mason, in *Recent Progress in Hormone Research*, G. Pincus, Ed. (Academic Press, New York, 1959), vol. 15, pp. 345–389.
3. R. W. Porter, J. V. Brady, D. G. Conrad, J. W. Mason, R. Galambos, D. McK. Rioch, *Psychosomat. Med.* **20**, 379 (1958); J. V. Brady, R. W. Porter, D. G. Conrad, J. W. Mason, *J. Exptl. Anal. Behavior* **1**, 69 (1958).
4. J. W. Mason, *J. Appl. Physiol.* **12**, 130 (1958); J. W. Mason, C. T. Harwood, N. R. Rosenthal, *Am. J. Physiol.* **190**, 429 (1957).
5. D. H. Nelson and L. T. Samuels, *J. Clin. Endocrinol. and Metabolism* **12**, 519 (1952); C. T. Harwood and J. W. Mason, *ibid.* **16**, 790 (1956).
6. M. L. Anson, *J. Gen. Physiol.* **22**, 79 (1938).
7. N. R. Rosenthal and J. W. Mason, *J. Lab. Clin. Med.* **53**, 720 (1959).
8. R. M. Rose, J. W. Mason, R. O. Poe, J. A. Jones, E. P. Lawrence, in preparation; E. Polish and J. V. Brady, unpublished observations.
9. M. H. F. Friedman, J. T. Magee, N. Telfer, D. Sandweiss, *Am. J. Physiol.* **183**, 617 (1955).
10. We are indebted to Benjamin Jackson and Jeanette Moss for skillful and valuable technical assistance.

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Hormonal Control of Sex Attractant Production in the Cuban Cockroach

Abstract. Virgin females of *Byrsotria fumigata* (Guérin) and several other species of Blattidae produce volatile substances which attract males and release in them characteristic precopulatory behavior. The removal of the corpora allata from females shortly after the imaginal molt results in a failure of production of sex attractant, as assayed by male behavior. Implantation of corpora allata can effect recovery.

The occurrence of sex attractants or sex pheromones (ectohormones) (*1*) in a wide variety of insect species of several orders, and their prominent role in assuring successful mating, are well-known phenomena (*1*, *2*). Their sites of production have been described in some species of Lepidoptera (*2*), and the isolation and chemical identification of two female sex pheromones—those of the moths *Bombyx mori* and *Porthetria dispar*—have recently been achieved (*3*). The physiology of the pheromone receptor organs in the males of several moth species is under investigation (*4*), but little is known of the control of pheromone production in the female.

A clue to possible physiological control was provided by the report of Engelmann (*5*) that a significant percentage of adult females of the cockroach, *Leucophaea maderae*, failed to mate when deprived of their corpora allata 1 day after emergence as adults. Engelmann noted that mating frequently occurred after the implantation of active corpora allata from young last-instar nymphs. Since virgin females of several cockroach species are known to produce sex pheromones (*6*), one may legitimately conclude that such substances are probably of considerable importance in sex recognition and the release of male precopulatory behavior. The present investigation was undertaken to examine the possibility that the failure of allatectomized females to mate might be mediated by failure of sex pheromone production.

To test this hypothesis, the Cuban roach, *Byrsotria fumigata* (Guérin), was used. All females in these experiments were kept singly in 250-ml beakers, the bottoms of which were lined with disks of Whatman No. 2 filter paper. Males were isolated from females and stored in groups in large containers. The testing procedure for pheromone production was as follows.

The filter paper was removed from the beaker containing the female and placed for 2 minutes in a container of males. If a positive response—indicating the presence of the pheromone—was to appear, it nearly always did so within 1 minute. A positive response is signaled by the "wing-raising display," characteristic of the precopulatory behavior of many roaches (*6*, *7*). Actual contact with other males at this time frequently results in the onset of copulatory movements. In the absence of the pheromone, the response is negative; that is, the filter paper is ignored or, at most, casually examined without any evidence of sexual excitement. Tests of this type show that normal virgin females begin to produce the pheromone 10 to 30 days after the imaginal molt and continue to do so for several weeks, sometimes for several months. Females that are permitted to mate show generally a gradual decrease in pheromone production, as indicated by the number of males responding during the tests. Mated or unmated females carrying öothecae rarely produce the pheromone.

To determine whether allatectomized females produce sex pheromone, corpora allata were removed 1 to 3 days after the imaginal molt. These animals, along with the controls, were tested for pheromone production every 5 to 10 days for 30 to 40 days. The results, summarized in Table 1, show that 90 percent of the unoperated controls and 86 percent of the sham-operated controls produced pheromone during the test period, while only 14 percent of the allatectomized individuals did so. Thus, the presence of corpora allata during the test period seems essential for the production of the pheromone.

The correlation between pheromone production and actual mating, as indicated by the presence of a spermatophore in the female's bursa copulatrix, is shown in Table 2. Animals from Table 1 were used in these tests except for an additional group of unoperated controls. After testing each female for pheromone production, a male was added to the female in each beaker, and then each female was examined daily for the presence of a spermatophore in the bursa copulatrix. If mating failed to occur within 3 or 4 days, pheromone production was retested and fresh males

Table 1. Number of *Byrsotria fumigata* females producing sex pheromone. The numbers in parentheses are percentages.

No. tested	Sex pheromone production	
	Presence	Absence
	Unoperated controls	
21	19 (90.4)	2 (9.6)
	Sham-operated controls	
50	43 (86.0)	7 (14.0)
	Allatectomized females	
90	13 (14.4)*	77 (85.6)

*Includes five animals that developed a limited sex pheromone production after the original test period, including the two which subsequently mated (see Table 2).