Table 1. Mean concentrations of unbound corticosterone (micrograms per 100 milliliters of plasma) in mice which were exposed to physical defeat by a fighter for 6 to 9 days (group 1), similarly treated for 5 days and then placed only in the fighter's presence on days 6 to 9 (group 2), or continually exposed to only the fighter's presence (group 3). The results are shown for four samples of plasma (each pooled from five mice) per daily average per group (12 samples when days pooled for each treatment).

Day	Group		
	1	2	3
6	9.0	11.1	3.8
7	9.5	11.2	1.2
9	3.5	10.9	4.5
	Mean ± standard error		
	7.3±1.6	11.1±1.1	3.2±0.9

trained mouse, and, within a few days, all of the behavioral symptoms of severe subordination on the part of the untrained, attacked mouse (*8*).

Mice were killed by decapitation 1 hour after the end of daily sessions with the trained fighter and within 15 seconds after their home cages were removed from the rack. Mice treated in each different way were divided into groups of five, the blood from each group being pooled. The plasma was then analyzed fluorimetrically for unbound (not precipitated by zinc) corticosterone (*9*).

Two-way analysis of variance revealed large differences which could be attributed to the experimental procedures ($p < .001$), but no significant differences which could be attributed to the days when blood was collected or to a possible interaction. Mice which had never been physically defeated by a fighter (group 3) had generally low concentrations of unbound hormone (Table 1). Those which had been exposed to the fighter's presence on the days of blood collection and which had experienced physical defeat (group 2) had continually high concentrations. Group 1, which was physically exposed to a fighter every day throughout the experiment, had relatively high concentrations, at least initially. Since variance analysis revealed no difference between results obtained on the 3 days on which blood was collected, the data within each type of treatment were pooled and the resulting means tested against each other by *t*-tests. The mean for group 3 was significantly lower than that for group 2 ($p < .001$) and, despite the overlap on day 9, was significantly lower than the mean for group 1 ($p < .05$. Group 1 was not significantly different from group 2.

The amount of unbound corticosterone is well suited as the measure of adrenal response in this type of experimentation because: (i) unbound corticosterone is present only in trace amounts in isolated controls; (ii) short daily exposures to fighters have little, if any, effect on bound corticosterone; (iii) the peak concentration of unbound corticosterone in the plasma is reached about 1 hour after exposure to the fighter; and (iv) after the 4th day of exposure to fighters, the concentrations return to trace amounts by 3 hours after each exposure (*10*). This last point is particularly important in the present experiment since differences in concentrations of unbound hormone which were found among the mice killed on days 6, 7, or 9 cannot be considered as having been carried over from the treatment on previous days.

The significant increase in the concentration of unbound corticosterone in mice placed in the presence of a trained fighter, given a background of physical defeat, when compared with those which had never experienced defeat, adequately demonstrates the reality of a psychological component in the adrenocortical response to defeat in a fighting situation. It is important that mice which had experienced defeat actually had somewhat higher concentrations of unbound hormone when exposed to a fighter's presence than did mice actively attacked by the fighter on the days of blood collection. This difference was not significant but does attest to the relative importance of psychological, as opposed to physical, stimuli. These data may, in addition, indicate that much the same type of stimuli could be responsible for hyperactivity of the adrenal cortex among crowded mice; that is, neurotropic stimuli elicited by the presence of the dominant animal in the group. Socially subordinate mice are known to have heavier adrenals than those which are dominants (*11*).

F. H. BRONSON
B. E. ELEFTHERIOU
The Jackson Laboratory, Bar Harbor, Maine, and *Department of Zoology, Kansas State University*

References and Notes

1. J. J. Christian, *Naval Med. Res. Inst. Lect. Rev. Ser. 60-2*, 49 pp. (1960).
2. E. Tobach and H. Block, *Am. J. Physiol.* **187**, 399 (1956); P. Lemond, *Proc. Soc. Exptl. Biol. Med.* **102**, 292 (1959).
3. D. E. Davis and C. P. Read, *Proc. Soc. Exptl. Biol. Med.* **99**, 269 (1959).
4. J. J. Christian and C. D. LeMunyan, *Endocrinology* **63**, 517 (1958).
5. K. Keeley, *Science* **135**, 44 (1962).
6. J. J. Christian, *Am. J. Physiol.* **182**, 292 (1955); S. A. Barnett, *J. Psychosomatic Res.* **3**, 1 (1958); F. H. Bronson and B. E. Eleftheriou, *Physiol. Zool.* **36**, 161 (1963).
7. C. H. Southwick and J. P. Bland, *Am. J. Physiol.* **197**, 111 (1959); J. J. Christian, *Proc. Soc. Exptl. Biol. Med.* **101**, 166 (1959).
8. J. P. Scott, *J. Comp. Psychol.* **39**, 379 (1946).
9. R. Guillemin, G. W. Clayton, H. S. Lipscomb, J. D. Smith, *J. Lab. Clin. Med.* **53**, 830 (1959); K. M. Knigge and R. M. Hoar, *Proc. Soc. Exptl. Biol. Med.* **113**, 623 (1963).
10. F. H. Bronson and B. E. Eleftheriou, *Proc. Soc. Exptl. Biol. Med.*, in press.
11. D. E. Davis and J. J. Christian, *ibid.* **94**, 928 (1957).
12. Supported by PHS research grant HD 00767 from NIH, and by grant No. 663 from the Agricultural Research Station, Kansas State University.

18 November 1964

Feeding Stimulants for the Female House Fly, *Musca domestica* Linneaus

Abstract. *Both casein and yeast hydrolysate contain feeding stimulants for the adult female house fly. Guanosine monophosphate is the major active component in yeast hydrolysate. Several amino acids, including leucine, methionine, lysine, and isoleucine, are also effective feeding stimulants and are presumed to be the active components in the casein hydrolysate. Solution in phosphate buffer is necessary in all instances to obtain maximum activity with the stimulants.*

During experiments on nutrition and reproduction in the house fly, the addition of yeast hydrolysate (*1*) to a dry semidefined adult diet appeared to make the diet more attractive to the flies, since both the incidence of feeding and the amount of food taken up increased. To determine whether this effect was due to an attractant or a feeding stimulant, or both, filter-paper discs were impregnated with solutions of the yeast hydrolysate and placed in cages of 4- to 7-day-old house flies which had fed on dry granular sucrose and water since emergence. Attraction from a distance could not be demonstrated by a variety of techniques (*2*); however, flies which came in contact with the disc extended

their probosces and fed vigorously, and within a few minutes a large cluster of flies accumulated on the disc. Of 280 individuals entrapped in such clusters, 98 percent were females; further trappings confirmed the high incidence of females in these clusters.

To isolate and identify the feeding stimulant(s) the following bioassay was devised. Three equidistant circles (3.2 cm in diameter) were drawn on a filter-paper disc (*3*) and the materials to be assayed were made up in 0.133*M* phosphate buffer (*p*H 7.2). A 0.1-ml sample of the solution was delivered to the center of a circle. The other circles served as blanks or as controls or for the comparison of compounds. The disc was air dried, placed in a 9-cm petri dish, and inserted into a screened cage (30 by 30 by 40 cm) containing 1400 to 2200 flies—4- to 7-day-old adults from a 1948 NAIDM strain (*4*). These flies were reared on CSMA larval medium (*4*) and after emergence were provided with dry granulated sucrose and water, which remained in the cage during the assay. When active materials were tested in this manner, the flies rapidly congregated and fed, forming an aggregate several flies in depth (Fig. 1).

Since the response of the female flies to some of the materials with stimulatory activity increased with the age of the fly and depended on the state of ovarian development, absolute values for the activity of these materials could not be obtained. However, relative values were obtained, or comparisons were made, by determining (i) the relative quantity of each different compound that caused congregation of flies, (ii) the priority of choice among the same quantities of different compounds tested on the same disc, and (iii) the time the flies would remain aggregated on a known quantity of a material. In addition, the compounds were tested by determining the percentage of flies extending their probosces when their tarsi were stimulated. For this test, groups of 30 to 50 flies were attached with soft beeswax to applicator sticks in a manner described for the blowfly (*5*), and their response recorded after immersion of their tarsi in the appropriate solutions.

The active compounds of the yeast hydrolysate were isolated by ion-exchange chromatography. Yeast hydrolysate, 10 g dissolved in 50 ml of water, was chromatographed on a 62.5- by 2.5-cm column of Dowex 50W — X8 (H^+) resin (20 to 50 mesh). The column was eluted with water (3 ml/min) and 100-ml fractions were collected. The first fraction with definite activity (fraction 4) yielded 285 mg of material that exhibited activity at 5 mg (*3*). Fractions 5 through 7 (combined weight 153 mg), tested at 1 mg, showed even greater activity. The ultraviolet absorption spectrum of the active fractions showed a peak of 257 mμ with a shoulder at 276 mμ (typical ultraviolet spectrum of the guanosine nucleotide or nucleoside).

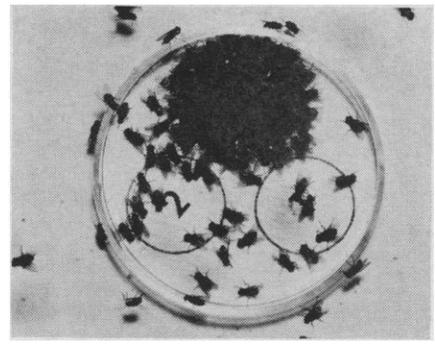

Fig. 1. Bioassay of a feeding stimulant on filter paper showing clustering of female flies. Circles *1* and *2* are the blank and the phosphate buffer control, respectively; circle *3* covered with flies contains 1 mg of L-leucine in phosphate buffer.

Calculations based on a molecular extinction coefficient of 14,000 for guanosine monophosphate (GMP) indicated that fractions 5 through 7 contained 110 mg of GMP. Further fractionation of fractions 5 through 7 on Dowex 50W — X8 resin yielded 91 mg of material that exhibited a molecular extinction coefficient of 14,150 at 257 mμ. The observed value from a quantitative phosphate analysis (*6*) after the sample was wet-ashed agreed with the calculated phosphate value for GMP. The material was analyzed by thin-layer chromatography on cellulose powder (*7*), by means of the solvent system—a mixture of saturated ammonium sulfate, 1*M* sodium acetate, and isopropanol (80:18:2) being used. Two spots that corresponded to the R_F values of the 2′- and 3′-isomers of guanosine monophosphate were detected. Quantitative separation of the isomers was accomplished by ion-exchange chromatography (*8*) on a Dowex 2 — X8 resin column (200 to 400 mesh, 12.5 by 1.7 cm; 20-ml fractions collected, flow rate 1.5 ml/min, chloride form; eluent, 0.005*N* hydrochloric acid). The 5′-, 2′-, and 3′-phosphate isomers were eluted from the column in fractions 75 through 100, 112 through 146, and 162 through 212, respectively, and were present in quantities of about 3, 33, and 64 percent, respectively. The ultraviolet absorption spectrum and the respective thin-layer chromatography R_F values for the authentic GMP isomers and the isolated GMP isomers from yeast hydrolysate were identical. The infrared spectra, taken in Nujol mull, of the major GMP isomer from the yeast hydrolysate, namely guanosine-3′-monophosphate, and of authentic guanosine-3′-phosphate were also identical.

Table 1. Feeding and clustering response (+) of 4-day-old female house flies presented with selected compounds (−, no response). (GMP, guanosine monophosphate; GDP, guanosine diphosphate; GTP, guanosine triphosphate; d-GMP, deoxyguanosine monophosphate; AMP, adenosine monophosphate.)

Compound	Quantity (mg)*		
	1.0	0.1	0.01
Nucleotides			
GMP-5′	+	+	+
GMP-3′	+	+	+
GDP-5′	+	+	+
GTP-5′	+	+	+
d-GMP-5′	+	+	+
AMP-5′	−	−	−
Amino acids			
L-Leucine	+	+	+
L-Methionine	+	+	+
L-Isoleucine	+	+	−
L-Lysine	+	+	−
L-Phenylalanine	+	−	−

* Milligrams per circle, see (*3*).

At quantities of less than 1 mg (*3*), all the isolated GMP isomers were very effective feeding stimulants for female flies, as were authentic samples (*9*) of the nucleotides. In equivalent quantities, guanosine-5′-monophosphate was a stronger stimulant than either GMP-3′ or GMP-2′. Guanosine-5′-diphosphate (GDP-5′), guanosine-5′-triphosphate (GTP-5′), and deoxyguanosine-5′-monophosphate (d-GMP-5′) were also active at less than 1 mg (Table 1). Certain other nucleotides, including those in yeast RNA (adenosine monophosphate, cytosine monophosphate, uridine monophosphate) and thymidine monophosphate, did not elicit a response at 2 mg. The di- and triphosphates of adenosine were likewise inactive on filter paper.

Despite intense feeding on quantities less than 1 mg of the active nucleotides on filter paper, these same nucleotides at high concentrations showed rather low activity in the tarsal stimulation test. The percentage of female flies

responding by proboscis extension to 1-percent solutions of these materials were as follows: 35.6 to yeast hydrolysate, 24.6 to GMP-5′, 12.4 to GMP-3′, and 30.0 to AMP-5′. All other monophosphate nucleotides tested for tarsal stimulation were inactive.

An enzymatic hydrolysate of casein (*10*) also stimulated feeding and clustering of female flies on filter papers, but the presence of GMP could not be detected by the same chromatographic and analytical procedures as used for yeast hydrolysate. Instead, the active fractions were eluted from the ion-exchange columns with 1*N* ammonium hydroxide solution and tested by the ninhydrin reaction which indicated the presence of peptides or amino acids, or both (*11*). When the constituent amino acids of casein were bioassayed on filter paper in phosphate buffer, five of these —leucine, isoleucine, methionine, lysine, and phenylalanine—were very active at 2 mg. Without the phosphate buffer these amino acids exhibited only slight activity, even at 2 mg. Several other anions, including sulfate, carbonate, and chloride, were tested as possible replacements for the phosphate; none were effective. Bioassay on filter paper showed that L-leucine and L-methionine in phosphate buffer were the most active of the amino acids (Table 1). The D-isomers of all the active amino acids showed slight activity when tested at 1 mg. However, when the D- and the L-isomers were compared on the same disc, the flies always selected the L-isomer even in the presence of more of the D-isomer. The peptide L-leucyl-L-leucine was inactive even at 2 mg. The active amino acids were also specific for female flies.

The stimulatory activities of the amino acids on the tarsi agreed well with the feeding and clustering tests on filter papers. Leucine, isoleucine, norleucine, lysine, and methionine at 1-percent concentrations stimulated more than 50 percent of the tethered flies. Phenylalanine, glutamic acid, and aspartic acid were stimulatory, but to a lesser number of flies, and the last two amino acids did not cause clustering on filter paper.

When tested for tarsal stimulation at a comparable molarity of 0.05*M*, the active amino acids stimulated more flies than the guanosine nucleotides. Amino acids at about 0.005*M* concentrations elicited responses from 50 percent of the flies. In contrast, even at concentrations higher than 0.05*M*, the guanosine nucleotides did not elicit responses from more than 40 percent of the flies. In tarsal stimulation tests adenosine monophosphate was as active as GMP-5′ and more active than GMP-3′, yet on filter paper it was inactive.

Differences in the relative activities of the nucleotides tested on free flies (filter-paper assay) or on tethered flies (tarsal stimulation) are not strictly comparable, since behavior of a group as opposed to that of an individual must be considered. Also, a large group of physiologically responsive flies is present during the filter-paper assay. Yet for reasons now obscure, some nucleotides that elicit proboscis extension when presented to the tarsi did not evoke feeding and clustering on filter paper. The active amino acids showed a better correspondence between tarsal stimulation and clustering of female flies. These amino acids also stimulated tarsi of males, but the clustering phenomenon occurred only with females. Phosphate buffering of all materials was necessary to obtain maximum feeding and clustering as well as maximum tarsal stimulation. Phosphate buffer alone was inactive by either assay.

Several adenosine nucleotides, including the mono-, di-, and triphosphates, are potent feeding stimulants for female mosquitoes of two species, *Culex pipiens* var. *pallens* Coquillett (*12*) and *Aedes aegypti* (Linnaeus) (*13*). However, the guanosine nucleotides were ineffective with these two species. In *C. pipiens* var. *pallens* the 3′- and 2′-isomers of adenosine monophosphate did not elicit responses and only the 5′-isomer caused feeding and engorgement. A comparable structure-activity relationship exists between the GMP isomers as flavor enhancers for human food in that the 5′-isomer alone is active (*14*). With the female house fly, although the 5′-isomer was the most active, the 3′- and 2′-isomers were also active.

Yeast and certain protein hydrolysates are extremely attractive foods as baits to several species of Diptera (*15*), including certain economically important species of fruit flies (*16*). Recently, a combination hydrolysate-pesticide poison bait was used extensively in eradication of the introduced Mediterranean fruit fly [*Ceratitis capitata* (Wiedemann)] (*17*). It will be interesting to determine whether the same components that stimulate feeding in the house fly are responsible for the effectiveness of the protein hydrolysates used as baits for other insects.

W. E. Robbins, M. J. Thompson
R. T. Yamamoto, T. J. Shortino
Entomology Research Division,
U.S. Department of Agriculture,
Beltsville, Maryland 20705

References and Notes

1. Yeast hydrolysate-enzymatic (an autolysate of Brewers yeast, USP), lot No. 8031, Nutritional Biochemicals Corp., Cleveland 28, Ohio.
2. V. G. Dethier, *J. Econ. Entomol.* **48**, 235 (1955).
3. Whatman No. 230, 9-cm diameter. Throughout this paper, the quantity of material tested refers to the number of milligrams delivered in 0.1 ml of phosphate buffer solution to a test circle 3.2 cm in diameter. This volume covers the entire circle.
4. "The Peet-Grady method," in *Soap and Chemical Specialties, Blue Book and Catalog 1959* (Chemical Specialties Manufacturing Association, New York, 1959), p. 219.
5. V. G. Dethier and L. E. Chadwick, *J. Gen. Physiol.* **30**, 247 (1947).
6. O. Lindberg and L. Ernster, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1956), vol. 3, p. 3.
7. K. Randerath, *Thin-Layer Chromatography* (Academic Press, New York, 1963), p. 188.
8. W. E. Cohn and J. X. Khym, in *Biochemical Preparations*, D. Shemin, Ed. (Wiley, New York, 1957), vol. 5, p. 40.
9. The nucleotides and amino acids used in these studies were purchased from Calbiochem, Los Angeles 54, Calif., and Nutritional Biochemicals Corp., Cleveland 28, Ohio.
10. Casein hydrolysate, enzymatic, lot No. 6610, Nutritional Biochemicals Corp.
11. Elution of the Dowex column with 1*N* ammonium hydroxide, after removal of the active GMP isomers, also yielded a minor active fraction from the yeast hydrolysate.
12. T. Hosoi, *J. Insect Physiol.* **3**, 191 (1959).
13. R. Galun, Y. Avi-Dor, M. Bar-Zeev, *Science* **142**, 1674 (1963).
14. A. Kuninaka, M. Kibi, K. Sakaguchi, *Food Technol.* **18**, 29 (1964); S. Hirao, *ibid.*, p. 36.
15. N. Green, M. Beroza, S. A. Hall, in *Advances in Pest Control Research*, R. L. Metcalf, Ed. (Interscience, New York, 1960), vol. 3, p. 129.
16. L. F. Steiner, W. C. Mitchell, K. Ohinata, "Fruit Fly Control with Poisoned-Bait Sprays in Hawaii," *USDA Rept. ARS 33-3* (1958).
17. L. F. Steiner, G. G. Rohwer, E. L. Ayers, L. D. Christenson, *J. Econ. Entomol.* **54**, 30 (1961).

23 December 1964

Preference for Shapes of Intermediate Variability in the Newborn Human

Abstract. *Newborn humans presented with pairs of shapes, each shape differing in number of turns (angles), prefer shapes with 10 turns to shapes with 5 turns or 20 turns, as inferred from photographic recordings of eye fixations.*

The possibility that newborn humans can perceive pattern has been suggested by recent evidence of Fantz (*1*) and Hershenson (*2*), although the results are by no means conclusive and the