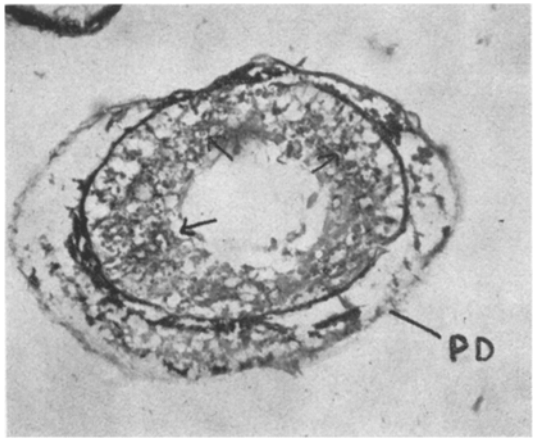

Fig. 4. Cross section (10 µm) of pedicle (PD) showing decrease in AF positive granules (arrows) AF × 360.

was reduction in the cell's size, which measured 30.0 × 20.5 µm with a nucleus of 9.5 µm diameter. During the last phase of vitellogenesis, when the oocytes were fully loaded with yolk spheres and measured from 2.17 to 2.25 mm in length, there is a recorded decrease in AF and PAVB positive granules in the pedicle, (Figure 4) as well as in the A-type cells. Simultaneously, the chorion formation takes place and the stainable granules in the pedicle come to a negligible concentration and some flaky mucoid substance was visible in this region.

It is difficult to trace the source of this AF and PAVB positive granules in the pedicle. But it is definite that the material is not secreted by the cells of the ovarian pedicle, because these cells always responded negatively to AF and PAVB stains. There could, therefore, be two likely possibilities. Either this material is other than the neurosecretory material, since, besides the NSM, a variety of other substances[6] are also revealed by these staining techniques, or this could be an additional storage site for the NSM elaborated by the A-type cells of the brain and ventral nerve cord ganglion. In *P. perpusilla*[3] the CHP, AF and PAAB positive material has been reported in pedicle and is interpreted as stored NSM[7]. WILLIAMS[8] also reported that the abdomen of male *Hyalophora cecropia* and *Cynthia* moths acts as a storage organ, for juvenile hormone (JH), which is secreted by the corpora allata. However, on the basis of the observations of WILLIAMS on the storage of JH hormone in certain abdominal organs of insects, the question of pedicle granules being a storage for some hormones in the insect under study cannot be ruled out. The maturation of the eggs in the absence of the median neurosecretory cells in *D. cingulatus*, *Rhodnius prolixus*[9] and *Oncopultus faciatus*[10] might probably be due to the presence of similar hormone deposits in the pedicles or in some other body tissues.

IVANOV and MESCHERSKAY[11] reported that the fat body produces a hormone which induces maturity of the ovaries in female cockroaches, *Blattella germanica* and *Blatta orientalis*. In *Iphita limbata*[12], a heat stable and water extractable material obtained from entire ripe ovarioles acts as an oyarian sex hormone which inhibits the neurosecretory supply to the corpus allatum, and simultaneously stimulates the discharge of neurosecretions from neurosecretory cells of the brain and thereby brings about oviposition. But the same result was not obtained when aqueous washings of freshly laid eggs were injected into other female bugs. The present observations suggest that the probable source of the ovarian sex hormone envisaged by NAYAR[12] and DOANE[13] might be related to the AF and PAVB positive granules of the ovarian pedicle of *D. koenigii* and may represent stored neurosecretory material.

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Lateral Hypothalamic 'Feeding' Sites and Gastric Acid Secretion

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Summary. Electrical stimulation within the lateral hypothalamus which had been effective in evoking stimulus-bound feeding in satiated cats did not produce any significant stimulating effect on gastric acid secretion in the same cats when hungry.

It is generally known that the lateral hypothalamic area contains structures responsible for the initiation of feeding[3,4]. It is reasonable to ask, therefore, whether the lateral hypothalamic feeding system also controls gastric acid secretion. So far, there have been only a few studies directly dealing with this problem[5–8]. It was found that gastric acid secretion increased as a result of electrical stimulation[5,6] or stimulation by 2-deoxy-D-glucose (2DG, a compound known to be effective in stimulating feeding)[7] within the lateral hypothalamus in acute rats. It was also observed that the stimulating effect of i. v. injection of 2DG on gastric secretion in chronic cats was abolished by bilateral lesions in the medial forebrain bundle, a hypothalamic structure known to be involved in feeding reactions[8].

[1] The author wishes to thank Dr. M. I. GROSSMAN for advice and valuable discussion and Dr. D. NOVIN for critical reading of the manuscript. Thanks are also due to Miss SUSAN DAVIS and to Mr. R. GARCIA for skillful technical assistance.
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The above data suggest that the lateral hypothalamic structures which govern the initiation of feeding may also influence gastric acid secretion. The present study was undertaken to further explore this possible relationship.

Method. The experiments were performed on adult cats, both male and female. In each animal 2 monopolar electrodes (made of stainless-steel wire, 0.3 mm in diameter, insulated except for 1 mm at the tip) were bilaterally implanted in the lateral hypothalamus. The coordinates of implantation were: A11, L3, H –4, according to the stereotaxic atlas by JASPER and AJMONE-MARSAN[9]. A reference electrode was placed in the calvarium over the frontal sinus.

Two weeks after the implantation a test for stimulus-bound feeding was performed. The cat was placed in an experimental compartment and allowed to eat ad libitum. When the animal stopped eating, electrical stimulation (1–3 V, 100 cps, 1 msec dur/imp, given either continuously or with 10 sec on and 10 sec off intervals) was applied unilaterally to the hypothalamic electrode tip. 7 cats in which electrical stimulation had resulted in eating were chosen for the experiment.

In each of these cats a gastric fistula was constructed, as described by EMÅS[10]. A few weeks later the experimental procedures started. First, during a few days the cat was trained to stay still in the stand (as shown by EMÅS et all.[11]). Then regular sessions were conducted 2 to 3 times a week. The cats were deprived of food for about 20 h before the session. Each session consisted of six 15 min periods of collection of basal gastric secretion. Immediately after the session the volume of each 15 min sample was determined and its acidity was measured by titrating a 0.2 ml sample to pH 7.0 with 0.2 *N* $NaHCO_3$ with the use of an automatic titrator ('Radiometer', Copenhagen). The total acid output was calculated by multiplying the volume of each sample by its acidity. After 5 control sessions, electrical stimulation of the hypothalamic site previously effective in producing feeding, was applied during the next 5 sessions. Stimulation was given with 10 sec on and 10 sec off intervals during the 3rd and 4th 15 min period of collection of gastric secretion in 5 cats (GH1–GH5) and either during the 3rd or 4th period in 2 other cats (GH7 and GH11). Parameters of stimulation were the same as those previously effective in evoking feeding.

After the completion of the experiments, the cats (except GH7 and GH11) were sacrificed and their brains were taken out for anatomical verification of the location of electrodes (Figure 1).

Results. It was found that the cats remained generally quiet during the hypothalamic stimulation, although licking, swallowing and salivation was frequently observed. The acid output was usually highest at the beginning of the session and tended to diminish toward the end of the session during both control and stimulation sessions. Figure 2 shows the diagrams of the mean values of the acid output in the periods before (A), during (B) and after (C) stimulation in stimulation sessions and in the corresponding periods of the control sessions (A′, B′, C′).

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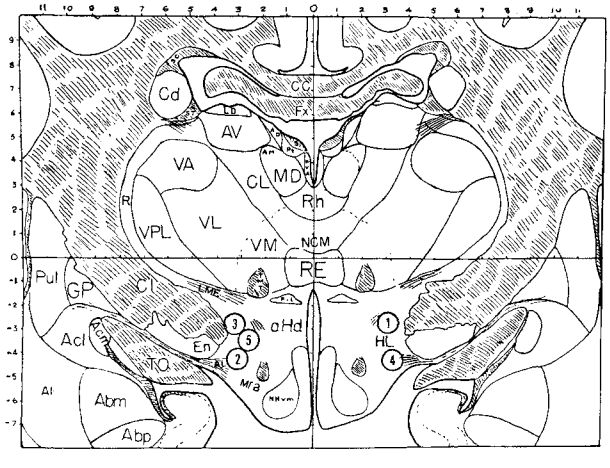

Fig. 1. A diagram of a frontal section of the diencephalon, after the atlas of JASPER and AJMONE-MARSAN[9], showing the sites of stimulation within the lateral hypothalamus. Numbers 1, 2, 3, 4 and 5 inside black circles show the location of the tips of stimulating electrodes in cats GH1, GH2, GH3, GH4 and GH5, respectively. The electrode tip locations for cats GH2 and GH3 were found 1 mm caudal to those shown above. HL, lateral hypothalamus; NHvm, ventro-medial hypothalamic nucleus; MFB, medial forebrain bundle; aHd, dorsal hypothalamus area. Other denotations refer to extra-hypothalamic structures.

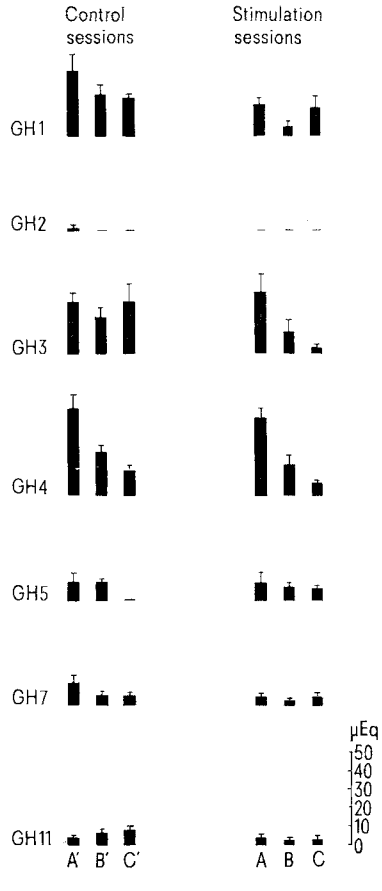

Fig. 2. The effect of electrical stimulation of the lateral hypothalamic 'feeding' sites on gastric acid secretion. Bars represent means ± SE for gastric acid output (μEq) in 15 min periods before (A), during (B) and after (C) the hypothalamic stimulation in 5 stimulation sessions and in the corresponding periods (A′, B′, C′) in 5 control sessions, for each cat (except GH5 in which 2 stimulation sessions were performed only). As shown above, the lateral hypothalamic stimulation produced no significant change or a slight decrease (in cat GH11) in gastric acid secretion.

Statistical analysis of the results was performed for each cat separately with the use of Wilcoxon rank sum test. Changes in the acid output from periods before to during, and from during to after stimulation in the stimulation sessions were compared to changes from the corresponding periods in the control sessions. No significant differences ($p > 0.05$) were found between the control and the stimulation sessions in 6 cats. In only one cat (GH11) a significant decrease ($p < 0.016$) in acid output was observed during stimulation sessions while an increase in acid output occurred during control sessions.

Discussion. This study showed that electrical stimulation within the lateral hypothalamus which had been effective in evoking feeding in satiated cats did not produce any significant increase in gastric acid secretion in the same cats in the hungry state.

These results are not consistent with the observations of the other authors[5–8] mentioned earlier in this paper. This difference in the results might be attributable to the difference in methods and in animal species used in their studies[5–7]. In addition, we cannot exclude a possibility that the sites of stimulation used by these authors were somewhat different than those used in our experiments. The electrolytic lesions within the lateral hypothalamus in studies of KADEKARO et al.[8] produced experimental conditions quite different from those of the present study. Therefore, the data obtained by those authors cannot be directly compared with our results.

On the other hand, the results of our experiments seem to be consistent with the observations of SEN and ANAND[12]. These authors found that electrical stimulation within the mid-lateral hypothalamus did not produce any significant change in gastric acid output in conscious cats. They also found that the sites effective in increasing acid secretion were located in the anterio-medial hypothalamus and preoptic area. In fact, in another study which is now in progress we did find sites within the antero-medial hypothalamus where electrical stimulation produced an increase in acid secretion in cats tested in the same experimental conditions as those of the present study; these effective sites corresponded to the loci indicated by SEN and ANAND[12]. Electrical stimulation of these sites did not, however, produce stimulus-bound feeding in these cats[13].

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[13] This study was supported by USPHS Grants Nos. AM 17328 and MH 13958.

Trypsin Activity in the Midgut of *Sarcophaga ruficornis* and *Musca domestica* (Diptera: Insecta)

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Summary. The pH and temperature for the optimum activity of trypsin from the midgut of *Sarcophaga ruficornis* and *Musca domestica* was 7.5 and 8.0 respectively and 50 °C. The enzymic activity increased with the increase in incubation period and enzyme concentration.

Proteins or their degradation products are essential dietary constituents for most insects[2]. The primary enzymes facilitating breakdown of complex proteins are proteinases or endopeptidases. Insect proteinases are generally active in neutral or alkaline medium, and thus resemble vertebrate trypsin. The present study deals with the nature of the midgut trypsin from the larvae and adults of *S. ruficornis* and *M. domestica*.

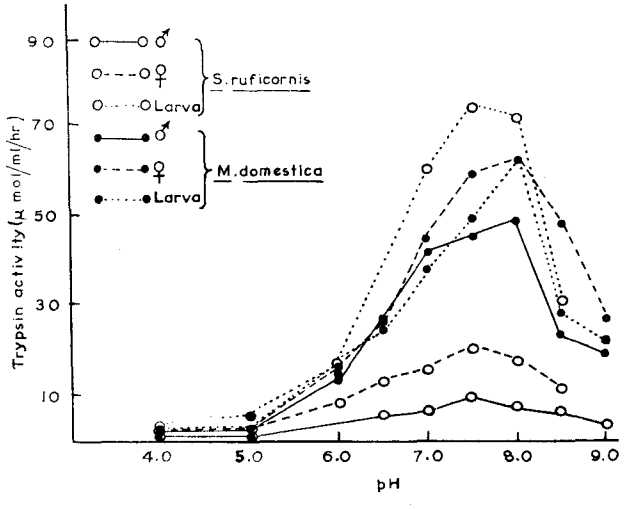

Fig. 1. Effect of pH on the activity of the midgut trypsin.

Materials and method. S. ruficornis was reared on cane-sugar and meat *M. domestica* on cane-sugar and milk[3]. The midgut homogenate was prepared as described earlier[4]. Enzyme homogenate (0.1 ml) was incubated with 0.2 ml of the 0.25 *M* substrate (*p*-tosyl-L-arginine methyl ester HCl) and 0.3 ml of appropriate buffer (Sörensen's phosphate buffer, 0.1 *M*, from pH 5.5–8.0; glycine-NaOH buffer, 0.1 *M*, from pH 8.5–10.5) at 37 °C. The enzyme concentration and incubation period was 1 gut/0.1 ml and 30 min in case of the larvae and 2 guts/0.1 ml and 2 h in case of the adults of *S. ruficornis*; 2 guts/0.1 ml and 1 h in *M. domestica*. After incubation, the enzyme was inactivated by adding 0.5 ml of 10% trichloroacetic acid (TCA), and the mixture was centrifuged at 2500 rpm for 10 min. Trypsin activity was measured by the colorimetric method of YANG and DAVIES[5].

Optimum pH for trypsin activity was determined first and then the effect of temperature, substrate concentration, enzyme concentration and incubation period on enzymic activity was studied at optimum pH.

Results and discussion. Effect of pH. The pH optima for midgut proteinase activity ranged from 7.5[6] to 8.5[7] in

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