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THE INTERACTIONS AMONG NUTRITION, ENDOCRINES AND PHYSIOLOGY ON THE REPRODUCTIVE DEVELOPMENT OF THE BLACK BLOWFLY, *PHORMIA REGINA* MEIGEN

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INTRODUCTION

Heterotrophic organisms, including insects, obtain energy and nutrients for their life processes by consuming and utilizing other organic substances. The quality and quantity of these organic foods can directly influence the performance of insects' growth, development, metamorphosis, aging, dispersal, overwintering, mating, reproduction and other important events. Nutritional requirements for insects are well studied (House, 1974; Dadd, 1985). On the other hand, at certain stages of development the special physiological and biochemical requirements of an insect also can influence dietary consumption and utilization. For example, many insects must consume and assimilate food of specific quality and quantity prior to oogenesis and oogenesis may in turn stimulate or inhibit further food intake (Slansky and Schriber, 1985; Slansky and Rodriguez, 1987). This also holds true for *Phormia regina*. The physiological mechanisms regulating both intake and cessation of feeding are best understood in this fly; consequently, it provides an ideal system to investigate how various factors influence food intake or *vice versa*. Thus, it is important to untangle the intertwining relationships among diet, nutrition, endocrines, physiology, and reproduction.

The principal source of energy for the adult black blowfly is carbohydrate (Dethier, 1969) in the forms of glycogen stored in the fat body and muscle and the blood sugar trehalose. In fact, trehalose is also found within the tissues of *P. regina* and its tissue concentration far exceeds that normally found in the total volume of blood (Anelli and Friedman, 1986). Demands for carbohydrates come mainly from flight and other locomotor activities plus the extra demand of developing 218-250 eggs per female at a time. Consequently, a general hunger for carbohydrate develops. The major sources in nature being nectar, honeydew, and ripe fruits. If the fly is to continue its locomotor activities, carbohydrate must be replenished before exhaustion or death results. Another nutritional demand on the fly is the need for a proteinaceous meal. A specific hunger for

protein becomes evident prior to egg maturation in female blowflies resulting in a cyclic pattern where there is a correlation between protein intake and egg maturation (Dethier, 1961; Belzer, 1978a; Greenberg and Stoffolano, 1977). In contrast, male *P. regina*, does not show cyclic consumption of protein and its spermatogenesis occurs independent of protein intake (Stoffolano, 1974). A protein meal is, however, essential for the complete development of male accessory reproductive glands (Tobin, 1979).

An excellent book, "The Hungry Fly", describes and discusses the physiological basis of behavior associated with dietary preference, food uptake and termination of feeding in *P. regina* (Dethier, 1976). Thus, the present article reviews the work conducted in the authors own laboratories on the interactions among dietary consumption, nutritional status, and physiological events of the adult, *P. regina*, and hopefully it will stimulate more research activities on the subject. We will discuss the influences of physiological conditions, such as sex, age, flight and diapause, on nutrition and the dietary influences on physiological events such as midgut activity, protein biosynthesis, vitellogenesis and hormonal activities.

Food consumption and utilization as controlled by physiology

The idea so aptly expressed by Kennedy (1967) that behavior is physiology is now well embedded in current biological thought. Because of this it is worthwhile to explore how various behaviors such as feeding, reproduction, etc. influence onging and future physiological processes. At the same time, it is equally important to examine how various physiological states such as sex, age, flight and diapause may influence a specific behavior such as feeding.

Sex related food intake

Adult *P. regina* is completly anautogenous; thus, the necessary raw materials for egg development must be obtained from the adult's environment. In fact, Dethier (1976) reports that a specific hunger, in this case for proteins, has been identified for female *P. regina*. Dethier (1976) notes that protein intake increased in a cyclical fashion and was correlated with each gonotrophic cycle. In males, however, only one initial peak of protein intake has been observed. There is little doubt that sex-related differences in food intake do exist in *P. regina* but the underlying physiological mechanisms still remain to be elucidated (Belzer, 1978a, b, c, 1979).

Age related food intake

The intake of protein and carbohydrate by non-diapausing *P. regina* has been measured for 40 days after adult emergence (Greenberg and Stoffolano, 1977). Each day, seven flies of each sex were removed from holding cages and were used

to measure the daily food intake. An individual fly was housed in a container made from a cellulose nitrate centrifugal tube equipped with a two-choice feeding apparatus. Results show that daily mean intakes of protein (ranging from 0 to 35 μl of 10% yeast extract in distilled water) and carbohydrate (2 to 85 μl of 0.1 M sucrose) are significantly influenced ($p<0.01$) by the age of the fly. Intake of carbohydrate is generally low during the first two days, increases on day 3 through day 16, and decreases gradually until day 40. Intake of protein, however, more or less follows a similar pattern to that of carbohydrate. The decrease in food intake as a function of age, is probably associated with either a decrease in general body metabolism or a decline in spontaneous locomotor activity or both.

Flight related food intake

In general, our knowledge concerning the effect of flight on feeding behavior in insects is extremely limited. In fact, none of the major reviews concerning insect feeding behavior even discuss this topic (Dethier, 1976; Bernays, 1985). This is surprising when one considers that the majority of adult insects use wings to disperse from the site of natality in search of mates, oviposition sites and food.

Hudson (1958) demostrates that the behavioral acceptance tarsal threshold of *P. regina* to glucose decreased with increased duration of filight. In her study she did not separate the sex of flies used. She evaluated all potential carbohydrate sources (e. g. hemolymph, glycogen, crop, and midgut) and showed that the carbohydrate reserves in all these regions decreased with increased flight durations (i. e., 15 to 180 min). However, she did not evaluate the effect of flight on carbohydrate intake. Based on her data and the research of Dethier (1976), a decreased tarsal acceptance threshold means that the fly will probably feed when it encounters a carbohydrate source. Because of the importance of flight, more research remains to be done on its influence on both carbohydrate and protein feeding.

Diapause related food intake

The intake of protein and carbohydrate by diapausing black blowflies has been measured for 40 days after emergence (Greenberg and Stoffolano, 1977). The daily mean intake of sugar by diapausing flies is significantly greater than protein as measured by volume. Females who weigh more (42.59 mg/fly) than males (40.38 mg/fly) consumed significantly more of both carbohydrate and protein than males on both individual and unit body weight basees. Comparisons between diapausing and non-diapausing flies show that the diapausing flies of both sexes fed significantly more on carbohydrate than their non-diapausing counterparts, although the protein consumption compares equally for the two groups of flies.

The increased intake of carbohydrate by diapausing flies may be viewed as a result of the changed metabolism in response to diapause inducing conditions, which is characterized by an accumulation of nutrient reserves in fat body and other tissues. This interpretation is further supported by the presence of hypertrophied fat body in diapausing flies and is consistent to the currently accepted concepts regarding preparation and maintenance of diapause in insects (Mansingh, 1971).

Physiological events controlled by dietary intake

What an animal eats often determines its ecology. At the same time, what an aminal eats also greatly influences subsequent physiological events. Using *P. regina* as our model system, we will discuss how dietary intake influences specific physiological processes and events in the life of the adult.

Hemolymph pH

Hemolymph pH values are measured *in situ* through a dorsal-thoracical opening at ambient temperature using a needle pH electrode and a microreference electrode. Results (Liu, 1985) show that, within five days of emergence, the hemolymph pH of *P. regina* of both sexes is around neutral with minor variations between 6.98 to 7.20. When flies feed on a sugar-water diet, no significant differences are found between sexes and ages. After a liver meal, on day 2 of adulthood, the hemolymph pH remains essentially unchanged on days 3, 4 and 5 in females, despite the fact that the liver juice shows an average pH of only 4.8. In contrast, the hemolymph pH in males declines slightly but significantly ($p<0.05$) from 7.14 at 3 hr after a liver meal on day 2 to pH 6.98–6.99 on days 4 and 5. What caused the sexual dimorphism remains to be studied.

Diet and midgut activities

Like many other insects, the epithelium of the midgut in adult, female *P. regina* is composed of numerous digestive cells, scattered regenerative cells, and endocrine cells (Stoffolano *et al.*, in preparation). According to the location, shape, and electron density, there are two types of endocrine cells (closed and open) in the posterior midgut. Close endocrine cells are located basally and are solitary and cone-shaped with cytoplasm of lighter electron density. They have a round nucleus and rough endoplasmic reticula are abundant. They do not possess a basal labyrinth and their apical surface does not extend to the lumen. The cytoplasm contains membrane-bound and electron dense secretory granules ranging in size from ca. 600 to 1,800 Å in diameter. Open endocrine cells are slender, vase-shaped and their apical surfaces are always extended to the lumen of the midgut. The cytoplasm has a darker electron density and contains membrane-bound electron dense secretory granules of a smaller size ranging from ca.

400 to 1,300 Å in diameter. Numerous secretory granules can be found in the closed cells from flies feeding only on carbohydrate. After a liver-meal, the number of these granules decreases significantly, while large vesicles of unknown nature appear. In contrast, ultrastructural changes in the open endocrine cells after a liver-meal are not as obvious as those of the closed cells. Nevertheless, after consuming proteins (liver), large lipid droplets appear in the cytoplasm of open endocrine cells. Future studies are needed, using mammalian immunological probes, to examine the nature of the secretory products of these cells.

Diet and endocrine activities

Brain neurosecretory cells—The neuroendocrine system and the stomatogastric nervous system of the adult female *P. regina* are examined using phase contrast and Nomarski light microscopy, plus transmission electron microscopy (Dai *et al.*, 1987)· No frontal ganglion or ganglion-like structure is found in *P. regina*. The nerve junction, where the frontal ganglion should be if present, is the site where two "labrofrontal connectives" and the recurrent nerve join. The junction site contains no perikarya, therefore, it is not ganglionic. Neurosecretory granules are present in some axons at the junction. Our observation agrees with Langley's conclusion (1965) that the frontal ganglion is absent in the groups of flies related to *P. regina* and also responds to the urge by Dethier (1976) to reconfirm that the frontal ganglion is indeed absent in *P. regina*. Evidence also shows that, in *P. regina*, the recurrent nerve is joined by a pair of nervi corporis cardiaci (NCC) shortly after the pair of NCC emerges from the brain (Dai *et al.*, 1987). Thus, from the point of joining, the "recurrent nerve", as so identified in some earlier studies, is really the cardiac-recurrent nerve (CRN)—a composite nerve consisting of the recurrent nerue and two nervi corporis cardiaci. Consequently, some earlier results generated from surgical manipulation of the "recurrent nerve" may need reinterpretation to include the possible effect from transecting, unintentionally, the nervi corporis cardiaci. special anatomical features of the stomatogastric nervous system of *P. regina* clearly suggest that transection of the recurrent nerve can only be done at the anterior course of the nerve (i.e., the course of the nerve posterior to the labrofrontal-recurrent junction and anterior to the beginning of CRN).

Hsiao and Fraenkel (1966) find six groups of neurosecretory cells in each hemisphere of the brain: the median, frontal, lateral A, lateral B, posterior I and posterior II groups. They also find neurosecretory cells in the subesophageal ganglion and the thoracico-abdominal ganglion. They report a diet-dependent size change in the nuclei and cell bodies of the median neurosecretory cells in both female and male *P. regina* adults. Considerable cell size increase is also found in flies fed on a diet of liver, sugar and water while only a slight cell size

increase is found when flies are provided with sugar and water. No cell size increase is observed in flies fed on water alone. Although, the nature of the dietary influence remains unknown, it is clear the quality of diet can affect the median neurosecretory cells.

Hsiao and Fraenkel (1966) find only one type of median neurosecretory cell (type A) in the protocerebrum of adult *P. regina*. This finding presents a situation that differs from that of other blowflies, which usually contained two types of protocerebral median neurosecretory cells. Dai *et al.* (1987), however, identify two types of median neurosecretory cells in the protocerebrum and find that *P. regina* is comparable in this regard to the other blowflies. How the diet of adult *P. regina* influences the size, thus biosynthesis, of these neurosecretory cells still remains unknown.

Juvenile hormone biosynthesis—Earlier works on juvenile hormone in *P. regina* are restricted to surgical manipulation of the corpus allatum and/or application of juvenile hormone analogues after different feeding treatments (Orr, 1964; Mjeni and Morrison, 1976; Pappas and Fraenkel, 1978; Fraenkel and Hollowell, 1979). Their results indicate that a protein meal somehow functions as a "trigger" for the activation of the corpus allatum. The first attempt to measure juvenile hormone biosynthesis by the corpus allatum of adult *P. regina* has been made by Liu and co-workers (Liu *et al.*, 1988). Using a radiochemical assay, they incubate the corpus cardiacum-corpus allatum complex of the adult female *P. regina*, in a small volume of a custom-made MEM solution containing known amounts of ^{3}H-methionine and cold methionine. After a given period of incubation, the organic solvent (isooctane) extractable radioactive materials are collected and the radioactivity in the materials determined by scintillation counting. The presence of the corpora cardiaca does not interfere with the juvenile hormone biosynthesis and release by the corpus allatum. Liu *et al.* (1988) report that the ability of the corpus allatum to incorporate ^{3}H-methyl function from the ^{3}H-methionine into its bioproducts increased 12-fold 24 h after a liver-meal. The rate of incorporation by the corpus allatum of flies fed on sugar-water is 0.07 ± 0.01 pmol/gland/h, while that of flies fed 24 h ago on sugar-liver-water is 0.86 ± 0.09 pmol/gland/h. Further, Liu *et al.* (1988) find that, in addition to juvenile hormone III, the corpus allatum of *P. regina* produces a considerable quantity of radioactive unknowns. Chromatographic separation and subsequent scintillation counting indicate that over 92% of the total isooctane extractable radioactive materials are unknowns; showing polarities higher than that of the juvenile hormones. These results not only serve as the first case where the radiochemical assay for juvenile hormone biosynthesis/release is successfully adapted to a dipteran system, but also serve as the first warning that researchers should be careful about the

common practice of considering all isooctane extractable radioactive materials as juvenile hormone (Feyereisen and Tobe, 1981). Only in certain species of insects, may such a practice be justifiable.

Using a different radiochemical assay, originally developed by Tobe and Pratt (1974), the release of juvenile hormone by the corpus allatum of *P. regina* is examined *in vitro* in the medium TC199 (Zou *et al.*, 1989). Again, feeding on liver results in a greater incorporation of ^{3}H-methyl function by the corpus allatum *in vitro*. The isooctane extractable radioactive materials of the corpus allatum-corpus cardiacum complexes are separated by HPLC equipped with either a normal phase Lichrosorb Diol column or a reverse phase C-18 column. Again, juvenile hormone III is the predominant juvenile hormone species and represents ca. 10% of the total isooctane extractable radioactivities. It appears that with TC199 medium, the overall incorporation of the ^{3}H-methyl group by the corpus allatum is slightly lower than that when the gland is incubated in MEM. Using the TC199 system, a time course study on juvenile hormone release after a liver meal reveals that immediately following a liver meal, the corpus allatum releases very little juvenile hormone (Fig. 1, open circles). Eight hours later (80 h of age), however, a significant increase in activity is observed; similar activity level is again observed at 24 h post-liver feeding. In the next 8 h, a rapid increase in hormone release is observed changing from ca. 0.09 pmol juvenile hormone/gland/h at 24 h to 0.33

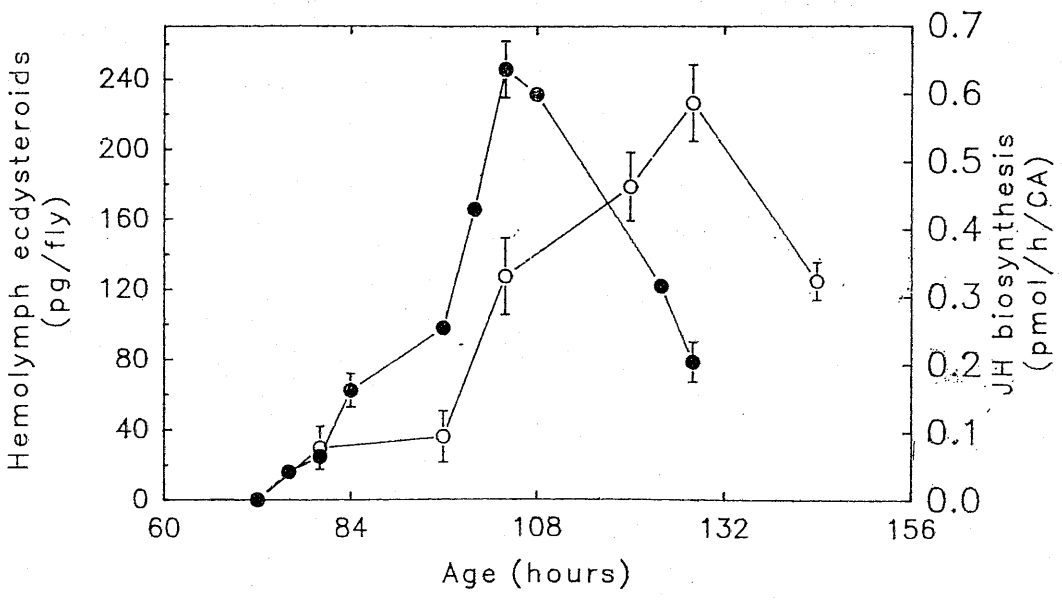

Fig. 1. Profiles showing the total hemolymph ecdysteroids (filled circles) and juvenile hormone biosynthesis (open circles) during the 1st gonotrophic cycle of the black blowfly, *Phormia regina*. Note that the juvenile hormone biosynthesis peaked 24 h after the highest quantity of ecdysteroids was detected. If error bars are too short to be drawn, they are not shown graphically.

pmol at 32 h after being liver-fed. At 56 h post-liver-feeding (128 h of age), the juvenile hormone release reaches its maximum of 0.6 pmol/gland/h and then declines rapidly thereafter as females start to deposit their eggs (Zou *et al.*, 1989).

When precocene II is topically administered to newly emerged flies, the corpus allatum decreases greatly in volume and its juvenile hormone biosynthesis becomes deminished (Yin *et al.*, 1989a, b). A dose related response is evident (Fig. 2).

Elucidating the complexity of the relationship among diet, juvenile hormone biosynthesis, and cessation of juvenile hormone biosynthesis is essential if we are to fully understand how diet impacts on egg production.

Ecdysteroid titers—Hemolymph ecdysteroid titers are determined for both sugar-fed and liver-fed females using the radioimmunoassay for ecdysteroids previously described (Yin and Chaw, 1984). All flies are maintained on a sugar-water diet for the first 72 h of adulthood. At 72 h, a liver-sugar-water diet is provided *ad lib* for liver-fed flies, whilst flies fed on sugar-water served as controls. Newly emerged females contain 5.5 pg (alpha-ecdysone equivalent) of ecdysteroids per microliter of homolymph. This amount declines to an undetectable level at 24 h post-emergence and remains so if flies only have access to sugar and water (Yin *et al.*, in preparation). In contrast, following a protein meal provided at 72

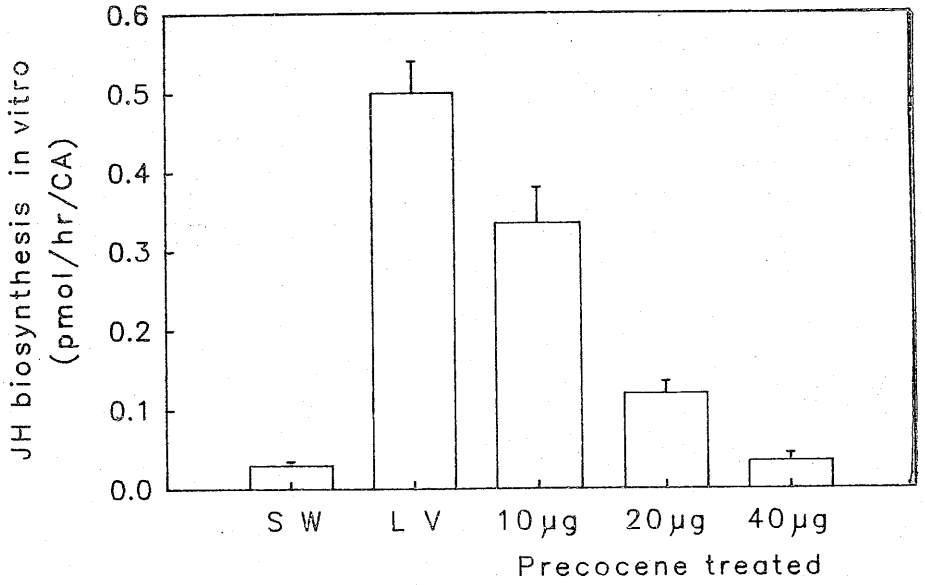

Fig. 2. The effect of precocene II on the juvenile hormone biosynthesis by the corpus allatum of female, *Phormia regina*. SW=control flies with sugar and water provided all the time. LV=control flies fed on liver at 72 h of age for 1 h only with sugar and water provided at all other times. Precocene treated flies received topically one treatment of 10, 20 or 40 μg of precocene II at less than 12 h of age and fed on liver at 72 h of age. Juvenile hormone biosynthesis was then determined, 48 h after the liver-meal, using a radiochemical assay.

h, the ecdysteroid level rises rapidly to 97.8 pg/fly 24 h later (Fig. 1, filled circles) and then peaks at 245.7 pg/fly at 104 h (32 h post-liver-feeding). Within the next 24 h, it declines to 78.5 pg/fly at 128 h post-emergence-a time when juvenile hormone release peaks (Yin *et al.*, in preparation). Thus, diet not only influences juvenile hormone biosynthesis but also greatly influences ecdysteroid biosynthesis. Without protein in the diet, ecdysteroids are not produced.

Diet and protein biosynthesis

Results by Zou *et al.* (1988) show that the growth of the ovary and increase of total ovarian protein (measured by using the Bradford method with bovine serum albumin as standard) are synchronous after liver feeding. The hemolymph total protein content increases rapidly to a peak value of 470 μg/fly 32 h after liver-feeding then steadily delines to 175 μg/fly by 56 h (Fig. 3, open circles). Total ovarian protein increases from barely detectable amounts (<20 μg/fly) at 4 h to over 1,200 μg/fly by 56 h post-liver-feeding (Fig. 3, filled circles).

Using an antiserum, specific to vitellogenin and vitellin, an enzyme-linked immunosorbent assay is developed to quantify the yolk protein and its precursor (Zou *et al.*, 1988). Vitellogenin in the hemolymph increases rapidly following an *ad lib* feeding of liver at 72 h of age (Fig. 4, open triangles). Its titer peaks 32 h later (104 h of age) and then declines as the quantity of vitellin (Fig. 4, filled triangles) starts to rapidly increase in the oocytes. These authors also show that there are four separable (i.e., using SDS-electrophoresis) yolk proteins instead of three as previously reported for *P. regina* (Huybrechts and De Loof, 1982). The

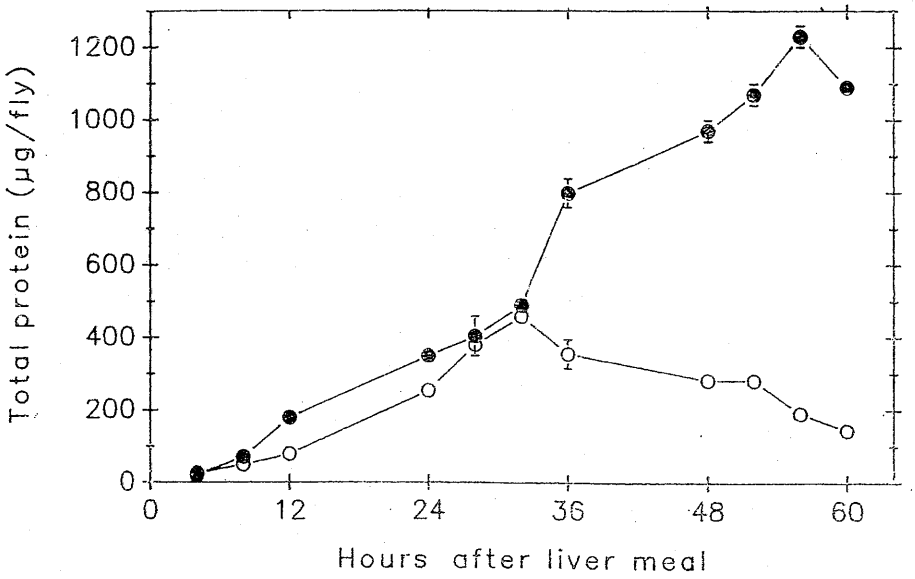

Fig. 3. Profiles showing the total hemolymph (open circles) and ovarian (filled circles) soluble proteins. Liver was was provided at 72 h of age.

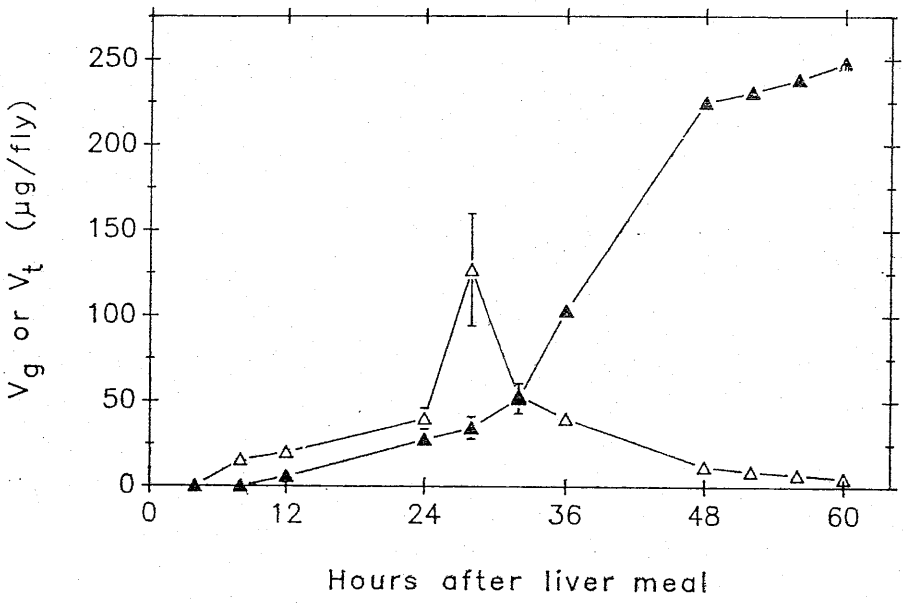

Fig. 4. Profiles showing the vitellogenin (Vg, open triangles) in the hemolymph or the vitellin (Vt, filled triangles) in the ovarian homogenates of the black blowfly, *Phormia regina*. Liver was provided at 72 h of age.

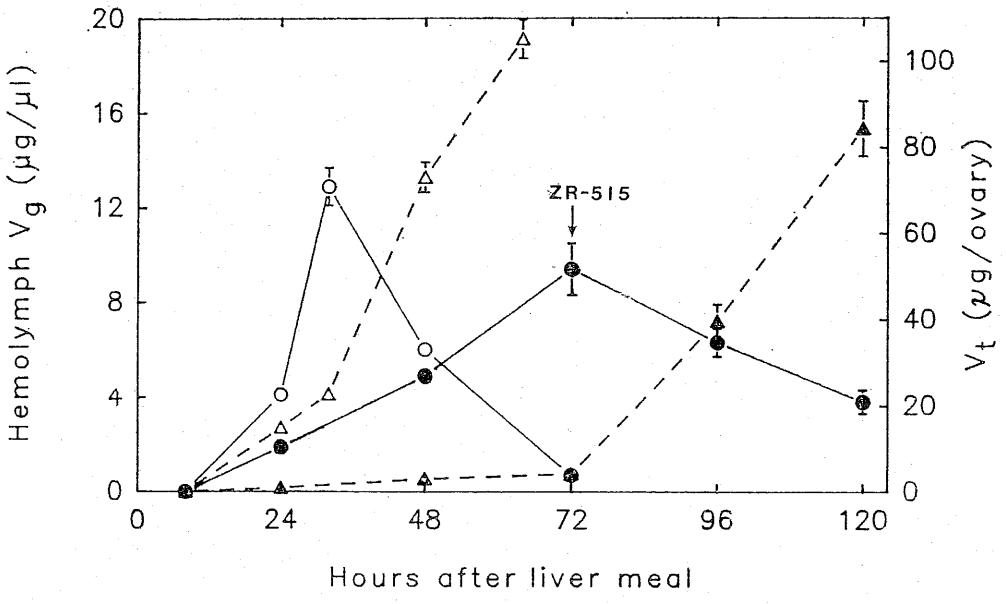

Fig. 5. Profiles showing the vitellogenin (Vg) and vitellin (Vt) in control (precocene untreated, liver fed at 72 h of age; Vg=open circles, Vt=open triangles) and precocene treated (20 μg precocene II at less than 12 h of age, liver fed at 72 h of age; Vg=filled circles, Vt=filled triangles) blowfly, *Phormia regina*. At 72 h after the liver-meal, each precocene II treated flies received a 10 μg of methoprene (ZR-515, arrow) topically to see if vitellogenin uptake and vitellin formation could be enhanced.

molecular masses of these four proteins are estimated as 42,000, 43,000, 44,000 and 45,500 (Zou *et al.*, 1988).

After flies are treated with 20 μg of precocene II, the liver meal starting at 72 h of age causes a slower appearance of vitellogenin in the hemolymph (Fig. 5, filled circles) when compared to controls (open circles). Vitellogenin does not peak at 32 h post-liver-feeding as in the controls and thus there is no decline in its titer. Practically speaking, there is no vitellin in the ovary at 72 h post-liver-feeding (Fig. 5, filled triangles) in precocene-treated flies. At this time the control flies have accumulated more than 100 μg vitellin per ovary (open circle). A single topical application of, a juvenile hormone analog, methoprene (also known as ZR-515) at 10 μg/fly (Fig. 5, arrow) at 72 h post-liver-feeding to flies previously treated with precocene II causes a decline of vitellogenin in the hemolymph and a corresponding increase of vitellin in the ovarian homogenates (Fig. 5, filled circles and triangles). The use of both precocene and methoprene to experimentally manipulate the corpus allatum and the juvenile hormone titer has facilitated the demonstration that the juvenile hormone regulates the uptake of vitellogenin while the ecdysteroids regulate its biosynthesis (Yin *et al.*, 1989a, b).

Diet and development of gonads and accessory reproductive glands

The dietary effects on the development of ovaries and female accessory reproductive glands are examined by Stoffolano (1974) in both non-diapausing and diapausing *P. regina*. Non-diapausing flies are reared at 26°C (50% R.H. and 16 h light: 8 h dark photoregime). Adults have free access to granulated sucrose, water, and powdered milk. Fresh beef liver is placed in the cage daily to serve as both a protein source and the oviposition medium. Under these conditions, yolk deposition begins on day 3 and considerable amount appears by days 4, 5, and 6. Increases in yolk deposition are accompanied by the concurrent increases in the size of follicles and ovaries. In contrast, follicles and ovaries of the same non-diapausing females, if fed only sugar and water, fail to deposit any yolk. Yolk deposition also fails to occur in diapausing females (reared at 18°C, 40% R.H., and 9 h light: 15 h dark photoregime) after feeding on protein. Fat body hypertrophy becomes obvious in diapausing females, but is lacking in non-diapausing flies.

Results also show that the developmental state of the female accessory reproductive glands reflects that of the follicle development. Protein-fed, non-diapausing flies with well developed ovaries invariably possess well developed accessory reproductive glands while the reverse is also true for the flies lacking ovarian development. The female accessory reproductive glands of sugar-fed, non-diapausing flies do not develop beyond that of a day 3 protein-fed fly. Examination of the accessory reproductive glands of protein-fed females under the phase

contrast microscope reveals that the glands of newly emerged flies (less than 24 hr old) to day-3 flies contain granular, cellular material and do not show a defined lumen. By day 4 (i.e., one day after the beginning of yolk deposition) a defined lumen becomes apparent for each accessory reproductive gland. The lumen is filled with a clear fluid while the cytoplasm of the glandular cells appears less granular. The lumen never develops and its clear fluid never appears in the glands of sugar-fed, non-diapausing and protein-fed, diapausing females.

Studies of the male accessory reproductive glands show that the glands of protein-fed males increase in length from 0.56 mm on day 1 to 1.0 mm on day 3. In contrast, the length of the accessory reproductive glands from sugar-fed males only increases to 0.82 mm even by day 6. The well developed gland from liver-fed flies has a clearly-defined lumen filled with a clear fluid. The cytoplasm of the glandular cells of sugar-fed flies appears granular, and the glands lack a well-defined lumen (Stoffolano, 1974).

The influences of diet on the ultrastructural changes of the ovaries of *P. regina* have also been investigated. Mazzini *et al.* (1987) show that in sugar-fed females, the undeveloped ovaries are each entirely enclosed by a sac-like muscle sheath of fenestrated appearance. These ovaries exhibit all the ultrastructural features of previtellogenic ovaries. Within all the ovarioles only the terminal follicles are to be developed during each ovarian cycle. Each follicle is characterized by a well developed follicular epithelium. The epithelial cells associated with nurse cells are squamous in shape while those surroundeding the oocyte are cuboidal cells tightly juxtaposed to each other. As in other dipterans, the cuboidal cells are connected to each other through intercellular bridges of 3.5 microns in width. Gap junctions of various size are of the macular type and are also found between adjacent follicle cells. The 15 nurse cells and the oocyte are all linked to one another through a highly organized network of ring canals that are intercellular bridges bordered by fibrous electron dense material. Each nurse cell contains a large nucleus characterized by highly convoluted nucleoli and a narrow fringe of cytoplasm composed mainly of free ribosomes and mitochrondria. The cluster of nurse cells is involved in three different cell interfaces: the nurse cell/nurse cell, nurse cell/follicle cell, and (for some nurse cells) nurse cell/oocyte. A number of macular gap junctions appears clustered in large aggregates at the nurse cell/nurse cell interface. In contrast, gap junctions found at the follicle cell/nurse cell interface are highly irregular in size and shape and show no aggregation. In sugar-fed females, oocyte cytoplasm does not differ significantly in ultrastructure from that of the nurse cells and lacks yolk granules. However, the nucleus of the oocyte is much smaller in size in comparison to that of the nurse cells. The small nucleus contains ring-shaped chromosomal material and no nucleolar material.

Conspicuous changes in the ultrastructure of the ovaries are induced by liver feeding. One of the earliest structural changes in the ovary after a proteinaceous meal is the appearance of wide intercellular spaces both between adjacent follicular cell/oocyte interface-a phenomenon known as patency. The spaces allow the passage of vitellogenin (i.e., the hemolymph precursor of the yolk protein vitellin) between the follicle cells thus permitting the protein to reach the cytoplasmic membrane (oolemma) of the oocyte. The onset of vitellogenin uptake is marked by the appearance of numerous coated pits and vesicles along the oolemma and newly formed yolk spheres in the cortical (peripherall) ooplasm. In addition, long microvilli (about 1.2 microns in length) start to project outwardly from the oolemma into the perivitelline space without interlocking with the plasma membrance of the facing folloicle cell. The ultrastructural evidence is consistent to the concept that vitellogenin uptake occurs by receptor-mediated internalization of vitellogenin containing coated pits from the oolemma to give rise to free intracellular free coated vesicles. Each yolk sphere is formed when several of these vesicles convey their load of membrane-bound vitellogenin together by a process of coalescence.

The growth of the ovary after a liver meal can be estimated by observing the ovarian wet weight changes. Before a liver meal, each ovary weighs less than 0.13 mg. From 8 h to 24 h after a liver meal, the weight increases from 0.28 to 2.17 mg/ovary and then to 10.87 mg/ovary at 56 h post-liver-feeding. After egg-laying, the weight decreases to 1.5 mg/ovary (Zou *et al.*, 1988).

DISCUSSION

It is clear from the above that an adequate protein meal by *P. regina* triggers the neuroendocrine and physiological cascades that eventually result in the completion of oogenesis. The success of oogenesis thus depends on the orderly execution of a large array of events of which only some are described here. There are many more details that remain to be learned before we obtain a truely comprehensive understaanding of oogenesis in *P. regina*.

Once an adequate protein meal is taken, significant changes occur in midgut activity, endocrine activity, protein synthesis, and gonad development. Knowledge of the timing of oocurrence for each event is essential if we hope to decipher the relationships among all events. At least it prevents us from assuming that an event occurring earlier in the sequence of events is to be driven by an event occurring later. Unfortunately, we do not know the precise timing for all the above mentioned changes and this lack of information necessitates certain speculations until more information becomes available.

Our current knowledge, although incomplete, still allows us to synthesize a series of working hypothese to describe the chain of events concerning the dietary initiated hormonal regulation of oogenesis in *P. regina*. The very beginning of the events leading to oogenesis is the "decision" by the fly to search and ingest food rich in proteins. The decision is made mainly around day 3 as mentioned above. Hormones have been demonstrated to change feeding behavior in blowflies. De Clerck and De Loof (1983) find that feeding μg amounts of 20-hydroxyecdysone to males of *Sarcophaga bullata* changes its preference from sugar to protein. The role of dietary 20-hydroxyecdysone in females is not examined by these authors. They also find that ZR-515 (juvenile hormone analogue) could increase both sugar and protein uptakes. Our data on ecdysteroid titer and juvenile hormone biosynthesis indicate that during the first two days of the adulthood, there is a decline in the ecdysteroid titer and there is no juvenile hormone biosynthesis in female flies. Unfortunately, the lack of juvenile hormone biosynthesis may not necessarily be taken as indicating that the absence of this hormone is required for switching the preference from sugar to protein, because absence of biosynthesis may not mean absence of titer. A slight juvenile hormone residue may be carried over from pupal events prior to eclosion. But because of the lack of an appropriate test for the hemolymph juvenile hormone, we are currently unable to measure the juvenile hormone titer. We hypothesize that there may be a hormonal basis for the change in the preference from sugar to protein on day 3 in female *P. regina*. Presently, our data seem to suggest that the decline in ecdysteroid titer may lead to protein hunger. It is also conceivable that the absence of juvenile hormone biosynthesis may permit the onset of protein hunger. This hunger elicits at least two major behavioral events involved in feeding: food seeking and ingestion.

P. regina with a hunger for carbohydrate shows a lowered tarsal acceptance threshold for sugar and an increased spontaneous locomotor activity (Dethier, 1976). These changes may be related to the qualitative and quantitative changes in neurotransmitters. One can manipulate the feeding behavior of *P. regina* by using various pharmacological agents that modify in various ways the neurotransmitters involved in the pathways that lead either to feeding initiation or cessation. Octopamine, demethylchlordimeform (octopaminergic drug), and clonidine (octopaminergic receptor stimulating drug) enhance tarsal responsiveness and induce hyperphagia of sucrose solution, while dopamine, 5-hydroxytryptamine, and DL-norepinephrine fail to cause hyperphagia (Long and Murdock, 1983). These results suggest that octopaminergic receptors in the nervous system of *P. regina* positively modulate carbohydrate ingestion. We do not know at present if similar mechanisms also modulate protein ingestion. It would be most interesting to examine

the possible roless of 20-hydroxyecdysone, juvenile hormone, demethylchlordimeform, D-octopamine, and DL-dopamine on protein ingestion in *P. regina*. In other words, *P. regina* provides a unique opportunity for us to study the interactions between hormones, neurotransmitters and the over behavior of protein ingestion.

If food supply is not limiting, protein ingestion will normally be terminated by some feedback via the recurrent and ventral nerves. Frontal ganglionectomy experiments (presumably remove a negative feedback via the recurrent nerve) lead to sugar or protein hyperphagia in both protein-deprived and gravid females when either food are provided (Belzer, 1978a, b, c and 1979). Operated, protein-deprived flies, given a chance, ingest more sugar than protein while control (unoperated) flies prefer protein to sugar. Belzer notes that the results show that the negative feedback executed through the recurrent nerve exerts stronger control for sugar intake than that for protein. Belzer (1979) also shows that the presence of developing or mature oocytes in the abdomen reduces protein ingestion because of negative feedback from abdominal stretch receptors. It is interesting to note that using *P. regina* females with sealed anuses and then feeding them a known volume of protein and sugar solutions each three times a day, Orr (1964) concludes that abdominal distension is not essential for the initiation of egg development.

In most protein feeding flies, after proteins enter the esophagus, they can be directed either into the midgut or the crop. Our unpublished data indicated that, when fed to repletion, the midgut and the crop each has the capacity of ca. 2 μl and 20 μl for protein meals, respectively. Instricate control mechanisms must exist to ensure orderly transport of proteins from the crop to midgut for digestion and absorption. Valves and pumps, in *P. regina*, are identified for this controlled transportation (Thomson, 1975). Our unpublished data also indicate that it takes *P. regina* ca. 48 h after the liver meal for a full crop to empty its protein content.

Ultrastructural study, mentioned earlier, indicates that the liver meal causes the release of secretory granules into the hemolymph from the closed endocrine cells in the midgut (Stoffolano *et al.*, in preparation). We do not know, however, what causes this release nor do we know what are the target organs (tissues) of the midgut secretion. It is conceivable, however, that such secretion may either convey messages to the brain or it may affect corpus allatum, corpus cardiacum, fat bodies, or ovaries directly. Studies on the mosquito, *Aedes aegypti*, demonstrate that after a replete blood meal the pressure of the undigested blood meal on stretch receptors of the midgut does not play any role in the induction of vitellogenin synthesis, rather the message of a blood meal is mediated by some digestion products of the blood (Lea, 1972; Van Handel and Lea, 1984). At present, we do not known if digestion products in *P. regina* trigger vitellogenesis. In

contrast, more recent data on mosquitoes show that midgut distension caused by a *diluted* blood meal may also play some role in the initiation of vitellogenesis. It is now known that in mosquitoes the degree of stretchness of the midgut by a *diluted* blood meal (rat blood in saline artificially given by enema) is an important factor leading to egg maturation (Klowden, 1987). Since the midgut of *P. regina* can only hold ca. 2 μl of liver at any one time, the degree of stretching is no where near that experienced by a mosquito after a blood meal. Based on this, can we then assume that midgut stretchness may thus play a lesser role in blowflies than mosquitoes with regard to the induction of egg maturation? At present, we can conclude from the past literature and our own results that midgut stretch receptors, digestion products, and midgut secretions (from the closed cells) and their combinations may all participate in the induction of vitellogenesis in *P. regina*. Further studies are required to sort out these possibilities.

Regardless of the nature of the message originating in the midgut and according to most literature, the brain seems to be the major target. In mosquitoes, an egg development neurosecretory hormone (EDNH) (Hagedorn *et al.*, 1979) is secreted, in response to the midgut message, by the median neurosecretory cells of the pars intercerebralis to stimulate the ovary to produce ovarian ecdysteroids. The ecdysteroids then stimulated the fat bodies to produce the yolk protein precusor-vitellogenin, which is then sequestered into the developing oocytes as vitellin. Hagedorn's model, invoking ovarian ecdysteroid, is developed from studies on mosquito vitellogenesis. Research on the higher dipterans suggests that ovarian ecdysteroids are also important to vitellogenin synthesis in *Sarcophaga bullata*, *Drosophila melanogaster*, *Calliphora vicina*, *Phormia terrae-novae* and *Lucillia caesar* (Postlethwait *et al.*, 1980; Briers and Huybrechts, 1984). Our data on the ecdysteroid titer and vitellogenin titer presented in this paper are consistent with the mosquito ovarian ecdysteroid model. The presence of an EDNH type of neurohormone, however, is not unequivocally demonstrated in blowflies.

Application of 20-hydroxyecdysone to male and female *D. melanogaster* results in an increased accumulation of yolk polypeptide-transcripts in a fat body preparation containing fat body cells, epidermis and oenocytes (Bownes *et al.*, 1983). They also show that this hormone-stimulated transcription is tissue specific but not sex specific. Testes, ovaries, Malpighian tubules, and gut do not respond to the same hormone treatment. Effects of ecdysteroids on yolk polypeptide transcripts in *P. regina* remain to be studied. In addition to hormonal regulation, it is also known that dietary components may modulate yolk polypeptide gene transcription in *D. melanogaster* (Bownes *et al.*, 1988). The yolk proteins of *D. melanogaster* begin to be produced by the fat bodies at adult eclosion. The transcription of the three yolk protein genes is regulated by the genes known as *tra*, *tra-2* and

dsx, and hormones. Bownes *et al.* (1988) show that females starved from eclosion exhibit only a low basal level of yolk protein gene transcription. Using a *ypl-Adh* fusion technique, these authors find that the DNA sequence required for the diet-enhanced transcription is different from the sequence involved in the hormone enhanced transcription of yolk protein genes. Again, no comparable information is available for *P. regina*.

The involvement of juvenile hormone in the regulation of oogenesis has long been known for *P. regina*. Earlier workers seem to support the notion that the corpus allatum (thus the juvenile hormone) is necessary to vitellogenin synthesis (Mjeni and Morrison, 1976; Fraenkel and Hollowell, 1979), although they have studied only oocyte development or total protein profiles but not specifically vitellogenin synthesis. Methoprene application to non-liver-fed *P. regina* stimulated oocyte development according to Fraenkel and Hollowell (1979). Again, they didn't attempt to specifically measure the quantity of vitellogenin or vitellin. In contrast, Briers and Huybrechts (1984) find that juvenile hormone fails to cause vitellogenin uptake in both non-liver-fed and in chemically castrated female *S. bullata*, although both received prior ecdysterone treatment to enhance vitellogenin biosynthesis and to elevate the concentration of ecdysteroids to normal liver-fed, non-castrated levels. Despite the above mentioned conflicting interpretations and inconsistent results, our data clearly show that juvenile hormone regulates the vitellogenin sequestrotion (uptake) by the oocyte. Additional minor involvement in the regulation of vitellogenin biosynthesis, if it exists, remains to be studied. Further, juvenile hormone may operate at two different levels in its regulation of vitellogenin uptake: it may act on the follicular cells to induce the patency (shape and/or size changes of the follicular cells to form intercellular spaces, which facilitate the passage of vitellogenin from hemolymph to the intercellular space between the follicle cells and ultimately into the developing oocyte); it may also induce the synthesis of a vitellogenin receptor protein to allow the formation of coated pits located on the surface of the developing oocyte. These two levels of actions need not be mutually exclusive. It is interesting to note that in mosquitoes, juvenile hormone biosynthesis is suppressed by a blood meal (Shapiro *et al.*, 1986; Readio *et al.*, 1988) indicating the roles of juvenile hormone in oogenesis are not comparable between the black blow flies and mosquitoes.

In summary, Fig. 6 illustrates a working model showing the various factors involved in the regulation of oogenesis in *P. regina*. The brain is involved in both the search and ingestion of proteins, which following feeding cause distension of the abdomen. The mechanoreceptors (i.e., stretch receptors) of the distended abdomen provide negative feedback to prevent excessive feeding or hyperphagia. In addition, the midgut may provide endocrine (i.e., from closed endocrine cells)

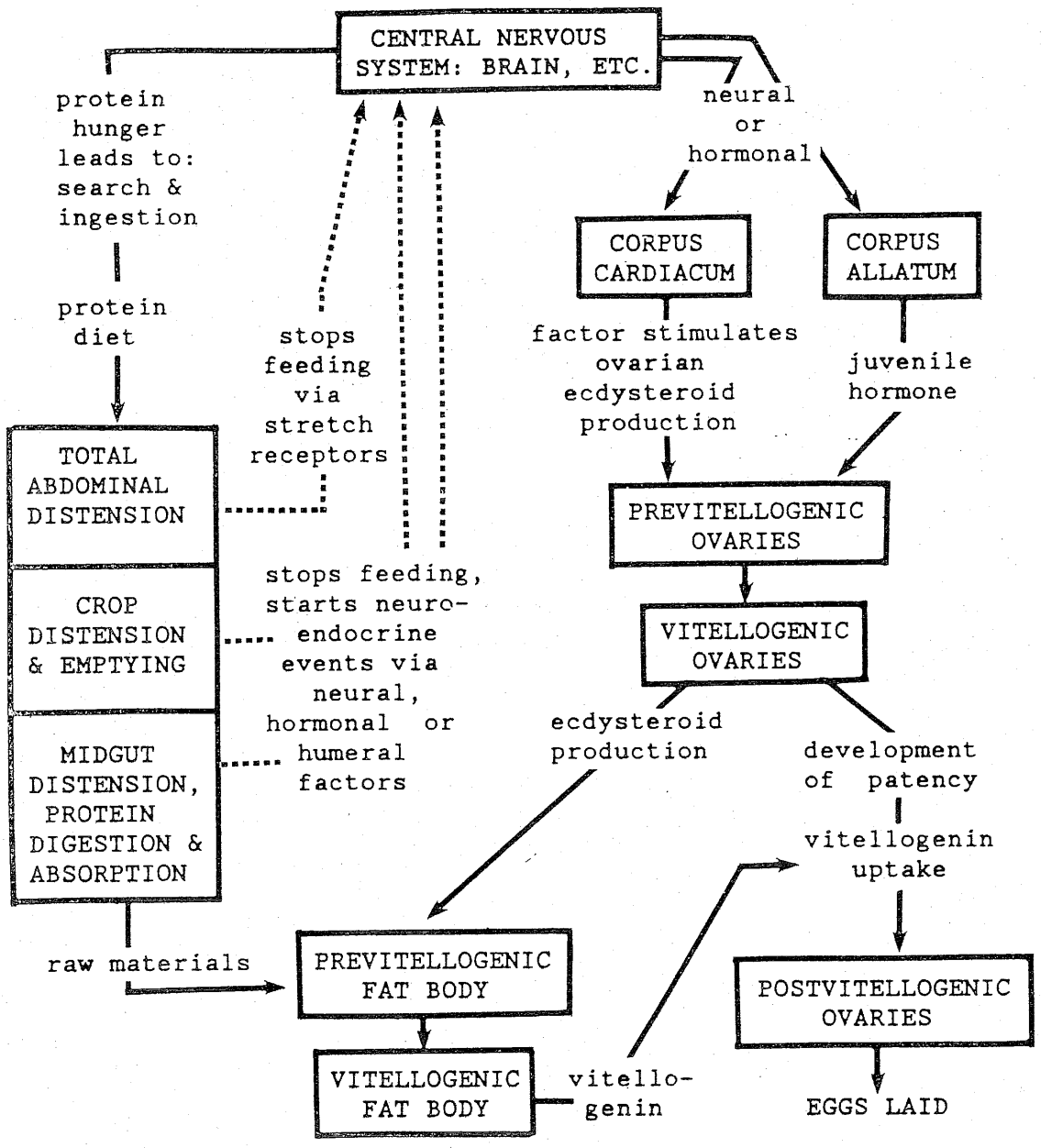

Fig. 6. Working model summarizing various factors governing the the oogenesis of the black blowfly, *Phormia regina*.

and/or humeral (i.e., from digestive products) messages to the brain to initiate the neuroendocrine cascade, which leads to complete oogenesis. In response to the message from midgut, the brain directs synthesis and release of the factor(s) causing the ovary to produce and release ecdysteroids. This brain factor may be released from the corpus cardiacum into circulation. A little later (as judged from the sequence of appearance of ecdysteroid and juvenile hormone biosynthesis), the brain may activate the corpus allatum (via neural or allatotropin

stimulation) to synthesize and release juvenile hormone III. The brain factor initiates the production of ecdysteroids (perhaps by the follicular cells), whereas juvenile hormone causes the development of patency between follicular cells and the appearance of coated pits on the oolemma for vitellogenin uptake. Meanwhile, the ovarian ecdysteroid reaches it target of previtellogenic fat bodies and turns them into vitellogenic a phase to produce vitellogenin. Vitellogenin enters the follicle through the intercellular spaces and is selectively taken-up by the oocyte via receptor mediated endocytosis. Since we find juvenile hormone is synthezised during vitellogenin uptake, our data do not support the notion suggested by Fraenkel and Hollowell (1979) that an oostatic hormone is secreted by the developing oocytes (follicles) to suppress the biosynthesis of juvenile hormone; the lack of juvenile hormone then disallows the development of the secondary set of follicles. We find there is juvenile hormone production while the secondary set of follicles remains undeveloped (Yin *et al.*, 1989), thus we propose that some unidentified mechanisms prevent oocyte development in the penultimate follicles.

EPILOGUE

Recent developments and progress in molecular biology, immunology, and computer-aided data analysis clearly points to new opportunities in the future, which will permit us to develop new techniques and strategies for insect pest management programs. Insect life processes are so complicated that the life and death of an insect is the result of the precise execution of a large number of highly sophisticated events. It is logical to deduce that through basic research, we should be able to identify areas of weakness in the insect's life procsses, especially egg production and exploit these weaknesses to our advantage. We believe that even with our present incomplete knowledge of *P. regina*, many possibilities exist for such exploitation. For example, we may be able to achieve considerable success in reducing egg production, if we thoroughly understand the mechanisms governing the protein hunger. By knowing the mechanisms, and when during the reproductive cycle they are expressed, we will be better able to trap flies at baits. Likewise, a thorough knowledge of yolk protein uptake may lead to measures that interrupt accumulation of protein yolk in the oocyte. The key to these biorational measures lies in a comprehensive knowledge of *P. regina*'s life processes at the molecular, cellular and organismic levels.

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