NUTRITIONAL EFFECTS UPON OVARIAN DEVELOPMENT AND REPRODUCTION IN THE FACE FLY, *MUSCA AUTUMNALIS* DEGEER (DIPTERA: MUSCIDAE)

by

STEPHEN MICHAEL VALDER

B. S. University of Nebraska, 1963

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1965

Approved by:

Theodore L. Hopkins
Major Professor

TABLE OF CONTENTS

INTRODUCTION AND LITERATURE REVIEW

The face fly, Musca autumnalis DeGeer, is widespread over parts of Europe, Asia, and North Africa, but for a long time it was believed that the only species of Musca indigenious to North America was the house fly, Musca domestica (L). West (1951) wrote that "This paucity of species in the new world is somewhat strange. . . . it would not be surprising if one or more additional species of Musca should sooner or later put in their appearance on the American scene. Vockeroth (1953) reported on the collection of five males and one female M. autumnalis from Middleton, Nova Scotia, in September, 1952. Thus West's prediction that another species of Musca would be found in North America was a good one. Walker (1871) had stated previously that M. corvina, a synonym of M. autumnalis, had been reported twice before from Nova Scotia, but later authors had assumed these reports to be misidentifications of M. domestica. At the time, Vockeroth seemed unable to decide if the face fly had been present in Nova Scotia, but not collected; or if it had been recently introduced. He thought it strange that the species had not disseminated to the northeastern United States, and thus considered it possible that the species had been recently introduced. He was probably correct in thinking the species was a recent introduction in North America, since following the time of its first report in 1953, the face fly has spread as far south as South Carolina and as far west as the Rocky Mountains in Wyoming and Colorado.

The face fly is now a recognized pest of cattle and horses in the central and eastern states. The females feed on the mucus secretions of the eyes, nose, and mouths of the host and on blood from wounds caused by blood-sucking flies or other injuries. Male face flies are seldom noted on cattle. The feeding females usually congregate around the eyes, nose, and mouths of cattle, and can cause severe irritation, swelling of the eyes, and excessive formation

of tears. The cattle may huddle in pastures, seek shade, and not feed, resulting in decreased milk production in dairy animals and lower gains in beef cattle.

Face flies come out of hibernation in the spring and can be found on cattle from May to October in the midwest. The most serious populations occur from July to September.

Face flies also can be a nuisance to humans during the fall when they may congregate in large numbers on, or in, dwellings prior to hibernation. They often hibernate in attics, between walls, and in other protected places. They may leave these places of hibernation between walls, etc., during warm days in the winter, and be found within, as well as outside of the dwellings during these times.

The face fly has never been shown to transmit any disease. It has, however, been suggested that it might transmit leprosy (Lamborn, 1937), infectious abortion (Hammer, 1942), and pink eye, or conjunctivitis (Thompson, 1938). Ershov (1956), showed that the face fly was an intermediate host of a mammalian eyeworm, Thelazia rhodesi in the Ukraine, and it was suggested by Sabrosky (1959) that should the face fly extend its range to the west coast, it might serve as a host for an American eye worm, T. californiensis.

In nature, face flies oviposit on fresh bovine manure, where the larvae develop. When the larvae reach maturity they migrate from the manure and pupate under it, or in the ground. A complete generation will develop in ten days to four weeks depending upon climatic factors. The life history and habits of the face fly in nature, as well as its taxonomy were reviewed by Tesky (1960). Wang (1964) studied the life history and habits of the face fly under laboratory conditions. He described all stages of the life cycle and observed certain ecological aspects. Hammer (1942), who made a lengthy study

of the biology and ecology of flies associated with pasturing cattle and their excrement in Denmark, devoted much attention to the face fly. He considered that the face fly is in the group of flies that are in transition between general secretion suckers and blood suckers. He observed that in Denmark, the face fly also fed on flower nectar, and believed that nectar was the principal diet of the flies before the cattle were pastured. Hammer (1942) and Derbeneva-Ukhova (1942) also observed face flies feeding on fluids on the surface of cow manure. The latter author, in his grouping of muscoid flies which was cited by West (1951), classified the face fly as a "facultative haematophagous species." "Thise seek blood when they can obtain it, but can and do make out on other food if circumstances require."

The nutrition of adult flies with this type of feeding habits has never been thoroughly studied, although it seems to represent a stage between polyphagous types such as M. domestica, which can utilize a large variety of foods, and obligatory blood feeders such as Stomoxys calcitrans, which require a blood meal before the ovaries can develop. The nutritional requirements for at least one species of insect in both of these groups has been elucidated. A chemically defined diet for M. domestica was developed by House and Barlow (1958). Although a defined diet has yet to be developed for Stomoxys or any other muscoid blood feeder, the nutritional requirements for Aedes aegypti, another obligatory blood feeder were determined by Dimond et al (1956).

The purposes of the study reported upon here are twofold. The first objective was to develop a chemically defined diet for M. autumnalis which would allow ovarian development, and if possible to rear an entire generation of flies from adults raised on this diet. Emphasis was placed upon the role of protein in the diet. The second objective was to obtain some idea of the manner of female reproductive system development from eclosion to maturity.

The first successful laboratory rearing of Musca autumnalis was reported by Fales, Bodenstein, and Keller in 1961. The larvae were reared on bovine manure, and the adults on bovine manure (used both as food and as an oviposition medium), ground raw stew beef, a skimmed milk-granulated sugar solution, and a solution of Diamalt enriched with mucoproteins, brain-heart infusion, and pollen. Since the publication of this paper, the diet has been modified as follows: citrated beef blood is used in place of raw beef, and the gut slime (mucoprotein) has been eliminated (J. H. Fales, personal communication).

It was discovered that there were several inherent problems in the laboratory rearing of the face fly, although little trouble was encountered in keeping adult flies alive and reproducing with a laboratory diet developed at Kansas State University and composed of the following: one part dried soluble bovine blood, one part dried, defatted milk, and six parts sucrose. Care had to be excercised that the relative humidity was kept high (70 %) or the manure used as a larval medium became crusted over and the larvae pupated under the crust instead of migrating into the sand provided for this purpose. Eclosion was also adversely affected by low humidity, possibly due to excessive dehydration of the pupae. It was difficult to keep the manure from becoming contaminated with eggs from other species of flies before it was collected in warm weather, and pupae of flies other than the face fly were encountered several times. The manure containing developing cultures of face flies was kept in a large screened-in cabinet to reduce possible contamination in the rearing room.

The role of nutrition in the ovarian development of the diptera will be briefly reviewed in order to show what has been done in this field and to state the classes of nutrients proven necessary or helpful to ovarian development in other species of flies.

Glaser (1923) theorized that longevity and reproduction of flies are correlated with certain types of food. He found that both sucrose and a protein source were necessary for oviposition in M. domestica. He also reported that a few eggs were deposited by flies fed sucrose and water, although Ascher and Levinson (1956) feel that this small amount of oviposition was made possible by nutrients obtained from cannabalism of dead flies. Greenberg (1959) proved that an egg-laying female housefly consumes two to three times as much protein as a male or virgin female, although no significant difference was found in sucrose consumption of egg-laying females, virgin females, and males. This seems to be quantitative evidence of the fly's need for an exogenous protein source to fulfill the reproductive function. Rasso and Fraenkel (1954) stated that the blowfly, Phormia regina Meig., showed no ovarian development on sugar and water, and when fed solely on water and a protein source, their maximum life span was only five days. When both sugar and a source of protein was provided, ovarian development was observed and longevity was increased. The necessity of protein for ovarian development has also been demonstrated in Lucilia sericata (Hobson, 1938) and Musca vicina (Ascher and Levinson, 1956). Thus it is probable that protein is necessary for ovarian development and reproduction in some flies.

Nutrients other than sugar and protein have also been shown helpful or necessary for fly reproduction. B-vitamins accelerated the ovarian development of P. regina (Rasso and Fraenkel, 1954), and are necessary in the ovarian development of M. domestica (House and Barlow, 1958), and Drosophila melanogaster (Sang and King, 1959). The addition of certain antivitamins to the diet of M. domestica caused ovarian atrophy (Levinson and Bergmann, 1959). Cholesterol has also been proven essential for ovarian development in M. domestica (Monroe, 1959, although it can be supplied in the larval diet and

carried over in the adult for the initial ova produced (Robbins and Shortino, 1962). Robbins and Shortino also showed that if sufficient cholesterol was supplied in the larval diet, mature ovaries could be observed in adult females which were held on an adult diet consisting only of sucrose and water. The eggs produced from these flys were viable and produced larvae which developed to adults.

Certain minerals have been shown to be helpful to ovarian development in Aedes aegypti (Lea et al, 1958), and D. melanogaster (Sang and King, 1959).

Thus it seems probable that a chemically defined diet for M. autumnalis should include at least a protein source, sugar, cholesterol, a B-vitamin mixture, and a mineral salt mixture.

METHODS AND MATERIALS

Face Fly Colonies-Rearing and Maintenance

The stock culture originated from pupae obtained from the University of Nebraska (C. M. Jones, U. S. D. A., at Lincoln) in the spring of 1963. Adults were maintained in cages approximately 12 inches long, 8 inches wide, and 10 inches high. The bottom and back of the cage were 1 inch boards 12 by 8 and 10 by 8 inches respectively, attached at right angles with wood screws, and finished with several coats of white enamel. The sides and top were of wire screen which was stretched over the two boards and stapled in place. The entrance end was a nylon sleeve approximately 18 inches long, which was stapled onto the screen and bottom. This was held closed with a rubber band when the cage was in use.

To start a new culture of adults, 800 to 2000 pupae were placed in each cage in a 3½ ounce soufflé cup. After the adult face flies had emerged, food and water were supplied in soufflé cups. The food consisted of a mixture of one part soluble dried bovine blood (Nutritional Biochemicals Corporation),

one part defatted dry milk, and six parts sucrose, mixed dry. A styrofoam chip was floated on the top of the water to minimize drowning.

The flies were egged at eight to ten days of age. Fresh bovine manure obtained from bulls at the Kansas Artificial Breeding Service Unit was put into eight by eight inch cake pans and introduced into the cage. The manure was left in the cages from one-half hour to 24 hours, depending upon the number and age of the flies in the cage to approximate optimum egg density. After the pans of manure were removed from the cage, they were placed in larger pans of fine sand where the larvae migrated in order to pupate. When the pupae were two to four days old, they were sifted out of the sand and used to start new colonies or for experimentation. The sifter was a one-pint ice cream carton that had the bottom replaced with a circle of wire screen.

The stock culture was maintained in a rearing room with the temperature held at 80 degrees Fahrenheit ± 4 degrees, and relative humidity at 70 per cent ± 5 per cent. Temperature was maintained with a thermostatically controlled electric heating unit and a refrigeration unit. Relative humidity was maintained by an automatic mist-type humidifier. The photoperiod used was eight hours of dark and sixteen of light. The light was supplied by four 48 inch fluorescent lights, and a 275 watt sun lamp which was trained on the fly cages.

Diet Testing Procedures

Small Scale Tests. Diet tests were conducted in one quart wide mouth mason jars with wire screen in the caps, which were laid on their sides during the tests. Twenty-five pupae from 2 to 4 days of age were introduced into each jar in a one-ounce shallow soufflé cup. The diet being tested and water were also introduced into the cages in soufflé cups. A small styrofoam chip

was floated on the water to minimize drowning. Food and water were replaced every 3-4 days. Dead flies were removed daily and mortality was recorded.

The experiments were conducted in a room maintained at the same temperature and relative humidity as listed above. The photoperiod used was eight hours of darkness and sixteen of light. Light was supplied by a bank of eight, 48 inch fluorescent lights.

Large Scale Tests. The procedure for conducting large scale diet tests was identical to that used in maintenance of the stock colonies. After eclosion, the adults were fed the diet being tested for a suitable period of time and then egged with bovine manure. The resultant pupae were weighed, counted, and the percentage of adults emerging from these pupae was calculated.

Synthetic Diet Formation

Method One. This method was identical to that described by Monroe (1960) except that proteins other than vitamin-free casein were used. The following diet components were ball-milled for four hours: sucrose, 47.0 parts by weight; protein source, 47.0 parts (more or less in the case of certain serum albumin diets discussed later); sodium oleate, 2.0 parts; salt mixture, 4.0 parts; and ribose nucleic acid, 0.1 part. The salt mixture, obtained from Nutritional Biochemicals Corporation, was the Wesson modification of the Osborn-Mendel salt mixture (Wesson, 1932). The following amounts of compounds give one kilogram of salt mixture.

Salt	Grams
NaCl	105.0
KCl	120.0
KH_2PO_4	310.0
$Ca_3(PO_4)_2$	149.0
$CaCO_3$	210.0

Salt	Grams
$MgSO_4$ (anhydr.)	90.0
$FePO_4 \cdot 4\ H_2O$	14.7
$MnSO_4$	0.20
$K_2Al_2(SO_4)_4 \cdot 24\ H_2O$	0.09
$CuSO_4 \cdot 5\ H_2O$	0.39
NaF	0.57
KI	0.05

After four hours this mixture was removed from the ball mill, and one milliliter of the B-vitamin mixture listed below was added for each ten grams of diet.

B-vitamin	mg./100 g. diet
thiamine hydrochloride	50.0
riboflavin	25.0
nicotinic acid	100.0
calcium pantothenate	50.0
pyridoxine hydrochloride	25.0
choline chloride	1000.0
inositol	500.0
folic acid	5.0
biotin	1.0

The vitamins were weighed on a Mettler balance to an accuracy of ± 0.1 milligram and then were dissolved in 10 milliliters of distilled water to which a few drops of conc. ammonium hydroxide had been added. This mixture was then pipetted onto 100 grams of the diet mixture. Also added to the mixture at this time was one milliliter per 100 grams dry diet of a 2.0 per cent zinc chloride solution, as it has been shown for at least one insect, Tenebrio molitor larvae, that zinc is essential for growth (Fraenkel, 1953), and the salt mixture used lacked zinc. The ingredients of the dry diet and vitamin solution were combined for ten minutes with an electric kitchen mixer, which gave a diet of uniform composition. The mixture was then spread out in the bottom of a pan and allowed to dry. A fan was used to speed up this process.

When the mixture was thoroughly dried, it was put through a 0.012 inch screen of a hammer mill. A 0.1 per cent solution of cholesterol in methylene chloride was then added at the rate of one milliliter of cholesterol solution per gram of diet. Excess methylene chloride was added to insure thorough wetting and even mixing of cholesterol with the dry diet. The methylene chloride was evaporated under nitrogen while the mixture was stirred occasionally. The diets were then ready for use and were stored in a deep freeze until needed. In the series of sixteen diets formulated by this method, only the protein or the protein concentration was varied.

Method Two. This method was similar to method one except that all ingredients but the protein were combined into a diet base, and the protein was added later as needed. The sucrose, sodium oleate, salt mixture, and RNA were ball milled, the vitamin mixture and zinc chloride were added, the diet was dried and hammer milled, and cholesterol added as described previously. The proteins used were run through the 0.012 inch screen of a hammer mill separately so that their particle size was the same as that of the diet base.

This method has two advantages over method one. When a large amount of protein-free base had been prepared, diets could be formulated more rapidly. The necessary amounts of the diet base and protein were weighed out on a torsion balance accurate to 0.1 gram, and thoroughly blended with a mortar and pestle. Many diets could be made up in a day using this type of formulation. A second advantage was that smaller amounts of diet could be prepared with accuracy and without excessive loss of material. Sixteen diets were prepared using this method.

Method Three. Method three was the volume-volume mixing of the diet components with a mortar and pestle. Five diets containing undefined com-

ponents were formulated in this manner. They were not hammer-milled and the particle size was subsequently larger.

Method Four. Three diets consisting of single materials (dried blood, dried milk, sugar) were fed uncombined to the flies. These diets were not hammer-milled.

The forty-one diets tested are tabulated in Table 1. Most of the proteins or other components listed are self-explanitory. The composition of the amino acid mixture used in diet 1 is given below.

Amino Acid	Grams
L-argenine	4.42
L-histidine	2.56
L-lysine hydrochloride	6.54
L-tyrosine	4.42
DL-tryptophan	1.06
DL-phenylalanine	5.66
L-cystine	1.41
DL-methionine	3.89
DL-threonine	3.98
DL-serine	6.46
L-leucine	8.49
DL-isoleucine	5.84
DL-valine	7.07
L-glutamic acid	17.25
L-aspartic acid	7.25
DL-glycine	2.56
DL-alanine	4.33
L-proline	6.72
	99.91

Source of Materials Used in the Diets. The components of the diets were all purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, with the following exceptions. L-leucine and L-aspartic acid were obtained from Mann Research Laboratories of New York, New York. Sucrose (granulated sugar), dried, defatted milk (Bordon's Starlac), and Fleischmann's frozen egg white were obtained locally. The salivary mucin was precipitated from bovine submaxillary saliva as described by Hawk, Oser, and Summerson (1947).

Table 1. A complete list of the diets tested.

Protein Source	% protein in diet	Method of formulation
A. Holidic Diet		
1. amino acid mixture	50	2
B. Meridic Diets		
2. lactalbumin	47	1
3. hemoglobin	47	1
4. bovine blood fibrin	47	1
5. serum albumin	14	1
6. serum albumin	24	1
7. serum albumin	39	1
8. serum albumin	47	1
9. serum albumin	56	1
10. serum albumin	61	1
11. serum albumin	66	1
12. serum albumin	50	2
13. hemoglobin	50	2
14. gelatin	50	2
15. lactalbumin	50	2
16. vitamin-free casein	50	2
17. salivary mucin	50	2
18. gastric mucin	50	2
19. sodium caseinate	50	2
20. egg albumin	50	2
21. hemoglobin-lactalbumin	14-33	1
22. hemoglobin-serum albumin	19-28	1
23. serum albumin-hemoglobin	40-10	2
24. serum albumin-hemoglobin	25-25	2
25. serum albumin-hemoglobin	10-40	2
26. gastric mucin-alphacel	40-20	2
C. Undefined Diets		
27. brewer's yeast	47	1
28. bovine blood plasma	47	1
29. dried egg white	50	2
30. egg solids	50	2
31. brewer's yeast-lactalbumin	14-33	1
32. hemoglobin-brewer's yeast	14-33	1
33. brewer's yeast-sucrose, 1:1	--	3
34. dried milk:sucrose, 1:1	--	3
35. dried blood:dried milk, 1:1	--	3
36. dried blood:sucrose, 1:1	--	3
37. dried blood:dried milk:sucrose, 1:1:6	--	3
38. dried milk	--	4
39. dried blood	--	4
D. Control Diets		
40. dried blood:dried milk:sucrose, 1:1:6 (hammer milled)	--	3
41. sucrose	--	4

Techniques Used in Testing for Non-nutritional Factors that Could Have Influenced Results. There were possibly factors involved other than the diet that could have influenced the results of the mason jar tests. These factors were eliminated whenever possible, but some might still have been present.

Several replicates of the diets that contained lactalbumin became moldy, but the results did not differ from the non-moldy replicates. No mold was noticed on any other diets unless they were spilled and then got wet. All such tests were discarded.

A few tests were carried out using liquid diets, and while these gave better results than the same diet dry, it was difficult to determine whether these results were due to the physical state of the diet, or to bacterial growth, as a very definite odor was developed by the diets after about twelve hours. No conclusions were drawn from these tests, but it seems feasible that liquid diets might be equal to or better than the dry diets tested. It might be reiterated here that the face fly in nature ingests a large part of its food in the form of liquids, such as blood, saliva, mucus secretions, and manure juices.

There was also a possibility that some nutrient material might have been obtained from dead flies or empty puparia. House fly puparia have been shown to contain sterols (Monroe, 1960), which have been proven to be essential nutrients to many insects. Ascher and Levinson (1956) reported cannabalism in Musca domestica which could also serve as a source of nutrients. Tests were therefore undertaken in which empty puparia, dead flies, or both, were allowed to remain in the cages. Sucrose, which could support life for long periods of time while allowing no ovarian development, and water were also supplied. Dissections made during a two week test period showed no ovarian development

in any of the cages tested in this fashion. Also, no flies were observed attempting to feed on puparia or dead flies. It was therefore concluded that there was little possibility that the flies derived any nutrients from dead flies or puparia.

Glaser (1923) showed that in M. domestica and Stomoxys calcitrans the presence of the male furnishes an important stimulus to oviposition. He did not speculate upon the nature of this stimulus. Adler and Theodor (1935) later showed that this stimulus might be fertilization, as the development of eggs in Phlebotomus perniciosus depended upon both a blood meal and fertilization. An uneven sex ratio might at times develop in the mason jar tests and influence fertilization, due to the small number of flies used in each test. Therefore, the rate of ovarian development of virgin females was compared with that of females from a cage containing an equal number of males and females. The flies for these tests were obtained from pupae that had been placed individually in small vials stoppered with small pieces of diSPo-plugs (Scientific Products, Evanston, Illinois). When the flies emerged from the puparia, they were sexed. Forty females were put in one cage and 20 males and 20 females were put in another. A series of dissections showed that the presence of males made no appreciable difference in the amount of ovarian development, so it was concluded that the presence of males had no effect on ovarian development in the face fly.

Determination of Ovarian Development

Dissection. The following procedure was used to determine whether or not the ovaries were mature. The mason jars were put in a deep freeze and left until the flies were dead. The jars were then removed, the males counted and discarded, and the females dissected under a low power dissecting scope.

They were grasped with a pair of fine curved-tip forceps held in one hand and the tip of the abdomen was sliced off with a razor blade held in the other hand. Pressure was then applied to the abdomen with the razor blade, forcing the contents out. If mature ovaries were present they could be immediately discovered. The criterion used in determining the maturity of the ovaries was the darkening of the respiratory masts of the eggs.

Fixation and Storage. If more detailed dissections were desired, the flies were killed with modified Carnoy's solution, consisting of 6 parts absolute isopropanol, 3 parts chloroform, and 1 part glacial acetic acid. A small vial about half full of the solution was introduced into the cage and the flies were trapped between the vial and the screen of the cage, as in all such cases the flies were reared in cages rather than jars. If the flies were to be dissected immediately, they were taken directly out of the solution without being rinsed. If there was a time lapse of more than a few hours between sacrifice and dissection, the flies were taken out of the Carnoy's solution after one to two hours, and given 3 one-minute rinses in 70 per cent isopropanol. They were then stored in 70 per cent isopropanol until dissected. This method preserved the structures well. although they became somewhat brittle. A more detailed account of methods used in dissection is found in the results and discussion.

RESULTS AND DISCUSSION

Morphology of the Female Reproductive System

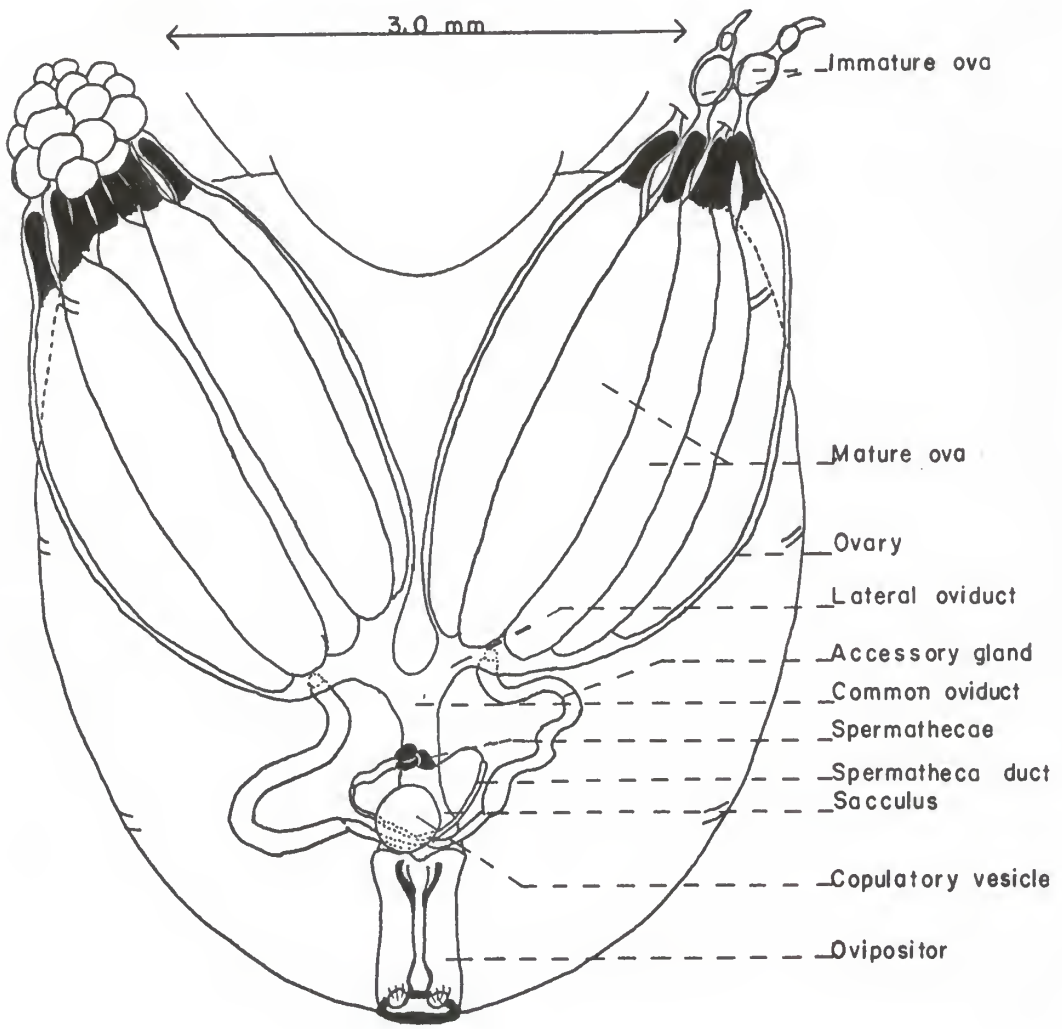
The Mature Reproductive System. The reproductive system of a mature female Musca autumnalis is diagrammed on Plate I. The ovaries are the most conspicuous structures of the system and occupy most of the abdominal cavity. They lie ventral and lateral to the alimentary tract, which rests in the space

EXPLANATION FOR PLATE I

This is a diagrammatic sketch of the reproductive system of a mature female Musca autumnalis. It has been drawn as if the dorsum of the abdomen and all abdominal viscera but the reproductive system have been removed. The ovaries have been pulled anteriorly to show the rest of the reproductive system, much of which would otherwise be obscured by them. The left ovary has been drawn with the immature ova in place, resembling a cluster of grapes. Two egg tubes of the right ovary have been dissected, to show the relationship between mature and immature ova in the egg tube.

PLATE I

Female Reproductive System



between them, except for the crop, which may rest dorsal to the respiratory masts and immature ova when distended. The number of egg tubes varies between five and fifteen per ovary. The proximal portion of each egg tube is distended by a single mature ovum if oviposition has not taken place. One to three immature gametes are also present in each egg tube and are indicated by successively smaller bulges in the distal portion. The egg tubes are thin, delicate, transparent structures, that connect anteriorly with the thin-walled lateral oviduct situated in the fifth abdominal segment. No terminal ligament could be demonstrated, but the egg tubes are held together in a vairly coherent manner by numerous tracheae. The lateral oviducts are short and unite almost immediately to form the common oviduct, which curves ventrally and approaches the ovipositor at a point ventral to the rectum. Shortly anterior to the ovipositor, the common oviduct dilates to form the sacculus, where the proximal ends of the accessory glands and the spermathecal ducts are attached. The sacculus is continuous with the vagina, which is within the ovipositor and opens into the genital aperture, probably behind the subanal plate of segment 9. The accessory glands are narrow tubular structures, with the posterior portion reduced to about one-half the diameter of the rest of the gland. The glands are attached at both ends, the posterior end being inserted into the sacculus and the anterior end being connected on the ventral side of the calyx of the ovary. There are three spermathecae present, two on the left side and one on the right. These are sclerotized, ovoid, black capsules, which are attached to the sacculus by slender ducts. The spermathecae are held loosely together, probably by connective tissue or tracheae. Each spermatheca is partially inclosed in a cuplike distal expansion of the wall of its duct. The copulatory vesicle is a spherical, transparent organ positioned dorsal to the sacculus and

attached to it. It often obscures the distal connections of the accessory glands and spermathecal ducts. Two of these structures are present, one on each side, in the house fly, Musca domestica (West, 1951), but this has not been demonstrated in the face fly, where only a single copulatory vesicle appears to be present. The above description assumes the ovipositor to be retracted within the body of the fly, when the four segments that comprise it are telescoped within each other, an arrangement made possible by the flexibility of the intersegmental membranes. When the ovipositor is extended for oviposition, most of the reproductive system posterior to the lateral oviducts is pulled into it due to its attachment to the genital opening in segment 9 (see Plate III, figs. 16 and 17).

Development and Maturation of the Ova. It was desired to give an unsophisticated description of the changes observed in the female reproductive system from eclosion to maturity, in order to have a series of reference points to refer to in the discussion of dietary tests which follows. A series of flies from eclosion to maturity was sacrificed as described earlier, dissected, and photographed. The results appear in plates II and III. Some of the reproductive systems were photographed in situ, but the majority were removed from the body of the fly and the ovaries were stretched anteriorly so the entire system could be seen. All figures are dorsal views. The systems photographed against a white background were stained orange with metanil yellow for contrast. Such staining was unnecessary for systems photographed against a black background.

The most prominent structures seen upon opening the abdomen of a newly emerged fly are the huge abdominal air sacs, which are well illustrated by the dissection of a one day old fly in figure 1. The sacs get smaller as

the ovaries increase in size and are much harder to demonstrate in mature flies. Evans (1933), who studied this change in Lucilia sericata, theorized that the abdominal air sacs of muscoid flies have little respiratory function but are chiefly concerned with the preservation of increased volume of newly emerged flies, and with the provision of ample space within the abdomen for growth of the fat body in the males and growth of the ovaries and the fat body in females. Figure 2 shows a newly emerged female with the air sacs and digestive system removed. The ovaries are barely discernible in the thick mass of fat body-like material that lines the body wall. Each ovary is extensively tracheated. The rest of the reproductive system is poorly defined, but the spermathecae and ovipositor are distinguishable. Figure 3 shows the system of the same fly as in figure 2 removed from the body and stretched out. The ovaries are smaller than the ovipositor and somewhat resemble small clusters of grapes. The reproductive system of a 6 hour old female shown in figure 4 shows no difference in size and shape from that shown in figure 3. By two days, however, marked changes begin to appear in the system, as shown by figure 5. The ovipositor has assumed a definite shape and is no longer the almost amorphous mass of tissue that it is in the newly emerged fly. The rod-like tergites of segments 6 and 7 are clearly visible and appear as black lines on the photograph. From this age to maturity, there is little change in the size and shape of the ovipositor. The copulatory vesicle is also easily seen, just anterior to the proximal end of the ovipositor. This structure is small and somewhat resembles an uninflated balloon in newly emerged flies, but here is seen as a clearly defined spheroid structure. The ovaries have enlarged and are more spherical. There is little change in size of the lateral and common oviducts, but the accessory glands

EXPLANATION FOR PLATE II

Fig. 1. One day old female with the dorsum of the abdomen removed. Note the large abdominal air sacs.

Fig. 2. Dissection of female just emerged from the pupal case. The digestive system was removed and the highly tracheated ovaries are exposed (arrows).

Fig. 3. Reproductive system of the newly emerged female of Fig. 2 removed from the body.

Fig. 4. Reproductive system of six hour old female.

Fig. 5. Reproductive system of two day old female. Note the copulatory vesicle (arrow).

Fig. 6. Reproductive system of three day old female.

Fig. 7. Reproductive system of four day old female.

Fig. 8. Reproductive system of five day old female. Note the difference in transparency between the copulatory vesicle (arrow) of this fly and that of the fly in Fig. 5.

Fig. 9. Reproductive system of six day old female.

PLATE II

are approximately the size that they attain in the mature fly. Figure 6 shows the system of a three day old fly and was included to show that there is a variation in the rate of growth of the reproductive system. It appears to be less developed than the two day old fly in figure 5. The system of a four day old fly is shown in figure 7. Here the ovaries have increased in size, although retaining their spherical conformation. The oviducts have also increased in size. This figure shows the entire reproductive system to be the approximate size and shape as that of the mature fly, with the exception of the ovaries, and it is primarily to these structures that the rest of the discussion will be devoted. By the time the flies are five days old the ovaries begin to assume a more ovoid general conformation, due to the lengthening of the individual ova with little or no corresponding increase in diameter. This lengthening trend is continued in the 6 and 7 day old flies (figures 9 and 10). The difference in size between the ova in the posterior egg tubes and the smaller anterior ova is clearly shown in figure 10. The anterior ova will show little increase in size until the posterior ova have matured and have been oviposited. Figure 11 shows the system of a 9 day old female. The ova are the size of mature ova (approximately 3.0 mm long and 0.5 mm wide). The respiratory masts have been formed and are beginning to darken, but are not yet as dark as those of a mature ova.

Figures 12 to 17 show various features of mature reproductive systems from flies ten days of age. It should be noted that although these particular reproductive systems are from ten day old flies, face flies normally mature at any time between seven and ten days under the rearing room conditions and diet used. Figure 12 shows the abdominal tergum removed and the viscera *in situ*. The ovaries can be seen on either side of the digestive

system, which lies in the hollow between them. In figure 13, the digestive system is removed and the ovaries are pulled upward, to show the details of the anterior end of the ovaries. The immature ova are visible as a mass of round translucent bodies clustered anterior to and among the black respiratory masts of the fully developed ova. The individual egg tubes are fragile transparent structures that are not visible in any of these figures, but it is by these tubes that the mature and immature ova are connected. Figure 14 shows the posterior portion of the reproductive system. Both of the accessory glands are shown, one appearing more transparent than the other, however in most cases the two glands are identical in appearance. The spermathecae are visible as three small black bodies dorsal to the median oviduct. The eggs are spread out more than usual due to ruptures of the egg tubes. A more complete picture of the entire reproductive system is given in figure 15, but the structures posterior to the median oviduct are unclear. Figures 16 and 17 are included to show the relative positions of the organs of the reproductive system during oviposition. When the ovipositor is extended, the system is pulled into it until the lateral oviducts and ovaries are at the proximal end of the ovipositor, as shown in figure 16. In figure 17, the ovipositor has been cut open by a ventral incision. The spermathecae are visible about one third of the distance within the ovipositor, and the sacculus and vagina are distal to these structures.

A number of females from the stock colonies reared on the standard diet were sacrificed at ten days and both mature and immature ova were counted. The results are shown in Table 2 below, as are some values found in the literature.

EXPLANATION FOR PLATE III

Fig. 10. Reproductive system of seven day old female.

Fig. 11. Reproductive system of nine day old female. The respiratory masts of the eggs are darkening (arrow) but are not yet as black as they will become when maturity is attained.

Fig. 12. Mature female with the dorsum of the abdomen removed to show the relative position of the mature female reproductive system to the other abdominal viscera.

Fig. 13. View of the distal end of the ovaries of a mature female. Note the black color of the respiratory masts of the mature ova, and the immature ova, which somewhat resemble a cluster of grapes (arrow).

Fig. 14. View of the proximal end of the reproductive system of a mature female.

Fig. 15. Another view of the proximal end of the reproductive system of a mature female, at a smaller scale than the other figures on this plate.

Fig. 16. View of the reproductive system of a mature female with the ovipositor extended, to show the position of the reproductive system in the abdomen during oviposition.

Fig. 17. Further dissection of the fly in Fig. 16 showing the extent to which the common oviduct and the structures posterior to it are pulled into the ovipositor during oviposition. The arrow points to the spermathecae.

PLATE III

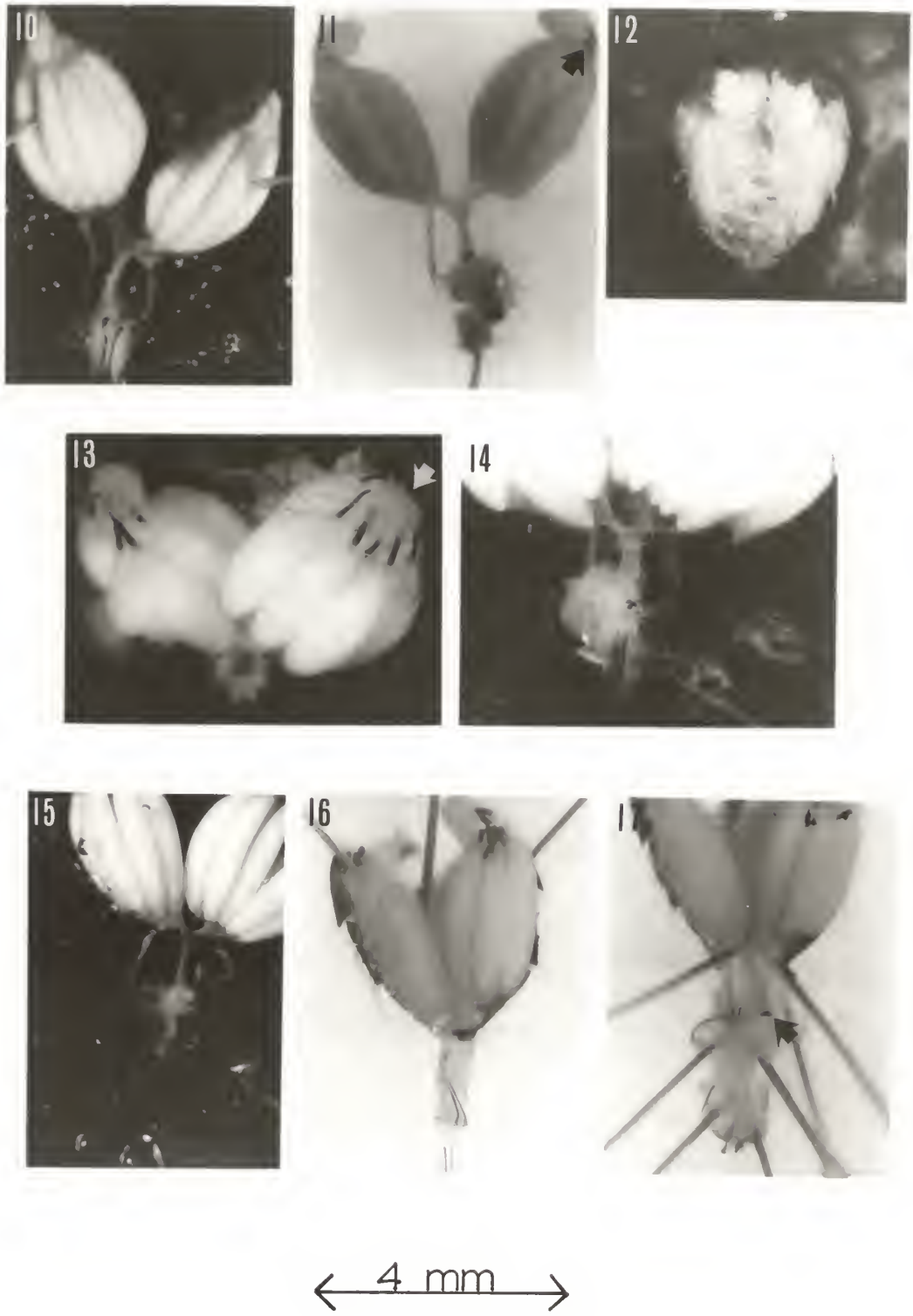


Table 2. Number of Eggs in Face Fly Ovaries: Literature and Personal Observations

Source	Mature eggs	Immature eggs	Batches of eggs	Average no. eggs per batch	Total eggs per fly
Hammer (1942)	--	--	--	--	24.6
Wang (1964)	--	--	3-7	10-22	30-128 (lifetime)
K. S. U. Culture	14	20	--	--	34.0

Nutrition Experiments

Small Scale Nutrition Experiments. A total of forty-one diets were tested by the mason jar method to determine their utility in inducing ovarian development in the face fly. On the basis of these tests, the diets could be divided into three general classes with regard to the amount of ovarian development induced, as follows: (A) diets inducing no formation of mature ovaries in a ten day test period; (B) diets inducing only slight to moderate development of mature ovaries (less than 40 per cent); and (C) diets that induced good development of mature ovaries (over 70 per cent). The per cent ovarian development induced by the diets in each class is summarized in Table 3. The control diet included as a comparison was a mixture of one part dried soluble beef blood, one part defatted dry milk solids, and six parts sucrose. These components were thoroughly blended and run through the 0.012 inch screen of a hammer mill. Sucrose alone served as a control for no ovarian development. The blood:milk:sucrose control gave less apparent ovarian development than several of the other diets tested. This was thought to be due to a higher degree of spontaneous oviposition by the gravid females in the food and on the side of the jars. Although flies fed on the seven diets that induced

Table 3. Comparative ovarian development in face flies fed on synthetic diets containing different protein sources.

Diet no.	Protein source	% protein in diet	% ovarian development
A.	Diets that promoted no ovarian development		
4.	fibrin	47	0
5.	serum albumin	14	0
27.	brewer's yeast	47	0
29.	dried egg white	50	0
31.	brewer's yeast-lactalbumin	14-33	0
33.	brewer's yeast-sucrose	--	0
39.	dried blood	--	0
B.	Diets that promoted slight to moderate ovarian development		
1.	amino acid mixture	50	29
2.	lactalbumin	47	8
3.	hemoglobin	47	3
6.	serum albumin	24	12
7.	serum albumin	39	21
8.	serum albumin	47	20
9.	serum albumin	56	16
10.	serum albumin	61	4
11.	serum albumin	66	20
12.	serum albumin	50	13
13.	hemoglobin	50	28
14.	gelatin	50	3
15.	lactalbumin	50	4
16.	vitamin-free casein	50	3
17.	salivary mucin	50	5
21.	hemoglobin-lactalbumin	14-33	10
22.	serum albumin-hemoglobin	28-19	36
23.	serum albumin-hemoglobin	40-10	14
24.	serum albumin-hemoglobin	25-25	19
25.	serum albumin-hemoglobin	10-40	30
28.	plasma	47	3
32.	hemoglobin-brewer's yeast	14-33	3
34.	dried milk:sucrose, 1:1	--	6
35.	dried milk:dried blood, 1:1	--	31
38.	dried milk	--	6
C.	Diets that promoted good ovarian development		
18.	gastric mucin	50	90
19.	sodium caseinate	50	82
20.	egg albumin	50	81
26.	gastric mucin-alphacel	40-20	81
30.	egg solids	50	82
36.	dried blood:sucrose	--	76
37.	dried blood:dried milk:sucrose, 1:1:6	--	77
D.	Control diets		
40.	dried blood:dried milk:sucrose, 1:1:6 (hammer-milled)	--	77
41.	sucrose	0	0

good ovarian development did show spontaneous oviposition seven to ten days after eclosion in at least one replicate, this phenominon was usually observed to a much higher degree in the control than in any of the other diets tested. Among the group B diets, spontaneous oviposition was also observed in the 50 per cent hemoglobin, 10 per cent serum albumin-40 per cent hemoglobin, and amino acid diets, and occurred from seven to ten days after eclosion.

Diets Containing Undefined Components. Thirteen diets containing one or more undefined components were tested in an attempt to gain an insight in the formulation of meridic diets at a later date. These diets could be placed in two broad categories; those which were formulated by methods one or two and contained known percentages of other well defined components in addition to the undefined protein source; and those which were volume:volume mixtures of one or more unknown components, with or without sucrose, or with unknown components fed alone. The results given by these diets are tabulated in Table 4.

Ovarian development was generally low except for the egg solids, dried blood:sucrose, and dried milk:dried blood:sucrose diets, which apparently contained utilizable proteins. It is not surprising that good development was possible on the egg solid diet, as the egg albumin meridic diet also gave excellent results. The dried blood:sucrose diet was also adequate. The dried blood:dried milk:sucrose diet gave almost exactly the same results as the control diet, which was expected, as the only difference between the two diets was that the control diet was passed through the 0.012 inch screen of a hammer mill.

The extreme high mortality encountered among flies fed the dried blood, dried milk, and dried blood:dried milk diets was undoubtably due to the low

Table 4. Ovarian development and survival of face flies fed on diets containing undefined components.

Diet no.	Diet components	No. of replicates	Average % ovarian development	Range	Average % survival, 10 days	Range
27	brewer's yeast	4	0	-	87	75-97
28	bovine blood plasma	4	3	0-11	93	83-98
29	dried egg white	4	0	-	86	68-100
30	egg solids*	6	82	71-100	71	40-88
31	brewer's yeast-lactalbumin	4	0	-	96	92-100
32	hemoglobin-brewer's yeast	4	0	-	90	80-100
33	brewer's yeast:sucrose, 1:1	4	0	-	91	81-100
34	dried milk:sucrose, 1:1	6	8	0-25	98	96-100
35	dried milk:dried blood, 1:1	4	31	0-67	14	0-41
36	dried blood:sucrose, 1:1*	6	76	60-86	88	70-100
37	dried blood:dried milk:sucrose, 1:1:6*	6	77	50-100	95	75-100
38	dried milk	6	6	0-25	4	0-13
39	dried blood	2	0	-	0	-
40	control*	18	77	50-100	93	74-100
41	sucrose	4	0	-	97	92-100

* denotes spontaneous oviposition

amount of carbohydrate contained in these diets. Flies fed on dried blood alone always died in less than four days. Some of the flies on the milk or blood:milk diets were able to survive through the ten day test period, and even develop mature ovaries, but mortality was invariably high. The concentration of carbohydrate in bovine milk and in bovine blood are given by Altman (1961). Liquid milk contains 4.9 grams of lactose per 100 milliliters of milk, and liquid blood contains only 47 milligrams of glucose per 100 milliliters of blood. The carbohydrates would be much concentrated by the drying of the liquid blood and milk to powder. Although the milk contains about 100 times as much carbohydrate as does blood, the amount is probably still insufficient for viability. Galun and Fraenkel (1957) in their studies of the physiological effects of a number of carbohydrates on three species of flies, found that glucose was as good a carbohydrate source as sucrose for the three flies experimented upon. Lactose was well utilized by Musca domestica but unutilizible by Sarcophaga bullata or Aedes aegypti. It seems probable that the amount of glucose in the dried blood is so low compared to the amount of protein that the flies could not consume enough total food to obtain an amount of carbohydrates sufficient to support life. This is probably also the case with the lactose supplied by the dried milk diet. Dried milk can support life somewhat better than dried blood due to its higher carbohydrate content, but this content probably falls short of the required amount. Lactose has at least some utility to the face fly, as dried milk will allow a few flies to survive long inough to develop mature ovaries.

Meridic Diets Containing a Single Protein Source. Thirteen meridic diets containing a single protein source were tested and the results tabulated in Table 5. Ovarian development obtained on the diets varied from nil to

Table 5. Ovarian development and survival of adult face flies fed meridic diets containing a single source of protein.

Diet no.	Diet components	No. of replicates	Average % ovarian development	Range	Average % survival, 10 days	Range
2	lactalbumin (47 %)	4	8	0-14	90	81-100
3	hemoglobin (47 %)	6	3	0-9	91	84-96
4	bovine blood fibrin	4	0	-	97	87-100
12	serum albumin (50%)	5	13	0-30	88	77-100
13	hemoglobin (50%)*	6	28	0-67	84	69-100
14	gelatin	6	3	0-20	46	30-68
15	lactalbumin (50 %)	6	4	0-22	89	84-96
16	vitamin-free casein	4	3	0-13	84	74-92
17	salivary mucin	2	5	0-10	53	48-58
18	gastric mucin*	6	90	67-100	39	14-56
19	sodium caseinate*	6	82	73-88	86	73-100
20	egg albumin*	6	81	57-100	87	70-100
26	gastric mucin-alphacel*	4	81	67-100	70	39-92
40	control*	18	77	50-100	93	74-100
41	sucrose	4	0	-	97	92-100

* denotes spontaneous oviposition

excellent, with four diets giving results comparable to the control diet. These four diets (gastric mucin, egg albumin, sodium caseinate, and gastric mucin-alphacel) are discussed below.

The amino acid content of sodium caseinate (as casein) and egg albumin is known, and the amino acid composition of the holidic diet tested was an average of the percentages of each amino acid contained in these two proteins (see page 11). Gastric mucin is a glycoprotein of the sulfomucin type that contains sulfuric acid, uronic acid, and glucosamine in addition to protein (Hawk, Oser, and Summerson, 1947). The amino acid composition of the protein moeity is apparently unknown. The purpose of the alphacel in the gastric mucin-alphacel diet will be explained later.

It is interesting to note the differences in ovarian development promoted by the three albumins tested. Fifty per cent lactalbumin induced only 4 per cent ovarian development; 50 per cent serum albumin induced 13 per cent; and 50 per cent egg albumin induced 81 per cent ovarian development. The difference in utility of these proteins in promoting ovarian development could be due to differences in their amino acid content, the linkages of the component amino acids, or to possible nutrient impurities in the protein source.

Fifty per cent sodium caseinate induced excellent ovarian development (82 per cent) while 50 per cent vitamin-free casein induced only 3 per cent ovarian development. There may have been differences in the flies' ability to digest or absorb the diet, or sodium caseinate may have contained trace impurities of nutrient value which the more highly refined vitamin-free casein lacked. To obviate the latter possibility, all of the known essential dietary nutrients of insects were included in the diet base.

The 47 per cent hemoglobin diet formulated by method one induced only 3

per cent ovarian development, compared with the 28 per cent induced by the 50 per cent hemoglobin diet formulated by method two. No evidence was found to explain the differences between the diets.

There were also differences in the ability of the flies to survive on the diets tested. Although in most cases the survival of flies was 70 per cent or above, three diets, (gastric mucin, salivary mucin, and gelatin), caused very high mortality. Some of this mortality was directly attributable to rearing room conditions. Upon exposure to the high relative humidity used in the rearing room, the gastric mucin absorbed water and became extremely sticky. Flies often became entrapped in the diet, were unable to extricate themselves, and died. Two methods were used in an attempt to remedy this situation. Wire screen was fitted over the diet, in direct contact with it. This provided the flies with a substrate to rest on during feeding, while keeping most of the body from direct contact with the diet. Although this method prevented the flies from becoming stuck in the diet, many dead flies with bloated abdomens were noticed. Dissection of these flies showed the crop to be greatly distended with a pale yellow fluid. The consistancy of the diet was then changed by the addition of alphacel (non-nutritive cellulose) to the gastric mucin. The diet tested consisted of 40 per cent gastric mucin, 20 per cent alphacel, and 40 per cent of the diet base of method two. This reduced the mortality of the flies, prevented crop bloating, and continued to induce good ovarian development.

The high mortality in flies fed on salivary mucin cannot be readily explained, because the consistancy of the diet remained unaltered in the rearing room. Possibly toxic materials were introduced due to rumen juice contamination of the saliva from which the mucin was precipitated.

Mortality was also high on the gelatin diet. In this case, the high humidity of the rearing room could possibly have caused a small amount of gelling, making it difficult for the flies to ingest enough of the diet to meet their needs for maintenance.

Meridic Diets Containing Two Protein Sources. Several diets containing a mixture of two protein sources were tested to determine if such mixtures could induce a higher percentage of ovarian development than either of the component protein sources when used alone. Table 6 shows the results of these tests. Two combinations of two protein sources (28 per cent serum albumin-19 per cent hemoglobin, and 10 per cent serum albumin-40 per cent hemoglobin) induced more ovarian development than the 50 per cent hemoglobin diet, and all of the mixtures tested except the hemoglobin-lactalbumin diet gave a higher percentage of ovarian development than the 50 per cent serum albumin diet. Due to the somewhat erratic results obtained, caution should be taken in drawing conclusions. There is, however, an indication that diets containing mixtures of serum albumin and hemoglobin as a protein source can induce slightly more ovarian development than either of these protein sources when used alone.

Effect of Varying Protein Concentration. A series of diets containing varying amounts of serum albumin was tested to determine the per cent protein composition that induced the most efficient ovarian development and survival. These were formulated by method one. Although serum albumin was not the most effective protein for ovarian development, it was used as a protein source in the series because it was the first meridic diet tested that induced any ovarian development. The results of the tests are shown in Table 7. It can be seen that the best ovarian development was obtained from flies supplied with

Table 6. Ovarian development and survival of face flies fed meridic diets containing mixtures of two proteins.

Diet no.	Diet components	No. of replicates	Average % ovarian development	Range	Average % survival, 10 days	Range
21	hemoglobin-lactalbumin 14 per cent-33 per cent	6	10	0-25	99	96-100
22	serum albumin-hemoglobin 28 per cent-19 per cent	6	36	11-80	94	88-100
23	serum albumin-hemoglobin 40 per cent-10 per cent	6	14	0-55	90	77-100
24	serum albumin-hemoglobin 25 per cent-25 per cent	6	19	0-46	94	86-100
25	serum albumin-hemoglobin 10 per cent-40 per cent*	6	30	0-67	79	61-96
40	control*	18	77	50-100	93	74-100
41	sucrose	4	0	-	97	92-100

* denotes spontaneous oviposition

Table 7. Ovarian development and survival on meridic diets containing varying concentrations of serum albumin.

Diet no.	Per cent dietary protein	No. of replicates	Average % ovarian development	Range	Average % survival, 10 days	Range
5	14 per cent serum albumin	4	0	-	89	70-100
6	24 per cent serum albumin	6	12	0-32	87	82-96
7	39 per cent serum albumin	6	21	8-44	93	79-100
8	47 per cent serum albumin	6	20	8-33	89	71-100
9	56 per cent serum albumin	6	16	0-22	84	63-100
10	61 per cent serum albumin	6	4	0-16	80	49-96
11	66 per cent serum albumin	6	20	0-38	68	34-92
40	control*	18	77	50-100	93	74-100
41	sucrose	4	0	-	97	92-100

* denotes spontaneous oviposition

diets containing 39, 47, and 66 per cent protein. The mortality of flies fed on the 66 per cent protein diet was greater than that on diets of lower protein concentration, so the optimum per cent protein concentration for both ovarian development and survival was considered to be in the range of 40 to 40 per cent. The sudden rise in per cent ovarian development observed in flies fed the 66 per cent protein diet, after the steady drop in development from the peak observed in the 39 per cent protein diet cannot be explained on the basis of availiable evidence. The higher mortality which occurred with the 66 per cent serum albumin diet was probably due to a low concentration of sucrose or other nutrient in the food ingested. Although this diet contained 66 per cent serum albumin by weight, the percentage by volume was much higher, as serum albumin is an extremely light, fluffy material with small particle size and low weight per given volume. Thus, non-protein nutrients might have been dispersed in a less than optimal concentration resulting in a nutritional deficiency.

Holidic Diet. Only one holidic, or completely defined, diet was tested. An amino acid mixture was substituted for protein, and the diet was formulated by method two. Fifty per cent of the amino acid mixture listed on page 11 was utilized. The diet absorbed much water from the humid atmosphere of the rearing room, and became somewhat sticky, although not to the degree observed in the gastric mucin diet. Because some of the flies did become entrapped in the moist diet, wire screen was utilized as a substrate for the flies to rest on while feeding.

At the end of the ten day test period, flies fed on the holidic diet showed 77 per cent survival and 29 per cent ovarian development. A significant difference was observed in the degree of ovarian development obtained with the amino acid diet and that obtained with the other diets giving slight or mod-

erate development. In almost all of the flies fed the amino acid diet in which the ovaries were not completely mature, there was still a uniform amount of ovarian development which approximated the ovaries of the five to seven day old females depicted on figures 8 to 10. On other diets, ovarian development was almost always arrested at the stage depicted by figure 5 (two days old).

Due to the number of flies with nearly matured ovaries, it was decided to evaluate ovarian development at fourteen rather than ten days to ascertain if the percentage of ovarian development was increased. At the end of the fourteen day test period, the flies showed 49 per cent ovarian development, an increase of 20 per cent over that observed at ten days. It was concluded that the amino acid diet is capable of promoting ovarian development at a slower rate than the better meridic diets, but there is a much greater uniformity of ovarian development than observed in the other flies fed on diets giving only slight to moderate development

Large Scale Nutrition Experiments. Five diets were tested on a larger scale to determine their nutritional value for reproduction. The diets tested were amino acids, sodium caseinate, egg albumin, serum albumin-hemoglobin (10 per cent-40 per cent), and gastric mucin-alphacel. A cage containing the control diet was also included. All of these diets were either holidic or meridic, and all had induced spontaneous oviposition in the mason jar tests. Although the gastric mucin diet caused spontaneous oviposition in the mason jar tests, it was not used due to its undesirable physical characteristics.

The tests were conducted in cages rather than jars, with approximately 1300 flies per cage in the first replicate of the experiment and 800 flies in the second replicate. After eclosion, the flies were supplied with water and the test diet. On the tenth day after eclosion, bovine manure was put in

each cage in a 7 ounce food cup for oviposition. The manure was removed from the cages after 24 hours and placed in a pan of fine sand for pupation. The pupae from each cage were counted and weighed, and placed in mason jars, where the adults emerged. The adults were counted and the per cent emergence from the puparia calculated. These results are summarized in Table 8. Each cage could be egged only once, as the flies were observed to imbibe manure juices which would bias any further tests.

It can be seen from Table 8 that the number of pupae, average weight of pupae, and percentage emergence of adults varies widely. This is best explained by examination of the ecology of the larvae. Few eggs were laid by flies supplied with the amino acid, serum albumin-hemoglobin, and gastric mucin-alphacel diets, while the flies fed sodium caseinate, egg albumin, and the control produced many eggs. Thus, in these latter three diets, overcrowding due to a limitation of the availiable food resulted in stunted larvae and subsequently, smaller pupae. This phenominon had previously been observed in Lucilia cuprina (Webber, 1955). It is also conceivable that the small size of the larvae might limit the emergence of the adults. Thus, no conclusions should be drawn with regard to the average weight of the pupae and the per cent emergence of the adults, but it can be definitely stated that all of the diets tested are capable of promoting survival and reproduction for at least one generation. The egg albumin and sodium caseinate diets appear to give excellent results in this respect.

SUMMARY AND CONCLUSIONS

During the previously described experimints a total of 41 diets (including the control diets) were screened to determine their utility in promoting ovarian development in the face fly, Musca autumnalis DeGeer. Survival of

Table 8. Results of large-scale tests of adult face fly diets.

Diet no.	Protein component	Replicate 1			Replicate 2		
		Number of pupae	Average wt. of pupae	% emergence of adults	Number of pupae	Average wt. of pupae	% emergence of adults
1	amino acids	5	27.6 mg	100	--1/	--	--
2	sodium caseinate	297	12.8	69	306	16.9	84
20	egg albumin	446	13.6	85	640	8.1	45
25	serum albumin-hemoglobin 10 per cent-40 per cent	58	24.9	76	--2/	--	--
26	gastric mucin-alphacel	17	25.9	94	--3/	--	--
40	control	421	8.7 mg	77	310	16.1 mg	98

1/ Insufficient diet for second replicate
2/ No data due to high mortality
3/ No second replicate due to poor physical characteristics of the diet

flies fed on these diets was also noted. The most promising of these diets were tested on a large scale to determine their nutritional value for reproduction. The types of diets tested ranged from those whose composition was undefined, to one in which the exact chemical composition was known. The majority of the diets tested were chemically defined except for the protein source, which was only approximately defined (meridic). A gross study of the anatomy and development of the female reproductive system was made as an adjunct to the nutritional studies.

It was found that a diet of sucrose was entirely adequate to support adult life during the ten day test period, but there was no ovarian development. No diets were tested in which protein was the sole food source, but dried blood could not support life for more than four days, and dried milk also resulted in high mortality, although a few flies developed mature ovaries. Thus it appears that the flies require sucrose or another carbohydrate source to support life, while protein is necessary for ovarian development. This is in accord with the work of earlier authors who studied the nutrition of muscoid flies (Glaser, 1923; Hobson, 1938; and Rasso and Fraenkel, 1954). When both sucrose and a suitable protein source were provided, ovarian development and good adult survival were observed. The essentiality of the other materials included in the holidic and meridic diets was not determined, but it can be assumed from the data pertaining to other insects that at least some of these components are essential or beneficial to the face fly.

The face fly appears to require specific proteins in the diet to produce mature ovaries. Many of the protein sources tested induced at least a small amount of ovarian development, but only a few promoted development equal to or greater than the control. There was an interval of 30 per cent between the

best of the mediocre diets, and the poorest of the diets which allowed good ovarian development. Two meridic diets (egg albumin and sodium caseinate) promoted more apparent ovarian development than did the control used, and two others (gastric mucin and gastric mucin-alphacel) also induced good development, but adult survival was lower due to their undesirable physical characteristics.

There were indications that diets containing mixtures of hemoglobin and serum albumin promoted slightly more ovarian development than equivalent amounts of either protein fed separately.

The optimum concentration of the meridic diets appeared to be between 40 and 50 per cent. The physical characteristics of the protein used may influence the optimum protein concentration by affecting dispersal and dilution of other essential ingredients. The optimum concentration also may vary with each protein in relation to its nutritional value for ovarian development and survival.

The holidic diet promoted 29 per cent ovarian development during the ten day test period. It was observed however, that flies fed on this diet exhibited a uniform degree of ovarian development which could not be demonstrated with the meridic diets which induced only a small to moderate amount of ovarian development. When the period between eclosion and dissection was extended to fourteen days, development of mature ovaries increased to 49 per cent, an increase of 20 per cent over the amount of ovarian development observed at ten days. It seems probable that the concentration of one or more of the amino acids contained in the mixture was below the optimum concentration for maximum ovarian development.

Five diets were tested under rearing room conditions in an attempt to determine their utility in promoting oviposition and reproduction. The diets

tested were amino acid (holidic), egg albumin, sodium caseinate, gastric mucin-alphacel, and serum albumin-hemoglobin (10 per cent-40 per cent). All of these diets allowed oviposition and the majority of the eggs produced appeared to be viable. These tests were not as useful as they perhaps could have been, due to a definite limitation of availiable larval food in some diets, which resulted in smaller larvae and pupae, and probably caused a reduction in percentage emergence of adults from the puparia. The egg albumin and sodium caseinate diets appeared to be as good as the control diet, while the others were less useful. The amino acid diet would probably have given better results if the flies were given a longer period of feeding to allow time for the retarded oogenesis.

ACKNOWLEDGMENTS

I wish to thank my major professor, Dr. T. L. Hopkins, for his untiring suggestions and assistance, and for his availiability whenever such suggestions or assistance were needed.

I should also like to extend my appreciation to Dr. R. E. Monroe, now at the Department of Entomology at Michigan State University, for valuable advice during the formative stages of the research, and to Dr. C. W. Pitts for helpful suggestions throughout the course of the work. The efforts of Larry David and Larry Cox in caring for the stock colonies are also appreciated.

I am indebted to Sue Shelton for patience and understanding far beyond the call of duty during the preparation of the thesis.

The funds used for the research were in part from the National Science Foundation Grant GB-684.

LITERATURE CITED

Adler, S., and O. Theodor.
Investigations on Mediterranean kala azar. VII. Further observations on Mediterranean sandflies. Proc. Roy. Soc. (B). 116(801):505-515. Feb., 1935.

Altman, P. L.
Blood and Other Body Fluids. Washington, Federation of American Societies for Experimental Biology. 1961. 540 p.

Ascher, K. R. S., and Z. W. Levinson.
The influence of protein addition to the larval diet on oviposition of the housefly. Riv. di Parassit. 17(4):217-222. Oct., 1956.

Derbeneva-Ukhova, B. B.
On the development of ovaries and on the imaginal nutrition in dung flies (in Russian). Med. Parasit. 11(5):85-97. 1942.
Abstract in Rev. Appl. Entomol. (B). 32(6):118. June, 1944

Dimond, J. B.
The amino acids required for egg production in Aedes aegypti. Canad. Ent. 88(2):57-62. Feb., 1956.

Ershov, V. S., ed.
Parasitology and Parasitic Diseases of Livestock (English Translation). Moscow, State Publishing House for Agricultural Literature. 1956. 466 p.

Evans, A. C.
Some notes on the biology and physiology of the sheep blowfly, Lucilia sericata Meig. Bull Ent. Res. 26(1):115-125. March, 1935.

Fales, J. H., O. R. Bodenstein, and J. C. Keller.
Face fly laboratory rearing. First successful rearing in the laboratory of Musca autumnalis, a serious pest of livestock, is major step forward in developing controls. Soap and Chem. Spec. 37(4):81-83. April, 1961.

Fraenkel, G. S.
The effect of zinc and potassium in the nutrition of Tenebrio molitor, with observations on the expression of a carnitine deficiency. J. Nutr. 65(3):361-395. July, 1958.

Galun, R., and G. S. Fraenkel.
Physiological effects of carbohydrates in the nutrition of a mosquito, Aedes aegypti, and two flies, Sarcophaga bullata and Musca domestica. J. Cell. Comp. Physiol. 50(1):1-23. Aug., 1957.

Glaser, R. W.
The effect of food on longevity and reproduction in flies. J. Expt. Zool. 33(3):383-412. Nov., 1923.

Greenberg, B.
House fly nutrition. I. Quantitative study of the protein and sugar requirements of males and females. J. Cell. Comp. Physiol. 53(2):169-197. April, 1959.

Hammer, O.
Biological and ecological investigations on flies associated with pasturing cattle and their excrement. Vidensk. Medd. Dansk. Naturh. Foren., Kbh. Bind 105:141-393. 1942.

Hawk, P. B., B. C. Oser, and W. H. Summerson.
Practical Physiological Chemistry. New York, Blakiston. 1947. 1439 p.

Hobson, R. P.
Sheep blow-fly investigations. VII. Observations on the development of eggs and oviposition in the sheep blow-fly, Lucilia sericata, Mg. Ann. Appl. Biol. 25(3):573-582. Aug., 1938.

House, H. L., and J. S. Barlow.
Vitamin requirements of the house fly, Musca domestica (L.) (Diptera: Muscidae). Ann. Ent. Soc. Amer. 51(3):299-302. May, 1958.

Lamborn, W. A.
The haematophagous fly, Musca sorbens Weid., in relation to the transmission of leprosy. J. Trop. Med. Hyg. 40(1):37-42. Jan., 1937.

Leas, A. O., J. B. Dimond, and D. M. DeLong.
Some nutritional factors in egg production by Aedes aegypti. Int. Congr. Ent. X (Montreal, 1956). 3:793-796. 1958.

Levinson, Z. H., and E. D. Bergmann.
Vitamin deficiencies in the housefly produced by antivitamins. J. Ins. Physiol. 3(3):293-305. Oct., 1959.

Levinson, Z. E.
Nutritional requirements of insects. Riv. di Parassit. 16(2):113-138. April, 1955.

Monroe, R. E.
Role of cholesterol in house fly reproduction. Nature 184(4697):1513. Nov., 1959.

Monroe, R. E.
Effect of dietary cholesterol on house fly reproduction. Ann. Ent. Soc. Amer. 53(6):821-824. Nov., 1960.

Rasso, S. C., and G. Fraenkel.
The food requirements of the adult female blow-fly, Phormia regina (Meigen), in relation to ovarian development. Ann. Ent. Soc. Amer. 47(4):636-645. Dec., 1954.

Robbins, W. E., and T. J. Shortino.
Effect of cholesterol in the larval diet on ovarian development in the adult house fly. Nature 194(4827):502-503. May, 1961.

Sabrosky, C. W.
Musca autumnalis in the central states. J. Econ. Ent. 52(5):1030 Oct., 1959.

Sang, J. H., and R. C. King.
Nutritional requirements of axenically cultured Drosophila melanogaster adults. J. Expt. Biol. 38(4):793-808. Dec., 1961.

Sang, J. H., and R. C. King.
Nutritional requirements for normal oogenesis in D. melanogaster. Drosophila Inform. Serv. 33:156-158. Nov., 1959.

Tesky, H. J.
A review of the life-history and habits of Musca autumnalis DeGeer (Diptera:Muscidae). Canad. Ent. 92(5):360-367. May, 1960.

Thompson, M.
The housefly (Musca domestica) and the stable fly (Stomoxys calcitrans). Investigations on their biology and control, with a review of other species of flies associated with domestic animals or dwellings. (in Danish). Veretn. Vet.-og Landbohojsk Kbn., no. 176. 1938. 352 p. Cited by Tesky, 1960.

Vockeroth, J. R.
Musca autumnalis Deg. in North America (Diptera: Muscidae). Canad. Ent. 85(11):422-423. Nov., 1953.

Walker, F.
Notes on some insects of Nova Scotia and Canada. Canad. Ent. 3(8):141-144. Oct., 1871.

Wang, C. M.
Laboratory observations on the life history and habits of the face fly, Musca autumnalis (Diptera: Muscidae). Ann. Ent. Soc. Amer. 57(5):563-569. Sept., 1964.

Webber, L. G.
The relationship between larval and adult size of the Australian sheep blow-fly, Lucilia cuprina (Weid.). Aust. J. Zool. 3(3):346-353. Oct., 1955.

Wesson, L. G.
A modification of the Osborne-Mendel salt mixture containing only inorganic constituants. Science 75(1943):339-340. March, 1932.

West, L. S.
The Housefly. Ithaca, Comstock. 1951. 384 p.

NUTRITIONAL EFFECTS UPON OVARIAN DEVELOPMENT AND REPRODUCTION IN THE FACE FLY, *MUSCA AUTUMNALIS* DEGEER (DIPTERA: MUSCIDAE)

by

STEPHEN MICHAEL VALDER

B. S. University of Nebraska, 1963

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1965

A total of 41 diets were tested to determine their utility in promoting ovarian development in the face fly, Musca autumnalis DeGeer. Survival of flies fed on these diets was also noted. The most promising of these diets were tested on a large scale to determine their efficiency for reproduction and for producing viable offspring. Types of diets fed ranged from those whose composition was unknown to one which was chemically defined (holidic). The majority of the diets tested had a chemically defined diet base to which was added the protein source, which was only approximately defined (meridic). A general study of the anatomy of the female reproductive system, and of oogenesis, was made as an adjunct to the nutritional studies.

Sucrose could support life during the ten day test period, but did not promote ovarian development. No diet with protein as the only nutrient was tested, but dried blood could not support life for more than four days, and dried milk also caused extremely high mortality, although a few flies survived long enough to develop mature ovaries. Thus it appears that face flies need sucrose or another carbohydrate source to support life, while protein is necessary for ovarian development. When both sucrose and a suitable protein source were provided, ovarian development and good viability were observed.

The face fly appears to require specific proteins in the diet to produce ovaries. Many of the protein sources tested induced at least a small amount of ovarian development, but only a few promoted over 70 per cent ovarian development comparable to the control laboratory diet of dried blood, dried milk, and sucrose. The whole proteins tested could be divided into three groups: those allowing no ovarian development (certain concentrations of lactalbumin and serum albumin); those allowing slight to moderate ovarian development (certain concentrations of lactalbumin and serum albumin, gelatin,

hemoglobin, vitamin-free casein, and salivary mucin); and those allowing good ovarian development (gastric mucin, sodium caseinate, and egg albumin).

The optimum protein concentration of the meridic diets appears to be between 40 and 50 per cent. The physical characteristics of the protein source may have influenced optimum dispersal of other nutrients in the diets.

There were indications that diets utilizing certain mixtures of serum albumin and hemoglobin as a protein source induced slightly better ovarian development than did either of these two protein sources when fed alone.

The holidic diet formulated with an amino acid mixture promoted only 29 per cent ovarian development during the ten day test period. It was observed however, that flies fed on this diet exhibited a uniform amount of ovarian development which was not demonstrated in the flies being fed meridic diets that allowed slight to moderate ovarian development. When the test period was extended to fourteen days, development of mature ovaries increased to 49 per cent.

Five diets giving good ovarian development were tested on a large scale in an attempt to determine their utility in promoting oviposition and reproduction. All of these diets allowed oviposition and the majority of the eggs produced appeared to be viable. These tests were not as useful as they perhaps could have been due to a wide variation in numbers of eggs produced with subsequent overcrowding of larvae and starving when egg densities were high. The smaller larvae and pupae may have resulted in the decreased emergence from the puparia. Diets containing 50 per cent egg albumin or 50 per cent sodium caseinate appear to be equal to the control diet in promoting development of mature ovaries, formation and oviposition of viable eggs, and excellent adult survival for at least ten days.