INFLUENCE OF LARVAL NUTRITION ON HOUSE-FLY REPRODUCTION

AXENIC CULTURE OF HOUSE-FLY LARVAE AND THE INFLUENCE OF STORED NUTRIENTS ON ADULT DIETARY REQUIREMENTS FOR SURVIVAL AND REPRODUCTION

By

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SCOPE AND CONTENTS:

Larvae of the house fly, Musca domestica L., were reared axenically on semi-synthetic media and their performance both as larvae and as adults was assessed in comparison to that of flies reared on an undefined wheat-bran medium. Three components of a standard sterile casein-base medium, i.e., sodium oleate, RNA and cholesterol, were assessed as to their effects on both the larval and adult stages. All improved larval growth, with cholesterol being essential. It has been substantiated that specific larval nutrient reserves were transferred to the adult. Larval nutrition influenced fecundity through its effect on adult survival and vigour, number of eggs developed, and the adult hormonal system.

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INTRODUCTION

In the course of evolution, some orders of insects, such as Lepidoptera, Diptera and Hymenoptera have developed a life cycle in which the larval stage is morphologically and physiologically very different from the adult stage. This holometabolous type of development has enabled the larva to occupy, in most cases, an entirely different ecological niche than that of the adult, and to eat food different from that eaten by the adult. In contrast, the young of many hemimetabolous insects, as exemplified by the Orthoptera and Hemiptera, eat much the same food and live in the same environment as the adults. Since the adult holometabolous insect develops from the larva via a quiescent pupal stage, it utilizes during postlarval development the food stores built up by the larva. Therefore, conditions which affect the larval growth will doubtless have some affect on the adult (Wigglesworth, 1953).

In recent years, a number of authors have investigated nutritional requirements in holometabolous insects, the Diptera being most intensively studied (Lipke and Fraenkel, 1956; House, 1961, 1962). These studies have shown that the larval forms of the Diptera

have approximately the same requirements in nutrition throughout the order. Requirements for protein or amino acids differ quantitatively, but not qualitatively, while requirements for trace amounts of other substances, such as certain vitamins or fatty acids, may differ to a higher degree, depending on the individual species' ability to synthesize the substances needed in trace amounts.

Adult dietary requirements are quite dissimilar among holometabolous insects, however, even within the Diptera. Generally, the needs for maintenance of body tissues and general activities are quite different from, and less demanding than, those for reproduction. For example, adults of Musca domestica L. will live on a diet of sugar solution alone, but in order to induce oogenesis, protein must be added to the diet (Wigglesworth, 1953). Evidently, factors other than protein can be carried over from the larva to the adult and therefore need not be supplied.

Nevertheless, requirements for reproduction differ markedly within the same family, genus, species or even within one race or strain of the same species. Most mosquitoes require a source of protein, in this case blood, in order to reproduce. On the other hand, a few require only a carbohydrate source of energy and are capable of developing their eggs without dietary protein. This was first shown by Roubaud and Toumanoff (1930) for one of two different strains of the same species of mosquito, Culex pipiens L. This phenomenon is called autogeny.

As House (1958) pointed out, the variability in nutritional requirements between adults of different species could be caused in part by the kind and amount of nutrient substances carried over in body materials from immature stages. Even within a single species, there has been the tacit or expressed assumption on the part of most authors studying adult nutritional requirements that factors of diet influencing the larva may well have an effect on the reproductive ability of the adult. This is shown by their concern in using insects reared as larvae by a standard procedure on an 'optimum' diet, in order to have, when studying the adults, comparable results between experimental diets. Dadd (1961) found, in the course of studying carotene requirements in the Orthoptera, that it was necessary to rear the insect on a sterile diet containing no carotene for one complete generation, before a carotene requirement could be shown for adult reproduction in the next generation. Sang and King (1961) also emphasized that they found it necessary, in studying the adult reproductive requirements of Drosophila, to have the larvae reared on a sterile diet of known composition, in order that differences shown would be due only to adult diets and that differences between experiments would be kept to a minimum. Dimond et al (1956) found that the requirements of adult Aedes aegypti L. included a partial requirement for cystine, differing slightly from that found by Singh and Brown (1957) and Akov (1961) for the same species. This suggests that differences noted in reproductive ability

between closely related species or races of the same species may be due to the amount of the carry-over of food materials from the larval stage. Gordon (1961) points out that it is possible to account for a differing susceptibility to insecticides in insects of the same strain by taking into consideration differing nutrition in the immature stages. Thus, although the importance of larval nutrition in adult requirements is acknowledged, little work in any insect group has been directed specifically to this aspect of nutrition.

Titschack (1926) found that underfeeding the larva of a moth, Tineola, reduced the number of eggs laid by the adult. A similar result was obtained by Alpatov (1932) using the fruit fly, Drosophila. He allowed some of the larvae to feed for a certain length of time, then removed some to an inert agar-water medium, leaving others to complete normal feeding. The total egg production was greatest in the well-fed larvae. Mackerras (1933) noted that small mature blow flies collected in the field with an average weight of 11.4 mg. produced 83 eggs per female while large mature flies reared in the laboratory with an average weight of 42.0 mg. produced 175 eggs per female. She therefore concluded that the amount and kind of food eaten by the larva determines the size of the fly and consequently the number of eggs which the female could produce. Norris (in Wigglesworth, 1953) fed the larvae of two moths, Ephestia and Plodia, on white flour, comparing their fecundity in the adult stage with adults fed as larvae on whole meal. On white

flour, their fecundity was reduced. This was probably due to a lack of one or several vitamins of the B group in the white flour. Since most Lepidoptera require only an energy source in adult life for both maintenance and reproduction (Wigglesworth, 1953), any lack in the larval diet would be expected to have an immediate effect on adult reproduction. The influence of larval nutrition on adult fecundity in mosquitoes was studied by Mathis (1938) who reared two groups of Aedes aegypti comprising several hundred larvae each; the first group was fed a diet of algae, Euglena, dried fish powder, and sheep brain, while the second was reared on an aqueous mixture containing three slices of bread. Those on the diet of bread laid only 226 eggs per female compared to 357 eggs per female for those reared from the rich diet. No record was kept of the difference in weight between the two groups. Thus, these early observations and experiments indicated that larval nutrition influenced adult reproduction, but controlled experiments with defined diets, and sterile rearing techniques were needed to elucidate the actual factors concerned and the role played by each.

At about the same time that these early nutritional experiments were being carried out, medical entomologists became interested in the problem of autogenous development of eggs, first noted by Roubaud and Toumanoff (1930) in Aedes aegypti. Transmission of yellow fever and

malaria by mosquitoes and of onchocerciasis by black flies, requires two successive meals; if races of these insects were capable of developing eggs without or with only one protein (blood) meal, the races which were capable of doing so could be neglected in programs of elimination of the arthropod vectors of these diseases. Physiological studies related to this aspect have been until now the source of most elucidation of the problem of the role of larval nutrient stores in the reproduction of the adult.

Since autogenous adults take no proteinaceous nutrients before developing eggs, Roubaud (1932) suggested that the larva stored up enough nutrients for later ovarian development in the adult. Roubaud and Toumanoff (1930) reported that larvae of the autogenous type contained more reserves than those of the anautogenous type reared under the same conditions. De Boissezon (1933) claimed that Culex pipiens could reproduce without a blood meal if fed iron, but Roubaud (1932) recognized that C. pipiens was actually a complex of several races, one of which was autogenous.

After emergence, the larval fat body and certain of the larval abdominal muscles still often remain within the adult. Roubaud (1932) suggested that it was these organs which provided the necessary nutrients in autogenous forms for yolk deposition. Möllring (1956) stated that anautogenous larvae of Culex had less reserves than autogenous larvae. Hocking (1954) reported that one form of Aedes com-

munis De Geer at Churchill, Manitoba, could develop eggs without a blood meal and that, as eggs developed, the longitudinal flight muscles disappeared. He found, however, by nitrogen analysis of eggs and of muscles, that these muscles contained enough nitrogen for about eighteen eggs, only 1/4 to 1/3 of the average 65 eggs developed. Beckel (1954), making a more careful histological analysis of this same race of mosquito, found that the flight muscles were not autolysed. However, he found that the larval fat body and the larval muscles disappear as eggs are developed, as Roubaud suggested. But in Aedes hexodontus Dyar, an anautogenous species, they also disappear, making no contribution to egg production (Beckel, 1954). Beckel therefore concluded that the physiological mechanism governing the utilization of larval reserves was different in the two groups. Terzian et al (1956) analyzed the tissue nitrogen of sucrose-fed and of blood-fed mosquitoes. They found that the tissue nitrogen remained constant in both groups, indicating that all protein which is fed to adults is used for eggs, while the protein accumulated by the larva is destined for the maintenance of body nitrogen metabolism. An analysis of the food reserves of the anautogenous strains of Culex pipiens showed that the fraction of total lipid and of glycogen was 2/3 and 1/2 respectively, as great as that in the autogenous strain (Twohy and Rozeboom, 1957). Surprisingly, the protein nitrogen was higher in the anautogenous strain than in the autogenous strain, suggesting that it was the lack of lipid

and glycogen rather than of nitrogen which limited development of eggs. Glycogen content was lower in the anautogenous strain than in the autogenous one even after oviposition had taken place, suggesting that only enough was present in the former for adult maintenance. Glycogen in the autogenous strain was utilized before oviposition normally began in the anautogenous strain. A similar situation with reference to lipids was found in the two forms (Twohy and Rozeboom, 1957). Another analysis of two similar forms of C. pipiens, an autogenous strain and an anautogenous one (C. p. fatigans Wied.) was undertaken by Chen (1959), who found that the anautogenous form had 30% more protein per unit volume of haemolymph but less free amino acids than did the autogenous form. The total quantity was the same in the two strains, thereby agreeing with Twohy and Rozeboom (1957) that the protein or its lack was not responsible for the development or lack of development of eggs in anautogenous forms. These two studies tend to support those of earlier workers (Gaschen, 1932; Hecht, 1933; Buck, 1935; Müllring, 1956), who considered that the nutrition of the larva influenced only the number of eggs laid by the adult rather than the initiation of autogeny, which occurred even when autogenous females were reared on a limited amount of food. Thus, the autogenous strain of C. pipiens was able to draw on stores from the larval muscles and fat body, while no matter how rich the larval food, the anautogenous strain could not do so (Roubaud and Mezgar, 1934). Bettini

(in House, 1958) also found that the autogenous strains of C. pipiens differ from the anautogenous strains in the deposition of protein in the fat body and its later mobilization.

Rozeboom and Kitzmiller (1958) have provided a recent review on the taxonomy and speciation of the difficult C. pipiens complex, and have summarized the data obtained by Spielman on the inheritance of autogeny among the different strains of this group. In a series of crossmatings between the autogenous and the anautogenous strains of C. pipiens, Spielman showed the existence of three autogeny factors on two chromosomes, one of which was the sex chromosome. These genes were not inherited as either a simple dominant or a simple recessive; there was a continuous relationship between chromosome dosage and appearance of autogeny.

Clements (1956) working with the same strains as Spielman provided evidence that the genes governing autogeny acted by a hormonal mechanism. Comparison of the fat body of the two strains showed that sufficient reserves were present to develop at least some eggs in the anautogenous forms. In the autogenous strain, Clements (1956) found that decapitation or ligation of the female earlier than seven hours after emergence prevented ovarian development. Gillette (1957) obtained a slight development of eggs in the anautogenous strain by transfusing haemolymph from the autogenous strain to

the anautogenous strain, indicating that a hormonal mechanism was involved.

As early as 1936, Wigglesworth showed that the hormone produced by the corpus allatum was necessary for the formation of yolk in Rhodnius prolixus Stål. The corpus allatum was also the site of production of neotenin, the juvenile hormone responsible for growth and differentiation of the nymph. Later experiments have shown that both effects are produced by the same hormone (Wigglesworth, 1953, 1961). Wigglesworth (1953) showed that in R. prolixus, this hormonal mechanism was triggered by distention of the midgut after a blood meal. Larsen and Bodenstein (1959) confirmed this finding for the mosquito. A cycle of hormonal secretions from the corpus allatum was set off by injection of blood into the midgut. Similar results were obtained by sealing the anus with wax, an operation which resulted also in the distention of the midgut with sugar solution accumulated from feeding. The autogenous form of mosquito did not require such a stimulus, its hormonal secretions occurring automatically a certain number of hours after emergence. These authors concluded that the utilization of nutrient reserves was controlled by this hormonal mechanism.

Scharrer (1958) has provided a recent review on neuroendocrine mechanisms in insects, in which the relationship between the median neurosecretory cells of the brain, the corpus allatum and

ovarian development is outlined. Wigglesworth (1954) points out that the corpus allatum has a general effect on metabolism, controlling the mobilization and synthesis of materials necessary for the growth of the egg. Thomsen and Møller (1959) and Strangeways-Dixon (1961) showed that protein digestion was controlled by hormonal activity. Thus, the early observations that anautogenous strains of mosquitoes had some reserves but evidently did not use them, may now be associated with the control of metabolism exerted by the hormone of the corpus allatum, neotenin, and the control of the production of this hormone by the neurosecretory cells of the brain.

The possibility that autogeny is determined environmentally within one species or one race cannot be disregarded. Weyer (1934) claimed that in a certain strain of C. pipiens, autogeny occurred when the larval diet was rich in protein, but that anautogenous development of eggs was the rule when the larval diet was rich in carbohydrate. However, other anautogenous strains of C. pipiens were not similarly affected. In black flies, one species, Simulium vittatum Zett. varied in the first ovarian cycle from almost complete autogeny in the early spring generation to 1% autogeny in the summer generation (Davies et al, 1962). This could be due either to nutritional differences or to physiological differences arising from lower winter temperatures. In certain Russian simuliids, Rubtzov (1958) postulated facultative autogeny in response to environmental conditions. Scharrer (1958)

points out that external factors of the environment such as nutrition act upon the brain neurosecretory cells, which in turn act upon the corpus allatum which then releases a hormone controlling ovarian development. Rozeboom and Kitzmiller (1958) also suggested the utilization or synthesis by autogenous forms of a specific food factor, which acted on the hormonal system and in this way produced partial or complete development of ovaries. Robbins and Shortino (1962) reported that their strain of Musca domestica showed about 2% autogeny when reared on CSMA medium (see Materials and Methods); this proportion was increased on an enriched larval diet. Addition of protein to the larval diet of the oriental house fly, M. vicina Macq. did not cause the autogenous development of eggs (Ascher and Levinson, 1956) but these authors did not investigate whether other improvements in fecundity, short of actual egg laying, had occurred.

With a view to the clarification of the role of larval nutrition in the reproductive capacity of the adult female insect, the nutritional requirements of the house fly, Musca domestica, were investigated in this study. This species proved to be well adapted to such studies, since it was easily reared, and the nutritional requirements of the females reared on CSMA, under xenic conditions, had already been worked out by Morrison (1963). It was also advantageous that it was a member of the Diptera, the order in which most chemical and nutritional work has been done.

House-fly larvae were first reared under sterile conditions by Glaser (1938). Greenberg (1954) succeeded in rearing them on sterile CSMA which had previously been incubated for 48 hours to allow bacterial growth. The first successful sterile rearing on a semi-synthetic diet (casein base) was accomplished by Brookes and Fraenkel (1958). Levinson and Bergmann (1957), and Chang and Wang (1958) also reported moderate success rearing the oriental house fly, M. vicina, on a semi-synthetic diet. House and Barlow (1958) were successful in rearing M. domestica on a completely synthetic diet containing 19 amino acids, in addition to other components; these flies were capable of reproducing, although they were about half the normal size. Monroe (1962) developed a semi-synthetic diet which he claimed gave pupae of almost the same size as pupae from CSMA, rather than 30-40% lighter which had been found by the previous authors using a semi-synthetic casein-base diet.

House-fly larvae, like all other insects, require the ten essential amino acids first shown by Rose to be necessary for vertebrates (House, 1958). In addition, they require for good growth, a number of non-essential amino acids. The only other essential components of their diet are certain of the B vitamins and cholesterol, a substance which is an essential requirement only for insects (Bergmann, 1962). In addition, RNA and certain fatty acids have a growth

promoting effect. House (1962) has provided a recent review of larval and adult nutrition.

The adults of M. domestica used in this study are anautogenous, when fed as larvae on CSMA. However, requirements for nutrition in the first ovarian cycle proved to be different from those of succeeding cycles (Morrison, 1963). Only amino acids, sucrose and salts were needed in the adult diet for a first cycle of eggs, while vitamins, cholesterol and RNA had to be added to the basic diet if further cycles were to be obtained. This suggested that carry-over from the larva was occurring, causing a sparing action on these substances during the first period of reproductive activity.

The nutritional differences arising in the adult house fly between the first and succeeding cycles of egg production resemble the partial autogeny of certain blood-feeding insects, discussed above. Because of this interesting relationship, a program of research was undertaken to provide a preliminary survey of those substances which might conceivably be stored by the larva and later used by the adult. Since rather small differences were expected, a method had to be established to detect them, in spite of high biological variability. These studies would then form part of the foundation for an eventual linkage of the nutritional requirements of the larva with those of the

adult, which would lead to an understanding of the role of nutrition in the reproduction of holometabolous insects, particularly in those displaying autogeny.

MATERIALS AND METHODS

Stock Culture

The strain of Musca domestica used throughout the work was obtained in 1958 from Dr. H.L. House, Canada Department of Agriculture, Belleville, and maintained for several years (78 generations) in this laboratory using a standard rearing procedure developed by Morrison (1963). Eggs were collected from stock cages, and new stock cultures established about twice a week. Eggs were washed in distilled water, those that floated being presumed inviable and decanted off. A volume of 0.5 ml. of eggs was measured out according to the method of Moreland and McLeod (1956) using the bottom of a 10 ml. graduated pipette of 8 mm. bore closed at one end by bolting silk. This gave 3500 eggs. Plastic containers, 20 cm. in diameter and 18 cm. high, were filled to a depth of 3-5 cm. with the larval medium. Larvae were reared on a standard alfalfa-bran mixture, known as CSMA [1.], to which a small amount of yeast and NaOH were added (Table I). The 0.5 ml. of eggs were washed into this mixture and the containers were kept in an incubator at 25°C ±1°C. More medium was added each day from the second to the fifth day until a depth of about 13 cm. was reached. Pupa-

[1.] Chemical Specialties Manufacturing Association.

tion occurred on the sixth day; emergence took place on the twelfth day. Three days before emergence, the drier middle and top layers of the medium were removed along with most of the pupae and further dried by spreading in shallow flat pans for 24 hours. The pupae were then separated from the medium by blowing the dry chaff from the heavier pupae with unheated air from a hand hair dryer. The pupae were then placed in beakers and adults were allowed to emerge in wooden cages for a short time so that the earliest emerging flies, a high proportion of which were males, could be removed and discarded (Sawicki and Holbrook, 1961). As the peak of emergence approached, the beakers with pupae were placed in stock wooden or acrylic plastic cages (approximately 1 cubic foot in volume) for 12-24 hours until 500-1000 adults had emerged. Adults were kept in a constant temperature room at a temperature of approximately $22^{\circ}C \pm 2^{\circ}C$. For most of the work the relative humidity in this room varied from 70-90%. Fluorescent lights, at the same distance above each cage, were kept in continual operation. The adults were fed 0.1 M sucrose [1] solution ad libidum. The solution was contained in two, four or eight ounce, wide-mouthed sample bottles equipped with absorbent cotton wicks which extended from the bottom of the bottle, through a small hole in the screw cap to approxi-

[1] Certified Reagent, Fisher Scientific Co. Ltd., Toronto.

mately one half inch above the cap. A new bottle of sucrose solution was provided daily. In addition, for the first three days after emergence, the flies were fed powdered skim milk (Carnation R), reconstituted by adding two thirds of a cup of water to one third of a cup of milk powder. Two layers of paper tissue (Cellucotton R) were placed in a stender dish and soaked with the skim milk. From the fourth to the sixth day, females laid first cycle eggs on the milk-soaked pads. These eggs were used both for stock and for experiments.

Design of Experiment

The main purpose of the research was to explore the linkage between larval diets and the substances required by the adult for survival and reproduction. Larvae were reared axenically using a slight modification of the synthetic medium reported by Monroe (1962) as the standard larval medium (Table II). The value of different components in the synthetic medium was tested by deletion or alteration in concentration. Two or three media, one being the standard and the others being a modification of one component of the standard, were used in each experiment. Each medium was assayed by observing larval rearing success, adult survival and reproduction on at least one adult diet. Four adult diets were used in this assay: (i) milk, (ii) complete synthetic diet (Table VI), (iii) incomplete synthetic diet (Table V), (iv) 0.1 M sucrose solution.

Experimental Procedure

Preparation of Larval Medium

The dry components of the media to be used in each experiment (Table II) were weighed out and ground in an electric mortar grinder in 8 gm. lots for three to eight hours.

3.0 gm. of powdered medium were then weighed out into 150 ml. pyrex large-mouthed flasks (Monroe, 1962) chosen because of their ability to withstand repeated autoclaving, their optimum surface-to-volume ratio, their low cost and their availability. Twenty-five milliliters of agar-vitamin solution (see Tables III and IV) were added to each flask. The flasks were plugged with non-absorbent cotton wrapped around a glass rod with flattened ends. In order to strengthen the plug, a piece of gauze, 20 mesh per inch, covered the outside of the plug, with a glass rod inserted through a hole in the gauze to the medium below (Fig. 1).

After autoclaving at 15 lb. pressure and 121°C for 20 mins. the medium was swirled and placed in an ice bath to promote rapid jelling while solid particles remained suspended. When the medium had hardened, it was broken up by moving the rod slightly up and down and from side to side with the plug still in place. Movement of the rod did not introduce contamination from the air since the focal point of movement was in the centre of the plug, making displacement at that

point slight in comparison to displacement at the surface of the medium, and since the cotton of the plug had been wound in such a way as to compensate for these slight movements so that no opening was produced. This mixing process was necessary because the small amount of liquid (less than 1 ml.) introduced along with the eggs would form a thin film over the hard agar surface and prevent the newly hatched larvae from gripping the medium with their mouthparts, thus causing death, apparently from starvation, within 48 hours. The broken, uneven surface of the medium allowed the liquid to disappear into the interstices, leaving the eggs behind in cracks near the surface.

Sterilization and Introduction of Eggs

First cycle eggs were collected within four hours after the onset of oviposition to insure uniformity of hatching and were washed with distilled water several times to remove all traces of milk and to help break up clumps of eggs. Eggs were allowed to rest on a wet Whatman No. 3 filter paper disc in a petri dish for up to 6 hours after oviposition. This was helpful because freshly laid eggs were hydrophobic and tended to float, making sterilization difficult. The eggs were then suspended in 0.1% solution of sodium hypochlorite (Monroe, 1962) and agitated vigorously for several minutes. This procedure broke up most of the egg clumps and the majority of the eggs sank to the bottom of the container. Those that floated were assumed to be inviable and were decanted off. A total of 200 ml. of sterilizing solution was used in 7

rinses of 25-30 ml. each, over a period of 20 minutes. Viability of the eggs was found to be seriously affected by prolonged total immersion in any liquid, viability dropping from 67% after 1 hr. to 37% after 2 hrs. With the 'hypochlorite' method, eggs for the experimental flasks were immersed in the solution for only 20 to 40 mins. and those for the CSMA rearing only 45 to 50 minutes. A suspension of eggs for each experimental flask was drawn up with a sterile 1 ml. pipette. These eggs were then counted and added to the previously prepared medium. By this method, 8-10 flasks could be seeded in 20 mins.; if more than this number were required, the eggs were divided after collection from the stock cages into the requisite number of batches and the whole process was repeated for each batch. The flasks of each diet (series) were arranged for seeding in such a way that each series had an equal number of flasks whose eggs had been in the liquid an equivalent period of time (i.e., 20-25 mins., 25-30 mins., etc.). Standard bacteriological practices were observed throughout the seeding operation. Transfers were usually conducted in a microbiological hood whose interior had previously been sterilized by ultraviolet light. Flasks were then placed in an incubator at 25°C ± 1°C during larval development.

Three viability tests were set up for each batch of eggs used:

1. viability before immersion in liquid, as a test for normal hatching percentage.
2. viability after 40 mins. in hypochlorite solution, as a test for hatching percentage after sterilization.
3. viability after 50 mins. in hypochlorite solution, as a test for larval rearing success after sterilization.

The first two tests for viability were determined by the same method as that used for viability determinations on eggs laid by experimental females (see page 27). The third viability was set up by placing about 300 eggs in a small battery jar approximately 11 cm. in diameter, containing 50 gm. of CSMA medium. After two days another 75 gm. of CSMA medium was added. The number of pupae formed was counted after seven days, and three samples were weighed. An estimate of the hatching percentage could be obtained by comparing the hatch in the untreated eggs with the hatch after immersion in hypochlorite solution.

Testing Sterility of Larval Medium

The media were tested for maintenance of sterility after the larvae were mature, but just prior to their migratory phase in preparation for pupation. Those flasks which were not sterile were discarded. In early work, a technical difficulty was encountered with the thioglycollate medium used in standard sterility testing techniques. There-

fore the presence of bacteria was tested by direct smears and by using nutrient agar plates. An acidified agar plate was used to detect molds. If bacterial contamination was present, it was always visible before the time for testing arrived, as a dark greyish area in the yellow medium; the sterility test merely confirmed visual evidence of contamination. In later work, thioglycollate broth was inoculated with a suspension of the medium, incubated for 48 hrs. and tested by direct microscopic examination, staining with Gram stain. The presence of mold was detected by the streak plate method using Bacto Potato Dextrose Agar medium [1].

Pupation

Larvae ceased feeding 24 hrs. before pupation and migrated to a drier environment. When movement of larvae up the sides of the flasks began, each flask was placed in a 400 ml. beaker. The area in the beaker surrounding the flask was then filled to a depth of 1/2" with small pieces of alcohol-extracted filter paper. Some of the larvae pupated on the medium, while the rest migrated out of the flask into the beaker and pupated among the pieces of paper. Two days after pupation began (one day after most of the larvae had pupated), the pupae (in their puparia) were removed from each flask, counted, and weighed in lots of ten. They were then stored in the

[1] Difco Laboratories Inc., Detroit, Mich.

incubator in gauze-covered stender dishes or beakers until the adults began to emerge.

Experimental Procedure for Adults

The adults were allowed to emerge in beakers overnight and were then released, one set at a time, into a small wooden cage where they were caught individually in vials (60 x 15 mm.). Records of percentage emergence and sex ratio were thus obtained for each flask originally set up. The males were usually discarded and the females were released into experimental chambers in the constant temperature room, following the technique of Morrison (1963). Each experimental chamber consisted of a glass cylinder. Small cylinders, each containing only one female, were used in most experiments to give data on egg production per experimental female, number of females laying, number of ovarian cycles per female, average length of time required to develop each cycle, viability of each cycle and estimates of variance between individuals. Large cylinders, with 10 to 18 females in each, were used to obtain supplementary data on viability and survival, and to feed flies on sucrose solution, to be used later for dissection. Males were provided from stock cultures, in a ratio of one male to every three females in the large cylinders, and three males in each small cylinder.

The small glass cylinders, 52 mm. in diameter and 64 mm. in height, were mounted in three rows of four each on sheets of acrylic plastic (1 ft. x 1 ft. x 3/8 in.). In the centre of the floor of each chamber, a hole (26 mm. in diameter) was drilled. The surface of the plastic around each hole was recessed about 3 mm. to accommodate the cylinder. The floor of each chamber was lined with a filter paper disc, 7 cm. in diameter, with a hole in the centre to correspond to the hole in the plastic base. The top of the chamber was closed by nylon netting (approximately 20 mesh/inch) held in place with a rubber band. Two milliliters of the diet to be fed was placed in a shell vial, 12 mm. x 35 mm., containing a short length of cotton wick, which protruded a few mm. above the lip of the vial. The wicks were cut from a piece of absorbent surgical cotton, that had been extracted for 24 hours in alcohol and 24 hours in ether. Each vial of diet was then fitted into the bottom of a No. 6 rubber stopper that had been specially bored to accommodate it. The stopper, holding the vial, was then inserted from below, into the hole in the plastic base - the diet was now accessible to the flies within the chamber. A maximum of 96 small chambers were used in an experiment.

The large glass cylinders, 15.5 cm. in diameter and 18 cm. high, were accommodated in pairs on aluminum trays (21 cm. x 46 cm.).

Each tray was covered with chromatography paper, and both tray and paper were provided with two holes (one per cylinder) for the insertion of feeding vials as described above for the small cylinders. The top of each chamber was closed with nylon netting, held in place with a spring-released embroidery ring. Feeding was accomplished in the same way as described for the small cylinders. The trays for both large and small chambers were supported, in the constant temperature room, by metal frame shelves, permitting access to the underside of the trays for the removal and insertion of feeding vials. The fluorescent lights, at a standard distance above the cylinders, were in operation continually.

The stoppers, and vials with diet were renewed each day. Vials were washed in Alconox[1]. and in alcoholic NaOH before being rinsed three to five times each in tap water, deionized water and distilled water. Stoppers were scrubbed in Alconox solution and rinsed overnight in a pipette washer before being rinsed in deionized and in distilled water. Between experiments, the experimental apparatus was dismantled and washed thoroughly.

The two synthetic diets (Tables V, VI) were prepared before the experiment and 200 ml. aliquots were stored in polyethylene bottles in a deep freezer at minus 25°C. These diets were kept frozen until just before use, and were refrozen between daily

1. Canadian Laboratory Supplies Ltd., Toronto

feedings, to prevent bacterial growth. Powdered milk was reconstituted daily by adding 6 gm. to 43 ml. glass distilled water. Sucrose solution, 0.1 M, was made up about once a week and stored in the refrigerator.

The female flies laid their eggs on or near the cotton wick in the feeding vial. After each daily feeding, the vials and wicks were examined under a stereoscopic microscope for the presence of eggs. All eggs were transferred by means of a wash bottle and forceps to a 7 cm. filter paper disc (Carl Schleicher and Schuell Co., No. 8, 'ruled'). This filter disc was placed on a larger 9 cm. filter disc (Whatman, No. 3) in a plastic petri dish, moistened to the desired degree with carbon-filtered tap water, and kept at room temperature (approximately 23°C) for 48 hours. Hatching occurred during this time; the egg shells remained in situ on the filter paper, and most of the hatched larvae remained in the dish. Counting was usually not convenient until after the experiment, and thus each petri dish with egg shells and hatched larvae was stored in a deep freezer until after the experiment. The viability was assessed by counting the egg shells and relating them to the sum of shells and unhatched eggs. This count was supplemented, in later experiments, by a count of larvae, because it was often difficult to distinguish dead eggs with poorly formed shells from the empty shells. This count was always somewhat lower because a few larvae were able to escape from the dish.

RESULTS

In this investigation, it was necessary to develop first an axenic culture procedure and then a semi-synthetic larval medium which fostered a larval development comparable with that found using CSMA medium. The performance of this axenic culture, as indicated by criteria of growth, survival and reproduction, are reported in part I of the Results. In the following parts the effect of modifications in the larval medium on the various criteria is explored. Part II deals with the effect of modifications in the quantity of one of the components, the sodium salt of a mono-unsaturated fatty acid, oleic acid. In part III, the effect of RNA on larvae and adults is considered, while part IV is concerned with the role of cholesterol in larval and adult nutrition.

I. Comparison of Flies Reared on CSMA and on a standard Sterile Semi-synthetic Medium

In all previous work on adult dietary requirements in this and in other laboratories, CSMA was used as the larval rearing medium (Morrison, 1963). Therefore, it was desirable to compare both the larval and adult fly when larvae were reared on the CSMA medium, and on the sterile medium, which could then serve as a standard in future experiments.

Comparison of the Immature Stages

Larvae reared on standard sterile medium were used as controls in almost all experiments. In addition, in most experiments, eggs sterilized in sodium hypochlorite solution were set up on the CSMA medium. The results from these two larval media are compared. The most notable difference between the two media was the reduced weight attained by pupae from the sterile medium (Table VII). These pupae were only 75% as heavy as pupae from the CSMA medium. Flies reared on the standard sterile medium were also slower in developing, taking 13 1/2 days to reach the time of emergence rather than 11 days required by those from CSMA medium. This difference in time of development was more marked in the larval stage than in the pupal stage. There was little difference between the two media in larval survival (larvae produced per unit number of eggs seeded) (Table VII). The percentage of pupae from which adults emerged and the sex ratio of the flies were also not significantly different, being approximately 87% for the former and 1:1 for the latter.

Comparison of Adult Survival and Reproduction

Thirty females from each larval medium were divided into three groups of ten, and each group of adults was fed a different diet; (1) milk, (2) an incomplete synthetic diet of amino acids, salts and sucrose and (3) a complete diet of amino acids, salts, sucrose, vitamins, RNA and cholesterol (see Materials and Methods). Females

were maintained individually in the small cylinders. The influence of the sterile larval medium on adult survival and reproduction was assessed in comparison to that of the CSMA medium.

1. Survival

In general, adults from larvae reared on the standard sterile medium survived an average of 10.7 days, which was 2.2 days, or 20% longer than for flies from larvae reared on the CSMA medium (Table VIII). The difference in survival was greatest when the adults were fed milk and the incomplete synthetic diet.

2. Fecundity

On each adult diet, females from larvae reared on the standard sterile medium laid a larger total of eggs, and 29% more eggs per female, than those from larvae reared on the CSMA medium (Table IX). The difference was most marked on the incomplete synthetic diet (40%) and least marked on milk (15%). Since this difference might be caused by any of several factors, such as differences in pupal weight or in survival, it was desirable to express the number of eggs as an average per female alive at the time the eggs were collected - henceforth called the "average number per living female". When the average number of eggs laid per living female per cycle was determined (Table X), it was apparent that the increase in survival, reported above, of flies from larvae reared on the standard sterile medium was partly responsible for the greater total number of eggs laid. Although the milk diet appeared

to induce longer survival among flies from larvae reared on the CSMA medium, so few individuals are involved that the effect of adult diet is not clear.

Dissection of most flies after they died revealed that mature eggs were often retained in the ovaries. These unlaid eggs were added to those that had been already laid, giving the average number of eggs developed per living female per cycle (Table XI). In nearly every case, the average number of eggs developed is higher than the average number of eggs laid. It is now evident that on all three adult diets, the flies from larvae reared on the CSMA medium developed more eggs in the first two cycles (although not laying more) than those from larvae on the standard sterile medium, and on the milk diet more eggs in the third and fourth cycles as well. On the synthetic adult diets, however, reduced survival counterbalanced this increase.

The greater number of eggs developed by females from the CSMA medium, as compared to females from the standard sterile medium, can be attributed in part to the greater weight of the pupae (1.36 times). Pupal weight is one of the main criteria indicating the number of ovarioles which will develop eggs.

The surprising observation that axenically reared flies fed the incomplete synthetic diet developed and laid four cycles of eggs contradicted earlier work in this laboratory. Previously when adults from larvae reared on the CSMA medium were fed an incomplete diet,

only a first cycle of eggs developed, with a subsequent cycle developing in very few cases; moreover when a second cycle was laid, the viability was very low (Morrison, 1963).

Since the number of flies in this experiment was too small to permit a statistical interpretation of these results, a second experiment was set up using 24 flies of each type individually fed an incomplete diet. In this experiment, it was confirmed that second cycle eggs were laid by almost all females from axenically reared larvae, third cycle eggs by over half the flies, and fourth cycle eggs by a few flies. As in the first experiment, the average number of eggs developed per female for each cycle was calculated by adding the number of unlaid mature eggs, as found by dissection, to the number of eggs laid (Table XII).

For the first cycle, the average number of eggs developed was proportional to the average weight of the pupae. The ratio of eggs developed was

$$\frac{E_{D\text{-}CSMA}}{E_{D\text{-}Sterile}} = \frac{161.6}{120.4} = 1.34.$$

As seen above under "The Comparison of Immature Stages", the pupal weight ratio equalled 1.36. Thus both figures were almost identical.

In the second cycle, three of ten CSMA-reared females developed no eggs although they lived sufficiently long to do so. Discounting these flies, the average number of eggs developed in the second

cycle was still very high, although 11 eggs per female lower than in the first cycle. Six flies reared as larvae on the CSMA medium lived long enough to, but did not, develop third cycle eggs. All had retained a part of their second cycle eggs which appeared to inhibit development of the third cycle.

All seventeen living flies from larvae reared on the standard sterile medium developed a third cycle, and six out of eight females, still living, developed a fourth cycle.

Thus, in general, the same response to an incomplete synthetic adult diet was obtained in both experiments (Figs. 2 and 3), with the number of eggs developed per female dropping off sharply to zero after the second cycle in the case of flies reared as larvae on the CSMA medium, and with only a slow drop from the first cycle to the fourth cycle in the case of flies from larvae reared on the standard sterile medium.

3. Viability of eggs

It was possible that the increase in the number of eggs laid represented no real increase in the reproductive potential of the adult. Therefore, viability records for each group of eggs were kept. In addition to establishing viabilities of all eggs from the individual cylinders, three large cylinders were used each containing 20 females from larvae reared on the CSMA medium. In one of the large cylinders,

flies were from the same population as in the second individual experiment, but the other two contained different sets of flies. In addition to these three cylinders, there were two large cylinders each containing 19 flies from larvae reared on the standard sterile medium. The results were somewhat variable, even with strictly comparable groups (Table XIII). Viabilities for the eggs of those females from larvae reared on the CSMA medium were unusually low even for the first cycle, varying between 21% and 47%, with an average of 28% (of 7034 eggs). The viability of second cycle eggs was only 1/4 as high, varying between zero and 16.2% with an average of 7% (of 2068 eggs). On the other hand, viabilities for eggs of flies reared as larvae on the sterile medium were moderately high in the first cycle (65%) and in the second cycle dropped somewhat less sharply to half the early value, but in the third cycle they were 1/4 the first-cycle value. By the fourth cycle, the egg viability had fallen to zero. Thus, the egg viability data also tended to support the conclusion that with at least the second cycle, in addition to the first cycle of eggs, a real increase in reproductive potential was achieved in females from larvae reared on the standard sterile diet.

II. Effect of Dietary Oleate on Immature Stages and on Adults

Comparison of Flies from Larvae Reared on CSMA and on a Sterile Medium Lacking Oleate

In the previous experiment no significant difference in fecundity, other than that caused by weight, was observed between flies reared as larvae on CSMA and the standard sterile medium, whether the adults were fed milk or a complete synthetic diet. It was of interest to observe whether any difference would arise between flies from larvae reared on CSMA or on the standard sterile medium lacking sodium oleate. Milk was chosen as the adult test diet because flies from different larval media had shown little difference in fecundity on this diet while a difference had been found on the incomplete diet. Females were reared individually for 27 days in small cylinders.

1. The Effect on the Immature Stages

The larval response (Table XIV) to the two media was the same as that observed in the previous experiments; the weight of the pupae from the CSMA medium was 1.56 times as great as that of pupae from the sterile medium lacking oleate; the total time required for the flies to develop to adulthood was greatly increased for the flies from larvae reared axenically ($\frac{14.5}{10.3}$ = 1.41 times as long).

Both these effects were more pronounced than in the first set of experiments (cf. Table VII).

2. The Effect on Adult Survival and Fecundity

Adults from larvae reared on the sterile medium lacking oleate did not live as long as those from larvae reared on the CSMA medium; the 50% survival level was reached in 15.6 and 20.7 days respectively at 19°C. This was in contrast to the previous experiment (in part I) where adults from larvae reared on the sterile medium supplemented with 2% oleate was beneficial to survival of the adult female.

The flies from larvae reared on the CSMA medium laid a significantly greater number of eggs than those from larvae reared on the sterile medium lacking oleate (Table XV). A consistently lower value for the average number of eggs laid in each of the four cycles was obtained with flies from larvae reared on the sterile medium (Fig. 4). The mean values were not significantly different for each cycle individually ($P > 0.05$) but the cumulative effect, measured as the average total number of eggs per female, was significant ($P < 0.025$). Since no dissection data was available, only the number of eggs laid could be compared to the previous experiment.

This comparison of the average number of eggs laid per female per cycle obtained on the milk diet in the first experiment and on the same milk diet in this experiment indicates that the standard sterile larval medium with oleate increases fecundity over that obtained with an oleate deficient larval medium.(Table XVI). The average total number of eggs per female was not significantly different ($P = 0.40$) between flies from larvae reared on the CSMA medium and flies from larvae reared on the standard sterile medium (part I), while there is a significant difference ($P<0.025$) between flies from CSMA-reared larvae and those from larvae reared on a sterile medium lacking oleate.

Comparison of Flies from Larvae Reared on Sterile Diets Containing Different Amounts of Oleate

A difference had been found previously in the response between flies from larvae reared on the standard sterile medium (which contained 2% oleate) and on the same medium lacking oleate, when each was measured against CSMA-reared flies. An experiment was designed to test the previous results directly, by setting up three sets of sterile larval media with 0%, 0.5% and 2% oleate, the set containing the 2% oleate being the standard medium.

1. The Effect on the Immature Stages

Rearing success, measured as the percentage of mature larvae produced from the eggs seeded, dropped slightly as the per-

centage of oleate in the medium decreased (Table XVII), but this difference was not significant at the 95% level ($P \approx 0.20$).

The pupae reared from the sterile medium containing 2% oleate were significantly heavier than pupae from either the 0.5% or the 0% oleate media ($P < 0.005$). Although the pupae from the 0% oleate medium were heavier than the pupae from the 0.5% oleate medium, the difference in weight was not significant ($P = 0.11$). The difference between the pupae from the 2% oleate and from the 0% oleate media was slight compared to the difference obtained in the first experiment between pupae from either of these media and the pupae from the CSMA medium. Nonetheless, it confirmed earlier observations that the ratio of the average pupal weight from the CSMA medium to the average pupal weight from the standard sterile medium containing 2% oleate was greater (1 : 0.74) than the ratio of the average pupal weight from the CSMA medium to the average pupal weight from the sterile medium lacking oleate (1:0.64). The predicted value for the weight ratio was thus 1.15 (0.85-1.29) and the observed value from Table XVII was 1.08 (0.99-1.25).

No significant difference was noted in the percentage of adults emerging from the pupae in the three media.

Flies from larvae reared on the sterile medium lacking oleate required an average of one day longer to reach adult emergence than did flies reared on the standard sterile medium. Flies

from a 0.5% oleate medium were as slow to emerge as flies from the medium lacking oleate.

2. The Effect on Adult Survival and Reproduction

A previous experiment, using milk as the adult diet, and comparing females from larvae reared on a sterile medium lacking oleate had some reduction in fecundity. Such an impairment was not evident in adults reared from larvae on the standard sterile medium containing oleate, in comparison to adults reared from the CSMA medium. Thus, there was indirect evidence that the presence of dietary oleate in the larval stage enhanced fecundity in adulthood. This suggested that oleate in the larval medium might have been responsible for the increase in the number of cycles laid on an adult diet of amino acid, by females from larvae reared on a sterile medium containing oleate, in comparison to the number of cycles laid by females from CSMA-reared larvae.

Twenty-four females from larvae on each of the three concentrations of oleate were maintained individually on the incomplete diet for approximately 17 days, or 400 day-degrees, in order to test the role of oleate in the production of third and fourth cycles.

Only a small difference was noted in the survival of adults from the three different larval media. Adults from larvae reared on the standard sterile medium lived an average of one day longer

than those from the medium lacking oleate, and two days longer than those from the 0.5% oleate medium (Table XVIII). The survival curves of females from the three larval media were alike within biological variation (Fig. 5).

Females from all three larval media were capable of developing and laying four cycles of eggs, although the percentage of living females laying eggs dropped sharply in all three cases after the second cycle.(Fig. 6). This indicated that oleate was not the factor responsible for the presence or absence of the second, third and fourth cycles noted in the previous experiment on an incomplete synthetic adult diet. However, 2% oleate in the larval medium resulted in 14% more females laying second, third, and fourth cycles.

Females from a larval medium containing 2% oleate laid more eggs per living female in each cycle than females from either the 0.5% or the 0% oleate medium, but there was no significant difference between the fecundity of females from 0.5% and 0% media (Table XIX, Fig. 7). The increased number of eggs laid by each female from the 2% oleate medium was due partly to the effect of oleate in increasing the percentage of females which laid (Fig. 6), and partly to its effect in increasing the actual number of eggs per laying female (Table XX, Fig. 8). An approximation of the actual number of eggs developed in each cycle was made by studying the number of eggs per female lay-

ing a full cycle of eggs and, using dissection data, excluding the few flies in each group, which had an aberrant laying pattern. Again, the females from the 2.0% oleate medium developed, in all cycles, a significantly higher number of eggs than the other two groups ($P < 0.05$).

An analysis of this data showed that the number of eggs developed and laid in the first cycle was directly proportional to the weight of the pupa from which the female had come (Table XXI). This proportionality was maintained in succeeding cycles, although the number of eggs developed in succeeding cycles dropped in all three cases. An analysis of the ratios $\frac{E_{D\text{-}2.0\%\ oleate}}{E_{D\text{-}0.5\%\ oleate}}$ and $\frac{E_{D\text{-}2.0\%\ oleate}}{E_{D\text{-}0.0\%\ oleate}}$ (where E is the number of eggs developed (D) per female per cycle) shows that the number of eggs developed per female per cycle is maintained as well by females from larval media with 0.5% or 0% oleate as by females from the medium containing 2% oleate, since these ratios never, even in the fourth cycle, exceed the extreme limits of the weight ratio.

Viability records were kept for all eggs laid by females in individual cylinders. In addition, 15 large cylinders, each containing 18 females and six males, were set up, using the flies from the same three larval media as those used in the individual cylinders. Flies in six cylinders, two from each group, were fed the incomplete

adult diet, while flies in the remaining nine cylinders, three cylinders from each group, were fed the complete synthetic adult diet. Viability records were kept for eggs laid in these cylinders. On both adult diets the viability of the eggs varied greatly from lot to lot. However, the larval medium showed no effect on viability (Table XXII).

III. Effect of Dietary Nucleic Acid on Immature Stages and on Adults

Part II demonstrated that oleate was not the factor responsible for the greater number of cycles developed by females from larvae reared on a sterile medium as compared to the number laid by females from larvae reared on the CSMA medium when fed an incomplete synthetic adult diet. It was possible that the presence of RNA in the larval medium was responsible for this observation. The effect on fecundity of RNA in the larval medium was tested by an experiment in which larvae were reared in flasks on sterile media, each group of six flasks having a different concentration of RNA (by dry weight), i.e., 0%, 3% (standard) and 6%.

Effect on the Immature Stages

An analysis of the data showed that as in previous experiments, a lack of a non-essential, but advantageous, component of the larval medium had no effect on the percentage of eggs resulting in mature larvae, or the percentage of adults emerging from the pupae (Table XXIII).

This lack during larval growth did affect the average weight of the pupae from each of the larval media. Pupae from the standard medium containing 3% RNA were 2 mg. heavier than pupae from the medium lacking RNA ($P < 0.005$). Pupae from larvae reared on the medium containing 6% RNA, double the concentration of the standard medium, were the same weight as the standards.

The time required for completion of pre-imaginal stages was also increased by one day for flies from larvae reared on the medium lacking RNA. Thirteen days were required to reach the adult stage for larvae reared on the 3% and 6% RNA media, while fourteen days were needed for flies on the larval medium lacking RNA.

Effect on Adult Survival and Reproduction

Since no differences in the immature stages had been found between groups reared on larval media containing 6% and 3% (standard) RNA, only adults from the latter medium were considered further. Females were maintained in individual cylinders, each with three males from stock CSMA culture. Three diets fed to the adults were used to compare the response of females from larvae reared on media containing 0% and 3% (standard) RNA. Twelve adults from each larval medium were fed on milk, eighteen others from each larval medium were given the complete synthetic diet and eighteen from each larval medium were fed the incomplete synthetic diet, making a total of 96 cylinders.

1. Survival

On milk and on the complete diet, there was no difference in the survival of females from larvae reared on media with RNA or without it (Table XXIV). However, a difference appeared when the two groups of females were fed the incomplete synthetic diet; in this case the adults reared from larvae on the standard sterile medium (3% RNA) lived 1.5 days longer than those from larvae reared on the medium lacking RNA.

2. Time Required by Females to Develop and Lay each Cycle

Females from the larval medium lacking RNA lagged slightly behind females from a 3% RNA medium in the time required to develop the first cycle of eggs when the complete synthetic adult diet was fed ($P < 0.005$). The development time, in succeeding cycles, is the same for the two groups, as is shown graphically by the similar slopes of the lines in Fig. 9. Females fed milk or the incomplete diet required the same time to develop the first cycle of eggs no matter from which group of larvae they came. However, on the incomplete diet, the time required to develop the first cycle of eggs was retarded in both groups.

3. Fecundity

On the incomplete diet, both groups of females laid only two cycles of eggs. Thus, the question of whether RNA was the factor

responsible for an increase in the number of ovarian cycles in females fed an incomplete diet remained unanswered. The actual survival (expressed as 50% survival) and the time required to develop and lay the various cycles of eggs must always be considered together because the interaction of the two affects to some degree the accuracy of the figures for the average number of eggs laid per female in the third and fourth cycles. This is because the 50% survival time will cut the curve for the development time at an earlier point if the curve is displaced to the left (i.e., if development of each cycle is slower), and fewer flies will be alive to lay succeeding cycles. This was particularly evident when the incomplete diet was fed to the adults; in this case both groups of flies were late in laying the first cycle of eggs. Few flies from either larval medium were alive to lay a second cycle and none to develop a third cycle.

On all three adult diets, the average number of eggs laid per living female was considerably higher for females from larvae reared on the 3% RNA medium than for those reared from larvae on the medium lacking RNA (Fig. 11). Over the four ovarian cycles, the former laid twice as many eggs on every adult diet as the latter (Table XXV).

However, the presence of RNA in the larval medium also affected the percentage of females which laid eggs (Fig. 10). By using the number of eggs per laying female, differences caused by

variation in the percentage of females which oviposited were eliminated. Such an analysis showed that the variation in the number of ovipositing females accounted for an average of 26.4% of the above-mentioned increase in the number of eggs per living female (Table XXVII). For all three adult diets, females from larvae reared on the sterile medium containing RNA laid over twice as many eggs per experimental female as females from larvae reared on the medium lacking RNA (Tables XXV and XXVII). The number of eggs per laying female, on the other hand, is only one and one half times as great for females from the larval medium containing RNA, indicating that approximately three quarters of the increase in fecundity per living female from the standard larval medium (containing 3% RNA) is due to the actual increase in the number of eggs laid, while the other quarter results from an increase in the percentage of females ovipositing.

The average number of eggs developed by each female is an approximation of the reproductive potential of the two different groups of flies (using both laying and dissection data), and it shows that the adults from the larval group reared on the 3% RNA medium develop more eggs on all adult diets, in all cycles, than those from larvae on a medium lacking RNA (Table XXVI). The number of eggs developed in the first cycle both by females fed milk and those fed the

complete synthetic diet were proportional to the weight of the pupae. Pupae from larvae reared on the medium containing 3% RNA were 1.06 to 1.18 (average 1.13) times as heavy as pupae from the medium lacking RNA, and eggs developed per female from the larval medium with 3% RNA were 1.14 and 1.12 times as many when adults were fed milk and a complete synthetic diet respectively. This proportionality was not apparent in the females maintained on the incomplete diet where the ratio was 1.35 times as many for those from RNA-fed larvae. This is well outside the extreme limits of the weight ratio.

The number of eggs developed per female remained proportional to the weight, at least in the second cycle (Fig. 12). On the other hand, in the third cycle, the fecundity of flies from the larval medium containing RNA was slightly greater than that of flies which received no RNA as larvae. The data for the fourth cycle are unreliable for both the milk and the complete synthetic adult diets because of the few flies still alive.

Viability of eggs in all cycles from flies reared on both larval media was similar within each diet fed the female.

IV. Effect of Dietary Cholesterol on Immature Stages and on Adults

During the course of this work, a report appeared stating that high levels of cholesterol in the larval medium allowed adults fed only on sucrose solution to develop eggs and lay them (Robbins and

Shortino, 1962). In part I above, evidence was obtained that adults from larvae reared on the sterile medium, which contained 0.25% cholesterol, developed more ovarian cycles than adults from CSMA-reared larvae, when both groups were fed an incomplete synthetic diet. In parts II and III, two other larval dietary factors, oleate and RNA, were found to be unsuccessful in eliciting this development. Two experiments were carried out to verify the response to cholesterol reported by Robbins and Shortino.

In the first experiment of this series with cholesterol, larvae were reared on the CSMA medium and on two sterile media, one containing no cholesterol and the other containing cholesterol in a concentration of 0.25% of the dry constituents (which was, in fact, the same as the standard sterile medium). In the second experiment, CSMA and a sterile medium containing 0.50% cholesterol, twice the normal concentration, were used as the larval rearing media.

Females in both experiments were kept in large cylinders (each containing 12-18 females) and were fed only a 0.1% solution of sucrose. Five females were selected at random and removed for dissection from each of two cylinders on the eighth and thirteenth days in the first experiment, while in the second experiment, two females from each of five cylinders were removed for dissection on the sixth, eighth, tenth, twelfth and fourteenth days.

Effect on Immature Stages

Because the results from CSMA-reared larvae were similar in both experiments, both sets of data have been grouped together (Table XXVIII). Cholesterol is an obligatory requirement for larval growth, as is shown by the larval survival approaching zero for the group reared on the medium lacking cholesterol. Most larvae on this medium died between 7 and 20 days after hatching, not growing beyond the second instar. Only two extremely small pupae were obtained after 20 days; each had an incompletely hardened puparium with an abnormal shape and colour. No adults were produced.

Both sterile media containing cholesterol produced pupae of approximately the same weight, but about 1.6 mg. less than the average weight (i.e., 18.6 mg.) obtained in other experiments with sterile media (cf. Table VII). However, as the CSMA-reared flies were also slightly lighter, it was felt that the difference was the result of the population rather than the medium. The usual lag in growth rate between larvae reared on the CSMA medium and on any sterile medium was observed, with CSMA-reared flies requiring only 12 days, or 20% less time, to reach adult emergence than those reared on the standard sterile medium with 0.25% cholesterol.

Doubling the cholesterol did not improve the medium for the immature stages. Indeed, indications of adverse effects were in evidence (Table XXVIII). Pupae from one flask which contained 0.50%

cholesterol had the lowest adult emergence of any experiment, and the average, 75.0%, was also somewhat lower than usual (cf. Table VII). The growth rate for flies reared on the medium containing 0.50% cholesterol, i.e., the double concentration, was also slower with an additional day required to reach the time of emergence, lengthening the total pre-imaginal period to 16 days.

Effects on the Adults

Of the flies from the CSMA larval medium fed only a sugar solution as adults, 20 females in the first experiment and 42 females in the second were removed for dissection beginning on the sixth and ending on the fourteenth day after emergence. None had oocytes developed beyond the initial stage of yolk deposition. The greatest length attained by any oocyte in both experiments was 0.34 mm. Previous experience in this laboratory with flies from larvae reared on the CSMA medium, and fed sucrose solution as adults strengthened the conclusion that none of these flies were capable of developing eggs on an adult diet containing only carbohydrate and no protein.

In the first experiment, sugar-fed females from larvae reared on the the standard sterile medium containing 0.25% cholesterol showed only the initial stages of yolk deposition after eight days and were similar to sugar-fed adults from larvae reared on the CSMA medium. However, after 13 days, one fly from a total of 20 dissected up to that time showed almost complete yolk deposition, and the 36 eggs averaged

0.65 x 0.20 mm. in size.

In the second experiment, using sugar-fed females from larvae reared on the sterile medium containing 0.50% cholesterol, oocyte development, which had previously been found to be related to oocyte length, was measured every second day from the sixth to fourteenth day. By the end of the fourteenth day, 8.6% or five flies out of a total of 58 dissected, had oocytes with at least half of the total yolk deposit required for full development (Table XXIX). Development may begin in some cases before the eighth day, as is shown by the presence in one fly of eggs almost fully developed on this day. A second fly contained eggs about one half developed by the eighth day.

Even after 14 days, however, no fly developed eggs to the stage where they were ready to be laid. Although development proceeded almost to completion in two cases, a small cap of nurse cells, 0.08-0.12 mm. long, remained at the top of eggs that were 0.80 and 0.83 mm. long, indicating that further growth was necessary before oviposition could take place.

Of the six flies in both experiments with some oocyte development, three, one from a larva reared on the standard sterile medium containing 0.25% cholesterol and two of five from larvae reared on the medium containing 0.50% cholesterol, had eggs sufficiently developed,

with vitelline membranes and partly formed chorions, that they could be counted easily. The three had approximately the same number of developed oocytes, 36, 37 and 45 respectively. The rest of the ovarioles in both ovaries of each fly were undeveloped, and had remained at the stage of the initial stage of yolk deposition, appearing similar to undeveloped oocytes in flies reared from the CSMA medium. The number of oocytes developed was approximately 1/3 the number ordinarily developed by females from larvae reared on the standard sterile medium when fed an incomplete synthetic adult diet (cf. Table XII).

In the second experiment, in order to obtain viability data, three additional large cylinders containing females from larvae reared on the high cholesterol medium and two containing females from CSMA-reared larvae were fed the incomplete synthetic diet. On the incomplete adult diet, females from larvae reared on both these media laid eggs on which viabilities were determined. In general, the viability was the same in first cycle eggs of both groups, whereas in eggs of the second ovarian cycle, viability was higher for females reared from the sterile medium containing 0.50% cholesterol than for females reared from the CSMA medium (Table XXX). The egg viabilities in the first two cycles for flies from a larval medium containing 0.50% cholesterol were the same as those noted previously

for females reared from the standard sterile medium containing 0.25% cholesterol (cf. Table XIII). This similarity has some reliability because in both experiments, eggs from flies reared on the CSMA medium had the same viability also.

DISCUSSION

When studying the nutrition of an insect, one must select a control diet as close to an optimum as possible (Sang, 1956; Akov, 1961). Only then can the effect of changing a component of the diet be correctly interpreted without the masking effects of disturbed metabolic pathways. In order to evaluate the effects of larval nutrition upon the adult, the larval medium must be at least semi-defined chemically. In addition, larvae should be reared axenically because bacteria in the medium contribute compounds not originally present, or otherwise alter the definition of the medium.

The semi-defined medium developed by Monroe (1962) for the axenic culture of house-fly larvae was closer to an optinum than any reported previously. The formula for Monroe's medium was used essentially unchanged in this study, and gave uniform results in replicate experiments. In addition, with the sterile medium the average weight per pupa was only 10% less than that obtained by Monroe (1962), although it was considerably less than the weight of pupae reared on the CSMA medium. The small difference between the present result and that of Monroe may have arisen from slight differences in the way in which he prepared his sterile medium or from strain differences in the flies.

There were, however, slight indications of metabolic imbalance. The puparium was of a redder colour than those of the controls, and puparia of the pointed type (formed when the larva fails to retract the mouthhooks) appeared somewhat more frequently. These abnormalities occurred too infrequently to reduce emergence significantly. One may conclude that this diet, while not yet optimum, is sufficiently close to an optimum that it could be used to test the effect of variations in it upon adult reproduction.

Larvae reared on the CSMA medium grew faster and were larger than those reared on the standard sterile medium. The decrease in growth rate on the sterile medium may be related to its physical properties. Sang (1956) found for D. melanogaster that the first and early second stage larvae had difficulty in feeding on a non-particulate medium in an agar gel. When the larvae were larger, they were physically able to do so. On the CSMA medium, by the end of the second day, the larvae were in late second or early third instar, while the experimental larvae did not reach this stage until about the fourth day, after which they proceeded to grow at the normal rate. A medium which is judged optimum by one criterion may not be optimum by another, and therefore, several criteria must be used. The CSMA medium, judged by larval growth rate and pupal weight, was better than the sterile semi-synthetic medium. How-

ever, it appeared that certain nutrients supplied by the sterile medium, and not by the CSMA medium, produced adults that lived longer and laid more eggs than those from the CSMA medium even though females from the latter developed more eggs in proportion to their increased weight. For all females on the adult diets the number of eggs laid was lower than the number of eggs developed, because a certain percentage of females which developed eggs, retained them without laying. This occurred more often with females reared from the CSMA medium, thus explaining why the females from the standard sterile medium laid more eggs than those from the CSMA medium. This may be an indication of more vigorous flies resulting from the sterile diet, since it coincided with the improved survival of adults from that group.

One major difference in performance was found between females reared on the CSMA medium and those reared on the standard sterile diet. The latter, when fed on an incomplete synthetic diet, developed, and in some cases, laid four cycles of eggs while the former laid only first cycle eggs, and some of these did not even develop a second cycle. This contradicted the findings of Morrison (1963) who showed that the house fly required a complete synthetic adult diet in order to lay more than one cycle of eggs. This was thought to be because his larvae,reared on the CSMA medium, stored

enough of the nutrients required to develop the first cycle but no more. In studies reported here, flies from the sterile semi-synthetic medium evidently did possess enough nutrients to develop more than first cycle eggs, even though they were lighter than the flies from the CSMA medium. The specific constituents of the larval medium involved were not yet apparent, because, although the constituents of the sterile medium were known, those of the CSMA medium were not. Furthermore, bacteria and yeast probably provided the actual source of food of the larva in the CSMA medium, since freshly prepared sterile CSMA medium did not support larval growth.

The first experiment on the role of oleate compared a sterile larval medium lacking oleate to the non-sterile CSMA medium. It indicated that the house-fly larva benefitted in rate of growth and in increased weight by the presence of oleate in its diet. The second experiment was a comparison of larvae reared axenically on the standard sterile medium containing 2% oleate with those reared on the same medium lacking oleate. This also showed that the rate of larval growth was improved and that there was a weight increase. These results agreed with those found by House and Barlow (1956), for the sarcophagid, Agria affinis (Fall.), whose growth rate and survival were increased by the addition of lard to the larval diet.

A mixture of palmitic, stearic, oleic, linoleic and linolenic acids was found to work equally well, most of the activity of the mixture being due to oleic acid, with some benefit from stearic and palmitic acids. It is assumed here that sodium oleate and oleic acid had the same activity.

In general, fats are important in the metabolism of insects, comprising 20% of the dry weight of the mature larva, 13.6% of the late pupa, 9% of the newly-emerged adult and 12% of the egg (calculated from data for Musca vicina given by Levinson and Silverman (1954): corroborated by Pearincott (1960) for his strain of M. domestica). Fats provide most of the energy for metamorphosis (Wigglesworth, 1953; Levinson and Silverman, 1954). However, most of the lipids found in the insect body, with the exception of a sterol, can be synthesized from protein (Sedee, 1956; Brookes and Fraenkel, 1958; Levinson and Bergmann, 1957). This is in contrast to other animal groups, such as, the mammals, which require several fat-soluble vitamins and unsaturated fatty acids (Prosser and Brown, 1961).

That lipids, other than cholesterol, are not an essential dietary factor of the house-fly larva is shown by the moderately good results obtained in these experiments on the casein-base synthetic medium without added oleate. However, the possibility that fats were present as a contaminant in the sterile media lacking oleate cannot be

completely excluded, even though the vitamin-free casein was alcohol and ether extracted for twenty-four hours as was the small amount of cellulose powder used, because a commercial preparation of yeast RNA (Calbiochem) was used as obtained.

The improvement in pupal weight noted when 2% oleate was present in the larval medium was similar to that obtained by Sedee (1956) for another cyclorrhaphous fly, Calliphora vicina; in his study, fatty acids added to a semi-defined larval medium (casein-base) induced an improved growth that could not be attributed to this constituent being merely a source of carbon. Unsaturated fatty acids with one or more ethylenic bonds were more effective in their growth-promoting action than saturated fatty acids. Other authors, however, have disagreed that dietary fatty acids exert any beneficial effects. Some lower molecular weight saturated fatty acids, having from six to twelve carbon-atoms, were toxic to larvae of Musca vicina, acting as contact or stomach poisons (Levinson and Ascher, 1954). In the house-fly, Brookes and Fraenkel (1958) found that caproic, caprylic, capric and myristic acids (with six, eight, ten and fourteen carbon-atoms respectively) all retarded growth while palmitic and stearic acids, although non-toxic, had no effect on growth whatever. Brookes and Fraenkel also tried oleic acid, but found that it had no effect. The contradiction between their results and those obtained in this study have several explanations; their strain of flies was different and may have

had differing requirements. However, a more likely explanation may be that their synthetic medium had quite a different proportion of casein, as well as additional compounds. Indeed, as Sang (1959) has pointed out, the optimum requirements for one chemical will depend on the concentrations of the other compounds included in the diet, since the several compounds may be metabolically interrelated.

The metabolic role of dietary oleic acid in the house-fly larva is unknown, but there are indications in Agria affinis that it is unconnected with the need for biotin (House and Barlow, 1960). On the other hand, biotin has been implicated in other organisms in the conversion of saturated fatty acid to unsaturated fatty acid (Gilmour, 1961). The improvement noted in this study was obtained in the presence of a small, but adequate, supply of biotin.

The promoting effect of oleate on adult survival and reproduction was not as marked, however, as it was on larval growth. Adult survival was least affected; there was an increase of one day in the 50% survival time between females reared on a larval medium containing 2% oleate and that of females reared on one with 0.5% oleate, and a difference of two days between females from larval media containing 2% and 0% oleate. Oleate in the larval medium increased the weight of the pupa and presumably also of the adult fly, thus increasing its capacity to develop a proportionally larger number of eggs because of a proportional increase in the number of ovarioles. Thus, although

the total number of eggs developed per female increased, the number of eggs developed per unit of female weight was unchanged, as was egg viability. The number of eggs actually laid was increased, also, because the percentage of females laying more than one cycle of eggs was greater. This was interpreted as an indication of a slight increase of vigour in these flies, accompanying the slight increase in survival mentioned above. The fact that oleate in the larval medium did not result in an increase in the number of eggs developed per unit of adult weight suggests that exogenous fatty acid was neither stored in an increased amount nor involved in a metabolic process bringing about the increased storage of another compound such as glycogen or protein in larvae, pupae or newly-emerged adults reared on a larval medium containing fatty acid.

In the insect's own body, the larva has 75% unsaturated fatty acids and 25% saturated acids (Sedee, 1961a). Sedee reared Calliphora vicina on a medium containing only casein, indicating that the ratio of saturated to unsaturated fatty acid was under genetic rather than dietary control. Since the degree of unsaturation, principally found as oleic acid, is so high in the dipterous larva, an exogenous source of oleic acid could conceivably aid the larva's own synthetic mechanism in supplying its requirements.

The lack of utilization of unsaturated fatty acids by the adult noted in the results may be related to the observations of Levinson and Silverman (1954), on the degree of saturation of fatty acids in the oriental house fly, M. vicina. The iodine number dropped from 98.0 in the third stage larva to 63.8 in the newly-emerged adult and to 55.3 in the egg, indicating a breakdown and utilization of unsaturated acids during metamorphosis and a later utilization of predominantly saturated acids in reproduction. Thus, the demand for unsaturated acids would be kept within the synthetic capacities of the adult, making an exogenous supply, either in the diet or carried over from the larval stage, unnecessary.

The addition of RNA to the sterile medium in this study had a growth promoting effect on the larva of the house fly, similar to that with oleate, increasing its weight by 15%. An increase in the rate of development, with pupation and emergence occurring one day, or 14%, sooner in the presence of RNA was also noted. These results are similar to those obtained by previous workers with other dipterous larvae (Sedee (1956) for Calliphora vicina; Hinton (1956) and Sang (1956) for Drosophila melanogaster; Brookes and Fraenkel (1958) for Musca domestica). House and Barlow (1957) found that the larva of Agria affinis grew faster when a small amount of RNA was added to its medium, but they found that concentrations above 0.1 gm. per 100 ml. exerted an adverse effect, by lowering pupation and adult emergence.

Brust and Fraenkel (1955) had already observed similar adverse effects with Phormia regina. In the experiment in this study, adult emergence did not go below 90%, regardless of the concentration of RNA (0 to 6%) used, indicating that RNA in the larval medium does not affect adult emergence of the house fly. It is possible that the inhibitory effect of RNA in the larval medium, noted for P. regina and Agria affinis, was manifested only because their basic larval medium was much below optimum, as was shown by the low emergence (41%) obtained for A. affinis even under the best of conditions. In a near optimum medium, such as that used in this experiment, a non-optimum concentration of RNA would not put a metabolic load, in addition to those already present, upon the organism.

Although the results obtained in this study indicate that the house-fly larva grows faster and larger when its diet contains RNA, it can also grow successfully without it, although more slowly. One would conclude that it can synthesize its requirements for RNA, but that synthesis is not fast enough to allow the maximum rate of growth.

Sang (1957) has shown that the larva of Drosophila melanogaster also benefits from the presence of RNA in its medium, but that adenylic acid, adenine, adenosine, guanosine and guanylic acid each had a stimulatory effect as well. He concluded that it was actually these compounds, as purine bases in RNA, that exerted a stimulatory effect.

Brookes and Fraenkel (1958) also found that adenine and guanine could partly replace RNA in the larval medium of the house fly. Other animals, as a rule, do not exhibit a dietary requirement for RNA or its bases, except for some specialized Protozoa, in which obligatory requirements for a purine, adenylic acid or adenosine, and a pyrimidine, cytidylic acid or cytosine, has been shown (Kidder, in Bourne and Kidder, 1953).

The need for RNA in insects depends on the level of folic acid in the diet (House, 1962). In the present study the improvement noted in rate of larval growth and in pupal weight when RNA was present in the larval medium was obtained in every case in the presence of folic acid. Brookes and Fraenkel (1958) found that folic acid could partly replace the need for RNA in the larval house fly. Sang (1959) showed that the need for RNA in Drosophila melanogaster was doubled when folic acid was lacking in the larval medium. Folic acid is important in methylation and group-transfer reactions; one type of reaction in which it takes place is the conversion of uridine to thymidine, and Sang (1959) showed that, in D. melanogaster, the ill effect of aminopterin was reversed by addition of thymidine to the larval medium. Folic acid is also involved in the direct synthesis of the purine ring structure from formate (Brown and Roll, 1955); since dietary experiments have indicated that it is the purine ring which cannot be syn-

thesized at a rate sufficient for the demand, this reaction is probably the one most concerned with folic acid and RNA requirements.

Because a large amount of RNA is formed by nurse cells during the development of an egg (King and Sang, 1959) and because insect eggs contain an unusually large amount of RNA, one would expect that RNA or its purines would be required for adult reproduction as well as for larval growth. However, few studies have shown its necessity. Sang and King (1961) reported that adults of D. melanogaster required no RNA, and that egg production and viability was not reduced in its absence. If no folic acid was supplied, however, a need for RNA became apparent, and faulty oogenesis resulted. In the strain of Musca domestica used in this laboratory, RNA was required in the adult diet for continued egg production; folic acid alone was insufficient to produce more than one cycle of eggs on an amino acid diet (Morrison, 1963). He observed even better fecundity when adenylic and cytidylic acids were substituted for RNA. Previous workers, however, have not investigated the effects of RNA in the larval medium on the adult after emergence.

In this study, the reproduction of adults from larvae reared on the sterile medium containing RNA was compared with that of adults from a larval medium lacking RNA. Those female flies that had been deprived of RNA as larvae were slow to begin ovipositing when as adults

they were fed the incomplete synthetic diet (lacking RNA) and even the complete synthetic diet (with RNA), but did not show a delay when fed on milk. In addition, adults that had received RNA as larvae were also slow to begin ovipositing when they were fed the incomplete synthetic diet, but showed no delay when fed the complete diet or milk. These results suggest that the addition of RNA to the larval medium enhanced later adult reproduction, and that some carry-over occurred; the inadequacy of this carry-over was only apparent, however, when the adult diet was deficient. Once deposition of the first cycle had been initiated, however, the deposition of the second, third and fourth cycles followed at a constant rate that was independent of the presence or absence of RNA in the larval medium. One may tentatively conclude that the rate of synthesis of adenine and cytidine in the adult is unaffected by the presence or absence of RNA in the larval medium.

The presence of RNA in the larval medium resulted in a striking increase in the number of eggs laid. This increase was apparent on all three adult diets, and was due to at least two factors. The largest factor was the increase in weight of the pupa and presumably also of the adult fly, thus increasing its capacity to develop a proportionally larger number of eggs. The other factor was the increase in the percentage of females laying each cycle of eggs. Both these factors recall the beneficial effect of oleate in the larval medium, in that the actual reproductive capacity per unit of female weight as

measured by the eggs developed, was not increased. On the complete synthetic adult diet and on milk, the increase in weight accounted for 75% of the increase in number of eggs, while the remaining 25% of the increase was a result of the increased number of females laying more than one cycle. On the incomplete adult diet, however, a third factor was in evidence. Eggs developed by females reared from larval media lacking RNA were fewer than expected on the basis of the weight of the fly. The number of eggs developed by females that were reared on larval medium containing RNA, was more comparable to the number laid by females fed milk and the complete diet. This indicated that a deficiency of RNA in the larval medium resulted in some reduction in the reserves of the pupa and adult. These flies were unable to develop eggs in the number of ovarioles that would be expected on a weight basis. However, if the adult diet contains RNA, this deficiency can be rectified in the adult stage, almost all of the ovarioles producing fully developed eggs. Therefore, one may conclude that storage and later utilization of RNA takes place when it is present in the larval medium.

Sang and King (1961) showed that for Drosophila melanogaster, RNA was not required in the adult stage, while Morrison (1963) found that the adult house fly required RNA in its diet after the first cycle of eggs had been laid, in order that oogenesis could proceed. Sang and

King explained their results as being due to a difference in the quantitative RNA requirements between adults and larvae, but they might also be explained by carry-over from the larva of RNA in an amount sufficient to supply the adult needs. This study lends support to the hypothesis that such a storage in the larva can take place, influencing the fecundity of the resultant adult, particularly if the component is lacking from the adult diet. From qualitative preliminary dissection data, Sang and King were aware that newly emerged adults had differing amounts of fat and other storage products, depending on the larval nutrients, but they did not investigate whether the RNA or other adult requirements were changed by a different larval medium.

The present results might have quite a different explanation than that the purine and pyrimidine bases accumulated by the larva were utilized directly. It is well known that RNA is involved in the synthesis of protein within living cells, and the effect of RNA in the larval medium may have resulted from increased levels of other substances in the larval and pupal bodies. This possibility should be checked by chemical analyses of groups of larvae, pupae, newly emerged adults, later adults, and newly-laid eggs, testing particularly for total nitrogen, tissue nitrogen, lipids, and glycogen.

A lack of cholesterol in the larval medium prevented full development of the immature stages. This result, establishing the essential nature of cholesterol, has been obtained for all insects thus

far studied (Horning, 1958; Grant, 1962). In the present study most house-fly larvae on the medium lacking cholesterol were unable to grow beyond the second instar, and died after a larval period of 7 to 20 days. Sedee (1956) using Calliphora vicina and Levinson (1960) using Musca vicina also found that lack of sterol was manifested early in larval development. Levinson stated that the need for cholesterol decreased with increasing larval age.

The formation of two very small improperly formed puparia, from which adults did not emerge, was probably due to cannibalism which was noticeable with a medium deficient in nutrients. Since the eggs from which the larvae hatched contained cholesterol, a small amount of cholesterol would be present in each larva, although none was present except as a very small amount of contaminant in the medium itself. Cannibalism would thus tend to give the larva the greatest possible concentration of cholesterol. A similar phenomenon was noted by Levinson (1960) for Dermestes vulpinus Fabr., a carnivorous larva. House (1958) grew his house-fly larvae in individual cylinders to prevent cannibalism, but this effect was ordinarily so slight for Musca domestica that it could be neglected.

While this research was in progress, Robbins and Shortino (1962) found that with their strain of M. domestica the addition of cholesterol in a concentration of 0.125% to 0.500% of the dry weight

of the larval medium, caused over half the adults from that medium to develop eggs when fed on sugar solution only. This result was not confirmed in our work when a concentration of 0.25% cholesterol in the larval medium (the standard for all previous experiments) was used, although there was some indication that egg development did proceed farther than was the case in females from CSMA-reared larvae. When the concentration of cholesterol was doubled (to 0.5% dry weight) this effect on oogenesis was more marked, thus partly substantiating the results of Robbins and Shortino (1962). However, in our work, no eggs were laid and dissection data showed that egg development never reached completion, even after 14 days. This may have been due to a strain difference; the strain used by Robbins and Shortino (1962) already displayed 2% autogeny, indicating that some genes for this character were already present in the gene pool. As mentioned in the introduction, autogeny in mosquitoes is partly hereditary, being conferred by one or several genes. In our strain, reared for 60 generations, no autogeny had ever been manifested, indicating that the gene or genes were probably not present and that the strain was homozygous for their lack. Therefore, the effect noted was believed to be a response to a nutritional, rather than a genetic cause.

There were no indications that other substances tested in the larval medium, RNA and oleate, caused autogeny or partial autogeny. Differing casein levels were not tried, but the results of

Ascher and Levinson (1956) on Musca vicina indicated that higher protein levels in the larval medium were not involved in the autogenous production of eggs.

Consistent differences were noted, however, when two groups of females fed amino acids (the incomplete diet) were compared, one from CSMA-reared larvae and the other from larvae reared on the standard sterile medium even when it lacked RNA or oleate. The production of second, third and fourth cycle eggs by these axenically reared flies, and the lack of production by CSMA-reared flies is suspected to be linked to the level of cholesterol in the larval medium since this factor was sufficient to produce such striking differences between CSMA-reared and axenically reared flies when the adults were fed sucrose solution. Further experiments should be performed, using only axenically cultured flies, with cholesterol levels in the larval medium varying from subminimal to very high levels, in order to establish this point more clearly.

It should be pointed out that cholesterol alone, when administered to the adult, did not have the effect of producing autogeny (Morrison, 1963). There have been reports that cholesterol is important in the reproduction of insects (Chauvin, 1949 - for Blatella germanica; Levinson, 1960 for Musca domestica; Monroe, 1960 for M. domestica). It is known that the adult of M. domestica is unable to synthesize choles-

terol from C^{-14} acetate (Robbins et al, 1960). Monroe (1960), using an adult diet of milk, found that egg viability rather than egg production was affected by lack of cholesterol in the adult diet. Results in this laboratory using the complete synthetic adult diet (containing cholesterol) did not confirm this, probably because the concentration of cholesterol used was much lower than that used by Monroe. A further series of experiments testing the role of cholesterol alone or in combination with other substances, in adult reproduction, are needed.

In view of the essential nature of dietary cholesterol or some closely related sterol for insects and its non-essentiality for all other groups of organisms, with the exception of a few specialized Protozoa, it is surprising that few biochemical studies have dealt with sterol metabolism and utilization in this group.

Dietary experiments have shown that the specificity of this compound is rather high, with very few alterations in the molecule being accepted by any insect. To some extent, the compounds capable of being utilized are governed by the normal diet of the insect, zoophagous insects such as Dermestes vulpinus being capable of utilizing only cholesterol and 7-dehydrocholesterol, the normal sterols found in animal bodies, and phytophagous insects being capable of utilizing, in addition, compounds such as ergosterol and β-sitosterol, the normal sterols of plants (Levinson, 1960a; Horning, 1958). Levinson and

Bergmann (1957), studying Musca vicina a species in which the larva eats both plant and animal material, found that both the ring structure and R group at C_{17} were required, with a few minor changes in each area being tolerated.

In mammals, much of the synthetic pathway for sterols is known, with synthesis proceeding from acetate by way of mevalonic acid to squalene, a triterpene, and thence to cholesterol (Grant, 1962). In insects, Clark and Bloch (1959) investigated the effect of mevalonic acid and squalene, finding that neither had any sparing effect on the need for cholesterol. Sedee (1961) fed squalene to the larva of Calliphora vicina, finding that in this fly there was also no mechanism for proceeding from squalene to cholesterol. Sedee and the preceding two authors conducted studies with radioactive tracers concurrently with the dietary experiments and both found that C^{-14} acetate was not incorporated into mevalonic acid (Clark and Bloch, 1959a), into squalene nor into cholesterol (Sedee, 1961; Clark and Bloch, 1959a). This indicates that almost the whole of the known pathway is absent in insects.

In mammals, the only group on which extensive studies have been performed, cholesterol plays both a structural and a metabolic role and it is reasonable to suppose that it does this in insects also. In its structural role, bound cholesterol forms a part in all lipid layers of the cell, particularly in protein phospho-lipids. Free cholesterol

is essential for the maintenance of membranes such as those forming the mitochondria of the cell (De Robertis et al, 1960). The sparing action of analogues of cholesterol with side chains shorter than eight carbons, noted by Bergmann et al, (1959) for Musca vicina larvae was attributed by Levinson (1960) to the use of these compounds in a structural role. A like conclusion was reached by Clark and Bloch (1959a) for the sparing action of β-sitosterol (which ordinarily cannot be used at all by Dermestes vulpinus). Labelled β-sitosterol was recovered unchanged after feeding. One milligram of cholesterol per gram of medium was ordinarily needed by this insect, but when 1 mg. of β-sitosterol was fed, only 0.03 mg. of cholesterol was needed in the medium. Clark and Bloch (1959b) pointed out that this amount was now within range of values expected for a substance with a metabolic function, such as, a vitamin or a hormone.

Two major hormones, neotenin or juvenile hormone, and ecdyson or moulting hormone, are involved in the growth and metamorphosis of insects. The juvenile hormone, neotenin, has been obtained in pure form in ether extracts from many sources by a number of workers. This hormone is responsible for larval growth and the differentiation of larval characters (Wigglesworth, 1961), but it, or a very closely related compound, is also necessary for adult reproduction. Recently, Wigglesworth (1961) reported that one of the compounds in a crude hormone extract, farnesol, an open-chain terpene

alcohol, had all the attributes of the juvenile hormone when applied externally to Rhodnius prolixus, causing the retention of larval characters in the moulting fifth stage nymph, and inducing yolk formation in the adult.

Levinson (1960) pointed out that cholesterol also acts in a manner similar to the juvenile hormone; as was shown, cholesterol is required in a metabolic role in the larva, and excess amounts in the larval stage have the effect of inducing yolk formation in the adult female.

Farnesol, or its acid, farnesenic acid, has been implicated in the biosynthetic pathway of cholesterol in mammals, two units of farnesenic acid possibly condensing to make one unit of squalene. This compound would thus be a good starting point for an investigation into the hormonal action of cholesterol in insects.

Of the six components of the larval medium, three, RNA, a mono-unsaturated fatty acid (oleate), and a sterol (cholesterol) were tested for their effects on adult survival and reproduction. The first two affected the survival and vigour of the adults and the number of eggs laid, but neither the number of cycles produced nor the attainment of autogeny. The third, cholesterol, influenced the last two features, both evidently related to one another. In the house fly, therefore, adult reproduction cannot be explained solely on the basis of adult

nutrition. The adult must have already, before feeding, some nutrient stores, sufficient to develop at least a few eggs. Indeed protein, given in adulthood, is required not only to replenish the supply carried over from the larval stage, but to release or trigger the release of a hormone or hormones. The workers reviewed in the introduction, were correct in their belief that the larval medium did play a role; however, the sterol content of the larval medium probably acts as a hormone or hormone precursor causing the utilization of available reserves.

SUMMARY

(1) Larvae of the house fly, Musca domestica L., were reared axenically on semi-synthetic media and their performance both as larvae and as adults was assessed in comparison to that of larvae and adults reared on the CSMA medium, an undefined medium used in most previous work on adult house-fly nutrition. The standard sterile larval medium contained casein, RNA, sodium oleate, cholesterol, B-vitamins and inorganic salts.

(2) The major difference arising from the two larval diets was in the number of ovarian cycles completed. On an incomplete synthetic adult diet, containing only amino acids in addition to sugar and salts, females from larvae reared on the standard sterile medium laid as many as four cycles of eggs, whereas females reared from the CSMA medium completed a maximum of two cycles.

(3) Three components of the standard sterile medium, i.e., oleate, RNA and cholesterol were assessed as to their effects on both the larval and adult stages.

(4) Sodium oleate and RNA were non-essential but their presence in the larval medium improved larval growth rate and pupal weight. The third component, cholesterol, was an essential dietary requirement for larval growth.

(5) Larval nutrition influenced the reproductive ability through its effect on three adult features, the adult survival and vigour, the number of eggs developed, and the adult hormonal system.

(6) The number of eggs laid was affected by adult survival and vigour, since less vigorous flies more often retained mature eggs without laying them. The presence of oleate slightly improved both adult survival and the percentage of females laying eggs, while RNA in the larval diet produced a greater effect.

(7) The number of ovarioles present in the female was found to be proportional to the pupal weight. All three larval dietary factors investigated influenced the pupal weight, thus affecting the number of ovarioles.

(8) The proportion of the ovarioles present in a female which are active in developing eggs depended both on the adult diet and certain components of the larval medium.

(9) Oleate in the larval medium did not affect this proportion while RNA affected it when the adult was fed an incomplete synthetic diet, but not when fed milk or a complete synthetic diet (one with B-vitamins, cholesterol and RNA added to the incomplete diet).

(10) With cholesterol in the larval medium and with no source of protein in the adult diet, there was a partial development of eggs in some ovarioles.

(11) The hypothesis that the dietary factor, cholesterol, acts as a hormone, triggering the autogenous, or partially autogenous, development of eggs, is discussed in the light of previous work on the utilization of larval nutritional stores for autogenous oogenesis.

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TABLE I

Composition of the CSMA medium

Constituent	Amount	Source
CSMA [a]	600 gm.	Ralston Purina Company, St. Louis, Mo.
Brewers' yeast	22 gm.	Nutritional Biochemicals Corp., Cleveland, Ohio.
NaOH 5 N.	20 ml.	Fisher Scientific Co. Ltd., Toronto, Ont.
Water	1205 ml.	carbon-filtered tap water

[a]. Chemical Specialties Manufacturing Association.

TABLE II

Composition of dry ingredients of the standard sterile medium for house-fly larvae

Constituent	% of dry weight	gm. per 100 ml. diet	Source of component	Grade
casein	87.50	10.50	General Biochemicals, Chagrin Falls, Ohio.	"vitamin free" alcohol extracted fine grind
RNA	3.75	0.45	California Corp. for Biochemical Research, Los Angeles, Calif.	"yeast RNA" grade C
salts-W	4.75	0.57	Nutritional Biochemicals Corp., Cleveland, Ohio.	Wesson modification *
celluflour	1.25	0.15	" " "	powdered, non-nutritive fibre
sodium oleate	2.50	0.30	Fisher Scientific Co., Toronto, Ont.	purified
cholesterol	0.25	0.03	California Corp. for Biochemical Research, Los Angeles, Calif.	grade C; m.p. 149°C

* Wesson modification of Osborne and Mendel salt mixture (Science, 75:339. 1932).

TABLE III

Composition of agar - vitamin solution

Constituent	Amount
agar-agar, B.B.L. [a]	3 gm.
glass-distilled water	85 ml.
vitamin solution (see Table IV)	15 ml.

a. Baltimore Biological Laboratories, Baltimore, Md.

TABLE IV

Composition of solution of B vitamins

Constituent[(a)]	mg./100 ml. glass-distilled water
thiamine	50.0
riboflavin	25.0
niacin	100.0
Ca-pantothenate	50.0
pyridoxal hydrochloride	25.0
choline chloride	1000.0
inositol	500.0
folic acid	5.0
biotin	1.0

(a) all from California Corporation for Biochemical Research, Los Angeles.

TABLE V

Composition of the incomplete synthetic diet for adult house flies

Constituent	gm./litre glass-distilled water
L-arginine hydrochloride	3.8
L-histidine hydrochloride	1.5
L-isoleucine	2.5
L-leucine	7.5
L-lycine hydrochloride	7.5
L-methionine	1.5
L-phenylalanine	12.0
L-threonine	1.5
L-tryptophan	3.0
L-valine	5.0
L-glutamic acid	10.0
salt mixture 'W'	1.5
sucrose	34.2

All amino acids were obtained from California Corp. for Biochemical Research, Los Angeles, Calif.

Salt mixture 'W' was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

Sucrose was obtained from Fisher Scientific Co. Ltd., Toronto, Ont.

TABLE VI

Composition of the complete synthetic diet for adult house flies

Constituent	Amount/litre
RNA	2.0 gm. dissolved in 5 ml. 1N, NaOH
vitamin solution (Table IV)	10.0 ml.
cholesterol	0.1 gm.
incomplete synthetic diet (Table V)	to make up 1 litre

TABLE VII

Comparison of rearing success when larvae are reared on CSMA and on standard sterile medium

Larval medium	Larval survival a.	Average weight of pupae after 2 days	Emergence of adults e.	Sex ratio fem/total x 100 e.	Days as larva	Total days to emergence
CSMA	62.17% (14) b. (41.9-75.4)c.	25.22 mg. (14) (23.0-27.5)	86.0% (3) (78.5-90.7)	49.0% (5) (46.6-50.6)	5.6 (14) (5-7)	11.2 (14) (11-12)
Standard sterile medium	59.6% d. (23) (31.1-80.0)	18.56 mg. (23) (15.0-21.9)	87.5% (18) (62.5-96.7)	51.2% (15) (37.7-59.5)	7.4 (23) (7-9)	13.5 (23) (12.5-17.0)

a. - Survival, measured as a % of mature larvae per unit number of eggs

b. - Number in brackets indicates the number of flasks observed

c. - Numbers in brackets indicate the range of values obtained; CSMA flasks contained approximately 250 eggs and sterile flasks approximately 80 eggs

d. - In one experiment some larvae escaped from the flasks, lowering the % of mature larvae

e. - This data recorded for only some of the experiments

TABLE VIII

Survival of adults from larvae reared on

CSMA and on standard sterile medium

Larval medium	Adult Diet			All diets
	Milk	Complete synthetic	Incomplete synthetic	Average
CSMA	7.1 days	9.9 days	8.6 days	8.5 days
Standard sterile medium	9.9 days	10.4 days	11.8 days	10.7 days

Figures shown are average survival of females on each diet.

TABLE IX

Total number of eggs laid and average number of eggs per female; a comparison of females fed three different adult diets, from larvae reared on CSMA and on standard sterile medium

Larval medium	Adult Diet					
	Milk		Complete synthetic		Incomplete synthetic	
	Total	Eggs/fem. (a)	Total	Eggs/fem.	Total	Eggs/fem.
CSMA	1372	171.5	589	74.1	684	97.7
Standard sterile medium	1834	201.6	884	110.5	984	164.0

(a) average eggs/female was calculated here by using the total number of females living at the beginning of the experiment.

TABLE X

Average number of eggs laid per living female per cycle; a comparison of females fed three different adult diets, from larvae reared on CSMA and on standard sterile medium

Adult diet	Larval medium	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Total 3 cycles	Total 4 cycles
Milk	CSMA	70.4 (8)	68.4 (5)	120.0 (2)	106 (1)	258.8	364.8
	sterile	107 (8)	80.0 (8)	56.5 (4)	56.5 (2)	243.5	300.0
Complete synthetic	CSMA	48.4 (8)	50.5 (4)	0 (2)	-	98.9	98.9
	sterile	40.8 (8)	40.1 (8)	52.8 (4)	8.7 (3)	133.7	142.4
Incomplete synthetic	CSMA	73.9 (7)	33.4 (5)	0 (1)	0 (1)	107.3	107.3
	sterile	86.4 (6)	67.3 (4)	48.3 (3)	17.3 (3)	202.0	219.3

Figures in brackets indicate the number of living females used to obtain the average.

TABLE XI

Average number of eggs developed per living female per cycle; a comparison of females from larvae reared on CSMA and on standard sterile medium. First experiment.

Adult diet	Larval medium	Cycles				Total 3 cycles	Total 4 cycles
		1	2	3	4		
Milk	CSMA	143.0(7)	140.0(3)	120.0(2)	113.0(2)	403.0	516.0
	sterile	117.4(7)	109.6(7)	113.0(2)	98.0(2)	340.0	438.0
Complete synthetic	CSMA	126.8(6)	106.5(2)	0 (1)	-	233.3	233.3
	sterile	111.0(7)	98.0(3)	102.6(3)	99.0(1)	311.6	410.6
Incomplete synthetic	CSMA	127.8(6)	112.5(2)	76.0(1)	-	316.3	316.3
	sterile	103.6(5)	77.3(3)	72.5(2)	52.0(1)	253.4	305.4

Figures in brackets indicate the number of females included. Females whose cycles were irregular, or for which no dissection data were available, were disregarded.

TABLE XII

Average number of eggs developed per female on an incomplete synthetic adult diet; a comparison of females from larvae reared on CSMA and on standard sterile medium. Second experiment.

Larval medium	Cycle 1	2	3	4
CSMA	161.6(17)	149.4(7) 104.5(10)[a]	0(6)	-
Standard sterile medium	120.4(23)	96.0(20)	90.8(17)	66.3(6)

Figures in brackets indicate the number of females included in the analysis. Both dissection data and laying data were used.

a. - Three out of ten flies did not develop any eggs although they lived six to eight days after they laid the first cycle. The average time until laying was four days (two to five days). Therefore the three flies should be taken into account when the development of the second and following cycles is considered; the lower figure is regarded as more accurate.

TABLE XIII

Viability of eggs laid by females from larvae reared on CSMA and on standard sterile medium

Larval medium	Type of cylinder	Percentage of viable eggs in each cycle			
		1	2	3	4
CSMA	IND	21.6%	0%		
	IND	22.8%	10.8%		
	LG	29.3%	16.2%		
	LG	47.0%	1.4%		
	LG	25.7%	4.4%		
		av. 28.2%	6.8%		
Standard sterile medium	IND	39.2%	40.2%	14.8%	3.8%
	IND	79.7%	29.5%	8.1%	0%
	LG	49.4%	16.2%	8.8%	0%
	LG	53.5%	46.0%	35.2%	0%
		av. 65.2%	28.3%	15.3%	0.4%

IND - adults maintained in individual cylinders.
LG - adults maintained in large cylinders (10 to 18 females per cylinder)

TABLE XIV

Comparison of rearing success when larvae are reared on CSMA and on sterile medium lacking oleate

Larval medium	Average weight of pupae after 2 days	% of eggs producing mature larvae	Time as larva (days)	Time as pupa (days)	Total time to emergence
CSMA	27.0 mg. (3) a (26.9-27.5) b	53.5% (3) (51.4-59.2)	5.3 (3) (5-6)	5.0 (3) (5)	10.3 days
Sterile medium lacking oleate	17.3 mg. (2) (16.9-17.6)	52.4% (2) (47.9-56.8)	9.0 (2) (9)	5.5 (2) (5-6)	14.5 days

a. - number in brackets indicates the number of flasks included.
b. - numbers in brackets indicate the range of values obtained.

TABLE XV

Average number of eggs laid per living female; a comparison of females from larvae reared on CSMA and on sterile medium lacking oleate

	Adult diet - Milk			
Larval medium	Cycle			
	1	2	3	4
CSMA	89.0 (6)	109.3 (6)	46.5 (6)	45.4 (4)
Sterile 0 % oleate	68.4 (14)	57.8 (13)	31.4 (12)	35.4 (10)

Figures in brackets indicate the number of living females used to obtain the average.

TABLE XVI

Average number of eggs laid per female; a comparison of females from larvae reared on CSMA, on standard sterile medium and on sterile medium lacking oleate

	Expt.	larval medium			Probability
		CSMA	ster.-2% oleate	ster.-0% oleate	
Total 3 Cycles	Part I (Table X)	258.8	243.5	-	>0.05
	Part IIa (Table XV)	244.8	-	157.6	<0.025
Total 4 Cycles	Part I (Table X)	364.8	300.0	-	>0.05
	Part IIa (Table XV)	290.2	-	193.0	<0.025

TABLE XVII

Comparison of rearing success when larvae are reared on standard sterile medium, on sterile medium containing 0.5% oleate and on sterile medium lacking oleate

Larval medium	% of eggs producing mature larvae	Average weight of pupae	Days as larvae	Days to emergence	% emergence	% females
standard 2.0% oleate	70.4% (8) a. (62.5-83.2)b.	18.7 mg. (8) (18.1-20.8)	7.4 (8) (7-8)	13.4 (8) (13-14)	89.3% (8) (81.7-92.3)	50.6% (8) (37.7-59.5)
0.5% oleate	65.5% (9) (50.5-79.6)	16.8 mg. (9) (14.7-18.9)	7.9 (9) (7-9)	14.1 (9) (13-15)	82.5% (9) (48.6-100)	48.4% (6) (44.4-52.1)
0% oleate	62.2% (9) (27.7-76.8)	17.3 mg. (9) (15.8-18.3)	7.9 (9) (7-9)	13.9 (9) (13-15)	91.9% (9) (77.8-97.7)	48.5% (9) (39.5-56.9)

From the above table,

$$\frac{W_{2.0\%\ \text{oleate}}}{W_{0.0\%\ \text{oleate}}} = \frac{18.7}{17.3} = 1.08\ (0.99\text{-}1.25)$$

a. - Number in brackets indicates the number of flasks observed.
b. - Numbers in brackets indicate the range of values obtained; each flask contained approximately 80 eggs.

TABLE XVIII

Survival of adults; a comparison of females from larvae reared on standard sterile medium, on sterile medium containing 0.5% oleate and on sterile medium lacking oleate

Survival	Larval medium		
	0% oleate	0.5% oleate	2.0% oleate
ST_{60}	340[a.] - 14.2[b.]	320 - 13.3	360 - 15.0
ST_{50}	365 - 15.2	340 - 14.2	385 - 16.0

ST_{60} - time when 60% survival is reached.

ST_{50} - time when 50% survival is reached.

a. - time expressed in day-degrees.

b. - time expressed in days at 24°C.

Temperature - 24°C ± 2°C.

Relative humidity - 55% ± 5%.

TABLE XIX

Average number of eggs laid per living female on an incomplete synthetic adult diet; a comparison of females from larvae reared on standard sterile medium, on sterile medium containing 0.5% oleate and on sterile medium lacking oleate

Larval medium	Cycle				Total 4 Cycles
	1	2	3	4	
0.0% oleate	107.2 (24)	60.7 (24)	32.4 (21)	5.5 (17)	205.8
0.5% oleate	104.9 (23)	67.8 (24)	35.3 (21)	5.7 (14)	213.7
2.0% oleate	117.3 (23)	79.8 (22)	46.9 (21)	18.1 (13)	262.1

Figures in brackets indicate the number of living females.

TABLE XX

Average number of eggs developed per female on an incomplete synthetic adult diet; a comparison of females from larvae reared on standard sterile medium, on sterile medium containing 0.5% oleate and on sterile medium lacking oleate

Larval medium	Cycle				Total 3 Cycles	Total 4 Cycles
	1	2	3	4		
0.0% oleate	110.6 (23)	82.3 (17)	74.4 (9)	72.0 (1)	267.3	339.3
0.5% oleate	107.4 (22)	86.1 (18)	78.7 (9)	80.0 (1)	272.2	352.2
2.0% oleate	119.8 (22)	92.8 (18)	90.9 (10)	73.8 (4)	303.5	377.3

TABLE XXI

Ratio of pupal weights compared to ratio of eggs developed per female per cycle; a comparison of females from larvae reared on standard sterile medium, on sterile medium containing 0.5% oleate and on sterile medium lacking oleate

		Cycle		
		1	2	3
$\frac{W\ 2.0\%}{W\ 0.0\%}$ = 1.08 (0.99-1.25)	$\frac{E_{D-2.0\%}}{E_{D-0.0\%}}$	1.08	1.13	1.22
$\frac{W\ 2.0\%}{W\ 0.5\%}$ = 1.11 (0.96-1.15)	$\frac{E_{D-2.0\%}}{E_{D-0.5\%}}$	1.12	1.08	1.16

TABLE XXII

Viability of eggs laid by females on an incomplete synthetic and on a complete synthetic adult diet; a comparison of females from larvae reared on standard sterile medium, on sterile medium containing 0.5% oleate and on sterile medium lacking oleate

Adult Diet	Larval medium	Percentage of viable eggs in each cycle					
		1		2		3	
		Egg Count	Larval Count	Egg Count	Larval Count	Egg Count	Larval Count
Complete synthetic	2% oleate	48.6		42.2		46.4	
		52.8		35.4		39.3	
		47.9		28.0		2.8	
		49.7	48.1	34.7	22.6	26.2	14.8
	0.5% oleate	39.6		11.4		23.0	
		61.9		26.3		6.3	
				20.8		3.6	
		48.1	39.7	21.7	19.2	9.7	6.2
	0% oleate	60.2		32.8		10.7	
		70.1		13.7			
		35.0		47.4		33.3	
		54.3	46.8	28.4	23.7	17.1	11.2
Incomplete synthetic	2% oleate	79.7		29.5		8.1	
		49.4		16.2		14.0	
		53.5		46.0		35.2	
		60.9	50.8	30.6	19.9	19.1	14.1
	0.5% oleate	80.1		45.4		8.0	
		41.9		24.9		15.4	
		39.2		46.2		10.7	
		53.7	43.6	38.8	29.9	11.4	6.8
	0% oleate	67.7		41.5		25.7	
		34.7		22.9		21.4	
		50.0		31.6		0	
		50.8	35.5	32.0	18.5	15.7	12.4

TABLE XXIII

Comparison of rearing success when larvae are reared on standard sterile medium, on sterile medium containing 6% RNA and on sterile medium lacking RNA

Larval medium	% of eggs producing mature larvae	Average weight of pupae	Emergence	Days as larvae	Time to emergence
0% RNA	63.7% (4) a (35.4-74.6) b	16.70 mg. (4) (16.5-16.9)	89.0% (4) (78.2-95.5)	8	14
3.0% RNA (standard)	57.4% (4) (40.9-64.6)	18.91 mg. (4) (17.9-19.5)	90.0% (4) (87.1-92.2)	7	13
6.0% RNA	62.4% (6) (54.9-71.6)	18.75 mg. (6) (18.2-19.0)	91.1% (6) (76.9-95.0)	7	13

a. - number in brackets indicates the number of flasks included.

b. - numbers in brackets indicate the range of values obtained.

TABLE XXIV

Survival of adults; a comparison of females from larvae reared on standard sterile medium and on sterile medium lacking RNA

Larval medium	Adult Diet		
	Milk	Complete	Incomplete
Sterile-0% RNA	10.1 days	11.7 days	9.4 days
Sterile-3.0% RNA	10.1 days	11.7 days	10.9 days

Value given is the average survival.

Temp.: 24° - 25°C.
RH: 85% (74-912).

TABLE XXV

Average number of eggs laid per living female; a comparison of females fed three different adult diets, from larvae reared on standard sterile medium and on sterile medium lacking RNA

Adult Diet	Larval medium	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Total 4 cycles
Milk	Sterile 0% RNA	61.5(12)	37.0(9)	27.0(3)	2.0(2)	127.5
	Sterile 3.0% RNA	112.3(9)	97.0(9)	82.5(8)	15.6(5)	307.4
Complete synthetic	Sterile 0% RNA	76.4(17)	48.8(13)	29.8(9)	0 (5)	155.0
	Sterile 3.0% RNA	110.3(17)	105.2(13)	63.8(12)	41.5(8)	320.8
Incomplete synthetic	Sterile 0% RNA	35.4(13)	6.0(9)	0 (6)	0 (3)	41.4
	Sterile 3.0% RNA	64.4(17)	18.1(12)	0 (9)	0 (7)	82.5

Figures in brackets indicate the number of females living during the cycle

TABLE XXVI

Average number of eggs developed per female; a comparison of females fed three different adult diets, from larvae reared on standard sterile medium and on sterile medium lacking RNA

Adult Diet	Larval medium	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Total 4 cycles
Milk	0% RNA	117.8(10)	115.5(6)	81.5(2)	40.0(1)	354.8
	3% RNA	134.7(7)	124.7(7)	117.3(6)	110.0(4)	486.7
Complete synthetic	0% RNA	112.6(15)	97.7(7)	91.0(4)	91.5(2)	392.8
	3% RNA	126.0(16)	119.4(13)	124.4(9)	107.7(4)	477.5
Incomplete synthetic	0% RNA	87.0(12)	69.3(3)	-	-	156.3
	3% RNA	117.1(14)	88.3(3)	-	-	205.4

Figures in brackets indicate the number of females included in the analysis. Females whose ovarian cycles were irregular or for whom no dissection data was available were not included.

TABLE XXVII

Influence, on total eggs laid per living female, of females failing to oviposit; a comparison of females from larvae reared on standard sterile medium and on sterile medium lacking RNA

Adult Diet	Ratio $\frac{E_{Li-3\%}}{E_{Li-0\%}}$	Ratio $\frac{E_{La-3\%}}{E_{La-0\%}}$	Difference between ratios	% of E_{Li} accountable to differences in % of females laying
Milk	2.41	1.65	0.76	$\frac{0.76}{2.41} \times 100 = 31.5\%$
Complete synthetic	2.07	1.68	0.39	$\frac{0.39}{2.07} \times 100 = 19.0\%$
Incomplete synthetic	1.99	1.44	0.55	$\frac{0.55}{1.99} \times 100 = 27.6\%$
Average	2.16	1.59	0.57	$\frac{0.57}{2.16} \times 100 = 26.4\%$

E_{Li} is average of total eggs laid per living female.

E_{La} is average of total eggs laid per laying female.

TABLE XXVIII

Comparison of rearing success when larvae are reared on CSMA and on varying concentrations of cholesterol

Larval medium	% survival	Average weight of pupae	Emergence of adults	Days as larvae	Days to emergence
CSMA	69.8% (3) a. (64.1-75.4)b	23.5 mg. (3) (22.5-25.0)	84.5% (1)	6 (3)	12 (3)
Sterile medium with 0% cholesterol	0.4% (4) (0-1.2)	6.1 mg. d.	0 %	20 (most died sooner)	-
Sterile medium with 0.25% cholesterol	40.9% c.	17.1 mg. (4) (15.0-19.5)	80.4% (4) (73.3-96.4)	7.8 (4) (7-9)	15.0 (4) (14-17)
Sterile medium with 0.50% cholesterol	62.1% (7) (51.6-76.6)	16.9% (7) (16.2-19.0)	75.0% (7) (50.0-93.7)	no data	16.0 (7) (15-17)

a. - Number in brackets indicates the number of flasks

b. - Numbers in brackets indicate range of values observed

c. - An unknown number of larvae escaped

d. - Only two pupae are involved.

TABLE XXIX

Oocyte development in females fed sucrose solution; a comparison of females reared on CSMA and on a sterile medium containing 0.50% cholesterol

Days after adult emergence	Average length of oocytes (mm.)		Length of largest oocyte (mm.)		% of females showing advanced stages of yolk deposition	
	CSMA	Sterile medium	CSMA	Sterile medium	CSMA	Sterile medium
6	0.29	0.28	0.34	0.38	0	0
8	0.25	0.33	0.31	0.80	0	3.4
10	0.24	0.22	0.25	0.24	0	3.4
12	0.24	0.32	0.28	0.83	0	6.9
14	0.24	0.28	0.30	0.51	0	8.6

TABLE XXX

Viability of eggs laid by females on an incomplete synthetic adult diet; a comparison of females from larvae reared on CSMA and on sterile medium containing 0.50% cholesterol

Larval medium	Cylinder no.	Percentage of viable eggs per cycle			
		1		2	
		egg count	larval count	egg count	larval count
CSMA	1	29.3%	20.5%	16.2%	13.3%
	2	47.0%	44.1%	1.4%	0.8%
	average	36.9%	30.6%	6.8%	5.3%
sterile medium with 0.50% cholesterol	1	27.4%	17.8%	1.9%	1.6%
	2	47.0%	44.1%	39.8%	36.5%
	3	40.1%	36.1%	26.8%	20.3%
	average	37.5%	30.2%	28.2%	25.3%

Fig. 1. Rearing flask for axenic culture of larvae.

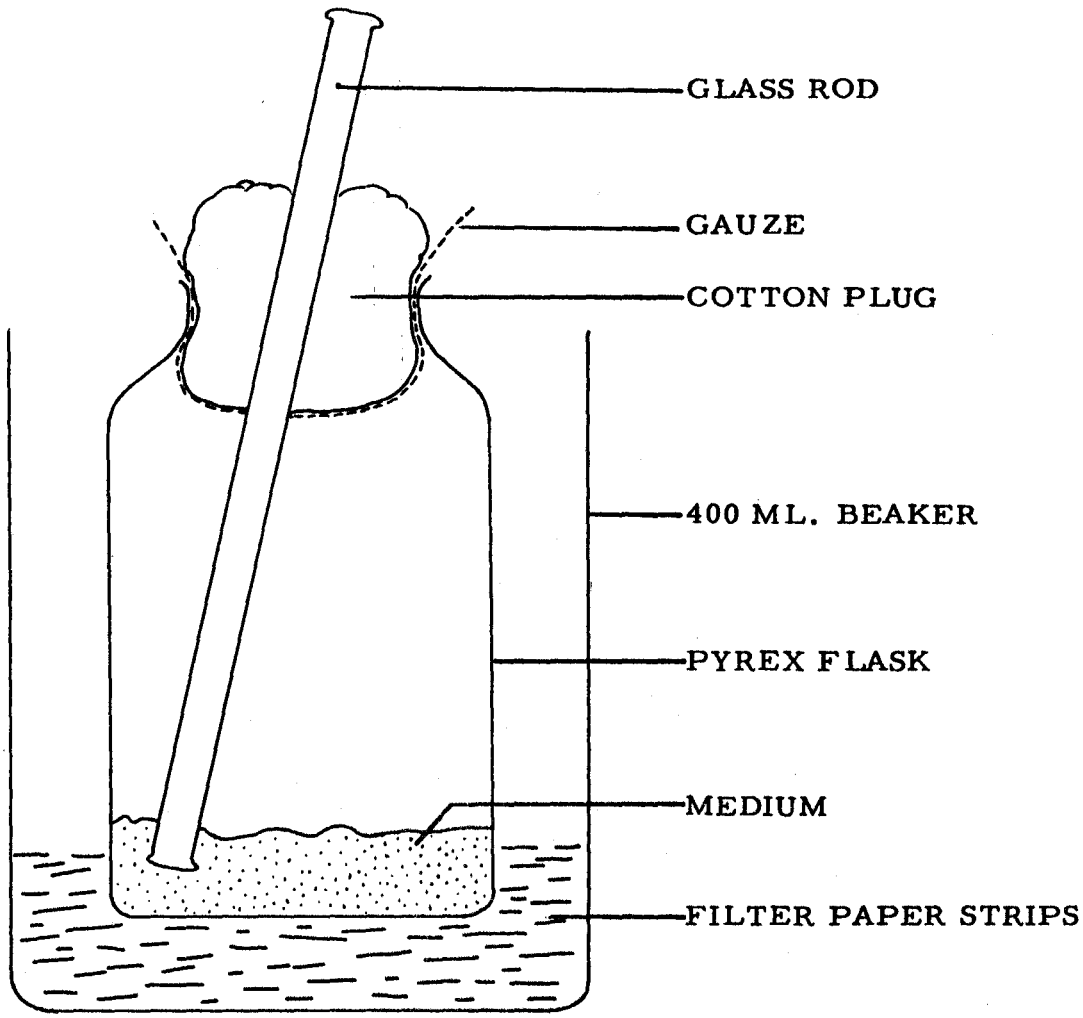
GLASS ROD
GAUZE
COTTON PLUG
400 ML. BEAKER
PYREX FLASK
MEDIUM
FILTER PAPER STRIPS

Fig. 2. Average number of eggs developed per female in each ovarian cycle; a comparison of flies reared from CSMA medium with those from standard sterile medium. First experiment.

a. Adults fed milk

b. Adults fed complete synthetic diet

c. Adults fed incomplete synthetic diet.

Fig. 3. Second experiment (similar to first experiment). Adults fed incomplete synthetic diet.

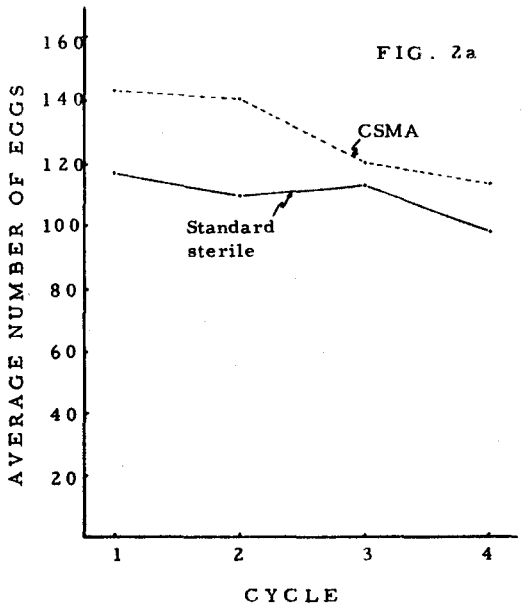
FIG. 2a
AVERAGE NUMBER OF EGGS
160
140
120
100
80
60
40
20
CSMA
Standard sterile
1
2
3
4
CYCLE

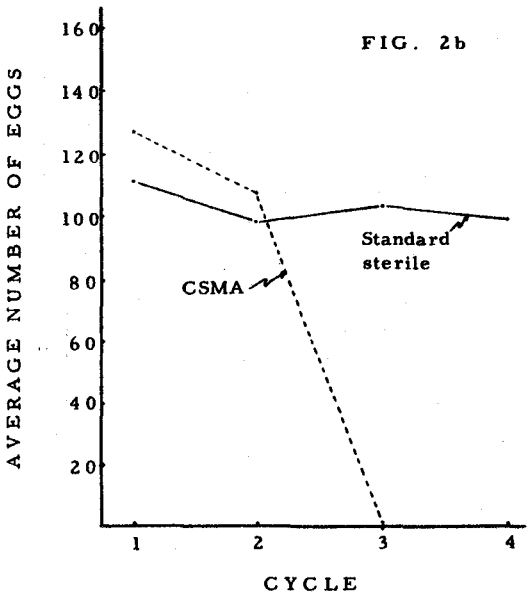
FIG. 2b
AVERAGE NUMBER OF EGGS
160
140
120
100
80
60
40
20
Standard sterile
CSMA
1
2
3
4
CYCLE

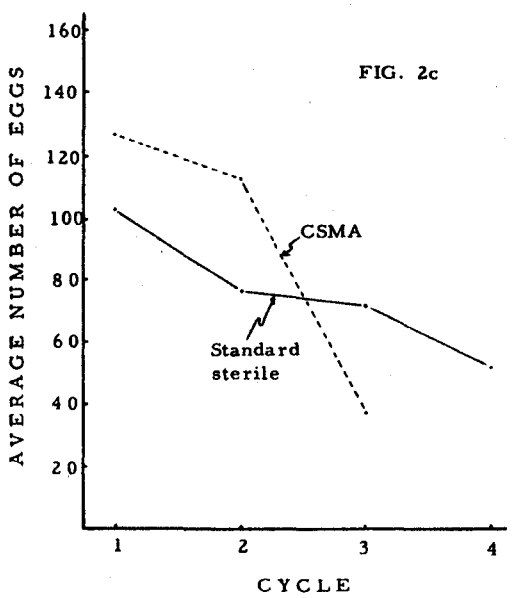
FIG. 2c
AVERAGE NUMBER OF EGGS
160
140
120
100
80
60
40
20
CSMA
Standard sterile
1
2
3
4
CYCLE

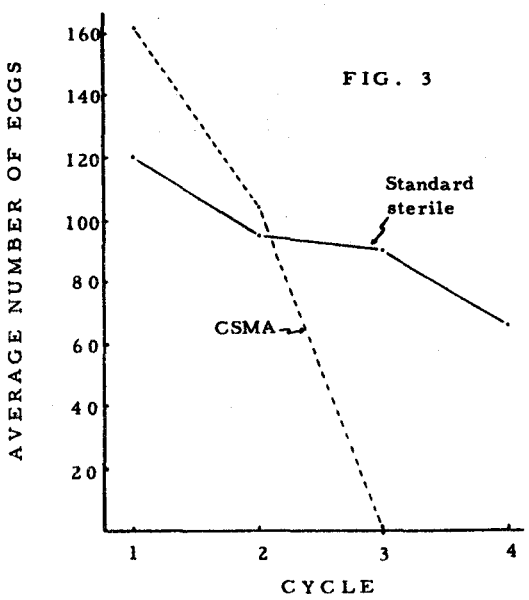
FIG. 3
AVERAGE NUMBER OF EGGS
160
140
120
100
80
60
40
20
Standard sterile
CSMA
1
2
3
4
CYCLE

Fig. 4. Average number of eggs laid per living female; a comparison of flies reared from CSMA medium with those from a standard sterile medium lacking oleate.
Adults fed milk.

Fig. 5. Survival of adults; a comparison of females reared from sterile media containing 0%, 0.5%, and 2.0% sodium oleate.

Fig. 6. Percentage of living females laying eggs in each cycle; a comparison of flies reared from sterile media containing 0.0%, 0.5% and 2.0% sodium oleate.

FIG. 4

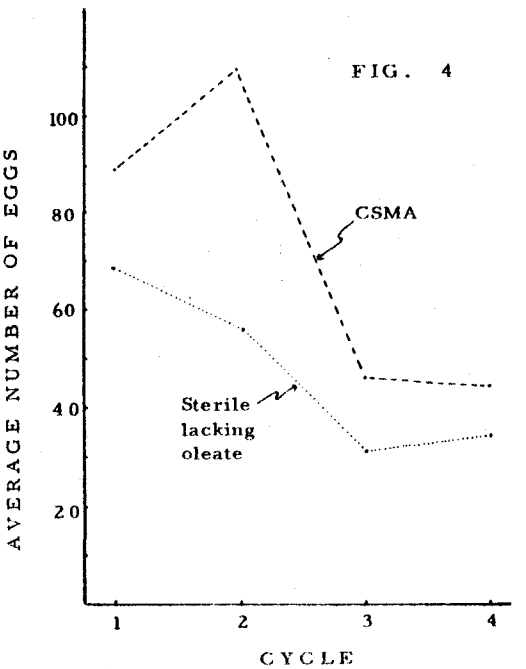

FIG. 5

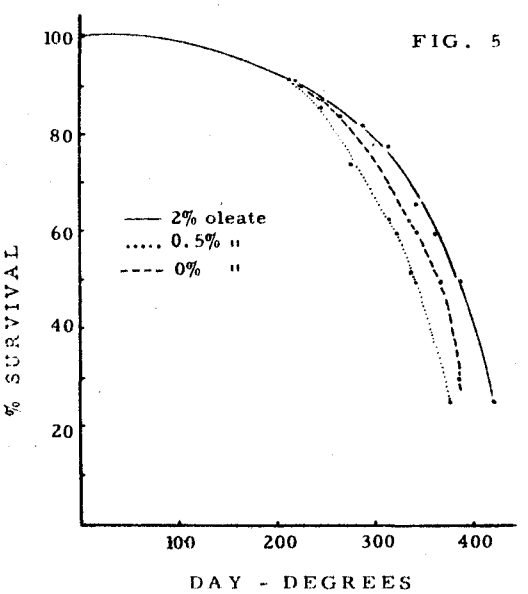

FIG.. 6

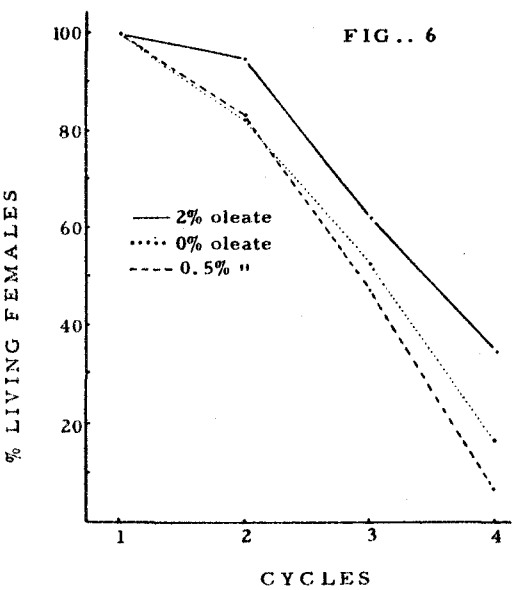

Fig. 7. Average number of eggs laid per living female in each ovarian cycle; a comparison of flies reared from sterile media containing 0.0%, 0.5% and 2.0% sodium oleate. Adults fed incomplete synthetic diet.

Fig. 8. Average number of eggs developed per female in each ovarian cycle; a comparison of flies reared from sterile media containing 0.0%, 0.5% and 2.0% sodium oleate. Adults fed incomplete synthetic diet.

FIG. 7

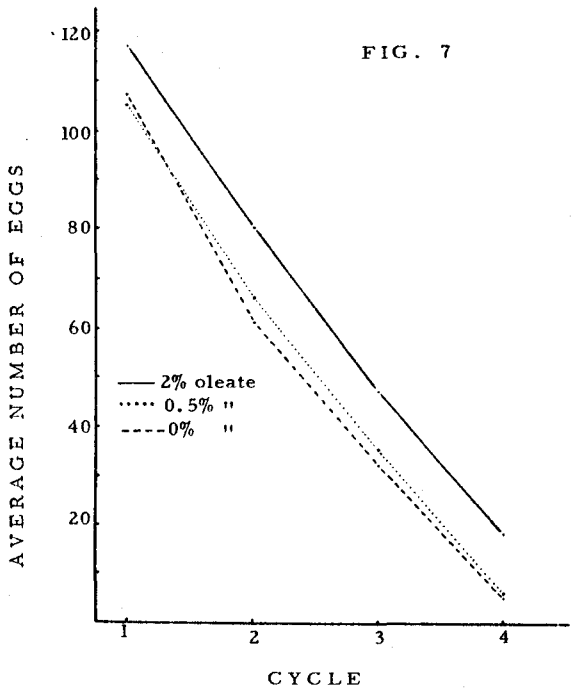
AVERAGE NUMBER OF EGGS
120
100
80
60
40
20
2% oleate
0.5% "
0% "
1
2
3
4
CYCLE

FIG. 8

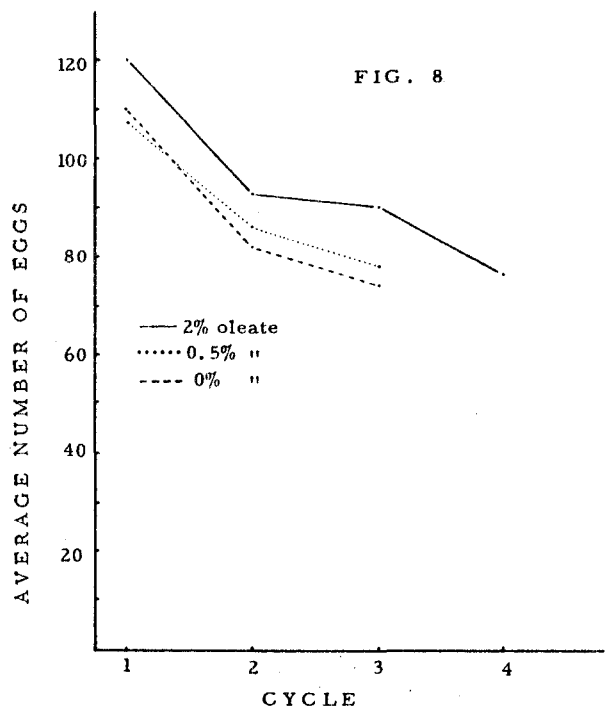
AVERAGE NUMBER OF EGGS
120
100
80
60
40
20
2% oleate
0.5% "
0% "
1
2
3
4
CYCLE

Fig. 9. Average time required to develop and lay each cycle of eggs: a comparison of females reared from standard sterile medium with those from sterile medium lacking RNA.

a. Adults fed milk

b. Adults fed complete synthetic diet

c. Adults fed incomplete synthetic diet.

NOTE: The figures plotted are the averages for the time of laying each cycle of eggs. In 9c, seven females laid a first cycle whereas only one female laid a second cycle; this female laid the first cycle after 4 days, then the second cycle after 7 days.

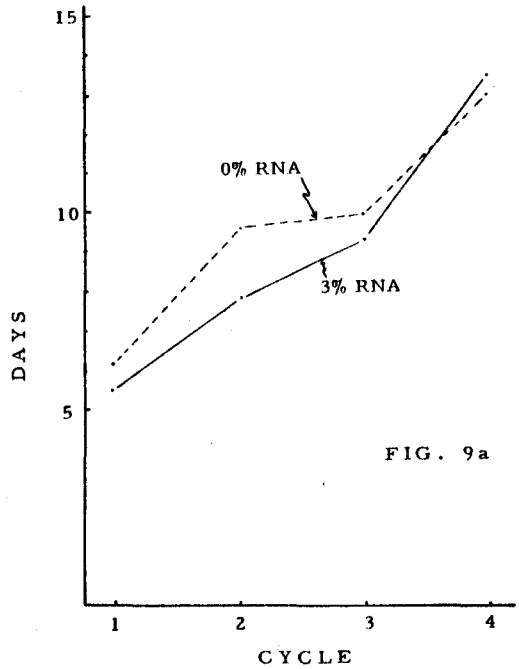

FIG. 9a

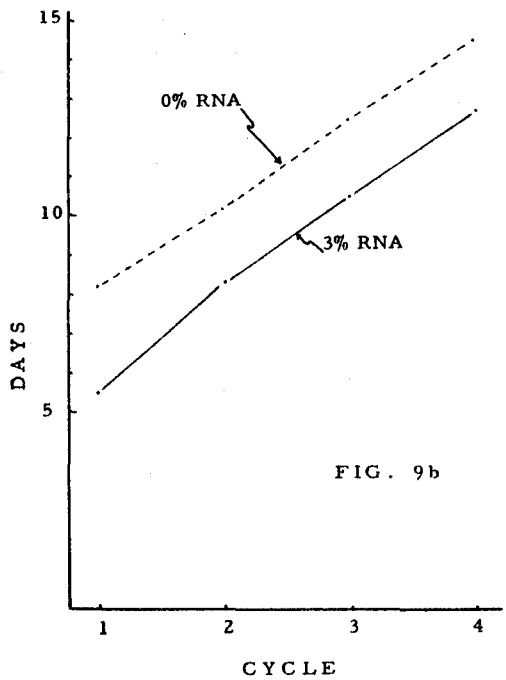

FIG. 9b

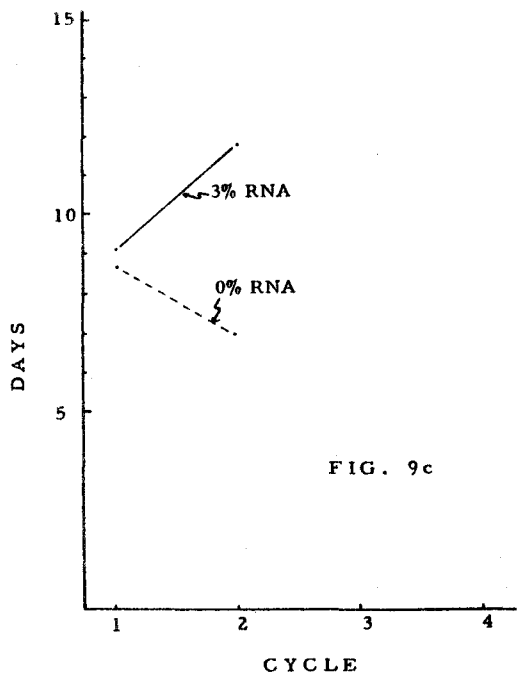

FIG. 9c

Fig. 10. Percentage of living females laying eggs in each ovarian cycle: a comparison of flies reared from standard sterile medium with those from sterile medium lacking RNA.

a. adults fed milk

b. adults fed complete synthetic diet

c. adults fed incomplete synthetic diet.

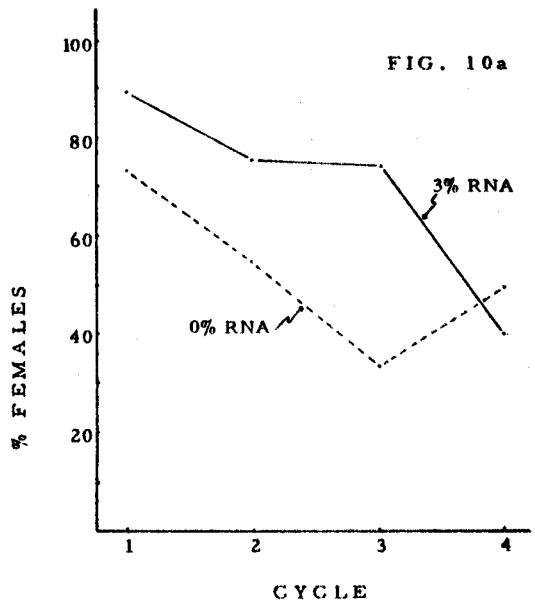

FIG. 10a

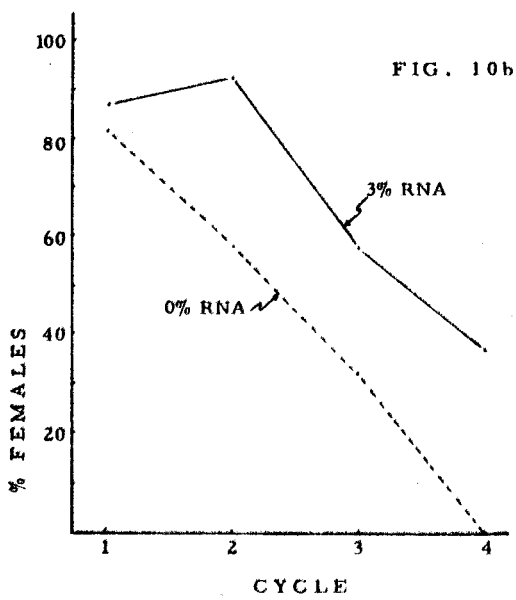

FIG. 10b

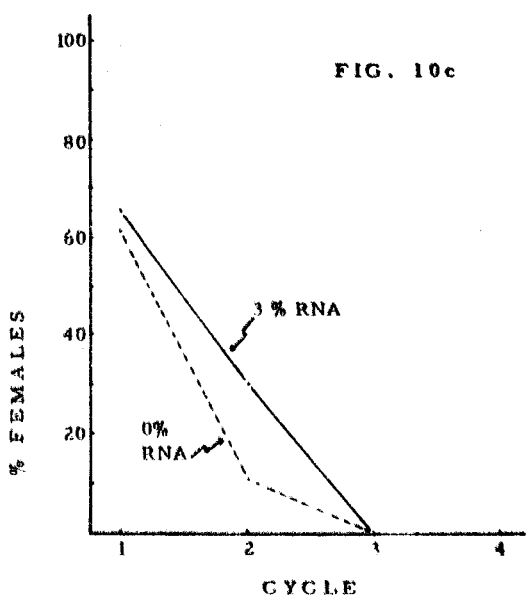

FIG. 10c

Fig. 11. Average number of eggs laid per living female in each ovarian cycle: a comparison of flies reared from standard sterile medium with those from sterile medium lacking RNA.

a. adults fed milk

b. adults fed complete synthetic diet

c. adults fed incomplete synthetic diet.

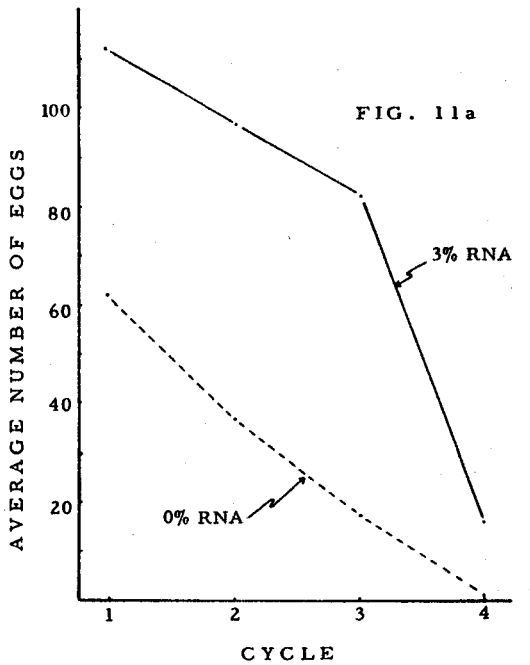

FIG. 11a

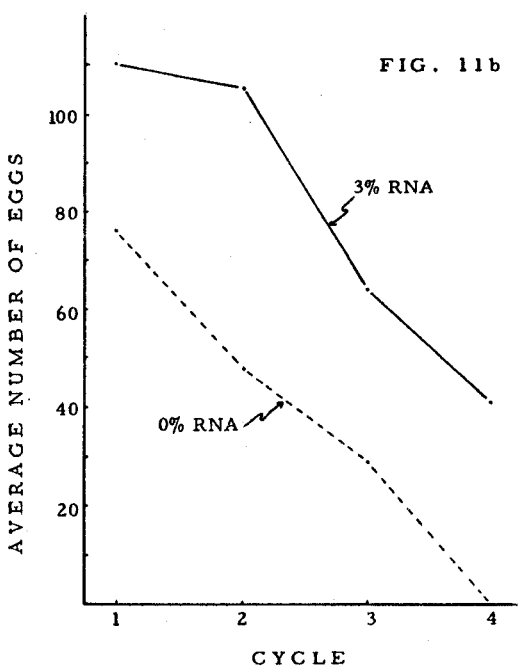

FIG. 11b

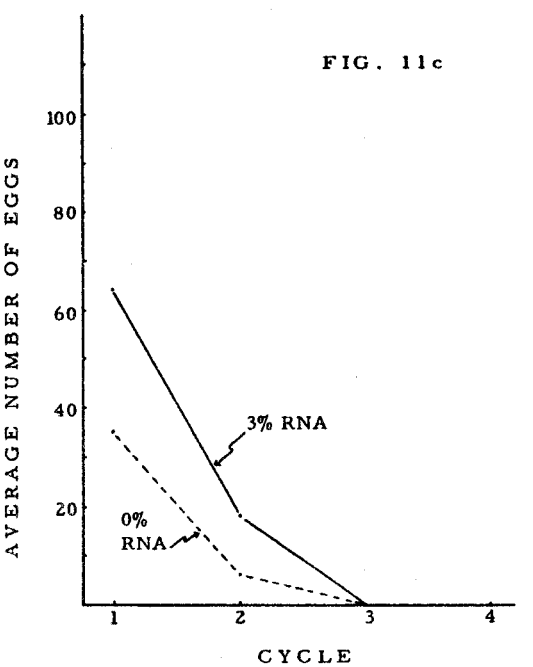

FIG. 11c

Fig. 12. Average number of eggs developed per female, in each ovarian cycle: a comparison of flies reared from standard sterile medium with those from sterile medium lacking RNA.

a. adults fed milk

b. adults fed complete synthetic diet

c. adults fed incomplete synthetic diet.

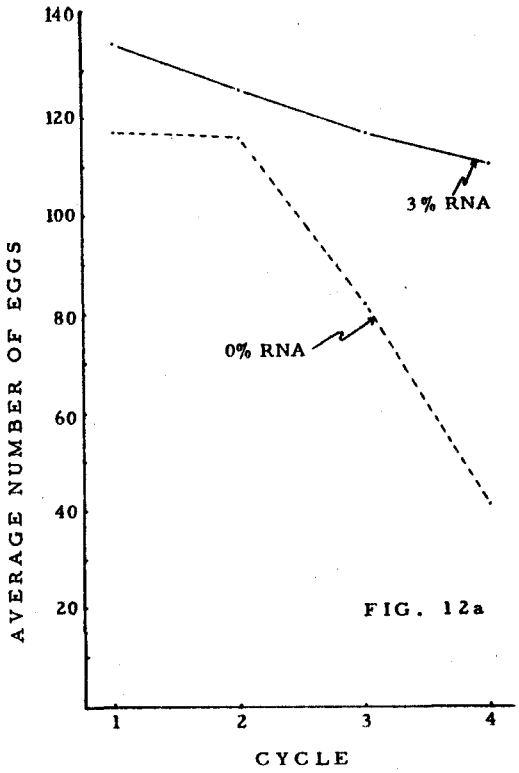

FIG. 12a

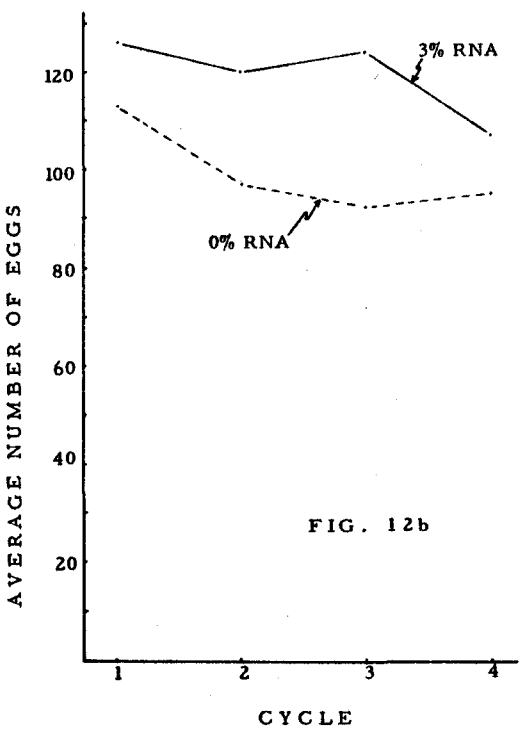

FIG. 12b

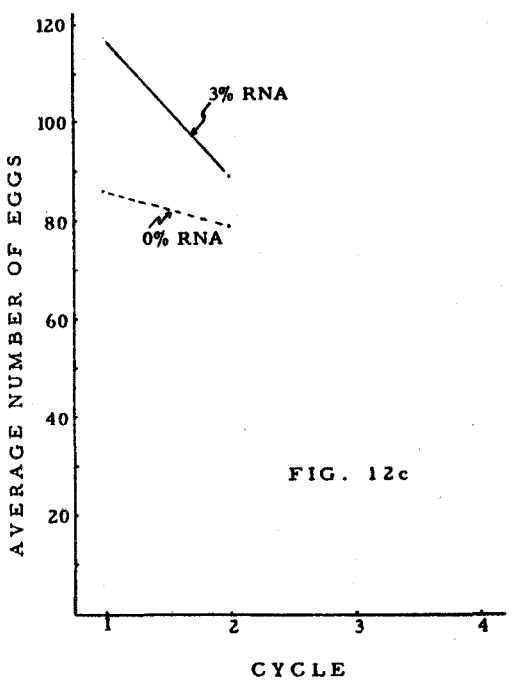

FIG. 12c