NUTRITION AND OÖGENESIS IN THE ADULT HOUSE FLY

THE FIRST AND SUBSEQUENT OVARIAN CYCLES OF THE HOUSE FLY, *MUSCA DOMESTICA* L., IN RELATION TO CHEMICALLY DEFINED NUTRITIONAL REQUIREMENTS OF THE ADULT

By

PAUL ELMOR MORRISON, M.Sc.

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

May 1963

DOCTOR OF PHILOSOPHY (1963) McMASTER UNIVERSITY
(Biology) Hamilton, Ontario

TITLE: The first and subsequent ovarian cycles of the house fly, Musca domestica L., in relation to chemically defined nutritional requirements of the adult.

AUTHOR: Paul Elmor Morrison, B.Sc., M.Sc., (University of Western Ontario)

SUPERVISOR: Professor D. M. Davies

NUMBER OF PAGES: vii, 180

SCOPE AND CONTENT:

A study of the nutritional requirements of adult house flies has shown that liquid diets of natural and purified products are capable of supporting continued oögenesis.

Chemically defined liquid diets containing nine l-amino acids fed to newly emerged female flies were necessary for ovarian maturation and oviposition. Water, salts and carbohydrate were the other basic dietary requirements for this process and for survival. The addition of cholesterol to this synthetic diet also influenced fecundity.

For the maturation and oviposition of more than one ovarian cycle the synthetic diet had to be supplemented with the amino acid l-methionine, certain B-vitamins and nucleic acid bases. Flies fed diets deficient in these supplements showed a lower fecundity and survival.

A dry synthetic diet was developed which supported egg production.

ACKNOWLEDGMENTS

I wish to acknowledge my deepest thanks to Dr. D. M. Davies for his supervision and generous assistance throughout this study, and to Dr. P. F. Nace and Dr. I. D. Spenser for their continued interest and advice. I am indebted to Dr. D. B. DeLury, Head, Department of Mathematics, University of Toronto, and to Dr. J. D. Bankier, Professor of Mathematics, McMaster University, for their advice in the statistical treatment of the data. To Miss M. Moule and Mrs. T. Goodman particularly, and also to others, I am grateful for technical assistance. I acknowledge with gratitude the encouragement and assistance given by my wife during this study and in the preparation of this thesis.

I am indebted to the Department of Biology for an assistantship and to the Ontario Research Foundation for scholarships.

TABLE OF CONTENTS

Page

DESCRIPTIVE NOTE ii

ACKNOWLEDGMENTS iii

TABLE OF CONTENTS iv

INTRODUCTION 1

METHODS AND MATERIALS 23

Constancy of the Fly Strain and Larval Medium 23

Preparation of Larval Medium 24

Seeding the Culture Medium with Eggs 24

Larval Rearing 27

Separation of Pupae 27

Stock Cages of Adults 28

Experimental Adult Flies 29

Cleaning of Experimental Apparatus 30

Preparation of Diets 32

Dissection and Measurements of Ovaries 36

Histological Techniques 38

RESULTS

Part I Determining Experimental Standards and Ovarian Development in Relation to Undefined Diets 39

[a] The appropriate ratio of males to females in experiments 39

RESULTS (Continued)

Part I Determining Experimental Standards and Ovarian Development in Relation to Undefined Diets (Continued)

[b] Variation in fecundity of individual milk-fed females 41

[c] Rate of ovarian development in adult females fed milk or sugar-water diets 42

[d] Some morphological changes in the ovary during the first gonotrophic cycle 43

[e] Delayed presentation of the milk diet to newly emerged flies and the effect on fecundity and survival 46

[f] Fecundity and survival in relation to adult diets of yeast extract, two purified preparations of casein, and milk 47

Part II Egg Production, the Essential Amino Acids and Other Dietary Supplements 50

[a] The need for salts, a carbohydrate and water in the adult diet 50

[b] Determination of the amino acids necessary for ovarian maturation 51

[c] The substitution of nitrogen compounds for glutamic acids in the defined diet 53

[d] The effect of eliminating cystine or methionine in the synthetic diet 54

RESULTS (Continued)

Part II Egg Production, the Essential Amino Acids and Other Dietary Supplements (Continued)

[e] The role of the sulphur-containing amino acid, methionine, in egg production 55

[f] Variation in fecundity of groups of females fed on amino acid diet C or milk 57

Part III The Role of Supplementary Nutritional Factors on the First and Subsequent Gonotrophic Cycles 57

[a] The fecundity of individual females fed on amino acid diet C or milk 58

[b] The value of cholesterol added to amino acid diet C 59

[c] The value of eight B-vitamins added to amino acid diet C 60

[d] The value of combining cholesterol, B-vitamins and ribonucleic acid with the amino acid diet C 62

[e] The importance of vitamins in amino acid diet D for repeated oögenesis and female survival 64

[f] The replacement of ribonucleic acid by nucleic acid bases in diet D and its effect on repeated oögenesis and female survival 68

RESULTS (Continued)

Part III The Role of Supplementary Nutritional Factors on the First and Subsequent Gonotrophic Cycles (Continued)

[g] Feeding dry chemically defined diets to adult flies 71

DISCUSSION 74

SUMMARY 106

PLATES 108

TABLES 124

FIGURES 160

REFERENCES 167

INTRODUCTION

In the last decade research on insect nutrition has been increasing. Earlier studies were concerned with the effect of natural diets on growth and development of the rapidly growing immature stages but the more recent research has emphasized the use of defined diets. This is evident from the reviews in this field (Uvarov, 1928; Craig and Hoskins, 1940; Brues, 1946; Wigglesworth, 1950; Trager, 1953; Lipke and Fraenkel, 1956; Fraenkel, 1959; and House, 1958, 1961).

There exist few studies on the nutritional requirements for insect reproduction and those that have been conducted are almost exclusively confined to the Diptera. However much work has been done on the nutritional requirements for growth and development of the immature stages. Since the environment of the immature stages ultimately affects egg production in the adult females, a review of the major studies on the nutrition of immature as well as of mature insects is presented.

The number of eggs laid by the adult female ('fecundity' as used by Robertson and Sang, 1944; and Hagen, 1958) change under varying conditions, and further a part of the environment, the diet of the adult, may be considered as a major influence. This influence may act directly, or indirectly through its effect on the survival of the adult. However, as Wigglesworth (1950) and Trager (1953) concluded, fecundity may be greatly influenced by the nutrition of the immature stages. Alpatov (1932) showed that underfed fruitfly (Drosophila) larvae had a reduced number of eggs. Boissezon (1934) observed that larval

nutrition of the mosquito, Culex pipiens L., has a marked influence on adult fecundity. Webber (1955) also noted that the sheep blow fly, Lucilia cuprina (Wied.), laid fewer eggs when the larval food was restricted, and Mellanby (1938) observed that the fecundity of another blow fly, L. sericata Meig., is greater in larger flies. These larger adults can be obtained if the larvae drink water before pupation. Similarly Golightly (1940) reported that for two species of Psychoda, if food supply is quantitatively limited, the larvae will develop into small adults with a potential egg output lower than would occur in larger flies reared under better feeding conditions. Adkisson et al (1960) have recently shown that one artificial diet fed to the pink bollworm larva, Pectinophora gossypiella (Saund.), can produce a more fecund female than another diet which is efficient in producing a few more adults. In Anopheles elutes Edw., ovarian development up to stage 2 can be achieved either at the expense of reserves or by feeding on raisins (Mer, 1936). However, with poor larval food, development to stage 2 was impaired, and a single blood meal was insufficient to complete oögenesis. Hecht (1933) reported that with certain races of Culex pipiens the unfed female will produce eggs if the larvae receive a diet rich in proteins, but no eggs will develop on a predominately carbohydrate diet. Thus autogeny and anautogeny in the same species may be influenced by the adequacy of the larval diet, and the quality of the larval diet will determine the level of egg production.

It was soon recognized by those interested in nutrition and reproduction that a wide range of nutritional requirements for the adult exist in holometabolous insects. However, generally the adult male

insect is less affected by nutrition (Wigglesworth, 1950). In fact, many reach the adult stage with the testes and the spermatozoa developed or almost fully developed. Yet Buxton (1930) found that the males of Rhodnius appear less fertile if they are starved in the adult stage. Christophers (1960) concludes that in the male Aedes aegypti (L.) the formation of new sperm after successive matings appears to depend on either reserves or the food fed. Hagen (1952) has shown for the oriental fruit fly, Dacus dorsalis Hendel, that only after protein and minerals are ingested along with carbohydrate are the males able to copulate. These reports disagree with the unqualified statement by Trager (1953) that it is only among female insects that special nutritional requirements for reproduction have been observed, that is, in addition to dietary needs for energy. Much more information is needed to clarify the effect of age and nutrition on male fecundity and ultimately on female reproduction. It is these apparent conflicts which serve to emphasize the paucity of information on insect reproduction.

The great differences existing in the nutritional needs for reproduction among adult female insects have been noted repeatedly (Wigglesworth, 1950; Trager, 1947; Roeder, 1953). Some insects have simple requirements for food during the adult stage as evidenced by the saturniid moths and cattle grubs which take neither food nor water during the adult stage and yet develop hundreds of eggs to maturity (Roeder, 1953 and Scharff, 1950 respectively). The adult phycitid moth, Ephestia kuhniella Z. needed only water, and the absence of a carbohydrate (sugar) had no effect on fecundity; also two related species E. cautella Wlk. and E. elutella Hb. required only water without which

fecundity was about halved (Norris, 1934). The autolysis and mobilization of body tissues in the adult may also eliminate the need of a protein meal for ovarian development. For example a northern species of mosquito, Aedes communis (DeG.), and Mochlonyx culiciformis (DeG.) are considered to provide nitrogen from the autolysis of the adult flight muscle to ensure ovarian development (Hocking, 1952, 1954). However Beckel (1954) considered that A. cummunis utilized mainly those reserves stores in the fat body for egg development. Recently it was observed that embryonic development in alate aphids is unaffected by products of muscle breakdown, yet ovary development is considered to be associated with these products in ants and termites (Johnson, 1957).

In most insects however, the adult female requires a protein diet for egg production. For example, as early as 1923 Glaser reported that the house fly, Musca domestica L., and the blow flies, Calliphora erythrocephala (Meig.) and Phormia regina (Meig.) all required a protein meal if eggs were to develop. Kobayashi (1934) and Derbeneva-Ukhova (1935) both confirmed that a protein was essential for reproduction in the adult house fly. This protein requirement for egg production has been established for the mosquito, Aedes aegypti (Yeoli and Mer, 1938), the fruit fly, Drosophila melanogaster (Meig.) (Robertson and Sang, 1944; Bodenstein, 1947), the apple maggot, Rhagoletis pomonella (Walsh) (Dean, 1938), the oriental house fly, Musca vicina (Macq.) [1] (Ascher and

---

[1] Now known as Musca domestica vicina Macquart, Bull. Ent. Soc. Am. 1960. 6:185 and 201.

Levinson, 1956) and the blow flies, Protophormia terrae-novae (R.D.) (Harlow, 1956), Lucilia sericata (Mackerras, 1933; Hobson, 1938), Phormia regina (Rasso and Fraenkel, 1954).

The fact that the house fly, M. domestica, exhibits indiscriminate feeding habits followed by the development of mature eggs had been noted by Hewitt (1914) and West (1951). From the literature, it is evident that the food of the adult house fly was important in two main ways: (1) for the maintenance of body functions necessary in survival and (2) for those requirements essential in development and maturation of the reproductive products. Glaser (1923), Derbeneva-Ukhova (1935), and Kobayashi (1934) all confirmed that the house fly required an assimilable carbohydrate, in the form of a sugar or starch if the fly was to survive. There is little evidence that carbohydrates play an essential role in insect reproduction (House, 1958) but they may be important to egg production through their effect on survival. On the other hand House (1958) concluded that proteins, or products of protein hydrolysis, were needed if insect eggs were to mature. Furthermore, the rate of ovarian development in the house fly was shown to vary with the type of food ingested (Derbeneva-Ukhova, 1935). Not only is the right food important for reproduction but Tischler (1931) also found that the number of times the adult house fly feeds is important for survival. Fecundity and survival of the adult house fly are then closely related.

The type of protein required varies in different insects, and insects normally feeding on a specific diet show varied responses when changes are made in the normal diet. It has been found that diets

differing from the normal, although allowing for varying levels of fecundity, are seldom as successful as the natural diet. Woke (1937) reported that for egg development in the blood-feeding mosquito, Aedes aegypti, the ingested protein could be in many forms, either as washed blood cells, serum, or whole blood. Lea et al (1956) have shown that A. aegypti will also lay eggs after a meal of milk, casein, or egg albumin, but the number of eggs were fewer than when whole blood was the protein source. Glaser (1923) showed that the blood-feeding stable fly, Stomoxys calcitrans (L.), only laid eggs when on a diet of whole blood or defibrinated blood, and not on a diet of serum or washed whole blood cells alone.

The type of host also has a bearing on fecundity in blood-sucking mosquitoes. Tate and Vincent (1936) observed that when adult female mosquitoes, Culex pipiens, fed on various vertebrates, differences in egg production occurred. On man, fewer eggs were produced than when the mosquitoes fed on rats, rabbits, or guinea pigs. More recently, Jordan (1961) reported similar differences in the quality of blood and egg production for the mosquito, C. quinquefasciatus Say. She found that the source of blood had a bearing on the average number of eggs produced; fewest eggs and egg rafts were laid when feeding was on the hog-nosed snake (Heterodon platyrhinos Latrielle) while the greatest number of eggs came from mosquitoes fed on the domestic chicken.

The amount of food taken by the adult influences the number of eggs laid. Working with another mosquito, Aedes aegypti, Roy (1936) found that the female required at least 0.82 mg of blood at a meal to produce eggs. More eggs were laid, however, if the mosquito ingested

a larger quantity of blood, an average of 2.0 mg. per meal. Woke et al (1956) also established that A. aegypti feeding on human blood showed a marked increase in egg production if successively larger quantities of blood were ingested. He also found that, if the mosquitoes took their blood meal 28 days after emergence, significantly fewer eggs (a mean of 56 eggs) were laid than if they took blood 5 or 14 days after (a mean of 89 and 86 eggs respectively). From the above at least two factors, the quality and quantity of the ingested food, are considered to have a direct bearing on fecundity in the mosquito.

To appreciate present concepts and recent advances in insect nutrition it is desirable at this stage to present a short discussion of early work on protein and amino acid metabolism in animals.

As is evident from the literature the relation of protein and amino acid metabolism to growth has received considerable attention, more than any other field of nutrition. The first major advances in the metabolism of proteins and amino acids were made at the beginning of this century by Osborne and Mendel (1917) and Rose (1937). The early observation that some proteins failed to support growth, substantiated the implication that these proteins also failed to provide the constituents for the synthesis of tissue proteins (Fisher, 1954). However it was not long before a direct relationship between diet and nitrogen metabolism was demonstrated in the animal and this relationship was related to changes in cytoplasmic proteins (Addis et al, 1936; Luck, 1936). They showed that the protein content of an organ (liver) reflected the immediate diet. From the effect that certain dietary proteins had on growth, these proteins became classified nutritionally

as complete or incomplete. Incomplete proteins were only able to support growth when certain amino acids were added to the diet. It was these amino acids that were designated as "essential, or indispensable amino acids" (Osborne and Mendel, 1917; Fisher, 1954). The means of determining the value of these dietary proteins were made by measuring animal growth, longevity, erythrocyte number, change in tissue protein, urinary urea and urinary amino acids.

The certainty that amino acids could replace proteins in the diet and provide adequate growth awaited the contribution of Rose (1937). It is now accepted that the amino acids required for growth were needed mainly for the synthesis of proteins (Mitchell, 1959). Rose (1937) was the first to demonstrate that certain amino acids could be considered as essential or non-essential for normal rat growth. In this respect the 10 found essential were arginine[2], histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Rose, 1937, 1938). Of these the adult rat does not require arginine. In comparison to the young rat, the young chick requires in addition to the above 10, the amino acid, glycine (Almquist, 1959), while the adult human does not require arginine and histidine for nitrogen balance (Berg, 1959; Frost, 1959). It is apparent then that this requirement for amino acids, whether in vertebrates or insects (Gilmour, 1961), depends upon the physiological process being measured, and the concept implied by the term "essential amino acid" is dependent upon the criterion

---

[2] Arginine can be synthesized by the young rat but not at a sufficient rate for normal growth (Rose, 1937, 1938).

employed. Thus the classification of "essential" or "indispensable amino acid" is distinctly a matter of definition (Rose, 1938).

Although growth of young rats fed these 10 essential amino acids occurred, it was found that growth was slow. The fact that an increased rate of growth was possible when the essential amino acids were supplemented with ammonium citrate, ammonium acetate or l-glutamic acid suggested to Rose et al (1949) that some conversion of these to the non-essential amino acids occurred. Significant but smaller increases in growth were also produced by urea and glycine supplements. Frost (1959) has pointed out in his review that to replace all other non-essential amino acids, glutamic acid is an efficient source of nitrogen. It was evident however that the conditions of experimentation are important in assessing the utilization of non-specific nitrogen (Frost, 1959). One conclusion, based on the addition of a non-specific nitrogen source to an amino acid diet, has been that the animal can synthesize most or all the other required non-essential amino acids from a suitable nitrogen supply (Fisher, 1954; Frost, 1959).

In recent years, by use of chemically defined synthetic diets, it has been established that certain amino acids are also essential for complete insect growth. These amino acids found essential, in a wide representation of insects, have been tabulated by Albritton (1954) and the more recent reviews summarizing the amino acid requirements for insect growth have also been presented by Wigglesworth (1950), Roeder (1953), Lipke and Fraenkel (1956), Levinson (1955), and House (1961). From the information presented by these authors it is evident that insects usually require the same 10 amino acids classified as essential for

growth in the rat (Rose, 1937; Maddy and Elvehjem, 1949). This substition of amino acids for proteins, required by the rat for growth, did not necessarily establish that the same amino acids were needed by all insects. It is known, and Williams (1959) has emphasized, that there exist fundamental qualitative and quantitative differences in the amino acid needs of an organism because of age or individual variation. It is necessary then to ascertain whether and which amino acids are satisfactory for the process of reproduction in insects.

For reproduction in the mosquito, Aedes aegypti, the work of Dimond et al (1956) indicated that a mixture of 12 amino acids: arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and glutamic acid along with a carbohydrate (sucrose) and salts, were all that was required by the mosquito, Aedes aegypti, for reproduction. Dimond (1957) considered that the inclusion of glutamic acid in the diet acted principally as an additional nitrogen source which contributed to the synthesis of non-essential amino acids. He found that the stimulatory action of glutamic acid on egg production could be matched by aspartic acid or ammonium acetate.

As for the amino acid cystine it was found that although not absolutely essential, it was required to maintain a high level of fecundity (Dimond et al, 1956; Dimond, 1957). Because of this, cystine was considered essential for fecundity. In fact he and his colleagues were unable to obtain eggs when arginine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan or valine were eliminated individually from the amino acid diet mixture; when histidine, cystine, methionine and glutamic acid were omitted, a significant decrease in

fecundity occurred although a few eggs were laid.

In contrast, the work of Singh and Brown (1957) indicated that only 10 of the above amino acids were required for reproduction in this mosquito and that the omission of cystine and glutamic acid were without effect on reproduction. They were concerned only with the production of eggs, however, and not a high level of egg production.

At first glance, the simple fact that amino acids were the principal dietary requirements for egg production seems striking. In comparison, Harlow (1956) concluded from her work on the blow fly, Protophormia terrae-novae, that a mixture of 19 amino acids substituted for a homogenized protein diet was inadequate for maturation of the ovaries. Some other accessory factor(s) was required. Of the amino acids she used to substitute for protein, the 10 essential amino acids required for mammal and insect growth were included. Hagen (1952) found that for the oriental fruit fly, Dacus dorsalis, egg production followed when a mixture of 13 amino acids[3], plus minerals and a carbohydrate, were fed to the adults. There was no reference to which of the 13 amino acids were absolutely essential for egg production in this fruit fly. Although it appears that the same 10 amino acids are required by insects for growth as by mammals, it is not clear whether the same amino acids are the only requirements for continued reproduction in insects. It would not be surprising to find that for insect reproduction these 10 amino acids were either all that was required or the basic amino acid

---

[3] Arginine, cystine, isoleucine, leucine, lysine, methionine, histidine, phenylalanine, threonine, tryptophan, tyrosine, valine, and glutamic acid.

requirements of any synthetic diet for reproduction.

In addition to the inclusion of proteins or amino acids in the adult diet a number of dietary supplements have been found to contribute to the fecundity of insects. Considerations of the specific role that minerals play as requirements for reproduction have been few (House, 1958). In 1934, Boyce reported that the walnut husk fly, Rhagoletis completa Cresson, showed an increase in fecundity and longevity when zinc and copper were added to the adult diet. Akao (1935) found that zinc had an important role in egg formation in the silkworm, Bombyx mori L. Dean (1938) working with the adult of the apple maggot, Rhagoletis pomponella, similarly found that potassium phosphate slightly increased egg production, but a mixture of calcium, sodium, iron and zinc chlorides, magnesium sulphate and potassium phosphate, roughly in the same proportions found in cow's milk, gave the greatest increase. In 1951, Barker and Tauber reported that there was a significant reduction in fecundity of the pea aphid, Macrosiphum pisi (Harris), when this insect was reared on garden peas deficient in sodium, potassium, phosphorus, calcium or magnesium. The greatest reduction in fecundity resulted when nitrogen or phosphorus was lacking whereas a deficiency in potassium, calcium or magnesium was less effective in reducing egg production. For the blow fly, Phormia regina, Rasso and Fraenkel (1954) concluded that potassium phosphate by itself had the same effect as a complete salt mixture in the diet. Ovarian development was unaffected by other individual minerals, such as sodium chloride, potassium carbonate, magnesium sulphate, potassium chloride, and tricalcium phosphate. More recently Finlayson (1961) observed that significant

differences occurred in egg production when different levels of manganese sulphate were added to the diet of the adult hymenopterous parasite, Aptesis basizona (Grav.). However the above author reported that ferric citrate had no significant effect on reproduction in this parasite. It is worth noting that many of the semi-synthetic diets, available or those synthetic diets formulated in the laboratory, contain as impurities sufficient quantities of some minerals to meet or mask the true picture of mineral requirements. This is certainly why investigations on the exact mineral requirements for ovary development are so few. House (1961) considers that in the field of insect nutrition knowledge of the mineral requirements of insects probably remains the most neglected. However, it is probable that minerals have a definite role in the relationship between the adult diet and fecundity.

Since the larval stages of most insects are considered to store much of their energy supplies as lipids (Gilmour, 1961), it is not surprising that lipids have been found to play such an important role in the diets (Trager, 1947). Some of the lipid-soluble factors known to be required by immature or adult insects include the fatty acids, phospholipids and sterols. From reports in the literature, the requirements of immature insects for lipids during growth is not always the same (Trager, 1947).

In regard to the fatty acids, House and Barlow (1960) reported that for the growth of the parasitoid, Agria affinis (Fall.), oleic acid promoted growth, and this requirement was independent of other natural lipids. Yet linoleic and arachidonic acids, but no oleic acid, promoted growth of the moth, Ephestia kuehniella (Fraenkel and Blewett, 1946, 1947). In the pink bollworm, Pectinophora gossypiella (Saund.), linoleic

and linolenic acids had a definite effect on the moth emergence although linolenic acid appeared more beneficial (Vanderzant et al, 1957). Yet linoleic and linolenic acids were not required in the diet by the larvae of Aedes aegypti (Goldberg and DeMeillon, 1948). Recently Dadd (1961b) reported that good growth and development of the locusts, Locusta migratoria L. and Schistocerca gregaria (Forsk.) was only possible when the unsaturated fatty acids, linoleic and linolenic, were supplied in the diet. Yet for larval growth of the oriental house fly (Silverman and Levinson, 1954) and the house fly (Brookes and Fraenkel, 1958) no fatty acids are required.

This lack of evidence supporting the requirement for a specific fatty acid does not exclude the possibility that fatty acids may be required for some other role in the insect, for instance in fecundity or fertility. In this connection, the work of Pepper and Hastings (1943) on the sugar beet webworm Loxostege stiticalis (L.), is of interest. In their analysis of the immature stages of this webworm they showed that linoleic acid was essential as a body lipid for metamorphosis and reproduction. No linoleic acid was found in sterile females when the webworm adults were fed only a diet of water and nectar which lacked any fatty materials. They concluded that lipids were required for growth and the development of fecund females. It is quite clear, then, that insects show varying nutritional requirements for fatty acids.

Although it has been established that many immature insects require a dietary source of lipids for growth and development (Trager, 1947; Albritton, 1954; Levinson and Bergmann, 1957), the substances

known as sterols are, to date, by far the most important lipid soluble factors required by insects. House (1954) and House and Barlow (1960) found that cholesterol promoted larval growth of Agria affinis. The lack of this sterol was most evident during the growth of the second instar. Silverman and Levinson (1954) reported that for the oriental house fly, Musca vicina, the steroids appear to act as both growth and pupation factors. The sterol found in the medium was sitosterol, but cholesterol, cholesterol acetate, and cholest-4en-3-one give the same biological activity. Similarly it has been found that the lipid needed during growth and metamorphosis of the larvae of Aedes aegypti may be supplied by cholesterol or ovolecithin (Goldberg and DeMeillon, 1948). Brookes and Fraenkel (1958) also reported that for larval growth of the house fly cholesterol could satisfy the sterol requirements. Levinson (1960) in his paper on the evolution of sterol requirements in animals concluded that the larvae of insects may be divided into two major groups: the phytophagous insects capable of converting the C_{28-29} sterols found in their food plants to cholesterol, and the carnivora adapted to cholesterol utilization only.

There are few reports in the literature of adult insects requiring lipids for reproduction. In 1948, Grison found that the potato beetle, Leptinotarsa decemlineata (Say), showed an increase in fecundity when young potato leaves were painted with a sucrose and lecithin emulsion. However, the research of Singh and Brown (1957) and Dimond (1957) indicated that for reproduction, the adult mosquito, Aedes aegypti, required no lipid, whether a sterol or fatty acid. Yet, recently Monroe (1959, 1960) reported that for the adult house fly the sterol, cholesterol,

was necessary for the sustained reproduction of viable eggs although the quantity of eggs oviposited was little reduced by a lack of cholesterol in the diet. When the cholesterol concentration was lowered the viability of the eggs decreased. More recently, Monroe et al (1961) established that if the larval medium was supplemented with cholesterol, viable egg production was almost double that found when a non-supplemented medium was used; however, there was no increase in total fecundity. From his observations Monroe (1960) suggested that the female house fly lacks the biochemical mechanisms for sterol biosynthesis. This was confirmed by Robbins et al (1960). Kaplanis et al (1960) substantiated Monroe's observations by injecting 4-C^{14}-cholesterol into adult house flies and establishing that it was efficiently utilized in egg production.

Unsaturated fatty acids in the diet may satisfy the requirements of an insect during growth (Dadd, 1961; House and Barlow, 1960), and of the sterols cholesterol can meet, at different developmental stages, the requirements for a dietary sterol while the need for sterols by insect larvae differs (Levinson, 1960a). Furthermore there are marked differences between insects and vertebrates in their requirements for fat-soluble-factors during growth (Trager, 1947). It is of interest that adults of dipterous insects have as yet shown no requirement for fatty acids during reproduction.

Relatively few reports can be found in the literature that indicate the direct requirement for vitamins by the adult insect for egg production. This is surprising for the water soluble B-vitamins play such an important role in insect growth. It is interesting that

Rasso and Fraenkel (1954) should state that vitamin-free casein was inadequate for ovarian development in the adult blow fly, Phormia regina but the casein's inefficiency was due only to its insolubility in water. They did note, however, that the addition of B-vitamins accelerated egg development. As early as 1944, Robertson and Sang noted that different strains of yeast will influence female fecundity and egg viability of Drosophila melanogaster. They associated these reproductive differences with the different vitamin contents of the yeast strains fed to the fruit flies. Working with the tephritid fruit fly, Dacus dorsalis, Hagen (1952) indicated that there is for the male fly a subtle fertility factor present in the enzymatic protein hydrolysate of soy. He indicated that this fertility factor may be one or more of the tocopherol vitamins.

More recently the use of antimetabolites, and in particular antivitamins, have become one of the standard procedures in the study of nutritional requirements in many organisms (Wooley, 1952). Egg formation was inhibited in Drosophila melanogaster (Goldsmith and Frank, 1952) and Musca domestica (Mitlin et al, 1957) by feeding of 4-aminopteroylglutamic acid, a folic acid antivitamin. Levinson and Bergmann (1959) noted that ovaries were undeveloped in Musca vicina when the flies were fed 4-aminopteroylglutamic acid, 3-acetylpyridine (a nicotinic acid antivitamin), desoxypyridoxine (a pyridoxine antivitamin), neopyrithiamine (a thiamine antivitamin), and pantothenol (the pantothenic acid antivitamin). This use of antivitamins in relation to the nutritional requirements is enlightening, but further research is needed. It would be of interest to compare, during the

active period of oogenesis, the need of an organism for vitamins in the diet and the consequences of feeding antivitamins.

The general list of dietary vitamins required by insects for growth includes thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, pteroylglutamic acid, p-amino-benzoic acid, choline, and inositol (Trager, 1953). It was shown that growth of the beetle, Tribolium destructor, was influenced by the nutritional value of the flour given to the parent (Reynolds, 1945). Individual larvae of this insect fed a wholemeal flour and larvae from parents fed this diet developed more rapidly than those larvae in tests in which a white flour, known to be deficient in B-vitamins, was used. He concluded that the rate of development of the offspring of this grain beetle was influenced by the nutritional value of the food on which their parents fed. The larvae of the flour beetle, Tenebrio molitor L., do not grow if thiamine, riboflavin, pyridoxine, nicotinic acid, or pantothenic acid is omitted from the medium (Martin and Hare, 1942). For sometime then vitamins have been known to be required for insect growth.

Until recently, it was considered that insects required for growth only the water soluble B group of vitamins, while the fat soluble vitamins A, D, E, and K, and the water soluble vitamin C were not required. No nutritional requirement for vitamins other than those of the B group had ever been substantiated (Lipke and Fraenkel, 1956). Yet earlier Day (1949) identified vitamin C histochemically in the tissues of some insects represented by the orders Orthoptera, Coleoptera, and Diptera. Recently Dadd (1957, 1960) was able to show

that the locusts Schistocerca gregaria and Locusta migratoria required vitamin C for growth. Even the vitamin requirements can be shown to vary for an insect. That is, the level of the vitamin, folic acid, required in the diet of Drosophila melangaster for optimum growth depends on the levels of protein (casein) and amino acid (glycine or serine) in the diet.

For egg production the bed bug, Cimex lectularius L., required thiamine and folic acid (DeMeillon and Goldberg, 1946, 1947). They found that this bed bug laid a markedly reduced number of eggs when fed on rats deficient in folic acid and thiamine. From their studies on nutrition and reproduction of the adult mosquito, Aedes aegypti, neither Dimond (1957) nor Singh and Brown (1957) found that vitamins were necessary for egg production. However when B-vitamins were present in the diet fed to Dacus dorsalis adults, longevity was improved (Hagen, 1952). Since the survival and fecundity of an insect are closely interdependent, vitamins were important indirectly to reproduction in this fly. Even from such meager information it is evident that in insects vitamins bear directly on adult reproduction and indirectly influence reproduction through survival.

The requirement for nucleic acids by insects has been shown by those experiments in which growth was the unit of measurement. The first suggestions of nucleic acid requirements came when it was observed that yeast, yeast extract, or wheat-germ extract, were all found to have nutritional value. This was evident early in this century when insects were grown on partially purified diets (Delcourt and Guyenot, 1910; DeMeillon and Goldberg, 1945; Trager, 1947; and

Fraenkel, 1959). It was only when semi-synthetic or synthetic diets were devised that nucleic acids were clearly established as growth factors. That the nucleic acids should be found important for growth might be expected because the rate of growth in insects is extremely rapid and heterogonic (Roeder, 1953). In the house fly, Musca domestica, the larva increases its original weight 54 times in 4 days (Larsen and Thomsen, 1940). Just which part of the nucleic acid molecule, consisting of phosphoric acid, pentose sugars, and heterocyclic bases of purines and pyrimidines, is responsible for the stimulation of growth was not immediately established. Only with the work of Villee and Bissell (1948), Hinton (1956), and Sang (1959) on Drosophila was it certain that the growth promoting effect lay in the "nucleic acid" purine and pyrimidine bases, particularly adenine, rather than in the entire ribonucleic acid molecule. In the growth studies of the mosquito larvae, Aedes aegypti, Singh and Brown (1957) found that although nearly all the larvae reached the 4th instar, growth and pupation were inhibited when ribonucleic acid was absent from their synthetic diet. Generally ribonucleic acid cannot be substituted by deoxyribonucleic acid, however, House and Barlow (1957) have reported that for growth of Agria affinis these nucleic acids have an equivalent effect.

On the other hand when the adult dietary needs for reproduction are considered it is of interest to note that for fecundity and fertility of three tephrited fruit flies, there was no need for nucleic acids (Hagen, 1952). Also neither Dimond et al (1956) nor Singh and Brown (1957) reported that the nucleic acids or their bases were

required in the adult diet for egg production in Aedes aegypti. In fact Dimond et al (1958) reported that no stimulation to fecundity was observed in this species upon the addition of vitamins, nucleic acid and sterols to the amino acid diets.

Studies on insect nutrition have passed through a transitory, purely descriptive phase, when raw materials or refined natural foods were used (Uvarov, 1929), to the present basic quantitative stage when synthetically pure chemicals comprise the test diet. It is anticipated that dietary differences between species, in addition to those now evident will be elucidated at the molecular level. For example, differences in nucleic acid synthesis have been reported between two strains of the fruit fly, Drosophila melanogaster (Hinton, Noyes, and Ellis, 1951; Hinton, Ellis and Noyes, 1951). Most physiologists now consider nutrition as a chemical process and the apparent genetic basis for these metabolic differences substantiate this concept (Albanese, 1959). In addition to dietary differences of genetic origin, it is likely that differences in nutritional requirements will be found in insects harbouring microorganisms or larger parasites. Some intracellular symbiotes are already known to synthesise and provide those nutrients lacking in the normal diet and so indicating a certain self-sufficiency.

In the field of insect nutrition it is obvious that most of the attention has focussed on the basic requirements for growth and that studies on chemically defined nutrients for reproduction are rare. Since reproduction is considered as the most characteristic feature of living systems, it is surprising then that this process and those known

to be intimately associated with it have been so neglected.

It is commonly known that the house fly, Musca domestica, is of considerable medical and economic importance (Hewitt, 1914; Patton and Evans, 1929; West, 1951) and is in the adult stage a polyphagous feeder (West, 1951). Other muscid flies have diverse adult feeding habits. For example the stable fly, Stomoxys calcitrans, is considered an obligatory blood feeder, whereas the face fly, Musca automnalis De Geer, shows somewhat intermediate feeding preferences. Since these divergent feeding habits occur in this family it will be of interest to investigate whether such behavioural differences also indicate a significant difference in the nutritional needs for ovarian growth and fecundity.

A beginning to this problem is made here by evaluating the specific nutritional requirements for egg production in the house fly. In this thesis the chemically defined dietary requirements for the first gonotrophic cycle are compared with those for subsequent cycles. These results will lead to further studies on the influence of changing larval environments on adult dietary requirements for reproduction. This research is thus basic to an understanding of the vital process, reproduction, and of the other basic functions associated with fecundity.

METHODS AND MATERIALS

Constancy of the Fly Strain and Larval Medium

To aid in comparing experiments and to insure their reproducibility, variables in the methods were reduced to a minimum. Firstly, a single strain of house flies was used throughout. These were obtained in 1958 from Dr. H. L. House, Entomology Research Institute for Biological Control, Canada Department of Agriculture, Belleville, Ontario[4]. The maintenance of house flies in the laboratory for use in these experiments continued through 48 generations. The culturing techniques were a synthesis of features from several reports but followed those of Moreland and McLeod (1956) most closely.

For the culture of the immature stages of the house fly, the standard C.S.M.A. medium (Standard Chemical Specialties Manufacturers Association)[5] was used. The standard dry larval medium used in the Peet-Grady Method (Anonymous, 1955) is this C.S.M.A. mixture, composed of alfalfa meal, dried brewers' grains, and soft-wheat bran in equal amounts by weight. It has been shown that the size and the number of pupae and, of course, adults produced from a fixed quantity of the medium is largely a function of the interrelation of the

---

[4] Dr. House received his house-fly culture from the Science Service Laboratory, London, Ontario, where it had been maintained for three years (House and Barlow, 1958).

[5] Ralston Purina Company, St. Louis, Missouri, U.S.A.

amount of water mixed in the medium,and the number of larvae growing in the medium (Moreland and McLeod, 1957). Since it was regularly observed that the pupae at different times varied in size and numbers, a slight modification was made in our original C.S.M.A. mix in an attempt to reduce this variation. This later modification was used at the time that experiments on individual female flies commenced.

Preparation of Larval Medium

The first medium used for rearing larvae contained the following ingredients:

Dried Brewers' Yeast	22 g.
C.S.M.A.	600 g.
Water	1250 ml.

The dry components of the medium, dried brewers' yeast and C.S.M.A., were mixed together, then moistened with the water by thorough hand-mixing. These proportions gave a mix whereby a few drops of water could be readily squeezed from a handful of the mixture.

The second type of rearing medium was a slight modification of the above but gave a more uniform yield of pupae. The change made to the medium consisted of the addition, to the water portion, of 20 ml. of 5 N sodium hydroxide solution so that the final liquid remained at 1250 ml. This was then added to the mixed brewers' yeast and C.S.M.A. The resulting medium had an initial pH of about 7.

Seeding the Culture Medium with Eggs

From our observations and others (Basden, 1947; Wilkes et al,

1948; Smith and Harrison, 1951; and Moreland and McLeod, 1957), it has been demonstrated that a medium seeded with large numbers of eggs produces small flies. Moreland and McLeod also showed that emergence and sex ratios change with the actual numbers of pupae per jar.

Since the last authors established that a more uniform number and size of pupae could be obtained if the quantity of eggs seeded in a standard amount of medium were constant, rearing procedures were standardized by following their pipette-tube method of seeding house-fly eggs to the culture medium. Newly laid house-fly eggs were collected from 'milk' dishes placed in the stock cages 18 to 24 hours earlier. The egg masses were then transferred by spatula to a 50 ml. beaker containing tap water at room temperature, and mixed to separate the egg clumps. Floating eggs were poured off with the surplus tap water. The remaining eggs in the beaker containing about 25 ml. of tap water were poured into the open end of a 3-ml. portion of a cut 10-ml. graduated pipette. The other end was closed with a circle of bolting silk. Water was drained from the pipette tube by placing the base on a cellulose sponge about ½ inch thick. This was held upright by a strip of 'plexiglas'[6] holed to fit the measuring tube (Moreland and McLeod, 1957). The dispersed eggs were poured into the pipette tube to give the desired 0.6 ml. volume of eggs. These measured eggs were added to the correct amount of prepared C.S.M.A. medium. The initial amount of fresh C.S.M.A. medium added to the clear plastic

---

[6] An acrylic resin product of Rohm and Haas Co., U.S.A.

rearing jars[7] at the time of seeding was only 520 g. (wet) or 84 g. (dry) and this covered the bottom of the jar to a depth of approximately 2 inches. A volume of 0.6 ml. of eggs seeded into each plastic jar gave consistent yields when a total of 736 g. (dry) of the medium over the entire rearing period was used. With any change made in the number of eggs seeded to the medium there was a corresponding change in the amount of medium presented to the growing larvae. All rearing jars were covered tightly with double weight, tightly woven cheesecloth to prevent the possible introduction of eggs from stray flies. When the C.S.M.A. medium is not neutralized, molds regularly grow throughout the medium forming a moldy crust on the surface, as was reported also by Moreland and McLeod (1957). However, with neutralized medium the larvae work through the fresh medium and a dried crust does not form. On the 2nd, 3rd, 4th, and 5th days after seeding, a minimum of 390 g. (wet) or 38 g. (dry) of newly prepared C.S.M.A. medium was added to the top of the old medium so that the total larval rearing medium was at least 2080 g. (wet) or 736 g. (dry). Within 4 to 6 hours larvae work actively through the medium which by this time shows evidence of heat of fermentation. This amount of C.S.M.A. medium added to each jar ensured optimum rearing conditions. On the day that the first pupae are observed no medium is added. The larvae then pupate in the loose upper layer of the old medium and are collected from this region only.

---

[7] These were 7½ inches high and 8 inches in diameter and were obtained from Tri State Plastic Molding Company, Henderson, Kentucky, U.S.A.

In the experiments determining the requirements for production of the first batch of eggs, the adults used were reared from the first, second, or third egg collections from a stock cage. However, in those test where requirements for second and third gonotrophic cycles were investigated, the females used were all reared from stock eggs of the first gonotrophic cycle unless indicated otherwise.

Larval Rearing

Rearing jars, containing eggs or newly hatched larvae, were placed in an incubator maintained a 78 ± 0.2 F [Plate 4]. Both growing larvae and pupae were kept at this temperature and at a mean relative humidity of approximately 55% varying occasionally between 30 to 99%. Under these conditions the length of the larval period lasted 6 days, and pupae first appeared near the end of the sixth day[8]. The pupae were usually separated from the larval medium on the eighth or ninth day. On the eleventh day the first adults appeared (males were regularly the first to emerge), while peak emergence began one day later.

Separation of Pupae

Pupae and the upper dry layer of the C.S.M.A. medium were placed in flat trays to dry, and then transferred into a wooden-framed plastic sieve, 10½ inches by 12 inches and 2½ inches high. The nylon mesh of

---

[8] All ages in the life cycle of the house fly refer to the time of egg laying as the first day in the age of the fly unless noted otherwise.

the sieve contained rectangular holes (1/8 inch by 5/32 inch). Gentle sieving of the dried medium and pupae left large clumps of C.S.M.A. medium in the sieve while pupae and loose dried medium were collected below. The sieved pupae and medium were transferred into a slightly tapering polyethylene bowl 6 inches high and 13 inches inside diameter. With gentle agitation and unheated air from a hand hair dryer the medium was blown off and cleaned pupae remained in the container. These pupae were placed in coded 6-oz. jars, covered with cheese cloth and stored in the incubator until the beginning of emergence.

Stock Cages of Adults

When the first adults began to emerge the jars of pupae were placed in stock cages and the cheesecloth covering was removed until sufficient numbers had emerged. Each stock cage held between 300 and 400 flies [Plate 1]. All stock cages were kept in the same incubator with the growing larvae under constant fluorescent lighting from a 30-watt, 35-inch General Electric Fluorescent light. Emerging adults were fed a 0.1 M sucrose solution by a cotton wick through a stoppered 20-ml. sample bottle and given fresh milk or dissolved powdered milk (Carnation powdered milk) within 24 hours after emergence. Milk feeding was continuous, with changes made every 24 hours until the end of the third day. This is the day before the eggs of the first gonotrophic cycle are mature. Should the maintenance of the stock colony or experimental tests require that eggs be collected on the fifth or sixth day, then further milk feeding and subsequent egg laying of the stock culture was delayed until that time. This meant that females,

although ripe with eggs on the fourth day, retained their eggs until a suitable substrate was presented for oviposition on the fifth or sixth day. By this method of feeding and egg collecting, a stock fly cage receiving milk in the morning would yield by afternoon sufficient eggs to seed a new cylinder of medium; or if feeding was withheld until late afternoon, sufficient eggs would be deposited by the following morning.

In the course of the preparation of experimental diets for the adults, it was noted that individuals within a given lot varied less in size than individuals from different lots. This made it desirable to set up a series of tests and replicates with flies from a single lot of males and females. Thus daily rearing was standardized and each lot set up was large enough both for nutritional experiments and for stock culturing. Since under our rearing conditions the life cycle of the house fly from egg to adult was 12 days, experiments were planned two weeks in advance of actual emergence and feeding of the adults.

Experimental Adult Flies

When flies were to be used for experimental diets, male and female adults were allowed to emerge in a stock cage until adult emergence was at a maximum. The remaining pupae were transferred to a clean cage and emergence continued for 4 to 6 hours until there were sufficient adults in the new cage for experiments. These young male and female adult flies were then caught in shell vials, 50 mm. by 20 mm. diameter, and transferred to experimental cylinders [Plate 2], set up

in the rearing incubator.

Cleaning of Experimental Apparatus

The pyrex glass cylinders used to confine the test flies during these nutritional studies were of two sizes, 18.0 cm. high by 15.0 cm. outside diameter [Plates 2 and 4], and 6.0 cm. high by 5.0 cm. outside diameter [Plate 4]. The former will now be referred to as large and latter as small cylinders in this thesis. The large cylinders were used for those diets that determined the amino acid requirements for egg laying and contained 15 adults, 12 females and 3 males. The small cylinders were used to determine the nutritional requirements for individual female flies and contained 1 female and 2 or 3 males. All cylinders were washed first with Alconox (sold by Canadian Laboratory Supplies Ltd., Toronto) and rinsed clean, then just before use, rewashed with Express (Proctor and Gamble) in an automatic glassware washer rinsed for 2½ minutes with hot tap water, ½ minute with deionized (resin-filtered) water and then drained dry. All cylinders were topped with Alconox-washed nylon netting. This was held in place with 7-inch expansion hoops, while the smaller cylinders had the nylon netting held in place with elastic bands.

The large cylinders were placed on aluminum trays covered with two layers of Whatman No. 1 filter paper cut to size from 46 cm. by 57 cm. sheets. Each aluminum tray, 20.5 cm. by 46 cm., was long enough to hold three large glass cylinders and in the centre of each section, holding each cylinder, a 2.8 cm. diameter hole was bored to take a No. 6 rubber stopper [Plate 2]. The small cylinders were arranged on clear

plastic trays, 30.5 cm. square and 0.8 cm. thick with 12 cylinders on each tray, and, as above, there was a hole in the tray under each cylinder. In the centre of each rubber stopper a hole was bored, into which a 12 mm. by 35 mm. shell vial was easily introduced. The bottom of this vial was pushed level with the large end (bottom) of the rubber stopper. In the rearing incubator the experimental cylinders and trays were placed on shelves which were 50 cm. below two 40-watt, 47-inch long fluorescent lights.

Between experiments rubber stoppers were brushed with a solution of Alconox, rinsed over night in running tap water, then thoroughly rinsed five times in deionized water and five times in distilled water[9] before drying at 105 F. The shell vials used to contain the diets were similarily washed in Alconox with a test-tube brush and rinsed in tap water. These vials were cleaned further in stender dishes in units of 15 by immersion in a dichromate cleaning solution for 3 or more hours. They were then rinsed individually in tap water before being placed in a battery jar and rinsed for 6 to 12 hours in running tap water. The vials still in stender dishes were rinsed repeatedly in deionized water and five time in distilled water before being dried in a drying oven with the rubber stoppers.

In presenting the liquid diet to adult house flies, wicks provided the feeding surface [Plate 3]. These were prepared from absorbent cotton. To remove possible dietary contaminants, such as unsaturated

---

[9] Always refers to distilled water redistilled in a glass still.

fatty acids (Pollock, 1948), the absorbent cotton was washed by Soxhlet extraction as described below. Wicks were prepared from a 3 inch by 6 inch piece of absorbent cotton cut from a 1 lb. roll. The 3 inch by 6 inch piece of absorbent cotton was then rolled in Kleenex tissue, to prevent packing, and inserted into a Soxhlet extraction thimble (35 mm. by 80 mm.). The cotton was next washed in a Soxhlet extractor with two washings of distilled water and two final washings of 95 per cent ethyl alcohol, each washing lasting for at least 8 hours. These washings always removed a soluble yellow substance from the cotton. The washed cotton was then removed, unrolled with clean forceps and dried in the drying oven. This cotton was stored in large 20 mm. by 150 mm. glass dishes. When wicks were required they were cut from this cotton.

Preparation of Diets

[a] Preliminary tests with more or less modified natural products fed to adult flies:

In these experiments adults were fed natural foods, or their products, as diets. In some cases fresh homogenized whole milk was used as a 'control' diet and hereafter is known as milk diet or simply milk. The milk diet was fed to adult flies to determine whether egg maturation, oviposition and adult survival were consistent rather than as an absolute standard within or between experiments. The variations evident on this diet resemble the differences found when blow flies were fed on milk (Harlow, 1956). When 3.42 g. of sucrose[10] (a 0.1 molar

---

[10] Fisher Certified Reagent.

solution), and 0.15 g. of salt mixture W[11] were dissolved in 100 ml.[12] of solution, this was known as sugar-water diet[13]. All purified casein preparations were dissolved in 0.1 N sodium hydroxide[10], and with the above amounts of sucrose and salt mixture added, this was known as casein diet. A yeast diet[14] was prepared by dissolving various amounts of yeast and the above quantities of sucrose and salt mixture W to make 100 ml. of solution. The amount of casein or yeast extract employed in each diet is indicated in the results. All the constituents of these diets were weighed in the dry state and dissolved in enough distilled water or sodium hydroxide solution to make 100 ml. of diet.

[b] Later tests with chemically defined diets fed to adult flies:

The diets used in these experiments, were all solutions of dietary

---

10 Fisher Certified Reagent.

11 A Wesson modification of a salt mixture of Osborne and Mendel (1932) and purchased from Nutritional Biochemical Corporation, Cleveland, Ohio as the prepared salt mixture W containing calcium carbonate, copper sulphate, ferric phosphate, manganous sulphate, magnesium sulphate, potassium aluminum sulphate, potassium chloride, potassium dihydrogen phosphate, potassium iodide, sodium chloride, sodium fluoride, tricalcium phosphate.

12 All water used in the preparation of diets was distilled water redistilled in a glass still and hereafter is referred to as distilled water.

13 Sold as "vitamin free" casein by Nutritional Biochemicals Corporation, Cleveland, Ohio or vitamin-free or vitamin-test casein by General Biochemicals, Chagrin Falls, Ohio, or as low-vitamin casein by Genatosan Ltd., Fison Chemicals, England.

14 A product of Nutritional Biochemicals Corporation sold as yeast extract powder.

mixtures in 100 ml. quantities. All diets contained 3.42 g. of sucrose and 0.15 g. of salt mixture W as in the above diets [Part 1] unless otherwise specified. All amino acids used in the diets were of the l-form and of the highest purity[15]. In the first experiment they were mixed in the quantities listed in Table I (diet A) in the same proportions as in the diet 'D' of Dimond et al (1956) and Dimond (1957). In all further experiments the proportions of amino acids used were as in Table I (diet B). Only distilled water was used in the preparation of all chemically defined diets. Those components of the diets, i.e. sugar, salts, and amino acids, requiring continual stirring to dissolve them, were mixed by a glass-covered magnetic stirrer and heated by a hot plate warmed to 50 to 60 F. This initial mixing was performed in a 150 ml. glass beaker covered with a clean 100 mm. by 15 mm. petri dish. After the various components of the diet were dissolved and cooled, the diet in solution was neutralized[16] with about 5 ml. of 5 N sodium hydroxide[17]. This neutralized solution was then diluted to 100 ml. in a volumetric flask with 15 to 25 ml. of distilled water, rechecked for its pH, and immediately stored at 10 C. Diets were replaced daily and

---

[15] California Corporation for Biochemical Research, Los Angeles, California.

[16] Neutrality was determined when the Beckman model Zeromatic (R) pH meter indicated a pH of 7. The initial pH of the diet before neutralizing was usually about 4.

[17] The 5 N sodium hydroxide solution was prepared from Fisher reagent grade pellets dissolved in distilled water.

freshly made for each experiment as a precaution to obviate contamination (Fraenkel, 1940) and old diets were never reused.

When nucleic acids were added to the diets at concentrations of 0.4 g. per 100 ml. diet they were dissolved in about 2 ml. of 2N sodium hydroxide before being added to the amino acid mixture and before neutralization of the prepared diet (House, 1954a). Nucleic acid bases, at 0.2 g. per 100 ml., were used in other diets.

The amount of vitamins added to the diets were chosen from those concentrations found by Sang (1959) and House and Barlow (1958) as suitable for growth of the larvae of the fruit fly, Drosophila melanogaster, and the house fly, Musca domestica, respectively. The vitamin solution used in these studies was first prepared as a concentrated solution (Table II) and kept at 5 C. To obtain the desired concentration of vitamins a 1 ml. sample of the concentrated vitamin solution was pipetted into the non-neutralized amino acid diet under preparation. For each new series of test diets a fresh vitamin solution was prepared.

Because of the importance of cholesterol in egg viability, as shown by Monroe (1959, 1960) for the house fly, this sterol was added to some of the test diets of the adults. The addition of cholesterol to the liquid test diets, at the concentration we found optimal for egg laying, was conducted as follows. In 5 ml. of hot 95% ethyl alcohol was dissolved 0.25 g. of cholesterol[18]. Then to a weighed 50 ml. volumetric

---

[18] California Corporation for Biochemical Research, Los Angeles, California.

flask were added 0.25 g. of the emulsifier Tween 80[19] (polyoxyethylene sorbitan monooleate) and approximately 20 ml. of hot distilled water. To this solution was added the hot cholesterol solution. Care was taken to prevent the precipitation of the cholesterol on the sides of the volumetric flask during the addition of the cholesterol solution. This was prevented by pouring the cholesterol solution through a warmed glass funnel into the detergent water mixture. The resulting fine cholesterol suspension was diluted with hot distilled water, then cooled to room temperature before final dilution to 50 ml. To obtain a 0.01% cholesterol suspension in a test diet, 2 ml. of the above cholesterol solution were pipetted into the amino acid solution while it was being stirred. Other concentrations of cholesterol used in test diets were similarly prepared and added in 2 ml. quantities, so that the solvent, ethyl alcohol, and emulsifier Tween 80, were in the same quantity for all test and control diets.

Dissection and Measurements of Ovaries

Measurements similar to those of Harlow (1956) on ovary growth of blow flies were made on house flies selected from individuals that emerged within a 6-hour period. Each cage contained both male and female test flies which were fed milk every 24 hours. A 0.1 M sucrose solution and fresh milk each in separate containers, were the only nutrients fed these flies. Random samples of these flies were taken and dissected

---

[19] A sample from the Atlas Powder Company, Canada, Ltd., Brantford, Ontario.

at 24-hour intervals. Another cage containing flies fed only sugar water, a 0.1 M sucrose solution, were similarly fed and selected for dissection. All adults used for a series of dissections were reared together. Dissections and measurements of ovaries were made[20] using insect Ringer's solution[21] as dissecting medium and at a magnification of 50 times. Each ovary was removed and manipulated by the branches of the trachea entering the ovary. Ovary measurements were then made on their in situ shape. This was not the technique used by Harlow (1956); she flattened each ovary before taking measurements. Our measurements of the ovarian length and width were made on the medial surface of the ovary and thickness was measured from the medial to lateral tracheated surface. The product of these measurements, although having no absolute meaning, gives an approximate volume measured in arbitrary units (Harlow, 1956).

All eggs deposited by the flies (on the feeding wicks and the few laid on the stoppers) were counted and included in the values reported as fecundity. Before eggs were counted the wick was removed from the vial and placed on the dissecting dish containing wax blackened with carbon. The white illuminated eggs were then readily counted under a binocular microscope against the black background. (Eggs were collected from each cylinder once every 24 hours at the time of replacement of diets and later counted.)

---

[20] With a linear ocular micrometer.

[21] Drosophila Ringer's solution according to Bodenstein (Roeder, 1953).

Most of the eggs of the first gonotrophic cycle are laid on the fourth or fifth day after emergence, and eggs laid after this time can be ascribed to further cycles. This information was used when studying fecundity of individual flies. In some cases the determining of relict eggs by dissection further aided in establishing gonotrophic cycles.

Histological Techniques

The ovaries of adult female house flies were removed in Bodenstein's insect Ringer solution (Roeder, 1953) at periods immediately following emergence until after oviposition of the first gonotrophic cycle. The ovaries were fixed in Bouin's, Zenker's, Helley's or Carnoy and Lebrun's fixatives (Kennedy, 1949). In these studies Bouin's was the fixative used most extensively. The tissue was dehydrated in three changes of cellosolve (ethylene glycol monoethyl ether), cleared in benzene and embedded in Tissuemat 60-63 C. Microtome sections were made at 10 microns. Delafield's hematoxylin gave the most satisfactory results for routine staining of the sectioned ovaries.

RESULTS

To facilitate the presentation of the results, this section is divided into three parts. Parts I and II deal with undefined and chemically defined diets in relation to general fecundity. Part III establishes the role of supplementary nutritional factors in the first and subsequent gonotrophic cycles.

Before the nutritional requirements for ovarian development could be determined, parameters applicable to this line of experimentation had to be chosen. More precisely it was necessary to measure and record some evidences of physiological processes in the house fly, processes whose values could be observed and compared. These processes had to be sensitive to the experimental conditions. The nutritional experiments in this thesis were concerned therefore, with three values: female survival, ovarian growth and egg production.

Consequently, some of the following preliminary tests were undertaken, firstly to develop satisfactory experimental methods and secondly, to determine the optimum values under the experimental conditions designed for this study.

PART I

Determining Experimental Standards and Ovarian Development in Relation to Undefined Diets

[a] The appropriate ratio of males to females in experiments :

Twelve adult females per large cylinder were chosen arbitrarily

as the experimental number. Fresh homogenized milk was fed daily. Generally the number of eggs laid over a 14-day period under these conditions was approximately 2800 eggs. It was now necessary to determine whether the number of males present in the cylinder had an effect on egg deposition. Thus this experiment was concerned with the number of eggs deposited and not with the viability or size of the eggs. Since it was possible that impregnation of the females had a bearing on ovarian development and on egg laying, 5 adult males were placed with the 12 females. Test flies were from one group of larvae and therefore of the same age. This association of males to females was the same in all cylinders until the third day. On the third day males were removed in most cylinders so that in each set of three experimental cylinders there remained a male to female ratio of 0:12, 1:12, 3:12 or 5:12.

The results indicate that the ratio of males to females in a cylinder has a bearing on the number of eggs laid. To remove the bias of mortality, comparisons were made on the number of eggs per surviving female. More eggs were deposited after a 6-day period, when three males were present than with any other number. This difference is not as marked after 10 days, when the cylinders with 3 and 5 males respectively, are considered (Table III). However a lower fecundity is evident after 10 days in cylinders containing 0 and 1 male as compared to those with 3 and 5 males. These calculations confirm that greater egg deposition occurred when 3 or 5 males were present in the large cylinders.

Because of these data, a ratio of 3 males to 12 females was used in all experiments with large cylinders. To ensure that males will be present throughout an experiment to stimulate egg deposition, each small

cylinder had an initial 3:1 ratio of males to females and this ratio was never allowed to fall below 1:1.

[b] Variation in fecundity of individual milk-fed females:

Preliminary experiments had indicated that it was impossible to distinguish between gonotrophic cycles and the variation of individual female egg laying when the large cylinders contained 12 female and 3 male flies. For these two reasons the oviposition of individual females was investigated.

Experiments using eighteen small cylinders, each with one female and 3 males of the same age which were fed fresh milk daily, continued for 22 days (Table IV). Under these conditions 15 of the 18 females laid eggs (84%) but of the total number of females, only 7 of the 18 laid two or more cycles of eggs (39%). When considering only laying females, 7 of 15 (49%) laid eggs beyond the first cycle. A little more than half (56%) laid eggs within 7 days after emergence. The commencement of the second egg laying cycle in the individual females began between the 6th and 13th days. This extensive period indicates a noticeable overlap in the gonotrophic cycles between female flies. This accounts for the difficulty in distinguishing the cycles by observations on oviposition during those experiments using groups of females in large cylinders.

The exact reason for these differences between females is obscure. Differences in the rate of ovarian development may be a factor. These observations on individual milk-fed flies indicated that females can develop two or more gonotrophic cycles and that mean survival under these conditions was two weeks. More than half the females survived two

weeks or more. This variation in reproduction and survival made it advisable to continue the use of small cylinders for observations on nutrition and ovarian development of individual female flies.

[c] Rate of ovarian development in adult females fed milk or sugar-water diets:

To answer a number of questions concerning the rate of ovarian growth and the effect of diet on oögenesis in the house fly, measurements were made and volumes calculated, of dissected ovaries from freshly killed flies only. Observations and measurements on both ovaries were conducted daily on 7 to 9 females fed milk or sugar-water. To insure adult uniformity, flies were chosen from larvae reared and pupating together in the same rearing cylinder.

Any growth of the ovaries occurring in sugar-water fed females was evident only between the first and second days; no change in size in the ovaries was measurable after the second day. In milk-fed flies this same early growth pattern was evident. Ovarian change in milk-fed flies was little during the first day and showed the first measurable increase during the second day. With a milk diet the time of greatest yolk deposition, which accounts for most of the ovarian growth, occurs during the second and third days (Table V, Fig. 1). Any yolk deposition at the end of the third day was past the period of greatest oöplasm increase. Between the third and the fourth days only a slight growth is noticeable; this is the time of chorion deposition (see next section). Egg laying of the first gonotrophic cycle generally commenced on the fourth day under our experimental conditions. Many other dissections of females fed milk, and also the amino acid diets, substantiated these

results.

The course of ovarian growth in the second gonotrophic cycle follows very closely that of the first. But the ovary in older flies, which have just oviposited, is slightly larger than those of sugar-water fed flies of the same age (Table V). This may be because the yellow body (see next section) is now part of the second cycle ovary. In addition, nurse and follicular cells of the first gonotrophic cycle are still present although degenerating (see next section).

[d] Some morphological changes in the ovary during the first gonotrophic cycle:

Since this work is principally concerned with the nutritional requirements during oogenesis, it was considered that a histological study of the general changes during the maturation of the ovary would establish some characteristics of this process. Moreover, this should more clearly illustrate those factors contributing to the growth of the ovary outlined in the previous section.

The general anatomy of the reproductive system of the female house fly has been described by Hewitt (1914) and West (1951). In 1921 Verhein outlined the basic histological changes taking place in the maturing ovary of muscid flies. He established that each maturing ovariole of the house fly contains an ovum (oocyte) and 15 nurse cells, the result of four mitoses. Each ovary contains about 50 to 75 ovarioles (West, 1951). Three typical zones or regions are recognizable in an ovariole (Imms, 1957): the slender thread-like apical terminal filament, the germarium containing the primordial differentiating germ and nutritive cells, and the follicle or vitellarium consisting of the

major portion of the ovariole. The last region contains the developing nutritive nurse cells and oocyte, and is bounded by a layer of follicle cells (sometimes referred to as the follicular epithelium). These cells primarily function in the secretion of the chorion of the egg.

Examination of the histological sections showed that there were marked general changes in each maturing follicle of an ovariole. Principally these changes are responsible for the growth of the ovary during each gonotrophic cycle. All the plates, except 8 and 12, illustrate directly the growth of the ovary since these microphotographs were taken at the same magnification (62.5X). Plates 8 and 12 are at a higher magnification (107.5X). Under the rearing conditions described here changes in the ovary of milk-fed house flies appeared as follows.

In the ovary of the newly emerged fly the round follicle is noticeably enveloped in a layer of follicular epithelium cells. In comparison to the nuclei of the follicular cells those of the nurse cells are large (Plate 7), being also large in relation to the size of the cell. At a higher magnification the chromatin material of the nurse cell nuclei appears either diffuse or in clumps (Plate 8). At this time differences between the nurse cells and oocyte are less apparent than in older follicles. Tracheoles are easily noticeable between the ovarioles.

After about 24 hours the follicular and nurse cells show further differences. The former cells are now much smaller than the nurse cells. This difference is the result of the growth undergone by the nurse cells. Nuclei of these cells are also larger than at the time of emergence and

now contain diffuse chromatin. Generally the largest nurse cell nuclei lie adjacent to the oocyte and all nurse cells are about the same size. In cross section the follicle is now oval rather than round. Up to this time ovarian growth is slight as a result of these changes in ovarioles (Plate 9).

After 48 hours the most obvious change in each follicle is the growth of the nurse cells, and the evidence of much yolk deposition in the oocyte. These changes mark the beginning of active vitellogenesis. The follicular cells of the developing follicle noticeably enclose the oocyte. Visible elongation of the follicle is now evident. The nuclei of the nurse cells have greatly enlarged and still contain a diffuse chromatin (Plate 10).

By the time the female is 3 days old (72 hours), the nurse cells and their nuclei have reached maximum size. This is probably the beginning of the most active period of vitellogenesis. This is characterized by oocyte growth overtaking nurse cell growth. In cross section the oocyte and the nest of nurse cells have grown to about the same size (Plate 11). Yolk material is apparently transferred directly from the cytoplasm of the two nurse cells adjacent to the oocyte, into the growing oocyte (Plate 12).

At approximately 84 hours the most characteristic change evident is the degeneration of the nurse cell nuclei. Those most adjacent to the oocyte become irregular. These changes are also accompanied by a decrease in nurse cell size. In contrast the oocyte has grown markedly and approaches the maximum size. Evidently vitellogenesis is maximum between 60 and 84 hours under these experimental conditions. Evidence

of the chorion can be seen (Plate 13). These conditions in the follicle (and oocyte) indicate that oocyte maturation is almost complete. Oviposition by the female of eggs approximately one millimeter in length (West, 1951), generally occurs on the fourth day (96 hours).

However, just prior to egg deposition, at about 94 hours, the egg is mature and vitellogenesis is complete. This stage in the maturation of the ovary is characterized by further degenerative changes in the nuclei of the nurse cells and a decrease in size of these cells. Initial changes in the nurse cells are apparent in those follicles destined for the second ovarian cycle (developed from the former germarium) and adjacent to maturing oocytes (Plate 13 and 14).

After oviposition the remaining follicle cells and degenerated nurse cells appear as a mass of tissue which Lineva (1953) called the yellow body. The adjacent follicle, containing the nurse and follicular cells next in line for maturation, appear to be in the initial stages of oogenesis (Plate 15).

[e] Delayed presentation of the milk diet to newly emerged flies and the effect on fecundity and survival:

The time at which adult female flies were fed, may have a bearing on the rate of ovarian growth or on the numbers of eggs developing. Therefore, individual adult female flies, kept with 3 males in the small cylinders, were fed milk at intervals of 1, 2, 10, and 37 hours after emergence. Under all experimental conditions flies generally began feeding 20 to 24 hours after emergence and when flies were presented with milk 37 hours after emergence, they immediately fed.

During this experiment no appreciable differences were evident in the mean total number of eggs laid, the mean time of first egg laying, and female survival (Table VI). Thus feeding shortly after emergence or after a day and a half (37 hours) did not affect the fecundity or survival of the females. Although these results indicate that withholding food from the adult female has little effect, in subsequent experiments flies were always fed within 30 hours after emergence.

[f] Fecundity and survival in relation to adult diets of yeast extract, two purified preparations of casein, and milk:

After the above results were known, further tests were designed to determine the effect on survival and fecundity when female flies were fed on various protein preparations.

[i] Therefore in this experiment two casein preparations, a 4% vitamin-test casein[22] and a 4% low vitamin casein[23] were compared with, a sugar-water protein free preparation and a fresh milk control. The sugar-water diet contained the usual minerals. Both caseins were dissolved in 0.1 N sodium hydroxide to give 100 ml. of diet, and contained the usual minerals and sucrose. The diets were evaluated by comparing both the number of eggs laid and female survival for each diet. All four dietary preparations were fed to individual female flies placed with 3 males, all of the same age and stock. Small cylinders were used, 18 cylinders per test.

---

[22] A preparation supplied by General Biochemicals, Chagrin Falls, Ohio, and sold as vitamin-free or vitamin-test casein.

[23] A product of Genatosan Ltd.: Fisons Chemicals, England, and sold as a low vitamin casein.

Although those flies that fed on the low vitamin casein were slightly more fecund in the first cycle, the nutritional value of both casein diets for this cycle is considered similar to that of milk (Table VII). It is of interest to note that the average number of days between the first and second gonotrophic cycles were 4 to 5 days on the casein diets and 2 days on the milk diet.

With the vitamin-test casein diet, 7 of the 14 laying females deposited second cycle eggs whereas with the low vitamin casein diet only 4 of the 14 laying females did. In comparison the latter represents a lower number of females capable of developing a second gonotrophic cycle. Thus the two casein preparations for the first gonotrophic cycle were similar, but the vitamin-test casein diet seemed nutritionally more successful for subsequent cycles.

Adult house flies fed a sugar-water diet did not oviposit; this was expected. However, when female survival was compared (Table VIII), this diet was far superior to either of the casein diets or to milk. In general female survival on both casein diets showed a marked similarity. However, with both casein diets the mortality was similar throughout the experiment, the last flies dying in each case between the twelfth and thirteenth day (Figure 2). On the other diets (milk, sugar-water) female survival was at 100% at least until the sixth day after emergence. In this experiment female survival on all these diets was poor in comparison to other experiments in which survival values were similarily graphed.

Both these casein diets satisfy the nutritional requirements

for ovarian development and maturation. This fact would tend to confirm that proteins are the sole requirement for the initiation of egg development in the adult female house fly. However one casein preparation, "vitamin free" casein, gave higher fecundity.

[ii] In investigations on nutrition and growth, yeast or a yeast fraction has been often used together with more basic components in a partially defined diet. However yeast extract alone known to be rich in vitamins is seldom, if ever, used as the sole source of food.

To establish whether the female house fly is able to feed, lay eggs and survive on a diet of 6% yeast extract[24] , as successfully as on milk or 6% "vitamin free" casein[25], 2 large cylinders containing 12 females and 3 males, and 7 small cylinders containing 1 female and 3 males were used for each diet.

Data from the large cylinders showed that of the two diets, 6% yeast extract or 6% casein, the former diet was the better (Table IX). For example, if after 18 days the mean number of eggs per cylinder is compared, the milk, the casein, and the yeast extract diets gave values 3353, 636 and 1105 eggs respectively. The data from individual females are similar (Table X). The mean number of eggs per laying female on the milk, the casein, and the yeast extract diets

---

[24] A product of Nutritional Biochemicals Corp., Cleveland, Ohio, and sold as yeast extract powder.

[25] This casein was a product of Nutritional Biochemicals Corp., Cleveland, Ohio, and sold as "vitamin free" casein.

over 14 days was 212, 34 and 77 respectively. This difference between the yeast and casein diets was apparent when fecundity, expressed as oviposition ratios, was compared (Table IX and X). However, this casein is less successful than the two used in the previous experiment (Table VII) which gave oviposition ratios of 0.66 compared to that of the present experiment, 0.16.

Both 6% yeast extract and 6% casein diets, fed to adult females, supported ovarian development and oviposition. The yeast extract diet was the better of the two by far. However, fresh milk, as the control diet, proved to be the best of the three diets tested.

PART II

Egg Production, the Essential Amino Acids and Other Dietary Supplements

Once experimental conditions, permitting continued ovarian development and egg laying were established, it became possible to determine chemically defined nutritional requirements for oögenesis and oviposition for the adult house fly. Under the rearing conditions used in these experiments this strain of house fly was anautogenous. For this reason both ovarian development and the number of eggs laid served as criteria of the nutritional adequacy of a diet fed to the adult.

[a] The need for salts, a carbohydrate and water in the adult diet:

In our formulation of a basic amino acid diet (Table I) used in these experiments, it was found that sucrose, salts (Table XI) and water were the only other constituents required for egg production. The elimination of any of the last three basic components of the diet resulted in adverse effects to egg production (Table XI) and to

survival. Any salts present in the amino acids as impurities would have reduced the effect of these deletions. Even when Wesson's salt mixture or sucrose were eliminated from a "vitamin-free" casein[26] diet, no eggs were laid. From these observations it was evident that the addition of both the salt mixture and carbohydrate to the diets was essential.

[b] Determination of the amino acids necessary for ovarian maturation:

Preliminary test with a purified casein hydrosylate supplemented with 3% tryptophan (dry weight) fed to adult house flies gave viable eggs. Therefore further experiments employing a chemically defined amino acid diet were attempted.

The first problem was to determine whether the amino acid diets, which had been fed to other dipteran adults, allowed successful ovarian maturation in the house fly. Dimond (1957) had prepared his synthetic amino acid diets on the basis of adequate natural protein sources, casein and blood (Table XII), and had further established the levels which gave the best egg production for the adult female mosquito, Aedes aegypti, (Table XII,right column). Therefore, our first synthetic test diet was prepared with the naturally occurring l-form of the amino acids. This diet (diet A, Table I) contained the same concentrations of the amino acids as found optimum by Dimond (1957)(Table XII), but the l-form of isoleucine, methionine, phenylalanine, threonine and valine were used instead of the dl-forms used by Dimond.

---

[26] Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Since the amino acid diet A (Table I) gave sufficient and consistent numbers of eggs (Tables XI and XIII), the next problem was to determine which of these 12 amino acids were essential for consistent egg maturation. Therefore tests were made deleting single amino acids from diet A. Those amino acids found to be required ('essential'), as indicated by no ovipostion, were arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan and valine (Table XIII; section "e" below). Dissections of the females fed on these synthetic diets indicated incomplete ovarian development when these required amino acids were lacking. The role of a sulphur containing amino acid, cystine or methionine is not apparent from these results. However, if either of the sulphur-containing amino acids, methionine or cystine, were eliminated from the diet, eggs were deposited (section "d" below). It is certain then that only nine of the original twelve amino acids were necessary for egg production.

Once the essential amino acids for ovarian maturation were established a second synthetic diet, diet B, was prepared and fed to adult house flies. This diet differed from diet A by containing only half the amount of the l-forms of isoleucine, threonine and valine. The amount of these amino acids was halved in our second synthetic diet, diet B (Table I), since these d-forms were not utilized by the mosquito, Aedes aegypti (Dimond, 1957).

The results obtained by feeding diet B were noticeably better than with the original diet A, when comparing egg production after 10 and 15 days (Table XV). After 15 days 1007 eggs were produced on the latter and a comparable 1239 eggs in the former.

[c] The substitution of nitrogen compounds for glutamic acid in the defined diet:

As shown above (Table XIII), glutamic acid is not essential for fecundity in the adult female house fly but without it egg production is less. Whether this amino acid can be replaced by other non-specific nitrogen compounds in the adult house fly is unknown.

Therefore experiments were tried in which ammonium citrate or urea replaced glutamic acid in diet A. Both these diets were compared with diet A and with diet A lacking glutamic acid. Three large cylinders were set up containing 12 females and 3 males for each diet. Apparently ammonium citrate can be more effectively utilized than glutamic acid by the female adult house fly for egg production (Table XIV). Urea cannot be considered a non-specific nitrogen source, but on the other hand appears to interfere with fecundity, for, when glutamic acid is totally lacking from the synthetic diet, more eggs are produced than when urea is substituted.

The data also show a high survival value for the females fed ammonium citrate (Table XIV). All twelve of the females were alive in all three cylinders after 7 days. This was slightly better than for the milk control and for those fed the amino aicd diet A. In both cases 10 of 12 females survived for the same period.

Although further tests should be undertaken to have a clearer picture of the utilization of these non-specific nitrogen compounds, the above results indicate differences in the use of these two compounds, ammonium citrate and urea, by the adult female for egg production.

[d] The effect of eliminating cystine or methionine in the synthetic diet:

It was necessary, since eggs were laid with either cystine or methionine in the diet, to determine just what part either of these amino acis played in oogenesis. Therefore a series of experiments were designed to determine which of these two sulphur amino acids were essential to the house fly.

In the first experiment the amino acid diet A (Table I) was used and compared with cystine or methionine deficiencies from this diet. Diet B (Table I) was used as another comparison in this experiment. Three large cylinders, with 12 females and three males in each cylinder comprised a group. After 10 and 15 days, egg production, although increasing for each diet up to the 15th day, was always lower when methionine was lacking (Table XV). There was no meaningful increase in egg production per experimental female after 10 days on the methionine deficient diet,while with all other diets marked increases were evident after 10 days. On diet A, egg production per experimental female was 89 eggs on the 10th day and 99 on the 15th day. Total egg production of flies fed the methionine deficient diet was much reduced. This did not occur when cystine was lacking. In fact the cystine deficient diet gave a better mean total production than the other synthetic diets. With all the synthetic diets there was little egg production after the 10th day, differing from the fecundity with milk.

In a second experiment cystine and methionine were deleted individually from the diet B and methionine from the diet A. The

results (Table XVI) confirmed the first experiment. With the cystine deficient diet high fecundity occurred, similar to that with the control diet B over the same period, indicating that cystine was a non-essential amino acid. Again a methionine deficiency in diet A or diet B was followed by a marked reduction in fecundity, evident after the 9th and 16th day (Table XVI). Therefore methionine, although not essential when cystine is present, is required if egg production is to continue at a high level.

An additional observation was the notable difference in fecundity with the two methionine deficient diets, the deletion of methionine from the diet A resulting in a much greater decrease in egg production. In both series of experiments with females fed amino acid diets A and B there was a noticeable decline in fecundity after the 9th or 10th day as compared with that of milk-fed flies (Tables XV and XVI).

These results suggested that cystine need not be a constituent of further synthetic amino acid based diets. Therefore in all further experiments amino acid diet B lacking cystine will be referred to as diet C (Table I, Methods and Materials).

[e] The role of the sulphur-containing amino acid, methionine, in egg production:

The requirement of adult house flies for a particular sulphur-containing amino acid was not evident from the earlier amino acid studies (section "b" and "d" and Table XIII). Cystine, according to the metabolic studies of Cotty et al (1958) was not essential for the house fly but methionine was. However the exact need of dietary

methionine for ovarian maturation has been obscure. To clarify the role of the sulphur-containing amino acid, methionine, the following tests were performed.

In this experiment for each of the diets tested, five large cylinders were set up in replicate. Each cylinder contained 15 flies, 12 females and 3 males. Except for a specific amount of methionine in each diet, the synthetic diet fed to the flies in the cylinders were based on diet C. The amounts of methionine in each of the four test diets were 0, 0.01, 0.04 or 0.15 g.

The fecundity of house flies fed on diet C, in which varying amounts of methionine were present, are shown in Table XVII. The results indicate the role of dietary methionine in house fly egg maturation. That is, some eggs were laid even when methionine was lacking from the diet. On this deficient diet egg laying all but stopped by the 7th day, yet by the 5th day egg laying had not reached a peak. When increasing amounts of methionine were added to the diet fecundity also increased respectively. However as the increase in fecundity was less when the methionine was raised from 0.04 g. to 0.15 g., it was evident that an asymptote was being reached (Table XXXIII in Appendix). The level of 0.15 g. of methionine used throughout the rest of the experiments was therefore close to optimum.

This procedure of eliminating and varying the amount of the sulphur-containing amino acids from the synthetic diet resulted in three important observations. The first that eggs were laid by females fed a synthetic diet lacking the sulphur amino acids and second, the number of eggs laid by females fed on this diet were

by comparison considerable. Thirdly, fecundity increased with the addition of increasing amounts of methionine.

[f] Variation in fecundity of groups of females fed on amino acid diet C or milk:

It became apparent after a number of experiments that the fecundity of females in the large cylinders was relatively consistent, but depended on the diet fed. In experiments comparing an amino acid diet with milk the mean total number of eggs per cylinder and the number of eggs per surviving female were more consistent with diet C than with the milk after 14 days under the same conditions (Table XVIII). With diet C, although the number of eggs laid was more consistent, it was markedly lower. The repeated low fecundity with amino acid diet C indicated that this diet was nutritionally deficient and prompted further experiments using individual females.

PART III

The Role of Supplementary Nutritional Factors on the First and Subsequent Gonotrophic Cycles

At this time it may appear that, once the amino acids essential for ovarian maturation in the house fly were determined, further studies in this field would add little. Moreover the literature does not suggest the need for investigations into the requirements for continued oögenesis. However from our investigations, two points required further research. The first was that, when adult house flies were fed the amino acid diet C, the fecundity was below that of females fed milk over the same period. That is, as previously noted, this synthetic diet was deficient. The second point was that egg laying

declined noticeably, after an initial period, with flies fed on an amino acid diet but not with those flies on milk. Therefore, it seemed that if egg production was to continue some specific additions to the amino acid diet were required. To recognize effectively gonotrophic cycles and the nutritional requirements for each, the fecundity of individual females was emphasized. It was this fact that led to the preparation and feeding of the experimental test diets presented in the next section.

[a] The fecundity of individual females fed on amino acid diet C or milk:

These experiments are presented to elucidate the details of the differences in egg production between individual females fed the amino acid diet C or fresh milk. The decline after the initial period of egg laying in flies fed diet C was evidently due to the lack of gonotrophic cycles beyond the first (Table XIX). Of those individual females fed amino acid diet C, 8 of the 12 oviposited but only one female laid a second batch. Even after the dissection of these 11- to 16-day old females, the ovaries of only one other fly (cylinder 7a, Table XIX) showed an indication of a second gonotrophic cycle. On the other hand this fly may have retained some first cycle eggs. Of those females fed on milk, 47% deposited two or more gonotrophic cycles (Table XIX). Eggs of second and third gonotrophic cycles are regularly laid by milk-fed flies.

In addition to the above observation, it was often noted that the total number of developing or mature eggs in the ovaries of flies fed the amino acid diets (A, B or C) is similar to that deposited by milk-fed females when both relict and laid eggs of the first cycle are

counted (Table XIX). This indicates that the amino acid diet fed to the female house fly is adequate for the maturation of eggs of the first cycle, with very few follicles failing to mature.

Thus the synthetic amino acid diet known as diet C is adequate yet limited. It is adequate, in that it allows, in the majority of females, the maturation and laying of eggs of the first gonotrophic cycle. However, this diet is obviously limited because eggs of the second gonotrophic cycle rarely develop.

[b] The value of cholesterol added to amino acid diet C:

In an attempt to improve the cystine-deficient amino acid diet (diet C), cholesterol was used as a supplement. To test the role of cholesterol three different concentrations of cholesterol 0.05%, 0.01%, and 0.001%, were added to amino acid diet C. These diets were fed to 12 females and 3 males set up in large cylinders over a 14-day period. Each concentration of cholesterol was run in triplicate. In the first test, using a concentration of 0.05% cholesterol, it was evident from the number of eggs laid and by comparison of the oviposition ratios that the concentration of cholesterol fed to the flies was less favourable than the control (diet C plus Tween and ethyl alcohol) or even diet C alone (for results and calculations of oviposition ratios see Table XX). When the oviposition ratio of the control (amino acid diet C plus Tween and ethyl alcohol) was 1, the same diet containing 0.05% cholesterol produced a ratio of 0.73. As a further comparison the oviposition ratio on the amino acid diet C was 1.96, considerably better than the diet containing cholesterol. It would appear that Tween 80 and ethyl alcohol together had deleterious effect on fecundity. The

milk diet in this experiment produced more than five times as many eggs (5.22) as the control diet.

The poor egg production with cholesterol at the concentration of 0.05% suggested that a lower level might confirm the picture reported by Monroe (1960). Therefore another series of experiments were similarly set up in triplicate using two different cholesterol concentrations, a 0.001% and a 0.01%. Two other diets were used for comparison with the earlier experiment, a control amino acid diet C preparation containing Tween 80 and ethyl alcohol, and diet C alone. This experiment indicated a much different picture of fecundity than with the higher cholesterol concentration (Table XXI). When either 0.01% or 0.001% cholesterol was fed, egg production was above that of the control, (Table XXI). Similarly this improved fecundity is apparent when a comparison is made with Monroe's data and diet C (Table XXII, Figure 3). The addition of 0.01% cholesterol to diet C gave the best egg production. Once again with the addition of the emulsifier and solvent alone, the oviposition ratio was below that of the amino acid diet C (Table XXI).

Therefore cholesterol at concentrations of 0.01% or 0.001%, are conducive to increased fecundity while at a higher concentration, 0.05%, egg laying is below that of a diet containing no cholesterol.

[c] The value of eight B-vitamins added to amino acid diet C:

Because even when diet C was supplemented with cholesterol the fecundity of flies was much below that found with milk as a diet, additional dietary supplements were sought. Studies of insect growth have repeatedly shown the importance of the B-vitamins. Because of this requirement for B-vitamins by immature and mature insects it was con-

sidered that some increase in fecundity might result in the house fly if the B-vitamins were added to the synthetic diet.

Two experiments were set up to test the effect of vitamins on fecundity and survival. In the first, diet C supplemented with vitamins was fed to 12 females and 3 males in large cylinders. The other experiment was concerned with the effect of vitamins on the fecundity of individual females confined to small cylinders. Three large cylinders and five small cylinders were used with both the amino acid diet C and diet C supplemented with eight B-vitamins (Table II).

With the large cylinders little difference exists in the egg production between the two diets (Table XXIII), i.e. the addition of eight vitamins is of little effect in mean total egg production after 7 days. The vitamin supplemented diet produced more eggs after 14 days. If female survival is considered, then some difference is noticed between the amino acid diet C and diet C supplemented with vitamins from the 7th to 10th day over the same period (Table XXIV). Almost twice as many females are surviving on the latter diet, 59% as compared to 37% on the 10th day.

In the second experiment a comparison of individual females shows that little difference exists in the number of eggs laid in the first gonotrophic cycle (Table XXV). Two females completed a second cycle when vitamins were in the diet, while only one female did on diet C alone. It is because of these second cycle eggs that a difference is apparent between the above two diets, i.e. the mean number of eggs after 14 days was 106 eggs per female fed diet C and 132 eggs per female fed the vitamin-supplemented diet. Differences in female survival were less

marked in this experiment (Table XXV).

It is of interest that the addition of vitamins to the synthetic amino acid diet C was of some value when fecundity in the first and subsequent gonotrophic cycles was followed over a 14-day period. Moreover, female survival was improved initially when vitamins were present.

[d] The value of combining cholesterol, B-vitamins, and ribonucleic acid with the amino acid diet C:

Although various supplements increased fecundity when added to diet C, in no case did this improvement approach the level with the milk diet. Therefore it was evident that even the supplemented diet C lacked some nutrient factor(s). Oögenesis as we have shown involves a rapid growth of the egg cell. To accomplish this, large amounts of cytoplasmic material must be deposited by the nurse cells of each oöcyte. In each house-fly egg there must be stored those metabolic products required for the development of a first instar larva. The limiting nutrient factor for continued egg production was, at this stage in the problem, likely some nucleic acid(s) which the adult fly was unable to synthesize, or in insufficient quantity.

To test the role of nucleic acids in reproduction, amino acid diet C was supplemented with yeast nucleic acid (RNA), vitamins and cholesterol (diet D see Table I) and compared with diet C itself. For each of the two diets tested 28 small cylinders were set up, each containing one female and three males all from the same rearing jar. Individual females were tested so that for each gonotrophic cycle the individual as well as the group fecundity could be compared.

[i] First gonotrophic cycle: The figures in Table XXVI have

been condensed from the data and are arranged so that they show gonotropic cycles and egg production. With diet D which contains RNA, the fecundity per experimental female during the first cycle shows a marked increase over that with diet C, i.e. 103 to 30 eggs per experimental female respectively. An analysis of the sample means, by the student's 't' test, showed this increase to be statistically significant [$P<0.001$]. When the number of eggs per laying female are calculated the difference is still evident, i.e. 116 to 85 respectively. The difference between these two sets of calculations actually indicates that female survival is better with diet D than with diet C. This difference in survival is shown in Figure 4. Moreover females fed diet D have a fecundity almost identical with those fed milk. The number of females laying on diet C is slightly more than one third of the total number of females used, while on milk or diet D it reaches 75 and 89% respectively. This indicates again that diet D is as good as, if not better than, milk during the first gonotrophic cycle.

[ii] Subsequent gonotrophic cycles: By studying gonotrophic cycles beyond the first, the adequacy of diet D for continued oviposition can be determined. In the second cycle with diet D, the number of eggs per experimental female and the number of eggs per laying female are 61 and 123 respectively, while the percentage of laying females was 50. In sharp contrast, diet C gave fecundity values of 9 and 132 respectively, but only 7% of the females oviposited. This large value of 132 eggs per laying female with diet C resulted from eggs laid by two females only and as previously observed, two gonotrophic cycles may occasionally be completed. A student's 't' test for the significance of the difference

between these two chemically defined diets, gave a P value < 0.01 for the second gonotrophic cycle when considering eggs per experimental female. This P value was considered significant.

Further evidence of the adequacy of diet D was noted from the record of individual females laying more than two gonotrophic cycles (Table XXXIV in Appendix). Of females fed this diet 35% laid three or more gonotrophic cycles while 50% laid two or more egg cycles. No females laid more than two cycles when fed diet C.

Although earlier, diet C seemed to satisfy the requirements for fecundity, it appears now that it is usually adequate just for the first cycle of eggs. Only when diet C is supplemented with RNA, B-vitamins and cholesterol are subsequent cycles regularly produced. Thus the real difference, when females are fed these two chemically prepared diets, is evident in three ways:

[a] the number of eggs laid per experimental, or laying female, was greater on the diet D,

[b] the number of ovipositing females was also greater on this diet, and,

[c] egg laying continues past the second gonotrophic cycle when females were fed this diet over a 15-day period.

[e] The importance of vitamins in amino acid diet D for repeated oögensis and female survival:

From the results of the previous test, a question arose as to the role of vitamins in diet D. Thus two diets were studied, one deleting the B-vitamins from diet D (number 58, Table XXVII) and the other adding inositol to diet D (number 69, Table XXXI) and each compared with diet

D and a milk diet. Eighteen small cylinders, each containing one female and three males were used for a diet. Six small cylinders were set up as milk 'control'.

[i] Experiment with diet D lacking B-vitamins: In the first gonotrophic cycle the mean number of eggs laid by the experimental females fed on the vitamin-deficient diet was noticeably lower than when flies fed on diet D or milk (Table XXVIII). The difference was less marked when considering the number of eggs per laying female. However, when examining the number of eggs per laying female, diet D appeared to be as successful as the milk diet. On the milk diet all the females laid the first batch of eggs, while only 75% laid when fed diet D and 67% when fed the vitamin-deficient diet (Table XXVIII). Although for the first gonotrophic cycle the above differences in fecundity and survival were apparent, a statistical analysis of egg laying per experimental female fed the vitamin-deficient diet D or diet D by itself, showed no significant difference ($P < 0.5$, student's 't' test).

When egg production beyond the first gonotrophic cycle was followed, only one of 15 females fed the vitamin-deficient diet (number 58) completed the second cycle, whereas with diet D (number 59) and with milk, 8 of 16 and four of the six experimental females respectively laid a second batch of eggs (Table XXIX). When females fed the vitamin-deficient diet were dissected between the 10th and 16th days, ovaries were less than a quarter the mature size. In addition, with each of these last two diets, two females completed three or more cycles. When considering egg production per experimental female in the

second cycle, the difference between diet D and the vitamin-deficient diet D was greater than in the first cycle, yet was not statistically significant ($P < 0.1$) when comparing the number per laying female. However, when the second and subsequent cycles were considered together, the difference between these two diets was significant ($P < 0.05$). Thus the B-vitamins become increasingly more important in the diet after the first gonotrophic cycle in most flies. On the other hand, the fecundity of individual flies laying a second batch of eggs with the vitamin-deficient diet, was somewhat similar to the mean fecundity of laying females fed diet D (Table XXIX, column 3).

The female survival with the vitamin-deficient diet when compared with the milk diet or the other synthetic diets indicates an inadequacy (Figure 5). However, female survival with diet D with adenine replacing RNA was similar to that with milk.

This experiment was repeated in the same way by using adults reared from second rather than first cycle eggs (Table XXX). Again egg production for the first and subsequent gonotrophic cycles was generally similar to that in the first experiment. Females were fed the vitamin-deficient diet (number 61) and diet D (number 62) and were compared with those fed milk. However in this experiment a statistical evaluation of the fecundity of the experimental females, during the first cycle, showed a significant difference between the vitamin-deficient diet and diet D ($P < 0.02$, $t = 2.575$). As in the first experiment this difference during subsequent cycles was also significant ($P = 0.05$, $t = 2.038$), but considering the second cycle only this difference had a P value < 0.06 ($t = 1.980$). This difference between the vitamin-deficient diet

and diet D is also reflected in the total fecundity (Table XXX, last column).

The survival of females (not graphed) fed on the vitamin-deficient diet was equivalent to that with diet D and milk. A comparison of the time in days at which 50% of the females were alive was of interest (the median time for survival, hereafter referred to as ST_{50}). For the vitamin-deficient diet, diet D and milk the ST_{50}'s were 7, 6.5 and 8 days respectively. These supporting observations emphasize a different criterion for dietary adequacy in addition to that of fecundity.

[ii] Experiments with diet D supplemented with inositol: It was anticipated that some improvement in egg production might be possible if the vitamin inositol was added to diet D.

Therefore an experiment was conducted using 18 small cylinders with diet D (number 68) and 18 with diet D plus inositol (number 69), and six as milk controls.

For the first gonotrophic cycle the fecundity of the females fed the two synthetic diets is similar (Table XXXI), although females fed the inositol supplemented diet laid a slightly higher number of eggs than those females fed on diet D. This is reflected in the total number of eggs laid in the first cycle, 1302 to 1189 respectively[27], and the number of eggs per experimental female. A similar result was seen in the second gonotrophic cycle (Table XXXI). Records of individual females laying eggs after 16 days showed that more than two cycles

---

[27] This was established from the daily records and by dissecting the individual females at the end of the experiment.

occurred on these two synthetic diets. For diet D and diet D plus inositol the number of females laying three to five gonotrophic cycles was 2 and 4 of 18 respectively; this was reflected in the total fecundity of females on these diets (Table XXXI, last column).

Female survival was clearly better with diet D than with the inositol-supplemented diet, ST_{50} 11.9 and 7.5 days respectively (Figure 6). The fact that egg production is better with the inositol supplemented diet than with diet D, in spite of poorer survival with the former, points up the value of inositol in the diet for fecundity although this was not shown to be statistically significant.

[f] The replacement of ribonucleic acid by nucleic acid bases in diet D and its effect on repeated oogenesis and female survival

[i] Experiments with adenine substituted for RNA in diet D: It was considered possible that oogenesis in the house fly could proceed when RNA was replaced by one or more of the nucleotides, and in particular the purine and pyrimidines bases. In the first experiment to test this possibility, adenine was substituted for RNA in diet D (number 60, Table XXVIII and XXIX) and this diet was compared with diet D (number 59) and milk. Eighteen small cylinders were used with each of the synthetic diets and six with milk.

In the first gonotrophic cycle the average number of eggs laid by the experimental females fed the diet containing adenine was noticeably lower than that by females fed diet D or milk (Table XXVIII). This difference was even more marked when considering the number of eggs per laying female. Of the synthetic diets the diet containing adenine gave the highest percentage of females laying first cycle eggs.

Although the diet containing adenine gave a lower egg production in comparison with diet D, this difference was not statistically significant ($P < 0.5$).

When the second gonotrophic cycle was considered one third of the experimental females fed on the diet containing adenine (number 60) completed this cycle, whereas with diet D (number 59) one half of the females laid a second batch (Table XXIX). When considering more than two cycles, only one female fed on this diet containing adenine laid more than two batches of eggs whereas with diet D two females completed three or more cycles. Total egg production on the former diet was similar to flies fed milk, and noticeably poorer than diet D although this difference did not prove significant. Flies fed the diet with adenine replacing RNA laid less than half the number of eggs (334) after the first cycle than did flies fed diet D (734).

In comparison to those females fed other synthetic diets, the survival of females fed on the diet containing adenine was noticeably better (Figure 5). This increased longevity is unexplained. However the decline in fecundity was of major interest.

A second experiment in which adenine replaced RNA in diet D was carried out in the same way but using adults reared from second rather than first cycle eggs (Table XXX). Generally the number of eggs per experimental female in both the first and second gonotrophic cycles and the total number of eggs laid were similar to these results of the first experiment. However again the differences in the number of eggs per experimental female between the two synthetic diets was not statistically significant for either cycle. In this experiment

the percentage of females laying first and second cycle eggs was noticeably lower, which meant a slightly greater number of eggs per laying female in both cycles. The insufficiency of the diet containing adenine (number 63), compared with diet D (number 62), was more evident when considering the total number of second cycle eggs (188 and 591 respectively). These results confirmed the first experiment.

Again the diet containing adenine gave a better survival (not graphed) than either diet D or milk as shown by ST_{50}'s of 10.5, 6.5 and 8 days respectively. This extended survival when adenine is substituted for RNA is of obvious value to fecundity, but is apparently offset by other effects.

[ii] Experiments with adenine and cytidylic acid replacing RNA in diet D:

The question arose as to whether a purine and pyrimidine base together might produce an improved fecundity and survival when they replaced RNA. In the previous experiment the substitution of adenine for RNA resulted in a reduced fecundity, but an improved survival. Thus, the addition of another nucleic acid base, cytidine (in place of cytidylic acid), might improve egg laying and still maintain a high adult survival.

In this experiment, diet D with adenine and cytidylic acid replacing RNA (number 67, hereafter diet E, see Table I) was compared with diet D (number 68) and milk. For each of these synthetic diets, eighteen females were set up individually in small cylinders. Milk, as control, was fed to six females, each in a small cylinder.

In the first gonotrophic cycle with diet E the number of eggs

per experimental female was greater than that with diet D (Table XXXI). This is the result of more females ovipositing when fed diet E rather than a greater number of eggs per laying female. However with milk although fewer females laid eggs than with diet E the number of eggs per laying female was higher than found with either of the synthetic diets. Moreover the total number of eggs laid during the first cycle was greater with diet E (1324) than with diet D (1189).

In the second gonotrophic cycle the greater number of eggs per experimental female fed on diet E was due to a higher fecundity per laying female as well as to more females laying eggs (Table XXXI). In this experiment both synthetic diets, especially diet E gave better fecundity for the second cycle than the milk control. On diet E four females laid three to five cycles of eggs whereas on diet D only two did.

Survival of females fed diet E was somewhat better than diet D, the ST_{50}'s being 13.0 and 11.9 respectively (Figure 6).

Thus it is notable that the substitution of these two nucleic acid bases, adenine and cytidylic acid for RNA improved overall fecundity (Table XXXI, last column) more than in previous experiments where only adenine was substituted and also more than when RNA was used, i.e. diet D.

[g] Feeding dry chemically defined diets to adult flies: Although the present techniques using xenic liquid diets resulted in no discernible effects of biotic contamination, the development of dry synthetic diets in studies of house-fly nutrition would make a useful comparison and might ultimately be preferable. Because of the

polyphagous habits of the adult house fly, the feeding of dry synthetic diets alone, generally considered impractical, seemed worthy of investigation. Unattractiveness and unpalatability of a dry diet was considered the likeliest cause for failure rather than a dry diet being nutritionally inadequate. Whether house flies would oviposit on such a dry medium was uncertain. After initial test powdered sucrose was found to stimulate feeding and was the necessary ingredient in the preparation of further dry synthetic diets.

To determine that a dry synthetic diet was adequate for egg development, experiments were designed so that feeding and any egg laying could be observed. Twelve females and three males, all reared identically, were introduced into large cylinders (Plate 5). Six cylinders were employed to test each diet. At the time of the daily introduction of the dry diet, a 0.1 M sucrose solution in a vial with a cotton wick was also presented (Plate 6). Two diets were used and ground separately in a mortar. Dry diet no. 73 contained all the components previously included in liquid diet C (Table I). Diet no. 72 consisted of all the dry components present in liquid diet D (Table I) but with cholesterol omitted. The fresh dry diets were refrigerated and portions presented daily in small glass dishes, 12 mm. high, by 22 mm. inside diameter. Four large cylinders were also set up with liquid milk as a "control." From the mean number of eggs per cylinder after 7 and 17 days it was evident that fecundity on both synthetic diets was exceptionally low when compared to that with milk (Table XXXII). Also the mean number of eggs per surviving female was unusually low for diets no. 72 and no. 73; 15 and 11 after 7 days, and

21 and 18 after 17 days respectively. By comparison during the same period the milk diet gave values of 66 and 138. From earlier experiments, it is evident that with this group of experimental flies, egg laying on all diets was poor. However, the interest here was the comparison between the dry diets and the milk diet. It should be mentioned that when these females were dissected, they contained additional mature eggs. Thus both dry synthetic diets were capable of supporting oögenesis.

In contrast to fecundity female survival on these dry diets was much better. For both dry diet C (number 72) and dry diet D less cholesterol (number 73) the ST_{50}'s were 12.2 and 13 days respectively (Figure 7). The survival values on both these dry diets were comparable to the best ST_{50}'s for all other diets and the initial survival after emergence was better than any other amino acid diet.

From these tests two valuable observations are evident. Firstly, adult house flies fed chemically defined dry diets can mature and deposit eggs. Secondly, female survival was as good as with the best liquid diet.

DISCUSSION

The elucidation of the chemically defined dietary needs of the house fly, Musca domestica, in relation to fecundity was considered a fruitful beginning to further research aimed ultimately at relating the larval and adult nutrition to the reproduction of blood feeding and non-blood feeding muscid flies. Information gained from earlier work on the nutritional requirements for growth and reproduction in the adult house fly and in other dipterous insects indicated that there existed certain definite food requirements for these processes. For example Ascher and Levinson (1955) concluded that protein reserves laid down in the larval stages cannot be mobilized for egg development in adults of the oriental house fly, Musca vicina, but that another source of protein was required. In support of this it had been reported that for ovarian development adults of various species of flies and other insects needed amino acids, carbohydrates, minerals, vitamins or nucleic acids (Rasso and Fraenkel, 1954; Harlow, 1956). Yet the food reported as required for reproduction represents only part of the picture. Many other factors are known to contribute to fecundity in insects.

The adult house fly, unlike many insects, is polyphagous[28].

---

[28] Recently Robbins and Shortino (1962) demonstrated that adults show autogeny when the larvae are reared on a high cholesterol diet.

It is this character that certainly facilitates the feeding of artificial diets to an immature or adult insect (Dadd, 1960b). Fortunately other characteristics of the adult female house fly are favourable for studies on nutrition and reproduction. Of obvious value is the short gonotrophic cycle. Other, almost equally important, characteristics of the female are the need for only one mating (Zingrone et al, 1959) and the unspecialized needs for oviposition sites (West, 1951), and the saccharides, non-specific nutrients, which function as attractants and gustatory stimuli (Galun and Fraenkel, 1957; Thorsteinson, 1960).

It was important initially, to determine the basic rate of ovarian maturation when a diet adequate for egg production is fed. For, if other environmental conditions are favourable, the rate of oögenesis in the adult house fly and related insects should depend on the suitability of the food. It was also evident in other Diptera (Harlow, 1956; Hosoi, 1954) that variations in the rate of gonadal development of the female occur when different diets are fed to the adult. Kobayashi (1934) observed similar large irregularities in female house flies. These physiological variations were found in the present studies on the house fly, but these female variants were considered a normal part of the data.

Although axenic techniques can present the most reproducible picture of insect nutritional requirements, it has been repeatedly demonstrated that under xenic conditions many specific dietary needs can be determined (Noland et al 1949; Fraenkel and Printy, 1954; Rasso and Fraenkel, 1954; Harlow, 1956; Dimond, 1957; Dadd, 1957). In those cases where xenic techniques were employed investigators have

attacked the problem by inhibiting the gut flora in the insect (Fraenkel, 1959), by feeding metabolic antagonists (Levinson and Bergmann, 1959), by feeding dry diets (Frobrich, 1953; Dadd, 1957), or by maintaining rigid techniques (Harlow, 1956; Dimond, 1957). It is well known that by using axenic culturing methods the feeding conditions of the insect and the synthetic foods, in many ways,do not present the best growing conditions. Secondary defects, such as the consistency of the diet, may become a primary complication. It is of significance that Fraenkel (1959), in a survey of the dietary requirements in insects, should conclude that he was less convinced about the importance of intestinal sterility in many insects. Indeed the results of recent nutritional studies, in which septic flies and unsterilized media were used, have contributed in many ways to the understanding of insect nutrition. In the present study contamination was kept to a minimum by storing diets at low temperatures until needed although they were initially unsterilized, a procedure used by Dimond (1957). The daily renewal of the diet fed to the adults and the short time required for oögenesis, when adults are fed an adequate diet[29], assisted further in establishing the diet needed for egg production.

In preparing and feeding these xenic liquid test diets to house flies, the most acute complication would be to feed diets changed by contamination, for example changes resulting from contamination by microorganisms. The contributions of symbiotic microorganisms in the

---

[29] An adequate diet, in this case, is one supporting ovarian maturation.

insect are also important (Fraenkel, 1959). Dimond et al (1958) reported that accessory growth factors in addition to the amino acids, contributed no stimulation to egg production in Aedes aegypti. They considered that undoubtedly microorganisms within this insect were able to provide the necessary growth factors. However this interpretation may not be justified for Lea et al (1956) using aseptic techniques, reported that for A. aegypti egg production occurred with an amino acid diet alone. In any case in a recent paper, Greenberg (1959) observed that in house flies there is a general process of gut sterilization before adult emergence. If microorganisms were to make an obvious contribution, then two main observations would be evident. Firstly, erratic and inconsistent results would arise and secondly, insignificant differences in fecundity would be found between different diets. This was not the case in the present study. Marked consistency in egg laying was found during feeding of identical diets (Table XVIII) and marked differences were evident between certain other diets.

The adequacy of our test diets were chiefly assessed in terms of the fecundity of the adult fly rather than by changes in weight, survival or ovarian growth. This means of measuring the adequacy of a diet is not without complications. When food, in a sufficient number of cases, fails to support ovarian maturation, this failure may result either from inadequate feeding or the quality of the food. It is well known that house flies (West, 1951), blow flies (Fraenkel, 1936, 1940) and flesh flies (Dorman et al, 1938) require an assimilable carbohydrate for survival, otherwise death results even before ovarian maturation is possible. Therefore, female longevity or survival may be an effective

criterion to determine whether sufficient food is eaten by the adult house fly. The use of adult survival is not new to nutritional studies, for in 1933 McCay compared growth rate and longevity of rats in his experiments. In the present research, then, it was crucial to determine at what stage factors other than the quality of the diet affected egg production. Such factors were prevented from operating or were evaluated during these studies on house fly nutrition and fecundity.

Robertson and Sang (1944), working with adult Drosophila, found that when the quantity of available food is restricted oviposition rate is reduced. Because of this, the experimental food, including sugar, was always available in excess to the adult house fly and feeding was ad libidum.

Harlow (1956) noted that for adult female blow flies the presence of males encouraged egg laying. She pointed out however that males had no effect on ovarian growth. Other research workers (Dean, 1938; Glaser, 1923; Hagen, 1952) found that unmated female flies, whether the apple maggot fly, house fly or fruit fly, all laid fewer eggs than mated females. Browne (1958) showed that for the sheep blow fly, Lucilia cuprina, the state of the ovaries is important in determining whether females will mate. For this reason it was considered essential to establish and maintain under experimental conditions, the number of males for optimum egg laying. As we have outlined, males were always part of our experiments. Yet it should be noted that the absence of males from the experimental cylinders does not prevent egg laying by the house fly.

Previous research on defined diets and reproduction in dipterous

insects was concerned with the preparation of diets adequate for the production of eggs or total numbers of eggs laid by large groups of adults (Hagen, 1952; Dimond et al, 1956; Singh and Brown, 1957). The female house fly is known to lay discrete batches of eggs regularly when fed a satisfactory diet (Glaser, 1923; Kobayashi, 1934; Derbeneva-Ukhova, 1935). However under our experimental conditions the number of eggs per fly, the number of cycles, the egg laying rate and longevity vary between individual flies. This, as we noted previously, was also found for the house fly by Kobayashi (1934) under different experimental conditions. Thus individual females must be followed if semi-synthetic or defined diets are fed to adults and reproductive cycles are measured. For this and other obvious reasons fecundity and reproductive cycles were reported rather than the period of each oviposition or the time between them. Data comparing female reproductive cycles, after the milk control or supplemented amino acid diets (diet D and E) are fed, show surprising similarity. Our data consistently show a decline in fecundity with continued egg laying, as was also reported by Greenberg (1955).

A decline in general fecundity of a house fly population may result from lowered oögenesis in each female because of inadequate diet or from aging or mortality. The longevity of house flies in the laboratory has been studied extensively by Rockstein (1956) and Rockstein and Lieberman (1959), and is known to depend on the conditions of maintenance. Under much different conditions Kobayashi (1934) found variation in house-fly survival. Such factors as temperature, humidity, diet, and age of parents are found to have the most effect on survival

(Rockstein, 1957). Rockstein and Lieberman (1958) reported that under laboratory conditions adult males had a mean longevity of 17.4 days and females a mean longevity of 29.4 days. Under the present experimental conditions female house flies fed milk had a median survival (ST_{50}) of 14 days. However these flies were fed fresh homogenized milk only, much different than the diet of sugar, water and powdered milk used by the above authors.

To establish in this research whether the eggs laid indicated the full expression of oögenesis, a portion of the females were dissected and examined for stage of ovarian maturation and for relict eggs. The various stages of ovarian change have been reported for the fruit fly, Drosophila (King et al, 1956), the mosquito, Aedes aegypti (Christophers, 1960), the black fly (Wanson, 1950), the blow fly, Protophormia terraenovae (Harlow, 1956) and the house fly (Derbeneva-Ukhova, 1935). These changes classified into as many as 14 stages, have been reported from observations made either microscopically or macroscopically.

Ovaries in the house fly are of the polytrophic type containing between 50 to 75 ovarioles per ovary (West, 1951). The rate of ovarian development depends primarily on two main factors, diet and temperature (Derbeneva-Ukhova, 1935). In this work temperature was maintained approximately constant so that attention could be directed entirely to dietary influences. Generally complete ovarian maturation of the milk-fed house fly was accomplished within 4 days at 78°F. The greatest and most striking increase in the size of the ovariole was due to the growth of nurse cells and primarily to yolk deposition between the 2nd and 3rd day (Figure 1 and Plates 7 to 14). Similarly

in the blow fly, Harlow (1956) found greatest volume change over a one-day period, but between the 3rd and 4th day, with oviposition occurring on the 7th day, and for Drosophila the oocyte undergoes an increase in volume of over 100,000 times in 3 days (King et al, 1956). This rapid yolk formation indicates a notable mobilization of food reserves and/or the active utilization of ingested food. This was evident in the present study through dissections and observations of those changes in the fat body of females exhibiting rapid oogenesis contrasted to that of females fed only sugar water which showed limited ovarian growth. From the appearance of our values and the results of Derbeneva-Ukhova (1935) for sugar-water flies, ovarian growth for the first and some of the second day is due to reserves from the larva.

Recently it has been established for the mosquito that ovarian development is under control of humoral factors produced in the head (Gillett, 1958; Larsen and Bodenstein, 1959). The work of Day (1943) and Thomsen (1940,1956) indicated that changes in metabolism, in the fat-body cells, oenocytes, and ovaries in the blow flies, Calliphora, Lucilia, and Sarcophaga are under control of the median neurosecretory cells and corpora allata of the brain. More recently Thomsen and Møller (1959) showed that these neurosecretory cells influenced as well the production of proteinase by the midgut cells, protein synthesis and in some way protein metabolism. However, flies without neurosecretory cells were able to utilize the specific protein of the larval fat body. Scharrer (1958) questioned whether the central nervous system represented an obligatory way-station for all factors controlling the activity of the female reproductive organs, or whether some of them

acted more directly, i.e. on the corpora allata or even on the gonads themselves. That ovaries develop little if diets are inadequate suggests that, in addition to insufficiency of nutrients, one or more of these neurosecretory processes may not have been initiated. However in this study attention was focussed on the effect of diet on the process of ovarian maturation, only after these processes were operating. There still remains the question of the relationship between an adequate diet and the neuro-endocrine process.

The adults of the apple maggot fly, oriental fruit fly, mosquito and blow fly have all been fed casein successfully as a protein source for egg production (Dean, 1938; Hagen, 1952; Lea et al, 1956; Rasso and Fraenkel, 1954 respectively). For studies on insect growth other investigators have supplemented purified casein, the protein source, with other factors determined essential for growth. Yeast or yeast extract added to the diet generally supplied the required vitamins (House, 1961). Neither of these last two prepared products were considered as an adequate diet in themselves.

In RESULTS (Part I, section "f") it is evident that yeast extract or various brands of low vitamin or "vitamin free" casein added to sugar-water, were alone adequate for egg production, but one brand of "vitamin free" casein was much less successful even than the yeast. It must be remembered that a purified casein is never entirely free of vitamins and may contain enough vitamins to influence ovarian development (Rasso and Fraenkel, 1954). However differences in the ratio of protein to carbohydrates in the diets may have been responsible. Recent investigations on selective feeding have shown that quantitative protein and

carbohydrate requirements account for differences in fecundity during the reproductive cycle in the house fly (Greenberg, 1959) and a blow fly (Strangways-Dixon, 1961).

Differences between these casein preparations were more evident when female survival and continued ovarian cycles were observed. Previously it was supposed that only proteins or amino acids were essential for continued egg production when fed to the adult house fly. Indeed, the results of Singh and Brown (1957), and Dimond (1957) seem to attest to this conclusion. The fact that there were differences in fecundity with flies fed the yeast extract and the 6% casein (Nutritional Biochemicals Corporation, - "vitamin free") might be considered as due to the quantity of food ingested by the fly, or that yeast was a more suitable diet. Also there is the chance that different impurities are present in the casein preparations. This points to the possible importance of other factors in addition to protein. With all the above questions in mind, a study was initiated to establish the chemically defined diet adequate for egg production in the adult house fly.

As previously reported for the mosquito, Aedes aegypti, only amino acids, in addition to water, minerals and a carbohydrate, were considered essential for egg production when fed to the adults (Singh and Brown, 1957; Dimond, 1957). Yet Harlow (1956) reported that a mixture of 19 amino acids were ineffective in producing mature ovaries in the blow fly. All the amino acids found essential for A. aegypti by the above authors were included in the diet fed to the blow fly. The lack of success with the blow fly she ascribed to an unidentified accessory factor or factors, and suggested these to be certain other

mineral salts and B-vitamins. With this background it was considered that for the adult house fly the essential amino acids might be identical to those established as indispensable for reproduction in the mosquito.

The first indication of essential or indispensable amino acids in animal nutrition was established by various researchers studying vertebrates (Osborne and Mendel, 1917; Rose, 1937). It was soon recognized by those in this field of nutrition that the absolute quantities of each of these essential amino acids, which must be supplied in the diet, will change with the species of animal and will vary rather widely with the physiological state of the animal (Block, 1957). The nutritive classification of the amino acids by Block (1957), with regard for the young growing mammal, lists nine amino acids as indispensable, namely; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Arginine is included if maximum growth is desired (Frost, 1959).

The data accumulated by previous workers on the amino acid requirements for reproduction in Diptera show an interesting uniformity. In the present study, nine amino acids were considered as the "essential amino acids," i.e. they are required in the diet of the adult house fly for the first oögenesis, and include arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan and valine. When any one of these is omitted singly from the diet, egg laying fails. This is not due to the lack of an ovipositional response, because the ovaries are immature on dissection and appear similar to ovaries of flies fed sugar-water only. Except for methionine these are the same

amino acids listed above as indispensable for the growing rat (Rose, 1937, 1938). Apparently arginine cannot be synthesized by the growing chick, pigeon or turkey (Block, 1957; Almquist, 1959). Thus, the amino acid requirements for reproduction in the adult house fly include arginine as with the avian species.

The amino acids determined in this study as essential for ovarian development and oviposition in the house fly, although generally similar to those found necessary for egg laying of the haematophagous mosquito, Aedes aegypti, do show several important differences.

Singh and Brown (1957) working with the same mosquito disagree with Dimond et al (1956) as to its requirements for cystine. Singh and Brown (1957) did not consider cystine essential. Dimond (1957) considered that cystine, although not absolutely essential, is required to maintain a high level of egg production in A. aegypti, and because of this, he includes cystine as an "essential amino acid." As far as egg laying is concerned in the house fly, our observations agree with the conclusion of Singh and Brown (1957) that the omission of cystine was without effect, providing that methionine was present in the diet. Initially it was noted that when either these amino acids were deleted separately from the diet fed to the house fly, eggs were laid. Also when both cystine and methionine are present in the diet, no increase in egg production is evident; in fact if cystine is deleted, fecundity improves.

These observations indicated a need for further information on the dietary requirements of the house fly for the sulphur amino acids during oogenesis. Rose (1938) had reported that for the rat methionine

amino acids listed above as indispensable for the growing rat (Rose, 1937, 1938). Apparently arginine cannot be synthesized by the growing chick, pigeon or turkey (Block, 1957; Almquist, 1959). Thus, the amino acid requirements for reproduction in the adult house fly include arginine as with the avian species.

The amino acids determined in this study as essential for ovarian development and oviposition in the house fly, although generally similar to those found necessary for egg laying of the haematophagous mosquito, Aedes aegypti, do show several important differences.

Singh and Brown (1957) working with the same mosquito disagree with Dimond et al (1956) as to its requirements for cystine. Singh and Brown (1957) did not consider cystine essential. Dimond (1957) considered that cystine, although not absolutely essential, is required to maintain a high level of egg production in A. aegypti, and because of this, he includes cystine as an "essential amino acid." As far as egg laying is concerned in the house fly, our observations agree with the conclusion of Singh and Brown (1957) that the omission of cystine was without effect, providing that methionine was present in the diet. Initially it was noted that when either these amino acids were deleted separately from the diet fed to the house fly, eggs were laid. Also when both cystine and methionine are present in the diet, no increase in egg production is evident; in fact if cystine is deleted, fecundity improves.

These observations indicated a need for further information on the dietary requirements of the house fly for the sulphur amino acids during oogenesis. Rose (1938) had reported that for the rat methionine

was essential and was capable of completely replacing cystine. Yet in the mammal a certain level of dietary cystine will spare methionine and for some mammals cystine is an important dietary constituent (Almquist, 1959). For the house fly Hilchey et al (1957) and Cotty et al (1958) indicated that methionine was converted to cystine but the adult could not reverse this reaction. For this reason and from the reports that methionine is required for growth of other Diptera, it seemed that methionine was the most likely of the sulphur-containing amino acids to exhibit essential characteristics.

However the problem of the role of dietary methionine for oogenesis in the house fly is not as clearly defined as other studies on Diptera fed the essential amino acids. Since a number of eggs were laid when both cystine and methionine were deleted from the synthetic diet, then certainly methionine cannot be considered as an essential amino acid. Certainly the value of methionine for a high fecundity in the first ovarian cycle was evident in these studies. For when this sulphur amino acid was added to our synthetic diet at increasing concentrations, fecundity increased respectively. Because a good number of eggs were laid when methionine was deleted, it is probable that the newly emerged fly must possess utilizable stores of sulphur metabolites carried over from the larva. It has been reported that the adult house fly is known to metabolize dietary or injected isotopic sulphur compounds in a manner resembling that of vertebrates (Hilchey, 1958; Cotty et al, 1958). However prior to this work the significance of methionine for oogenesis was not established. In conclusion then, from these experiments involving methionine deletion

and in those concerned with the requirements for continued egg production methionine is evidently an "essential amino acid." Certainly the means of determining its essentiality were unusual in comparison to the other essential amino acids for house fly oogenesis. Briefly dietary methionine is not required for the first ovarian cycle, but is essential for continued egg production.[30]

Other differences between the house-fly and mosquito requirements are found in the work of Dimond (1957). He obtained a few eggs when one of the amino acids, arginine, histidine or methionine (with cystine present) were lacking from his test diet. He suggested the hypothesis that adults of Aedes aegypti possessed store of amino acids sufficiently large in these three amino acids to satisfy the requirements for a lower level of egg production. However we were unable to obtain eggs in the house fly when either histidine or arginine were omitted from the amino acid diet.

In addition to the essential amino acids certain nitrogen containing compounds are able to provide the extra nitrogen required for the synthesis of non-essential amino acids. That the female house fly was able to utilize the non-essential amino acids and other sources of nitrogen during oogenesis is not unusual. Other investigators have been able to show that, for growth, mammals and insects can utilize glutamic acid, glycine, diammonium citrate, ammonium acetate or urea

---

[30] It is appreciated that some would consider that methionine be designated as an essential amino acid for egg production, whether considering first or repeated oogensis.

(Rose et al, 1949; Frost, 1959; DeGroot, 1953). It is not surprising then that in this study the house fly, when fed the "essential amino acids," was able to utilize glutamic acid, ammonium citrate or urea as a source of nitrogen. The absence of these was followed by a reduction in fecundity. Of these urea was the least effective. For egg production in the mosquito fed an amino acid diet, Dimond (1957) reported that the stimulatory effect of glutamic acid was matched when aspartic acid or ammonium acetate were substituted. Dean (1938) also showed that reproduction of the adult apple maggot, Rhagoletis pomonella, was increased when non-protein nitrogen was added to a sugar and water diet and few, if any, eggs were produced on a sugar solution alone. The young adult honey bee significantly increased its nitrogen content when fed ten essential amino acids along with other nitrogen compounds (DeGroot, 1953). He found the nitrogen compounds capable of supplementing the amino acids to be urea, glycine, glutamic acid, di-ammonium citrate and ammonium acetate, and all induced an improvement in growth. Thus the ability of adult insects to utilize ammonium compounds agrees with the growth response of mammals fed similar compounds. Gilmour (1961) points out that glutamic acid occupies a central role in amino acid metabolism, it is likely the major point of entry of ammonia nitrogen into the metabolic pool.

A suitable carbohydrate and minerals (salts) along with the essential amino acids and water, are the only other substances required under these conditions if ovarian development in the house fly is to be initiated and oviposition achieved. The essential nature of these has been tested and is discussed below. A carbohydrate solution (sucrose)

was found necessary to maintain survival after emergence, and must be part of the diet if eggs are to develop. Also without minerals ovaries did not develop, but survival was better on a mineral deficient diet than when sucrose was deleted. It is essential therefore, if egg production is to occur, that water, a carbohydrate, and minerals be included in the diet. This is similar to the mosquito, Aedes aegypti, and the blow flies, Phormia regina and Protophormia terrae-novae (Dimond, 1957; Rasso and Fraenkel, 1954; and Harlow, 1956 respectively).

By the time that the above observations were recorded it was becoming increasingly evident that the requirements for egg development during the life of the adult house fly were more than the simple amino acid diets initially fed to emerging adults. In xenic studies on larval growth and nutrition it is necessary to eliminate specific differences in the quality and quantity of the diets in order to compare growth requirements, but in adult nutrition it is necessary in addition, to consider stored nutrients transferred from the larva (Dimond, 1957). As Trager (1947) pointed out, when an insect reaches the adult stage with reproductive organs only partially developed, its reproductive ability is affected by its nutrition as an adult as well as by that during its growing stages.

Earlier observations on the oriental housefly, Musca vicina, (Ascher and Levinson, 1956) led to the conclusion that in some Diptera, protein reserves laid down in the larva cannot be mobilized for egg production. This fecundity of adult house flies, M. domestica, fed methionine-deficient diets, but not with cystine-deficient diets, suggested two points for consideration: either some cystine was being

converted to methionine, or sufficient methionine or utilizable sulphur was transferred from the larva to support the maturation of a few eggs. The first point seemed unlikely since Cotty et al (1958) had established by autoradiography that cystine after injection or by feeding is not converted to methionine. The second point was more likely and suggested quite conclusively that food reserves retained from the larvae, were implicated in continued egg development. Earlier Gordon (1959) realized the importance of reserves in insects and concluded that one of the most striking features of research in insect nutrition is the difficulty of producing deficiency symptoms such as those observed in mammals. It is interesting to speculate as to why a few flies were capable of laying a second cycle on an amino acid diet (diet C) and that some first cycle eggs were laid on the amino acid diet (diet B) less methionine. This may indicate that more nutrients can be stored by some larvae and transferred to the adult. In the light of recent reports on the house fly (Robbins and Shortino, 1962) it is undetermined whether this is due to genetic or environmental causes, and this is in contrast to the observations of Ascher and Levinson (1955) for the oriental house fly, that the protein for oogenesis is not supplied by the larval reserves.

These results led to a consideration of the role that other chemicals transferred from the larvae, might play in ovarian growth. However, the possibility existed that once these nutrient stores were depleted, probably by egg production, there might be found some factors that, when added to the amino acid diet, would help to compensate for this depletion and to maintain egg production. Since the amino acid

diet (diet C) still produced fewer eggs than the protein diet (milk), the addition of even a single nutritional factor might be all that was required. The course to follow then was to supplement the amino acid diet so that an optimum egg production would result.

Earlier we had discussed the general dietary requirements of insects for lipids, now we shall consider the lipid requirements for house-fly reproduction. Both Hammen (1956) and Monroe (1960, 1961) established the value of cholesterol for improved adult weight and egg viability when in diets fed to house-fly larvae and adults. In fact Monroe (1960) reported that the lack of cholesterol in the diet fed to the adult caused nearly an 80% reduction in egg viability. Of much interest is the recent report by Robbins and Shortino (1962); they found that the addition of an unusually high level of cholesterol to the larval medium, resulted in ovarian maturation in about half the house-fly adults. Sucrose and water only served as their food. Because of Hammen and Monroe's findings cholesterol was the first supplement added to the amino acid diet which gave an increase in egg production, with an optimum around 0.01%. As a result of this increase in fecundity, cholesterol at this concentration was used in all further diets during this study. Nevertheless, even with this increase, egg production was much less than that obtained with milk, neither were there repeated gonotrophic cycles. In contrast Sang and King (1961), using axenic techniques, concluded that for the fruit fly, cholesterol seemed unnecessary for egg production.

Nothing was found in the literature pertaining to research on the factors necessary for continued oögenesis in adult Diptera.

Obviously these factors were entirely overlooked or never considered. Current interpretations of the nutritional requirements for reproduction are based, in part, on earlier studies with natural and synthetic diets fed to groups of flies. For this reason it was hoped that observations on individual females might supply more detailed information on egg production. Thus it was important to establish whether the female house fly fed amino acid diets, laid the usual separate batches of eggs as with the milk diet. From experiments using individual flies, two main conclusions were evident. The first was that female house flies on a synthetic diet did lay eggs in cycles. Secondly, an individual female fed an amino acid diet (diet C) generally lays only one batch of eggs, which is the consequence of the maturation of the first gonotrophic cycle only. Rarely does a female develop another batch of eggs. This is of great significance and explains the reason for the low egg production previously obtained from groups of flies maintained in large cylinders.

It was repeatedly observed in the house fly (from dissections) that all the eggs of a single gonotrophic cycle tend to mature simultaneously and that if gonodal development reaches completion the number of mature eggs varies within narrow limits. This characteristic of the female house fly we are designating as ovarian synchrony. Although there was evidence in our histological sections of ovarian degeneration in an occasional fly, never did we observe the maturation of one or a few eggs separately in the ovary as Larsen and Bodenstein (1959) did for the mosquito, Aedes aegypti. Also, Hosoi (1954) reported that in another mosquito, Culex pipiens, the number of mature

follicles and the rate of follicle development varied considerably, depending on the amount or kind of blood ingested. It may be that for the female house fly, one meal is adequate for continued maturation of both ovaries if it is sufficient to initiate ovarian growth. This is in contrast to the observations of the above authors who showed that the maturation of a mosquito ovariole can proceed independently of the other ovarioles within the same ovary. Bodenstein (1947) concluded from his observations on Drosophila that the external nutritional factors are not determiners of growth and maturation. We consider that these factors merely represent one source of raw materials on which these processes in the house fly depend. The reasons and significance of the balance between nutrition, hormones and oögenesis at this time are not yet clear.

Earlier experiments indicated an inadequacy in the synthetic diets. This was apparent with the study of individual females, or even groups of females, which were fed amino acid diet C supplemented with cholesterol. These diets allowed the laying of first cycle eggs but not the maturation of subsequent ovarian cycles. It seemed then that to maintain a high level of egg production some other dietary factor(s) must be required by the adult female. For ovarian maturation in the adult blow fly, Protophormia terrae-novae, Harlow (1956) reported that vitamins were not satisfactory, yet earlier it was reported for another blow fly, Phormia regina (Rasso and Fraenkel, 1954) that the addition of B-vitamins to a basic carbohydrate-protein diet influenced the rate of ovarian maturation. Harlow (1956) failed to consider rate of ovarian development which might have accounted for this conflict. Thus,

if this was to be clarified the influence of vitamins for ovarian development in the house fly needed investigation. The observations resulting from feeding an amino acid diet (diet C) supplemented with eight of the B-vitamins (folic acid, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, thiamine, biotin, and choline) to the adults clearly indicates that they were of little value to fecundity (Table XXIII). Certainly these B-vitamins were not the answer to continued egg production.

Up to this time no additives to the amino acid diet C, produced a marked increase in egg production. The problem then appeared to be concerned with some dietary factor basic to vitellogenesis. The rapid increases in volume observed in the developing ovary of the house fly and blow fly was previously pointed out. It is well known that any increases in cell size or in cell secretion, particularly exocrine, involves an increase in ribonucleic acid (RNA), whether in the silk glands of the silk worm or in the salivary glands of Drosophila larvae (Brachet, 1957). Yet desoxyribonucleic acid (DNA) synthesis is independent of protein synthesis (Brachet, 1957). King and Burnett (1959) concluded from studies with tritiated uridine, that DNA synthesis in nurse cell nuclei of D. melanogaster, occurs in an asynchronous manner, whereas the protein synthesis under the control of RNA, occurs both in the nucleoplasm and cytoplasm of all the young nurse cells of the oöcyte (King and Burnett, 1959; Zalokar, 1960). King and Sang (1959), have reported that the adults of a hybrid strain of fruit flies were able, by de novo synthesis, to supply enough RNA for normal egg production when dietary folic acid is provided and when

folic acid is not supplied egg production is slowed even when RNA is fed. Therefore folic acid and RNA play a key role in yolk synthesis. Sang (1959) also reported that for growth of Drosophila larvae no extrinsic source of folic acid is needed, but when present in the diets, then less RNA is required for optimal growth. In the present studies the adult house fly was unable to produce eggs beyond the first cycle when either cholesterol or B-vitamins (of which folic acid was one) supplemented the amino acid diet C. Obviously then the house fly, unlike the hybrid strain of fruit fly, was unable to synthesize RNA, or synthesize enough for the requirements of oögenesis.

Once it was apparent that dietary supplements, such as cholesterol and B-vitamins, were of little value to the adult house fly in producing repeated ovarian cycles and that the literature indicated a key role for nucleic acids during yolk synthesis, it became likely that the nucleic acids or their precursors might be effective as a dietary supplement. When diet C containing cholesterol and B-vitamins was enriched with yeast RNA (diet D), there was a statistically significant improvement in egg production (Tables XXVIII - XXX).

This significant increase in total fecundity, with RNA supplementing a chemically defined diet, is largely the result of the success of this diet in promoting repeated gonotrophic cycles. This increase in fecundity was statistically significant in both the first and second cycles compared with the synthetic amino acid diet C which lacked RNA. Indeed egg production continued into the third, fourth and even fifth gonotrophic cycle in flies fed the RNA supplemented diet. Therefore the importance of RNA as the

main dietary supplement necessary for continued egg production in the adult female house fly was obvious.

Now that continuous egg production could be maintained on an adequately supplemented diet (diet D), the question of the necessity of each component naturally arose. Those additives which seemed most likely to influence reproduction were the vitamins and the nucleic acid bases; B-vitamins, as Rasso and Fraenkel (1954) found, influenced rate of ovarian maturation in the blow fly, and the rate of larval growth in Drosophila (Sang, 1959).

It was reported above that the B-vitamins when added to diet C were of some value to fecundity and survival. But it was also important to know the value of B-vitamins in diet C which had been supplemented with cholesterol and RNA. Thus it was found when B-vitamins were deleted from the diet (diet D), that in the first cycle there was no significant decline in egg production (Table XXVIII and XXX). However, in all but one female, eggs of the second cycle grew little, producing a statistically significant decline in total fecundity (Tables XXIX and XXX). This indicates that under the conditions which these larvae were reared, sufficient nutrient reserves may be laid down and that the adult utilized these stores during egg maturation of the first gonotrophic cycle. On the depletion of these stores, if egg production is to continue, a diet must be fed the adult adequate to replace the depleted reserves, presumably identical to the needs for the first cycle. In the experiment using females reared from second cycle eggs the significantly lower fecundity in the first cycle with the vitamin deficient diet is probably not due to the source of

the original eggs; but to less satisfactory conditions during larval growth which was manifest by a poorer fecundity even with the milk diet. This is similar to the report of Hecht (1933) and Wyer (1934) on mosquitoes. These authors reported that, when Culex pipiens larvae were fed a low protein diet, the adults required a blood meal for egg production; on the other hand adults, reared from larvae fed a protein enriched diet, laid eggs without a blood meal. It is obvious then that although the rearing conditions of the larvae were standardized the bacterial flora of the medium was not. Levinson (1960b) has indicated that the bacteria in the larval medium become the diet of the growing larva of Musca vicina. A possible explanation for the differences when the vitamin depletion tests were repeated was that larvae of the second experiment laid down insufficient reserves because of a poor bacterial flora in the C.S.M.A. medium.

Hagen (1952) suggested that a tocopherol, present as an impurity in an enzymatic protein hydrolyzate of whey, was a fertility factor for the fruit fly, Dacus dorsalis. Forgash (1958) also reported the tocopherol, inositol, improved growth in the cockroach, Periplaneta americana. The nutritional value of inositol for the two roaches, P. americana and Blattella germanica, was re-affirmed by Gordon (1959) and Forgash and Moore (1960). In the present study the addition of inositol to the diet (diet D) slightly improved fecundity during the first and further gonotrophic cycles of the house fly. This is the first indication that inositol may bring about an improvement in reproduction in Diptera (Lipke and Fraenkel, 1956; House, 1961). However the significance of this improvement requires

further study.

Nucleic acid bases as well as RNA have been found to influence the larval growth of Drosophila (Hinton, 1956; Sang, 1958) and Phormia (Brust and Fraenkel, 1955). These findings together with the notable influence of RNA on house-fly fecundity (reported here) suggested that nucleic acid bases could take the place of RNA in promoting increased fecundity. Moreover, Hinton (1956) reported that although a strain of Drosophila larvae could grow without the purines, pyrimidines or nucleic acids, the rate of growth was considerably slower in their absence. The base, which he found most responsible for an increase in growth rate, was adenine. Villee and Bissell (1948) also grew these larvae on a semi-synthetic sterile medium, and found that the growth promoting effect was not in RNA as such but in its nucleic acid bases, especially adenine. Also desoxyribonucleic acid (DNA) inhibited growth. For this reason adenine was the first base investigated as a substitute for RNA.

The substitution of adenine for RNA in diet D was without effect during the first gonotrophic cycle but a statistically significant decline in fecundity was evident during the second gonotrophic cycle (Tables XXVIII to XXX). This difference, as a result of the substitution between the first and second gonotrophic cycles may again be a function of the amount of stored nutrients carried over from the larvae. However another possibility exists, that is, on aging the metabolism of the adult female fly changes after the first gonotrophic cycle to the extent that it is unable to synthesize the required nucleic acid bases. In this regard Rockstein (1956) has shown that certain enzymes change in the aging adult house fly, in particular, a degenerative cellular

alteration in phosphorus metabolism. In comparison to the first cycle, further continued ovarian cycles indicate that some basic change(s) has taken place, since more than one nucleic acid base must be provided if RNA is lacking from the diet. It is possible then that this change, as evident in the dietary requirement, may be a modification in metabolic processes.

Hinton (1956) reported that when Drosophila larvae were reared aseptically on a chemically defined medium, the adenine component of ribonucleic acid was responsible for much of the increase in the rate of growth. However, only when cytidylic acid or orotic acid was combined with adenylic acid in the medium was growth as rapid as with RNA. He suggested this to be an interdependence between adenylic acid and cytidylic acid perhaps for incorporation into the RNA molecule. Hinton (1956) found that for these larvae neither cytosine nor cytidine was as suitable as cytidylic acid for growth. For these reasons in the present experiments cytidylic acid in addition to adenine was used in place of RNA in diet D.

The results of these experiments showed that in the first and subsequent gonotrophic cycles fecundity of females fed diets containing adenine and cytidylic acid (diet E) was slightly better than of those fed an RNA containing diet (diet D), but that adenine alone was not as good when it replaced RNA in the diet.

The only reference in the literature to the feeding of RNA to adult diptera is in the work of King and Sang (1959) and Sang and King (1961). They found that the omission of RNA in the diet fed to adult Drosophila had no effect on fecundity over a 16-day period. On

the other hand, the effect of the omission of RNA from the diet fed to larvae is rate limiting due to the larva's inability to synthesize adenylic acid fast enough. These larvae are able to grow when fed the two nucleic acid bases, adenine and cytidylic acid, in place of RNA (Sang, 1959). Earlier both Hinton (1956) and Sang (1957) concluded that the larvae were able to synthesize the required pyrimidines more readily than the purines. Thus Sang (1959) generalizes that Drosophila larvae, like other insects can make its own RNA, but growth is better when a dietary supply is added. From the more recent studies noted above the fecundity of the adult is unaffected when RNA is omitted from its diet. This is in sharp contrast to the requirements found for adult house flies.

In recent years a number of generalizations concerning nucleic acid bases have been widely accepted (Swift, 1962). Of much significance to oögenesis is the broad generality of the requirement for RNA during the manufacture of proteins. Considerable attention has been drawn to studies of the role of RNA in protein synthesis. There is ample proof that RNA is required and directly involved in the incorporation of amino acids into proteins (Brachet, 1957; Berg, 1961; Swift, 1962). Moreover the synthesis of RNA is concomitant with protein synthesis and fresh RNA synthesis occurs whenever new protein synthesis has been induced (Brachet, 1957; Berg, 1961). Ficq (1955), in studies on amphibian oöcytes, found that the nucleic acid base, adenine, was incorporated faster into RNA than phenylalanine into proteins. Recently Mitlin and Cohen (1961) have reported that the composition of RNA in the growing ovaries of house flies was predom-

inately composed of two bases, the purine, adenine, and the pyrimidine, uracil. (It will be recalled that for house-fly fecundity the bases, adenine and cytidylic acid, were found as satisfactory as yeast ribonucleic acid in the diet.) Of further interest was their observation that growing house-fly ovaries, up to a period of 96 hours after emergence showed a two-and-one-half-fold increase in RNA content. King (1960) similarly speaks of a build-up of oöplasmic RNA in female fruit fly. That the present studies should show a significant improvement in fecundity beyond the first cycle when RNA or the nucleic acid bases were fed to the adult house fly is therefore not surprising. Yet the omission of RNA from the diet of the adult fruit fly for a 16-day period had no statistically significant effect on fecundity or egg viability (King and Sang, 1959) although they concluded that nucleic acids play a key role in yolk synthesis. This apparent difference in nutritional requirements for fecundity can be due to the amount of RNA (or its components) stored in the adult fruit fly, or to the rate of synthesis of these metabolites during oögenesis.

In this connection egg production and nutrition of Drosophila adults is of interest. King and Sang (1959) reported that folic acid was the vitamin most involved in RNA synthesis, and when removed from the diet both egg production and egg viability are lowered. For when folic acid is not fed,egg production is slowed down even when RNA is fed; this vitamin deficiency appears to inhibit vitellogenesis. The omission of RNA from the adult diet had no effect on Drosophila egg production (Sang and King, 1961). Furthermore they concluded that the adult has no requirement for the B-vitamins, choline and biotin, for

egg formation (Sang and King, 1961). On the other hand the omission of pyridoxine and RNA together results in a higher fecundity than found with the omission of pyridoxine alone (Sang and King, 1961). Earlier Levinson and Bergman (1959) reported that it was only the folic acid antivitamin, 4-aminopteroglutamic acid, fed at the lowest concentration of all the antivitamins tested, which gave a decrease in fecundity, i.e. an 82 - 99% decrease. Already these few observations on the importance of nucleic acid bases and vitamins are of intrinsic interest. Further studies in this field will undoubtedly reveal other pertinent relationships between the vitamins and the nucleic acid bases during oogenesis.

In regard to the feeding of vitamins and adult survival, Levinson and Bergmann (1959) reported that the presence of antivitamins in the diet fed to adult Musca vicina reduced significantly the longevity of both sexes. Hagen (1958) also observed that the B group vitamins gave greatest longevity when in the diet fed to tephritid fruit flies. In the present studies when the B-vitamins were omitted from the synthetic diet D or when diet D was supplemented with inositol, adult female house flies showed a poorer survival than those fed diet D. Diet D gave a female survival similar to a diet of milk alone. However when further substitutions were made to diet D, that is, adenine or adenine and cytidylic acid for RNA, the survival of female house flies was improved. The significance of improved survival when one or two nucleic acid bases were fed to female house flies is obscure. The actual influence that nucleic acid bases have on aging processes awaits further studies. There is the possibility, however, that by feeding the bases rather than RNA,

the insect is spared toxic metabolites arising from the breakdown of this nucleic acid. Generally during the evaluation of fecundity it is of intrinsic value to determine longevity or mortality since a high fecundity is impossible without good survival. Yet good survival is possible on a sugar-water diet without any egg production. It should not be overlooked that, as Lavoipierre (1961) observed, an increase in fecundity may be accompanied by an increase in death rate. Certainly of much importance in nutritional studies is the inclusion of more than one criterion when evaluating the value of various dietary constituents.

Dry foods are considered to offer some advantage over wet diets (Lipke, 1957; Fraenkel, 1959; Dadd, 1960b;House, 1961) although chemically synthetic dry diets had never been fed to Diptera. Investigators have always been of the opinion that the feeding of dry diets, under xenic conditions, are subject to less contamination than liquid diets, especially over periods of extended feeding (Fraenkel, 1959; House, 1961). As Beck (1956) has pointed out, the understanding of the relationship between feeding behaviour and nutritional factors is basic to any meaningful interpretation of the experimental results on insect nutrition and development. With this in mind a successful dry diet was developed after a number of attempts were made to get adult house flies to feed on dry synthetic diets. At this time and in the experiment reported here ovarian growth and maturation and not fecundity were considered the essential criteria. Initially dissections showed ovarian growth in most females. These observations confirmed that the dry diets were adequate for some ovarian growth. However, later

when a number of mature eggs were deposited, the dry diets were also considered nutritionally adequate for oögenesis. Yet few eggs were oviposited. This was probably due to an unsatisfactory oviposition site. An oviposition site, moistened with ammonium carbonate, similar to that used by Monroe (1960), might well result in increased egg laying. When a more suitable oviposition site is provided it is anticipated that a more adequate assessment might be made between synthetic liquid and dry diets.

This study has established that for the adult female house fly the essential components in the diet necessary for the first cycle of ovarian maturation are nine amino acids, a suitable carbohydrate, minerals and water. These dietary components are closely similar to the requirements considered necessary for egg production in the haematophagus adult mosquito, Aedes aegypti, by Dimond (1957) and Singh and Brown (1957). These authors were concerned only with gross egg production and did not suggest that differences might exist in the nutritional requirements for each gonotrophic cycle. We have noted that four to five gonotrophic cycles can take place on a fully supplemented diet fed to the adult female house fly, but not on the diet considered adequate for the first egg cycle. However whether this supplemented diet is an optimum synthetic diet cannot be established until a more thorough investigation of gonotrophic cycles has been made.

It may be concluded that, up to this time, studies were solely concerned with those nutritional needs that allowed general egg production in adult Diptera. However, we have shown that there are specific dietary requirements for different ovarian cycles. As yet for the house

fly no difference in dietary requirements for ovarian maturation is apparent between the second and third gonotrophic cycles. A number of physiologists have suggested in a general way that nutritional requirements for various processes such as growth, development, or reproduction may depend on reserves of substances stored in the body or egg sufficient for the needs of the insect for part or all of one generation (House, 1959, 1961; Fraenkel, 1959; Sang and King, 1961). The present research has established two concepts new to this field of insect nutrition. Firstly that the depletion of reserves during reproduction in adult house flies is apparent by the cessation of egg production, and a characteristic of a deficiency symptom. Previously it was considered that adult insects, unlike mammals, were unable to show nutritional deficiencies (Gordon, 1959). The present results answer also for the first time that problem which Dimond (1957) was aware of in his studies on reproduction and nutrition in adult mosquitoes, that is, the need to find a method to overcome differences in the amounts of nutrients transferred to the adult from an immature stage. The second new concept, now established, is that those dietary nutrients required and determined as adequate for the first cycle of a repeated life process are not necessarily satisfactory for the continuation of the same process. These findings allow a new and different physiological and biochemical approach to the problem of insect nutrition.

SUMMARY

First cycle oögenesis and oviposition in the house fly took place when nine essential l-amino acids: arginine, histidine, isoleucine, leucine, lysine, phenylalaline, threonine, tryptophan and valine were substituted for dietary protein in a liquid diet.

Dietary l-methionine influenced egg production greatly, whereas l-cystine another sulphur-containing amino acid had little influence. L-methionine was not considered strictly essential.

Experiments showed that nutrient stores transferred from the larva to the adult were utilized during oögenesis. For the production of first gonotrophic cycle eggs these reserves supplied the adult fly with methionine or sulphur-containing metabolites, purine and pyrimidine bases or precursors, vitamins, and other metabolites. Diets deficient in cholesterol or salts resulted in a lower fecundity.

For oögenesis to continue the synthetic diet must be supplemented with l-methionine, B-vitamins, and certain nucleic acid bases (or RNA). Flies fed these supplemented diets deficient in these nutrients showed a marked reduction in fecundity. The determination of dietary needs for each gonotrophic cycle was only possible because adult females were cultured individually.

In the female house fly, during each gonotrophic cycle, the synchronous maturation of oöcytes within ovarioles is termed ovarian synchrony. In the house fly this precision of ovarian maturation takes place only after the feeding of an adequate diet.

A marked increase in female longevity occurred in house flies fed a supplemented synthetic diet containing nucleic acid bases as compared to those fed a "milk" diet.

The adult house fly is capable of feeding on dry synthetic diets. Egg maturation was observed on one of these dry diets.

PLATE 1

Stock cages containing adult house flies. Adults are emerging from jar containing pupae in lower right cage. Milk dishes in lower cages and sugar-water in upper cages constituted the adult diet.

PLATE 2

A shelf of large cylinders. Two cylinders are arranged on aluminum trays covered with filter paper. Each cylinder contains 15 experimental adults fed a test diet.

PLATE 1

PLATE 2

PLATE 3

A house fly feeding on cotton wick moist with synthetic diet.

PLATE 4

A shelf of large and small glass cylinders containing test flies. In the background are plastic rearing jars partly full of C.S.M.A. medium.

PLATE 3

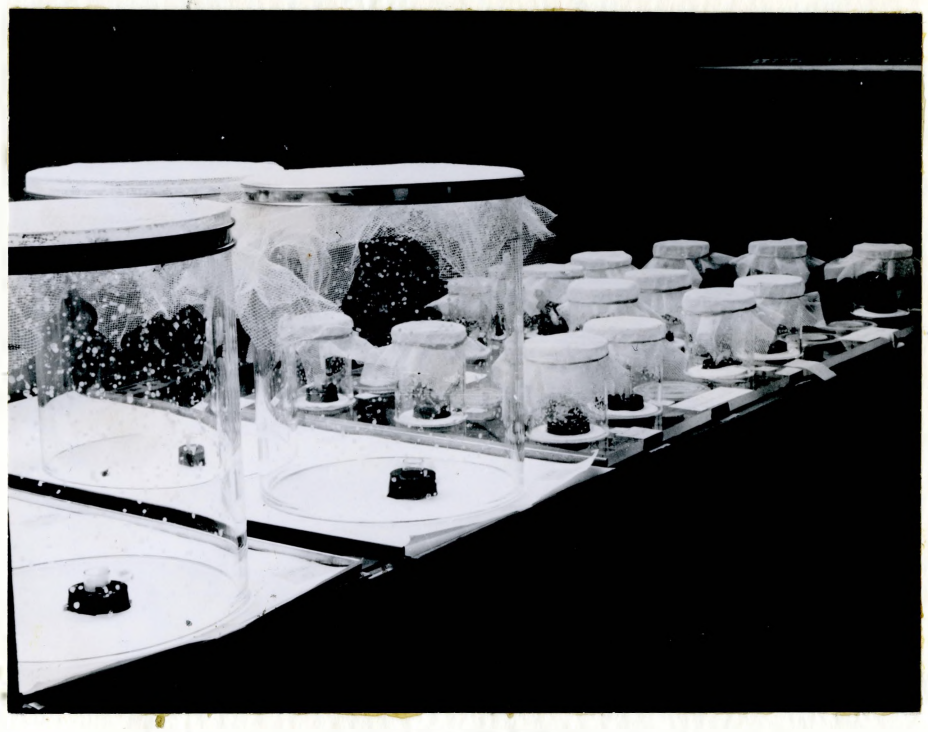


PLATE 4

PLATE 5

Large testing cylinders containing adults feeding on dry synthetic diets

PLATE 6

A large cylinder showing adults feeding on sugar-water and dry synthetic diets.

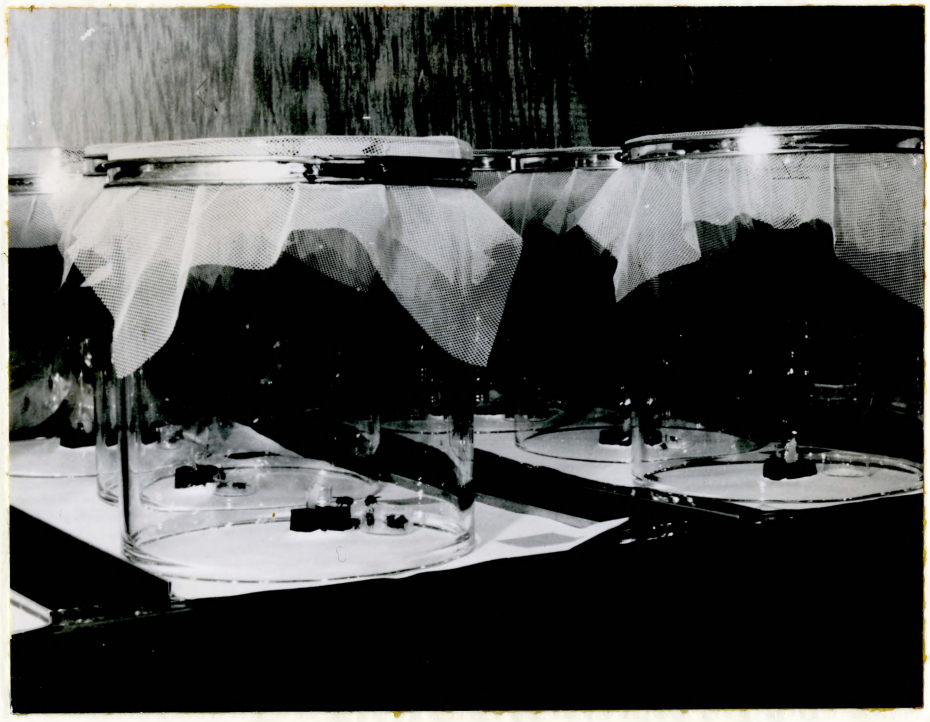


PLATE 5

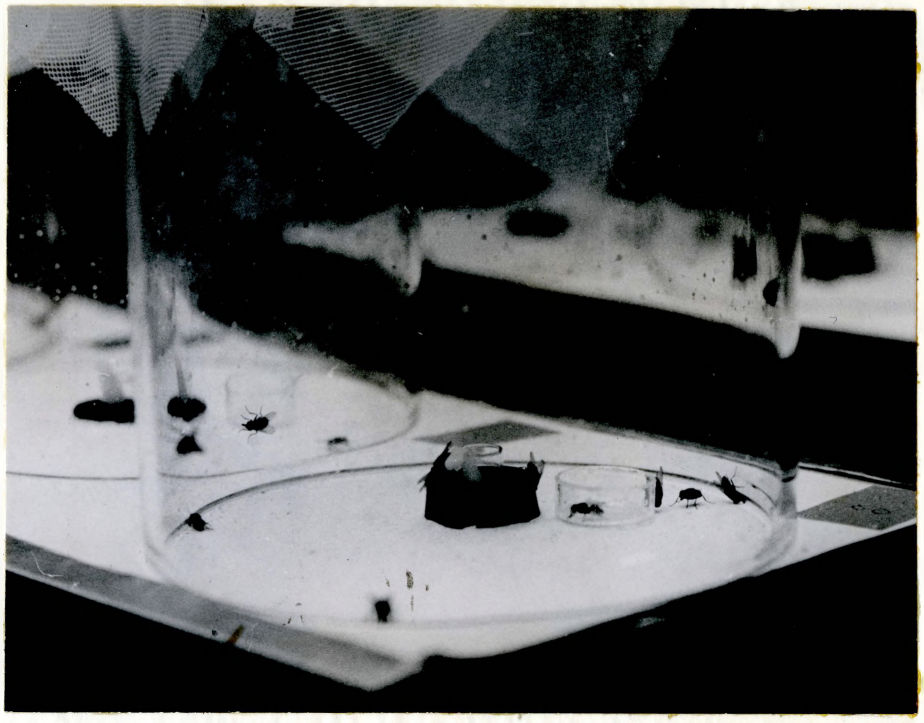


PLATE 6

PLATE 7

An ovary from a newly emerged adult containing developing follicles. Each follicle, bounded by follicular cells, contains nutritive nurse cells with large nuclei. Tracheoles enter the ovary at the lateral border.

PLATE 8

A higher magnification of the ovary of a newly emerged adult showing the chromatin in the nuclei of nurse cells in large clumps, although some diffuse chromatin is present. The attached germarium contains differentiating cells.

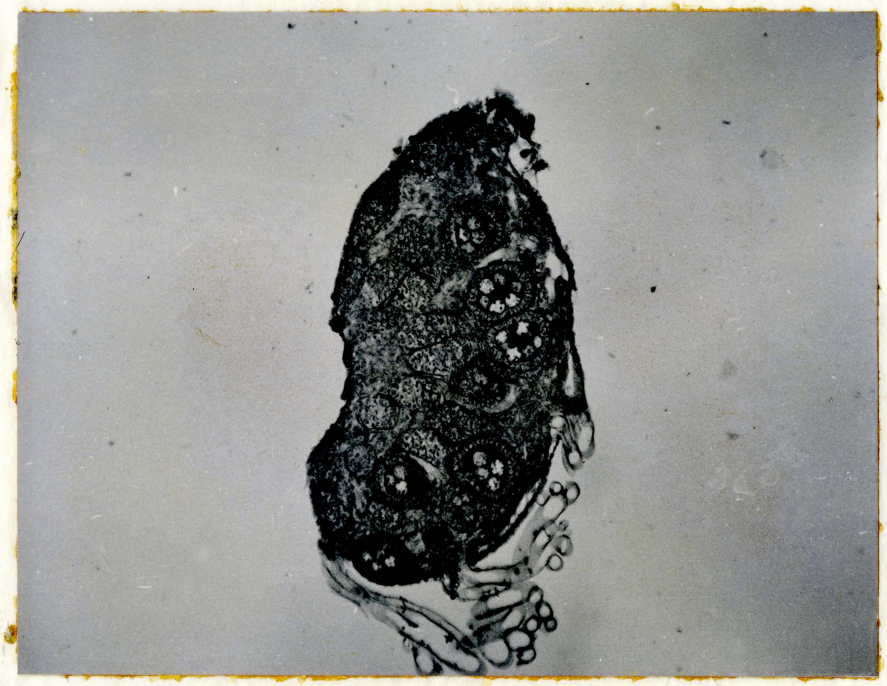


PLATE 7

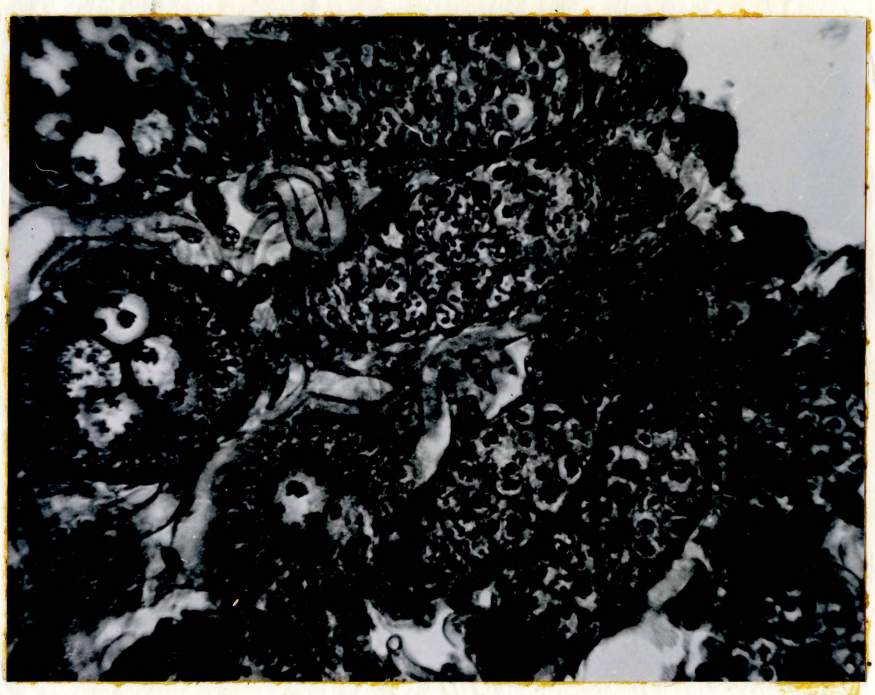


PLATE 8

PLATE 9

A section of a 24-hour-old (1 day) ovary. Nurse cells with large nuclei continue to show growth. Only diffuse chromatin fills these nuclei. Oocyles (egg cells) and nurse cells about the same size.

PLATE 10

A 48-hour-old (2 days) follicle showing some elongation. Growth of the oocyte indicates active vitellogenesis. Nurse cells and nuclei show further growth.

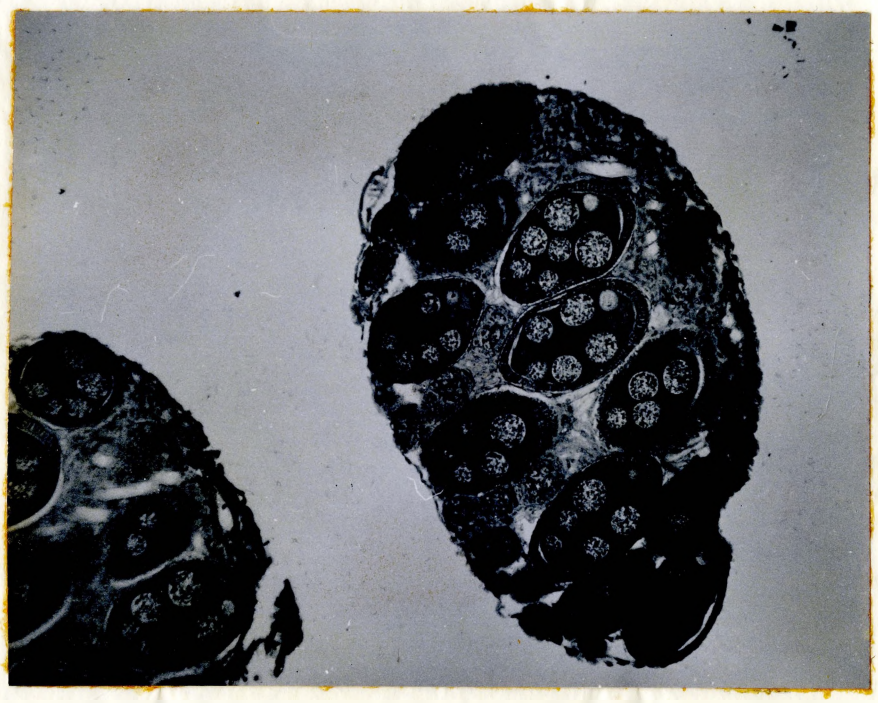


PLATE 9

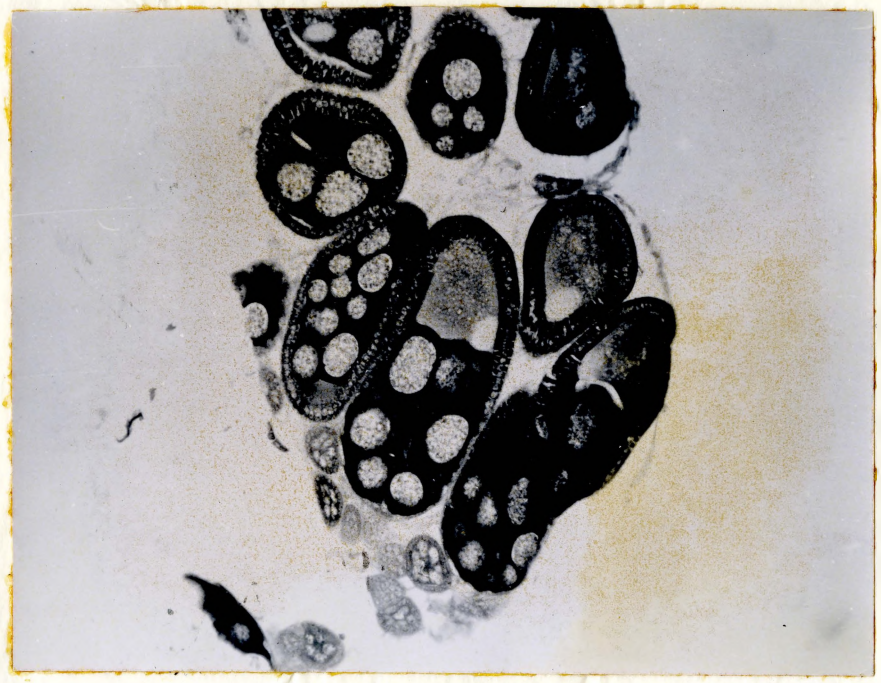


PLATE 10

PLATE 11

At 72 hours (3 days), the growing oocyte about equals the mass of nurse cells. Nurse cells in the developing follicle show maximum size. Vitellogenesis is still active.

PLATE 12

An enlargement of a portion of the follicle (about 70 hours) showing the way in which the nurse cells transfer nutritive material from the adjacent nurse cells into the oocyte during vitellogenesis.

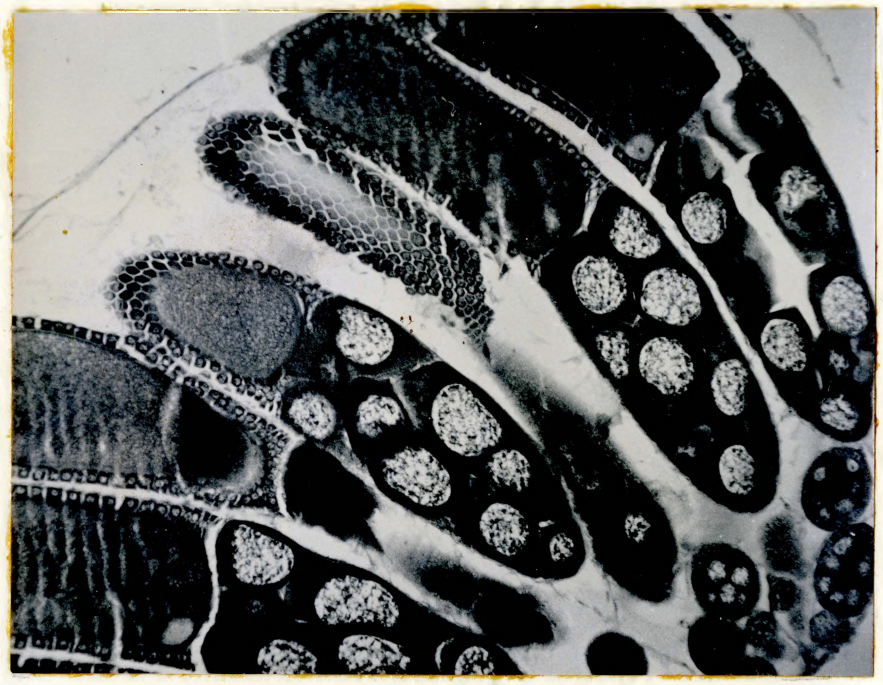


PLATE 11

PLATE 12

PLATE 13

The appearance of the follicle after 84 hours (3½ days) and the degeneration of some of the nuclei of the nurse cells. The chorion begins to appear around the elongated oocyte.

PLATE 14

This shows the follicle after 96 hours (4 days) with its smaller nurse cells and degenerated nuclei. The mature oocyte (with a maximum length of about 1 mm.) is now considerably larger than the rest of the follicle.

PLATE 13

PLATE 14

PLATE 15

The ovary shortly after oviposition. The remaining tissue of the old follicle forms a mass below the next developing follicle.

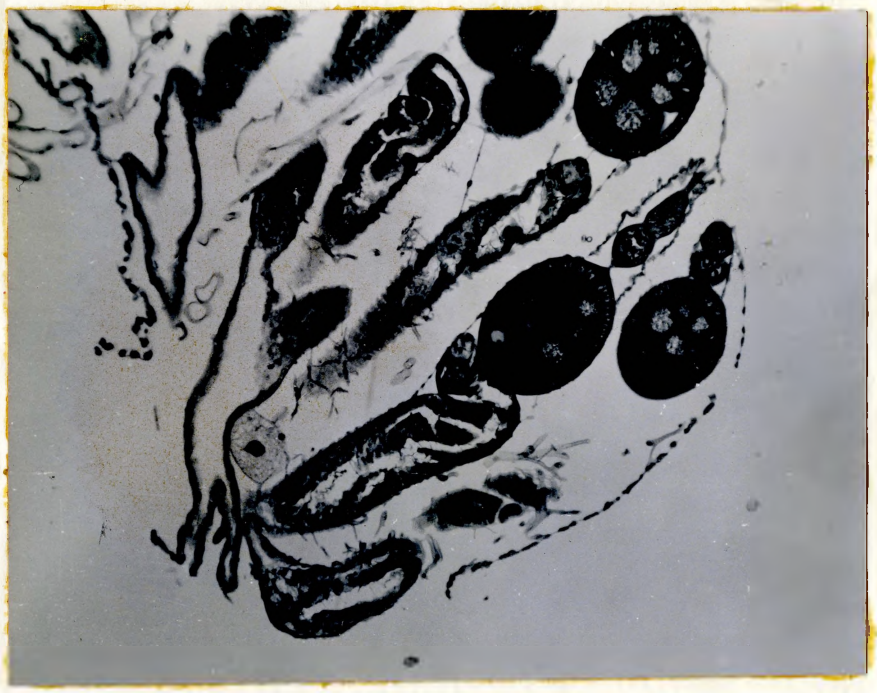


PLATE 15

TABLE I Composition of the chemically defined diets most frequently fed to the adult house flies. Values are in grams per 100 ml. of diet solution (except for B-vitamins in mg.).

Components	Diets A	B	C	D	E
l-arginine	0.38	0.38	0.38	0.38	0.38
l-cystine	0.15	0.15	-	-	-
l-histidine	0.15	0.15	0.15	0.15	0.15
l-isoleucine	0.50	0.25	0.25	0.25	0.25
l-leucine	0.75	0.75	0.75	0.75	0.75
l-lysine	0.75	0.75	0.75	0.75	0.75
l-methionine	0.15	0.15	0.15	0.15	0.15
l-phenylalanine	1.20	1.20	1.20	1.20	1.20
l-threonine	0.30	0.15	0.15	0.15	0.15
l-tryptophan	0.30	0.30	0.30	0.30	0.30
l-valine	1.00	0.50	0.50	0.50	0.50
l-glutamic acid	1.00	1.00	1.00	1.00	1.00
Sucrose	3.42	3.42	3.42	3.42	3.42
Salt mixture 'W'[a]	0.15	0.15	0.15	0.15	0.15
B-vitamins (in mg.)[b]	-	-	-	0.43	0.43
Cholesterol	-	-	-	0.01	0.01
Ribonucleic acid (yeast)	-	-	-	0.40	-
Adenine	-	-	-	-	0.20
Cytidylic acid	-	-	-	-	0.20

a See footnote in text.

b See Table II.

TABLE II Vitamin[a] quantities used in preparing 100 ml. of the concentrated vitamin solution. One ml. of this solution was added to the non-neutralized amino acid diets D and E presented in Table I.

Vitamin Mixture	Amount (grams)
d-Biotin	0.005
Choline chloride	0.200
Folic acid	0.034
Nicotinic acid	0.030
d-Pantothenate calcium	0.044
Pyridoxal hydrochloride	0.090
Riboflavin	0.016
Thiamine hydrochloride	0.012
TOTAL	0.431 g.

a All vitamins U.S.P. of highest purity available were purchased from California Corporation for Biochemical Research, Los Angeles, U.S.A.

TABLE III The relationship between the number of eggs laid within a 10-day period and the ratio of males to females.

Ratio of males to females after the 3rd day	Cylinder No.	Eggs/surviving female after 10 days
0:12	1	160
	2	84
	3	235
Mean per cylinder		160
1:12	1	166
	2	231
	3	81
Mean per cylinder		159
3:12	1	231
	2	230
	3	227
Mean per cylinder		229
5:12	1	140
	2	229
	3	300
Mean per cylinder		223

Mean total number of eggs laid after 6 days for each of the ratios in increasing values were 787, 630, 1287, and 840.

TABLE IV Individual egg laying of 18 female house flies fed a fresh milk diet for 22 days.

Cylinder No.	No. of eggs (fecundity)		No. of egg cycles	Mean[a] no. of eggs/cycle	Total no. of eggs laid
	First Cycle	Second Cycle			
1	75	-	1	-	75
2	14	-	1	-	14
3	93	-	1	-	93
4	108	-	1	-	108
5	105	-	1	-	105
6	-	-	-	-	-
7	90	94	2	92	184
8	102	-	1	-	102
9	117	104	4	83	332
10	109	102	4	84	336
11	111	-	1	-	111
12	85	-	1	-	85
13	-	-	-	-	-
14	152	129	5	122	609
15	115	114	3	113	340
16	104	110	3	99	298
17	89	94	4	78	312
18	-	-	-	-	-
Mean	98	107	2	96	206

a Arithmetic mean.

TABLE V Changes in relative ovarian volume of house flies fed either sugar-water or milk diets.

Days after emergence	Ovary Size[a]								
	"Sugar-water" flies			Milk fed flies					
				First Cycle			Second Cycle		
	Right	Left	Mean	Right	Left	Mean	Right	Left	Mean
0	99	101	100	99	101	100			
1	223	220	221	296	289	292			
2	600	663	631	1866	1783	1825			
3	644	660	652	7137	7530	7334			
4	720	630	675	7772	6687	7230	750[b]	770	760
5	714	729	722				1683	1390	1537
6	833	807	820				4396	5173	4784
7	923	818	871				7197	5253	6225

a Ovary volumes are based on measurements made on both right and left ovaries.

b Second gonotrophic cycle ovary volumes are based on 3 to 6 ovaries.

TABLE VI Delayed presentation of a fresh milk diet to newly emerged individual house flies and the effect on female fecundity and survival.

Time of first feeding (hours after emergence)	Mean time of first egg laying[a]	Mean no. of eggs		Female survival[a]
		Per laying female	Per exptl. female	
1	6	190	133	11
2	8	159	106	12
10	6	210	168	10
37	7	166	142	11

a Number of days after emergence.

TABLE VII Fecundity of individual female house flies fed on 4% low vitamin casein diets compared with milk and sugar-water diets.

Diet	Mean no. eggs in first cycle	Mean no. eggs in second cycle	Mean of total no.eggs/ laying female	% of females laying eggs	% of females laying more than 1 cycle	Mean female survival (Days alive after emergence)
Milk-fresh	127	119	268	68	33	7
4% Casein[a] salts and sucrose	114	96	177	78	68	9
Sugar-water (0.1 M sucrose and salts)	None	None	None	None	None	15
4% Casein[b] salts and sucrose	131	77	178	72	33	9

a Vitamin-test casein, General Biochemical, Chagrin Falls, Ohio.

b Low vitamin casein, Genatosan Ltd., Fisons Chemicals, England.

TABLE VIII A comparison of the survival of female house flies fed on different natural products. For each diet 18 small cylinders were used. Each contained one female and three males.

No. of days from emergence	% Survival			
	Milk	0.4% casein[a] salts and sucrose	Sugar-water and salts	0.4% casein[b] salts and sucrose
1	100	100	100	100
2	100	100	100	93.5
3	100	100	100	93.5
4	100	100	100	93.5
5	100	86.7	100	93.5
6	50	80.0	100	80.0
7	25	73.4	100	66.7
8	25	60.0	100	60.0
9	0	53.4	93.9	53.2
10		53.4	93.9	40.0
11		26.6	93.9	35.3
12		6.7	68.8	13.3
13		0	68.8	
14			62.5	
15			56.3	
16			31.2	
17			25.0	
18			0	

a Vitamin-test casein, General Biochemicals, Chagrin Falls, Ohio.

b Low vitamin casein, Genatosan Ltd., Fisons Chemicals, England.

TABLE IX A comparison of egg production from adult house flies fed a yeast extract, casein or milk diet for 18 days.

Diet	Egg production over 18 days		Oviposition ratio[a]
	Mean no. of eggs per cylinder	Mean no. of eggs per experimental female	
Milk	3353	280	1.00
6% Casein ("vitamin free"[b])	636	53	0.19
6% Yeast extract[c]	1105	92	0.33

a Oviposition ratio is calculated so that other egg laying values compare with milk. Over any limited period the

$$\text{oviposition ratio} = \frac{\text{Total no. eggs per cylinder - test diet}}{\text{Total no. eggs per cylinder - milk diet}}$$

b A product of Nutritional Biochemicals Co., Cleveland, Ohio and sold as "Vitamin Free" Casein.

c A product of Nutritional Biochemicals Co., Cleveland, Ohio and sold as Yeast Extract Powder.

TABLE X A comparison of egg production from individual adult house flies fed a yeast extract, casein or milk diet for 14 days.

Diet	Mean no. of eggs/ laying female	Oviposition ratio
Milk	212 (2 females laid 2 batches of eggs)	1.00
6% Casein ("vitamin free")	34	0.16
6% Yeast extract	77	0.37

TABLE XI Role of non-amino-acid components of the control diet for egg maturation and oviposition in the adult house fly.

Diet	Egg production	
	Mean no. of eggs/cylinder	Days experiment
Milk (control)	1136	7
Diet A (control)	336	7
Diet A - less salt mixture 'W'	None	9
Diet A - less carbohydrate (sucrose)	None	3[a]

a All flies dead after 3 days.

TABLE XII Amino acid composition of two preliminary diets and the optimum diet used by Dimond[a]. Values are in grams per 100 ml. of diet.

Components	Diet based on blood	Diet based on casein	Optimum diet Dimond's diet 'D'
l-alanine	0.60	0.55	-
l-arginine monoHCl	0.50	0.55	0.38
l-aspartic acid	1.00	0.60	-
l-cystine	0.20	0.04	0.15
l-glutamic acid	1.00	2.33	1.00
glycine	0.50	0.05	-
l-histidine monoHCl	0.70	0.21	0.15
dl-isoleucine	1.00	0.63	0.50
l-leucine	1.00	1.00	0.75
l-lysine monoHCl	0.90	0.90	0.75
dl-methionine	0.20	0.34	0.15
dl-phenylalanine	0.70	0.50	1.20
l-proline	0.50	0.50	-
dl-serine	0.20	0.77	-
dl-threonine	0.80	0.38	0.30
l-tryptophan	0.40	0.12	0.30
l-tyrosine	0.40	0.67	-
dl-valine	1.00	0.65	1.00

a Taken from Dimond (1957), p.29. The diets based on casein and blood contain the amino acids in the amounts reported by Hawk et al (1954) and Albritton (1 54).

TABLE XIII The effect of deleting individual amino acids from amino acid diet A upon house-fly fecundity.

Diet	Number of experimental cylinders	Egg production	
		Mean number eggs/cylinder	Days/experiment
diet A (control)	13	313	7
l-arginine deficient	3	none	8
l-cystine deficient	3	400	7
l-histidine deficient	3	none	8
l-isoleucine deficient	3	none	9
l-leucine deficient	3	none	9
l-lysine deficient	3	none	9
l-methionine deficient	6	366	7
l-phenylalanine deficient	4	none	9
l-threonine deficient	3	none	8
l-tryptophan deficient	3	none	8
l-valine deficient	3	none	7
l-glutamic acid deficient	3	240	8

TABLE XIV The effect on egg production and survival of substituting other non-specific nitrogen compounds for glutamic acid.

Diet	After 7 days	
	Mean total no. of eggs per/ cylinder	Mean no. of surviving females
Milk (control)	993	11 of 12
Diet A (control)	275	10 of 12
Diet A - glutamic acid deficient	196[a]	10 of 12
Diet A - ammonium citrate replacing glutamic acid	433[b]	12 of 12
Diet A - urea replacing glutamic acid	0(43)[c]	11 of 12

a In one cylinder of the group the first eggs were laid on the 8th day; these 131 eggs gave a mean for the 8th day of 260 eggs.

b In one cylinder of the group the first eggs were laid on the 9th day; these 381 eggs gave a mean for the 9th day of 560 eggs.

c By the 10th and 11th days, 129 eggs were laid giving a mean of 43 eggs per cylinder.

TABLE XV A comparison of egg production of house flies fed the basic and modified amino acid diets and diets deficient in the sulphur-containing amino acids, cystine or methionine.

Diet	Eggs/female (mean number)		Total eggs after 15 days (mean number)
	After 10 days	After 15 days	
Milk (control)	91	169	1761
Diet A (control)	89	99	1007
Diet A (cystine deficient)	86	117	1423
Diet A (methionine deficient)	52	54	605
Diet B	92	115	1239

TABLE XVI A further comparison of egg production from house flies fed the amino acid diets A or B and diets deficient in the sulphur-containing amino acids, cystine or methionine.

Diet	Mean no. of eggs/experimental female				Number of living females after 16 days
	After 9 days	Total	After 16 days	Total	
Milk (control)	77	923	181	2166	7
Diet B (control)	56	670	87	1044	4
Diet B[a] (cystine deficient)	46	553	73	879	4
Diet B (methionine deficient)	19	229	31	366	5
Diet A (methionine deficient)	7	80	9	112	2

a Same as diet C.

TABLE XVII Fecundity of house flies in which varying amounts of methionine were present in diet C.

Diet	Cumulative fecundity on days after emergence			
	5th day	7th day	9th day	11th day
Diet C (methionine deficient)	95	575	576	576
Diet C (containing 0.01 g. methionine)	224	813	857	940
Diet C (containing 0.04 g. methionine)	699	963	1118	1254
Diet C (containing 0.15 g. methionine)	614	1078	1131	1246

TABLE XVIII A comparison of the variations in egg laying when females are fed milk or amino acid diet C[a].

Experiment number	Mean total no. of eggs/cylinder after 14 days		Mean no. of eggs/ surviving female after 14 days	
	Diet C[b]	Milk diet	Diet C	Milk diet
52	1222	1351	113	116
59	820	2190	75	195
61	923	2815	97	276
90	883	1849	98	155
Average	962	2051	96	186

a Values are calculated from 2 to 4 large cylinders per diet.

b For composition of diet C see Table I.

TABLE XIX The adequacy of the amino acid diet C as compared to a milk diet for egg laying in individual adult house flies[a].

Diet	Cylinder number	Number of eggs laid		Total number of eggs laid after 14 days
		First cycle	Second cycle	
Milk (control) (from Table IV)	10	109	102	288
	14	152	129	403
	15	115	114	340
	16	104	110	298
Diet C	3a	131	0	131 (2 eggs relict)[b]
	6a	33	0	33 (108 eggs relict)
	7a	64	0	64 (135 eggs relict)
	1b	138	61	199
	2b	11	0	11 (132 eggs relict)
	3b	155	0	155
	4b	26	0	26 (20 eggs relict)
	5b	140	0	140 (6 eggs relict)

a These results presented here have been condensed from experiment numbers 60, 88 and 90.

b All dissections were made on the flies at death or when the experiments were terminated.

TABLE XX The effect of 0.05% cholesterol on house-fly fecundity over a 14-day period.

Diet	Mean no. of eggs/ surviving female	Mean no. of eggs/ cylinder	Oviposition ratio[a]	Oviposition ratio[b]
Amino acid diet C	75	820	1.0	1.96
Amino acid diet C + Tween and EtOH (control)	52	419	0.51	1.00
Amino acid diet C + 0.05% cholesterol + Tween and EtOH	34	307	0.37	0.73
Milk	195	2190	2.67	5.23

a This is calculated from the mean number of eggs per cylinder laid by flies fed the diet C divided into the number of eggs laid by a similar group of flies fed on test diets over a comparable period.

b The denominator of this ratio is the fecundity for the mean number of eggs per cylinder when flies are fed diet C plus Tween and ethyl alcohol.

TABLE XXI The results of feeding to adult house flies different dietary cholesterol concentrations and the effect on egg production over a 14-day period.

Diet	Mean no. of eggs/ surviving female	Mean no. of eggs/ cylinder	Oviposition ratio[a]
Milk	276	2815	4.65
Diet C	97	923	1.53
Diet C + Tween 80 and EtOH (control)	81	605	1.00
Diet C + 0.001% cholesterol + Tween 80 and EtOH	101	994	1.64
Diet C + 0.01% cholesterol + Tween 80 and EtOH	145	1163	1.92

a The denominator for this ratio is the fecundity for the mean number of eggs per cylinder when flies are fed diet C plus Tween and ethyl alcohol.

TABLE XXII A comparison of egg production from female house flies fed amino acid diet C, or a semi-synthetic diet, each supplemented with different concentrations of cholesterol[b].

Per cent cholesterol concentration in diet C or in Monroe's diet	Ml. of eggs/100 females (Monroe, 1960)	Oviposition ratio[a]
0.05	5.4	0.37
0.01	6.0	1.26
0.005	7.0	-
0.001	6.4	1.08
0	6.3	1.00

a Based on diet C and the mean number of eggs per cylinder after 14 days.

b Monroe (1960) fed adult house flies a semi-synthetic diet.

TABLE XXIII A comparison of fecundity from groups of 12 female house flies over 14 days when fed amino acid diet C, or diet C supplemented with eight B-vitamins.

Diet	Cylinder number	After 7 days		After 14 days	
		Total no. of eggs/ cylinder	No. of eggs/ experimental female	Total no. of eggs/ cylinder	No. of eggs/ experimental female
Diet C	1	1398	117	1408	117
	2	1127	94	1127	94
	3	1115	93	1150	96
	Mean	1213	101	1228	102
Diet C plus vitamins	1	898	90	1376	138
	2	1415	118	1758	146
	3	1226	102	1250	104
	Mean	1180	103	1461	122

TABLE XXIV Survival of female house flies fed amino acid diet C, or diet C supplemented with eight B-vitamins.

Diet	Cylinder number	Per cent female survival after		
		7 days	10 days	14 days
Diet C	1	67	25	25
	2	67	33	33
	3	75	42	8
	Mean	70	37	22
Diet C plus vitamins	1	70	60	8
	2	92	67	42
	3	75	50	8
	Mean	79	59	19

TABLE XXV A comparison of fecundity from individual female house flies over 14 days when fed amino acid diet C, or diet C supplemented with eight B-vitamins[a].

Diet	Cylinder number	No. of eggs in first cycle	No. of eggs in second cycle	Total no. of eggs laid	Female survival (days after emergence)	No. of relict eggs
Diet C	1	138	61	199	13	
	2	11	-	11	14	132
	3	155	-	155	10	
	4	26	-	26	11	20
	5	140	-	140	14	6
	Total	470		531		
	Mean	94	61	106	12	
Diet C plus vitamins	1	111	42	153	14	81
	2	132	-	132	12	-
	3	5	-	5	14	145
	4	162	77	239	13	151
	5	131	-	131	14	-
	Total	541	119	660		
	Mean	108	60	132	13	

a Only 5 of 12 females fed on each diet laid eggs within a 14-day period. The 7 of each diet, which did not lay contained eggs in ovaries.

TABLE XXVI Fecundity and gonotrophic cycles of individual house flies fed diet C, or diet C supplemented with cholesterol, B-vitamins and RNA[b] during a 15-day period.

Diet	First Gonotrophic Cycle			Second Gonotrophic Cycle		
	No. eggs/ exptl. female[a]	Per cent laying	No. eggs/ laying female	No. eggs/ exptl. female	Per cent laying	No. eggs/ laying female
Milk (control)	91	75	121	62	50	125
Diet C plus 0.01% cholesterol, B-vitamins and 0.4% RNA[b]	103	89	116	61	50	123
Diet C	30	36	85	9	7	132

a When the fecundity of females fed these two chemically defined diets were compared, P was < 0.001 for the first gonotrophic cycle. For a similar comparison of the second gonotrophic cycle, P was < 0.05 (student 't' test).

b Or diet D (for composition see Table I).

TABLE XXVII Composition of the chemically defined diets 58, 59 and 60 fed to adult female house flies.

Diet	Constituents[a] Made up to 100 ml. diet solution				
	Amino acids (Table I) (diet C)	B-vitamins (Table II)	0.01% cholesterol	0.4% RNA	0.2% Adenine
No. 58	+	-	+	+	-
No. 59[b]	+	+	+	+	-
No. 60	+	+	+	-	+

a Each 100 ml. of these diets also contained 3.42 g. of sucrose and 0.15 g. of salt mixture 'W'. For the basic ingredients see Table I.

b Diet D.

TABLE XXVIII Fecundity of individual house flies fed amino acid diet D lacking B-vitamins or diet D with adenine replacing RNA compared with diet D or milk during the first gonotrophic cycle.

Experimental diet (See Table XXVII for composition)	First Gonotrophic Cycle		
	Mean no. eggs/ experimental female	Per cent laying	Mean no. eggs/ laying female
Milk (control)	107	100	107
Diet D less B-vitamins (no. 58)	65	67 10 of 15 experimental females	97
Diet D (no. 59)	84	75 12 of 16 experimental females	111
Diet D with 0.2% adenine replacing 0.4% RNA (no. 60)	61	88 14 of 16 experimental females	70

TABLE XXIX Fecundity of individual house flies fed amino acid diet D lacking B-vitamins or diet D with adenine replacing RNA compared with diet D or milk during the second gonotrophic cycle.

Experimental diet	Second Gonotrophic Cycle			Total of eggs for all cycles
	Mean no. eggs/ experimental female	Per cent laying	Mean no. eggs/ laying female	
Milk (control)	75	67 4 of 6 experimental females	112	1354 2 females laid 3 or more cycles
Diet D less B-vitamins (no. 58)	6	7 1 of 15 experimental females	89	1062
Diet D (no. 59)	34	50 8 of 16 experimental females	69	2068 2 females laid 3 or more cycles
Diet D with 0.2% adenine replacing 0.4% RNA (no. 60)	18	31 5 of 16 experimental females	56	1311 1 female laid 3 or more cycles

TABLE XXX Fecundity and gonotrophic cycles of individual house flies fed an amino acid diet D lacking B-vitamins or with adenine replacing RNA compared with diet D or milk over a 14-day period.[a]

Experimental diet	First Gonotrophic Cycle			Second Gonotrophic Cycle			Total no. of eggs laid
	Mean no. eggs/ exptl. female	% laying	Mean no. eggs/ laying female	Mean no. eggs/ exptl. female	% laying	Mean no. eggs/ laying female	
Milk (control)	55	67	83	39	33	98	857
Diet D less B-vitamins (no. 61)	27	28	107	12	6	121 only 1 female laid	657
Diet D (no. 62)	75	67	112	39	33	99	2153
Diet D with 0.2% adenine replacing 0.4% RNA (no. 63)	65	67	97	14	17	63	1477

a Females were reared from second gonotrophic cycle eggs.

TABLE XXXI Fecundity and gonotrophic cycles of individual house flies fed amino acid diet D supplemented with inositol or diet D with adenine and cytidylic acid replacing RNA compared diet D or milk over a 14-day period[a].

Experimental diet	First Gonotrophic Cycle			Second Gonotrophic Cycle			Total no. of eggs laid
	Mean no. eggs/ exptl. female	% laying	Mean no. eggs/ laying female	Mean no. eggs/ exptl. female	% laying	Mean no. eggs/ laying female	
Milk (control)	85	67	128	11	17	67	579
Diet D with adenine and cytidylic acid replacing RNA[b] (no. 67)	74	78	95	38	39	97	2209
Diet D (no. 68)	66	61	108	24	28	71	1919
Diet D plus inositol (no. 69)	72	72	100	30	39	72	2497

a Compiled from daily observations and by dissections of individual females at the end of the experiment.

b Table I, diet E.

TABLE XXXII Fecundity of house flies fed dry snythetic diets plus sugar-water compared with a liquid milk diet during a 17-day period[a].

Experimental diet	After 7 days			After 17 days		
	Mean no. eggs/ cylinder	Mean no. eggs/ exptl. female	Female survival %	Mean no. eggs/ cylinder	Mean no. eggs/ exptl. female	Female survival %
Milk (control)	725	60	97	1208	107	47
Diet D dry, less cholesterol (no. 72)	130	10	100	200	17	47
Diet C dry, (no. 73)	176	15	98	235	20	28

a Flies fed diets 72 and 73 were also supplied with fresh 0.1 M sucrose solution daily.

APPENDIX

TABLE XXXIII Fecundity of house flies in test cylinders, fed diet C containing varying amounts of methionine.

Diet	Cylinder no. (replicates)	Total fecundity (cumulative) after			
		5 days	7 days	9 days	11 days
Diet C lacking methionine	1	-	635	635	635
	2	100	571	571	571
	3	-	513	513	513
	4	317	605	613	613
	5	59	550	550	550
Mean no. of eggs/ cylinder		95	575	576	576
Diet C (containing 0.01 g. methionine)	1	183	842	855	912
	2	515	533	556	557
	3	329	962	1117	1269
	4	86	1028	1057	1057
	5	6	702	702	905
Mean no. of eggs/ cylinder		224	813	857	940
Diet C (containing 0.04 g. methionine)	1	600	776	1033	1213
	2	689	829	1279	1279
	3	698	1381	1381	1581
	4	787	948	950	1250
	5	720	883	946	947
Mean no. of eggs/ cylinder		699	963	1118	1254

Continued.....

TABLE XXXIII Continued.

Diet	Cylinder no. (replicates)	Total fecundity (cumulative) after 5 days	7 days	9 days	11 days
Diet C (containing 0.15 g. methionine)	1	916	1461	1461	1759
	2	920	1539	1702	1970
	3	522	959	959	968
	4	612	612	901	901
	5	98	517	632	632
Mean no. of eggs/ cylinder		614	1017	1131	1246
Milk	1	1233	2354	2642	2646
	2	1444	2614	3518	3811
	3	1246	2462	3971	4520
	4	1297	2074	2747	3264
Mean no. of eggs/ cylinder		1305	2376	3220	3560

APPENDIX

TABLE XXXIV A comparison of egg production from individual house flies fed diet C or diet C supplemented with cholesterol, B-vitamins and RNA during a 15-day period.

Diet	Cylinder number	No. of eggs in first cycle	Days between cycle	No. of eggs in second cycle	Total no. of eggs laid
Diet C	1	118	-	-	118
	3	137	-	-	137
	4	121	2	121	141
	9	1	-	-	1
	10	78	-	-	78
	14	133	4	143	176
	18	46	-	-	46
	19	104	-	-	104
	21	109	-	-	109
	28	3	-	-	3
Total	28				
Mean[b]		85	3	132	111
Milk	1	97	2	83	180
	2	126	3	166	516[a]
(control)	3	139	-	-	139
	4	-	-	-	-
Total	4				
Mean		121	3	125	278

Continued.....

TABLE XXXIV Continued.

Diet	Cylinder number	No. of eggs in first cycle	Days between cycle	No. of eggs in second cycle	Total no. of eggs laid
Diet C plus 0.1% cholesterol, B-vitamins and 0.4% RNA	1	119	-	-	119
	2	67	-	-	67
	3	127	-	-	127
	4	121	3	100	298[a]
	5	130	6	130	260
	6	149	2	137	576[a]
	8	104	-	-	104
	10	67	-	-	67
	11	163	3	148	575[a]
	12	143	3	130	308[a]
	13	82	-	-	82
	15	137	5	34	171
	16	69	-	-	69
	17	176	3	134	413[a]
	18	1	-	-	1
	19	119	2	118	237
	20	140	3	135	541[a]
	21	159	3	149	454[a]
	22	109	-	-	109
	23	147	-	106	253
	25	183	3	152	451[a]
	27	32	-	-	32
	28	120	2	121	382[a]
Total	26	2664			
Mean[b]		116	3	123	256

a More than two gonotrophic cycles laid within the period of the test.

b The mean values are calculated from the number of females laying eggs in each gonotrophic cycle.

FIGURE 1: CHANGES IN OVARY VOLUME WHEN ADULT HOUSE FLIES WERE FED A SUGAR WATER OR MILK DIET FOR 7 DAYS.

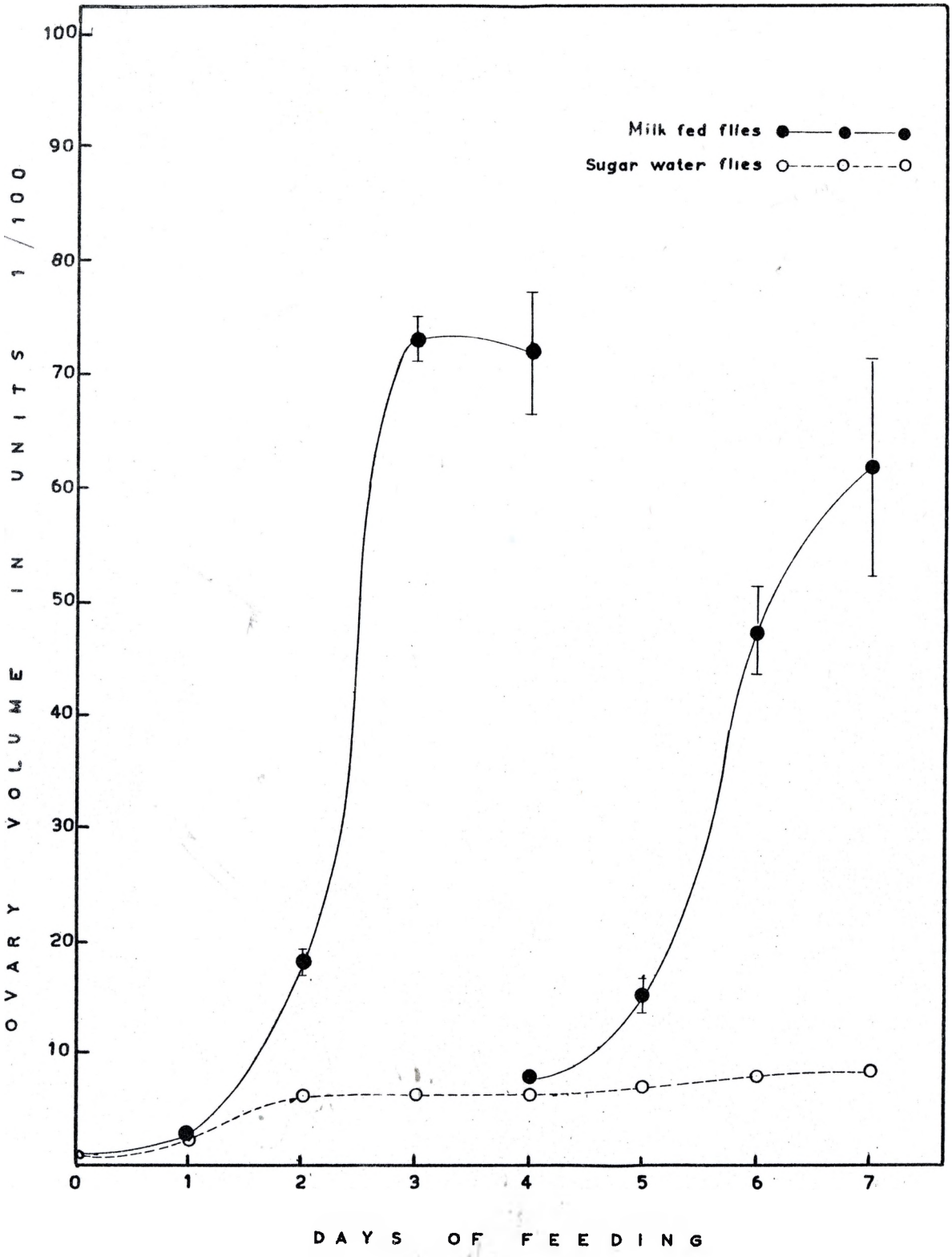


FIGURE 2: A COMPARISON OF ADULT SURVIVAL WHEN FEMALE HOUSE FLIES WERE FED VARIOUS NATURAL PRODUCTS.

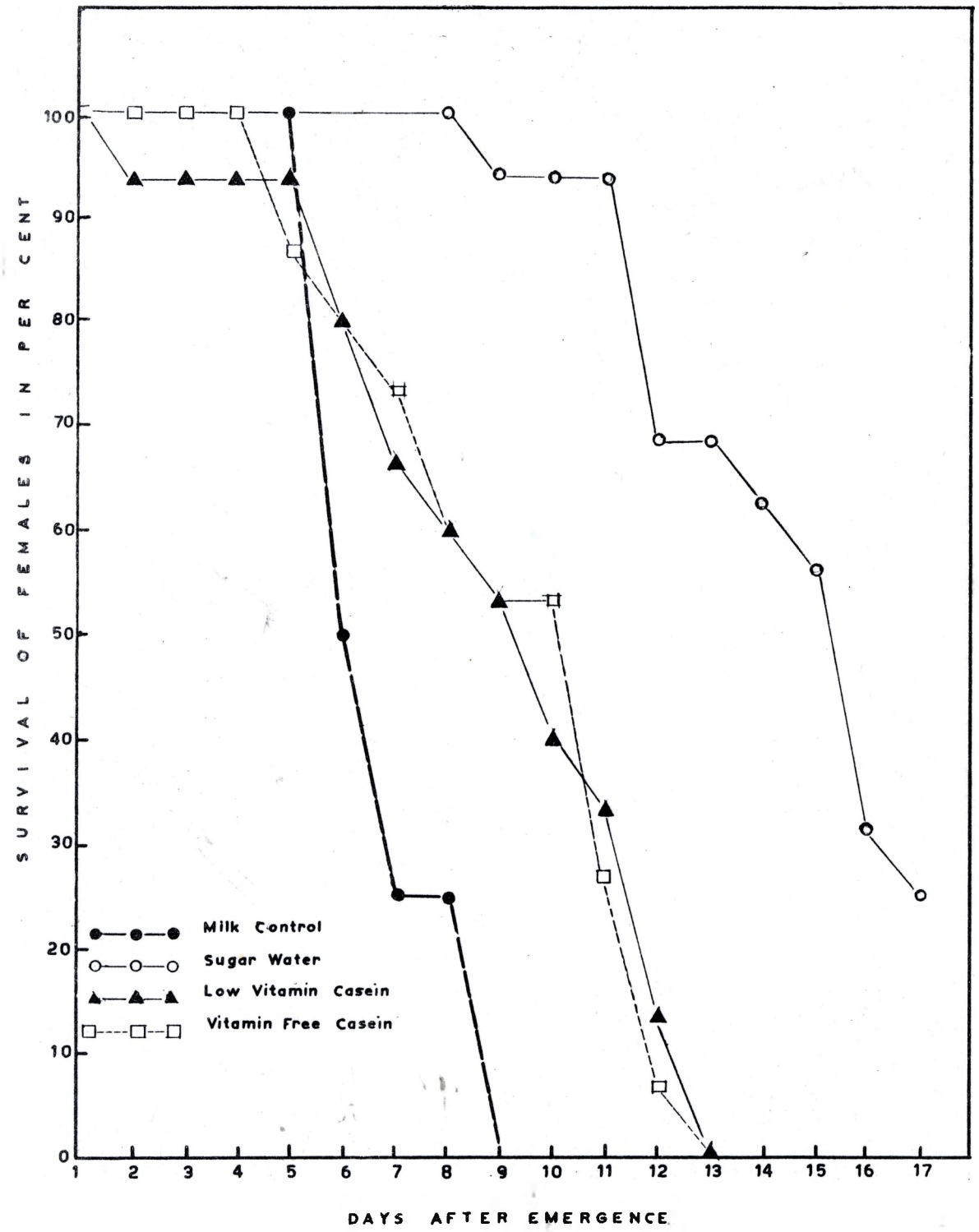


FIGURE 3: THE EFFECT ON HOUSE FLY FECUNDITY WHEN VARYING AMOUNTS OF CHOLESTEROL ARE PRESENT IN THE DIET FED TO ADULTS

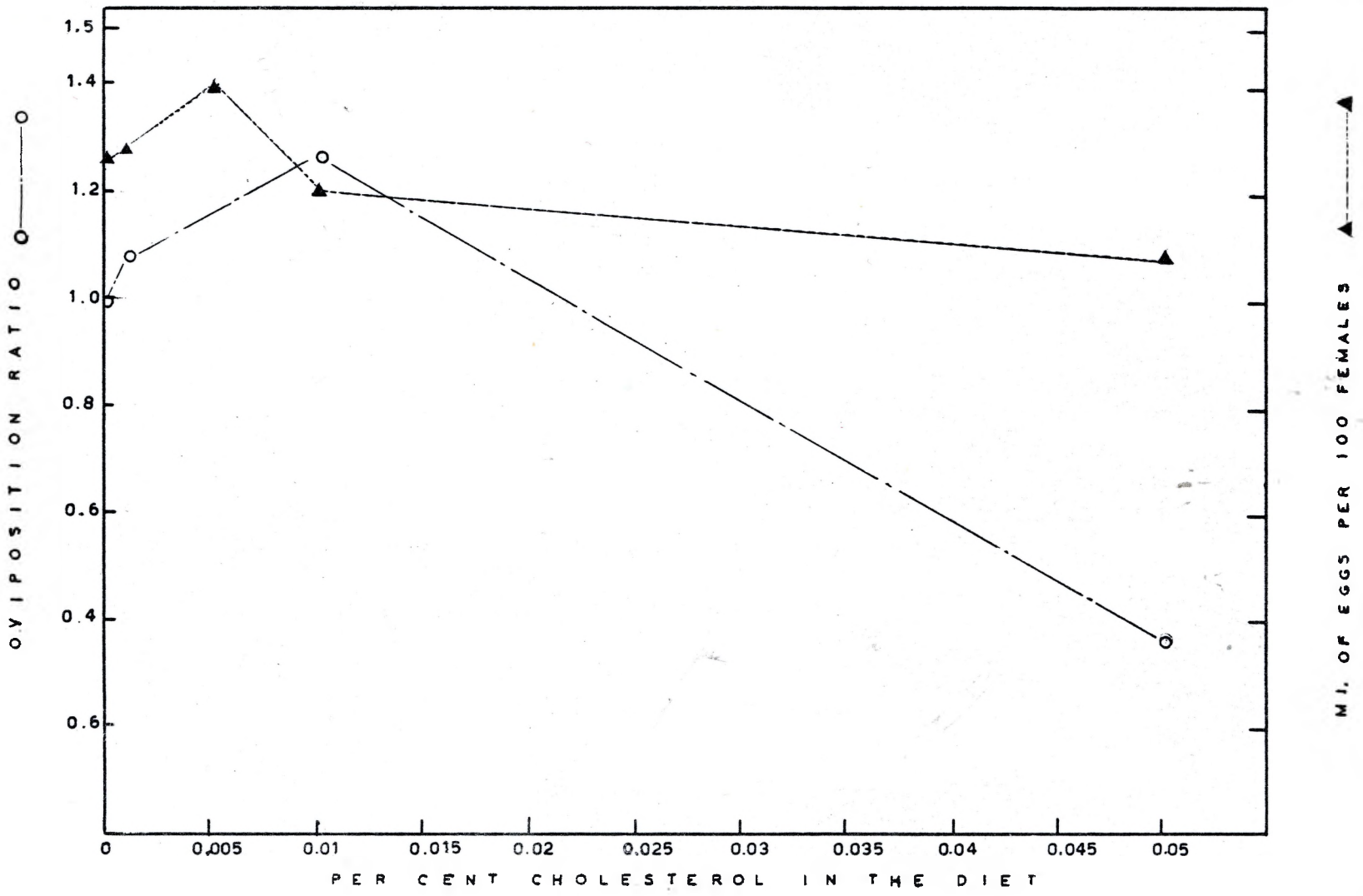


FIGURE 4: COMPARISON OF FEMALE SURVIVAL WHEN ADULT HOUSE FLIES WERE FED DIET C OR DIET D.

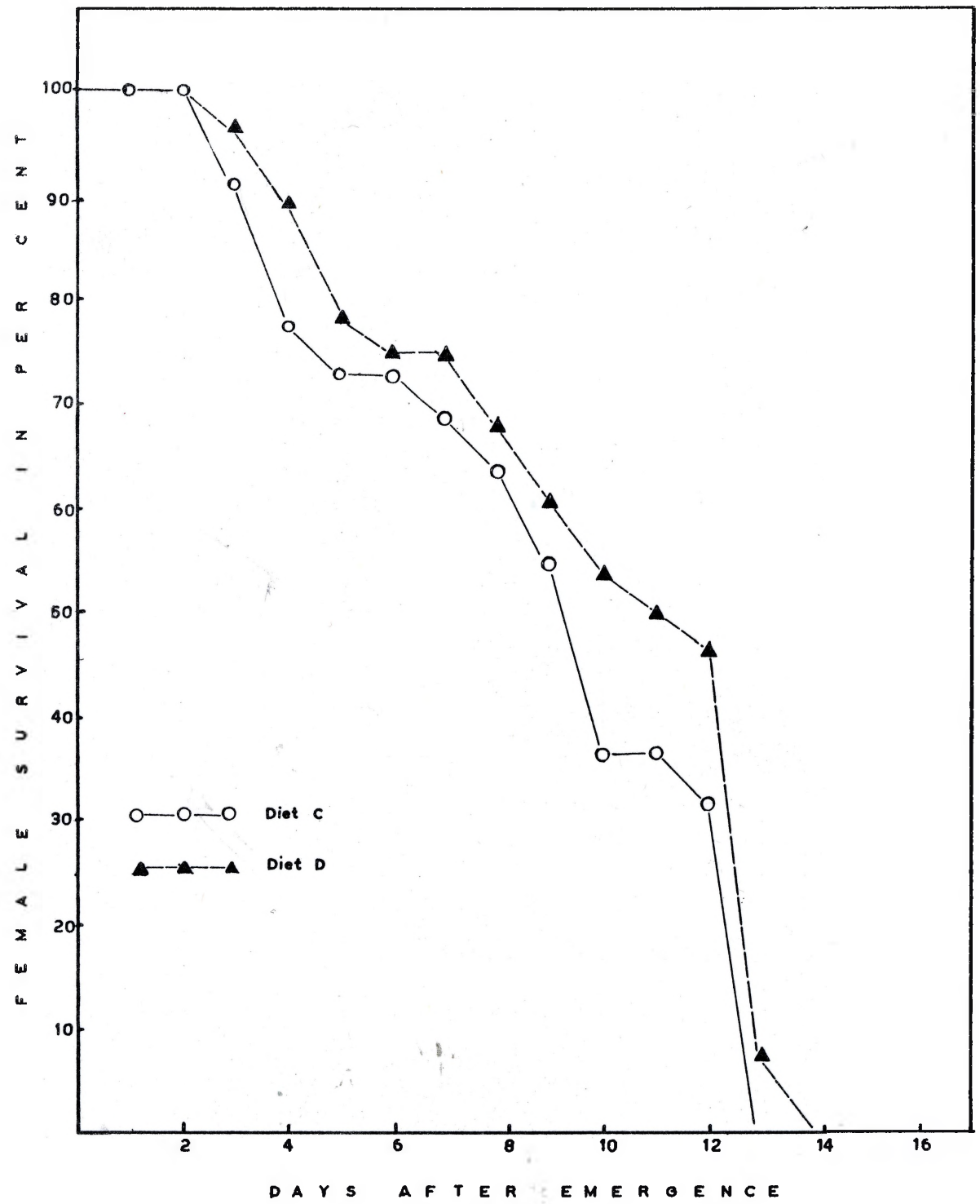


FIGURE 5: SURVIVAL OF THE ADULT FEMALE HOUSE FLY WHEN VARIOUS DIETARY SUPPLEMENTS ARE ADDED TO THE MODIFIED AMINO ACID DIET. CONTROLS WERE FED A FRESH MILK DIET.

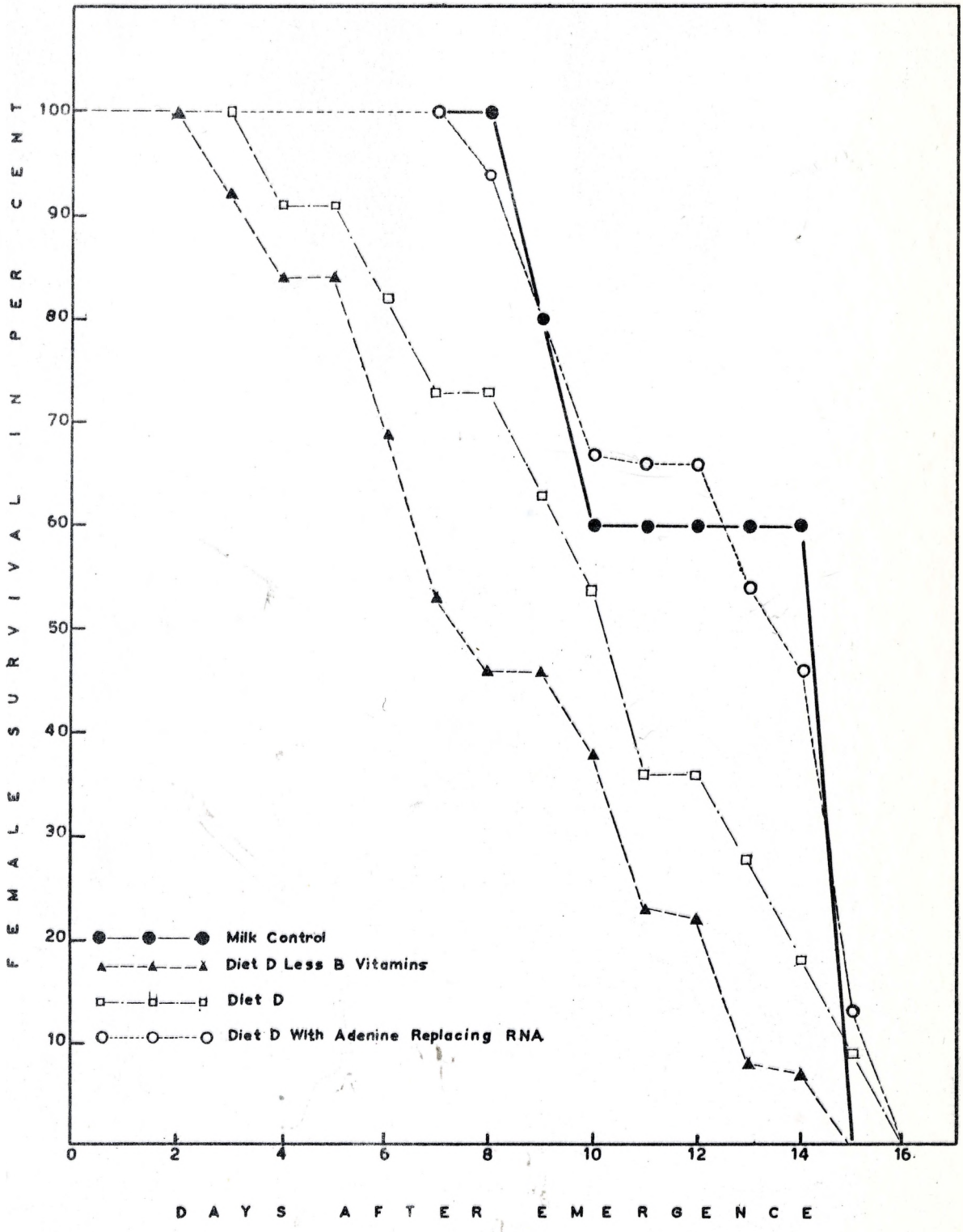


FIGURE 6: A COMPARISON OF FEMALE SURVIVAL WHEN ADULT HOUSE FLIES WERE FED DIET D SUPPLEMENTED WITH INOSITOL OR DIET D WITH ADENINE AND CYTIDYLIC ACID REPLACING R N A. COMPARED WITH DIET D AND MILK OVER A 14 DAY PERIOD.

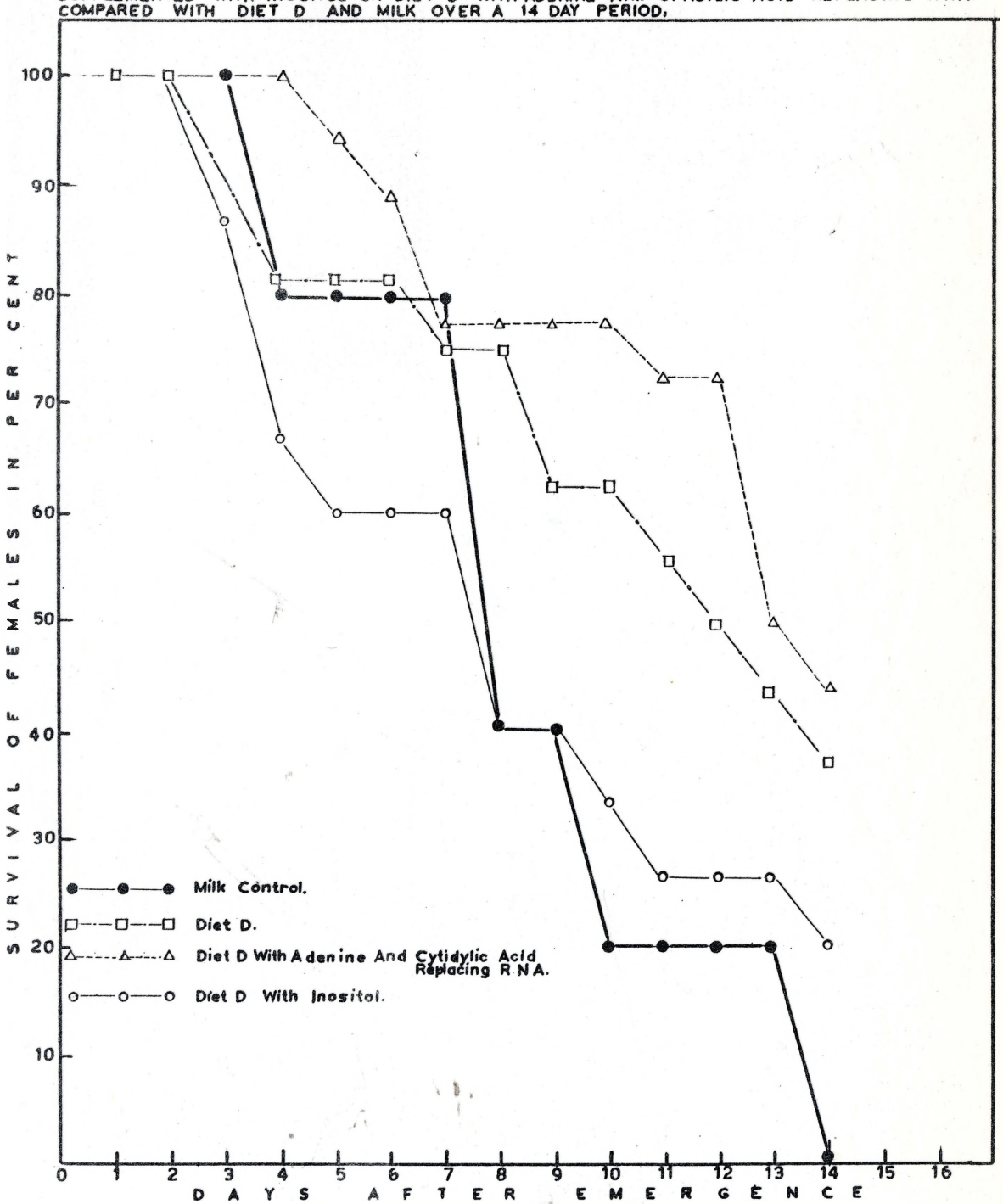
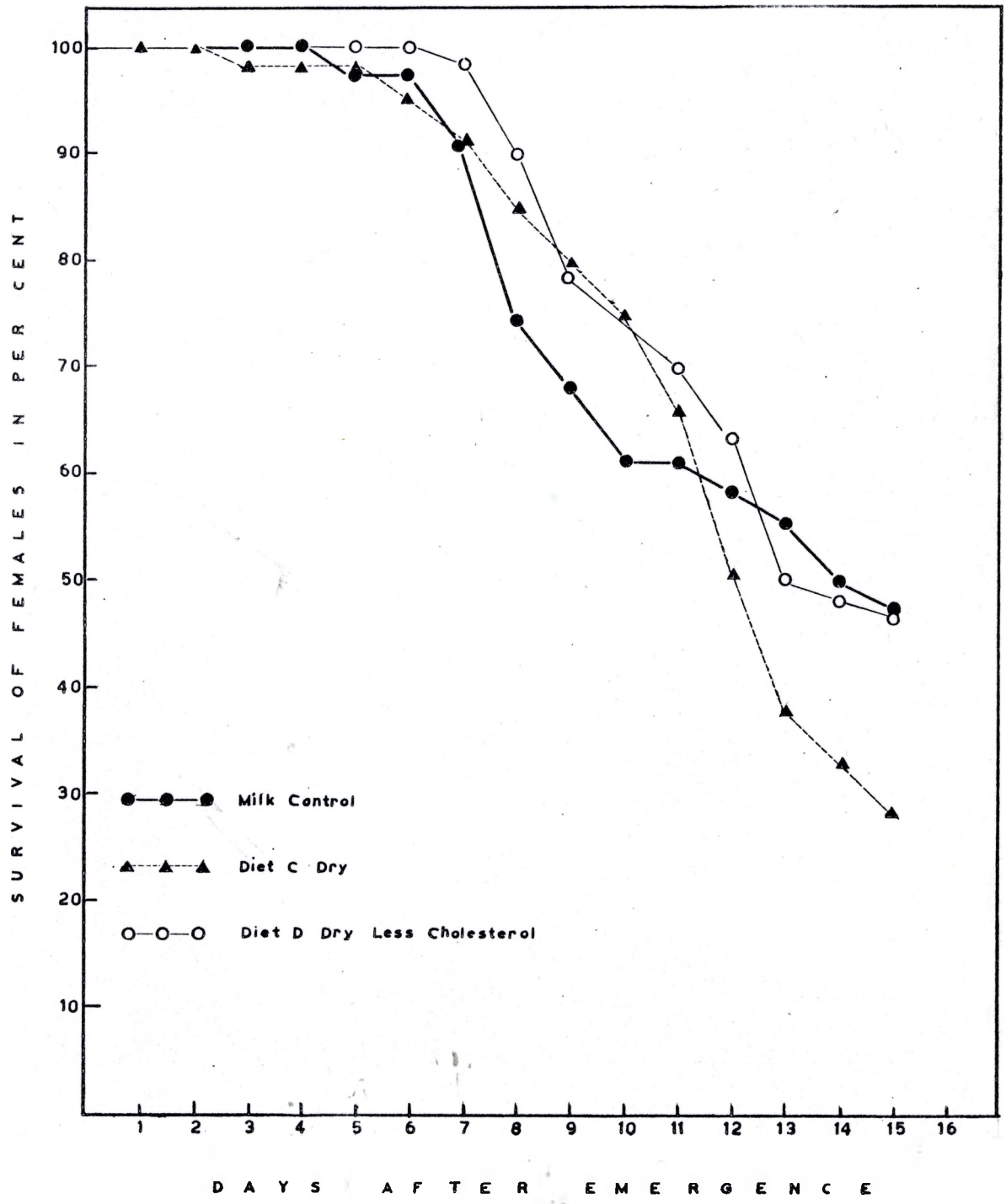


FIGURE 7: A COMPARISON OF FEMALE SURVIVAL WHEN ADULT HOUSE FLIES WERE FED DRY SYNTHETIC DIETS OR FRESH MILK.



REFERENCES

Albritton, E. C., 1954. Standard values in nutrition and metabolism. W. B. Saunders Co., Philadelphia, Pa.

Addis, T., Poo, L. J., and Lew, W., 1936. The rate of protein formation in the organs and tissues of the body; I After casein refeeding. J. Biol. Chem. 116:343-352.

Adkisson, P. L., Bull, D. L., and Allison, W. E., 1960. A comparison of certain artificial diets for laboratory cultures of the pink bollworm. J. Econ. Entomol. 53:791-793.

Akao, A., 1935. Preuve expérimentale sur le rôle du zinc dans la fonction de reproduction. Keijo J. Med. 6:49-60.

Albanese, A. A., 1959. Protein and amino acid nutrition. Academic Press, N.Y.

Almquist, H. J., 1959. The amino acid requirements of animals. In A. A. Albanese,(ed.), Protein and amino acid nutrition. Academic Press, N. Y.

Alpatov, W. W., 1932. Egg production in Drosophila melanogaster and some factors which influence it. J. Exptl. Zool. 63:85-111.

Anonymous, 1955. The Peet-Grady Method. Soap and Chem. Spec. Blue Book.

Ascher, K. R. S., and Levinson, Z. H., 1956. The influence of protein addition to the larval diet on oviposition of the housefly. Riv. Parasitol. 17:217-222.

Basden, E. B., 1947. Breeding the house-fly (Musca domestica L.) in the laboratory; I Introduction and technique. Bull. Entomol. Res. 37:381-387.

Barker, J. S., and Tauber, O. E., 1951. Fecundity of and plant injury by the pea aphid as influenced by nutritional changes in the garden pea. J. Econ. Entomol. 44:1010-1012.

Barlow, J. S., and House, H. L., 1960. Effects of dietary glucose on haemolymph carbohydrates of Agria affinis (Fall). J. Insect Physiol. 5:181-189.

Beck, S. D., 1956. Nutrition of the European corn borer, Pyrausta nubilalis (HBN.); IV Feeding reactions of first instar larvae. Ann. Entomol. Soc. Am. 49:399-405.

Beckel, W. E., 1954. The lack of autolysis of the flight muscles of Aedes communis (DeGeer)(Culicidae) in the laboratory. Mosquito News. 14:123-127.

Berg, C. P., 1959. Utilization of d-amino acids. In A. A. Albanese, (ed.), Protein and amino acid nutrition. Academic Press, N. Y.

Berg, P., 1961. Specificity in protein synthesis. Ann. Rev. Biochem. 30:293-324.

Block, R. J., 1957. Nitrogen requirements of animals and man: Comments on the Folin and Schoenheimar hypothesis. Proc. Intern. Symposium on Enzyme Chemistry. 444-449.

Bodenstein, D., 1947. Investigations on the reproductive system of Drosophila. J. Exptl. Zool. 104:101-152.

Boissezon, P., 1934. Nouvelles expériences sur la biologie de Culex pipiens L. Ann. Parasitol. 12:182-192.

Boyce, A. M., 1934. Bionomics of the walnut husk fly, Rhagoletis completa. Hilgardia. 8:363-579.

Brachet, J., 1957. Biochemical cytology. Academic Press Inc., N. Y.

Brookes, V. J., and Fraenkel, G., 1958. The nutrition of the larva of the house fly, Musca domestica L. Physiol. Zool. 31:208-223.

Browne, L. B., 1958. The frequency of mating of the Australian sheep blowfly, Lucilia cuprina. Aust. J. Sci. 20:185.

Brues, C. T., 1946. Insect dietary. Harvard University Press, Cambridge, Mass.

Brust, M., and Fraenkel, G., 1955. The nutritional requirements of the larvae of a blowfly, Phormia regina (Meig.). Physiol. Zool. 28:186-204.

Buxton, P.A., 1930. The biology of a blood sucking bug, Rhodnius prolixus. Trans. Entomol. Soc. Lond. 78:227-236.

Charbonneau, R., and Lemonde, A., 1960. Unidentified growth factors in brewer's yeast; I Necessity of these factors for Tribolium confusum (Duval). Can. J. Zool. 38:87-90.

Christophers, S. R., 1960. Aedes aegypti. Cambridge University Press.

Cotty, V. G., Henry, S. M., and Hilchey, J. D., 1958. The sulphur metabolism of insects; III The metabolism of cystine, methionine, taurine, and sulphate by the house fly, Musca domestica L. Contrib. Boyce Thompson Inst. 19:379-392.

Craig, R., and Hoskins, W. M., 1940. Insect Biochemistry. Ann. Rev. Biochem. 9:617-640.

Dadd, R. H., 1957. Ascorbic acid and carotene in the nutrition of the desert locust, Schistocerca gregaria Forsk. Nature. 179: 427-428.

Dadd, R. H., 1960a. Some effects of dietary ascorbic acid on locusts. Proc. Roy. Soc. Series (B). 153:128-143.

Dadd, R. H., 1960b. The nutritional requirements of locusts; I Development of synthetic diets and lipid requirements. J. Ins. Physiol. 4:319-347.

Dadd, R. H., 1961a. The nutritional requirements of locusts; IV Requirements for vitamins of the B complex. J. Ins. Physiol. 6:1-12.

Dadd, R. H., 1961b. The nutritional requirements of locusts; V Observations on essential fatty acids, chlorophyll, nutritional salt mixtures, and the protein or amino acid components of synthetic diets. J. Ins. Physiol. 6:126-145.

Day, M. F., 1943. The corpus allatum of the sheep bed, Melophagus ovinus L. Psyche. 50:1-8.

Day, M. F., 1949. The distribution of ascorbic acid in the tissues of insects. Aust. J. Sci. Res. B, 2:19-30.

Dean, R. W., 1938. Experiments on raising the apple maggot adult. J. Econ. Entomol. 31:241-244.

De Groot, A. P., 1953. Protein and amino acid requirements of the honeybee (Apis mellifica L.). Physiol. Comparata et Oecologia. 3:1-90.

Delcourt, A., and Guyénot, E., 1910. De la possibilité d'étudier certains Dipteres en milieu défini. Acad. Sci. Compt. Rend. 151:255-257.

De Meillion, B., Golberg, L., and Lavoipierre, M., 1945. The nutrition of the larvae of Aedes aegypti L. J. Exptl. Biol. 21:84-89.

De Meillion, B., and Golberg, L., 1946. Nutritional studies on blood sucking arthropods. Nature. 158:269.

De Meillion, B., and Golberg, L., 1947. Preliminary studies on the nutritional requirements of the bed bug (Cimex lectularius L.) and the tick, Ornithodorus moubata Murray. J. Exptl. Biol. 24:41-63.

Derbeneva-Ukhova, V. P., 1935. The influence of adult nutrition on the ovaries in Musca domestica L. (in Russian). Med. Parasit. 4:394-403.

Dimond, J. B., 1957. The amino acids required for egg production in the yellow fever mosquito Aedes aegypti L. Ph.D. Thesis, Ohio State University.

Dimond, J. B., Lea, A. O., Hahnert, W. F., and De Long, D. M., 1956. The amino acids required for egg production in Aedes aegypti. Can. Ent. 88:57-62.

Dimond, J. B., Lea, A. O., and De Long, D. M., 1958. Nutritional requirements for reproduction in insects. Proc. 10th Intern. Congr. Entomol. Congr. 2:135-137 (1956).

Dorman, S. C., Hale, W. C., and Hoskins, W. M., 1938. The laboratory rearing of flesh flies and the relations between temperature, diet and egg production. J. Econ. Entomol. 31:44-51.

Ficq, A., 1955. Etude autoradiographique du métabolisme des proteines et des acides nucleiques du cours de l'oogénèse chez les batraciens. Exptl. Cell Research. 9:286-293.

Finlayson, T., 1961. Note on effects of some minerals on fecundity of Aptesis basizona. (Grav.)(Hymenoptera: Ichneumonidae). Can. Entomol. 93:626-628.

Fisher, R. B., 1954. Protein metabolism. Methuen Ltd., London.

Forgash, A. J., 1958. The effect of inositol on growth, survival and maturation in Periplaneta americana (L.). Ann. Entomol. Soc. Amer. 51:406-409.

Forgash, A. J., and Moore, R. F., 1960. Dietary inositol requirement of Periplaneta americana (L.). Ann. Entomol. Soc. Amer. 53: 91-94.

Fraenkel, G., 1936. Utilization of sugars and polyhydric alcohols by the blowfly. Nature. 137:237.

Fraenkel, G., 1940. Utilization and digestion of carbohydrates by the adult blowfly. J. Exptl. Biol. 17:18-29.

Fraenkel, G., 1959. A historical and comparative survey of the dietary requirements of insects. Ann. N. Y. Acad. Sci. 77:267-274.

Fraenkel, G., and Blewett, M., 1946, 1947. Linoleic acid, vitamin E and other fat soluble substances in the nutrition of certain insects, Ephestia kuehniella, E. elutella, E. cautella, and Plodia interpunctella (Lep.). J. Exptl. Biol. 22:172-190.

Fraenkel, G., and Printy, G., 1954. The amino acid requirements of the confused flour beetle, Tribolium confusum, Duval. Biol. Bull. 106:149-157.

Fröbrich, G., 1953. Die Caseindosierung in synthetischen Diëten für die Aufzucht von Tribolium confusum (Duv.). (Tenebrionidae, Coleoptera.) Naturwissenschaften. 40:556

Frost, D. V., 1959. Methods of measuring the nutritive value of proteins, protein hydrolyzates and amino acid mixtures. The repletion method. In A. A. Albanese, (ed.), Protein and amino acid nutrition. Acad. Press, N.Y.

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

Glaser, R. W., 1923. The effect of food on longevity and reproduction in flies. J. Exptl. Zool., 38:383-412.

Gillett, J. D., 1958. Induced ovarian development in decapitated mosquitoes by transfusion of haemolymph. J. Exptl. Biol. 35:685-693.

Gilmour, D., 1961. The biochemistry of insects. Academic Press, N. Y.

Goldberg, L., and De Meillion, B., 1948. The nutrition of the larva of Aedes aegypti Linnaeus. 3 Lipid requirements. Biochem. J. 43:372-379.

Goldsmith, E. C., and Frank, T., 1952. Sterility in the female fruit fly, Drosophila melanogaster, produced by the feeding of a folic-acid antagonist. Am. J. Physiol. 171:726-727.

Golightly, W. H., 1940. Factors influencing the abundance and size of Psychoda species in sewage bacteria beds. Ann. Appl. Biol. 27:406-421.

Gordon, H. T., 1959. Minimal nutritional requirements of the German roach, Blattella germanica L. Ann. N. Y. Acad. Sci. 77:290-351.

Greenberg, B., 1955. Fecundity and cold survival of the house fly. J. Econ. Entomol. 48:654-657.

Greenberg, B., 1959. Persistence of bacteria in the developmental stages of the house fly; II Quantitative study of the host-contaminant relationship in flies breeding under natural conditions. Am. J. Trop. Med. Hyg. 8:412-416.

Grison, P., 1948. Action des lécithines sur la fecondité du Doryphora. Acad. Sci. Comp. Rend. (Paris). 227:1172-1174.

Hagen, K. S., 1952. Influence of adult nutrition upon fecundity, fertility and longevity of three tephritid species. (Diptera, Tephritidae). Ph.D. Thesis, University of California, Berkeley.

Hagen, K. S., 1958. Honeydew as an adult fruit fly diet affecting reproduction. Proc. 10th Int. Congr. Entomol. 3:25-30.

Hammen, C. S., 1956. Nutrition of Musca domestica in single pair culture. Ann. Entomol. Soc. 49:365-368.

Harlow, P. M., 1956. A study of ovarial development and its relation to adult nutrition in the blowfly, Protophormia terrae-novae (R.D.). J. Exptl. Biol. 33:777-797.

Hawk, P. B., Oser, H. L., and Summerson, W. H., 1954. Practical physiological chemistry. The Blakiston Company, Inc., N. Y. 13th ed.

Hecht, O., 1933. Die Blutnahrung, die Erzeugung der Eier und die Überwinterung der Stechmuckenweibchen. Arch. Schiffs. -u. Tropen- hyg. 37:3-87.

Hewitt, G. C., 1914. The house fly, Musca domestica Linn.; its structure, habits, development, relation to disease and control. Cambridge University Press, London.

Hilchey, J. D., 1953. Studies on the qualitative requirements of Blattella germanica (L.) for amino acids under aseptic conditions. Contrib. Boyce Thompson Inst. 17:203-219.

Hilchey, J. D., 1958. The relationship between nutrition and intermediary metabolism in studies of the sulfur amino acids in insects. Proc. 10th Intern. Congr. Entomol. 2:127-134,(1956).

Hilchey, J. D., Block, R. J., Miller, L. P., and Weed, R. M., 1955. The sulfur metabolism of insects; I The utilization of sulfate for the formation of cystine and methionine by the German cockroach, Blattella germanica (L.). Contrib. Boyce Thompson Inst. 18:109-123.

Hilchey, J. D., Cotty, V. F., and Henry, S. M., 1957. The sulphur metabolism of insects; II The metabolism of cystine-S^{35} by the house fly, Musca domestica L. Contrib. Boyce Thompson Inst. 19:189-200.

Hinton, T., 1956. Nucleic acid utilization by Drosophila. Physiol. Zool. 29:20-26.

Hinton, T., Ellis, J., and Noyes, D. T., 1951. An adenine requirement in a strain of Drosophila. Proc. Not. Acad. Sc. 37:293-299.

Hinton, T., Noyes, D. T., and Ellis, J., 1951. Amino acids and growth factors in a chemically defined medium for Drosophila. Physiol. Zool. 24:335-353.

Hobson, R. P., 1938. Sheep blow-fly investigations; VII Observations on the development of eggs and oviposition in the sheep blow fly, Lucilia sericata Mg. Ann. Appl. Biol. 38:383-412.

Hocking, B., 1952. Autolysis of flight muscles in a mosquito. Nature. 169:1101.

Hocking, B., 1954. Flight muscle autolysis in Aedes communis (De Geer) Mosquito News 14:121-123.

Hosoi, T., 1954. Egg production in Culex pipiens pallens Coquillett; III Growth and degeneration of ovarian follicles. Jap. J. Med. Sc. Biol. 7:111-127.

House, H. L., 1954a. Nutritional studies with Pseudosarcophaga affinis (Fall.), a dipterous parasite of the spruce budworm, Choristoneura fumiferana (Clem.); I A chemically defined medium and aseptic-culture technique. Can. J. Zool. 32:331-341.

House, H. L., 1954b. Nutritional studies with Pseudosarcophaga affinis (Fall.), a dipterous parasite of the spruce budworm, Choristoneura fumiferana (Clem.); II Effects of eleven vitamins on growth. Can. J. Zool. 32:342-350.

House, H. L., 1954c. Nutritional studies with Pseudosarcophaga affinis (Fall.), a dipterous parasite of the spruce budworm, Choristoneura fumiferana (Clem.); IV Effects of ribonucleic acid, glutathione, dextrose, a salt mixture, cholesterol, and fats. Can. J. Zool. 32:358-365.

House, H. L., 1958. Nutritional requirements of insects associated with animal parasitism. Exptl. Parasitol. 7:555-609.

House, H. L., 1959. Nutrition of the parasitoid Pseudosarcophaga affinis (Fall.) and of other insects. Ann. N. Y. Acad. Sci. 7:394-405.

House, H. L., 1961. Insect nutrition. Ann. Rev. Entomol. 6:13-23.

House, H. L., and Barlow, J. S., 1957. Effects of nucleic acids on larval growth, pupation and adult emergence of Pseudosarcophaga affinis (Fall.). Nature 180:44.

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

House, H. L., and Barlow, J. S., 1960. Effects of oleic and other fatty acids on the growth rate of Agria affinis (Fall.), (Diptera: Sarcophagidae). J. Nutrition 72:409-414.

Imms, A. D., 1957. A general textbook of entomology. Ninth ed. Methuen and Co. Ltd., London.

Jordan, H. B., 1961. The effects of the quality of blood and temperature on the production and viability of eggs in Culex quinquefasciatus. Mosquito News 21:133-135.

Johnson, B., 1957. Studies on the degeneration of the flight muscles of alate aphids; I A comparative study of the occurrence of muscle breakdown in relation to reproduction in several species. J. Ins. Physiol. 3:248-256.

Kaplanis, J. N., Robbins, W. E., and Tabor, L. A., 1960. The utilization and metabolism of 4-C^{14}-cholesterol by the adult house fly. Ann. Entomol. Soc. Amer. 53:260-264.

Kennedy, C. H., 1949. Methods for the study of the internal anatomy of insects. Ohio State University.

King, R. C., 1960. Oögenesis in adult Drosophila melanogaster; IX Studies on the cytochemistry and ultrastructure of developing oocytes. Growth 24:265-323.

King, R. C., Rubinson, A. C., and Smith, R. F., 1956. Oögenesis in adult Drosophila melanogaster. Growth 20:121-157.

King, R. C., and Burnett, R. B., 1959. Autoradiographic study of uptake of tritiated glycine, thymidine and uridine by fruit fly ovaries. Science 129:1674-1675.

King, R. C., and Sang, J. H., 1959. Oögenesis in adult Drosophila melanogaster; VIII The role of folic acid in oögenesis. Growth 23:37-53.

Kobayashi, H., 1934. The influence of foods on the fecundity of Musca domestica. Keijo J. Med. 5:1-33.

Larsen, E. B., and Thomsen, M., 1940. The influence of temperature on the development of some species of Diptera. Vidensk. Medd. fra Dansk naturk. Foren. 104:1-75.

Larsen, J. R., and Bodenstein, D., 1959. The humoral control of egg maturation in the mosquito. J. Exptl. Zool. 140:343-381.

Lavoipierre, M. M. J., 1961. Blood-feeding, fecundity and ageing in Aedes aegypti var. queenslandensis. Nature 191:575-576.

Lea, A. O., Dimond, J. B., and De Long, D. M., 1956. A chemically defined medium for rearing Aedes aegypti larvae. J. Econ. Entomol. 49:313-315.

Levinson, Z. H., 1955. Nutritional requirements of insects. Riv. Parasitol. 16:113-183.

Levinson, Z. H., 1960a. The evolution of sterol requirements. Proc. 11th Int. Congr. Entomol. 3:154-156.

Levinson, Z. H., 1960b. Food of housefly larvae. Nature 188:427-428.

Levinson, Z. H., and Bergmann, E. D., 1957. Steroid utilization and fatty acid synthesis by the larvae of the house fly, Musca vicina Macq. Biochem. J. 65:254-260.

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

Lineva, V. A., 1953. Concerning the physiological age of the housefly, Musca domestica L. (Diptera: Muscidae), (in Russian). Entomol. Obozr. 33:161-173.

Lipke, H., 1957. Nutritional considerations for rearing insects. Proc. Entom. Soc. Am., N. Central Branch 12:90-91.

Lipke, H., and Fraenkel, G., 1956. Insect Nutrition. Ann. Rev. Entomol. 1:17-44.

Luck, J. M., 1936. Liver proteins; I The question of protein storage. J. Biol. Chem. 115:491-510.

Mackerras, M. J., 1933. Observations on the life histories, nutritional requirements and fecundity of blow flies. Bull. Entomol. Res. 24:353-362.

Maddy, K. H., and Elvehjem, G. A., 1949. Studies on growth of mice fed rations containing free amino acids. J. Biol. Chem. 177:577-590.

Martin, H. E., and Hare, L., 1942. The nutritive requirements of Tenebrio molitor larvae. Biol.Bull. 83:428-437.

McCay, C. M., 1933. Longevity compatible with optimum growth? Science 77:410-411.

Mellanby, K., 1938. Diapause and metamorphosis of the blowfly, Lucilia sericata Meig. Parasitol. 30:392-402.

Mer, G. G., 1936. Experimental study on the development of the ovary in Anopheles elutus Edw. (Dipt. Culix.). Bull Entomol. Res. 27:351-359.

Mitchell, H. H., 1959. Some species and age differences in amino acid requirements. In A. A. Albanese, (ed.), Protein and amino acid metabolism. Academic Press, N. Y.

Mitlin, N., Butt, B. A., and Shortino, T. J., 1957. Effect of mitolic poisons on house fly oviposition. Physiol. Zool. 30:133-136.

Mitlin, N., and Cohen, C. F., 1961. The composition of ribonucleic acid in the developing house fly ovary. J. Econ. Entomol. 54:651-653.

Monroe, R. E., 1959. Role of cholesterol in house fly reproduction. Nature 184:1515-1514.

Monroe, R. E., 1960. Effect of dietary cholesterol on house fly reproduction. Ann. Entomol. Soc. Am. 53:821-824.

Monroe, R. E., Kaplanis, J. N., and Robbins, W. E., 1961. Sterol storage and reproduction in the house fly. Ann. Entomol. Soc. Am. 54:537-539.

Moreland, C. R., and McLeod, W. S., 1956. House fly egg-measuring techniques. J. Econ. Entomol. 49:49-51.

Moreland, C. R., and McLeod, W. S., 1957. Studies on rearing the house fly on a bran-alfalfa medium. J. Econ. Entomol. 50: 146-150.

Noland, J. L., Lilly, J. H., and Baumann, C. A., 1949. Vitamin requirements of the cockroach, Blattella germanica L. Ann. Entomol. Soc. Am. 42:154-164.

Norris, M. J., 1934. Contributions towards the study of insect fertility; III Adult nutrition, fecundity and longevity in the genus Ephestia. (Lepidoptera, Phycitidae). Proc. Zool. Soc. Lond. 334-360.

Osborne, T. B., and Mendel, L. B., 1917. Nutritive factors in animal tissues; I J. Biol. Chem. 32:309-323.

Patton, W. S., and Evans, A. M., 1929. Insects, ticks, mites and venomous animals of medical and veterinary importance. H. R. Grubb, Ltd., Croydon, Great Britain.

Pepper, J. H., and Hastings, E., 1943. Biochemical studies on the sugar beet webworm (Loxostege sticticalis L.) with special reference to the fatty acids and their relation to diapause and sterility. Montana Ag. Expt. Sta. Tech., Bull. 413.

Pollock, M. R., 1948. Unsaturated fatty acids in cotton plugs. Nature 161:853.

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

Reynolds, J. M., 1945. The inheritence of food effects in a flour beetle, Tribolium destructor. Proc. Roy. Soc. B. 132:438-451.

Robbins, W. E., Kaplanis, J. N., Louloudes, S. J., and Monroe, R. E., 1960. Uitilization of 1-C^{14}-acetate in lipid synthesis by adult house flies. Ann. Entomol. Soc. Am. 53:128-129.

Robbins, W. E., and Shortino, T. J., 1962. Effect of cholesterol in the larval diet on ovarian development in the adult housefly. Nature 194:502-503.

Robertson, F. W., and Sang, J. H., 1944. The ecological determinants of population growth in a Drosophila culture; I Fecundity of adult flies. Proc. Roy. Soc. Lond. B, 132:258-276.

Rockstein, M., 1956. Some biochemical aspects of aging in insects. J. Gerontology 11:281-285.

Rockstein, M., 1957. Longevity of male and female house flies. J. Gerontology 12:253-256.

Rockstein, M., and Lieberman, H. M., 1958. Survival curves for male and female house flies, Musca domestica L. Nature 181:787-788.

Rockstein, M., and Lieberman, H. M., 1959. A life table for the common house fly, Musca domestica. Gerontologia 3:23-36.

Roeder, K. D., 1953. Insect Physiology. John Wiley and Sons, Inc., New York. Chapman and Hall, Limited, London.

Rose, W. C., 1937. The nutritive significance of the amino acids and certain related compounds. Science 86:298-300.

Rose, W. C., 1938. The nutritive significance of the amino acids. Physiol. Rev. 18:109-136.

Rose, W. C., Smith, L. C., Womack, M., and Shane, M., 1949. The utilization of the nitrogen of ammonium salts, urea and certain other compounds in the synthesis of non-essential amino acids in vivo. J. Biol. Chem. 181:307-316.

Roy, D. N., 1936. Blood in ovulation. Aedes (Diptera) Bull. Entomol. Res. 27:423-429.

Sang, J. H., 1957. Utilization of dietary purines and pyrimidines by Drosophila melanogaster. Proc. Roy. Soc. Edinb. B, 66:339-359.

Sang, J. H., 1959. Circumstances affecting the nutritional requirements of Drosophila melanogaster. Ann. N. Y. Acad. Sci. 77: 352-365.

Sang, J. H., and King, R. C., 1961. Nutritional requirements of axenically cultured Drosophila melanogaster adults. J. Exptl. Biol. 38:793-809.

Scharff, D. K., 1950. Cattle grubs: their biologies, their distribution and experiments in their control. Montana Sta. Coll. Tech. 471:1-74.

Scharrer, B., 1958. Neuro-endocrine mechanisms in insects. Proceedings Second Internat. Sympos. Neurosecretion. Springer. Verlag, Berlin-Gottingen-Heidelberg, 79-84.

Silverman, P. H., and Levinson, Z. H., 1954. Lipid requirements of the larva of the housefly, Musca vicina (Macq.) reared under non-aseptic conditions. Biochem. J. 58:291-294.

Singh, K. R. P., and Brown, A. W. A., 1957. Nutritional requirements of Aedes aegypti L. J. Ins. Physiol. 1:199-220.

Smith, A. G., and Harrison, R. A., 1951. Notes on laboratory breeding of the housefly (Musca domestica L.). New Zealand J. Sci. Technol. B. 33:1-4.

Strangways-Dixon, J., 1961. The relationship between nutrition, hormones and reproduction in the blowfly Calliphora erythrocephala (Meig.); I Selective feeding in relation to the reproductive cycle, the corpus allatum volume and fertilization. J. Exptl. Biol. 38:225-235.

Swift, H., 1962. Nucleic acids and cell morphology in dipteran salivary glands. In J. M. Allen,(ed.), The molecular control of cellular activity. McGraw-Hill Book Company Inc., New York.

Tate, P., and Vincent, M., 1936. The biology of autogenous and anautogenous races of Culex pipiens. Parasitol. 28:115-145.

Thomsen, E., 1940. Corpus allatum hormone and insect reproduction. Nature 145:28-29.

Thomsen, E., 1956. Observations on the oenocytes of the adult Calliphora erythrocephala Meig. "Bertil Hanström, Zoological Papers in honour of his sixty-fifth birthday" (Lund, 1956).

Thomsen, E., and Møller, J., 1959. Neurosecretion and intestinal proteinase activity in an insect, Calliphora erythrocephala Meig. Nature 183:1401-1402.

Thorsteinson, A. J., 1960. Host selection in phytophagous insects. Ann. Rev. Entomol. 5:193-242.

Tischler, N., 1931. A satisfactory nutriment for adult house flies. J. Econ. Entomol. 24:558.

Trager, W., 1935. On the nutritional requirements of mosquito larvae (Aedes aegypti). Am. J. Hyg. 22:475-493.

Trager, W., 1947. Insect Nutrition. Biol. Rev. 22:148-177.

Trager, W., 1953. Nutrition. In K. Roeder, (ed.), Insect physiology. John Wiley and Sons, Inc., New York.

Uvarov, B. P., 1929. Insect nutrition and metabolism, Trans. Entomol. Soc. London,76:255-343.

Vanderzant, E. S., Kerur, D., and Reiser, R., 1957. The role of dietary fatty acids in the development of the pink bollworm. J. Econ. Entomol. 50:606-608.

Verhein, A., 1921. Die Eibildung der Musciden. Zool. Jb. (Anat.) 42:149-212.

Villee, C. A., and Bissell, H. B., 1948. Nucleic acids as growth factors in Drosophila. J. Biol. Chem. 172:59-66.

Wanson, M., 1950. Contribution à l'étude de l'Onchocerose africaine humaine. Ann. Soc. Belge. Méd. Trop. 30:667-863.

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

West, L. S., 1951. The housefly. Comstock Pub. Co., New York.

West, E. S., and Todd, W. R., 1961. Textbook of biochemistry. Third edition. The Macmillan Company, New York.

Weyer, F., 1934. Der Einfluss der Larvalernährung auf die Fortpflanzungsphysiologie verschiedener Stechmucken. Arch. Schiff - u. - Tropen - Hyg. 38:394-398.

Wigglesworth, V. B., 1950. The principles of insect physiology. Methuen and Co. Ltd., London.

Wilkes, A., Bucher, G. E., Cameron, J. W. MacB., and West, A. S., 1948. Studies on the house fly (Musca domestica L.). I The biology and large-scale production of laboratory populations. Can. J. Res. D. 26:8-25.

Williams, R. J., 1959. Individuality of amino acid needs. In A. A. Albanese, (ed.), Protein and amino acid nutrition. Academic Press, N. Y.

Woke, P. A., 1937. Effects of various blood fractions on egg production of Aedes aegypti Linn. Am. J. Hyg. 25:372-380.

Woke, P. A., Ally, M. S., and Rosenberger, R. C. Jr., 1956. The number of eggs developed related to the quantities of human blood ingested in Aedes aegypti L. (Diptera: Culicidae). Ann. Entomol. Soc. Am. 49:435-441.

Wooley, D. W., 1952. A study of antimetabolites. J. Wiley and Sons, New York.

Yeoli, M., and Mer, G. G., 1938. Nutrition and egg production in mosquitoes. Trans. Roy. Soc. Trop. Med. Hyg. 31:437-444.

Zalokar, M., 1960. Sites of protein and ribonucleic acid synthesis in the cell. Exptl. Cell Research, 19:559-576.

Zingrone, L. D., Bruce, W. N., and Decker, G. C., 1959. A mating study of the female house fly. J. Econ. Entomol. 52:236.