OBSERVATIONS ON THE RELATION OF MICRO-ORGANISMS TO THE NUTRITION OF *MUSCA DOMESTICA* L.

by

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It would be natural to assume that the food relations of such a well known insect as *Musca domestica* had been thoroughly studied. Such is not the case, however, and when all the available data are assembled the knowledge of the physiology of this species is surprisingly meager. As a matter of fact, there are very few insects, the actual food of which is reasonably well understood. General classifications of insects according to their feeding habits are often given but it is possible to characterize the nutritional requirements of only a limited number.

Entomological text books often contain, in the section dealing with nutrition, a statement to the effect that probably all insect nourishment is closely associated with microorganisms. The frequency of such an implication and the apparent ignorance as to specific food necessities in the case of most insects gave the impetus for the experiments herein described. An early conception of the work suggested the study of several saprophytic insects with the idea of comparing their varied relations to the microorganisms concerned. As such, the task would have been far beyond the limit of time allotted for this problem. The working basis was therefore altered to cover only one insect with the hope that more definite information might be gained than if several species were included.

Because of its short life cycle, its enormous reproductive powers, and its tendency to breed more or less continuously under favorable conditions of temperature, M. domestica proved ideal as the subject of these investigations. The larvae of the house fly, living as it does in a habitat so infused with a varied accumulation of microörganisms, both plant and animal, constitutes a typical example of an insect feeding in decaying organic matter.

HISTORICAL

The subject of food relationships between microorganisms and higher animals has long attracted the attention of workers in the field of physiology but as yet is little understood. Recent research with vitamines tends only to complicate our knowledge of general nutritional requirements and forces us to reconstruct the methods commonly used in measuring food values.

Naturally, the first observations concerned man and the higher vertebrates and it is only recently that insects have been studied from this angle. Early workers expressed the opinion that intestinal bacteria were essential to life in higher animals and this idea has greatly influenced the subsequent work with insects. During the past 25 years the literature on the subject has assumed gigantic proportions and, of late, the order Diptera has received much attention; due, no doubt, to the fact that such a great number of its species develop in conditions that teem with microorganisms. Other orders of the Hexopoda have not been neglected but most of the investigations have been confined to those insects feeding upon decaying or fermenting organic matter and usually classified as saprophytes. In order to eliminate a lengthy discussion of the nutritional requirements of the entire insect world the account of the literature here discussed will be confined to that on insects closely related to the house fly,

namely the order Diptera.

As a result of its popularity in the field of genetics, the fruit-fly Drosophila melanogaster has received much careful study from a multitude of workers but with a diversity of conclusions. In general, Delcourt and Guyenot (1910), Guyenot (1913, 1917), Loeb (1915, 1916), Loeb and Northrop (1916, 1917), Northrop (1917, 1926), and Baumberger (1917, 1919) agree in the opinion that yeast, living or dead, is an adequate food for the larvae of Drosophila and that the decaying fruit serves merely as a substratum for the yeast cells. Various artificial media for Drosophila have been devised but "the insect depends for its proteins on yeast and has no greater synthetic powers than the higher animals" (Baumberger). Glaser (1924) concludes that any bacterium that grows well on the medium will serve the purpose and that the importance of the yeast cells has been overestimated. This writer also bred two generations of Drosophila larvae on sterile orange and grapefruit juice.

The flesh flies have also been the subject of much study. Guyenot (1906, 1907), working with Lucilia and Phormia, found that there was no need for these larvae to produce digestive ferments as they fed not on the flesh, but on the products of bacterial decomposition. Calliphora larvae were noted by Weinland (1907, 1908) to contain a trypsin-like ferment which made them independent of bacteria in the digestion of meat and in building up fat from proteins. Bogdanow (1906, 1908) was unable to rear the larvae of Calliphora vomitoria

in the absence of bacteria but obtained good results in the presence of a definite and fairly simple bacterial flora, even on an artificial media. A series of experiments recorded by Woolman (1911, 1919, 1919a, 1921a, 1922), indicate that it is possible to carry on cultures of aseptic larvae of Calliphora indefinitely and that such larvae are able to liquify gelatin and grow in a perfectly normal way. He discovered that, even though most vitamins were destroyed by the high temperatures employed in sterilization, the larvae developed normally and either were able to concentrate these accessory food factors or to dispense with them altogether.

Most of the work with Musca domestica has consisted in experiments on the longevity and fecundity of adults as influenced by various foods. Bogdanow (1908) found that the larvae could be bred on starch paste or on gelatine, but only in the presence of mould and bacteria; concluding, therefore, that the latter were necessary for growth. Hewitt (1914) gives a long list of the breeding places of the house fly and notes that optimum development occurs on those substances in which fermentation appears. A complete absence of all material except bacteria, fungus spores, and yeast cells was found by Baumberger (1919) in the digestive tracts of larvae that had been reared on bran mash.

The attraction of gravid females to various substances for oviposition purposes is a field which not only arouses the interest of the physiologist but may have certain practical applications in concentrating the eggs of the house

fly in situations where they may be easily destroyed. Richardson (1916, 1916a, 1916b, 1917) tested the relative power of several attrahents and found that ammonia exerted the greatest influence. Crumb and Lyon (1917, 1921) believe that "the female house fly is attracted for egg laying by decaying organic matter in proportion to the amount of carbonic and acetic acids liberated in the fermentation process". Roubaud and Veillon (1922) conclude that the luring factor consists of a complex mixture of gaseous emanations produced at a certain stage of decomposition.

The larvae of mosquitoes have long been considered as consumers of small organisms, both plant and animal. Atkin and Bacot (1917) were able to rear adults of Aedes aegypi under sterile conditions in a few cases but concluded that the presence of bacteria or yeasts was a practical necessity. McGregor (1929), while investigating the significance of the pH, reared mosquito larvae aseptically in solutions of various food materials of which bread gave the best results.

Three species of the genus Hylemyia of the family Anthomyiidae have been reared aseptically from eggs that had been thoroughly washed in sterile water. Hylemyia antiqua and Hylemyia bassicae were bred on sterile agar jelly containing a high percent extract of their respective foods (onion and cabbage). "Bacterial contamination proved fatal to the larvae" (Eyre 1921). Leach (1926) while studying the relationship between Hylemyia ciliorura and potato blackleg in Minne-

sota, concluded that bacteria were essential for the development of the maggots after he was unable to obtain growth on sterile potatoes except when bacteria were added. Later Huff (1928) grew the larvae of this species on developing bean and pea seedlings in the absence of bacteria and upon partially decomposed potato plugs which had been sterilized by heat.

Many other Dipterous forms, less frequently or less completely studied, might be mentioned. Desmometopa m-nigrum, which feeds normally in decaying fish, produced normal adults from rich yeast food according to Baumberger (1919) and Sciara sp. larvae were grown on bran agar which they infected with microorganisms. Miastor larvae, unable to live on sterilized bark, will breed on an agar culture of organisms isolated from the wood (Harris, 1923). The larvae of the olive fruit-fly, Daucus oleae, were found by Petri (1905, 1910) to contain in their gastric caeca large colonies of bacteria which undoubtedly favor the digestion of the rich, oily food. Yet, he points out that many larvae living within seeds, rich in oil, do not contain bacteria. As noted by Schutte (1921) the larvae of Hydromyza livens burrow inside the leaves of Nuphar, emitting juices from the mouth which digest the plant tissue, extra-intestinally, without the aid of bacteria. Hering (1926) believes that infections by microorganisms are always possible in the burrows of leaf miners but so far no true symbionts are known.

The other orders of insects contain many examples of close association with microorganisms, some of which such as

the Termites, the Ambrosia beetles, and fungus-growing ants have received much attention. Others, not so well known, but none the less important and interesting, are also omitted as irrelevant to the present discussion.

TECHNIQUE

For the most part, the eggs of the house fly used in these experiments were gathered as needed from a stable on the university campus where an abundant supply was always available. These were usually collected during the morning and, although their exact age could not be determined, such a procedure was found adequate for the requirements of this work. Throughout the summer, the eggs deposited during the previous day usually had hatched by morning and those gathered at this time were sufficiently "fresh" to allow ample time for manipulation before the young larvae emerged. Where a more exact time of deposition was desired, eggs were obtained by inserting pans of fresh horse-manure, for short periods, in a large wire cage which contained a constant supply of flies. Unless the demand had previously been too heavy, one or more egg masses were supplied almost immediately.

At the outset of the study a satisfactory method of sterilizing and handling the eggs had to be developed. Individual eggs could most easily be handled by means of a camel's-hair brush but obviously this would not suffice for sterile eggs as such an instrument could not be satisfactorily sterilized. Consequently all eggs used, both untreated and sterile, were transferred on a drop of sterile water by means of a bulb-pipette. No doubt, this served to wash many

of the microorganisms from the surface of the untreated eggs but even so, countless millions invariably developed and it was thought best to sacrifice a few bacteria for the sake of uniformity.

Several methods of sterilizing the eggs were tried. Of the agents usually employed, namely alcohol, formalin and mercuric chloride solution, only the latter proved satisfactory. The percent sterility of eggs treated in alcohol or formalin was none too large and the mortality often great. The use of bichloride of mercury solution destroyed the surface organisms in most cases and seldom injured the egg itself. Bogdanov (1908) treated the eggs of Calliphora with bichloride of mercury solution (1:1000) for 15 minutes. Leach (1926) found that the eggs of Hylemyia cilicrura Rond could be emersed in such a solution for as long as two hours without injury to the embryo. As shown in table 1, which lists the results of a series of treatments in a 1:1000 solution of bichloride of mercury, such a prolonged soaking injured the developing larvae of M. domestica. In all probability this is due to the fact that the period of incubation is much shorter in the case of the house fly and, therefore, the inclosed embryo would be in a more advanced stage of development at the time of treatment.

TABLE 1.

Sterility and viability of eggs treated in bichloride of mercury (1-1000)

	Egg			Maggot
Number	Time	Sterility	Viability	Sterility
1	1 min.	Sterile	Hatched	Sterile
2	1 "	"	"	"
3	1 "	Not sterile	"	Not sterile
4	1 "	Sterile	"	Sterile
5	1 "	"	"	"
6	1 "	"	"	"
7	1 "	"	"	"
8	1 "	Not sterile	"	Not sterile
9	1 "	Sterile	"	Sterile
10	1 "	"	"	"
11	2 min.	Sterile	Hatched	Sterile
12	2 "	"	"	"
13	2 "	"	"	"
14	2 "	"	"	"
15	2 "	"	"	"
16	2 "	"	"	"
17	2 "	"	"	"
18	2 "	"	"	"
19	2 "	"	"	"
20	2 "	"	"	"
21	3 min.	Sterile	Hatched	Sterile
22	3 "	"	"	"
23	3 "	"	"	"
24	3 "	"	"	"
25	3 "	"	"	"
26	3 "	"	"	"
27	3 "	"	"	"
28	3 "	"	"	"
29	3 "	"	"	"
30	3 "	"	"	"
31	5 min.	Sterile	Hatched	Sterile
32	5 "	"	"	"
33	5 "	"	"	"
34	5 "	"	Not hatched	
35	5 "	"	Hatched	Sterile
36	5 "	"	"	"
37	5 "	"	"	"
38	5 "	"	"	"
39	5 "	"	"	"
40	5 "	"	Not hatched	
41	10 min.	Sterile	Not hatched	
42	10 "	"	"	
43	10 "	"	Hatched	Sterile
44	10 "	"	"	"
45	10 "	"	"	"
46	10 "	"	Not hatched	
47	10 "	"	Hatched	Sterile
48	10 "	"	Not hatched	
49	10 "	"	Hatched	Sterile
50	10 "	"	"	"

Eggs, treated as indicated in table 1, were washed in sterile water and placed on sterile beef-agar slants for hatching. After the resulting larvae were allowed to crawl about over the surface of the agar for a time, the tubes were then incubated at 27° C. An emersion of 3 minutes seemed to effect the desired results and this time was used throughout all subsequent treatments. In all cases the eggs were afterward washed in sterile water and checks on sterile agar provided for each series in order to determine their viability as well as sterility. Any series which showed contamination was discarded and those in which the checks were clear were rechecked by making inoculations from the food vials to sterile agar slants.

The various cages and equipment used in wholesale rearing work are the stock and trade of all entomologists and need not be described here. Pans of moistened manure served as a very satisfactory rearing medium for the larvae. These were placed within large screen cages with dirt flooring and thus, natural conditions for pupation and emergence were approximated. This type of propagation was used only to supply reserve material in all stages and to serve as time checks on the more exact rearings.

It was found necessary to develop a special technique for those larvae which were to be reared under sterile conditions. Several methods were tried and found wanting, principally because of the difficulty of maintaining sterility throughout the entire life of the larvae. The

container adopted and used consisted of 105 millimeter glass culture tubes, 25 mm. in diameter. Manure, placed in these tubes, retained its moisture for a sufficient time to allow complete development of larvae; especially if the tubes were kept in a chamber or room where a high relative humidity was maintained. These vials were fitted with snug, cotton plugs which excluded dust and bacteria but still permitted the passage of some air. This afforded a very efficient device for rearing larvae or small groups of larvae under isolated conditions where sterility could be sustained.

Mature larvae, while roving about in search of a suitable place in which to pupate, often crawled through the cotton plugs employed as barriers. After several experiments had been ruined by the escape of larvae, a tight fitting wire cap was supplied for each vial. Copper gauze was used for the screening and moulded to fit down over the sides of the tube.

Manure, gathered from fresh deposits, was placed in the vials and sterilized in an autoclave for 20 minutes under a pressure of 115 lbs. This procedure was chosen more or less arbitrarily for the first series of experiments and, as favorable results were obtained, was adopted for all subsequent work where sterile food and conditions were required. Approximately 7 grams of manure was used and this, with the cotton plug, occupied about half of the space within the tube. When the vials and their contents had thoroughly cooled, a

sterile egg was dropped into each and the stopple was not again removed until it could be determined that the larvae had formed its puparium. Agar slants were inoculated from the manure at the beginning and at the end of each trial and all that showed contamination were discarded and not figured in the results.

In the checks, where sterile conditions were not required, the vials and manure were handled in a manner identical with the above except that they were not heated in the autoclave.

During the development of the larvae, the vials were kept in a basement room where the humidity was high and the termperature varied but little from 22°C.; otherwise no particular means were taken to cohtrol conditions.

Pupariation usually occurred within the cotton plugs or in the drier parts of the food medium. The puparia were removed from the tubes as formed and placed in individual tin boxes for emergence. A dirt floor, which could be moistened as necessary, was provided and a copper-screen cap was fitted over the top of each to retain the emerging adults. No attempt was made to maintain sterile conditions after the puparium had been formed.

During cold weather when out-door development was retarded, large cages for the rearing of flies were constructed within the greenhouse of the insectary and thus an abundance of material in all stages was provided. No difficulty was

experienced in rearing flies in this manner so long as there was present plenty of natural food in a condition suitable for assimilation by both larvae and adults. Dishes of honey-soaked bread were also supplied for the adults and the dirt beneath the cages was sprinkled at intervals in order to maintain a high moisture content in the air. Other precautions seemed unnecessary and material for study was thus furnished throughout the winter.

EXPERIMENTAL DATA

Early in the sterility and viability tests, it was noted that the young larvae were unable to develop on beef-agar slants upon which the eggs had been placed. Larvae, although frequently living for several days on such a medium, showed no appreciable increase in size and usually spent the majority of their time wandering about within the tube. That the larvae attempted to feed was indicated by marks of the mouth hooks over the surface and the occasional burrowing of a larva into the agar. When newly hatched, the larvae crawled actively over the agar, repeatedly slashing at the surface with their oral hooks but were entirely unsuccessful so far as completed development was concerned. Larvae from unsterile eggs seemed to live longer and to show more development than those from eggs which had been sterilized, presumably because the surface of the agar was somewhat liquified by the action of the bacteria and thus more easily assimilated by the larvae. Larvae also failed to show any marked increase in size when allowed to feed in tubes of beef-agar which previously had been inoculated from manure and incubated until a luxuriant bacterial growth was evident.

With the idea in mind that some food requisite might be present in the manure not found in the beef agar, a medium

was made by boiling 500 grams of horse dung in 1 liter of distilled water for half an hour. This extract was filtered through cotton, 1.5 per cent of pure shredded agar added, and the solution then sterilized in an autoclave. Tubes of this manure-agar were used in a series of tests to determine the ability of the larvae to develop on such a food and were later used to check the sterility of eggs and larvae in subsequent experiments.

Table 2 shows the results of attempts to propagate larvae on such a medium compared with similar rearings on beef-agar. Little success was attained as will be seen by the fact that only 2 adults were obtained from approximately 50 larvae. A few of the remaining larvae that died without forming puparia managed to undergo one molt and showed a noticable increase in size but the majority died with little or no growth taking place. It is significant that all of the larvae which molted were reared on unsterile agar where considerable liquefaction had resulted from the action of the bacteria.

TABLE 2. DEVELOPMENT OF LARVAE ON NUTRIENT AGAR

Number	EGG: Egg Sterilization: Date	EGG: Egg Sterilization: Method	EGG: Egg Sterilization: Time	EGG: Date Hatched	LARVA: Growth	LARVA: Longevity: Days	LARVA: Longevity: Date Died	PUPARIUM: Date Formed	ADULT: Date Emerged	NUTRIENT MEDIUM: Agar Extract	NUTRIENT MEDIUM: Bacterial Growth	NUTRIENT MEDIUM: Liquefaction
III a 1		None		7-13	1 molt	14	7-27			Beef	Abundant	Considerable
2		"		7-13	1 molt	14	7-27			"	Abundant	Considerable
3		"		7-13	Slight	9	7-22			"	Abundant	Considerable
4		"		7-13	1 molt	12	7-25			"	Abundant	Considerable
5		"		7-13	Slight	6	7-19			"	Abundant	Medium
6		"		7-13	Slight	8	7-21			"	Abundant	Medium
b 1	7-12	$HgCl_2$	3 min.	7-13	None	1	7-14			Beef	None	
2	7-12	"	"	7-13	Slight	6	7-19			"	None	
3	7-12	"	"	7-13	Slight	6	7-19			"	None	
4	7-12	"	"	7-13	None	2	7-15			"	None	
5	7-12	"	"	7-13	None	1	7-14			"	None	
6	7-12	"	"	7-13	Slight	5	7-18			"	None	
IV a 1		None		7-27	None	2	7-29			Beef	Moderate	Light
2		"		7-27	None	4	7-31			"	Moderate	Light
3		"		7-27	None	4	7-31			"	Moderate	Light
4		"		7-27	None	3	7-30			"	Moderate	Light
5		"		7-27	Slight	6	8-2			"	Moderate	Light
6		"		7-27	1 molt	11	8-7			"	Abundant	Considerable
b 1	7-26	$HgCl_2$	3 min.	7-27	None	2	7-29			Beef	None	
2	7-26	"	"	7-27	None	3	7-30			"	None	
3	7-26	"	"	7-27	None	3	7-30			"	None	
4	7-26	"	"	7-27	None	4	7-31			"	None	
5	7-26	"	"	7-27	Slight	6	8-2			"	None	
6	7-26	"	"	7-27	None	3	7-30			"	Moderate#	Light
c 1		None		7-27	None	4	7-31			Manure	Scanty	None
2		"		7-27	Complete			8-5	8-11	"	Abundant	Considerable
3		"		7-27	None	4	7-31			"	Scanty	None
4		"		7-27	Complete			8-3	8-8	"	Abundant	Considerable
5		"		7-27	1 molt	15	8-11			"	Abundant	Considerable
6		"		7-27	1 molt	17	8-13			"	Abundant	Considerable
7		"		*								
8		"		7-27	None	4	7-31			"	Moderate	Light
9		"		7-27	None	4	7-31			"	Scanty	None
10		"		*								
11		"		7-27	Slight	6	8-2			"	Moderate	Medium
12		"		7-27	None	3	7-30			"	Moderate	Medium
d 1	7-26	$HgCl_2$	3 min.	7-27	None	4	7-31			Manure	None	
2	7-26	"	"	7-27	None	4	7-31			"	None	
3	7-26	"	"	*						"		
4	7-26	"	"	7-27	None	4	7-31			"	None	
5	7-26	"	"	7-27	None	2	7-29			"	None	
6	7-26	"	"	7-27	None	4	7-31			"	None	
7	7-26	"	"	7-28	None	3	7-31			"	None	
8	7-26	"	"	7-28	None	3	7-31			"	None	
9	7-26	"	"	*								
10	7-26	"	"	7-27	None	4	7-31			"	None	
11	7-26	"	"	7-27	None	4	7-31			"	None	
12	7-26	"	"	7-27	None	4	7-31			"	None	

* Failed to hatch. # Contaminated.

At various times throughout the course of the work, adult flies were reared from manure that had been previously sterilized as described above. Five sets of experiments were conducted with the express purpose of determining the effect of such conditions upon the growth of the larvae and on the emergence of the adults; each set consisting of 25 larvae accompanied by an equal number of larvae on unsterile manure along with agar checks to ascertain the sterility and viability of the treated eggs and the resulting larvae. All in all, including those from other but similarly conducted tests, a total of 242 flies were reared from egg to adult upon manure under absolutely sterile conditions as shown by the complete absence of bacteria on agar slants inoculated from each rearing tube. To tabulate the data for each of these would occupy much unwarranted space and consequently 25 individuals have been picked at random, 5 from each of the aforementioned series, and arranged with the dates and lengths of stages in statistical form in table 3. For the purpose of comparison, 25 individuals were selected from the adults reared under unsterile conditions in the checks of these series and are similarly treated in table 4.

TABLE 3

Rate of development in sterile manure

Number	LARVA		PUPARIUM		ADULT	TOTAL
	Date Hatched	Days Length	Date Formed	Days Length	Date Emerged	Days Length
II b 1	7-12	9	7-21	5	7-26	14
5	7-12	9	7-21	6	7-27	15
7	7-12	8	7-20	6	7-26	14
14	7-12	7	7-19	6	7-25	13
18	7-12	8	7-20	5	7-25	13
V b 3	7-26	7	8-2	5	8-7	12
11	7-26	7	8-2	6	8-8	13
19	7-26	7	8-2	6	8-8	13
27	7-26	7	8-2	5	8-7	12
32	7-26	6	8-1	6	8-7	12
VIII b 3	8-9	7	8-16	5	8-21	12
8	8-9	7	8-16	6	8-22	13
11	8-9	7	8-16	6	8-22	13
16	8-9	6	8-15	6	8-21	12
18	8-9	6	8-15	5	8-20	11
XII b 5	9-28	7	10-5	8	10-13	15
7	9-28	8	10-6	8	10-14	16
10	9-28	9	10-7	7	10-14	16
15	9-28	8	10-6	8	10-14	16
21	9-28	8	10-6	9	10-15	17
XV b 4	11-30	6	12-6	6	12-12	12
8	11-30	7	12-7	6	12-13	13
13	11-30	7	12-7	6	12-13	13
16	11-30	7	12-7	7	12-14	14
20	11-30	7	12-7	7	12-14	14

TABLE 4

Rate of development in unsterile manure

Number	LARVA		PUPARIUM		ADULT	TOTAL
	Date Hatched	Days Length	Date Formed	Days Length	Date Emerged	Days Length
II a 5	7-12	9	7-21	5	7-26	14
12	7-12	7	7-19	6	7-25	13
15	7-12	8	7-20	5	7-25	13
19	7-12	6	7-18	5	7-23	11
21	7-12	8	7-20	5	7-25	13
V a 4	7-26	6	8-1	6	8-7	12
8	7-26	6	8-1	5	8-6	11
17	7-26	5	7-31	5	8-5	10
23	7-26	6	8-1	5	8-6	11
29	7-26	6	8-1	5	8-6	11
VIII a 2	8-9	5	8-14	5	8-19	10
8	8-9	6	8-15	6	8-21	12
12	8-9	6	8-15	5	8-20	11
15	8-9	6	8-15	6	8-21	12
18	8-9	6	8-15	5	8-20	11
XII a 6	9-28	7	10-5	9	10-14	16
11	9-28	7	10-5	7	10-12	14
16	9-28	8	10-6	8	10-14	16
19	9-28	7	10-5	8	10-13	15
24	9-28	7	10-5	8	10-13	15
XV a 3	11-30	8	12-8	5	12-13	13
7	11-30	7	12-7	6	12-13	13
11	11-30	8	12-8	6	12-14	14
13	11-30	6	12-6	5	12-11	11
18	11-30	6	12-6	6	12-12	12

The increased length of the time spent in the resting stage in series IX was supposedly due to a drop in temperature which occurred at the time the puparia were being formed. The vials and cages were kept in a basement room which normally gave relatively isothermic conditions. Unfortunately the source of heat at this time was a central power plant doing daylight duty only, and as a result, the temperature within the room fell dangerously near the freezing point on several nights. This inconvenience was corrected for subsequent rearings but, even so, the temperature within the room varied more than it had during the summer and the duration of the pupal stage was slightly longer. No corresponding lengthening of the larval stage was noted.

TABLE 5

Summary of developmental periods

	LARVAL PERIOD		PUPAL PERIOD		TOTAL PERIOD	
	Sterile	Unsterile	Sterile	Unsterile	Sterile	Unsterile
Arithmetical Mean	7.28	6.68	6.24	5.88	13.52	12.56
Standard Deviation	0.8724	1.0088	1.0688	1.1771	1.5264	1.7223
Probable Error	±0.1176	±0.1360	±0.1441	±0.1587	±0.2059	±0.2323

Table 5 gives a summary and comparison of the sterile tests and unsterile controls. A study of these figures reveals that the lengths of all stages were slightly shorter for the controls but that the variation was not so great in the tests. Observations made on the development of larvae in the usual habitat indicate that the conditions within the glass vials were not so ideal as those furnished by nature and that the length of the developmental period was somewhat longer (1-3 days) in the artificial surroundings provided. This need not influence the deductions, however, as all checks were carried on with equipment identical to that used for the tests.

Some slight difference in size was noted in the puparia from sterile larvae as compared with those from untreated larvae. Adult flies in general show some slight differences in size within the species and Herms (1907) found that the dimensions of adult Lucilia caesar could be influenced by underfeeding the larvae. The exact size of adult flies in a species with a small deviation is difficult to measure and consequently the weights of puparia were taken as comparable units in determining the effect of sterile food upon individual flies. The weights of the puparia along with other data are recorded in table 6 for a series of flies reared under sterile and under natural conditions. It will be noted that the puparia of flies reared under sterile conditions are somewhat lighter but that the deviation is less

than is the case with the puparia reared under natural conditions. Each larva was reared in an individual tube in the presence of an excess of food and all eggs were treated in order that any resulting ill effects would be distributed over both series.

TABLE 6

Weights of puparia reared on sterile and unsterile manure

Number	EGG		LARVA	PUPARIUM		ADULT
	Date Treated	Date Hatched	Food Condition	Date Formed	Weight	Date Emerged
					grams	
VI a 1	7-26	7-27	Unsterile	8-1	0.205	8-7
2	7-26	7-27	"	8-1	0.197	8-7
3	7-26	7-27	"	8-1	0.187	8-6
4	7-26	7-27	"	8-1	0.170	#
5.	7-26	*				
6	7-26	7-27	"	8-1	0.155	8-6
7	7-26	7-27	"	8-1	0.197	8-7
8	7-26	7-27	"	8-1	0.187	8-6
9	7-26	7-27	"	8-1	0.170	8-7
10	7-26	7-27	"	7-31	0.165	8-5
11	7-26	*				
12	7-26	7-27	"	7-31	0.142	8-5
13	7-26	7-27	"	8-1	0.134	8-6
14	7-26	7-27	"	8-1	0.139	8-7
15	7-26	7-27	"	8-1	0.138	8-7
16	7-26	7-27	"	8-2	0.155	8-7
17	7-26	7-27	"	8-2	0.168	8-7
18	7-26	7-27	"	8-2	0.174	8-8
19	7-26	7-27	"	8-2	0.145	8-7
20	7-26	7-27	"	8-2	0.138	8-6
				Average	0.1647	
VI b 1	7-26	7-27	Sterile	8-2	0.150	8-7
2	7-26	7-27	"	8-1	0.189	8-7
3	7-26	7-27	"	8-1	0.173	8-7
4	7-26	7-27	"	8-2	0.157	8-8
5	7-26	7-27	"	8-1	0.151	8-6
6	7-26	7-27	"	8-2	0.148	8-7
7	7-26	7-27	"	8-2	0.160	8-7
8	7-26	7-27	"	8-2	0.182	8-7
9	7-26	7-27	"	8-1	0.151	8-6
10	7-26	*				
11	7-26	7-27	"	8-2	0.149	8-8
12	7-26	7-27	"	8-2	0.133	8-7
13	7-26	7-27	"	8-2	0.157	8-8
14	7-26	7-27	"	8-2	0.131	8-7
15	7-26	7-27	"	8-1	0.139	8-7
16	7-26	7-27	"	8-2	0.137	8-7
17	7-26	*				
18	7-26	7-27	"	8-2	0.135	8-7
19	7-26	7-27	"	8-2	0.150	8-7
20	7-26	7-27	"	8-2	0.130	8-7
				Average	0.1499	

* Failed to hatch
\# Injured

In order to test the fecundity and mortality of adults which had been reared as larvae under sterile conditions, one lot of about 50 such flies, newly emerged, were placed in a large wire cage on August 5th. Fresh manure, food scraps, and water were supplied in this cage daily. A similar cage containing flies from larvae reared under septic circumstances was carried on in conjunction. The daily mortality of the flies in the test cage was not so great as that of the flies in the control; a fact which probably has little bearing. No attempt was made to obtain eggs from the second cage and this was conducted only in order to compare the longevity of the adults.

On August 18th, 14 days after the emergence of the flies, a mass of 23 eggs was found in the manure within the cage and several masses, totalling over 600 eggs, were recovered during the next few days. As it is often difficult to locate scattered eggs or small masses within a pile of manure, many were doubtlessly overlooked. After this time the cage was discontinued and no attempts were made to obtain the total number of eggs laid or to determine the fecundity of single females.

One hundred eggs recovered from the above cage were sterilized on August 21st and placed in vials of sterile manure for development. Puparia were formed by the resulting larvae during the 28th and 29th of August and 63 adults emerged during the 3rd, 4th and 5th of September. Thus, al-

though aseptic conditions were not maintained through the pupal and imaginal stages, 2 cycles were reared from manure in which all life had been killed before the introduction of the sterile eggs.

DISCUSSION

At the outset of the work, it was hoped that something definite might be learned as to the exact food requirements of the larvae of Musca domestica; but, due to its magnitude, this task must remain for future work with the present observations serving as a preliminary consideration. Of the methods generally used in an experimental study of animal nutrition, namely the building up of synthetic media or the extraction of certain substances from the natural food, neither offers great possibilities in the case of the larvae of this species. The complex organic compounds of the plant material itself are certainly not simplified by the digestive processes of the vertebrates concerned and the microorganisms along with their products must also be given careful consideration. It was the hope of eliminating this last factor that gave rise to the experiments herein described.

Attempts to rear larvae from nutrient agar on which bacteria from manure were growing, have with two exceptions proven futile, in spite of the fact that a few of the larvae showed considerable development. Luxuriant growths of bacteria nearly always resulted when the surface of an agar slant was inoculated from manure, but such a condition was inadequate for the complete development of the larvae even with a medium made from an extract of manure as a substratum for the development of the bacteria. This bit of evidence is entirely

contrary to the general conception that the larvae of the house fly develop by feeding upon bacteria alone. That two larvae were reared to maturity is true but these were from trials where the manure-extract agar was used and serve only as evidence that the prime nutrient factor lies in the manure itself rather than in the bacteria or their products. It is also true that larvae developed better in the presence of bacteria on such a medium, than under sterile conditions. This may be due, in part, to the liquifying action of the bacteria upon the agar and, in part, to the accessory food factors concerned in such a process.

To deny the utility of the microorganisms to the larvae would be unwarranted in the light of such evidence, especially if the fact is considered that the natural foods furnish substrata already liquified by bacteria. Hewitt (1914) has given a long list of the materials in which larvae of the house fly can breed, all of which, from their very nature, are teeming with bacteria and most of which are deficient in protein. Richardson (1916), Crumb and Lyon (1917, 1921) and Roubaud and Veillon (1922) have pointed out the attraction of the chemical products of fermentation in such substances to the female fly. Certainly, this decomposition is accompanied by, if not resulting from bacterial action.

The element nitrogen enters into the chemical composition of all living organisms. Free nitrogen, however,

is the most difficult to acquire of all the essential food elements. There are two important natural processes which tend to keep up the available supply of nitrogen compounds. The first is the formation of nitric oxide during the passage of lightning through the air. This nitric oxide undergoes chemical reaction with the oxygen, water vapor, and ammonia of the air and, finally, in the form of ammonium nitrate, is carried to the soil by rain. The second process takes place through the aid of certain bacteria, known as nitrogen fixers. These forms seem to be universally distributed and are possessed with the power of transforming the nitrogen of the air into compounds which may be easily assimilated by higher organisms.

Ammonia is one of the products of the decomposition of organic substances and certain other bacteria are able to oxidize this compound into nitrites and nitrates. A third group of bacteria are able to reduce the nitrites and nitrates completely with the evolution of free nitrogen. These two groups, known respectively as nitrifiers and denitrifiers, carry on processes that are essentially different but often occur simultaneously.

This complicated biotic interdependency between microorganisms, higher plants, and higher animals is known as the nitrogen cycle and is graphically pictured in figure 1.

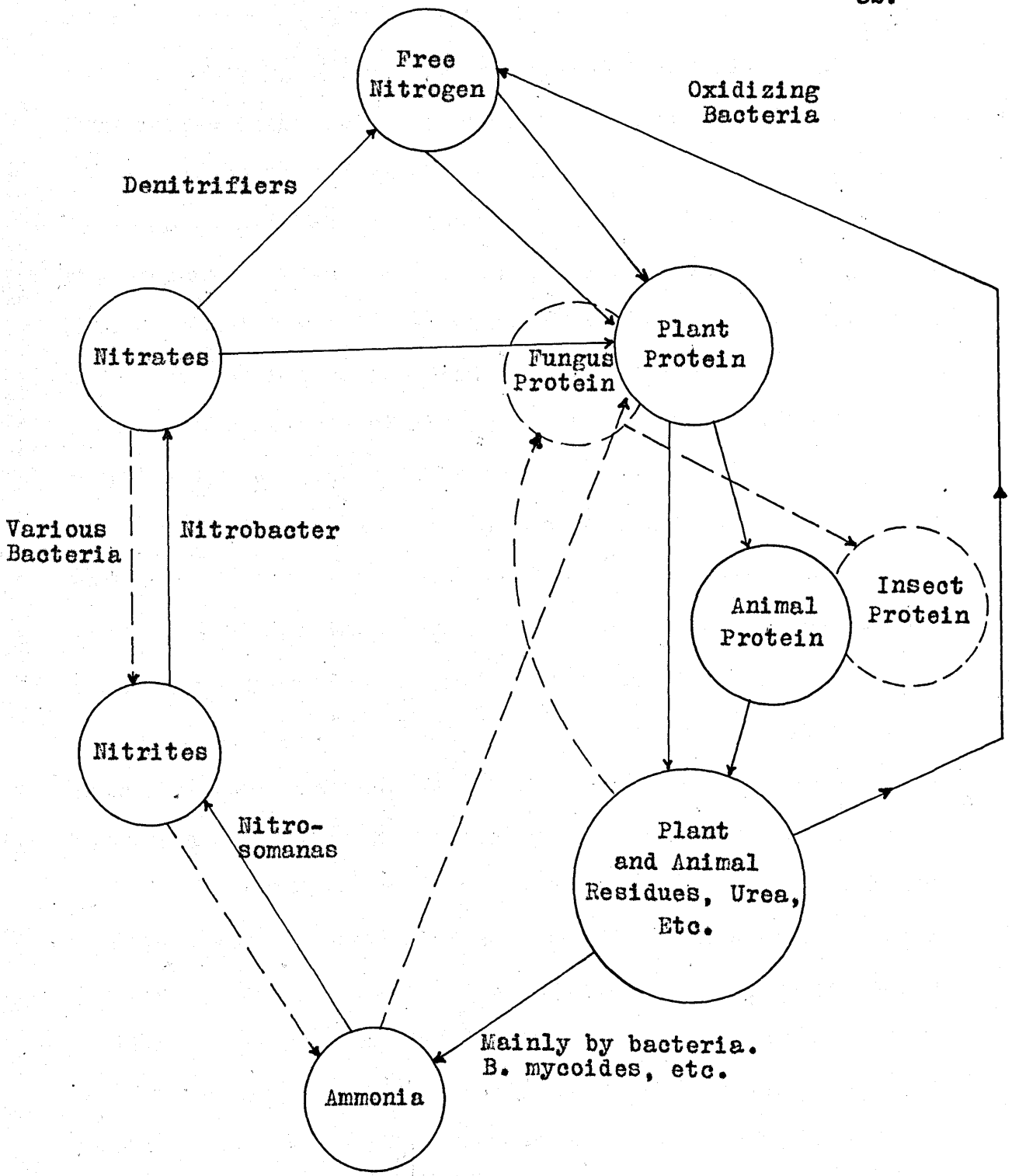

Figure 1. The Nitrogen Cycle (Baumberger)

During the course of this work 242 flies were reared from larvae which developed on manure under sterile conditions. Of course, it is impossible to absolutely exclude bacteria from manure and the only recourse lies in the sterilization of the product by heat, as soon as possible after its deposition. This does not prevent all action of the bacteria on the organic matter of the manure but does insure the cessation of such action and the absence of living organisms at the time of consumption by the larvae. Woolman (1919a, 1922) has recorded the fact that sterilization by heat often destroys the accessory food factors but concludes that such are not essential to the welfare of the larvae of Calliphora, and their loss not of momentous importance. Apparently if enzymes, ferments, or vitamins, necessary to the development of the larvae of the house fly were present, they were not complegely destroyed by the crude methods of sterilization adopted in this work.

As will be noted by an examination of tables 5 and 6, development on sterile manure was not without difficulties for the larvae concerned. Those feeding on such food required a slightly longer time for completed growth and on the average were somewhat lighter in weight than those enjoying their normal food. Thus there is a small but definite advantage in the development taking place in the presence of living bacteria.

One interesting feature that might be pointed out is the extent of the variation exhibited by the larvae in the

different situations. On the whole, the results obtained with larvae reared on natural food indicate more ideal conditions but the deviations from the mean were quite large, showing a variability in the food factors present. Growth upon sterile manure, although somewhat slower and producing smaller flies, was more stable and the extent of the divergence was considerably less. This fact denotes that the food constituents were more evenly distributed or more of a like character in manure that had been sterilized. No doubt the type and dispersal of the bacterial flora along with the extent and nature of their action would be responsible for such an unevenness of the accessory growth factors.

It has often been implied, and perhaps in some cases proven, that adult insects, reared under sterile conditions, were themselves sterile and unable to reproduce. Two generations of flies were reared as larvae under sterile conditions with no apparent ill effects. Females that had developed in such a manner, when allowed to feed naturally, produced normal eggs. These eggs developed into normal larvae which, by feeding on sterilized food, were able to transform into adults.

It can be easily seen that an insect whose eggs are mature at the time of emergence of the adult might be sterile so far as reproduction is concerned if fed as a larva upon food that had been sterilized and perhaps did not contain all of the factors necessary for complete growth. How-

ever, in an insect with a comparatively long pre-oviposition period during which the reproductive organs and eggs develop at the expense of nourishment taken as adult, the food of the larva can have no such great influence.

CONCLUSIONS

1. The theory that the larvae of Musca domestica L., are able to develop by feeding upon bacteria alone was proven fallaceous by the fact that they could not long subsist on bacteria growing on beef-agar slants.

2. Larvae of the house fly were reared from eggs to the formation of puparia upon manure which had been previously sterilized and which remained sterile throughout the procedure.

3. Larvae reared under sterile conditions, developed almost as rapidly and were only slightly smaller than those reared from untreated eggs on normal manure in similar containers.

4. Larvae, developing on sterile manure, were apparently normal and produced adults which, judging from their activity and fecundity, appeared normal in every way.

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C. R. Soc. Biol., 62; 914-917.
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C. R. Soc. Biol., 70; 702.

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Symbiose chez les larves xylophages. Etude des microorganism symbiotiques.
C. R. Soc. Biol., 70; 857.

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Developpment complet des larves de Tenebrio molitor, obtenu au mayen d'une nourriture sterilisee a'haute temperature (130 degrees).
C. R. Soc. Biol., 82; 59-60.
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A chemotropic response of the house fly, (Musca domestica L.).
Science, n. s., 43; 613-616.
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The attraction of Diptera to ammonia.
Ann. Ent. Soc., 9; 408-413.
Various flies frequenting the household attracted to ammonia and caught in traps.

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Jour. Econ. Ent., 10; 102-109.
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Etudes sur le sommeil d'hiver pre-imaginal des Muscidae.
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The attraction consists of a complex mixture of gaseous emanations produced at a certain stage of decomposition.

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Das Tonnchen der Musciden.
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Bacteriophage active against 4 species of bacteria was found in a salt solution extract of house flies.

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Uber den Polymorphismus bei den Larven von Miastor metraloas.
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On the influence of the metamorphosis of Musca domestica upon bacteria administered in the larval stage.
Jour. Hygiene, 12; 516-526.

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A general summary of the known larval food-habits of the Acalyptrate Muscidae.
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Excellent bibliography.

VATERNAHM, T. 1924
Zur Ernahrung und Verdauung unserer einheimischen Geotrupesarten.
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Dung bacteria play no important part in the nutrition of Geotrupes. Sterilized food has no harmful effects.

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(Physiology of nutrition of the house fly). (In Russian).
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Weitere Beobachtungen an Calliphora. IV. Ueber chemische Momente bei der Metamorphose (und Entwicklung).
Zeitschr. Biol., 49; 486-498.
Larvae of Calliphora contain a trypsin-like ferment which enables them to digest meat without the aid of bacteria.

WEINLAND, E. 1908
Uber die Bildung von Fett aus eiweissartiger Substanz in Brei der Calliphoralarven.
Zietschr. Biol., 51; 197-278.
Bacteria do not take part in the building up of fats from proteins by the larvae.

WERNER, E. 1926
Die Ernahrung der Larve von Potosia cuprea, Farb. Ein Beitrag zum Problem der Cellulosenverdauung bei Insektenlarven.
Zeitschr. Morph. Oekol. Tiere, 6; 150-206.
Larvae found in nests of ants where they feed on rotting vegetable matter. Digestive tract has a rich microflora and the bacteria are able to cause fermentation of cellulose.

WOLLMAN, E. 1911
Sur l'elevage des mouches steriles. Contribution a la connaissance du role des microbes dans les voies digestives.
Ann. Inst. Pasteur, 25; 79-88.
Larvae of Calliphora vomitoria can be raised in the absence of bacteria. Development is slower at first but the larvae attain normal size.

WOLLMAN, E. 1919
Elevage aseptique de larves de la mouche a viande (Calliphora vomitoria), sur milieu sterilise a haute temperature.
C. R. Soc. Biol., 82; 593-594.
Larvae bred on brains sterilized at 130 degrees developed much faster than if bred on meat sterilized at 115 degrees.

WOLLMAN, E. 1919 a
Larves de mouches (Calliphora vomitoria) et vitamines.
C. R. Soc. Biol., 82; 1208-1210.
Larvae are able to concentrate vitamins when feeding on a diet very poor in these accessory food factors.

WOLLMAN, E. 1921
Le role des mouches dans le transport de germes pathogenes etudie par la technique des elevages aseptiques.
C. R. hebdom. Acad. Sci., Paris, 172; 289-301.
Calliphora and Musca may be infected with typhoid, tubercle, or dysenteric bacilli in the larval stage and remain infective during the pupal stage. Pathogenic organisms are not passed to the adult - may become infected by organisms adhering to the outside of pupa, however.

WOLLMAN, E. 1921 a
La methode des elevages aseptiques en physiologie.
Arch. Internat. Physiol., 18; 194-199.
It is possible to breed sterile cultures of flesh flies indefinitely. Aseptically raised larvae liquefy gelatin which proves that they emitt proteolytic ferments. The larvae of Galleria melonella were also raised aseptiaclly on wax.

WOLLMAN, E. 1921 b
Sur le role des microorganismes dans le production des vitamines.
C. R. Soc. Biol., 85; 801-803.

WOLLMAN, E. 1922
Biologie de la mouche domestique et des larves de mouche a viande en elevages aseptiques.
Ann. Inst. Pasteur, 36; 784-788.
Sterile Calliphora and Lucilia larvae are able to liquefy gelatin. Nature of mouthparts allows the absorption of liquid food only. Sterilization may be done by heat which would destroy vitamins. This fact suggest that they are not necessary to the development of the larvae.

WOLLMAN, E. 1926
Observation sur une ligne aseptique de Blattes (Blattella germanica) datant de cinq ans.
C. R. Soc. Biol., 95; 164-165.
Cockroaches bred continuously for 5 years under sterile conditions on sterile food. Development was normal. This proves that they are able to dispense with vitamins.