NUTRITION AND METABOLISM STUDIES OF ARTHROPODS

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ABSTRACT

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By

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A preliminary study to define a laboratory diet on which to rear the larvae of Rhyacionia buoliana (Schiff.) was made. The rearing vials were very important and gaseous exchange was essential for proper rearing and moisture levels. It was found that wheat germ appeared to contain essential components or balance of nutrients necessary for rearing this insect. Ascorbic acid also appeared to be an important component either for its nutritional value or as an antioxidant protecting other labile dietary components. It also appeared, because of the feeding habits of this insect and the length of its life cycle, that the larvae may have to be periodically transferred to new diet formulations to insure the presence of changing essential nutrient(s). Aseptic rearing of the larvae seemed to be feasible. It was found that eggs, surface sterilized in 0.1% hypochlorite solution for 10-15 minutes, could be aseptically introduced

into containers without appreciable decrease in egg hatch; however, sustained asepsis was difficult to maintain in the rearing vials employed.

Female tarantulas, Aphonepelma sp., and female scorpions, Centruroides sculpturatus, were injected with acetate-1-^{14}C. On analysis, both species expired over 30 percent as $^{14}CO_2$. The tarantulas and scorpions had 6.3 and 3.6 times, respectively, the radioactivity in the fatty acids as in the unsaponifiable lipids, while they had 7.6 and 5.4 5imes, respectively, the weight in the fatty acids as in the unsaponifiable lipids. Oleic acid was the predominant unsaturated fatty acid in both species with 65.9 percent in the scorpions and 42.3% in the tarantulas. Palmitic acid was the predominant saturated fatty acid in both scorpions (13.3%) and tarantulas (19.8%). The tarantulas had nearly twice as much linoleic acid (22.7%) as the scorpions (12.1%), and the tarantulas had trace amounts of arachidonic acid. Cholesterol made up greater than 99% of the sterols in both species, but trace amounts of β-sitosterol and 7-dehydrocholesterol were also present.

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INTRODUCTION

In order to more fully understand the European pine shoot moth, Rhyacionia buoliana (Schiff.), physiologically, and for possible future host plant resistance studies, it was necessary to develop a defined diet on which to rear the larvae. Chawla and Harwood (1968) used a modification of Berger's (1963) diet which showed promise for further studies. A satisfactory mating technique has been developed by Daterman (1968, 1969). This paper concerns studies to define the synthetic diet both aseptically and nonaseptically to determine the nutritional requirements of the larvae.

MATERIALS AND METHODS

Diapausing larvae were collected from two Scotch pine plantations in the Cadillac-Traverse City area of Michigan. The infested buds were collected during the winter months and stored in a dark room maintained at 4.5°C until ready to use. The larvae were allowed to emerge from the buds by placing the buds in screened cages at 29-33°C for 4-8 days.

The eggs used to provide the first instar larvae for the aseptic experiments were obtained by catching single and mating females in the plantations during the first week of July, 1969. They were caught and held in cylindrical one pint containers lined with Whatman No. 1 filter paper. The females were allowed to lay eggs on the filter paper which was then removed and kept moist in closed Petri dishes until the eggs were ready to hatch.

The diets studied were based on Chawla and Harwood's (1968) modified Berger's diet. Table 1 lists the components of the diets examined. The vitamin mixture used was reported by Vanderzant and Reiser (1956) with the addition of choline (10 mg/ml) and inositol (5 mg/ml). This mixture was added to the diets as 1 ml/100 g solids except for diets 17 and 18 where it was added at 3 times that concentration.

Conversion of casein to its sodium salt was accomplished by wetting 1 kg of casein with 1 l of 1% aqueous sodium hydroxide. The casein-hydroxide mixture was spread evenly in pans and the water was evaporated with the aid of a fan. The dried sodium caseinate chunks were then cut into small pieces and pulverized by passing them through a 0.008 screen of a hammer mill (Monroe & Lamb, 1968).

The basic components of the diets were ball milled for 3-5 hours and stored dry. The cholesterol, ergosterol,

TABLE 1.--Composition of the diets used in rearing European pine shoot moth larvae.

Component[1]	1	2	3	4	5[2]	6	7	8	9	10	11	12	13	14[2]	15[2]	Aseptic diets 16	17	18
Casein[3]	28	--	--	28	28	28	28	--	--	--	--	28	28	--	--	28	--	--
Na caseinate[3]	--	28	28	--	--	--	--	28	28	28	28	--	--	28	28	--	28	28
Wheat germ[4]	24	--	--	--	--	--	--	--	--	--	--	--	--	--	--	24	--	--
Wesson salts[4]	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Sucrose[5]	21	21	21	--	--	--	--	--	--	--	--	--	--	21	21	21	21	21
Dextrose[6]	--	--	--	21	21	21	21	21	21	21	21	21	21	--	--	--	--	--
Alphacel[4]	12	44	44	44	44	44	44	44	44	44	44	44	44	44	44	12	44	44
Ascorbic acid[7]	2.8	2.8	2.8	2.8	2.8	--	--	2.8	2.8	2.8	2.8	--	--	2.8	2.8	2.8	2.8	2.8
22.5% KOH	4	--	--	4	4	4	4	--	--	--	--	4	4	--	--	4	--	--
15% methyl-p-hydroxybenzoate in alcohol	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	--	--	--
36.7% formaldehyde	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	--	--	--
Sorbic acid	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	--	--	--
Agar[4]	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
Cholesterol[8]	--	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	0.117	--	0.117	0.117
Linseed oil	--	--	0.117	0.117	0.117	0.117	--	--	0.117	--	0.117	0.117	0.117	--	0.117	--	--	0.117
Ergosterol[9]	--	--	--	--	--	--	--	--	0.117	--	0.117	--	--	--	0.117	--	--	0.117
α -tocopherol[4]											--	--	2.8	--	--	--	--	--
Vitamin fortification mixture[4]	8	--	--	--	--	--	--	--	--	8	8	--	--	--	--	8	--	--
Vitamin mixture	--	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	--	--	1.2	1.2	1.2	1.2	--	3.6	3.6
Water	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600

1. Dry ingredients in g; wet ingredients in ml.
2. Carbohydrate added after autoclaving.
3. Fisons Pharmaceuticals Limited, Loughborough, England.
4. Nutritional Biochemicals Corp., Cleveland, Ohio.
5. Cane sugar obtained locally.
6. Mallinckrodt Chemical Works, New York, N.Y.
7. Calbiochem, Los Angeles, Calif.
8. Fisher Scientific Co., Fairlawn, N. J.
9. Eastman Organic Chemicals, Rochester, N. Y.

(α-tocopherol, and linseed oil were added to the diets just prior to use as a dichloromethane solution and the solvent was allowed to evaporate. The vitamins, water, and potassium hydroxide were then added. The diets were autoclaved at 10 lbs. pressure for 10 minutes to insure mixing of the components.

The diets were cut into cubes and put into liquid scintillation vials intact with screw cap lids. A 5-7 mm hole was cut in the cap and the inside lined with a circle of 90 mesh brass strainer cloth. After the excess water had been allowed to evaporate for 1-2 days, 2 larvae were introduced into each vial.

For the aseptic experiments, tests were run to determine the effect of various concentrations of hypochlorite on the surface sterilization and hatching of the eggs. The filter paper and eggs were emmersed in the hypochlorite solutions for various time intervals after which some were inoculated into fluid thioglycolate medium to test for asepsis and some were put into Petri dishes and kept moist to determine percent hatch.

The aseptic vials and their controls were prepared by autoclaving the diets in the rearing vials. In addition to the strainer cloth a Whatman No. 1 filter paper lining was placed in the cap above the strainer cloth to filter out microorganisms. The vials were allowed to stand for 2 days before inoculation. From 2-5 eggs within

24 hours of hatching were treated with 0.1% hypochlorite solution for 10-15 minutes and aseptically introduced into each of the vials. Four larvae per vial were added to the nonaseptic control vials.

RESULTS

In preliminary experiments it was established that the larvae fed better on diced rather than poured medium and that the moisture in the vials was very important. Many larvae drowned even after the diets were allowed to stand for 1-2 days. Table 2 summarizes the results using post-diapause larvae on the various diets. In five tests with modified Berger's medium (diet 1), 7.4% pupation was achieved with 9 adults emerging. None of the other diets gave any pupation in tests totaling 40-60 larvae.

Diets 6, 7, and 12, in which ascorbic acid was omitted, turned a much lighter color than the others; but it was found that the substitution of α-tocopherol in place of ascorbic acid in diet 13 also resulted in a lighter colored diet.

Diets 14 and 15 and control diet 1 were chosen for the aseptic experiments with first instar larvae because the larvae remained alive longer and developed more rapidly on these than on the other diets studied. Table 3

TABLE 2.--Growth of post diapausing European pine shoot moth larvae on different diet formulations at 25°C.

Diet no.	No. trials	No. larvae	Percent feeding	Percent pupation	Percent adults emerging	No. adults emerged	
						Male	Female
1	110	231	31.6	7.4	3.9	7	2
2	40	80	43.8	0.0		-	-
3	40	80	47.5	0.0	--	-	-
4	40	80	7.5	0.0	--	-	-
5	40	80	18.8	0.0	--	-	-
6	40	80	2.5	0.0	--	-	-
7	40	80	1.2	0.0	--	-	-
8	30	60	13.3	0.0	--	-	-
9	30	60	10.0	0.0	--	-	-
10	30	60	25.0	1.7	0.0	-	-
11	30	60	26.7	3.3	0.0	-	-
12	30	60	3.3	0.0	--	-	-
13	30	60	8.3	0.0	--	-	-
14	30	60	21.7	0.0	--	-	-
15	30	60	40.0	0.0	--	-	-

[1]Percent based on number of larvae feeding after 4 weeks.

TABLE 3.--Growth of first instar European pine shoot moth larvae on different diet formulations at 25°C.[1]

Diet no.	No. trials	No. larvae or eggs	Percent feeding at 8 days	Percent feeding at 21 days	Percent feeding at 45 days
1[2]	95	380	56.3	42.4	28.7
14[2]	66	264	58.7	18.6	1.5
15[2]	67	268	50.0	17.9	0.7
16[3,4]	92	230	7.8	6.5	4.3
17[3,5]	60	142	4.9	0.7	0.0
18[3,6]	46	141	4.3	0.0	0.0

1. Experiment terminated after 45 days.

2. Larvae introduced onto diets.

3. Eggs aseptically introduced onto diets.

4. 46.7% contaminated after 8 days.

5. 83.8% contaminated after 8 days.

6. 84.8% contaminated after 8 days.

summarizes the results of these experiments. Due to the lid design mites infested the vials and these experiments had to be terminated after 45 days--none of the larvae could be reared to pupation. The aseptic diets, 16, 17, and 18, were contaminated in less than a week, thus the percent larvae feeding after 8 days is low for these diets. Diets 17 and 18 were 83.3% and 84.8% contaminated after 8 days with control diet 16 46.7% contaminated. This indicated that wheat germ retards contamination.

Control diet 1 supported the first instar larvae with 28.7% still feeding after 45 days. Diets 14 and 15 were as good through 8 days, but the larvae died sooner; so after 45 days, there were 1.5% and 017%, respectively, still feeding.

Table 4 shows the results of the surface sterility test for the eggs and the percent hatch using 11-30 eggs for each time interval. It was found that a 0.1% hypochlorite solution for 10-30 minutes would surface sterilize the eggs without reducing the percent hatch.

DISCUSSION

In preliminary experiments using a basic wheat germ diet, the percent pupation was far lower than reported for the same diet by Chawla and Harwood (1968). The difference could be that they calculated percent pupation on

TABLE 4.--Effect of sodium hypochlorite on egg hatch and surface sterility in fluid thioglycolate medium.

	Percent contaminated[1]					Percent hatch[2]				
Percent hypochlorite	5 min.	10 min.	15 min.	20 min.	25 min.	5 min.	10 min.	15 min.	20 min.	25 min.
Distilled HOH control	--	--	--	--	--	81	71	92	85	70
0.01	100	100	100	20	40	94	88	83	68	100
0.05	100	80	50	0	0	86	94	96	96	100
0.10	40	0	0	0	0	82	96	82	82	87

[1]Mean of 5 replications.

[2]Eleven to 30 eggs per test.

the number of larvae noted at the first observation (after 15-25 days), while in this study it was calculated from the number of inoculated larvae. It was felt, because the larvae were allowed to crawl from the buds on their own and by handling them carefully with a brush, that all larvae inoculated were healthy. It was found, however, that many larvae never started feeding and died within a couple of weeks. The reason for this could possibly be attributed to some physical or chemical property of the diet rather that mechanical injury. House (1961) indicated that unnatural food and feeding conditions may not be conducive to optimum feeding. Heron (1965) working with spruce budworm larvae found that phagostimulants and at least one feeding deterrent affected their feeding. Although Chawla and Harwood (1968) found that pine tissue was not a requisite for satisfactory growth, it was possible that there were phagostimulants present that would initiate feeding more rapidly.

Of the pupae that were obtained in these diets few adults were able to emerge. When 42 male and 35 female pupae were taken out of buds collected in the field, brought into the laboratory, and placed in the growth chamber with the others, only 12% and 23% adults, respectively, were able to emerge. It was observed that most of them attempted to break the pupal case but were not able to complete eclosion. Pointing (1961) noted that the pupae moved

through the pupal chamber to the exit hole and protruded through the hole before emergence. Green (1956) also noted that emergence dropped sharply at temperatures exceeding 25°C. Therefore, in addition to having a nutritional deficiency in the laboratory diets, there may be some physical aspect of movement through a tunnel that is conducive to adult emergence. It was also possible that a constant temperature of 25°C had some effect on the pupa so the initial mid-dorsal break in the pupal case was harder to make.

Of the diets tested the modification of Berger's wheat germ diet (Chawla and Harwood, 1968) appeared to give the best results. The larvae also seemed to do quite well on the diets in which the carbohydrate was added after autoclaving. It appeared, however, that there was an essential unidentified component in the wheat germ or a critical balance of nutrients that must be present for larval growth and development.

Vanderzant et al.(1962) working with three cotton insects noted that the diets deteriorated gradually, especially with the loss of ascorbic acid by oxidation. This resulted in either death of the prepupae or incomplete emergence of the moths. In the tests conducted with the European pine shoot moth it was found that by omitting ascorbic acid the diets became very light colored in a period of a week. When α-tocopherol was added in place

of ascorbic acid the same light color was evident. If ascorbic acid was an antioxidant for some essential labile dietary component, it would be expected that α-tocopherol might also act the same way; however, the natural color was not restored when it was added. These experiments were inconclusive as to whether ascorbic acid was an essential nutritive component or a protecting additive to the diet or a combination of both. It may be necessary to transfer larvae to new diets periodically to insure that necessary dietary components have not deteriorated.

Pointing (1963) reported that newly hatched larvae feed on needles before migrating to the buds where they feed during the late summer and fall. After overwintering in the buds he noted that they began their spring feeding in close correlation with shoot elongation. Because of these different feeding stages there may be a change in the dietary requirements with larval development. If this is so, it may be necessary to use several diets to insure the presence of necessary nutrient balances and components during the various stages of development.

The aseptic rearing of these larvae appeared to be feasible. The problem in the contamination of these preliminary studies was shown to be in lid design. Because the mites were not original contaminants and were able to infest the vials, microorganisms could also enter. Care should also be taken to introduce as little hypochlorite

solution as possible with inoculation because the first instar larvae would drown. Experiments showed that the eggs could be surface sterilized with 0.1% hypochlorite for 10-30 minutes without an appreciable decrease in egg hatch. The development of the larvae within the egg was easy to follow and eggs ready to hatch within 24 hours could readily be detected.

Nutritional studies of these phytophagus insects were impeded because it was difficult to collect necessary quantities of infested buds, and the eggs could only be collected in the field for about two weeks while the adults were flying. The length of the life cycle and the difficulties in laboratory mating of the European pine shoot moth also made it a difficult insect to rear on artificial media.

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PART II. UTILIZATION OF ACETATE-1-^{14}C BY THE TARANTULA, *APHONEPELMA* SP., AND THE SCORPION, *CENTRUROIDES SCULPTURATUS*, IN LIPID SYNTHESIS

INTRODUCTION

Information on lipid metabolism in several insect species has been accumulated in recent years (Fast, 1964; Gilby, 1965). In addition Zandee (1967) has studied cholesterol and fatty acid synthesis of crustacea, and he concluded that the absence of cholesterol biosynthesis may be characteristic of all arthropods. Zandee (1966b) working with the crayfish, Astacus astacus, Lamb and Monroe (1968) with the cereal leaf beetle, Oulema melanopus, Robbins et al. (1960) with the house fly, and Kodicek and Levinson (1960) with blowfly larvae found that these species utilized at least twice the amount of acetate in the fatty acids as in the unsaponifiable lipids. Because many similarities as well as differences have been found in the lipid metabolism of the arthropods studied, it was felt that diverse groups of arthropods should be examined. Tarantulas and scorpions represent two groups of arthropods in which little is known of their metabolism. These studies were designed to examine the incorporation of acetate into tarantula and scorpion lipids and to determine possible similarities and differences from other arthropod groups that have been studied.

MATERIALS AND METHODS

Experimental Animals. The animals used in these studies were female tarantulas, Aphonepelma sp., (obtained from the Southern Biological Supply Co., McKenzie, Tennessee) and female scorpions, Centruroides sculpturatus, (field collected by Lorin Honetschlager, Curator, University of Arizona, Tempe). Prior to testing all of the animals were kept at 25-27°C and fed Tenebrio molitor larvae.

Radiolabeled Acetate and Injection Techniques. The sodium acetate-1-^{14}C (200 µCi, obtained from Amersham/Searle Corp., Des Plaines, Illinois) was diluted to 10 ml with 0.134 M phosphate buffer (adjusted to pH 7.47). Radioassays performed during these studies were made with 15 ml of modified Bray's solution[1] per vial and were counted in a Nuclear Chicago Unilux I (model 6850) liquid scintillation spectrometer. After dilution the acetate-1-^{14}C solution had a specific activity of 1,595,892 dpm/µg.

The tarantulas, tested in 3 groups of 3 animals, were each injected with 10.8 µl acetate-1-^{14}C through the costal membrane between the third and fourth pairs of legs. The scorpions, tested in 3 groups of 10 animals each, were injected with 0.4 µl acetate-1-^{14}C through the dorsolateral intersegmental membrane of one of the posterior abdominal segments.

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[1] 1000 ml ethylene glycol monomethyl ether, 2000 ml toluene, 12 g PPO, and 150 mg POPOP.

$^{14}CO_2$ Analysis, Tissue Extraction, and Combustion

Analysis. The test animals were placed into a respiration train (Fig. 1) similar to that reported by Hopkins and Lofgren (1968). The $^{14}CO_2$ was collected for a 24 hour period in monoethanolamine and radioassayed. The groups of animals were then weighed live and frozen at -30°C until further analysis.

The animals were homogenized in water, refluxed for 90 minutes in acetone:ethanol (1:1) at 4 times the aqueous volume, and vacuum filtered (Kaplanis et al., 1960). The residue was dried, weighed, and stored for combustion analysis. The solvent pair was removed in vacuo, and the resulting aqueous transferred to a separatory funnel, acidified, and quantitatively extracted with ethyl ether to obtain the total lipids. The ether was dried over anhydrous sodium sulfate and removed in vacuo. The total lipids were analyzed gravimetrically and radiometrically and the aqueous fraction radioassayed.

The residue was analyzed by placing 100 mg samples into bags prepared from 1 inch dialysis casing, and combusting them to $^{14}CO_2$ in a combustion flask previously reported by Hopkins and Lofgren (1968). The $^{14}CO_2$ was trapped in 10 ml monoethanolamine:methyl cellosolve (1:2) and radioassayed.

Saponification and Column Chromatography. The total lipids were saponified under nitrogen in a glass-stoppered

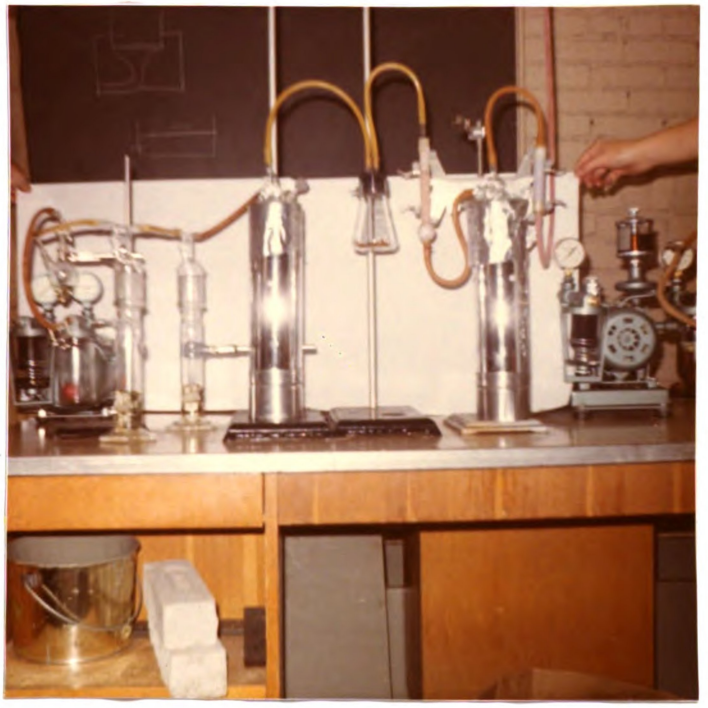

Figure 1.--The respiration train used to analyze the $^{14}CO_2$ production of tarantulas and scorpions injected with acetate-1-^{14}C.

tube with 10% potassium hydroxide in 95% ethanol for 90 minutes. The unsaponifiable lipids were extracted with ethyl ether, the aqueous acidified and the saponifiable lipids (fatty acids) extracted with ethyl ether. Both fractions were washed, dried over anhydrous sodium sulfate, and concentrated in vacuo. They were weighed and radioassayed.

The unsaponifiable lipids were fractionated by dual column chromatography on 1.1 X 7.5 cm columns each containing 7.5 g Woelm neutral aluminum oxide, grade 1, deactivated with 1.5% water (Kaplanis et al., 1960, as modified by Monroe et al., 1968). Each fraction was then weighed and radioassayed.

Sterol Purification and Analysis. The ether fraction from the aluminum oxide column was purified by 2 successive digitonin precipitations (Louloudes et al., 1961), and the purified sterols weighed and analyzed by a gas-liquid chromatograph equipped with a hydrogen flame detector (Research Specialities Corp., Series 600). Two stainless steel columns 3 ft X 4 mm ID were packed with 100-120 mesh Gas Chrom Q (Applied Science Laboratories, State College, Pennsylvania) coated with one of the following liquid phases: 1.0% QF-1 or 0.75% neopentyl glycol succinate. Detailed column conditions are presented under results. Ultraviolet absorption spectroscopy was also used to aid in the identification of 7-dehydrocholesterol.

Fatty Acid Analysis. The saponifiable lipids (fatty acids) were methylated with borontrichloride-methanol (Applied Science Laboratories, State College, Pennsylvania) in a test tube in a boiling water bath for 2 minutes (Metcalf and Schmitz, 1961). The fatty acid methyl esters were analyzed by gas-liquid chromatography employing a 3 ft X 4 mm ID stainless stell column packed with 100-120 mesh Gas Chrom Q coated with 12% HI-EFF 1B. Detailed column conditions are presented under results. The standards used were K 108 and N.I.H. Mix E fatty acid methyl esters, and mass spectroscopy was used to confirm the identities of the peaks when they deviated from the standards.

RESULTS

Table 5 summarizes the treatment data for the test animals. It presents the number of animals per test, the live weights, µg of acetate-1-^{14}C injected, and total dpm injected. Although the tarantulas out-weighed the scorpions considerably, an attempt was made to adjust the injected acetate-1-^{14}C per unit of body weight so that approximately the same amounts were injected in both species.

Table 6 presents the amounts of radioactivity recovered in the $^{14}CO_2$ residue, total lipids, and aqueous portions. After 24 hours 31.2% and 34.1% of the radioactivity were recovered as $^{14}CO_2$ from the tarantulas and scorpions, and

TABLE 5.--Summary of the number, live weights, and treatment data of tarantulas and scorpions injected with acetate-1-^{14}C.

Arthropod	No. in test	Live wt. (g)		µg of acetate-1-^{14}C injected		dpm of acetate-1-^{14}C injected	
		Total	Per arthropod	Total	Per g body wt.	Total	Per g body wt.
Tarantula	9	128.0059	14.2229	2.9781	0.0233	4,752,396	37,126
Scorpion	20	13.2927	0.6646	0.2460	0.0185	391,140	29,425

TABLE 6.--Recovery of radioactivity in the $^{14}CO_2$, residue, lipids, and aqueous fractions of tarantulas and scorpions injected with acetate-1-^{14}C.

	$^{14}CO_2$ after 24 hr.		Residue			Total lipids			Aqueous	
Arthropod	% of total dpm	µg-equivalents acetate-1-^{14}C	Total wt. (g)	% of total dpm	µg-equivalents acetate-1-^{14}C	Total wt. (g)	% of total dpm	µg-equivalents acetate-1-^{14}C	% of total dpm	µg-equivalents acetate-1-^{14}C
Tarantula	31.2	0.93	25.4353	13.9	0.41	2.5796	1.3	0.04	8.8	0.26
Scorpion	34.1	0.08	3.0353	9.1	0.02	0.7753	1.7	0.004	9.6	0.02

the residue, when combusted to CO_2 and water, gave 13.9% and 9.1% recovery, respectively. In both animals about 9% of the radioactivity was recovered in the aqueous fraction of the direct extraction while under 2% was recovered in the total lipid fraction. The total lipids comprised 2.0% of the tarantula live weight and 5.8% of the scorpion live weight.

After saponification of the total lipids, Table 7, it was found that the tarantulas had 7.6 times more fatty acids than unsaponifiable lipids while the scorpions had 5.4 times more fatty acids. The tarantulas and scorpions had 6.3 and 3.6 times, respectively, more radioactivity in the fatty acids than in the unsaponifiable lipids.

Table 8 shows that the tarantulas had more radioactivity, 42.1%, in the methanol fraction of the unsaponifiable lipids while most of the weight, 50.2%, was in the ether fraction (free sterols). The n-hexane fraction (hydrocarbons) had 20.5% of the weight but only 9.1% of the radioactivity. The scorpions had most of their radioactivity, 38.9%, in the benzene fraction with 23.2% in each of the ether and methanol fractions. The n-hexane fraction had 14.6% of the radioactivity. The ether fraction contained the most weight, 34.0%, while the n-hexane fraction had 30.8% of the weight.

TABLE 7.--Weights of the total lipids and the relative percents of the saponifiable and unsaponifiable lipids of tarantulas and scorpions injected with acetate-1-^{14}C.

Arthropod	Wt. total lipid (g)	Saponifiable			Unsaponifiable		
		Wt. (g)	% of total		Wt. (g)	% of total	
			Lipid	Radio-activity		Lipid	Radio-activity
Tarantula	2.1213	1.8739	88.3	86.3	0.2474	11.7	13.7
Scorpion	0.6909	0.5834	84.4	78.1	0.1075	15.6	21.9

TABLE 8.--Column chromatographic fractionation of the unsaponifiable lipids of tarantulas and scorpions injected with acetate-1-^{14}C.

	Relative % of unsaponifiable lipids							
	n-hexane (hydrocarbons)		Benzene		Ethyl ether (free sterols)		Methanol	
Arthropod	Wt.	Radio-activity	Wt.	Radio-activity	Wt.	Radio-activity	Wt.	Radio-activity
Tarantula	46.4	9.1	16.7	17.5	50.2	31.2	12.5	42.1
Scorpion	30.8	14.6	20.7	38.9	34.0	23.2	14.6	23.2

After analysis of the fatty acids, Table 9, it was found that arachidonic acid was present in trace amounts in the tarantulas, but was absent in the scorpion fatty acids. The C-18 acids were predominant in both species, although both had only traces of linolenic acid. Oleic acid was most abundant in both species with 65.9% and 42.3%, respectively, in the scorpions and tarantulas. The tarantulas had relatively more linoleic acid, 22.7%, as compared to 12.1% in the scorpions. Palmitic and palmitoleic acids were present in both species with 19.8% and 4.0%, respectively, in the tarantulas and 13.3% and a trace in the scorpions. Myristic acid was found in trace amounts in both species. Mass spectroscopy indicated that both of the species had trace amounts of two unidentified fatty acid methyl esters (MW of both was 284) which represented two C-17 branched or straight chain fatty acids. In addition two trace peaks with apparent molecular weights of 318 and 322 were present in the scorpion fatty acid methyl ester fraction.

Table 10 presents the gas-liquid chromatographic analysis data of the sterols of the two species. Cholesterol was the predominant sterol with greater than 99% in both species. The NGS column indicated that small amounts of β-sitosterol and/or 7-dehydrocholesterol were present. The β-sitosterol was finally identified in trace amounts on the QF-1 column and 7-dehydrocholesterol was determined

TABLE 9.--Gas-liquid chromatographic analysis and relative % of the methyl esters of fatty acids of tarantulas and scorpions injected with acetate-1-^{14}C.[1]

Carbon no.[3]	Relative %[2]	
	Tarantula	Scorpion
C-14:0	trace	trace
C-16:0	19.8	13.3
C-16:1	4.0	trace
C-18:0	11.2	8.7
C-18:1	42.3	65.9
C-18:]	22.7	12.1
C-18:3	trace	trace
C-20:8	trace	--
Unknowns	traces[4]	traces[5]

[1]Column: 12% HI-EFF 1B on 100-120 mesh Gas-Chrom Q, nitrogen 49.6 ml/min., column 180°C (stainless steel 3 ft X 4 mm ID), hydrogen flame detector.

[2]Computed by disc integration.

[3]The fatty acids were identified by comparison of the retention times with K 108 and N. I. H. Mix E standards of fatty acid methyl esters. Where the retentions times were significantly different, mass spectroscopy was used to confirm the identity.

[4]Mass spectroscopy indicated the presence of traces of two C-17 straight chain or branched fatty acid methyl esters (MW of both was 284).

[5]Mass spectroscopy indicated the presence of traces of two C-17 straight chain or branched fatty acid methyl esters (MW of both was 284) and two other peaks with apparent molecular weights of 318 and 322.

TABLE 10.--Gas-liquid chromatographic analysis and relative % of the free sterols of tarantulas and scorpions injected with acetate-1-^{14}C.

Compound of test animal	Retention times relative to cholestane				Relative %
	NGS[1]		QF-1[2]		
	Standard	Arthropod	Standard	Arthropod	
Tarantula:					
Cholesterol	5.5	5.6	3.0	3.0	99+
β-sitosterol			4.8	5.0	trace
7-dehydrocholesterol[3]					trace
Scorpion:					
Cholesterol	5.5	5.6	3.0	3.0	99+
β-sitosterol			4.8	5.0	trace
7-dehydrocholesterol[3]					trace

[1]Column: 0.75% NGS on 100-120 mesh Gas-Chrom Q, nitrogen 48 m./min., column 230°C (stainless steel 3 ft X 4 mm ID), hydrogen flame detector.

[2]Column: 1.0% QF-1 on 100-120 mesh Gas-Chrom Q, nitrogen 54.5 ml/min., column 208°C (stainless steel 3 ft X 4 mm ID), hydrogen flame detector.

[3]Minute quantities later determined by ultra-violet spectroscopy.

by ultra violet spectroscopy even though it was not present in amounts great enough to compute a finite retention time.

DISCUSSION

After injection of the acetate-1-^{14}C into the tarantulas and scorpions some of the compound should be eliminated as $^{14}CO_2$. These experiments showed that indeed greater than 30% of the radioactivity was expired as $^{14}CO_2$, and some preliminary tests of the respiration train used in these studies showed that 50% or more may be expired. Zandee (1966a) recovered 25% of the radioactivity from crayfish and Lambremont and Stein (1965) were able to recover 50.7% $^{14}CO_2$ from the boll weevil after treatment with acetate-^{14}C.

These experiments showed that both species utilized some of the radioactivity in the residue and aqueous fractions. It appeared that the tarantulas utilized slightly more in the residue than did the scorpions while both had about the same amount in the aqueous portion of the direct extract. In both species only 1-2% of the total radioactivity was found in the total lipid fraction.

These tests also showed that both species incorporated more of the radioactivity into the fatty acid fraction than into the unsaponifiable lipids, and is, therefore, similar to results found for insects (Louloudes et al., 1961; Robbins et al., 1960; Lamb and Monroe, 1968), and in other arthropods and vertebrates (Zandee, 1962).

Analysis of the fatty acids demonstrated that the C-18 series was predominant in both species. In the two spotted spider mite (Walling et al., 1968) and the cereal leaf beetle (Lamb and Monroe, 1968) the C-18 fatty acids were also predominant. In several insects and spiders, Barlow (1964) found that there were differences in the fatty acids, depending on the species studied. These studies only showed differences in the relative amounts of the fatty acids except arachidonic acid, which was present in the tarantulas but not in the scorpions. Oleic acid was far more predominant in the scorpions than in the tarantulas while linoleic acid was more predominant in the tarantula fatty acids.

Preliminary examination of the hydrocarbons by gas-liquid chromatography and mass spectroscopy demonstrated that the tarantulas had C-13, 14, 16, 17, 18, 23, 34, 25, and 26 straight chain saturated hydrocarbons. The scorpion had C-13, 14, 15 and 25 saturated and C-27 unsaturated straight chain hydrocarbons. In addition both species had unknown peaks representing other unsaturated straight chain and branched hydrocarbons. Louloudes et al. (1962) found that in house flies the odd numbered alkanes were predominant but that even number chains, alkenes, branched, and cyclic hydrocarbons were also present.

Both the tarantulas and the scorpions were found to have cholesterol as the predominant sterol. Although some

radioactivity was still present in the free sterols after two digitonin precipitations, it was possible that it represented higher molecular weight alcohols which precipitated with the sterols. This radioactivity was so low that it could not be attributed to sterol biosynthesis. Thus, the tarantula and scorpion, like other arthropods studied, cannot synthesize sterols from acetate. β-sitosterol and 7-dehydrocholesterol were found as trace sterols in both species only after huge quantities of the sterol fraction were injected into the gas chromotograph.

Both of these species demonstrated very similar patterns in the metabolism of acetate-1-^{14}C. It was shown that both species metabolized acetate-1-^{14}C into lipids in nearly the same proportions, although some differences did appear in the fatty acids and the various fractions of the unsaponifiable lipids.

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APPENDIX I

LITERATURE REVIEW FOR PART I

The European pine shoot moth, Rhyacionia buoliana (Schiff.) was a pest in Europe for more than a hundred years before it was introduced into America in 1914 (Friend and West, 1933). Since then much work has been done on the biology and behavior of this insect (Green and Pointing, 1962; Green et al., 1957, Pointing, 1961, 1963). The ecology of this insect has also been extensively studied (Green, 1962a, b; Haynes, 1961; Haynes and Butcher, 1961, 1962; Miller, 1967; Torgersen, 1964; Heikkenen, 1960, 1963). Tests have been published on European pine shoot moth control with the use of insecticides (Haynes, 1959; Kulman and Dorsey, 1962) and with the practice of shearing (Rudolph and Lemmien, 1963). Parasites and predators have also been studied by Watson and Arthur (1959), Juillet (1961), and Kulman (1965).

Chawla and Harwood (1968) have reared the larvae of R. buoliana in the laboratory on artificial media. They used modifications of a wheat germ diet (Berger, 1963) and a bean diet (Shorey and Hale, 1965), and had fair results (per their methodology) but were unable to define the diets further. A satisfactory technique for mating the adults in the laboratory has been developed (Daterman, 1968, 1969).

It has been difficult to eliminate wheat germ and yeast from many diets. Charbonneau and Lemonde (1960) showed that there was an essential factor in Brewer's yeast required by Tribolium confusum, and Ephestia kuehniella required substances in the water soluble and water insoluble fractions of yeast (Fraenkel and Blewett, 1943b). Adkisson et al. (1960) indicated that wheat germ contained sterols, fatty acids, tocopherols, protein, carbohydrates, vitamins, and minerals. The European corn borer, Pyrausta nubilalis, required an unidentified factor contained in corn leaves, grass juice concentrate, and other plant materials. The factor was not identical with the known B-vitamins, ascorbic acid, citrovorum factor, sodium nucleate, or carnitine. It was heat-stable, water soluble, and dialyzable (Beck, 1953). House (1965) showed that when insects were feeding on critical imbalances in the diet, they may have had metabolic difficulties resulting in a decreased food consumption and a slower rate of weight increment.

It may be possible that R. buoliana requires some chemotactic stimuli to initiate and maintain optimal feeding behavior; because Heron (1965), working with spruce budworm larvae, showed that foliage and staminate flowers contained chemical substances which were phago-stimulants and at least one feeding deterrent. The phagostimulants in staminate flowers were mainly sugars and L-proline. He

stated that the accumulation of the deterrent glucoside, pungenin, was perhaps the reason for the limited consumption of mature needles.

Leonard and Doane (1966) developed a wheat germ diet for the gypsy moth, Porthetria dispar, that gave larger specimens and more eggs that field collected ones. It was basically the diet reported by Vanderzant et al. (1962) except that the wheat germ content was increased and linolenic acid was added.

Water was a very essential component of insect diets (Fraenkel, 1943), and the amount needed varied tremendously (Trager, 1947). The amount of water required in insect diets depended on the rate at which the water was lost by the body (Wigglesworth, 1965). With housefly larvae too much water was detrimental. Too little water reduced the availability of the medium and resulted in a longer period of larval development and undersized pupae and adults (Brookes and Fraenkel, 1958).

Insects needed minerals and salts probably quite similar to those required by higher vertebrates (Trager, 1947). The osmotic properties of the medium were important to the ability of the insect to utilize the foodstuffs. There was little evidence that pH had much effect and most diets had good buffering capacities (House, 1959). Consistency was very important (House, 1959; Brookes and Fraenkel, 1958) and unnatural food and feeding conditions

may not have been conducive to optimum nutrition (House, 1961). Many diets also required chemical attractants in addition to the nutritive components (House, 1959, 1961).

The utilization of carbohydrates in the diet varied considerably among insects (House, 1961; Trager, 1947). Aedes aegypti larvae did not require a carbohydrate in their diet (Akov, 1962), and Vanderzant and Reiser (1956b) found that the pupation of the pink bollworm was accelerated when sucrose was reduced and/or Wesson salts increased. Brookes and Fraenkel (1958) stated that a few carbohydrates inhibited growth in housefly larvae. However, in spite of specific variations, all insects usually utilized glucose and fructose (House, 1961).

Protein amino acids were vital nitrogen sources in the diet of an insect (Wigglesworth, 1965), and the requirements were very complex (Trager, 1947; House, 1961). Singh and Brown (1957) determined that Aedes aegypti required arginine, isoleucine, leucine, lysine, phenylalanine, methionine, threonine, tryptophane, valine, and histidine in both larval and adult stages. These same ten essential amino acids appeared to be required by most insects (Wigglesworth, 1965). In many cases, however, these had to be supplemented with additional amino acids (House, 1961).

Nucleic acids were not essential in the diets of most insects although their addition could have accelerated growth (Burnet and Sang, 1963). Aedes aegypti larvae

required RNA for optimal growth (Akov, 1962; Singh and Brown, 1957). Brookes and Fraenkel (1958) found that RNA or adenine and guanine increased the growth of housefly larvae, while uracil and cytosine had no effect. Vanderzant and Reiser (1956b) found that the addition of nucleic acid had no effect on the pink bollworm.

All insects studied so far required a sterol in the diet (Fraenkel, 1943; Lipke and Fraenkel, 1956). Noland (1954a) found after studying 41 sterol derivatives with Blattella germanica that the no. 3 hydroxyl group, either free or esterified, was essential. The no. 5 double bond was not required for absorption and utilization. He concluded that the absorption of sterols in insects, as in vertebrates, involved the participation of a cholesterol esterase. After studying the effects of 31 nonutilizable cholesterol derivatives in German cockroaches in which the diets contained minimal optimal levels of cholesterol, Noland (1954b) concluded that there was a competitive inhibition of cholesterol by the other sterols. An excess of cholesterol in the diet of mosquito larvae inhibited pupation (Akov, 1962), and Sang (1956) reported that excess cholesterol had no effect on the mortality of Drosophila melanogaster but did affect the rate of development. In the nutrition of the pink bollworm Vanderzant and Reiser (1956b) found that cholesterol could be replaced by ergosterol, sitosterol, and stigmosterol.

Many insects have also required the addition of fatty acids to the diet. Ephestia kuehniella, E. elutella, E. cautella, and Plodia interpunctella required linoleic acid in the diet for wing development and adult emergence (Fraenkel and Blewett, 1946). Tamaki (1961) working with the smaller tea tortrix, Adoxophyes orana, found that linolenic acid was required for emergence, and that olive oil, stearic, oleic, and linoleic acids had no effect. The salt marsh caterpillar also required linolenic acid in the diet (Vanderzant, 1967). Rock et al. (1965) found that linolenic and linoleic acids gave better growth and adult emergence in Argyrotaenia velutinana. They found that oleic acid had a positive effect on growth but had no effect on adult emergence, while stearic and palmitic acids and methyl arachidonate had no effects on survival. Without linseed oil in the diet they found that larval growth was slow, mortality high, and that the adults were unable to emerge. They found that if the essential fatty acids were supplied as late as the fifth instar that normal adult emergence followed. Gordon (1959) noted that linoleic acid was required for the second generation of Blattella germanica. He found that linolenic and arachidonic acids were not effective, but possibly this was due to rapid deterioration in the diet.

All insects required B-vitamins in their diets, although symbionts may have supplied certain of them

(Fraenkel, 1943). Singh and Brown (1957) found that vitamins were not required for adult nutrition in Aedes aegypti. The larvae, however, would not grow in the absence of the B-vitamins: thiamine, riboflavin, nicotinic acid, pyridoxine, and patothenic acid. Folic acid or choline was required for pupation (Akov, 1962). Akov (1962) reported that riboflavin was the only vitamin that was detrimental in excess. Trager (1947) indicated that the insects tested thus far were all unable to utilize vitamin D. Vitamin B_{12} was not required by the pink bollworm (Ouye and Vanderzant, 1964), but Gordon (1959) stated that it was required for rearing the German cockroach past the first generation. The reason for this could have been that certain nitrients supplied in the egg were ample for growth and development of the progeny (House, 1959), but if not added to their diet, the next generation would be deficient. It was also found that choline and inositol were required in large enough quantities to be considered nutrients (Gordon, 1959). The omission of choline in the purified casein diet for pink bollworm prevented development (Vanderzant and Reiser, 1956b). Vanderzant and Reiser (1956a) presented a vitamin mixture for the pink bollworm. Gordon (1959) also stated that it was possible that α-tocopherol was either required or would improve growth in the German roach. Beck et al. (1949) suggested that it was essential in the diet of Pyrausta nubilalis, but

Fraenkel and Blewett (1946) suggested that α-tocopherol was an antioxidant preservative for the unsaturated fatty acids.

Sang (1962) reported that the vitamin requirements for Drosophila varied considerably on axenic diets with different amounts of protein. He suggested that the requirements depended partially on the kind of nutrients present that were substrates in which the vitamins acted as coenzymes. Fraenkel and Blewett (1943a) found that the B-vitamin requirements of Lasioderma and Sitodrepa varied considerably from aseptic cultures to normally reared ones. The aseptic cultures were free of symbionts, thus suggesting that the symbionts were responsible for supplying accessory food substances. Using aseptic casein medium, Ouye and Vanderzant (1964) found that the pink bollworm required calcium pantothenate, folic acid, nicotinamide, pyridoxine, riboflavin, and thiamine. The vitamin requirements of aseptically reared onion maggots, Hylemya antigua, have been studied by Friend and Patton (1956).

The diets for insects vary immensely and are extremely complex. Sang (1959), for example, found that there were a multiplicity of optimal diets for Drosophila melanogaster by partially substituting components. He also found that there appeared to be differences between various strains of Drosophila. Axenic techniques were also required to standardize test animals in order to separate specific metabolic needs from any host-symbiont relationships (House, 1961).

Another problem encountered while rearing some insects was that the diets gradually deteriorated, especially with the oxidative loss of ascorbic acid. This resulted in either death of the prepupae or incomplete emergence of moths with some cotton insects (Vanderzant et al., 1962). There is still a need in nutritional studies to define diets further and to develop techniques by which large numbers of insect species can be aseptically reared.

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APPENDIX II

LITERATURE REVIEW FOR PART II

Acetate metabolism has been found to be highly complex in arthropods. Because acetate forms a central position among many avenues of anabolism and catabolism, acetate is a useful compound to use for intital general metabolism studies, especially lipid metabolism.

After introducing acetate into a biosystem it would be expected that some of the compound would be eliminated as CO_2. Zandee (1966b) was able to recover 25% of the injected radioactivity as $^{14}CO_2$ when he gave 4 injections over a 4 day period in the crayfish, Astacus astacus. In work with the boll weevil, Lambremont and Stein (1965) were able to recover 25.3% of the radioactivity from acetate-1-^{14}C injection as $^{14}CO_2$ within 3 hours, and 50.7% after 12 hours. They found that the maximum $^{14}CO_2$ output was 1 hour after injection, and by 2 hours it had decreased to about half the rate found at 1 hour.

It has also been found that acetate-1-^{14}C was incorporated into the amino acids of the crayfish (Zandee, 1966a).

Louloudes et al. (1961) reported that acetate-^{14}C was incorporated into the unsaponifiable fraction of the

American cockroach at about the same rate as in the house fly, and most of the radioactivity was found to be in the hydrocarbon fraction. Robbins et al. (1960) found that with house flies, 80% of the unsaponifiable fraction's radioactivity from acetate-1-^{14}C was in the hydrocarbon fraction. Zandee (1962) was able to recover radioactivity in hydrocarbons of the crayfish, and Lamb and Monroe (1968a,b) working with the cereal leaf beetle, Oulema melanopus, found that 1% of the total lipid radioactivity was in the unsaturated hydrocarbons and 13% was in the saturated ones.

After analyzing the hydrocarbons of several adult fly species, Louloudes et al. (1962) found that the odd numbered compounds predominated over the even numbered ones. The alkanes were the major fraction, but alkenes were also present. All of the species had considerable branched chain compounds. The major distribution range was C-23 to C-29, but the relative distribution varied between species.

Beament (1955) found the cuticular "grease" of cockroaches consisted of a hard wax and a "solvent," and that a series of paraffins and alcohols extended into the short, C-8 - C-12, lengths to provide the "solvent." In analyzing the cuticular wax of the mormon cricket, Anabrus simplex, Baker et al. (1960) found that 48-58% of the wax was hydrocarbons of which 27-32% were C-30 and C-31. Free

acids made up 15-18% with the C-18 acids being the major components. Esters made up 9-11%, cholesterol 2-3%, polymers 12-14%, and 2-4% was unidentified.

It has been found that acetate-^{14}C was utilized for fatty acid synthesis in many insects to a much greater degree than for hydrocarbons. Kennedy (1957) stated that acetate was used in the synthesis of fatty acids and phospholipids. This was in agreement with that found for the intact rat in which acetate-1-^{14}C was incorporated into fatty acids (Hutchens et al., 1954). Louloudes et al. (1961) found that acetate-^{14}C incorporation in the American cockroach male was 14-17 times greater in the fatty acid fraction than in the unsaponifiable lipids. The male utilized more acetate for fatty acids than did the female. Kodicek (1960) found 8.8% of the radioactivity from dietary acetate-2-^{14}C in the fatty acids of blowfly larvae as compared to 1.0% in the unsaponifiable fraction. Robbins et al. (1960) found 2.5 times the acetate in the fatty acids of female house flies than in the unsaponifiable fraction, and they found that the females had 3.7-8 times the fatty acid synthesis as did the males. Lamb and Monroe (1968a) studying the cereal leaf beetle and Zandee (1962) studying the crayfish found that there were about 2 times the radioactivity in the fatty acids than in the unsaponifiable fractions after injections of acetate-^{14}C. This is in agreement with results obtained in vertebrates (Zandee,

1962). Lamb and Monroe (1968b) found radioactivity in all the fatty acids of the triglyceride fraction. Of these acids oleic and palmitic acids had the most radioactivity while linolenic acid had very little.

Barlow (1964) found that there were fatty acid differences among the 30 insect and 2 arachnid species studied. He found that aphids had a high concentration of myristic acid while Diptera had more palmitoleic acid and he concluded that species living in colder environments had more highly unsaturated fatty acids. Fast (1966) also found a high concentration of palmitoleic acid in the neutral lipids of most Diptera. Walling et al. (1968) working with the two-spotted spider mite, Tetranychus urticae, found that linolenic acid was the most abundant in both the neutral and phospholipids. They found other major acids to be palmitic, palmitoleic, stearic, oleic, and linoleic; and the C-18 series comprised 84% of the total fatty acids studied. Lamb and Monroe (1968a) found 10 fatty acids in the cereal leaf beetle. The radioactivity was found to be 25% in palmitic acid, 60% in oleic acid, and only 0.7% in linolenic acid even though it comprised 26% of the total fatty acids gravimetrically. Zandee (1966d) found large amounts of palmitic and oleic acids in crayfish, and he reported that palmitic acid had a central place in that animal's metabolism. He also found that the fatty acid composition varied from season to season.

The ability of animals to biosynthesize sterols varies throughout the animal kingdom. Holz et al. (1961) found that it was likely that cholesterol was the principal sterol or that it was the key intermediate to the formation of ciliate sterol in Tetrahymena corlissi Th-X. Squalene, mevalonic acid, and lanosterol could not replace cholesterol in the diet while certain other sterols as cholestanol, 22-dehydrocholesterol, β-sitosterol, and stigmosterol could partially replace the cholesterol; so they concluded that the impairment of the synthesis was between lanosterol and the ciliate sterol. Fagerlund and Idler (1960) found that a mussel and a clam could convert labeled squalene into sterols, and they also found that a clam could incorporate unsaturations at C-22 and C-25 (Fagerlund and Idler, 1961). In a carnivorous mollusc, Buccinum undatum, Voogt (1967b) found that it was unable to synthesize 3β-sterols from acetate. Howes and Whellock (1937) found that the snail, Helix pomatia, seemed to require cholesterol; however Voogt (1967a; 1968a,b) showed that archeogastropod and pulmonate snails can synthesize 3β-sterols from squalene and acetate. Wootton and Wright (1960; 1962) showed that Lumbricus terrestris could convert mevalonic acid to squalene but that there was a genetic block in the pathway of squalene to β-hydroxy and/or 5 αH sterols.

Van den Oord (1964) showed that in the crab, Cancer pagurus, neither acetate-1-^{14}C nor mevalonic acid-2-^{14}C was incorporated into cholesterol. Zandee (1966c; 1967) demonstrated that acetate was not utilized as a cholesterol precursor in Astacus astacus, Homarus gammarus, Avicularia avicularia (arachnid), or Graphidostreptus tumuliporus (myriapod). He suggested that the inability to synthesize cholesterol from acetate seemed to be characteristic of all arthropods.

Clayton (1964) outlined the functions of sterols in insects. He mentioned that intestinal symbionts supplied sterols to many insects, and that in the cockroach a dietary source of sterol was necessary even though there was a high content of symbionts. Pant and Fraenkel (1950; 1954) showed that symbiotic yeasts within two insects species supplied most of the sterols and B-vitamins. Monroe (1959; 1960) and Kaplanis et al. (1960) showed that cholesterol was required exogenously and that it was efficiently used for viable egg production in the house fly.

Clayton et al. (1962) showed that acetate-1-^{14}C was incorporated into cholesterol in a silver fish, Ctenolepisma sp., but suggested that it could have been due to the presence of symbionts. Kaplanis et al.(1963) working with a primitive insect, the firebrat, indicated that the incorporation of acetate-1-^{14}C was so low that the insect

was probably unable to synthesize sterols. Levinson (1960) suggested that phytophagous insects could convert plant sterols to cholesterol while obligatory carnivores could not.

The American cockroach may have been able to cleave the sterol side chain and resynthesize the isooctyl side chain of cholesterol (Casida et al., 1957). Clark and Bloch (1959b) indicated that the roach converted ergosterol to 22-dehydrocholesterol. Because cholesterol was not found in detectable quantities, they suggested that microorganisms supplied the insect with cholesterol. Louloudes et al. (1961) found low levels of radioactivity in the sterol digitonides of the American roach. Since they precipitated the sterols from the total unsaponifiable lipids, they suggested that possibly there were hydrocarbons present that were insoluble in the solvents used for digitonide formation so it resulted in decreased activity after reprecipitation of digitonides.

Bloch et al. (1956) found that the larvae of Dermestis vulpinus incorporated acetate-1-^{14}C into squalene but not into lanosterol or cholesterol; therefore, they concluded that cholesterol biosynthesis was interrupted at the squalene stage. Clark and Block (1959a,c) found that cholesterol could not be replaced by mevalonic acid, squalene, lanosterol, or Δ^8, 4,4-dimethyl cholestenol, which indicated that cholesterol biosynthesis was multiply

blocked. Ishii and Hirano (1961) found that cholesterol was essential in the diet of the rice stem borer, and Happ and Meinwald (1966) working with an ant found that labeled acetate was incorporated into citronellal and citral but not into the digitonide fractions.

Robbins et al. (1960) and Kaplanis et al. (1961) found that there was no sterol synthesis in the house fly and that a sterol was required in the diet. Levinson and Bergmann (1957) stated that a sterol was the only lipid required by Musca vicina for growth and metamorphosis. Agarwal et al. (1961) found "mucasterol" in house flies and that CSMA reared house flies contained at least 4 sterols but lacked cholesterol. Thompson et al.(1962) later identified this "house fly sterol" as campesterol. They found it originated from the CSMA medium and that the house fly showed a selective uptake of sterols that had the closest side chain to cholesterol. Kodicek and Levinson (1960) found that blowfly larvae were unable to synthesize sterols from acetate and that the cholesterol side chain was not formed by the re-synthesis of acetate. Sedee (1961) also found that blowfly larvae were unable to use squalene in place of cholesterol in their diet.

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