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Steroid Utilization and Fatty Acid Synthesis by the Larva of the Housefly *Musca vicina* Macq.

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Recent studies on the lipid requirements (Silverman & Levinson, 1954) of, and on the specificity of steroids as growth factors (Bergmann & Levinson, 1954) for, housefly larvae, have shown that a sterol (phytosterol or zoosterol) is the only lipid essential for growth and metamorphosis. The growing larva consumes the sterols of the wheat bran of the medium together with considerable amounts of unsaturated fatty acids; however, whilst the latter are metabolized during pupation, the sterol content remains relatively constant throughout this period (Levinson & Silverman, 1954). The importance of steroids in insect physiology has been known for some time, but relatively little work appears to have been done on the housefly.

In the present study, therefore, the utilization of different steroids by *Musca vicina* larvae has been investigated. A preliminary account of this work has been given by Levinson & Bergmann (1955).

MATERIALS AND METHODS

Musca vicina Macq. The strain of houseflies was the same as that used in previous investigations; the method of its maintenance has been described by Silverman & Silverman (1953).

Preparation of newly hatched larvae. Adult houseflies, 2 days of age, were fed for 2 days on a water suspension of 20% (w/v) powdered milk and subsequently sucrose and water. Six- to seven-day-old females were allowed to oviposit within 3 hr. on a pad of damp cotton wool. Approximately 750 eggs were collected, carefully washed with water, placed on damp blotting paper in a covered Petri dish (diam. 9 cm.) and incubated at 30°. Usually 80–90% of these eggs were fertile, and larvae hatched from them 11±1.5 hr. after oviposition. Soon after hatching, the larvae congregated inside the cover of the Petri dish.

Since up to the present time no satisfactory method for the sterile culture of houseflies is known, the experiments have been carried out in the presence of a controlled bacterial flora. Although this flora failed to provide utilizable steroids for larvae reared on a lipid-free diet, it is possible that the bacteria cause changes in the steroid structure before its ingestion.

Rearing method. The medium (approximately 1 g.) was placed in a test tube (150 mm. × 15 mm.), and 0.15 ml. of a suspension of stock bacterial flora (Silverman & Silverman, 1953; 1.5–2.5 × 10⁸ bacteria/ml., 70% of which were *Escherichia coli*, the balance *Sarcina*, *Lactobacillus* and *Bacillus subtilis*) was added together with one larva. The tubes were then incubated at 35°. The internal temperature of the medium was 36±0.5° during the period of larval growth. At an age of 72 hr., the larvae were removed from their diets in the same sequence as they had been inoculated, cleaned of adhering medium, and weighed to the nearest 0.1 mg. Immediately after weighing, they were returned to their media. For convenient observation of the appearance of pupae, a flock of cotton wool was loosely plugged into each test tube (Fig. 1). The larvae were reincubated (35°) and inspected daily at a constant time until pupation or death. Records were kept of the number and average weight of the larvae, as well as of the number of larvae pupating or perishing on successive days and the number of adults emerging from the pupae. In a few instances the fecundity of the females was determined.

Composition of the experimental media. No. 1. Wheat-bran diet: a quantity (25 g.) of sieved wheat bran (Golan-Flour Mills, Tel-Aviv) was covered with 50 ml. of pure ether and incubated at 35° for 2 days, at which time the ether had completely evaporated. Before use, 25 ml. of water was mixed uniformly with the bran. The ether treatment was applied in order to make the conditions as similar as possible for all three diets used.

No. 2. Casein diet: the chemicals listed in Table 1 were intimately mixed and the pH was adjusted to 7.0 by drop-wise addition of 5N-NaOH solution. Pieces of chloroform-

extracted cotton wool, weighing about 0.1 g., were soaked with portions of 1 g. of the resulting mixture. The vitamin mixture in this diet was selected in conformity with the vitamin content of several yeast species (Dunn, 1952). The large excess of cholesterol in this diet was applied in order to ensure that each larva would receive an adequate quantity of this compound.

No. 3. Steroid-free diet: a quantity (500 g.) of sieved wheat bran was extracted at least 20 times with 1.5 l. of CHCl_3 in a Soxhlet apparatus. These conditions were found to be sufficient to free the wheat bran from steroids. It should be mentioned here that the procedure formerly

employed for the preparation of a basic lipid-free diet (Silverman & Levinson, 1954) was found to yield media which, in spite of their negative Liebermann-Burchardt reaction, still promoted some larval growth. An amount (25 g.) of the steroid-free material was mixed with 25 ml. of a 5% (w/v) solution of dried-yeast extract (Baltimore Biological Laboratories, U.S.A.) in sterile water. On this diet, newly hatched larvae attained an average weight of 1.0 mg., but died at an age of 2-3 days.

Steroids. The following materials were commercial preparations: cholesterol, β -sitosterol (24b-ethylcholest-5-en-3 β -ol), stigmasterol, ergosterol, calciferol, 7-hydroxycholesterol, cholesteryl acetate, cholic acid, deoxycholic acid, oestrone, dehydroepiandrosterone acetate, 3 β -hydroxy-5 β -androstan-17-one acetate, strophanthidin, digitonin, saponin 'white'.

A number of steroids the authors owe to the courtesy of various donors: cholesterol, purified via the dibromide (Professor L. F. Fieser, Harvard University); 'sitosterol' and 2 α -chlorocholestan-3 β -ol (see Beerenboom, Djerassi, Ginsburg & Fieser, 1953) (Professor D. Ginsburg, Hebrew Technical College, Haifa); γ -sitosterol (24a-ethylcholest-5-en-3 β -ol) (Dr W. Graham, Organon Laboratories Ltd., England); diosgenin (Lepetit, Milano, Italy).

The remaining steroids have been prepared according to the references indicated in parentheses: Cholesteryl laurate, m.p. 90° (Page & Rudy, 1930). Cholesteryl chloride (3 β -chlorocholest-5-ene), m.p. 96° (Diels & Blumberg, 1911). Cholesterylaniline, m.p. 189° (Lieb, Winkelmann & Koeppl, 1934). Cholest-4-en-3 β -ol (*allocholesterol*), m.p. 132° (Windaus, 1927). Wheat-bran sterols (Silverman & Levinson, 1954). Cholestan-3 α -ol (*epicholestanol*), m.p. 181-182° (Ruzicka, Brüngger, Eichenberger & Meyer, 1934). Cholestan-3 β -ol acetate, m.p. 111° (Willstätter & Mayer, 1908). 3 β -Acetamidocholest-5-ene, m.p. 244° (Windaus & Adaml, 1911). Cholest-5-en-3-one, m.p. 127° (Butenandt & Schmidt-Thome, 1936). 6-Bromocholest-4-en-3-one, m.p. 130-131° (Ruzicka, Bosshard, Fischer & Wirz, 1936; Dane, Wang & Schulte, 1937). 3-Acetoxycholesta-2,4-diene (enol acetate of cholest-4-en-3-one), m.p. 80° (Ruzicka & Fischer, 1936). Cholestan-3-one, m.p. 128-129° (Diels & Abderhalden, 1906). 7-Oxocholesteryl acetate, m.p. 154° (Windaus, Lettré & Schenck, 1935). 7-Oxocholesteryl chloride, m.p. 145° (Marker, Kamm, Fleming, Popkin & Wittle, 1937). 7-Dehydrocholesterol (cholesta-5:7-dien-3 β -ol), m.p. 144° (Windaus *et al.* 1935). Cholest-5-ene, m.p. 90° (Mauthner & Suida, 1894). Dehydrocholic acid (3:7:12-trioxocholanic acid), m.p. 237° (Hammarsten, 1881). 17 β -Hydroxy-5 α -3-androstanone acetate, m.p. 151° (Butenandt, Tscherning & Hanisch, 1935). *iso*Strophanthidin, m.p. 274-276° (Windaus & Hermanns, 1915).

Fig. 1. Method of rearing single fly larvae. A flock of cotton wool plugged loosely above the larval medium constitutes the site chosen by the larva for pupation. The contrast of the colours of the two layers facilitates the observation of the appearance of pupae and adults inside the cotton wool.

Table 1. *Composition of casein diet (diet no. 2)*

	g.		mg.
Chloroform-extracted casein (vitamin-free, Hoffman-La Roche)	3.750	Thiamine chloride	0.250
Salt mixture no. 2 (U.S.P. XII)	0.150	Riboflavin	0.500
L-Cystine	0.100	Nicotinic acid	2.500
Cholesterol, purified via the dibromide	0.050	Pantothenic acid	0.500
Choline chloride	0.025	Folic acid	0.250
Inositol	0.010	Pyridoxine	0.250
Nipagin M	0.150	Biotin	0.0025
		<i>p</i> -Aminobenzoic acid	0.500
		Vitamin B ₁₂	0.001

Water up to 50 g.

In order to test the utilization or antagonist action of the steroids, they were dissolved in 50 ml. of ether, the solution was poured on 25 g. of the dry diets nos. 1 and 3, and the solvent (which was just sufficient to cover the media) evaporated at 35°, leaving a crystalline residue as evenly distributed as possible. The bile acids were tested as sodium salts, being added to the liquid components of the diets. As a rule, the liquid components were admixed with the dry diets just before the start of a series of experiments.

Determination of lipid content. For the determination of lipid content, larvae were reared at a density of two individuals/g. of diet (nos. 1-3, Table 2) at a temperature of 35°. Approximately 25 g. of larvae was collected from each experimental diet and analysed in batches of 5-10 g. The larval age at the time of analysis is indicated in Table 2. The larvae were desiccated, powdered and extracted exhaustively with ether, and the ether residue was saponified with 5N ethanolic KOH solution. The fatty acids were liberated by adding an excess of H₂SO₄, extracted with light petroleum (boiling range 40-60°) and titrated with 0.1N-KOH, with phenolphthalein as indicator. The resulting soap was dried and weighed. The amount of saponifiable fat was computed according to Wizoeff (1927) and is recorded as percentage of live weight in Table 2.

Inhibition of mould growth in the wheat-bran media. The wheat-bran diet, at a rearing temperature of 35°, would become infested with certain moulds which might provide the larvae with an additional supply of steroids. It was necessary, therefore, to add to the diet 0.3% of Nipagin M (methyl *p*-hydroxybenzoate), an antiseptic active against a wide range of moulds and bacteria (Aalto, Firman & Rigler, 1953), but apparently without effect on growing housefly larvae.

RESULTS

Table 2 shows that growing housefly larvae are able to synthesize body fat from diets consisting largely of carbohydrates (no. 3) or proteins (no. 2). The rate at which body fat is synthesized in these cases appears to be similar to that at which it is taken up from a fat-containing diet. The characterization of the fatty acids synthesized is reserved for a later publication.

The larvae grown on the fat-free diets developed into viable adults which reproduced normally when fed on sterile solutions of fat-free peptone and sucrose. The average number of eggs laid per female and life time (temperature, 30 ± 1°; relative humidity, 50 ± 5%) amounted to 300 ± 50, their fertility being similar to that of the controls. Under these conditions, the average length of life was 20 ± 1 days.

In order to ascertain whether the presence of such large amounts of sterols as contained in the wheat-bran diet (no. 1) is necessary for larval growth and metamorphosis, the experiments summarized in Table 3 have been carried out with diet no. 3. Each experiment represents a total of 100 larvae, employed in four parallel batches. The variation between such batches did not exceed 10%. Newly hatched larvae, which did not receive cholesterol, were able to gain 0.9 mg./larva, but died at an age of 2-3 days. Addition of 2 µg. of cholesterol/g. of diet

Table 2. *Synthesis of body fat by housefly larvae*

Diet no.	Diet				Larvae		
	Lipids contained in diet (% of wet wt.)		Lipids added to diet (% of wet wt.)		Age at time of analysis (hr.)	Average body wt. (mg.)	Average lipid content (% of live wt.)
	Total lipid	Sterol	Total lipid	Cholesterol			
1. Wheat bran	1.8	0.1	0.0	0.0	72	23.8	4.9
2. Casein	0.0	0.0	0.1	0.1	72	22.7	4.5
3. Steroid-free	0.0	0.0	0.1	0.1	48	18.7	3.8

Table 3. *Influence of increasing amounts of dietary cholesterol on survival, growth rate, pupation and emergence of the housefly*

Cholesterol added to diet no. 3 (µg./g.)	Larvae surviving after 72 hr. (%)	Average larval wt. at age 72 hr. (mg.)	Larvae pupating (%)	Percentage of pupation at subsequent age (hr.)					Larvae developing into adults (%)
				96	120	144	168	192	
0	4*	1.0	0	—	—	—	—	—	—
2	16	14.0	12	—	—	16	50	34	4
4	18	16.1	16	—	—	36	64	—	12
6	28	17.7	28	—	57	43	—	—	24
8	36	19.0	36	—	64	36	—	—	32
12	48	20.1	44	12	66	22	—	—	36
14	51	21.5	50	36	54	10	—	—	44
18	64	22.3	60	60	40	—	—	—	57
30	80	22.6	68	80	20	—	—	—	63
36	84	22.5	84	78	22	—	—	—	78
50	96	22.8	96	83	17	—	—	—	88

* Died within the following 3 hr.

Table 4. *Effect of steroids and certain other substances on housefly larvae*

Steroids were added to the steroid-free diet (no. 3) in a concentration of 0.1%.

Expt. no.	Steroid added	Average larval wt. at age 72 hr. (mg.)	Larvae pupating (%)	Mean time to pupation (days)	Larvae surviving at 72 hr. (%)
1	Cholesterol	24.2	96	4.1	—
2	Cholesterol, purified via dibromide	24.6	98	4.0	—
3	Cholest-4-en-3 β -ol	25.0	74	4.0	—
4	Cholesteryl acetate	24.0	95	4.0	—
5	Cholesteryl laurate	24.0	88	4.0	—
6	Wheat-bran sterols	23.9	82	4.5	—
7	' β '-Sitosterol	22.8	53	5.5	—
8	' γ '-Sitosterol	21.9	52	5.5	—
9	Enol acetate of cholest-4-en-3-one	19.5	59	5.0	—
10	7-Dehydrocholesterol	11.1	44	6.3	—
11	Ergosterol	19.0	39	6.4	—
12	Stigmasterol	18.1	28	6.8	—
13	Calciferol	3.5	0	—	44*
14	Cholest-5-en-3-one	3.0	19	8.1	—
15	6-Bromocholest-4-en-3-one	4.7	16	6.2	22
16	7-Hydroxycholesterol	—	0	—	0
17	7-Oxocholesteryl acetate	3.4	4	7.6	28
18	7-Oxocholesteryl chloride	—	0	—	0
19	Cholesteryl chloride	—	0	—	0
20	Cholesterylaniline	1.1	0	—	18
21	Cholest-5-ene	—	0	—	0
22	Dehydroepiandrosterone acetate	—	0	—	0
23	3 β -Hydroxy-5 β -androstan-17-one acetate	1.3	0	—	4
24	Diosgenin	3.0	4	8.0	27
25	Cholestan-3 β -ol acetate	7.0	0	—	68*
26	Cholestan-3-one	2.6	0	—	16*
27	2 α -Chlorocholestan-3 β -ol	—	0	—	0
28	3 β -Acetamidcholest-5-ene	—	0	—	0
29	Cholestan-3 α -ol	—	0	—	6*
30	17 β -Acetoxy-5 α -androstan-3-one	1.1	0	—	10
31	Oestrone	—	0	—	0
32	Cholic acid	1.5	0	—	6
33	Deoxycholic acid	1.3	0	—	4
34	Dehydrocholic acid	1.0	0	—	2
35	Saponin	—	0	—	0
36	Digitonin	—	0	—	0
37	Strophanthidin	—	0	—	0
38	<i>iso</i> Strophanthidin	—	0	—	0

* Died within the following 24 hr.

no. 3 fed to one larva permits an average weight gain of 13.9 mg., but this level is insufficient by far to promote normal growth and pupation. The larval weights increase proportionally with increasing concentrations of dietary cholesterol up to a level of 30 μ g./g. of diet, which is, however, not optimum for larval survival, pupation and adult emergence. The level of 50 μ g. of cholesterol/g. of diet was found to be the minimum concentration required for normal growth and metamorphosis of *Musca vicina*. Addition of very large amounts of cholesterol to the diet, e.g. 150 mg./g. of diet, did not affect its nutritional value for the larvae. The amount of cholesterol actually ingested by each larva is unknown.

The study of the utilization of thirty-eight steroids fed to housefly larvae at a concentration of 0.1% of the diet no. 3 is summarized in Table 4.

Values of less than 20% of the larvae reaching pupation are considered as indicating 'no utilization'; such values are probably due to contamination of the substances tested with traces of utilizable steroids.

Some of the steroids listed in Table 4 were not only non-utilizable for the development of the larvae, but inhibited the utilization of the wheat-bran sterols present in the diet. It can be seen (Table 5) that cholesteryl chloride and 7-oxocholesteryl chloride suppressed larval growth and pupation. Cholestan-3-one and cholestan-3 α -ol were even more active in this respect than 7-oxocholesteryl chloride. Digitonin decreased growth and the rate of pupation, proportionally to its concentration. It may be assumed that the latter compound is inhibitory because it can precipitate steroids possessing a 3 β -hydroxyl group. Since

Table 5. *Inhibitory effect of certain steroids and digitonin on the growth and pupation of larvae*

Steroid added to wheat-bran diet	Concn., % of wet diet	Average larval wt. at age 72 hr. (mg.)	Larvae pupating (%)
—	—	23.8	80
7-Oxocholesteryl chloride	0.20	20.6	42
Cholesteryl chloride	0.10	13.3	30
Cholestan-3 α -ol	0.20	9.0	3
Cholestan-3-one	0.20	7.6	2
Digitonin	0.20	—	0
Digitonin	0.17	—	0
Digitonin	0.10	15.2	29
Digitonin	0.05	23.0	55
Digitonin	0.02	24.0	82

Table 6. *Inhibitory effect of cholesteryl chloride on the growth and pupation of larvae*

Cholesterol in diet (%)	Cholesteryl chloride in diet (%)	Ratio	Average larval wt. at age 72 hr. (mg.)	Larvae pupating (%)
0.2	—	—	24.0	93
—	0.1	—	—	0
0.2	0.1	2:1	25.9	97
0.1	0.1	1:1	24.2	90
0.05	0.1	1:2	19.3	88
0.03	0.1	1:3	17.8	84
0.005	0.1	1:20	12.7	40*
0.001	0.1	1:100	12.4	16*
0.001	—	—	19.8	40
0.0002	0.1	1:500	5.0	0
0.0002	—	—	13.2	10

* These pupae did not develop into adult houseflies.

cholesteryl chloride has been found to inhibit the utilization of cholesterol by growing *Attagenus* (McKennis, 1947, 1954) and *Blattella* (Noland, 1952, 1954b), an attempt was made to establish the existence of such an antagonism for the housefly larva. Table 6 shows that larvae fed on cholesterol and cholesteryl chloride at a ratio of 1:1 grew and pupated normally; decreasing the concentration of cholesterol at a constant level of cholesteryl chloride in the diet suppressed progressively larval growth and pupation. Thus a ratio of 1:20 of cholesterol and cholesteryl chloride permitted only 40% pupation and prevented the emergence of adult flies.

DISCUSSION

It has been shown that not only are housefly larvae able to synthesize their body fat from diets consisting largely of carbohydrates or proteins, but also that they do not require dietary carbohydrate, as they can subsist on a casein diet (diet no. 2). In this respect they differ from a number of phytophagous larvae (Fraenkel & Blewett, 1943; Lafon & Teissier, 1939).

Furthermore, the housefly does not require the unsaturated fatty acids which are essential for growth and wing formation in *Ephestia* (Fraenkel & Blewett, 1947) and for the metamorphosis and

reproduction in *Loxostege* (Pepper & Hastings, 1943). Among the flies, *Phaenicia sericata* needs neither fat nor carbohydrate in the diet (Hobson, 1935), whereas the parasitic *Pseudosarcophaga affinis* requires both for optimum growth (House, 1954). On the other hand, the housefly is incapable of steroid synthesis.

From a quantitative aspect, the requirements of the housefly larvae for cholesterol appear to be similar to those of two other dipterous larvae, *Phaenicia sericata* (Hobson, 1935) and *Drosophila melanogaster* (Van't Hoog, 1936). It was surprising to find that *Musca vicina* tolerates as much as 15% of dietary cholesterol, since the presence of 6.3% of cholesterol in the diet had been found to be fatal to *Callosobruchus chinensis* (Ishii, 1951).

The experiments summarized in Table 4 show that the effect of the steroids depends on their molecular structure. The following modifications of the structure of cholesterol do not impede utilization: (1) Esterification of the 3-hydroxyl group with fatty acids, since cholesterol can be replaced by its acetate or laurate. This may be due to the ability of the larva to split these esters enzymically. (2) Presence of a second double bond in the polycyclic system or of a double bond in the side chain, since 3-acetoxy-cholesta-2:4-diene, 7-dehydrocholesterol, ergosterol and stigmasterol are utilized.

(3) Branching of the side chain, since ' β '- and ' γ '-sitosterol are utilizable. It is worthy of note that the natural mixture of wheat-bran sterols is better utilized than either ' β '- or ' γ '-sitosterol. (4) Presence of the double bond at 4:5 instead of at 5:6 as in cholest-4-en-3 β -ol.

On the other hand, there exist certain modifications of the molecule which result in loss of utilizability. These are: (1) Absence of the hydroxyl group in position 3 (cholest-5-ene). (2) Introduction of a hydroxyl or keto group in the 7 position. (3) Substitution of the hydroxyl group of cholesterol by the anilino or acetamido group or by chlorine. (4) Removal of the side chain (acetates of dehydroepiandrosterone and of 3 β -hydroxy-5 β -androstane-17-one and oestrone) or its replacement by the spiroketal structure (diosgenin). (5) Aromatization of ring A together with removal of the side chain (oestrone). (6) Conversion of the cholesterol into cholest-5-en-3-one or 6-bromocholest-4-en-3-one. (7) Saturation of the $\Delta^{5,6}$ double bond (cholestan-3 β -yl acetate, cholestan-3-one, 2 α -chlorocholestan-3 β -ol). (8) Epimerization of the C₃ hydroxyl group (cholestan-3 α -ol). (9) The more radical changes of the total structures expressed by the formulae of bile acids, strophanthidin, saponins and calciferol.

Recently, similar observations have been made in the protozoan *Paramecium aurelia*. A sterol (other than cholesterol or ergosterol) is required as growth factor, and it can be replaced by a steroid ketone (carbonyl in 3 position). However, the presence of more than one double bond in the ring or the more drastic changes required for the formation of cholic acid, sex hormones or steroid saponins destroy the growth-promoting activity (Conner, van Wagtenonck & Miller, 1953; Conner & van Wagtenonck, 1955). Similar investigations have been carried out before for other micro-organisms, namely, *Trichomonas columbae* (Cailleau, 1937), *Pleuropneumonia*-like organisms (Edward & Fitzgerald, 1951), *Labyrinthula vitellina* (Vishniac & Watson, 1953; Vishniac, 1955) and, recently, *Saccharomyces cerevisiae* and *Escherichia coli* (Adelberg, Roslansky, Myers & Coughlin, 1955).

Steroid utilization differs amongst insect species. So far about fifteen species, belonging to the orders Coleoptera, Lepidoptera, Orthoptera and Diptera, have been studied. They were all found capable of utilizing fatty acid esters of cholesterol, and Noland (1954b) has assumed that cholesterol absorption requires an esterase (ester-synthesizing enzyme). However, cholesteryl trimethylacetate, which is rather resistant to enzymic hydrolysis, was reported not to be utilizable by *Blattella* (Noland, 1954a).

Utilization of steroids containing more than one double bond differs among various species. The enol acetate of cholest-4-en-3-one (3-acetoxycholesta-

2:4-diene) is utilized by *Musca* and by *Drosophila* (Van't Hoog, 1936), but not by *Aedes* (Goldberg & De Meillon, 1948) and *Blattella* (Noland, 1954a). For the rat, cholestenone is not equivalent to cholesterol since the rat cannot reduce the unsaturated ketone to the sterol (Stokes, Fish & Hickey, 1955). 7-Dehydrocholesterol was found to be well utilized by the carnivorous *Dermestes* (Fraenkel, Reid & Blewett, 1941) and by the phytophagous beetles *Lasioderma*, *Ptinus*, *Silvanus*, *Sitodrepa* and *Tribolium*, and the moth *Ephestia kuehniella* (Fraenkel & Blewett, 1943), but it was less available to the zoophagous carpet beetle *Attagenus* (McKennis, 1947) and to the mainly coprophagous *Musca*. Among the phytophagous species, *Drosophila*, feeding mainly on living yeast, and the six above-mentioned species utilize ergosterol as well as cholesterol, and the zoophagous Dermestidae do not utilize it at all. *Phaenicia* (Hobson, 1935), *Aedes*, *Blattella* and *Musca*, which feed both on animal and vegetable matter, appear intermediate between phytophagous and zoophagous species: they utilize ergosterol, but poorly. *Callosobruchus* (Ishii, 1951), feeding preferably on leguminous seeds, utilizes stigmasterol well, whereas *Aedes*, *Drosophila*, *Blattella* and *Musca* utilize it less. The sitosterols are best utilized by the six flour insects, but they are less utilizable by *Drosophila*, *Blattella* and *Musca*, which feed only occasionally on cereals. However, the carnivorous *Dermestes* is unable to utilize sitosterols. All phytophagous species thus far investigated are able to utilize cholestan-3 β -ol. *Musca*, however, resembles the carnivorous *Dermestes* and *Attagenus* in being unable to utilize this compound. It may, therefore, be concluded that steroid utilization of the species studied is regulated by their feeding habits. The smallness of the structural changes which affect steroid utilization by insects suggest that the specificity of these growth-promoting substances is rather high. In this respect it should be recalled that the oestrone-induced uterine growth is also antagonized by steroids similar to oestrone (Huggins & Jensen, 1955), that the hydroxysteroid dehydrogenases of *Pseudomonas* are inhibited by other steroids (Talalay & Marcus, 1954), and that 3 β -cholestanol prevents the increase of plasma cholesterol in chicken reared on a high-cholesterol diet (Siperstein, Nichols & Chaikoff, 1953).

SUMMARY

1. Housefly larvae are able to synthesize body fat from dietary protein; they require neither fatty acids nor carbohydrate in the medium. Furthermore, they reproduce normally without any dietary source of fat during larval and adult life.

2. Cholesterol in quantities of 2 μ g./g. of diet stimulates growth of housefly larvae. Larval growth

is proportional to the concentration of dietary cholesterol, up to a certain limit. Excess of cholesterol in the diet has no adverse effect.

3. The influence of steroid structure on the utilizability has been studied. It has been found that some steroids are not only not utilized, but they inhibit the utilization of other steroids.

4. Cholesterol utilization by housefly larvae is reversibly inhibited by cholesteryl chloride.

5. The utilization of steroids by insects is discussed in connexion with their feeding habits.

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