A BIOCHEMICAL STUDY ON THE GROWTH OF THE MOSQUITO,

WITH SPECIAL REFERENCE TO THE CHANGES IN PROTEIN, FAT AND CARBOHYDRATE CONTENTS DURING ITS DEVELOPMENT

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(Received December 10, 1954)

Studies on the mosquito have hitherto been focused on its morphology and ecology, and attention has been paid so little to its biochemistry that even its content of chief bodily substances (protein, fat and carbohydrate) has remained unkown. A biochemical study on the mosquito was therefore attempted to supplement the defect of our knowledge in this realm. The mosquito was classified for its growth stages into the eggs, the first to fourth instar larvae, the pupa and the imago according to the morphological characters, taking advantage of the results of the research which had been presented in the previous report,[1] and they were determined for their content of nitrogen-containing substances (proteins), water-soluble reducing substances (free and combined sugars), and ether-alcohol-soluble substances (lipides).

The deta obtained in this way will be described in the hope that they may provide a starting point for the biochemical study of mosquitoes.

MATERIALS AND METHODS

Culex pipiens var. pallens Coquillet 1898 and Anopheles hyrcanus var. sinensis Wiedemann 1828 were used as materials, because they were among the most common of the mosquitoes found in this country. Mosquito collection were carried out at Ozuki-machi, Shimonoseki City, during the period from April to May, 1954. The eggs, larvae and pupae were obtained from the water of their natural breeding places (paddy-fields and ponds). They were scooped with a white metal tray, transferred into glass vials to be brought to the laboratory alive, and examined in fresh state under a binocular magnifier or a microscope for the identification of their species and growth stages. Author's criteria [1] for the determination of larval instars were employed. As soon as they were assorted for the species and the growth stages they were subjected to

chemical analysis. The larvae in the first and second instars were, however, hatched from the eggs and reared in the laboratory, and as for Anopheles hyrcanus var. sinensis, its imagines alone were used as materials, because the rice field, i. e. their natural breeding places, were treated with parathion, resulting in poor procreation of the larvae and pupae of this species.

I. *Nitrogen determination* [2]

Principle: The materials to be determined for nitrogen are introduced into a test tube with the digestion mixture (concentrated sulfuric acid containing mercuric oxide and potassium sulfate), heated until their N-substances (proteins for the most part) are completely converted into ammonium salt, allowed to stand to cool, diluted with water, and nesslerized for photoelectric colorimetry.

Reagents

1) Digestion reagent: Dissolve 0.8 g. of secondary mercury oxide HgO and 40 g. of anhydrous potassium sulfate K_2SO_4 in about 40 ml. of distilled water contained in a beaker which has a marking at 250 ml. (these salts do not dissolve perfectly), and warm it to complete transparency after adding 43.5 ml. of concentrated sulfuric acid (spec. gr. 1.84) drop by drop with constant agitation by means of a glass rod. To this add at once approximately 200 ml. of distilled water and dilute to the volume of 250 ml. when it has cooled to room temperature.

2) 20 mg./dl. N solution: 94.32 mg./dl. aqueous solution of ammonium sulfate

3) Nessler's reagent: Prepare mercuric iodide solution according to Koch and Mc Meekin,[3] preserve in a brown bottle, and mix one volume of it to five volumes of 10 g./dl. aqueous sodium hydroxide solution immediately before use.

Procedure

(1) Preliminary treatment

a) Imagines were killed by ether anethesia (absorbent cotton soaked with ether was introduced into the mouth of sucking tube in which imagines had been captured), and grouped into males and females. The females were further divided into those with eggs (they were characterized by swollen milk-white abdomen), those with blood (their abdomen looked dark red or black because of the fresh or half-digested blood) and those with empty abdomens (their abdomens were slender, having little indication of mature eggs and ingested blood). They were weighed individually in a balance.

b) Larvae and pupae were introduced individually into the distilled water contained in a beaker with a rubber-teated pipette. The beaker was emptied slowly by sucking the water up with a capillary-ended rubber-teated pipette, and refilled with fresh distilled water,..... the procedure was repeated until the larvae and pupae were completely freed of the dirty debris adhered to their body surface. Then they were transferred over a filter paper to absorb the excess of water as perfectly as they wriggled about on the paper. Inasmuch as such a procedure was not available for the younger larvae in the first and second instars, they were directly deposited on the tip of a small glass rod and tossed into a test tube containing digestion reagent, after they underwent the collective washing which was similar to that described above. The larvae and pupae were counted before washing and special care was taken lest any of them should be lost during the preliminary treatment.

c) Egg clusters were placed under a binocular magnifier to count the number of eggs exactly, and it was subjected to digestion as a whole. An egg cluster was usually composed of about three hundred eggs.

(2) Digestion. The materials treated as above were introduced into test tubes, each containing 1.5 ml. of digestion reagent. The imagines and pupae were put individually into separate tubes with a pincette, and the larvae were transferred into the tubes collectively in a number which was adequately large for the analysis by allowing them to adhere to the tip of a glass rod. (The optimum numbers of larvae are listed in Tables I, II and III.) Glass beads were added, two to each test tube, to prevent ebullition during the course of digestion, and the test tubes were inserted into a digestion rack made of metal wire.

The rack was placed upon an electric range (1 KW hour), and shaken incessantly to prevent the sudden outflow of the contents of tubes by ebullition. The contents began to boil within a few minutes, grow brown in color and was concentrated to form a caramel-like substance at the bottom of the tubes in about three minutes and became colorless in five minutes. The test tubes were rotated in their position with wooden forceps or gloved fingers so that their bottoms might change the parts which were directly heated over the fire. The brown substances which adhered to the lower inner surface of the tubes were washed down and oxidized by the flow of hot sulfuric acid condensed near the opening of the tubes. Additional heating was carried out for thirty minutes until small black residuum which might appear in the course of oxidation no longer remained. The electric range was switched off and the tubes were allowed to cool for three or four minutes.

(3) Nesslerization. To each of the digestion tubes were introduced

10.0 ml. of distilled water while they were warm, and the tubes were inverted several times to get clear solution, after they had been plugged. Aliquots of 1.0 ml. of the solution thus produced were diluted with 5.0 ml. of distilled water and colorized by adding 1.5 ml. of Nessler's reagent.

(4) Colorimetry. The colorized solutions were read in a photoelectric colorimeter set to zero with distilled water at 470 mμ of light wave length. Aliquots of 0.1, 0.3 and 0.5 ml. of 20 mg./dl. N solution were also subjected to the procedures (2), (3) and colorimetry, being regarded to represent the N contents of 20, 60 and 100 γ, respectively. A calibration curve was constructed, by plotting N concentration as abscissa and absorbance as the ordinate on rectangular co-ordinate paper. Then N content of each sample tube was got by the collation of its absorbance to the curve.

II. *Fat determination* [4]

Principle: Mosquitoes (eggs, larvae, pupae and imagines) are mashed in an agate mortar or in a homogenizer, and they are extracted with a hot mixture of ether and alcohol (1:3) to release fatty substances. A portion of extract is evaporated and the residue is dissolved in petroleum ether in order to remove the non-fatty substances contained in the extract. The solution is evaporated to complete dryness, a potassium dichromate solution in concentrated sulfuric acid is added to the residue, and heated to produce chrome alum by the reduction of dichromate with fatty substances. The chrome alum thus yielded is proportionate in amount to the quantity of fatty substances extracted, and it is easily determined by photoelectric colorimetry, because the absorbance of the chrome alum ion which is green in color is able to be measured at 610 mμ without any appreciable interference of the reddish-brown coloration of dichromate ion.

Reagents

1) Bloor's mixture for the extraction of lipoids: Mix ether with absolute ethyl alcohol in volume ratio one to three.

2) Dichromate mixture: Dissolve 20 g. of powdered potassium dichromate $K_2Cr_2O_7$ in 1000 ml. of concentrated sulfuric acid (spec. gr. 1.84)

3) Chrome alum solution: Dissolve 6.7871 g. of chrome alum $K_2Cr_2(SO_4)\cdot 2H_2O$ in 100 ml. of sulfuric acid (spec. gr. 1.84)

4) Petroleum ether (boiling pt. 33–60 C)

Bragdon [4] was followed in preparing these reagents.

Procedures

(1) Preliminary treatment: This was the same as in N determination except that the imagines were dried at room temperature for three days after they were killed by ether anesthesia. (They were thus reduced in weight to a third of the fresh state.)

a) Imagines with their wings undetached were mashed thoroughly in an agate mortar with one ml. of Bloor's mixture. The mashed materials were transferred into a test tube (2.0 cm. in diameter) which had a marking at 10 ml. through a glass funnel. The mortar and funnel were transferred to the test tube to be combined with the mashed materials.

b) The pupae and the third and fourth instar larvae were washed and dried in just the same way as in the case of N determination. They were taken by a pincette or allowed to adhere to the tip of a glass rod in order to be transferred into a homogenizer. The larvae in the first and second instars are pipetted into a centrifuge tube with sufficient amount of distilled water and centrifuged at 3000 rpm for twenty minutes. The supernatant was pipetted away and distilled water was added to the sediment which was composed of the agglomerate of larvae. The tube was again centrifuged at 3000 rpm for twenty minute,..... . The procedure was repeated thrice to wash the larvae completely. The agglomerate was transferred to a homogenizer with a glass rod, after the supernatant was removed as perfectly as possible. (The larvae and pupae were all alive when they were subjected to the preliminary treatment).

A volume of 0.5 ml. of Bloor's mixture was introduced into the homogenizer and the insects therein were mashed thoroughly with a pestle. The mashed material was washed away into a test tube which was graduated to 10 ml. with a volume of three ml. of Bloor's mixture.

c) Eggs. Egg clusters which had been counted for the number of eggs as in the case of N determination were subjected to a homogenizer to be treated in the same way as was the larvae. The homogenate was transferred to a test tube which had a marking at 10 ml., and it was made to about 3 ml. with Bloor's mixture which had been used to rinse the homogenizer.

The number of larvae, pupae, imagines and eggs which were used for analysis is presented in Tables IV to VI.

(2) Extraction. The homogenate in the test tube was made to about 7 ml. with Bloor's mixture, and it was heated in a boiling water bath (100 C) for about two minutes. (The tube was taken out of the bath as soon as its content began to bubble in order to prevent ebullition, and

it was again immersed in the bath when bubbling ceased. This procedure was repeated for the specified period.) The tube was then placed in running tap water to be cooled to the room temperature. Its content was made to volume (10.0 ml.) with Bloor's mixture, and filtered through a filter paper Toyo-roshi No. 7 to get limpid extract of fatty substance.

(3) Evaporation. An aliquot of 5.0 ml. of the extract was pipetted into a test tube (tube S), while 5.0 ml. of Bloor's mixture was transferred into another test tube (tube B). Both were evaporated to dryness in a boiling water bath. (It required about thirty minutes.) The tubes were placed in the bath until droplets of water were no longer visible on the dried residue of tube S.

(4) Purification. The residue of tube S was rubbed off completely from the wall with a glass rod after 5 ml. of petroleum ether was added, warmed in a boiling water bath for one minute to be brought into solution, and filtered into a 20 ml. volumetric flask (flask S) through a filter paper Toyo-roshi No. 7. The dissolution of residue was repeated thrice, and the filtrates were combined in the volumetric flask S. The tube B was also subjected to the same procedure, and its filtrates were collected in another 20 ml. volumetric flask (flask B). Both flasks S and B, were maintained at 70 C in a thermostat for an hour to evaporate the petroleum ether completely. A glass tube with its tip drawn out to a capillary was connected to a water stream pump, and it was introduced into the lumen of the flasks to aspirate the minute amount of petroleum ether vapor which might remain despite its complete evaporation, lest the trace of petroleum ether should lead to an undue promotion of reduction of dichromate in step (5).

(5) Oxidation (or reduction of dichromate to chrome alum by fatty substance). Dichromate mixture was pipetted into flasks S and B, 5.0 ml. each, while three 20 ml. volumetric flasks a, b and c were prepared as follows

a. dichromate mixt. 4.0 ml. + chrome alum solut. 1.0 ml.

b. dichromate mixt. 3.0 ml. + chrome alum solut. 2.0 ml.

c. dichromate mixt. 2.0 ml. + chrome alum solut. 1.0 ml.

They were all heated in a boiling water bath for thirty minutes. The solution in flask S turned in color from reddish brown to green or brown, whereas flask B remained unchanged. At the end of the specified time they were brought into running water to be cooled to room temperature, made to a volume of 20 ml. with distilled water, and mixed by inversion.

(6) Colorimetry. The contents of the flasks were measured for their absorbances in a photoelectric colorimeter at 610 mμ of light wave length. A calibration curve was constructed between the absorbance and the concentration of chrome alum, assuming that flasks a, b and c had the chrome alum in concentration 1, 2 and 3 units. The chrome alum concentration x units of flask S was thus read through the comparison of its absorbance with the calibration curve, and the content of fatty substance F of the material examined was calculated by the following equation.

$$F = \frac{6.7891}{100} \times \frac{294}{998} \times \frac{1}{17} \times x = 3.35x \text{ mg.}^*$$

III. *Carbohydrate determination* [5]

Principle: The homogenate of mosquito (eggs, larvae, pupae and imagines) is treated with hot water to extract water-soluble reducing substances including free and combined carbohydrates. The amount of free reducing substance is able to be determined when the extract is directly subjected to Somogyi-Nelson's procedure for the determination of blood glucose, while total carbohydrate is estimated when the extract has been hydrolysed by acid to release and decompose the combined carbohydrate before it undergoes the same procedure. Combined carbohydrate is calculated by subtracting the free from the total carbohydrate. Carbohydrates are thus obtained in terms of the weight of glucose.

Reagents

1) Concentrated hydrochloric acid (3.5 per cent, sp. gr. 1.18)
2) Alkaline Copper sulfate solution of Somogyi [5]
3) Arsenophosphomolybdate reagent of Nelson [6]
4) 0.3 N aqueous solution of barium hydroxide.
5) 5 g./dl. aqueous solution of zinc sulfate.
6) 100 mg./dl. glucose solution. This is diluted to 20–5 mg./dl. with distilled water before use.
7) Powdered anhydrous sodium carbonate.

Somogyi [5] and Nelson [6] are followed strictly in the preparation of reagent 1) and 6).

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* 6.7891: concentration of chrome alum in g./dl.
294: molecular weight of potassium dichromate
998: molecular weight of chrom alum ($K_2Cr_2(SO_4)\cdot 2H_2O$)
17: The equiavlent of potassium dichromate to lipoid. An amount of 17 mg. of potassium dichromate is reduced to chrome alum by 1 mg. of lipoid.

Procedure

(1) Preliminary treatment. An amount of 50 to 150 mg. of materials (eggs, larvae, pupae amd imagines) was treated as follows,

a) Eggs were mashed completely with a homogenizer.

b) Larvae and pupae were thoroughly washed in distilled water contained in a cup to be freed from dirty debris deposited on them, and the water, having been used for washing, was slowly aspirated off with a pipette which was plugged at its tip with a piece of absorbent cotton and connected to a water stream pump. The cup was repeatedly filled with distilled water and emptied in this way. The larvae and pupae were transferred to an agate mortar, after they were removed from water as completely as possible, in order to be mashed together with absorbent cotton to which some of the larvae had clung during aspiration.

c) Imagines were killed by ether anesthesia, weighed, and homogenated.

(2) Infusion. The mashed material was transferred to a graduated test tube with a small amout of distilled water which had been used to rinse the mortar or the homogenizer, made to volume of 5.0 ml. with distilled water, plugged with a rubber stopper, placed in a boiling water bath for twenty minutes, and filtered through a filter paper Toyo-roshi No. 7 while hot to obtain clear filtrate. The filtrate was allowed to cool to room temperature, and a 1.0 ml. aliquot of the filtrate was pipetted into test tube A, and another 1.0 ml. into test tube B.

(3) Acid hydrolysis. Test tube A was acidified with 0.1 ml. of concentrated hydrochloric acid, placed again in a boiling water bath for acid hydrolysis of combined carbohydrates, cooled, neutralized with powdered sodium bicarbonate (added little by little until bubble was no longer formed), and followed by the addition of 1.0 ml. of distilled water. In the meantime 1.0 ml. volumes of 20, 10 and 5 mg./dl. glucose solution were introduced to the new test tubes C, D and E, and aliquots of 1.1 ml. of distilled water added to the tube B, C, D and E.

(4) Removal of protein. Volumes of 1.0 ml. of zinc sulfate solution were added to the test tubes A to E, then 1.0 ml. volumes of barium sulfate solution were dropped into them, and their contents were filtered through filter papers Toyo-roshi No. 7 to get clear deproteinized filtrates.

(5) Reduction. Aliquots of 1.0 ml. of the filtrates were transferred separately into individual test tubes a,b, c, d, and e, 1.0 ml. volumes of alkaline copper sulfate reagent were added, heated in a boiling water bath for fifteen minutes, allowed to cool to room temperature, colorized by the addition of 1.0 ml. of arsenophosphomolybdate reagent, diluted

with 9.0 ml. of distilled water, and mixed by inversion for photoelectric colorimetry.

(6) Colorimetry. The blue color thus produced in the tubes a to e was measured for their absorbance in a photoelectric colorimeter at 660 mμ. A calibration curve was constructed, by assuming that the tubes c, d and e represented 20, 10 and 5 mg./dl. of glucose, in order to get the concentrations of reducing substance α and β in the tubes a and b, respectively. The amount of the total, free and combined carbohydrates contained in the material was calculated in terms of the weight of glucose by the following equation.

$$\text{Total carbohydrate} = \frac{5\alpha}{100}\text{mg} = 50\alpha\gamma$$

$$\text{Free carbohydrate} = \frac{5\beta}{100}\text{mg} = 50\beta\gamma$$

$$\text{Combined carbohydrate} = 50(\alpha - \beta)\gamma$$

Results

The data obtained in this study are listed in Table I–III (nitrogen content), IV–VI (fat content) and VII–IX (carbohydrate content). The average nitrogen content per one imago was, in Culex pipiens var. pallens, 173 γ for female with eggs, 119 γ for female without eggs, and 63 γ for male, while in Anopheles hyrcanus var. sinensis, it was 197, 132 and 67.7 γ, respectively. The nitrogen content was therefore larger in the latter species than in the former in proportion to the body weight. Correlation between nitrogen content and body weight is, however, not appreciable within an individual species, as readily seen from Tables I and II which disclose a marked variation. It is natural that females with eggs had a greater amount of nitrogen than those without eggs, because ovarium was mature (middle or high grade maturity, namely stage M or G_1–G_2 of Shibata [8]) and large in the formers whereas immature (Stage Y of Shibata [8]) and small in the latters. Nevertheless, little difference in the nitrogen content per mg. body weight is noted between females and males, as clearly witnessed by the same tables, since it was 49 (females without eggs) and 49 (male) γ/mg. in Culex pipiens var. pallens, and 46 and 48 γ/mg, in Anopheles hyrcanus var. sinensis.

The pupa of Culex pipines var. pallens had 43.7 γ of nitrogen on the average, an amount equaling the imago's nitrogen content, but the larvae were distinctly poor in nitrogen, and the egg contained nitrogen barely a four-hundredth as much as did imago.

The imagoes of Culex pipiens var. pallens contained 43.1 (female without eggs), 71.1 (female with eggs) and 27.3 (male) γ of fatty substances per one body on an average, while those of Anopheles hyrcanus var. sinensis had 45.5, 77.7 and 31.6 γ of them, respectively. The imago's fat content is, accordingly, two-fifths as much as its nitrogen content, and the same nearly holds true when they are compared in the amount per one mg. of body weight. However, eggs, larvae and pupae (except for the larvae in the first and second instars) had fatty substances in amount equaling their relevant nitrogen contents. They are therefore relatively more abundant in fat than are the imagoes.

In imagines the total carbohydrate content accords approximately in size with one-sixth to one-tenth of the nitrogen content. Male imago of Culex pipiens var. pallens contained 8.0 γ of carbohydrate on an average, while females of this species had 21 γ (without eggs) and 33.8 γ

TABLE I

Nitrogen content of the imagines. Culex pipiens var. pallens

Females				Males	
Without eggs		with eggs			
Body weight (mg.)	N (γ)	Body weight (mg.)	N (γ)	Body weight (mg.)	N (γ)
2.8	130	3.4	170	1.7	85
2.7	145	3.2	190	1.5	80
2.7	135	3.1	230	1.5	75
2.7	120	3.1	210	1.4	61
2.6	115	3.1	200	1.3	78
2.6	115	3.1	180	1.3	70
2.6	110	3.1	170	1.3	70
2.5	130	3.0	195	1.3	65
2.5	125	3.0	155	1.3	58
2.5	115	2.9	180	1.3	58
2.5	110	2.9	120	1.3	55
2.5	110	2.8	190	1.3	55
2.3	110	2.8	170	1.3	52
2.3	100	2.8	100	1.3	50
2.2	120	2.5	190	1.3	50
2.2	100	2.5	170	1.2	60
2.2	100	2.5	160	1.2	55
2.2	80	2.0	180	1.2	55
				1.2	50
				1.2	50
				1.2	45
Maximum 2.8	145	3.4	230	1.7	85
Minimum 2.2	80	2.0	120	1.2	48
Average 2.41	119	2.93	173	1.32	63
N(γ)/body wt.(mg.)	49		57		49

TABLE II

Nitrogen content of imagines. Anopheles hyrcanus var. sinensis

Females				Males	
without eggs		with eggs			
Body weight (mg.)	N (γ)	Body weight (mg.)	N (γ)	Body weight (mg.)	N (γ)
3.3	125	5.8	250	1.7	62
3.0	155	5.6	230	1.6	85
3.0	85	5.2	205	1.6	66
2.8	125	5.2	155	1.6	63
2.8	98	5.0	225	1.5	75
2.7	175	5.0	205	1.5	70
2.7	160	5.0	185	1.5	65
2.7	155	4.8	235	1.5	65
2.7	150	4.8	220	1.5	60
2.7	125	4.8	187	1.5	60
2.7	120	4.8	182	1.4	70
2.7	117	4.5	220	1.4	68
2.7	115	4.5	210	1.4	65
2.7	95	4.5	190	1.4	65
2.6	150	4.5	180	1.3	68
2.6	125	4.3	192		
2.5	127	4.0	185		
2.5	120	3.8	162		
2.5	120	3.5	182		
2.5	95	3.2	187		
2.4	170				
2.3	90				
Maximum 3.3	175	5.8	250	1.7	85
Minimum 2.3	85	3.2	155	1.3	60
Average 2.98	132	4.59	197	1.43	67.7
N(γ)/body wt. (mg.)	46		43		48

TABLE III

Nitrogen content of egg, larva and pupa. Culex pipiens var. pallens

Egg		1st inst. L.		2nd inst. L		3rd inst. L		4th inst. L		pupa	
Number used	N ($\gamma \times 10^{-2}$)	Number used	N ($\gamma \times 10^{-2}$)	Number used	N ($\gamma \times 10^{-2}$)	Number used	N ($\gamma \times 10^{-2}$)	Number used	N ($\gamma \times 10^{-2}$)	Number used	N ($\gamma \times 10^{-2}$)
200	13	165	90	50	358	20	700	5	3000	1	6000
180	11	100	85	50	291	20	630	5	2800	1	5000
208	10	150	80	50	288	20	630	5	2800	1	4500
		100	77	50	268	20	570	5	2700	1	4500
		100	70	50	248	20	600	5	2700	1	4500
								3	2600	1	4200
								3	2600	1	4200
								5	2500	1	4000
								5	2500	1	4000
								3	2500	1	3600
								5	2300	1	3500
								5	2200	1	3500
										1	3500
										1	3200
										1	3000
Average 11.3		80.5		297		650		2600		4370	

(with eggs) of it, respectively. The relevant figures were, in Anopheles hyrcanus var. sinensis, 10.7 (male), 13.8 (female without eggs) and 38.2 (female with eggs) γ. The carbohydrate to nitrogen ratio was nearly the same value also for the growth stages of the eggs and the first to third instar larvae, but in the fourth instar larva and in the pupa the carbohydrate content is somewhat high as compared with the nitrogen content, attaining to from one-fourth to one-third. The individual variation in carbohydrate content seemed to be as pronounced as was its nitrogen content, although it was not confirmed directly, because determination of carbohydrate required scores or hundreds of insects collectively at the least.

The combined to free carbohydrate ratio was about one to one with exception of the first instar larva and the female imago without egg of Culex pipiens var. pallens for which values of 7 : 3 and 3 : 7 were obtained, respectively (Figure 1).

TABLE IV

Fat content of imagines. Culex pipiens var. pallens

Females						Males		
without eggs			with eggs					
Number used	Body wt (mg.)*	Fat (γ)	Number used	Body wt (mg.)	Fat (γ)	Number used	body wt (mg.)	Fat (γ)
35	1.12	46	25	2.15	60	35	0.55	27
30	1.10	43	17	2.02	51	35	0.52	27
25	1.10	36	20	1.90	80	32	0.51	28
30	1.00	37	24	1.80	65	35	0.48	24
30	1.05	48	20	1.71	72	35	0.45	25
30	1.05	45	20	1.50	84	30	0.45	32
30	1.05	42	20	1.50	65	27	0.42	24
30	1.03	46	20	1.42	83	35	0.41	26
27	1.02	45	20	1.30	78	30	0.40	30
30	1.02	36	20	1.22	72	30	0.40	30
27	1.01	45	20	1.16	80	35	0.38	27
35	0.84	40				35	0.35	27
29	0.81	43						
25	0.71	42						
Maximum	1.12	48		2.15	84		0.55	32
Minimum	0.71	37		1.16	51		0.35	24
Average	1.00	43.1		1.61	71.1		0.45	27.25
F(γ)/body wt(mg.)**		17.9			23.6			19.6

* Dried weight

** Body weight at fresh state

TABLE V

Fat content of imagines, Anopheles hyrcanus var. sinensis

Females						Males		
without eggs			with eggs					
Number used	Body wt. (mg.)*	Fat (γ)	Number used	Body wt. (mg.)	Fat (γ)	Number used	Body wt. (mg.)	Fat (γ)
18	1.54	45	28	2.32	81	25	0.65	24
30	1.20	46	17	2.30	81	30	0.61	36
30	1.04	46	25	2.15	83	25	0.61	29
30	0.89	54	20	2.05	75	19	0.60	30
29	0.82	45	18	2.03	94	30	0.58	29
23	0.81	43	20	2.01	77	35	0.55	36
29	0.80	51	15	2.01	62	35	0.55	34
30	0.80	48	19	1.85	76	30	0.50	33
16	0.79	46	20	1.81	85	30	0.50	33
18	0.79	45	20	1.75	97	30	0.49	34
28	0.79	43	20	1.70	88	25	0.49	30
30	0.79	42	20	1.61	61			
31	0.77	43	20	1.58	61			
30	0.77	42	15	1.57	75			
23	0.75	48	20	1.40	72			
30	0.75	42						
Maximum	1.51	54		2.32	97		0.65	36
Minimum	0.75	42		1.40	91		0.49	24
Average	0.88	45.5		1.59	77.7		0.56	31.6
F(γ)/body wt(mg)**		15.2			16.7			21.9

* Dried weight

** Body weight at fresh state

TABLE VI

Fat content of egg, larva and pupa, Culex pipiens var. pallens.

Egg		First inst. L.		Second inst. L.	
Number used	Fat ($\gamma \times 10^{-2}$)	Number used	Fat ($\gamma \times 10^{-2}$)	Number used	Fat ($\gamma \times 10^{-2}$)
12000	8	3000	28	800	88
12000	8	3000	25	800	83
12000	8	3000	25	1000	82
12000	7	3000	25	1000	82
Average	7.4		26.7		83.2

Third inst. L.		Fourth inst. L.		Pupa	
Number used	Fat ($\gamma \times 10^{-2}$)	Number used	Fat ($\gamma \times 10^{-2}$)	Number used	Fat ($\gamma \times 10^{-2}$)
72	880	60	2900	29	6400
100	850	60	2700	53	5600
100	830	60	2700	60	5400
134	820	52	2500	60	5300
		50	2500	29	5100
				60	4900
Average	832		2660		5450

TABLE VII

Carbohydrate content of imagines, Culex pipiens var. pallens.

Females							
without eggs				with egg and blood			
Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb. (γ)	Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb. (γ)
70	2.67	8.9	18.1	33	4.75	10.0	1.5
70	2.66	2.6	17.3	45	4.49	8.0	8.0
80	2.64	8.0	20.5	35	4.46	6.3	5.4
11	2.67	3.3	7.6	56	4.44	11.4	7.0
50	2.54	12.5	20.5	36	4.42	2.8	5.6
69	2.52	9.9	16.7	41	4.36	17.8	18.7
15	2.50	2.3	26.3	36	4.36	7.5	7.5
50	2.48	19.7	19.7	33	4.24	9.5	8.1
80	2.36	10.5	10.1	50	4.24	5.2	4.0
54	2.36	9.8	18.2	55	4.16	8.3	5.1
30	2.31	3.1	7.7	30	4.03	14.4	3.0
49	2.21	6.2	12.4	28	4.00	22.2	4.5
50	2.15	5.4	5.0	16	3.90	7.2	0.6
60	2.14	6.5	15.1	28	3.78	1.8	6.8
68	2.03	1.6	9.2	30	3.70	17.2	5.5
40	2.01	4.3	8.2	40	3.58	8.8	14.0
43	1.92	0.2	9.1	54	3.50	12.2	18.4
Maximum	2.67	19.7	26.3		4.75	17.3	18.7
Minimum	1.92	0.2	5.0		3.50	1.8	0.6
Average	2.40	6.8	14.2		4.14	10.0	7.3
		21.0				17.3	
C(γ)/Body wt.(mg.)		8.7				3.9	

Females				Males			
with egg only							
Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb. (γ)	Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb (γ)
12	5.08	19.2	20.8	10	1.49	2.9	3.9
15	4.53	12.3	13.0	70	1.46	1.8	3.8
15	4.21	12.6	11.2	25	1.35	2.4	1.6
19	4.15	17.9	10.5	12	1.34	13.3	10.1
13	4.13	12.9	12.5	55	1.30	2.1	4.8
9	3.86	7.9	5.5	41	1.27	3.8	4.2
22	3.47	5.4	5.1	76	0.66	0.5	0.8
Maximum	5.08	19.2	20.8		1.49	13.3	10.1
Minimum	3.47	5.4	5.1		0.66	0.5	0.8
Average	4.21	12.6	11.2		1.27	3.8	4.2
		33.8				8.0	
C(γ)/Body wt.(mg.)		5.6				6.3	

TABLE VIII

Carbohydrate content of imagines. Anopheles hyrcanus var. sinensis.

Females							
without eggs				with eggs and blood			
Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb. (γ)	Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb. (γ)
10	5.87	2.1	18.9	50	6.00	13.0	12.0
55	3.04	9.6	12.2	70	5.84	9.1	6.6
42	3.00	3.9	1.6	45	5.82	2.8	4.1
30	2.94	12.9	5.5	69	5.80	5.1	18.2
48	2.90	7.4	3.7	63	5.69	13.5	11.6
31	2.74	4.6	5.1	67	5.64	6.7	13.2
46	2.55	3.8	5.8	50	5.60	15.5	4.1
24	2.44	2.3	12.7	60	5.44	7.9	8.9
50	2.36	5.6	6.0	97	5.22	2.9	11.9
				22	4.95	13.6	9.1
				25	4.70	13.4	4.2
				25	4.52	13.4	4.2
Maximum	5.87	12.9	18.9		6.00	15.5	18.2
Minimum	2.36	2.1	1.6		4.52	2.8	4.1
Average	3.10	5.8	8.0		5.44	9.7	9.0
		13.8				8.7	
C(γ)/body wt.(mg.)		4.5				3.4	

Females				Males			
with eggs only							
Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb. (γ)	Number used	Body wt. (mg.)	Combined carb. (γ)	Free carb. (γ)
12	3.61	24.9	11.7	23	1.00	4.4	7.8
17	3.54	10.8	29.2	25	0.98	6.7	6.1
				12	0.98	2.5	10.8
				54	0.97	3.4	0.9
Maximum					1.00	6.7	10.8
Minimum					0.97	2.5	0.9
Average	3.58	17.8	20.4		0.98	4.3	6.4
		38.2				10.7	
C(γ)/body wt.(mg.)		10.6				10.7	

TABLE IX

Carbohydrate content of egg, larva and pupa, Culex pipiens var. pallens.

Egg				First inst. L.			
Number used	Body wt. (mg)	Combined carb. ($\gamma \times 10^{-2}$)	Free carb. ($\gamma \times 10^{-2}$)	Number used	Body wt. (mg)	Combined carb. ($\gamma \times 10^{-2}$)	Free carb. ($\gamma \times 10^{-2}$)
10270	4.5	1.16	1.08	1860		7.94	0.88
17910	4.5	0.74	0.54	3700		3.58	1.01
10420	4.5	0.63	0.84	4500		2.40	2.71
Average	4.5	0.84	0.81			4.64	1.51
Total carb. (average)		1.65				61.5	

Second instar L.				Third inst. L.			
Number used	Body wt. (mg)	Combined carb. ($\gamma \times 10^{-2}$)	Free carb. ($\gamma \times 10^{-2}$)	Number used	Body wt. (mg)	Combined carb. ($\gamma \times 10^{-2}$)	Free carb. ($\gamma \times 10^{-2}$)
1100		23.6	41.9	360	540	33.4	44.4
1068		18.6	10.4	360	440	14.3	32.9
1300		15.2	8.6				
1450		10.0	10.0				
Average		16.8	17.7		490	23.8	38.6
Total carb. (average)		34.5				62.4	

Fourth inst. L.				pupa			
Number used	Body wt. (mg)	Combined carb. ($\gamma \times 10^{-2}$)	Free carb. ($\gamma \times 10^{-2}$)	Number used	Body wt. (mg)	Combined carb. ($\gamma \times 10^{-2}$)	Free carb. ($\gamma \times 10^{-2}$)
174	1910	440	340	45	2180	870	550
162	1230	150	190	30	2030	800	860
130	950	190	370	26	1970	290	1020
200	800	90	100				
Average	1220	220	250		2060	650	810
Total carb. (average)		470				1460	

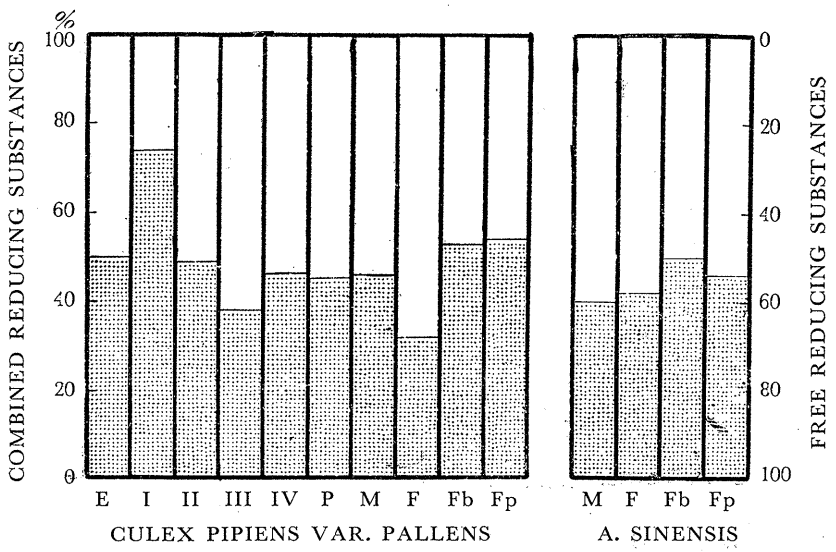

Fig 1. The combined to free carbohydrate ratio. E: eggs. I-IV: first to fourth instar larvae. P: pupa. M: male imago. F: female imago without eggs. Fb: female imago with blood in abdomen. Fp: female imago with eggs.

Discussion

The data described above contributes to reveal the characteristic feature of the vicissitude of the content of nitrogen, fatty substances and carbohydrate in mosquitoes with regard to their growth. Figures 2 (nitrogen), 3 (fatty substances) and 4 (carbohydrate) are the graphical representation in which logarithmic number of contents is compared with the advance in growth stages. These disclose the approximately linear lines, which indicate that the content of nitrogen, fatty substances and carbohydrate exhibit roughly exponential increase with the advance in growth stages, except at the time of the development of imagines from the pupae. Fatty substances and carbohydrate decreases, whereas nitrogen increases when a mosquito hatches from a pupa. Nutrition supplied by the ingestion of blood will be responsible for the fact that the female with eggs is superior to the pupa in the content of all the relevant body constituents.

The scrutiny of the figures reveals, however, that the exponential increase applies, in the strict sense, only either to the period of larval growth or to a restricted part of the whole mosquito growth. The increase in nitrogen content associated with the development of the first instar larvae from the eggs is more pronounced than that during the

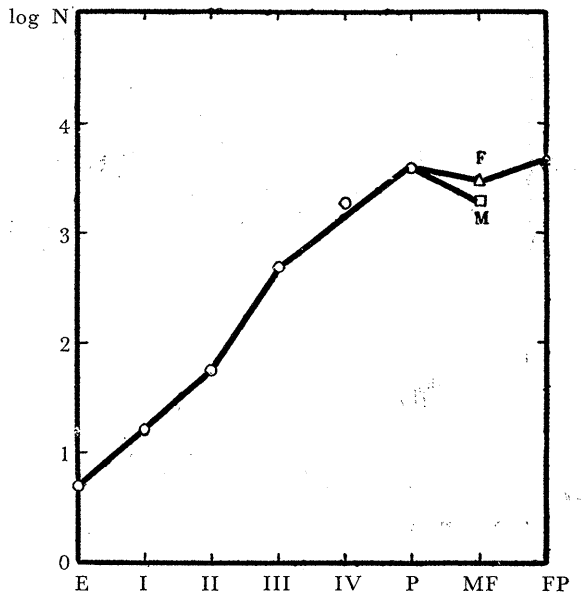

Fig. 2. Vicissitude of the content of nitrogen (N, expressed in terms of 1/100γ) during the course of mosquito growth. E: egg. I-IV: first to fourth instar larvae. P: pupa. M: male imago. F: female imgo without eggs. Fp: female imago with eggs.

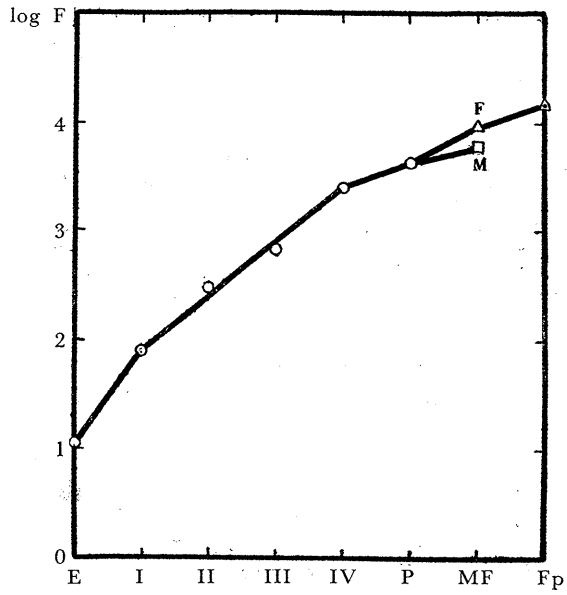

Fig. 3. Vicissitude of the content of fatty substances (F, expressed in terms of 1/100 γ) during the course of mosquito growth. E: egg. I-IV: first to fourth instar larvae. P: pupa. M: male imago. F: female imago without eggs. Fp: female imago with eggs.

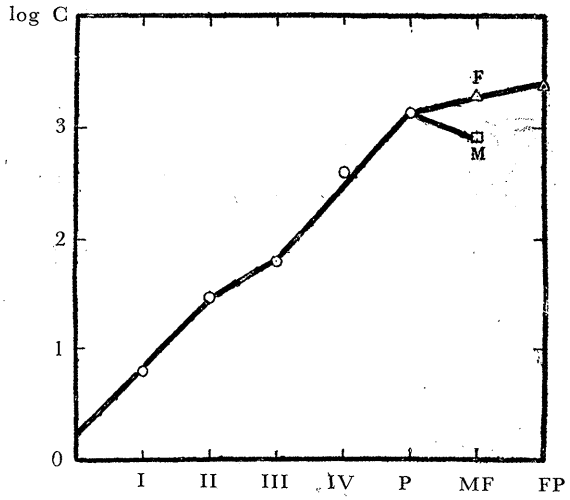

Fig. 4. Vicissitude of the content of carbohydrates (C, expressed in terms of 1/100 γ) during the course of mosquito growth. E: egg. I-IV: first to fourth instar larvae. P: pupa. M: male imago. F: female imago without eggs. Fp: female imago with eggs.

larval stages, whereas nitrogen content rises less markedly when the fourth instar larvae grow into the pupae. Exponential increase in fat content is also confined to the stages from the egg to the second instar larva and from the third instar larva to pupa with a steep fault at the stage from the second to the third instar, when prominent rise in fat content occurs. The vicissitude of carbohydrate content is quite similar, but increase is, on the contrary, depressed when the second instar larva develops the third. It seems, accordingly, to be most likely that fat increases with more speed at the expense of carbohydrate at the step from the second to the third instar than at the other period.

It will deserve special mention that the increase rate is largest in carbohydrate and smallest in nitrogen when contents of the bodily constituents are compared between the stages of eggs and pupae, being just in the reverse order of the absolute contents.

Conclusion

Mosquito's contents of nitrogen, fatty substances and carbohydrates were studied with Culex pipiens var. pallens and Anopheles hyrcanus var. sinensis as materials in order to elucidate the vicissitude of these constituents during the whole course of their growth.

1) The procedures for the determination of nitrogen, fatty substances and carbohydrates in mosquitoes were presented in detail.

2) The average contents (in γ) of the mosquito's body constituents

were as follows.

Culex pipiens var. pallens

Nitrogen: egg 0.11; larvae 0.81, 2.92, 6.50 and 26.0; pupa 43.7; imago male 63, female 119 (without eggs), 173 (with eggs).

Fat: egg 0.07; larvae 0.27, 0.83, 8.30, 26.60; pupa 54.5; imago male 27.3, female 43.1, (without eggs), 71.1 (with eggs).

Carbohydrate: egg 0.017; larvae 0.062, 0.35, 0.62, 4.70; pupa 14.6; imago male 8.0, female 21.0 (without eggs), 33.8 (with eggs)

Anopheles hyrcanus var. sinensis

Nitrogen: male 67.7; female 132.0 (without eggs), 197.0 (with eggs).

Fat: male 31.6; female 45.5 (without eggs), 77.7 (with eggs).

Carbohydrates: male 10.7; female 13.8 (without eggs), 38.2 (with eggs).

The fat to nitrogen ratio was about 2/5 in imagines, although it almost attained to 1/1 in the third and fourth instar larvae as well as in pupae. The carbohydrate to nitrogen ratio was 1/5 to 1/10, while somewhat higher ratio was obtained in the fourth instar larvae and the pupae.

3) These body constituents increased in an approximately exponential curve when the mosquito grows through the stage from egg to pupa. However, strictly speaking, exponential increase applied only to the larval growth, especially for its nitrogen content. Abrupt rise in the increase rate occurred for fat at the expense of the increase of carbohydrate when larva grows from the second to the third instar.

4) The increase rate was largest in carbohydrate content and smallest in nitrogen content. It was therfeore in reverse relation to the absolute contents of these substances, because body constituents were abundant in the order of nitrogen, fat and carbohydrate.

5) Fat and carbohydrate decreased when pupa developed imago, although increase in nitrogen was noted even at this period. The decrease was more marked when a male was produced than when a female was hatched. The ingestion of blood entailed a distinct rise in the content of nitrogen, fat and carbohydrate in female mosquitoes.

Grateful acknowledgments are due to Prof. Shigeki Mori for his advice in preparing the manuscript as well as to Prof. Susumu Shibata for his kind direction in this study.

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