ANNAL. BIOL. TIHANY | 27 | 9—14 | HUNGARIA 1960

CHANGES IN THE COMPOSITION OF FATTY ACIDS IN *BOMBYX MORI* L. IN THE COURSE OF ONTOGENY

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Received: 15th March, 1960.

Silkworm as a domestic animal occupies a special place among Insects and in theoretical works is often used as experimental animal. Thus many earlier publications refer to the fat content of *Bombyx mori* L. in various developmental stages, the most detailed accounts having been given in some studies carried out in the Nencky Institute. One of these (S. NIEMIERKO—WLODAWER—WOJTCZAK 1956) closely followed the changes in fat content throughout ontogeny. Fat content as per dry matter diminished in the caterpillars hatched out as against the egg and remained nearly constant through four consecutive moults. This value displayed already in the larval stage a difference according to sex, which later increased, the male imagines finally containing twice as many lipids as in dry matter than the female. Changes of fatty acid composition within the fats were only examined from the 4th moult until entering pupation with daily measurements of iodine and rhodan number (W. NIEMIERKO 1947). In this period a strong desaturation was observed and the absolute amount of oleic and linolenic acid further increased even after cessation of feeding.

In the series of examinations published in the present paper it was attempted to trace the changes of fatty acid composition with the aid of quantitative paper chromatography throughout ontogeny.

Material and method

Examinations were conducted in spring 1959 on the silk worm race "Itáliai Fehér" (White Italian). Eggs were examined in two different stages, *viz.* at the end of the diapause and immediately before the hatching, larvae were sampled in the larval stages invariably before moulting. After pupation the praepupa and the 3 and 10 days old pupae separated according to sex were examined. The imagines were analysed one or two hours after hatching, thus the females already before oviposition.

Samples were dried out in a vacuum-drier, ground in a mortar and extracted for two hours with warm petroleum ether in a nitrogen atmosphere. The extract evaporated to the volume required was saponified in the cold way and after having removed the unsaponifiable compounds the fatty acids

were released as usual. Saturated and unsaturated acids were separated with a selectivity suitable to the purpose with TWITSCHEL's method. After having determined the weight ratio of the solid and liquid fractions, the fatty acids were paperchromatographed according to KAUFMANN's method. A detailed description of the method is found in a paper on methodics (FARKAS—HERODEK 1959). As a variant: developing was carried out with rubeanic acid to obtain well-defined dark spots. Quantitative evaluation of the paper chromatograms was performed photometrically.

Results and discussion

The results of analyses are summarized in *Table 1.* When evaluating the data in this *Table* it should be taken into consideration that whereas in the case of pupae changes within a moderately diminishing quantity of fats

Table 1 — 1. táblázat

Fatty acid composition per cents in silkworm at various stages of ontogeny
A selyemhernyó %-os zsírsavösszetétele az ontogenezis különböző állapotaiban

Condition examined Vizsgált állapot	saturated — telített			unsaturated — telítetlen		
	arachidic	stearic	palmitic	oleic	linoleic	linolenic
Egg in diapause Diapuzáló pete	—	0,9	15,4	39,1	9,0	35,6
Egg before hatching Pete kikelés előtt	—	4,0	14,0	37,7	9,3	35,0
1st moulting — 1. vedlés	0,3	7,5	10,3	26,7	28,5	26,7
2nd moulting — 2. vedlés	0,8	12,1	7,1	14,7	44,2	21,1
3rd moulting — 3. vedlés	1,3	14,3	5,4	6,0	50,4	22,6
4th moulting — 4. vedlés	1,5	10,8	10,0	18,1	25,6	34,0
Praepupa — bekötött hernyó	—	4,1	26,9	20,8	14,7	33,5
3 day male pupa 3 napos hím báb	—	5,6	23,7	29,1	12,5	29,1
3 day female pupa 3 napos nőstény báb	—	7,2	23,6	22,4	14,5	32,3
10 day male pupa 10 napos hím báb	—	7,5	30,0	25,6	10,9	26,0
10 day female pupa 10 napos nőstény báb	—	3,5	17,8	28,0	15,1	35,6
Male imago — hím lepke	—	5,9	29,9	24,1	10,1	30,0
Female imago — nőstény lepke	—	2,5	14,8	29,2	16,2	37,3
Mulberry leaves — eperfalevél	—	2,3	13,5	11,0	36,4	36,8

are dealt with, in the case of larva stages, on account of the very rapid increase of the animals, the quantity of fats in the former stage is almost insignificant. Thus differences found in the larval stages should be interpreted as a change of the synthetic and assimilative processes. It is not known what changes take place within the larval stages since the end points were examined only, It is striking however that in the four morphophysiologically identical stages before moulting when fat content in dry matter was also the same (S. NIEMIERKO—WLODAWER—WOJTCZAK 1956) there are significant differences in the composition of fatty acids. As shown by the *Table* the change of the fatty

acid composition from the egg in diapause to the 3rd moulting exhibits a uniform trend asserting itself in a significant increase of the arachidic-stearic and linoleic-acid content, with the relative quantity of palmitic and oleic diminishing accordingly. Before the 4th moulting a reversion occurs in these changes as compared with the 3rd. When comparing the values obtained for the stage preceeding the 4th moulting with the data of NIEMIERKO referring to the first day after moult it is surprizing that linolenic acid completely disappeared while the percentage of the saturated acids is rising from 22.3 to 62.3 per cent, which can be only the result of the very intensive synthesis. Data obtained for saturated and oleic acid quantities in the praepupa, are in a good agreement with those of NIEMIERKO, while in our analyses substantially more linolenic acid was found as against linoleic acid. In our opinion the method used may be responsible for that, and the question arises whether it would not be advisable to show linolenic acid always present with paper chromatography also as to the days after moulting.

As to the origin of fat in the larvae already BIALASZEWICZ stated that the fully-developed larvae contains about twice as much fat as could have been taken up by it from food. It is not known however what part of fat originating from the food is deposited or used up respectively. The great amount of stearic acid as compared with the low value obtained from the leaves points to the intensity of synthetic processes.

The animals were only separated according to sex after pupation. Fatty acid composition in three days old male and female pupae and in praepupae is very similar, so it is not likely that more considerable differences might have been found as to sex in former stages. In ten days old pupae on the other hand very important differences were observed which still intensified in the imagines. The essence of this difference is that in the males the proportion of the saturated acids increased while in the females during egg formation it drops back to the low level found it the eggs. These conditions are clearly revealed in *Fig. 1*, showing the amount of saturated acids in the percentage of the total fatty acid in different stages of ontogeny.

DEMIANOVSKY and ZUBOVA (1956) examining the fats of *Antheraea pernyi* GUER. emphasized the analogies againts the *Bombyx mori* which is easy enough to understand in view of the many resemblances between the two "silk worms". When on the other hand these present data now are compared with the results obtained for *Antheraea* it is surprising that in *Antheraea* — as may be concluded from iodine value — there is no such change in fatty acids during the pupal period than in *Bombyx*. Iodine value in male and female *Antheraea* pupae are within a close range to each other, showing all the time but insignificant fluctuations. Fatty acid composition was only determined in the pupa in diapause and the values obtained are surprisingly similar to those found in the eggs of *Bombyx*. Fatty acid analysis was performed with oxidation according to BERTRAM and iodine value, rhodanine number measuring, making also fractioning according to TWITSCHEL's method. The value obtained for the solid fraction was 17.3—17.6 thus nearly the same as measured in the egg.

Since *Bombyx* hibernates in the form of egg while *Antheraea* in the form of pupa, the question arises whether the differences found in the pupa stage of the two animals do not cover identity n fatty acid composition of the hibernating forms. Recently THIELE (1959) in snails and the present authors

(HERODEK—FARKAS 1959) in Crustaceans demonstrated fatty acid composition assuring a lower melting point in winter than in summer. For insects but the data of SLIFER (1932) are known hitherto, who found in the case of some locust species that in eggs of the species hibernating in this form melting

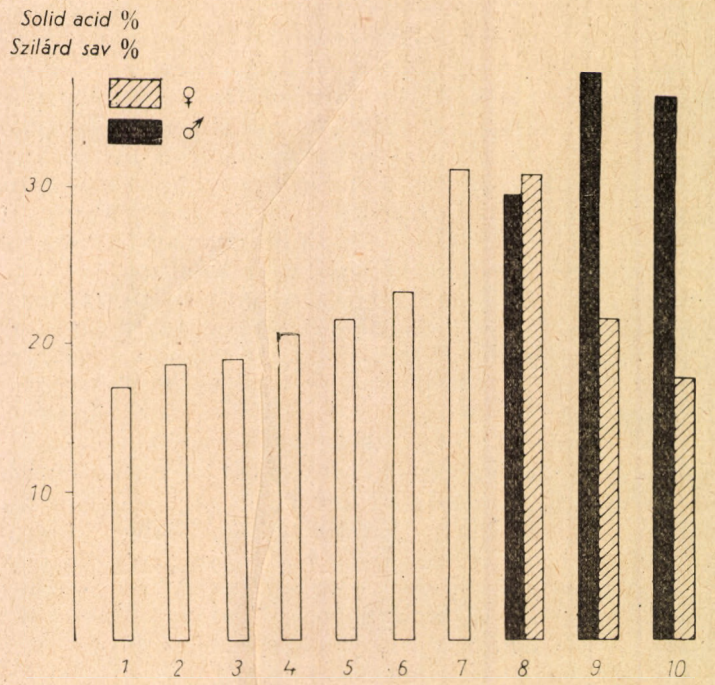

Fig. 1 The amount of the solid fraction in per cents of total fatty acid in various developmental stages of the silkworm.
1 — Egg (graine) in diapause, *2* — egg before hatching, *3* — 1st moulting, *4* — 2nd moulting, *5* — 3rd moulting, *6* — 4th moulting, *7* — praepupa, *8* — three day pupa, *9* — ten day pupa, *10* — imago

1. ábra. A szilárd frakció nagysága az összes zsírsav %-ában, a selyemhernyó különböző fejlődési állapotaiban.
1 — diapauzáló pete, *2* — pete kikelés előtt, *3* — első vedlés, *4* — második vedlés, — *5* harmadik vedlés, *6* — negyedik vedlés, *7* — bekötött hernyó, *8* — háromnapos báb, *9* — tíznapos báb, *10* — imágó

point of the fats is lower than in those hibernating in the nymph stage. As against cases known hitherto for the relation between composition of natural fats and temperature, the fats of low melting point in the hibernating forms of the insects must have been developed earlier, at the time of egg-laying or pupation, *i.e.* still under warm conditions. Anyway, it would be most interesting to know, how far the fatty acid composition warranting a low melting point may be considered as a general attribute of the hibernating forms.

The authors wish to express their gratitude to their fellow-worker ANDRÁS GUBICZA who kindly placed the silkworms to their disposal from his own cultures and helped ther with several information.

Summary

Fatty acid composition in the silkworm was determined is several ontogenetical conditions.

From the egg in diapause to the 3rd moult an identical trend was observed in the changes of fatty acid contents.

During metamorphosis a sharp difference arose according to sex, since in the males the amount of saturated acids increased while in the females it fell back to the low level in eggs.

When comparing the results with the data found in literature for *Antheraea* the authors were led to the conclusion that a substantial difference exists in the ontogenetical changes of fatty acid composition in the two species probably due to the different hibernation habit.

REFERENCES

Демяновский Л. Я и В. А. Зубова (1956) Жиры дубового Шелкопяда (Fats int he organism of the Chinese tussur moth) Биохимия **21**, 676—682.

Farkas T.—Herodek S. (1959): Bonyolult zsírsavkeverékek papírkromatográfiája (Paper chromatography of complex fatty acid mixtures). — *Annal. Biol., Tihany* **26**, 203—209.

Herodek S.—Farkas T. (1959): Évszakos változások az Astacus leptodactylus Esch. zsírsavösszetételében (Seasonal changes in the fatty acid composition of Astacus leptodactylus Esch.). *Biol. Közl.* **7**, 53—59.

Niemierko, S.—Wlodawer, P.—Wojtczak, A. F. (1956): Lipid and phosphorus metabolism during growth of the silkworm (Bombyx mori L.). — *Acta Biol. Exper.* **17**, 255—276.

Niemierko, W. (1947): Przemiany kwasow tluszczowych gasienic jedwabnika. — *Acta Biol. Exper.* 14, 137—150.

Slifer, E. H. (1932): Insect development. Quantitative studies on the fatty acids from grasshopper eggs. — *Physiol. Zool.* 5, 448—456.

Thiele, O. W. (1959): Die Lipide der Weinbergschnecke (Helix pomatia L.) I. Mitteilung: Jahreszeitliche Veränderungen in der Zusammensetzung der Lipide. — *Z. vergl. Physiol.* **42**, 484—491.

A *BOMBYX MORI* L. ZSÍRSAVÖSSZETÉTELÉNEK VÁLTOZÁSA AZ EGYEDFEJLŐDÉS FOLYAMÁN

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Összefoglalás

Kvantitatív papírkromatográfia segítségével meghatároztuk a selyemhernyó zsírsavösszetételét néhány egyedfejlődési állapotban.

A diapauzáló petétől a harmadik vedlésig a zsírsavösszetétel változásaiban azonos irányzatot észleltünk.

A metamorfózis folyamán a nemek között erős különbség lépett fel, a hímekben a telített savak mennyisége emelkedett, a nőstényekben visszatért a petéknek megfelelő alacsony szintre.

Eredményeinket az Antheraea-ra talált irodalmi adatokkal összehasonlítva arra a következtetésre jutottunk, hogy a kétféle selyemhernyó zsírsavösszetételének ontogenetikus változásában lényeges különbség van, melyet valószínűleg az áttelelés eltérő módja okoz.