VI. THE TYROSINE METABOLISM OF *BOMBYX MORI* (SILK WORM).

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IN view of the high tyrosine content of silk it was of interest to examine quantitatively the tyrosine intake of the silk worm and compare it with that of the silk produced. For this purpose one hundred eggs of the commercial silk worm, *Bombyx mori*, were purchased, from which 85 worms hatched. In six weeks 79 surviving worms had formed cocoons, varying in colour from a pale yellow to an intense orange-yellow. Throughout this period of six weeks the grubs were fed on mulberry leaves obtained from the Chelsea Physic Garden. The leaves were gathered in the morning and placed in a tin with well-fitting lid, weighed and fed to the grubs. The following morning all remains of the previous day's feed were removed, dried for 2 hours in a steam-oven and weighed. The weight of water removed from the mulberry leaves by this procedure was found to be on the average 77.95%, and the weight of leaves consumed by the 79 grubs corresponded to 125.71 g. of "dried leaves". The 79 cocoons and contained grubs were together dried and weighed and a portion was subsequently analysed for tyrosine. Some of the silk alone and also the mulberry leaves were likewise analysed. Both leaves and silk were placed before analysis in a steam-oven for 2 hours. The subsequent weights served as a standard and are referred to as "dried weight" throughout. The dried weight of the 79 cocoons *plus* contained grubs was found to be 21.10 g.

The mortality of 7% was surprisingly low, the dealer having led me to expect one of 50%.

Determination of the tyrosine intake.

Extraction preliminary to hydrolysis. Acetone, methylated spirit and a combination of the two were used in a Soxhlet apparatus to extract about 2 g. of dried leaves. Methylated spirit alone or followed by acetone was found to remove the greatest weight. Extraction of the fresh leaves with boiling methylated spirit in an open vessel proved to be a better method, as much as 32% of the dried weight being extracted as against 21% by the Soxhlet method. Although the chlorophyll and the other leaf pigments appeared to be removed completely, the leaves remained of a greyish tinge.

Hydrolysis of extracted leaves. For the hydrolysis and subsequent estimation of the tyrosine, the method of Folin and Ciocalteu [1927] was followed. The estimations of tyrosine were conducted on the day following the hydrolysis. Considerable difficulty was experienced in matching the unknown with the standard, owing to the yellow colour which persisted even after treatment with kaolin. In two of the following estimations caramel was added to the standard and the colour match was thereby much improved.

The figure given for tyrosine content is in each case the average of at least three colorimetric estimations. The estimations in which caramel was used were thought to be the more accurate. The tyrosine content was taken as the mean of these estimations, giving a value of 1.10 g. tyrosine per 100 g. dried leaves.

Weight of extracted leaves hydrolysed (g.)	Tyrosine content	
	% of dried weight of extracted leaves	% of dried weight of leaves
1.423	1.92	1.49
*2.166	1.59	1.33
*1.315	1.49	1.31
*1.203	1.69	1.23
*†1.206	1.55	1.09
	1.61	1.13
†1.567	1.59	1.07

* Hydrolysate shaken with kaolin. † Caramel added to standard.

Determination of the tyrosine content of silk together with contained grub.

The hydrolysis was performed as for the leaves without preliminary extraction; there was no necessity to employ kaolin or caramel since the colour of the hydrolysate did not interfere with the colour comparison. Some difficulty was experienced owing to the development of turbidities after heating with mercuric sulphate. This was obviated by adopting Folin and Marenzi's [1929] modification of the original Folin and Ciocalteu method [1927].

Hydrolysis number	Dried weight of silk <i>plus</i> grub hydrolysed (g.)	Tyrosine content % of dried weight
1	1.044	5.12
2	1.251	6.03
3	1.047	5.23
4	0.862	5.65
5	1.013	5.04
6	1.005	5.75
	Average	5.47
*7	1.043	5.05

* These cocoons were produced by a second batch of grubs whose eggs had been delayed from hatching by being kept in a cold place.

A number of cocoons of *Bombyx mori* from Cyprus were provided by the kindness of Dr Chandler of the Imperial Institute. The tyrosine content of the silk of these cocoons was estimated as above, and also that of the silk of the worms hatched here for purposes of comparison.

	Dried weight hydrolysed (g.)	Tyrosine content % of dried weight
Cyprian silk (1)	0.9915	10.0
(2)	0.9620	10.39
Own silk	0.731	10.3

The tyrosine content of silk of *Bombyx mori* obtained by this method by Silberman and Lewis [1932] was 9.75 % dry weight, or 11 % calculated on an ash-free basis. Whereas analysis of the silk alone gave a value of 10.3 %, analysis of the silk along with the contained grub gave the lower value of 5.47 %. The following figures show the weight of the grubs expressed as a percentage of the total weight of grubs *plus* silk:

Dried weight of six grubs (g.)	Dried weight of six grubs <i>plus</i> their silk (g.)	Dried weight of six grubs: % dried weight of grubs <i>plus</i> silk
2.056	3.497	58.80
2.150	3.645	58.98
1.991	3.310	60.14
1.831	3.028	60.47

Taking the average value of 59.6 g. of grubs to 40.4 g. silk, the grubs contained, by calculation, about 2% tyrosine. The variation in the tyrosine values expressed as a percentage of silk *plus* grubs (*viz.* 5.05 to 6.03 %) was expected, since as the above table shows, the grub does not form a constant weight of the cocoon.

*Comparison between tyrosine intake of worms and tyrosine of silk
and contained grub.*

Taking the tyrosine content of silk *plus* grub as 5.47 %, the 21.10 g. of silk *plus* grub from the 79 worms contained 1.15 g. tyrosine. The tyrosine content of the leaves was 1.10 %, so that the 125.71 g. of leaves ingested by the 79 worms represented a tyrosine intake of 1.38 g. That is considerably more than was found in the silk *plus* grubs. The excess of tyrosine intake over the output for the 79 grubs during the six weeks, was 0.23 g. No estimation was made of the loss of nitrogen by the excreta. In conclusion then, it is probable that the intake of tyrosine in the proteins of the mulberry leaves during the life history of the silk worm is sufficient to account for the high proportion of that amino-acid in the silk produced. There is no need therefore to have recourse to the supposition of tyrosine synthesis on the part of the worms.

SUMMARY.

The tyrosine intake in mulberry leaves ingested by silkworms (*Bombyx mori*) was found to exceed the output of tyrosine in silk and contained grubs; hence there is no need to postulate a mechanism for the synthesis of tyrosine.

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