On the Biochemistry of the Wild Silk-Moth, *Dictyoploca japonica*, Moore.
1. Chemical Development in the Growth of the Wild Silk-Moth.

By

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The *Dictyoploca japonica* (Saturnidae), commonly called Kusu-san or Shiraga-tarô, is widely spread in Japan. It lives mainly on the leaves of the poplar, camphor, walnut, chestnut, etc., and accordingly the worm is deemed noxious to the forestry. It is, however, regarded as a useful insect, in some respects, it being used as a substitute for the *Saturnia pyretorum* in obtaining catgut for fishery "Tegusu," and also its cocoon is availed of in the textile industry.

The nutritive physiology of most insects, especially the orders Lepidoptera and Hymenoptera, has aroused the present author's interest since they undergo complete metamorphosis, and also they are usually fed on certain definite sorts of food. The present experiment was, therefore, undertaken to find what, if any, relationship might exist between the chemical constituents of the insect and its food—the leaves of the chestnut.

Since the classical researches of Peligot[1] and of Kellner[2] on the silk-worm, many experiments in relation to the biological and chemical study of it were made by scientists[3], amongst which those of S. Kawase[4] and

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1 E. Peligot: C. R., **33**, 490-495 (1851); ibid., **34**, 278-282 (1852).

2 O. Kellner: Landw. Versuchsst., **30**, 59 (1884).

3 E. Bataillon & E. Couvreur: C. R. Biol., **44**, 669-671 (1892); E. Couvreur: ibid., **47**, 796-798 (1895); C. Vaney & F. Maignon: C. R., **140**, 1192-1195 (1905); ibid., **140**, 1280-1283 (1905); K. Kominato: Chosen Igakukwai Zasshi, No. **53**, 1-63 (1925); for other Japanese literature, see: K. Ishikawa: "Nippon Sanso Bunken Mokuroku" (List of Works on Japanese Sericulture) Tokyo. (1922).

4 S. Kawase: N gaku Kwaih , No. **136**, 21 (1923); ibid., No. **144**, 9 (1914).

E. Hiratsuka[1] seem to have the greatest value for the knowledge of the chemical development in the growth of the insect, and of the relation between the chemical constitution of the food material and the insect. There are, however, relatively few experiments made on other Lepidopterous insects[2].

MATERIAL

The eggs of the insects which were laid on the trunks of the poplar-trees in Hanazono near Kyôto were collected and brought up in the laboratory, the caterpillars hatched out being fed with chestnut leaves. The chemical analysis of the samples in the several stages of its life-cycle was made on the third day of each instar; but the first instar was not analysed owing to the small quantity of eggs obtained. The data of the life-cycle, and the mean weight of individuals at fixed times are given in the following table.

During the pupal stage of the insect, the decrease in the body weight is abrupt only just after pupation and before emergence[3]. In the following table, the pupae both on the lst. of August and on the 16th. of September show almost the same weight (a small variation is due to individual difference) whereas the first emergence is dated 16th. September, but this peculiar result appear since this emergence is an extraordinarily early one, it generally happening in late October. I can not agree with the idea of Linden[4] that the insects are able to assimilate carbon dioxide to sugar in an atmosphere rich in CO_2 and moisture during the pupation and thus the imagos are often heavier than the pupae.

Chestnut trees (*Castanea sativa*) were grown at Uriu-yama, Kita-shirakawa, Kyôto; their leaves were collected at 10 a.m. every day[5]. The samples used for analysis were always obtained on sunny days, except in the rainy season of June (5th. June).

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1 E. Hiratsuka: Sangyô Shikenjô Hôkoku (Bull. Imper. Seric. Exper. Sta.), **2**, 353-412 (1917).

2 E. Abderhalden: Zs. physiol. Chem., **127**, 93-98 (1923); W. S. Ssadkow & A. P. Winogradow: Biochem. Zs., **150**, 372-376 (1924); E. Weinland: Zs. Biol., **47**, 186-231, 232-250 (1906); ibid., **48**, 87-143 (1906).

3 W. Blasius: Zs. wiss. Zool., **16**, 135-177 (1866); F. Urech: Zool Anz., **13**, 254-260, 272-280 (1890); Vaney & Maignon: loc. cit.

4 Maria v. Linden: C. R. Biol., **57**, 692-694 (1904); Arch. (Anat. u.) Physiol., Suppl., (1906); L. Bounoure: C. R., **156**, 633-636 (1913).

5 Kawase, Saito & Kondo: Nôgaku Kwaihô, No. **173**, 13-18 (1916); Hiratsuka: Sangyo Shikenjo Hokoku, **3**, 321-338 (1918); Nogaku Kwaiho, No. **192**, 950 (1918); Ihobara & Fujise: Nogaku Kwaiho, No. **201**, 505-532 (1919); Kawase & Suda: Sanshi Kwaiho (Rep't. Jap. Seric. Assoc.), **28**, 34 (1919).

Table I.

Data of Life-Cycle.			Weight per 100 Individuals.		
Stage	from	to	Stage	Date	Gaammes
Hatching.	30/IV	10/V	2nd. Instar	19/V	7.8
1st. Moulting	11/V	21/V	3rd. 〃	29/V	17.0
2nd. 〃	19/V	29/V	4th. 〃	5/V	84.1
3rd. 〃	1/VI	8/VI	5th. 〃	19/VI	269.9
4th. 〃	10/VI	20/VI	Pupa	10 VII	256.9
Cocooning	25/VI	14/VII	〃	1/VIII	181.7
Pupation.	3/VII	14/VII	〃	16/IX	190.3
Emergence.	16/IX				

ELEMENTARY ANALYSIS

The content of carbon, hydrogen and ash of the materials–leaves and insects–were determined by the combustion-method after being dried to constant weight at 105° C. in an air bath, and the results are shown in Table II. These result show a constancy in percentage of these three elements through all stages in the life of the caterpillars, the pupae being a little higher in carbon and hydrogen compared with the caterpillers. An abrupt decrease in ash content after pupation is caused by the elimination in the faeces of mineral matters, especially alkaline carbonates, just before the cocooning[1]. This fact is very interesting with respect to the nutritive physiology, since it has some relation with the decrease of carbohydrate metabolism in the pupal stage.

Chestnut leaves show a constancy in the content of hydrogen and gradual decrease in that of carbon and ash, especially of the latter.

From these analytical figures of the insect and its food material, it was considered that, from the theoretical view point of nutrition, one part of food is enough for making up one part of the carbon and hydrogen constituents of the insects, assuming that all of the constituents of the former can be utilized for the forming of the latter, while two parts of the leaves would be required to form one part of the ash-constituent of the insect. As a matter of fact, the insect consumed much more of the leaves than necessary, and this phenomenon will be explained by the indigestibility of the vegetable fibres in the insect, which has already been shown analytically by Kellner[2], Hiratsuka[3], and Kawase[4] with the silk-

1 Peligot: loc. cit.; Verson: Zool, Anz., **13**, 558-559 (1890); Hiratsuka: loc. cit.
2 O. Kellner: loc. cit.
3 E. Hiratsuka: loc. cit.
4 S. Kawase: loc. cit.

worm; by means of the microscope also it can easily be recognized, the cell walls of the leaves, being noticed as remaining undigested in the rectum[1].

The ash of the insect of the 4th. and 5th. stages is coloured a light green; the cause of this coloration may be ascribed to the presence of manganese in it, and this hypothesis is very plausible as the presence of oxydase in blood of the insects was already shown by many investigators[2]; but not to copper contaminated in the course of combustion, a fact noticed by Zelinsky[3] in an anaerobic condition (Dumas' method for nitrogen).

The total nitrogen content was determined by Kjehldal's method with a fresh sample, taking methods of desiccation into consideration for the analysis, which should be affected on the content[4].

Nitrogen was regarded as an important element in the insect to form protein matter in the tissue, which by turn would be utilized, as we suppose, in forming the principal constituent of the cocoon. Consequently, the amount of this element should be increased with age, but the analytical results, kept constant through the whole of the instars, contradicted this assumption. To our astonishment, after cocooning, the nitrogen content increased from

Table II.

Dictyoploca.

Date	Stage	Weight	CO_2	H_2O	Ash	C	H	Atomic ratio C/H	Ash
19/V	2 Inst.	·0774	·1323	·0383	·0081	46·7	5·6	·70	10·5
29/V	3 〃	·0902	·1540	·0411	·0094	46·5	5·1	·77	10·4
5/VI	4 〃	·1271	·2184	·0644	·0126	47·1	5·7	·69	9·9
—	– –	·1299	·2222	·0650	·0132	46·7	5·6	·72	10·2
19/VI	5 〃	·1638	·2822	·0852	·0159	47·0	5·8	·68	9·7
—	– –	·1409	·2422	·0736	·0134	46·9	5·9	·67	9·5
10/VII[a]	Pupa	·1120	·2069	·0649	·0066	50·4	6·5	·65	5·9
—	—	·1160	·2141	·0663	·0073	50·4	6·4	·66	6·3
31/VII[b]	〃	·0740	·1345	·0414	·0047	49·6	6·3	·66	6·4
16/IX	Moth	·1033	·1909	·0606	·0071	50·4	6.6	·63	6·9

1 J. Suzuki: Sanshi Graphic, **2**, No. **5**, (1925).

2 J. Dewitz: C. R. Biol., **54**, 44–45, 45–47 (1902); M. Sawamura: Sanshi Kwaihō, No. **146**, 12 (1902); Kawase, Suda & Saitō: Tokyō Kwakaku Kwaishi (J. Chem. Soc. Tokyo), **42**, 118–130 (1921); R. Inouye, Iwaoka & Hiratsawa denied the presence of oxydase in the blood of the silk-worm (*Bombyx mori*, L.) &c., and only recognized the presence of tyrosinase (Nogaku Kwaihō, No. **221**, 99–114 (1921).

3 N. D. Zelinsky: C. R., **177**, 1041–1043 (1923); Biochem. Zs., **146**, 91–96 (1924).

4 K. Katayama: Sangyō Shikenjō Hokoku, **2**, 325–352 (1917); Chibnal: Biochem. J., **16**, 529–607 (1922); K. P. Link & E. R. Schulz: J. Amer. Chem. Soc., **46**, 2044–2055 (1924).

1·2% to 2·6% i. e. 9·2% to 10·4%: when calculated in dry materials.

On the other hand, the content of carbon and hydrogen as shown above, show a slight increase of from 47% to 50% and from 5·9% to 6·1% respectively; these results clearly show that the metabolism and respiration which are necessary for the maintenance of life during the cocooning, must have taken place mainly at the cost of fatty reserve materials.

Castanea

Date	Stage	Weight	CO_2	H_2O	Ash	C	H	C/H	Ash
30/IV	1 Inst.	·1271	·2336	·0607	·0061	50·2	5·3	·80	4·8
—	— —	·0948	·1748	·0428	·0049	50·3	5·0	·85	5·2
10/V	1–2 〃	·0875	·1561	·0422	·0038	48·7	5·4	·76	4·2
—	— —	·1225	·2170	·0600	·0050	48·5	5·5	·74	3·7
23/V	2–3 〃	·1638	·2971	·0800	·0063	49·5	5·5	·76	3·9
—	— —	·1690	·3060	·0822	·0063	49·4	5·4	·77	3·7
5/VI	4 〃	·1216	·2203	·0608	·0045	49·4	5·6	·74	3·7
—	— —	·1112	·2020	·0558	·0046	49·6	5·6	·75	4·1
13/VI	4–5 〃	·1398	·2517	·0677	·0052	49·1	5·4	·72	3·7
—	— —	·1012	·1834	·0509	·0039	49·4	5·7	·73	3·9
26/VI	5 〃	·1331	·2390	·0644	·0048	49·0	5·4	·72	3·6
—	— —	·1560	·2797	·0749	·0058	48·9	5·4	·72	3·7

a 5th day after the pupation.
b 25th. day after the pupaiion.

Table III.

Total Nitrogen

Dictyoploca

Sample		Nitrogen	
Date	Weight	Gram.	%
19/V	·7098	·00906	1·3
29/V	1·2246	·0148	1·2
5/VI	1·7512	·0180	1·1
—	1·6846	·0171	1·0
14/VI	1·5625	·0168	1·1
—	2·3699	·0272	1·1
19/VI	2·3073	·0278	1·2
♀10/VII[a]	3·0500	·0892	2·6
♀31 VII[b]	2·8728	·0863	3·0
♀ 1/VIII[b]	2·1388	·0673	3·2

a 5th. day after the pupation.
b 25th. day ofter the pupation.

Castanea

Sample		Nitrogen	
Date	Weight	Gram.	%
30/IV	·3463	·00417	1·2
—	·5520	·00556	1·0
10/V	1·2981	·0120	·9
—	1·7316	·0163	·9
23/V	1·5424	·0150	1·0
—	1·2650	·0139	1·0
5/VI	1·3088	·0119	·9
13/VI	1·5153	·0142	·9
—	1·7722	·0152	·9
26/VI	·7859	·0068	·9
—	·9231	·0082	·9

Table IV.
Analysis of cocoon

Date	Weight	N	CO_2	H_2O	Ash	N	C	H	C.H	Ash
1/VIII	·0901	—	·1626	·0480	·0006	—	48·7	6·0	·68	·7
〃	·1093	·0182	—	—	—	16·7	—	—	—	—

N. B. The percentage of moisture of the sample was 10.2%.

The great loss of weight during the pupation, as we actually observed, will indicate that there the biochemical reaction above-mentioned would have taken place in an active manner. Thus, the content of moisture, fatty substances, water-soluble matters and polysaccharides of the sample prepared from each stage of the life-cycle of the insect and that of the food material, was determined, to see, if possible, what biochemical change had occurred in these constituents.

PROXIMATE ANALYSIS.

Moisture.

The sample was dried at 105° C. to constant weight, and the loss of weight being assumed to be the moisture, the results in percentage for fresh materials are shown in Table V.

It is clear from these data that the moisture content of caterpillars is fairly constant (87%) while that of chestnut leaves decreases gradually from 78%, to 58%, and consequently water formed by some chemical change of some constituents of the leaves within the insect should have been stored in the body of the insect.[1]

During the pupal stage, the moisture content decreases gradually with age, and such a phenomenon indicates that respiration took place slightly.

1 S. M. Babock: Resear. Bull., No. **22**, Wisc. Agri. Exper. Sta., (1912).

Table V.

Moisture.

Dictyoploca			Castanea		
Date	Stage	Moisture	Date	Stage	Moisture
19/V	2 Inst.	86.	30/IV	1 Inst.	70.
29/V	3 〃	87.	——	——	70.
5/VI	4 〃	88.	10/V	1-2 〃	69.
——	——	88.	——	——	69.
14/VI	5 〃	87.	23/V	2-3 〃	64.
——	——	88.	5/VI	4 〃	61.
19/VI	5 〃	87.	——	——	61.
——	——	86.	13/VI	4-5 〃	61.
——	——	87.	——	——	62.
10/VII	Pupa	75.	26/VI	5 〃	58.
——	——	75.	——	——	58.
31/VII	〃	77.			
1/VIII	〃	75.			
16/IX[a]	〃	72.			

a 39th. day after the pupation.

Ether Extract.

The material dried at 105° C. above mentioned, had the fatty and other matters extracted with ether in Soxlet's apparatus until no more colouring matter came out into the solution. The results calculated on dry bases are tabulated below. The content of the ether extract in both the caterpillars and the chestnut leaves are constant, the former contains twice the percentage of the ether extract of the latter. In the stage of pupation, the formation of the fatty constituent was noticed.

Table VI.

Ether Extract.

Dictyoploca

Date	Extract
19/V	5·4
29/V	5·1
5/VI	5·3
—	5·0
14/VI	5·6
—	5·1
19/VI	4·8
—	5·5
10/VII	13·9
—	17 0
31/VII	13·1
1/VIII	18·6
16/IX	2·4

Castanea

Date	Extract
10/V	2·8
—	2·7
23/V	2·1
—	3·1
5/VI	2 2
—	2·4
13/VI	2·7
—	3·2
25/VI	2·1
—	2·4

Water Extract.

The residue separated from the ether extract was boiled with water for one hour, and filtered; the operation was repeated once more, and both filtrates being combined and made up to 100 c. c., of which the content of reducing sugar and of the water-soluble nitrogen was determined; the reducing sugar was estimated by means of Fehling's solution after eliminating some colouring matter in the extract with lead acetate, lead by sulphuretted hydrogen, and the nitrogenous matter was estimated by Kjehldal's method as usual. In the treatment with pupae, there occurred some difficulty in separating the insoluble matter from the aqueous solution, and a separation of a white crystalline substance, the quantity of which increases with age, was observed when the solution was concentrated after being clarified with lead salt.

The total amount of the extract of caterpillars is fairly constant (37%), but that of the chestnut leaves decreases markedly with growth (from 33% to 21%); the content of water-soluble nitrogen shows a gradual increase in the former, while in the latter it keeps constant, but the content of the reducing sugar shows no definite relation with age. The low percentage of sugar may, according to Link,[1] be due to the high temperature during the desiccation. The sudden decrease of the content of the water-soluble

1 Link & Tottingham: J. Amer. Chem. Soc., **45**, 439–449 (1923); Link: ibid., **47**, 470-476 (1925).

matter in the change from caterpillars to pupae, came to the author's attention, and it should be explained properly by connecting it with the metabolism of fatty matter in the life-cycle.

Table VII.

Water Soluble Constituents.

Dictyoploea

Date	Water Extract	Wat. sol. red. Sug.	Wat. sol. N
19/V	—	·74	3·0
29/V	37·4	1·12	2·9
5/VI	38·8	·04	—
——	36·1	—	4·1
19/VI	36 8	·10	4·6
——	37·1	·04	—
10/VII	—	·07	4·7
——	18·5	·06	4·2
31/VII	20·1	·08	2·3
1/VIII	25·2	·06	3·2
16/IV	33·3	·06	5·6

Castanea

Date	Water Extract	Wat. sol. red. Sug.	Wat. sol. N
10/V	33·1	—	—
23/V	28·6	·3	—
——	30·7	·3	·9
5/VI	24·7	—	·8
——	25·2	·3	·9
13/VI	23·8	·1	·9
——	23·3	·1	·8
26/VI	23·8	·0	·9
——	21·5	·0	·8

Hydrolysis.

The residue separated from the water-soluble matter was hydrolysed in a sealed tube with 1% HCl heated at 110°—120° C. for two hours. Reducing sugar thus formed was estimated by the usual method as in the above cases. In the case of the pupae, the solution shows only a deep crimson coloration when heated with the alkaline copper solution, though the occurrence of glycogene in measurable quantity was expected.

That the role of glycogene has much to do with the sexual activity can easily be ascertained by referring to the histo-chemical experiments of Kemnitz[1] on a parasitic worm, *Ascaris lumblicoides*, and also to reports by some other investigators on the sexual difference of the glycogene metabolism in the insect (the male consumes more glycogene than the female[2]). In any case, the absolute quantity is far smaller than that of fatty substances—the main reserve material.

1 Kemnitz: Arch. Zellforsch., **7**, 463—603 (1912).

2 Vaney & Maignon loc. cit.; Hiratsuka: Sanshi Kwaihô, **23**, 2—5 (1914).

The relative amount of polysaccharide which can be hydrolysed with dilute hydrochloric acid is greater in chestnut leaves than in caterpillars, keeping the relation in both cases through the whole life.

Table VIII.

Hydrolisable polysaccharide.

Dictyoploca

Date	Reducing Sugar
19/V	·1
29/V	—
5/VI	·2
—	·3
19/VI	·1
—	·2
10/VII	·2
—	·0
31/VII	·0
1/VIII	·2
16/IX	·0

Castanea

Date	Reducing Sugar
10/V	1·4
23/V	1·5
—	1·2
5/VI	·4
—	1·5
13/VI	2·0
—	·4
26/VI	1·2
—	3·4

N. B. The reducing sugar is calculated as d-glucose.

Polysaccharide.

The amounts of polysaccharides—cellulose or chitin—are estimated by Henneberg-Stohmann's method in the plant or animal tissues, and the crude polysaccharide thus obtained was then burnt in a crucible with an alcohol lamp and then ignited in an electric furnace to a constant weight.

As shown in the table below, the amount of the chitin in the caterpillars and pupae remains fairly constant through their life, while the cellulose content of the chestnut leaves show a marked abrupt increase with age. The marked decrease of the chitin in the change of stage from caterpillar to pupa, would be explained partly by the metabolic change of the "protochitin" from which chitin was formed into fatty substance in the caterpillar stage.

Table IX.

Insoluble Ash & Polysaccharide.

Dictyoploca

Date	Chitin. Subst.	Insoluble Ash	Total Ash
19/V	10·5	·2	10·5
29/V	—	—	10·4
5/VI	11·3	·1	9·9
—	10·9	·2	10·2
19/VI	11·6	·1	9·7
—	11·5	·2	9·5
10/VII	4·2	·1	5·9
—	—	·0	6·3
31/VII	4·5	·4	6·4
1/VIII	6·5	·1	—
16/IX	4·7	·1	—

Castanea

Date	Crude Fibre	Insoluble Ash	Total Ahs
10/V	11·8	·3	4·2
—	—	—	4·1
23/V	16·1	·2	3·9
—	15·0	·5	3·7
5/VI	19·1	·7	3·7
—	20·1	·3	4·1
13/VI	19·9	·3	3·7
—	21·6	·3	3·9
26/VI	19·1	·3	3·6
--	19·5	·4	3·7

SAMMARY & DISCUSSION

Chestnut leaves during growth show decrease in the content of water, total nitrogen, ether extracts, water extracts and ash, while increasing in that of water-soluble nitrogen, polysaccharides and fibres; on the other hand the amount of these chemical constituents in the catapillars fed with those leaves, at the same moment after each moulting was kept fairly constant.

This latter fact, being in contrast with what happened to the silk-worm (*Bombyx mori*), may be due to the silk gland not being so highly developed in this insect, and also the gonad develops so little during its larval life, as the pupal stage is extraordinarily long (about 100 days) and is enough to complete the development of it, that no marked anatomical change occurrs through its larval period (except in the later half of the 5th. instar, in which an enormous development of the fatty tissue is to be seen).

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