

sericologia

Journal of the silkworms.

Revue des vers à soie.

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EDITORIAL

In this second issue of *Sericologia* devoted to the Congress held in Bangalore all the papers presented in the "Bacology" section are published.

The papers accepted by the Chairman of the section are published in full, whereas abstracts only are given for the papers which were not accepted for an oral presentation. All the abstracts are translated either into French or into English.

The bibliography is particularly important since it could not be published in the previous issue.

As this issue is the last one to be published this year, the editorial board and all the persons who took part to its publication express to the readers their best wishes for 1985.

Dans ce deuxième numéro de *Sericologia* consacré au Congrès de Bangalore, sont publiées les communications présentées dans la section "Bacologie".

Comme dans le précédent numéro, les communications acceptées par le Président de section sont publiées en entier. Les communications non retenues pour une présentation orale sont publiées sous forme de résumé seulement. Pour des raisons de place, seuls les résumés font l'objet d'une traduction en français ou en anglais.

La revue signalétique est particulièrement importante du fait qu'elle n'avait pas trouvé de place dans le précédent numéro.

Ce fascicule étant le dernier numéro de *Sericologia* pour l'année 1984, la rédaction et tous ceux et celles qui ont participé à sa publication adressent aux lecteurs leurs meilleurs vœux pour l'année 1985.

XIV^E CONGRES SERICICOLE INTERNATIONAL
XIVTH INTERNATIONAL SERICULTURAL CONGRESS

BANGALORE - 21-25 MAI/MAY 1984

INDIA

SECTION BACOLOGIE
BACOLGY SECTION

★

Chairman : Prof. J-M. LEGAY

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BACOLGY - SCIENCE OF THE SILKWORM

BACOLOGIE - SCIENCE DU VER A SOIE

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ABSTRACT

Although the word Bacology was coined and used by Pasteur, it had not found place in common use until very recently, when efforts were made by the International Sericultural Commission to revive it. Bacology is the science of the silkworm which covers many bioscientific fields such as biochemistry, physiology, genetics, molecular biology, pathology and others.

In the current paper investigations on some biochemical, physiological and pathological aspects of the silkworm conducted in different laboratories in India have been reviewed.

Some significant findings have been made on the mechanism of absorption and transport of sugars and amino acids in the mulberry silkworm. Intestinal transport of glucose and amino acids was shown to be of a facilitated diffusion type. Glucose and α -methyl glucoside were transported by an energy-independent, passive mechanism in the fat body and posterior silk gland tissue. Extensive studies conducted on the uptake of glycine by posterior silk gland indicated an active uptake against a concentration gradient. Neither presence nor absence of Na^+ and K^+ ions had any effect on the transport. However, the requirement for phosphate was indicated. A glycine binding protein which is serologically distinct from fibroin was identified and partially purified from the silk gland extracts.

The fat body also showed the presence of an active mechanism for glycine accumulation.

Laboratory studies on silkworm pathology in India have concentrated on the elusive but economically damaging disease, flacherie. 'Kenchu' and 'Sappe' are the most prevalent forms of flacherie seen in Karnataka. Bacteriological investigations have revealed several bacterial species which can cause typical symptoms of Sappe. *Serratia marcescens* was found in some cases of Kenchu. However, other likely etiological agents like rickettsia and viruses have also been detected in Kenchu. Recently,

a small, non-occluded, spherical virus has been found to be the causal agent for a highly virulent form of Kenchu. Epizootiological studies have revealed many features of the disease which are useful in the control of the disease under field conditions.

RESUME

Bien qu'il ait été créé et utilisé par Pasteur, le mot Bacologie n'était pas utilisé couramment jusqu'à ce que récemment, la Commission Séricicole Internationale s'efforce de le faire revivre. La Bacologie est la science du ver à soie et couvre de nombreux domaines scientifiques tels que la biochimie, la physiologie, la génétique, la biologie moléculaire, la pathologie etc.

Cet article rend compte des recherches entreprises dans différents laboratoires indiens sur les aspects biochimiques, physiologiques et pathologiques du ver à soie.

Quelques découvertes significatives ont été faites sur le mécanisme de l'absorption et du transport des sucres et des acides aminés chez le ver à soie mûrier. Le transport à travers l'intestin du glucose et des acides aminés s'effectue par diffusion facilitée. Le glucose et l' α -methyl glucoside sont transportés par un mécanisme passif, indépendant dans le corps adipeux et la partie postérieure de la glande séricigène. Des études approfondies sur la captation de glycine par la partie postérieure de la glande séricigène, indiquent une entrée active qui s'effectue contre le gradient de concentration. Nila présence ni l'absence d'ions Na^+ et K^+ n'ont d'effet sur le transport. Cependant, le phosphate est nécessaire. Une protéine qui fixe la glycine, sérologiquement distincte de la fibroïne, a été identifiée et partiellement purifiée à partir des extraits de la glande séricigène.

Le corps adipeux montre également la présence d'un mécanisme d'accumulation de la glycine.

Des études effectuées en laboratoire en Inde sur la pathologie du ver à soie se sont concentrées sur la maladie insaisissable mais désastreuse du point de vue économique : la flacherie. 'Kenchu' et 'Sappe' sont les formes dominantes de flacherie rencontrée au Karnataka. Des recherches bactériologiques révèlent plusieurs espèces de bactéries qui peuvent provoquer les symptômes typiques de Sappe. *Serratia marcescens* apparaît dans quelques cas de Kenchu. Cependant, d'autres agents susceptibles d'être la cause de la maladie, tels que des rickettsia et des virus, sont aussi détectés dans le Kenchu. On a découvert récemment un virus sphérique, petit et non-occlus, responsable d'une forme très virulente de Kenchu. Des études épizootiques utiles pour le contrôle de la maladie sur le terrain, révèlent plusieurs composantes de la maladie.

SCIENCE AND STUDY OF THE SILKWORM

SCIENCE ET ETUDE DU VER A SOIE

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The silkworm *Bombyx mori* is a holometabolous insect belonging to the family Saturniidae - Order Lepidoptera class Insecta. It has been raised since times immemorial and has been the target of intensive scientific study both on fundamental problems as well as on applied aspects for its industrial importance. The silkworm requires care and considerable labour for rearing. However, it possesses excellent characteristics as an experimental organism.

The life cycle consists of four different phases: egg, larva, pupa and adult (moth). Normally, the larva passes through five instars, the larval stage is the only feeding stage.

When the larva attains maturity, it emits silk thread from a spinneret to spin the cocoon. The material is emitted in a liquid state but immediately turns into a fibril. This is attributed to the phenomenon of friction between the protein molecules due to tension applied by the larva.

The growth of the body weight is remarkable during the larval stage. There is a progressive increase in weight throughout each larval instar specially during the Vth instar. The maximum weight is reached 1 or $\frac{1}{2}$ days before the onset of cocoon spinning. It is nearly 12000 times the weight of the newly hatched worm. The rate at which the silkworm grows is influenced by temperature and normally development proceeds rapidly on a higher temperature, within the limits of temperature which permits growth - higher than 10°C and lower than 28°C. Humidity also affects the rate of development, optimum temperature and humidity for growth are 26-27°C 80-90% at the 1st-2nd instar, 25°C 70-80% at the 3rd instar, 23-24°C 60-75% at the 4th and 5th instar.

In the male silkworm, sterility is induced by high temperature 32°C or 33°C. When the males are kept at high temperature within 36 hours after the initiation of spinning no sterilization was obtained. However, the rate of sterilization increased rapidly by treating after 48-50 hours. The majority of the males were sterilized when kept at 33°C for 72 hours or more after start of spinning. In addition when there are periodical changes of temperature (32.25, 32.20, 32 and 16), high percentage of sterility occurs with high temperature treatment beyond 19 hours in a day. A large num-

ber of eupyrene silkworms sperms was ejaculated into the bursa copulatrix of the female moths when copulated with the sterilized male moth, while the amount of apyrene sperms were markedly reduced. Neither eupyrene nor a pyrene sperms was found in the the receptaculum.

Histological observation on spermiogenesis after high temperature treatment show marked abnormality in the apyrene sperms and many of them remain in the testicular chamber, without penetrating the basement membrane.

The effects of the temperature on larval development differ in the young and following instars. When the larva of the bivoltine race is maintained at high temperature (28°C) or low (24°C) during total larval stages, the total larval durations in hours are 424 and 508 respectively. But there is very little difference in the cocoon weight of both groups, the former being 1.51 gms and latter 1.55 gms. With high temperature (28°C) in the early instars and low (24°C) in the advanced ones, cocoons are the heaviest (1.65 gms) while in one with low temperature (24°C) at the early, and high (28°C) at the late instar, cocoons are the lightest (1.48 gms).

Different races of silkworm are characterised by undergoing 1, 2 or several uninterrupted generations during the year. Such races are called uni, bi or multivoltine. A variety of hypotheses have been advocated as to the inheritance of the voltinism. It is supposed to be genetically controlled and also influenced by environmental factors. When normal tetramoltine and bivoltine silkworms are kept at low temperature (17°C) during incubation some of them tend to become trimoltors and most of them lay non diapausing eggs.

Molting and voltine characteristics are the main physiological parameters concerned with self regulation. There are tri, tetra and penta molting strains, and there are uni, bi and multivoltine strains. Univoltine female lay diapausing eggs irrespective of temperature. Bivoltine female incubated at high temperature (24°C) lays diapausing eggs. But when incubated at low temperature (17°C) it lays non diapausing eggs and multivoltine female lays non-diapausing eggs irrespective of temperature. The genetic dominance of relationships are : Tri - Tetra - Pentamolting and Uni - Bi - Multivoltine.

The normal larvae are tetramolting. But when they are fed well, some of them tend to become trimoltors. If normal larvae are under fed or fed with mulberry leaves to which volcanic ash has been added they become pentamolters. Pentamolting is recessive to tetramolting.

In the tetramolting bivoltine race, when eggs are incubated at low temperature 17°C some trimolting larvae are often obtained and all the eggs easily change to non-diapausing. However, the larvae will remain as tetramolters and enter diapause at a high incubating temperature - 24°C.

In the younger and the older larval instar, the larvae show quite distinct relationships to temperature, light and nutrition due to an antagonistic physiological balance. The corpus allatum secretes a metabolism accelerating hormone and its titre gradually diminishes with age. The suboesophageal ganglion secretes a metabolism decelerating hormone and its titre gradually increases with age. These two hormones act antagonistically to each other thus a critical point throughout the larval life exist in the 3rd instar. Though molting and voltine characteristics are strictly determined by the function of the corpus allatum during early stage of the development and voltine characteristics are predominantly determined by the function of the suboesophageal ganglion during the late stage of the development.

Nutrition is one of the most important determinant of physiological characters like molting and diapause. Bad nutrition during the larval stage often causes additional molting. Incubation at low temperature (17°C) and rearing of the younger age larvae and the good nutritional condition (1-3 instar 24°C good quality tender leaf) result in the production of trimolters and at certain time non-diapausing egg laying moths.

Light :

Detailed study on the relationship between light and voltinism has been made. A threshold of intensity and duration of the illumination as well as a sensitive stage in the development of embryo has been established. The length of illumination was found to be critical for the determination of diapause. Bivoltine when incubated at 23.5°C in the light gave rise to hibernating egg laying moth, whereas incubation in the dark produce 25% non-hibernating egg laying moths. Exposure to light for 15 hours or more per day is as effective as exposure for 24 hours, while less than 12 hours is comparable to 24 hours in the dark.

The effect of such environmental factors was found to vary according to the developmental stage, the embryonic stage especially after last blastokinesis being the most sensitive. In the larval and pupal stages, the effects were reserved, so that high temperature produced non-diapausing egg layers, whereas temperature below 20°C resulted in the production of diapausing egg layers. When the larvae from trimolting strain are reared from 1-2, 1-3 instar in the dark a few larvae change into normal tetramolters.

Photoperiodic cycles play an important role in determination of the timing of hatching and emergence. The timing of hatching of the embryo is determined fundamentally by the daily cycle of their own biological rhythm (circadian rhythm) and hatching usually occurs in the morning. The degree of synchronisation can be changed by illumination. The most effective illumination for synchronisation is a periodic light and dark regime consisting of 12-18 hours light and 6-12 hours dark. The timing of emergence is also directly correlated with the daily cycle of light and dark and usually occurs at 4-8 in the morning. The time can be changed by illumination as in the case of hatching. Under total dark condition emergence is low and irregular, while sudden illumination causes rapid and synchronous emergence. The mature larva has positive phototaxis. Most effective wave length is 555 mμ.

Metamorphosis :

When the larva reaches the end of the 5th instar ecdysone is accumulated in the body, feeding stops and the spinning of silk starts. The changes in the integument during this process has been studied in great detail. The processes of larval-pupal ecdysis resemble those of larval molting. During the formation of a new cuticle the following changes occur :

1. Mitotic division of the epidermal cells which become closely packed.
2. Secretion of the molting fluid.
3. Secretion of the outer layer of cuticulin.
4. Secretion of the homogeneous layer of cuticulin.
5. Activation of a chitinase which digests the endocuticle.
6. Absorption of the digested substances of the old cuticle.
7. Start of secretion of a new procuticle.
8. Ecdysis of the old cuticle and expansion of the new cuticle.
9. Sclerotization of the cuticle.
10. Start of wax secretion.

Fat tissue :

The adult fat body originates from some larval fat cells that have survived histolysis at the pupal stage. The process of formation of the adult fat tissue is as follows :

Most of the cells of the pupal fat tissues dissociate during the histolytic phase due to the breakdown of the basement membrane covering each lobe of the fat body, but some of them reassociate during the later histogenetic phase and form the adult fat body.

The fat tissue is found in larger quantities in the female than in the male. The pupal fat cells in the female pupae are destroyed and their content utilized for egg maturation, while the cells in the male pupae survive.

The silk gland at the pupal stage gradually degenerates and is destroyed. The wet weight and the amounts of protein and RNA of the gland during the pupal stage decrease rapidly and markedly while the amount of DNA is reduced slowly.

From an endocrinological point of view, extensive studies have been made on the mechanism controlling growth and development. Recently an attempt has been made to prepare a developmental time table for the silkworm using the spiracle index as the criterion for the staging of molting silkworm larvae. Kiguchi hopes to clarify the mutual relations among the endocrinological events, genetical, nutritional and environmental factors.

Other organs :

Except the alimentary canal and the reproductive organs, changes in the others have not been well investigated and further investigations have to be made.

Sensory physiology :

Electron microscopic studies and electrical studies of response to photic stimulation have been studied on the photo receptive mechanism of silkworm. No electrical response to photic stimulation was recorded from the pupal compound eye which showed no sign of the differentiation of the rhabdom. With the differentiation of the rhabdom in the pupal compound eye, electrical responses could be recorded and their amplitude increased with the progress of development of rhabdom. Probably the rhabdom is the site of the photo chemical reaction which leads to the generation of the retinal action potentials.

The electrical responses of olfactory stimulation of the sensilla basiconica on the antennae of the larva have been studied by means of the microcapillary electrode technique.

The electrical responses in single optic ganglion cells of the larval ocellus and adult compound eye were also examined using microelectrodes. The patterns of electrical responses to photic stimulation between ocellus and ommatidium in the compound eye are largely the same. Furthermore, the response patterns of neurons of optic ganglion cells depended upon the intensity or duration of stimulating light and also upon the dark adaptation of the eye.

Ishikawa and his co-workers have carried out electrophysiological studies on sensory hairs of the maxilla. They found that one of the two sensilla styloconica on the maxilla is associated with contact chemoreceptors and the other sensilla styloconicum responds to different stimulus. They called the former "sugar sensory hair" and the latter "water sensory hair". The sugar sensory hair is composed of three

receptor cells in the base of the hair, which are called Ls, Li and G cells. The Ls, Li and G cells are stimulated by sucrose, inositol and glucose respectively. On the other hand the water sensory hair has four receptor cells which respond to stimuli by repellent substance (R Cell) water (W cell) and salts and acids (N_2 and N_2' cells). The silkworm represents useful material for studies in the field of neurophysiology.

Relationship of carbohydrate metabolism to organ development :

Trehalose is synthesised in the fat body and released into haemolymph and the synthesis is controlled by feed back inhibition. There is a study level of trehalose in the larval blood. The control of carbohydrate metabolism not only function to attain homeostasis but also to establish specific organs of metabolism depending upon developmental stages. The glycogen is very effectively transferred from the fat body to the ovaries. In the young pupa at first glycogen is synthesised but very soon a degradative phase is switched on - there is synthesis of trehalose which is released into the haemolymph - the ovaries in turn utilise the blood trehalose for synthesis of glycogen. When the ovaries are removed from the individual the high rate of glycogen synthesis is continued suggesting that the ovaries cause the fat body to convert its metabolic pattern from glycogen synthesis to trehalose synthesis. In normal pupa the volume of the fat body decreases in parallel with the diminution of the glycogen. The histolysis of the fat body is controlled by the ovary. There is a relationship between the metabolic switch over and the initiation of histolysis under the influence of other organ. It is possible that some humoral factors released from the ovaries may affect the carbohydrate metabolism.

Significant changes occur in the DNA, RNA and proteins during pupal-larval transition. The pupal fat body acts as an intermediary receiving these substances from the larval constituents and distributes them to the adult organs.

The increase in the DNA contents of fat body coincides with the DNA decrease in the silkgland. The DNA is transferred between these organs without degradation. This type of DNA transfer system is unique to the silkworm and is accelerated in *Bombyx* strains selected for higher production of silk resulting in a high DNA content of the silkgland.

Histolysis of the silkgland depends upon a kind of physical coupling of the fat body. But its growth is independent. The anterior part of the silkgland undergoes histolysis even in a culture medium without coexistence of the fat body. On the other hand the middle and posterior parts of the gland is degraded only when the fat body continuously removes the degradation products.

Protein contents in the ovaries of *Bombyx mori* increases at the middle stage of the pupal level. This is due to vitellogenesis in which blood proteins that have previously originated from the larval fat body are massively accumulated in the ooplasm. The vitellogenesis is controlled by follicular epithelial cells which form a uni layer surrounding the oocyte.

The follicle cells make channel that connect the blood on the surface of the ooplasm. After vitellogenesis is completed the follicle cells close the channel and in time initiate the synthesis of egg shell protein. Each egg enters a terminal growth phase which lasts for some time. At the end of this stage the follicular epithelial cells stop all the previous activities and suddenly begin to synthesize large amounts of the chorion nearly 70% of the egg population is engaged in synthesising chorion on the 9th day after larval-pupal ecdysis.

The chorion synthesis is controlled at the level of transcription. The follicular cells acquire a full population of active ribosomes mainly during the terminal

growth phase and the synthesis of chorion mRNA switches on choriogenesis.

The nutritional physiology of the silkworm is very interesting. It is a very fastidious monophagous feeding only on mulberry leaves. Under exceptional and unusual conditions it feeds on other leaves. It is possible to grow the silkworm on artificial diet.

Laboratory studies have been extensively made and the nutritional requirement of this insect has been elucidated by rearing larvae on artificial diets and the effects of supplementation or omission of specific substance on larval growth development and survival.

The requirements of carbohydrates, proteins and amino acids, lipids, vitamins, minerals have been determined. Quantitative requirement for various nutrients has been established and minimum, optimum level determined for each nutrient. The proportion of nutrients is very important for good development specially for growth of silk glands and cocoon production. The ratio of carbohydrates vs. proteins influenced not only larval growth and development but also cocoon production.

The digestive juices of the silkworm are interesting. Tizuka and Takizawa have reported antibacterial substances and have isolated caffeic acid which inhibits the growth and multiplication of the pathogen streptococcus faecalis.

Antimicrobial factors :

In addition, the digestive juices of silkworm have antiviral activity. One of the antiviral substance is a red fluorescent protein which is produced in the larval midgut epithelium. When the larvae are reared on synthetic diets RFP is not produced. The enzyme chlorophyllase is necessary for RFP synthesis. The exact mechanism of antiviral action of RFP is not known. But evidently it reacts with NPV and agglutinates the virus. The haemolymph of the silkworm larva also has bactericidal principle and can be resolved into two factors. One is similar to the lysozyme, the other is an anionic factor of low molecular weight. The bactericidal activity is inhibited by magnesium and calcium.

Aizawa has reported defense reaction against the nuclear polyhedrosis. There is a viral inhibitory factor in the haemolymph of silkworm larvae which can be extracted from the infected larvae and not in the uninfected.

In vitro studies show that this VIF inactivates the virus strongly at 27°C and 37°C for 60 minutes. The inactivation of the virus terminates at least within 7 minutes at 27°C. A reversible interaction between the virus and VIF could not be recognised when the VIF was mixed with healthy silkworm haemolymph the activity of the VIF is reduced. The silkworm haemolymph had an inhibitory effect on VIF. The VIF did not produce therapeutic effect when inoculated into pupae. This result is probably caused by the presence of an inhibitory substance in the haemolymph. The VIF inactivates the virus occurring within infected cell obtained from the pupa at an early viral multiplication. Even though the VIF inactivates the virus in vitro, this action differs from that of an interferon like substance. The irreversible interaction between the VIF and virus indicates the VIF is not a receptor substance for virus attachment. The VIF is not inactivated by proteinase digestion ultra violet irradiation and is relatively resistant against heat treatment. Judging from its physical and chemical characteristics the VIF does not seem to be an enzyme which plays a role in the uncoating of the virus.

Although the VIF does not show a therapeutic effect by injection and the inactivation of the virus by VIF is inhibited by the addition of insect haemolymph the VIF nevertheless is a substance that experimentally exhibits a defense reaction against insect virus infection.

Endocrine system in the silkworm :

There are three types of hormones in the insect :

1. The neuro hormones :

Synthesised in different cells of the nervous system and neuro-hemal organ.

2. The steroid hormone :

Ecdysteroids produced in different tissues.

3. The juvenile hormone :

Synthesised by the corpora allata. These hormones regulate many phases of the development.

1. Neuro hormones :

The neuro secretory cells of the pars-intercerebralis and neuro secretory cells of suboesophageal ganglion are well known. A neuro hormone synthesised by the cells of the suboesophageal ganglion is implicated in the determination of embryonic diapause in the female, while the cells of the pars-intercerebralis synthesise brain hormones.

Larval development :

There are a number of neuro secretory cells in the pars-intercerebralis and are termed as A cells and synthesise a prothoracotropic hormone. The number of neuro secretory granules per surface unit is maximum at each larval ecdysis where after it decreases, the activity of the cells can be measured by the number of granules present. The neuro secretory product is released with the first food intake and indicated by an increase of granules number, this number is stable during middle stage and before larval ecdysis the granules accumulate in the cells.

Pupal development :

The brain is required for further development. Brainless pupae of Bombyx become "dauer" pupae or permanent pupae. These pupae resume development after implantation of brain. The neuro hormone content in head, thorax and abdomen of Bombyx pupae is always greater in female than in male. The brain neuro secretory cell releases its secretion as soon as the larval pupal molt occurs. During pupal development brain hormone content is always higher in early pupae. In the female after the removal of the ovaries during the 4th instar, the brain hormone level is greater than in the normal female, while male castration has no effect. A part of the brain hormone seems to be used by ovary for maturation and or vitellogenesis.

The first insect hormone to be isolated and crystalized from the pupa of the silkworm was ecdysone. The prothoracic gland synthesises ecdysone and the maximum release activity takes place at the end of the 5th instar. In addition, a possible intermediate between cholesterol and ecdysone is detected. The ecdysone is converted into ecdysterone if the abdomen is ligated. This ecdysterone is recognised as a 20 - hydroxy ecdysone.

Larval development :

Changes of haemolymph ecdysone titer are observed as the larva develops. During each larval instar feeding period, a low level is observed; a brief peak occurs a short time before the beginning of molting when the larva stops feeding and then ecdysone falls before ecdysis, the level again increases soon after the silkworms start spinning their cocoon at the end of the 5th instar. Intensive investigations have been made on the steroids content variations at hatching and first food intake. The embryos eat yolk and serosa which contain ecdysteroids. The ecdysteroid level is high at hatching but falls with first food intake.

Pupal development :

The haemolymph of the pupae show a large peak of ecdysone and ecdysterone during the first 9 days, the content is the same in the male and the female. The ecdysteroids synthesised in the ovary are accumulated in oocytes.

The egg which is a closed system is formed of two parts, yolk and embryo. Embryo development occurs in the middle yolk, unfertilized egg yolk contains ecdysteroids. Embryogenesis begins in the presence of ecdysteroids.

In the univoltine eggs at the early stages of development ecdysteroids level is high, during diapause this level decreases and variations of few amplitude were observed during post diapause. Most part of the ecdysteroids present are localised in the yolk and serosa, while the remaining part is found in the embryo of the late stages.

The juvenile hormone :

The corpora allata synthesise the Juvenile hormone. There are two glands with lymphoid structure and have a secretory cycle. The juvenile hormone activity has been studied by highly sensitive assay system in other insects. In the silkworm it has been studied as follows :

1. Induction of larval molting in allatectomised 4th instar larva.
2. Induction of prolonged larval stage of the 5th instar larva.
3. Action as a lethal agent on silkworm eggs.
4. Induction of an additional larval instar.
5. Induction of special physiological changes.

Anti juvenile hormone substances have been isolated in the silkworm. When the 4th instar larvae are reared with an artificial diet containing 1% kagic acid for 3 days after the 3rd molt the larvae become trimolters and then pupae. 14 chemicals related to kagic acid have the same effect.

Anti growth promoting substance has been obtained from the haemolymph infected larva from cultures of *Spicaria prasina*. The active substance can be extracted with organic solvents. When heated at a temperature of 50°C for ten minutes this substance becomes inactivated.

The diapause hormone was extracted from brain oesophageal ganglion by Hasegawa. This hormone has been extracted from the brain, prothoracic ganglion of diapause egg produces pupae and the suboesophageal ganglion.

However, the activity of the extract from the suboesophageal ganglion was higher than that from the brain. This suggests that the diapause hormone is synthesised in the brain and transferred to the ganglion, where it accumulates or is released and the active principle exists as an inactive part in the brain but is activated during the extraction procedure. When diapause hormone extracts were injected into a non-diapausing egg-producing pupa the moth lays diapause eggs after emergence. Embryonic development proceeds irrespective of diapausing or non-diapausing nature of the eggs up to 24 hours after oviposition. Development of the embryo is suspended in diapausing eggs until the termination of diapause. The glycogen content increases from 0-24 hours after oviposition then decreases until the end of diapause. The amount of sorbitol and glycerine increases with decrease of glycogen content suggesting the conversion of glycogen to sorbitol and glycerine through trehalose. The onset of diapause is characterised by the accumulation of energy sources like glycogen and lipids.

3 hydroxy kynurenine a precursor of serosa pigment accumulates in the diapause eggs while the quantity is less in non-diapause eggs.

When diapause hormone extracts are injected into non-diapausing egg producing pupa the blood trehalose was actively transported into the ovary and converted to glycogen in the organ, while it decreased in the fat body. The hormone seems to act as a hypoglycemic factor, stimulating insulin action. This suggests that the target organ of the diapause hormone was the ovary and the changes in the other tissues were due to feed back phenomenon on glucose metabolism in the ovary.

It is well known in the silkworm *Bombyx mori* that larval molting is determined by the cooperative action of the juvenile hormone secreted from the corpora allata and the molting hormone secreted from the prothoracic gland activated by the brain hormone, and that larval-pupal and pupal-adult transformations are determined mainly by the function of the molting hormone. On the other hand, it has been found genetically that the number of molts is controlled by an allelic major gene series on the 6th chromosome. Morohoshi proposed from a physiogenetic view point that the corpora allata and suboesophageal ganglion hormones act antagonistically in the developmental processes. According to this view, the secretory activity of the corpus allatum varies with molting type and is strongest in tri-molting, intermediate in tetramolting and weakest in pentamolting silkworms. The function of the suboesophageal ganglion also differs with voltine type, and is strongest in univoltine, intermediate in bivoltine and weakest in multivoltine silkworms. The secretory activity of the corpora allata gradually decreases with age, whereas the activity of the suboesophageal ganglion gradually increases with age. The brain controls the functioning of the organs, so molting and voltine characteristics are strictly determined by the antagonistic balance of the two hormones. The molting characteristic would be predominantly determined by the function of the corpus allatum hormone during early development and voltinism would be predominantly determined by the function of the suboesophageal ganglion hormone during late development.

The synthesis of fibroin in the silk gland is controlled by the administration of hormones, specially ecdysone and juvenile hormone. High doses of ecdysone induce histolysis of the cell accompanied by a temporary high activity of silk protein synthesis. The juvenile hormone controls the larval period and silk production by both factors supplied among and developmental stages. High dose of the hormone inhibits the synthetic activity of silk proteins and the development of intra cellular transport system in the cell, while lower doses prolong the larval period by one or two days accompanying additional 10-20% of the silk material. In very effective cases cocoons with a shell weight of 940 mg have been produced.

Practical application of insect hormones to sericulture :

The utilization of the molting hormone and related substances to increase cocoon yield was first carried out by Kobayashi in 1963 in *Bombyx*. Since then, similar experiments have been carried out by several workers using phytoecdysones. Though phytoecdysones proved useful for the acceleration of larval maturation difficulties remain regarding practical application.

The induction of prolonged larval instar by applying juvenile hormone was first reported by Akai and Kobayashi. They found in *Bombyx* that the most effective stage for injection of the juvenile hormone was about 72 hours after the 4th molt with doses between 1 to 10 μg per g body weight. In the case of 10 μg administration, some animals survived for 26 days in the 5th instar. Silk glands of these animals contained large amounts of liquid silk in the lumen, and the gland cells were well developed, showing no signs of histolysis. Akai et al. reported that a single injection of the juvenile hormone during the first half of the 5th instar brought about increases in the duration of the feeding stages, RNA synthesis and fibroin synthesis. The cocoon shell weight increased about 30% as compared with that of the control. Similar work has been carried out using 3, 4-methylenedioxyphenyl derivatives, which are structurally related to cecropia juvenile hormone and juvenile hormone analogue.

Topical application of a juvenile hormone analogue during the first half of the 5th instar induces a prolongation of this instar and increases larval weight and silk secretion. If the treatment is done at a later stage it leads to an extra larval molt. The hormone analogue stops either the development or the secretory activity of the silk gland depending on the period of treatment. When juvenile hormone is administered at the beginning of the 5th instar, the silk gland growth, RNA and protein synthesis are first inhibited and later resumed and amplified. silk production increases up to 150% of controls.

The use of juvenile hormone analogues to increase silk productivity has also been investigated not only experimentally but also on a practical scale. However, considerable further work is required as regards both fundamental physiological and biochemical aspects and practical sericultural technique.

Genetics :

There are only two species of silkworm, *Bombyx mori* and *Theophila mandarina*. By using electrophoretic and serological methods Yoshitake claims that duplication of genes have played an important role in the differentiation of *Bombyx mori* and *Theophila mandarina* from the same ancestor. The species *B. mori* has differentiated into a number of races and strains. Mutants have arisen spontaneously and also have been induced by physical and chemical methods. More than 400 mutant genes have been described so far, and reviewed by Tajima (1964), Chikushi (1972), Tajima Yata (1975) and Doira (1978). In the female, crossing over is absent and it has been possible to draw linkage maps of the silkworms. Recently concrete partition of the mutants into one of 28 limited groups corresponding to the pairs of chromosomes have been determined. Though the number of chromosomes is large it has been possible to isolate 182 gene loci distributed among 25 linkage groups.

Morphological traits :

320 hereditary traits of the silkworms have been analysed. These are related to morphologically recognisable characters and have been classified into groups according to the developmental stages they affect. The serosa lying underneath of chorion and covering the yolk and embryo consists of cells which are derived from cleavage nucleus and unlike the chorion the genotype coincides with that of the embryo. The large number of egg colour mutants due to serosa pigment is a unique fea-

ture of the silkworm. The serosa colour normally is the same as the eye colour of the imago but it is not always true. Tajima has evolved a specific locus method for detecting mutations based on the egg colour mutants. Larval colour patterns depend on the nature and distribution of the pigment. The larval markings are not clear in the younger instars but from the 4th instar onwards the marking pattern becomes visible and is of three types.

1. Eye spots of the second thoracic segment.
2. Crescents on the second abdominal segment.
3. Star spots on the 5th abdominal segment.

The genes responsible for larval markings translucent skins, body, colour, blood colours, body shape, cocoon colours, cocoon shapes and structures, silk production have been reviewed by Tajima.

From the developmental point of view E-Allelic group of genes in the silkworms are very interesting. 17 genes belong to this group, located either at the proximal end or in its vicinity of the 6th chromosome. They are responsible for the extra markings, supernumerary legs, abnormality of gonads and genital organs. In the homozygous condition they are lethal. They show pleiotropic action on a number of tissues and organs of ectodermal origin producing morphological as well as physiological disorders. Experimental evidence has proved that the E group is located in the same region but at separate sites in close proximity to each other constituting a pseudo allelic complex region.

The affected character due to the pleiotropism of E group of genes, range from the early stage of development to that of the adult stages.

The E group genes are concerned with the number of ovarian tubules in the female and testicular follicles in the male. In the normal type their number is invariably four per ovary or testis. Extreme cases where gonads are completely lacking have been found in E/E^N and E^{Ca}/E^{Ca} embryos. On the contrary in E^{CO} , E^{OI} and E^H an increase in the number of ovarian tubules and also testicular follicles has been observed.

Chromosome aberrations :

A translocation between W and autosome as a tool for sex determination has been evolved. The chromosomal formula for the sexes is 2W for the female and 2Z for the male. W chromosome plays a decisive role in the determination of sex. It is assumed that the female determinant is found on a restricted region of W chromosomes. Several strains in which a dominant gene bearing fragment of an autosome was translocated with W chromosomes, first by Tajima, by Hasimoto and later by others. In these strains sexes can be separated with marker characteristics on the translocated piece such as egg colour, larval marking or cocoon colours.

Heat shock treatment of the female pupa has resulted in a recombination between the W and Z chromosomes by a complex translocation.

Many translocations between the 6th and the 14th chromosomes have been obtained, indicating that the genes located separately on these two chromosomes are closely related.

The other types of aberrations reported are, attachment inversion, fragmentation, polyploidy, trisomy or tetrasomy, attachment and trisomy. Methods for identification of tetraploids in silkworms has been determined by Ruban V.C.

Numerous attempts have been made to induce beneficial mutations for industrial application. The radiation mutagenesis of silkworm has been reviewed by Tajima.

The radiation sensitivity is measured by using LD₅₀ in a modified form. The LD₅₀ for hatching, LD₅₀ from hatching to pupation or emergence, or LD₅₀ during the pupal stage from pupation to emergence. In general the sensitivity to radiation is very high in the early embryo stages, moderately high in the larval stage increasing rapidly as the pupa develops. The adult is strikingly resistant. Using the egg coloured gene as recessive marker many mutants, red (re), pink (pe), and white - 2 (W2) genes have been identified and the specific locus method evolved for measuring mutation frequency. As one female moth lays 500 eggs, a large number of eggs can be collected at a time from only a few female and is highly useful for statistical analysis.

From a worldwide collection of silkworm strains, Murakami and Tajima have extracted several radio sensitive and radio resistance strains using embryonic killing as the criterion for selection. They have established a maximum difference in LD₅₀ as 3 - fold between Tokuishin and Ascoli.

The genes responsible for physiological and biochemical traits have been summarised by Tajima. Polyploid silkworms have been compared with diploid ones from an economic point of view. The sizes of eggs, silk gland cells and the first spermatocytes are larger in the polyploids than in the diploids. But they do not show advantages in the weight of cocoons or in the number of eggs laid.

The number of silk gland cells is rather smaller in the polyploids than in the diploids. The maintenance of a polyploid strain is difficult as there is irregular chromosomal behaviour in meiotic division, and is not economically useful in sericulture.

Maternal inheritance :

The first case of maternal inheritance in silkworms was reported by Toyama. Since then several cases have been added. They concern various stage, namely, egg colours, voltinism, hereditary mosaic and yellow lethal - all these characters are found in the developmental stage and none in the adult stage.

The silk fibroin gene is present in a single copy per genome and its expression is exclusive to the posterior silk gland during well defined periods of development.

A physical map of the DNA containing the gene has been developed from direct hybridisation analysis. The fibroin coding region contains an enormous number of sites for enzymes predicted to have activity from known fibroin in RNA sequences. The fibroin gene core is a large homogeneously repetitive block on DNA with very little sequence divergence or qualitatively different sequences.

The fibroin coding sequence resembles a "Satellite" in that it is highly repetitive and therefore may be subject to the generation of duplication, deletions and inversions by recombination.

Bacterial plasmids containing silk gene sequences have been produced. A recombinant plasmid containing about 1 200 base pairs of fibroin gene sequence a repetitive satellite like DNA was found stable during many generations of growth in bacteria.

Synthesis of proteins :

The most important feature in the physiology of the silkworm is the synthesis of silk proteins which consist of two groups, sericine and fibroin secreted in the middle and posterior silk gland respectively.

During the last two decades molecular biological studies on the bio-synthesis of silk proteins have been made. The metabolic activity of the silk gland of the 5th instar is highly and specifically directed to the synthesis of silk proteins. This feature makes it simple to establish a cell free system for protein synthesis, isolation of messenger RNA of silkworm proteins and genetic study of silk protein genes. The silk fibroin is composed of large and small subunits, both these are synthesized in the posterior gland. At a ratio of 1:2 $1/4$ the large subunit has a molecular weight of approximately 350 thousand and an amino acid composition of the so called fibroin type rich in glycine, alanine and serine. The small subunit is rich in acidic and hydrophobic amino acids as well as glycine and alanine. The main feature of the large subunit of fibroin is that glycine residues alternate with alanine serine, tyrosine and other amino acids almost throughout its sequence. This feature of the fibroin large subunits makes it possible for identifying nascent peptide of fibroins and also predict the nucleotide type composition and sequence of fibroin mRNA. This contributes greatly to the advancement of molecular biological study on silk formation.

The molecular weight of fibroin may vary between 3.4 and 3.8×10^5 dalton among species. Native fibroin splits into large and small subunits after treatment with thiol compounds such as to mercaptoethanol. The molar ratio of large and small subunits was estimated to be 1:1 assuming that the molecular weights of large and small subunits are 3.5×10^5 and 2.5×10^4 dalton respectively. Digestion of the large subunit of the fibroin by chymotrypsin give an insoluble precipitate and the mixture of soluble peptide. The precipitate pattern is composed only of glycine, alanine, and serine with little tyrosine and have average weight of molecular weight of about 4 000 corresponding to about 60 amino acid residues.

Its structure has been to be Gly. Ala. Gly. Ala. Gly. (Ser. Gly. (Ala. Gly)ⁿ Ser. Gly. Ala. Gly. Tyr) where n is usually 2 and have mean value of 2 (Lucas et al.). The soluble fraction (Cs) of the chymotryptic digest consists of various kinds of peptides which are smaller in size than the Cp peptides mentioned above. The chain lengths of the peptides are in the range of about 25-35 (group 1) 8-15 (group 2) and 3-6 (group 3) respectively by Sephadex G-15 gel filtration. Among them the peptides of group 1 peptide was about one half of the Cp peptides.

Group 2 peptide was composed essentially of two kinds of octapeptides that is G-A-G-A-G-A-G-T and G-A-G-V-G-A-G-T (G Gly; A. Ala; T. Tyr; and V.Val). Groups 1. 2 and 3 peptides occupied about 32%, 50% and 18% of a total soluble peptide recovered.

Although the construction of a whole primary structure of the fibroin large subunit is still incomplete at present, the experimental data indicates that the large subunit has a structure in which glycine residues occupy alternate positions almost throughout the whole molecule. The pattern will thus be X-Gly-X-Gly-X-Gly-X-Gly, where X is either Ala. Ser. Tyr. Val. or some other minor amino acid.

The ratio of Cp to Cs fractions obtained from the large subunit is about 55:45 in terms of amino acid residues. Taking all these into account, together with the results observed with X-ray diffraction analysis and acid partial hydrolysis, it is assumed that the large subunit has a copolymer-type structure in which the Cp peptide region (about 60 amino acid residues) and the Cs peptide region to mean value of about 50 amino acid residues alternate with each other.

The largest increase in the tRNA acceptor activity in the middle silk gland was observed for serine, a major amino acid in sericin. A functional adaptation of the tRNA population in the cell to the amino acid composition of the protein being synthesised among the various tissues examined thus for most striking is the silk gland. Amino acid tRNA synthetase activity in the posterior silk gland also changes in a way corresponding to the fibroin composition. If the specific activity at the 4th molt stage is taken as a reference the activities of glycyl alanyl and seryl tRNA synthetases increase 11, 4 and 6 times respectively during the 7 days in the 5th instar. Whereas leucyl-tRNA synthetase activity decreases to some extent during the 5th instar. The changes of the enzymatic activity represents quantitative changes in the intracellular concentration of synthetases and not merely a modification of the embryonic activity.

This suggests the existence of one of the regulates mechanism in protein synthesis at the level of t.RNA at its amino acid acceptor. A possible mechanism for such functional adaptation may be a specific amplification of the t.RNA gene. However, there is no evidence to support this hypothesis in the silk gland. The molecular mechanism controlling the expression of the t.RNA gene however still remains unexplained.

The occurrence of the hereditary variants has been detected recently. From the value of their electrophoretic mobility fibroin large subunit from various were classified into three groups, fast, moderate and slow, the large subunit of a hybrid strain shows two branch having two different mobility, which was the same as those of its parents silkworms. This fact indicates that the alleles which control the fibroin phenotype are codominant. Using the electrophoretic technique a linkage analysis of the fibroin gene was found to be linked with the ND gene located on the 23rd chromosome. The posterior silk gland of the ND silkworm is markedly degenerate whereas the middle silk gland is almost normal.

The ND mutant produces only a trace of fibroin. Fifteen recombinants were obtained between the fibroin and ND genes suggesting a fairly close distance between the two genes.

It has been suggested that a comparative study of the fibroin gene of ND and wild strains of the silkworms will provide a promising system for the study of the regulation of specific gene expression.

The m.RNA for silk fibroin has been isolated successfully from the posterior silk gland of the silkworm. The major codons for the amino acid are GGU & GGA for glycine and GCU for alanine. Based on the codon assignment, the fibroin m.RNA is predicted to have a minimum guanine plus cytosine content of 57% with a guanine content of 40%. The RNA component isolated from the silk gland has a base composition in accord with that predicted for the m.RNA. The amount of fibroin m.RNA has been measured during larval development. After its differentiation during the 4th molt the fibroin m.RNA appears and resumes again its activity in the 5th instar larva. In the mature larva the amount of fibroin m.RNA in the silk gland increases to 3.5% of the total cellular RNA. The hybridization between fibroin m.RNA and silkworm DNA shows that 0.0022% of the DNA is homologous to the fibroin m.RNA. The molecular weight of the fibroin m.RNA is estimated to be 5.5×10^6 daltons. In the mature posterior silk gland which consists of nearly 1 000 cells per larva each cell contains 0.2 μ g of DNA. The amount of DNA in the posterior gland varies with the silk producing ability of the races. On the basis of calculation of the DNA content polyploidization digestion of the silk gland involves the replications of all its genes to at least 4×10^5 times their numbers in a haploid speron nucleus. There are nearly 17 or more rounds of complete genome replication without cell division during larval development. DNA synthesis in the posterior silk gland has been studied enzymatically. The production rate of fibroin in the 5th instar larva is 2 500 fibroin molecules per gene per second. The calculations of this high rate of fibroin synthesis is based on the

transcriptional and translational yields of fibroin m.RNA that is 25,000 m.RNA molecules per gene, 100,000 proteins per m.RNA.

The t.RNA composition in the silk gland corresponds with the amino acid composition of fibroin. 80% of the total t.RNA is composed of four major species of the t.RNA specific for glycine, alanine, serine and tyrosine. This suggests that the t.RNA population in the posterior gland is well adapted for fibroin synthesis. Glycine t.RNA is made of two species, the arrangements of the nucleotide sequences of these two t.RNA confirms the cloverleaf models. Three enzyme fractions are involved in the synthesis of fibroin from amino acyl - t.RNA in cell free polysoma systems of the silk glands. Glycine activating enzymes in the posterior silk gland have been measured by ATP - PP exchange and glycyl - t.RNA formation. The two glycyl t.RNA synthetase found in the gland probably correspond to the two kinds of glycine t.RNA mentioned.

During metamorphosis to pupa, the quantity of RNA and protein in the silk gland decreases rapidly, whereas the DNA decreases rather slowly.

Cytolytic process in the posterior silk gland has been studied. Usual lysosomes which contain myelin figures appear in the cytoplasm and are followed by smooth membrane bounded vacuoles in the cytoplasm of the silk gland. After the larval pupal ecdysis the DNA of the silk gland is transported to fat body. The main DNA components in the pupal fat body consists of DNA. This has a smaller molecular weight than that of the DNA in the larva.

Quantitative measurements on fibroin m.RNA show that it is present in small amounts at the feeding stages of the 3rd and 4th instars and disappears during the molting stage. The fibroin m.RNA is synthesised in the 5th instar and continues to the 6th day and constitutes 3.5% of the cellular RNA weight.

In the posterior silk gland an additional m.RNA smaller in size responsible for the synthesis of a small size component - the P25 protein has been isolated whose function is not known. It is suggested that this protein may be responsible for processes of secretion and spinning of silk proteins.

Many questions regarding the silk protein synthesis have to be answered. The mode of transcription, translation and regulation of the synthesis of huge amounts of fibroin during the 5th instar requires further investigation.

Rodakis and Kafatos have demonstrated the origin of evolutionary novelty in proteins; how a high cysteine chorion protein has been evolved.

The chorion of the silkworm has an unusual high cysteine content in its protein. This H.C. protein sequence has been evolved from a family of more ordinary chorion genes through fixation of mutation leading to increased cysteine content. This has been brought about by both base substitution and expansion or deletion of tandemly repetitive elements which are important in the evolution of this type of protein. In this functional constraints of the various proteins domains dictate which class of the mutation can be accepted.

Mutagenicity testing :

Intensive investigations have been made to develop and exploit a sensitive system in the silkworm that can detect the mutagenic potential of substances. The mechanisms of mutation production at the molecular level is not known. The metabolic activation of chemical mutagens are important not only for the screening of environmental chemicals but also for the elucidation of the silkworm metabolic system.

The silkworm offers valuable bio-assay material for the toxicity testing of the chemicals. Most of the work on insecticide toxicity to the silkworm has been done on larvae and experimental work on toxicity testing using eggs and pupae is required. Since the physiology and genetics of the silkworm have been well studied the silkworm may be useful in studies on the mode of action of toxicants detoxication mechanisms and the genetics of the developmental of resistance.

Insect tissue culture :

A viable medium for the silkworm tissue culture has been established and it is possible to investigate virus growth and development very precisely. Screening test of the chemicals and insect hormone which affect virus multiplication has been studied in the silkworm in vivo. The cells of the tissue of the silkworm can be used for the study of virology and also in other fields of entomology.

Pheromones :

Chemical communication is found in many insects. The attraction of the male emperor moths to a caged virgin of female was first observed by Jean Henri Fabron. Research on olfactory communication was intensified, the chemical substance that is responsible for attraction was identified first in the silkworm and was named bombykol.

The antennae of the male moth are large with many side branches and can detect the presence of female emitting pheromone. They are sensitive to tactile stimuli, specially air movement, only the male responds to the female sex pheromone and female has no receptor for it.

The long multiporous hairs in the silkworm are the odour receptors and respond identically to a series of stimuli. The pheromone perception is a complex interaction between the receptors and central nervous system, integrating and translating receptor information into appropriate behaviour responses.

The silkworm larva produces a Kairomone that attracts the uzi fly. This substance is produced in large quantities from the fourth instar onwards to the spinning of cocoon. The chemical nature of this substance is not known and needs investigation. If this Kairomone is identified, it could be used for control of Uzi fly.

Recently cloning and establishment of a gene library of silkworm DNA has been established by Gopinathan and Patel in the Indian Institute of Science.

A genomic library of silk moth (*Bombyx mori*) DNA was isolated from the posterior silk glands of the 5th instar larvae of *Bombyx mori*, by gentle methods of isolation. The DNA was digested partially with EcoR I and an average 20-40 kb size DNA was purified by agarose gel electrophoresis. These fragments were cloned in the chanoon 4 A phage using the separated phage DNA endfragments and the invitro packaging techniques.

From the genomic library thus established, fibroin specific clones have been identified by using 125 I - labelled fibroin m.RNA. The new clones thus isolated are now being screened for the size of gene inserts. These clones will be used to find out the regulatory signals in the control of fibroin gene expression.

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AN ATTEMPT TO IMPROVE THE ORGANIC CONSTITUENTS IN THE BODY
OF SILKWORM LARVAE, BOMBYX MORI L.

TENTATIVE D'AMELIORATION DES CONSTITUANTS ORGANIQUES
DANS LE CORPS DES LARVES DU VER A SOIE BOMBYX MORI

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The multivoltine breeds of silkworm larvae were exposed to topical application of prolactin and biochemical analysis of the larvae was conducted. Prolactin treatment elevated the levels of structural and soluble proteins along with the total carbohydrates and free amino acids in the body, haemolymph and silk gland of the larvae. The increase in biochemical constituents was correlated with the reported increase in the body weight and cocoon size of the silkworms.

Les larves de ver à soie provenant de croisements multivoltins sont soumises à une application topique de prolactine et une analyse biochimique des larves est effectuée. Le traitement à la prolactine augmente le niveau en protéines solubles et celui des hydrates de carbone totaux et des acides aminés libres dans le corps, l'hémolymphe et la glande séricigène. L'augmentation des constituants biochimiques est accompagnée d'une augmentation du poids corporel et de la taille des cocons.

INTRODUCTION

Multivoltine silkworm breeds were found to be suitable for regions such as India (Sidhu *et al.*, 1968). Various hormones exerted profound influence on the carbohydrate and lipid metabolism of insects (Mansingh, 1972; Chen and Friedman, 1971). However, information pertaining to the impact of vertebrate hormones on the growth and silk yielding potential of silkworm is scanty. Our previous studies revealed that the vertebrate pituitary extract and prolactin induced accelerated growth and advancement in the period of pupation and increased the weight of cocoons (Bharathi *et al.*, 1983; Bhaskar *et al.*, 1983a). Hence it will be essential to analyse the effects of individual pituitary hormones. In the present study an attempt is made to analyse the effect of prolactin on the biochemical constituents of silkworm larvae.

MATERIALS AND METHODS

Multivoltine breeds of *Bombyx mori* (L) were used for the present experiment. Healthy layings of these breeds were reared at 28°C with RH 85 % in the month of November. Feeding was given after every 6 hours with equal quantity of leaves. Topical application of ovine prolactin (NIH, Bethesda, USA μ g PRL per ml of physiological saline) daily from first to fifth instar on a group of larvae consisting of 50 individuals. The control larvae were sprayed with physiological saline (0.9 % NaCl in distilled water). The whole body, haemolymph and silk glands of the larvae at the last instar period have been used for biochemical analysis. The total protein content (Lowry *et al.*, 1951), total carbohydrates (Carroll *et al.*, 1956) and free amino acids (Moore and Stein, 1954) were estimated. Dry weight and water content were estimated gravimetrically.

RESULTS AND DISCUSSION

The data are presented in tables 1-3. The growth rate and fecundity of the silkworm were reported to be increased by the topical application of vertebrate pituitary hormone, prolactin (Bhaskar *et al.*, 1983b). The cocoon weight was also significantly increased after the hormone treatment. In the light of this information, an attempt has been made in the present study to analyse the effect of prolactin (PRL) on the organic constituents of the larvae body, haemolymph and the silk gland.

The dry weight of the larvae body was increased with least change in the water content indicating active accumulation of organic components of the experimental larvae in response to PRL treatment. Since prolactin mimicks the growth hormone like influence (Keenan and Thomas, 1975; Thomas and Keenan, 1976) and in the presence of growth hormone, biosynthetic activities are accelerated (Grayhack *et al.*, 1955; Grayhack and Lobowitz, 1967). Increased dry weight of the present observation was indicative of PRL mediated activation of synthetic activities at the cellular level. The total protein content of the body was markedly elevated over control level after PRL administration indicating such possibility of enhanced protein biosynthesis in experimental larvae. In view of significant increase in both soluble and structural protein fractions, it can be suggested that PRL was inducing accelerated accumulation of both soluble and structural protein components in the larvae body. The free amino acids content was also markedly increased in the body of experimental larvae which might suggest their active uptake from the blood. The total carbohydrate content of the body was also elevated over the controls, suggesting their active uptake from the haemolymph. Since carbohydrates form essential energy source, their accumulation

in the body under influence of PRL was also suggestive on increased energy metabolism of the larvae. Prolactin induces active uptake of both carbohydrates and amino acids into the cells, mimicking the growth hormone like impact (Keenan and Thomas, 1975; Thomas and Keenan, 1976), the present observation indicates that the prolactin was responsible for increasing the uptake of carbohydrates and amino acids from the blood, which seems to be responsible for decreasing the haemolymph carbohydrate content. However, the free amino acid content of the haemolymph was significantly increased in the PRL administered larvae, which might be due to increased uptake of amino acids from the blood. Hence the reported increase in the growth of larvae (Bhaskar *et al.*, 1983b) under the impact of PRL seems to be due to the increased synthetic activities in the body.

The cocoon weight in the PRL administered animals was significantly increased, which indicates accelerated potentiality of the silk gland. Hence the biochemical analysis of silk gland of PRL administered larvae was undertaken. The dry weight of silk gland was increased with no change in water content from that of control in the presence of PRL. This observation indicates increased accumulation of biochemical components in the silk gland of the larvae after PRL administration. The total, soluble and structural protein contents of the gland were markedly elevated after PRL administration which was suggestive of increased structural and dynamic organization of silk gland of the larvae administered with prolactin. In view of increased carbohydrate content of the gland, elevated energy reserve can also be expected. The free amino acid content was elevated, which may be due to their active uptake from the haemolymph. Since free amino acids form the structural units for the formation of silk, increased free amino acid content of the gland might be responsible for the elevated potential of the larvae to increase the cocoon size reported earlier (Bhaskar *et al.*, 1983a).

In general it can be suggested that the topical application of PRL was inducing enhanced biosynthetic activities in the body of the larvae leading to the accumulation of structural and soluble protein components which form the structural units for the growth of the larvae. The exact mechanism by which PRL was inducing the biochemical alterations in the larval body is yet to be elucidated. Further work in this direction is in progress.

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S.No.	Components	Control	Experimental (prolactin treated)
1	Dry matter (mg/g wet wt)	101.39 ± 7.16	126.70 ± 9.43 +24.96 P<0.001
2	Water content in percent	90.07 ± 6.41	88.44 ± 5.67 -1.81 NS
3	Total proteins (mg/g wet wt)	161.80 ± 12.63	189.66 ± 11.32 + 17.22 P<0.001
4	Soluble proteins (mg/g wet wt)	52.95 ± 3.86	68.13 ± 6.77 + 28.67 P<0.001
5	Structural proteins (mg/g wet wt)	104.85 ± 6.43	129.13 ± 7.36 + 23.16 P<0.001
6	Free amino acids (μ mol of tyrosine equivalents/g wet wt)	18.47 ± 1.63	24.26 ± 1.18 + 31.35 P<0.001

Table 1. Levels of biochemical components in the whole body of control (saline treated) and experimental (prolactin treated) silkworm larvae. The values are the mean of 8 individual observations. Mean $\pm$ S.D., + and - indicate percent increase and decrease over control respectively. 'P' denotes the statistical significance and 'NS' indicates non-significance.

S.No.	Components	Control	Experimental
			Prolactin
1	Total proteins (mg/ml)	56.94 ±3.13	69.44
			±5.12
			+21.95
			P<0.001
2	Free amino acids (μ mol of tyrosine equivalents/ml)	4.33 ± 0.21	19.21
			± 1.23
			P<0.001
3	Total carbohydrates (mg/ml)	2.64 ± 0.17	1.05
			± 0.07
			- 60.23
			P<0.001

Table 2. Levels of biochemical components in the haemolymph of control and experimental silkworm larvae. The values are the mean of 8 individual observations. Mean ± S.D. + and - indicate percent increase and decrease over controls respectively. 'P' denotes the statistical significance.

S.No.	Component	Control		Experimental
1	Dry matter (mg/g wet wt)	216.48 ±17.51	+16.70 P<0.001	253.06 ±21.46
2	Water content (percent)	78.35 ±5.17	-4.67 NS	74.69 ±4.39
3	Total proteins (mg/g wet wt)	89.19 ±6.38	+252.90 P<0.001	314.75 ±25.16
4	Total carbohydrates (mg/g wet wt)	2.89 ± 0.17	+52.60 P<0.001	4.41 ± 0.32
5	Free amino acids (μ mol of tyrosine equivalents/g wet wt)	11.24 ±1.36	+ 92.26 P<0.001	21.61 ±2.17
6	Soluble proteins (mg/g wet wt)	41.24 ±3.68	+187.96 P<0.001	136.06 ±12.83
7	Structural proteins (mg/g wet wt)	47.25 ±3.68	+ 276.06 P<0.001	177.69 ±15.36

Table 3. Levels of biochemical components in the silk gland of control (saline treated) and experimental (prolactin treated) silkworm larvae. The values are the mean of 8 individual observations. Mean $\pm$ S.D., + and - indicate percent increase and decrease respectively. 'P' denotes the statistical significance and 'NS' non-significance.

GENE EXPRESSION DURING DEVELOPMENT: STUDIES ON THE ROLE
OF RIBONUCLEOPROTEIN PARTICLES IN FIBROIN SYNTHESIS

EXPRESSION DES GENES AU COURS DU DEVELOPPEMENT :
ETUDES SUR LE ROLE DES PARTICULES RIBONUCLEOPROTEIQUES
DANS LA SYNTHÈSE DE LA FIBROINE

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The silk gland differentiation in NB₄D₂ strain of silkworm, in terms of macromolecular changes during the different days of the fifth instar development was followed by monitoring the incorporation of ³²P-phosphate into the posterior silk gland. The synthesis of acid precipitable macromolecules was maximum on the 3rd day. However, the synthesis of RNA showed a peak on the 5th day prior to a rapid decrease, though the total RNA content of the posterior silk glands showed a maximal quantity on the 5th day and no significant decline thereafter. But the poly (A) RNA showed continuous synthesis as shown by the incorporation pattern into poly (U) filters. This implied that the synthesis of rRNAs and tRNAs was terminated on the 5th day of development and that there was a stabilization of the different species of RNA until they were needed for the massive synthesis of fibroin in the second phase of the 5th instar development. Labelling studies of the nuclear, free cytoplasmic and polysomal RNPs showed high phosphorylation during the early days which decreased gradually later. The poly (A) RNP showed maximum labelling on the 5th day. The protein moieties of the nuclear and cytoplasmic RNP fractions on different days of development were analysed by SDS polyacrylamide gel electrophoresis. The nuclear RNP gave a complex protein pattern consisting of about 8 polypeptides in the 25,000 to 80,000 molecular weight range in addition to a high molecular weight polypeptide of 400,000 dalton. The free cytoplasmic and polysomal RNPs showed the presence of a large number of proteins of molecular weights ranging from 25,000 to 400,000 and 25,000 to 102,000 daltons respectively. The number of proteins associated with the various RNP species decreased from the third day to the 7th day quantitatively as well as qualitatively. The radioactive pattern of the different polypeptides of the nuclear, free cytoplasmic and polysomal RNP showed high labelling on the first day and decreased thereafter. The increased synthesis of fibroin and

simultaneous removal of certain proteins associated with the mRNP particles during later days of development suggest that removal of peptides (unmasking) associated with the mRNP particles leads to enhanced availability of messengers for translation.

La différenciation de la glande séricigène en termes de changements macromoléculaires chez la souche de ver à soie NB4D2, est suivie pendant les différents jours du 5ème âge par l'incorporation de phosphate ^{32}P dans la partie postérieure de la glande séricigène. La synthèse des macromolécules acido-précipitables est à son maximum le 3ème jour. Cependant la synthèse de RNA présente un maximum le 5ème jour, suivi d'une diminution rapide, alors que le contenu total en RNA des glandes séricigènes présente un maximum le 5ème jour et ne montre pas de baisse ultérieure significative. Les RNA poly(A) sont synthétisés de manière continue comme le montrent les fixations sur filtres poly (U). Ceci implique que la synthèse des rRNAs et des tRNAs est achevée le 5ème jour du développement et qu'il y a stabilisation des différentes espèces de RNA jusqu'à ce qu'elles deviennent nécessaires pour la synthèse de la fibroïne dans la seconde phase du développement au 5ème âge. Des études de marquage des RNPs nucléaires, cytoplasmiques libres et polysomiques, indiquent une phosphorylation élevée pendant les premiers jours, qui décroît ensuite graduellement. Les poly(A)RNP ont un marquage maximum le 5ème jour. Les fractions protéiques des RNP cytoplasmiques et nucléaires sont analysées aux différents jours du développement par électrophorèse sur gel de polyacrylamide SDS. Les RNP nucléaires donnent un profil protéique complexe qui consiste en 8 polypeptides environ dont les poids moléculaires se situent entre 25 000 et 80 000 daltons, et d'un polypeptide de poids moléculaire très élevé (400 000 daltons). Les RNPs cytoplasmiques et polysomiques montrent la présence d'un grand nombre de protéines de poids moléculaires variant respectivement de 25 000 à 400 000 et de 25 000 à 102 000 daltons. Le nombre de protéines associées aux différentes sortes de RNP diminue du 3ème au 7ème jour, en quantité comme en qualité. Le profil radioactif des différents polypeptides des RNPs nucléaires, cytoplasmiques libres et polysomiques montre un marquage élevé le 1er jour et une diminution ensuite. La synthèse accrue de la fibroïne et la disparition simultanée de certaines protéines associées aux particules de mRNP pendant les derniers jours du développement suggèrent que la disparition des peptides (démasquage) associés aux particules de mRNP entraîne une disponibilité accrue des messagers pour la traduction.

INTRODUCTION

At the end of larval life, the posterior silk gland (PSG) is highly specialized for the secretion of the major protein of the cocoon, the silk fibroin. It has been demonstrated that the massive synthesis of fibroin which takes place during the 5th instar could not be due to amplification of the fibroin gene (Suzuki *et al.*, 1972). There is only a single fibroin gene per haploid complement of DNA in the PSG at the time of fibroin synthesis (Suzuki *et al.*, 1972; Lizardi and Brown, 1975). This focuses attention on transcriptional and translational controls. One means of trans-

lational control that is important for eukaryotes is the stability of mRNA. Stabilization of eukaryotic messengers is ascribed to the poly (A) stretches of their 3' terminal and the associated proteins. Alteration in proteins specifically bound to messengers could reveal the regulatory role of these proteins in the expression of messengers.

The present study attempts to unravel the post-transcriptional regulatory processes during the silk gland differentiation of NB₄D₂ strain of *Bombyx mori*. As the pattern of RNA synthesis varies for different species of silkworm (Kuliyev *et al.*, 1964; Daillie, 1965; Fournier, 1974; Okabe *et al.*, 1975 and Sridhara *et al.*, 1977) an initial analysis of RNA metabolism in PSG of NB₄D₂ strain was done.

MATERIALS AND METHODS

Rearing of the silkworm:

Fifth instar silkworms of NB₄D₂ race were obtained from the Sericultural Department, Madurai. The larvae were fed with local mulberry leaves. Batches of synchronous larvae which were selected by their uniform emergence from the moult within a period of an hour were used for all the studies.

Radioactive labelling of animals and RNA extraction:

Animals were labelled by intracoelemic injection of neutralized carrier free ³²P-phosphate (20 microcurie per animal) on different days of the 5th instar. After 24 hrs of labelling, the animals were immobilized in ice-cold SSC (0.15M NaCl, 0.015M Sodium-citrate) and the posterior silk glands were dissected out. PSGs were homogenized in buffer containing 0.01M Tris-HCl, pH 7.5, 0.01M NaCl, 3mM MgCl₂, 1% Triton-X 100, 0.1% DEP and 0.002% polyvinyl sulphate. Subcellular fractions were obtained from the homogenate and RNA was extracted with phenol-sodium dedecyl sulphate at pH 9. The proportion of radioactivity in the poly (A) RNA was determined by poly (U) filter binding assay as described by Sheldon *et al.*, 1972.

RNP preparation:

For RNP preparation, the labelled PSG were homogenized in buffer containing 20mM Tris-HCl, pH 7.5, 25mM NaCl, 5mM MgCl₂, 2mM reduced glutathione, 2mM PMSF, 0.1% DEP, 1% Triton-X 100. The RNP particles from the subcellular fractions were isolated essentially by Mg⁺⁺ precipitation method of Palmiter (1974). The kinetics of labelling of total RNP in the cellular fractions during development was assessed. Poly (A) RNP particles were isolated by oligo-dT cellulose chromatography (Lindberg and Sundquist, 1974), using a batch procedure (Kumar and Pederson, 1975).

Polyacrylamide gel electrophoresis:

The polypeptides of RNP were separated by SDS polyacrylamide gel electrophoresis using the gel system of Laemmli (1970). The gels were sliced and the radioactivity determined.

RESULTS AND DISCUSSION

Distribution of ³²P-phosphate in the acid precipitable fractions of the nucleus and cytoplasm:

³²P-phosphate incorporation into the acid precipitable fractions of the nucleus and cytoplasm were followed separately on the first, third, fifth and seventh days of the 5th instar to elucidate the overall pattern of synthesis in the fractions

(Fig. 1). Both nuclear and cytoplasmic fractions showed maximum incorporation on the 3rd day of development. The incorporation declined after the fifth day. This suggests the synthetic activity of macromolecules is high during the labelling period of 48 to 72 hrs of development.

Changes in the specific activity of RNAs of PSG:

The patterns of labelled nuclear and cytoplasmic RNAs, per milligram of total RNAs (Fig. 2) showed an increase from the first day to the fifth day and then a decline till the seventh day. As the larvae were allowed to incorporate ^{32}P -phosphate during 24 hrs period, on various days of the fifth instar development, the steep increase in incorporation into RNA during the 72-120 hrs of development indicated higher extent of synthesis of RNA during this period of development as observed by most authors (Kullyev *et al.*, 1964; Daillie, 1965; Fournier, 1974). The decline in RNA synthesis after the fifth day could be attributed to the cessation of ribosomal RNA and tRNA synthesis since earlier observation indicates transcription of rRNA and tRNA upto the fifth day of the final instar only.

^{32}P -phosphate incorporation into the Poly (A) RNA of PSG:

The poly (U) binding capacity of ^{32}P -phosphate labelled RNA isolated from the PSG (Fig. 3) showed a sharp increase from the first day to the fifth day followed by a levelling off till the end of the larval period. This is in accordance with the previous observation by Suzuki and Giza (1976) that fibroin messenger is transcribed continuously till the last day of larval development. When the poly (U) binding capacity of ^{32}P -phosphate labelled RNA was expressed in terms of milligram RNA, it showed a steep increase from the first day to the third day and declined thereafter.

Changes in the level of RNA content of the PSG:

The total RNA content in the nuclear and cytoplasmic fractions of the PSG was determined on different days of development (Fig. 4). The nuclear fraction showed comparatively higher RNA content on the fifth day and decreased levels of RNA during the following period of development. The decline in RNA content after the fifth day might indicate decrease in the synthesis of major RNA species, ribosomal and tRNAs and their mobilization into the cytoplasm to support the massive fibroin synthesis. Accordingly, the cytosol showed continuous increase in its RNA content till the fifth day and also maintained its high level during the following days of development. This tempted us to suggest that ribosomal RNA, tRNA and also fibroin mRNA remain stable following a period of active synthesis to support the production of fibroin during the later phase of the 5th instar. This phenomenon was also recognized by others (Kafatos, 1972; Suzuki, 1974) who observed an extensive accumulation of RNA in the cytoplasm during the early growth phase.

^{32}P -phosphate incorporation into the RNP-particles of the PSG:

It is well established that most of the eukaryotic RNA species are complexed with various proteins and exist as RNP particles and that the RNA and protein components of RNP can vary along with the time axis of the development. The repression/derepression of RNP must be accompanied by a change in the protein moiety probably by mechanism such as phosphorylation or binding to cAMP (Obrig *et al.*, 1975). Hence in all the experiments ^{32}P -phosphate precursors were used. Fig. 5 shows the pattern of ^{32}P -phosphate incorporation into the RNP, nuclear, polysomal and free cytoplasmic RNP particles of the PSG. The ^{32}P -phosphate labelling of the nuclear RNP showed a slight increase from the 1st day to the 3rd day and a gradual decline thereafter. The polysomal RNP labelling decreased gradually with the increase in the age of the larvae. The labelling of the free cytoplasmic RNP showed a sudden decline from the 1st day to the 3rd day and then an increase on the fifth day followed by a decline. This pattern shows that phosphorylation or the binding of protein to the RNA is more

during the early days, and during the later days there is less phosphorylation. According to Takeyama *et al.*, 1958, the synthesis of fibroin is less during the early phase of development and there is a steep increase in the synthesis during the later phase of development. The decreased synthesis of fibroin observed during the early days may be due to low synthesis of mRNA or the presence of mRNA as mRNP particles.

The free RNP and polysomal RNP showed a similar labelling pattern. The most likely explanation for these observations is that the two types of RNP (free RNP and polysomal RNP) exist in dynamic equilibrium as observed by Jain and Sarkar (1979); in this study, poly (A) RNP particles isolated by oligo (dT)-cellulose chromatography (Table 1) showed high labelling on the fifth day in all the sub-cellular fractions. However the total RNP in all the fractions showed maximum labelling on the first day. The observation that radioactivity associated with the poly (A) RNP on the first day is less and the increase of radioactivity in the same fraction on the fifth day suggests that the increase in the radioactivity with poly (A) RNP on the fifth day can be attributed to the increased labelling of poly (A) RNA on the fifth day.

Protein components of RNP-particles:

The protein moieties of the nuclear and cytoplasmic fractions on different days of development were analysed by SDS-polyacrylamide gel electrophoresis (7.5%). The nuclear RNP gave a complex protein pattern consisting of about eight polypeptides in the 80,000 to 25,000 molecular weight range (Fig. 6, panel A) in addition to a high molecular weight polypeptide of 400,000 dalton. The lower molecular weight proteins include five major polypeptides of molecular weights of 14,000; 21,000; 31,000; 53,000 and 63,000. The 400,000 molecular weight protein which was present on the third day was absent on the fifth and seventh days; the other low molecular weight proteins were also decreased from the third day to the seventh day.

The free cytoplasmic RNP (Fig. 6, panel B) showed the presence of a large number of proteins of molecular weights ranging from 25,000 to about 400,000. The polysomal RNP (Fig. 6, panel C) contained mainly seven proteins of molecular weights 25,000 to 102,000 dalton molecular weight range. The number of proteins associated with the RNA were less on the third day (than on 5th and 7th days) in the case of free cytoplasmic RNP and polysomal RNP. The proteins which were present in hnRNP were absent in free cytoplasmic RNP; in the latter, completely new protein complexes were present except for the 400 K protein. The polypeptides associated with free cytoplasmic RNP on different days of development were similar except for the appearance of 400 K polypeptide on the seventh day and decrease in the quantity of 69 K protein. On the third day, in addition to other polypeptides a 56 K protein was also present. In the case of polysomal RNP the number of proteins were more in the third day and fifth day and a loss of six major bands ranging from 51 K to 82 K was seen on the seventh day.

The most likely interpretation of these results is that the three types of RNPs represent three distinct classes of nucleoprotein complexes. The free cytoplasmic RNPs are relatively protein rich while the hnRNP and polysomal RNPs are comparatively protein deficient particles. The results presented here support the model of translational control involving mRNP as suggested by Jain *et al.*, (1979). Accordingly, proteins which are present in free cytoplasmic mRNPs but absent in polysomal mRNPs participate in the *in vivo* regulation of the entry of mRNAs from the non-polysomal pool to the polysomes. The mRNA associated proteins accordingly may exist as a pool. The size and availability of this pool of proteins may function as a subtle regulatory parameter in controlling translation as in the case of embryonic muscle cells (Jain and Sarkar, 1979) and other differentiated eukaryotic cells (Cervera *et al.*, 1981; Geoghegan *et al.*, 1979; Mc Mullen *et al.*, 1979), where post transcriptional modification of gene expression involving mRNAs is believed to operate.

To unravel whether modifications of the RNP associated proteins are through phosphorylation, the larvae were labelled with ^{32}P -phosphate, the RNP proteins were isolated and the gels containing the phosphorylated RNPs were sliced and counted. Fig. 7 shows the labelling patterns of the different polypeptides of the nuclear, free cytoplasmic and polysomal RNPs on the first, third and fifth days of development. The high molecular weight polypeptides associated with polysomal, cytoplasmic and nuclear RNPs were labelled to a larger extent during the first day. During the third day only 128 K, 124 K and 99 K polypeptides of the nuclear RNP were highly labelled while other proteins showed decreased labelling. Though the presence of certain proteins associated with different RNP species were observed by commassie brilliant blue staining after the fifth day, the slicing and counting pattern demonstrated the decreased labelling of these polypeptides suggesting the cessation of synthesis of these polypeptides after the fifth day. These results show that as the days of development progressed, the labelling of proteins associated with RNA decreases which in turn shows no addition of new proteins through phosphorylation. The polypeptides pattern in the SDS gel showed a loss of certain peptides during the later days of development. The increased synthesis of fibroin (Tashiro *et al.*, 1968) and simultaneous removal of certain proteins associated with the mRNP particles during later days of development suggest that removal of peptides (unmasking) associated with the mRNP particles leads to enhanced availability of messengers for translation.

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TABLE 1.

Distribution of labelled poly (A) RNP
(expressed as percentage cpm of ^{32}P -phosphate incorporated in each fraction
in comparison with the total incorporation in all RNPs)
of the subcellular fractions on different days
of 5th instar larval development.

Subcellular fractions	Days of development			
	1st	3rd	5th	7th
Nuclear RNP	3.72	10.20	32.72	6.20
Free cytoplasmic RNP	5.33	12.32	27.33	10.45
Polysomal RNP	0.65	6.96	25.87	-

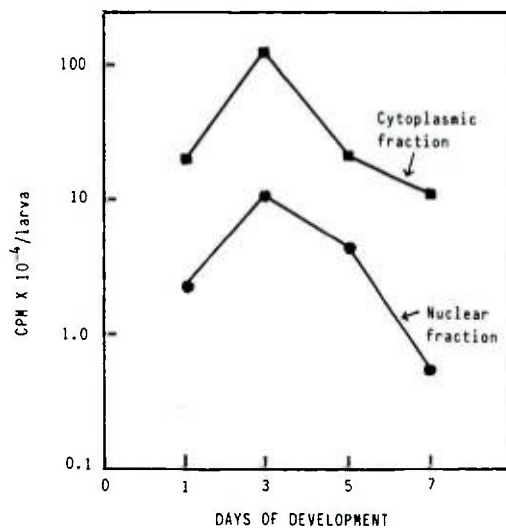


FIG. 1

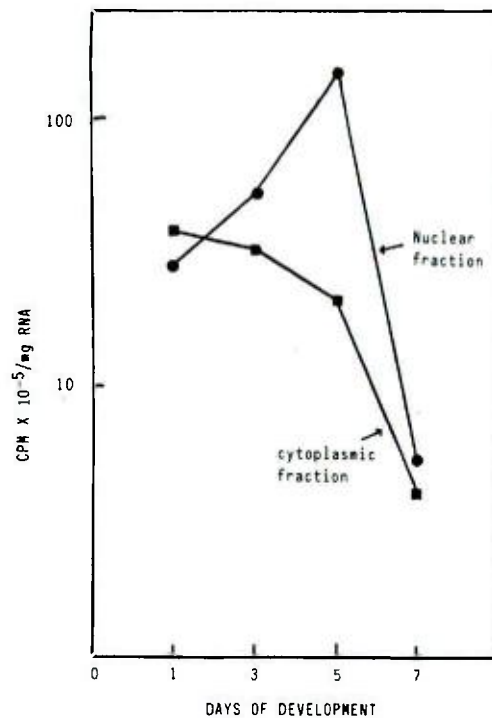


FIG. 2

Fig. 1. Distribution of ^{32}P -phosphate in the acid precipitable fractions of the nucleus and cytoplasm.

Fig. 2. Changes in the specific activity (radioactivity per mg RNA) of the nuclear and cytoplasmic RNAs.

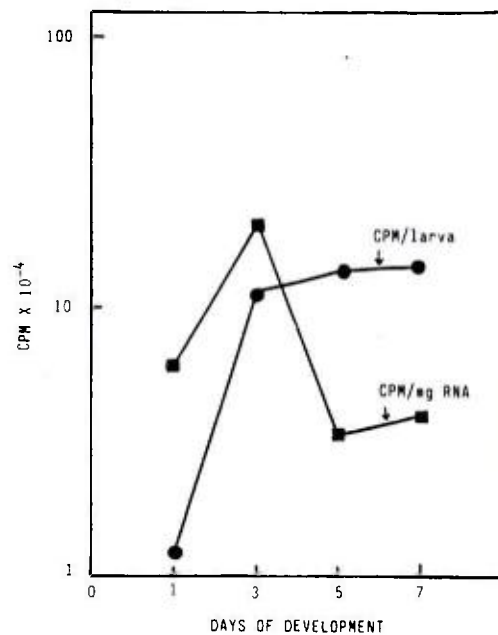


FIG. 3

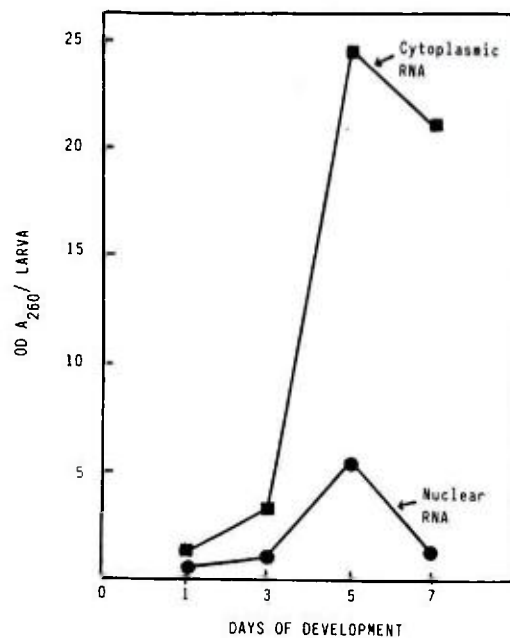


FIG. 4

Fig. 3. Incorporation of ^{32}P -phosphate into poly (A) RNA of posterior silk gland.

Fig. 4. Changes in the level of RNA on different days of the fifth instar.

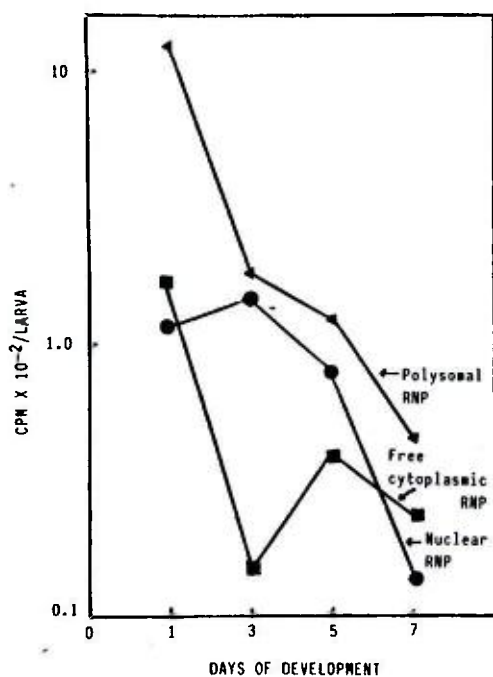


FIG. 5

Fig. 5. Incorporation of ³²P-phosphate into the ribonucleoprotein particles of the different sub-cellular fractions.

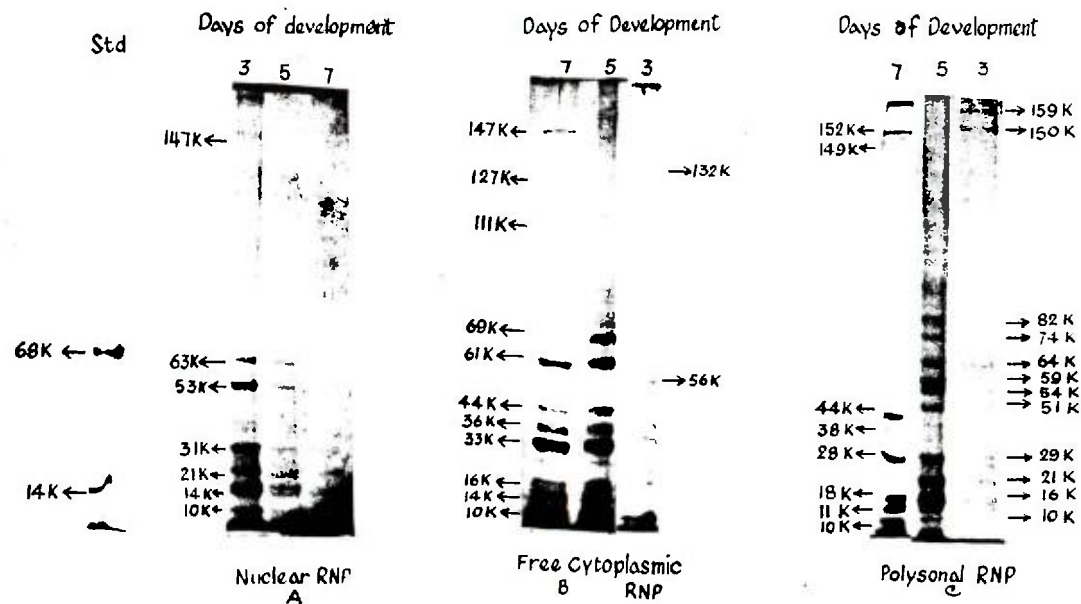


Fig. 6. SDS-polyacrylamide gel electrophoresis of the nuclear RNP, free cytoplasmic RNP and polysomal RNP of the 3rd, 5th, and 7th days of the fifth instar.

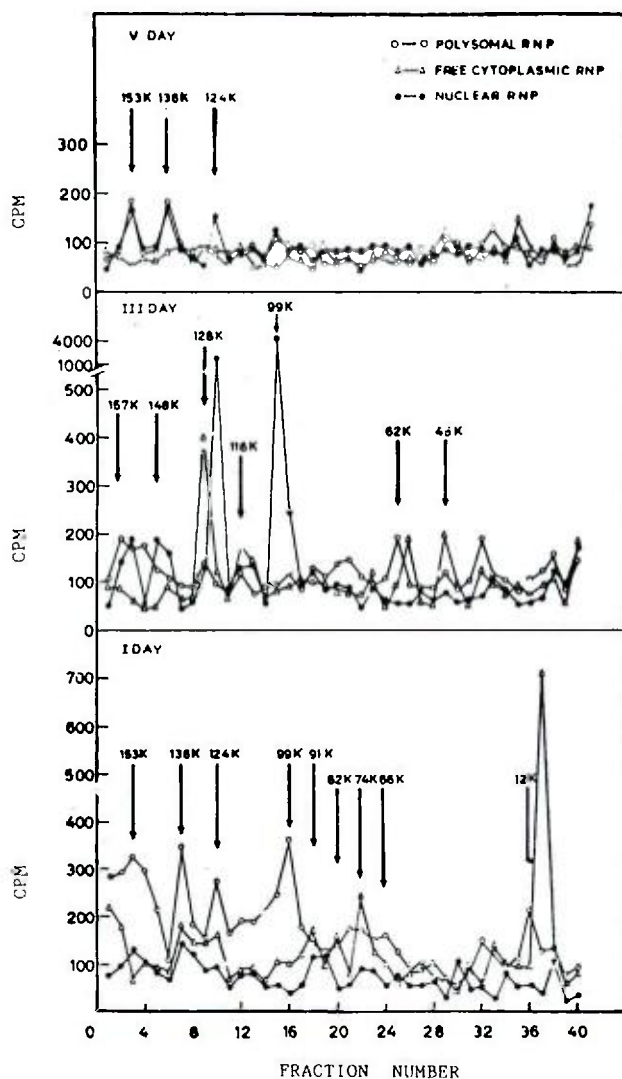


Fig. 7. Radioactivity pattern of the nuclear RNP, free cytoplasmic RNP and polysomal RNPs on the 1st, 3rd, and 5th days of the 5th instar as determined from SDS-gel electrophoresis.

INCORPORATION OF SINGLE CELL PROTEIN IN THE SILKWORM
BOMBYX MORI

INCORPORATION DE PROTEINE D'UN SEUL TYPE CELLULAIRE
DANS LE VER A SOIE BOMBYX MORI

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Use of Spirulina fusiformis and Chlorella protothecoides as additional/supplemental source of protein for the silkworm larvae, was studied by tracing the pattern of incorporation of labeled algae. Radioactive labelling studies showed that about 15% of the Spirulina and Chlorella proteins were directly incorporated into the silk gland. Scope for the utilization of SCP in silkworm nutrition as an additional/supplemental protein source is discussed.

Nous avons étudié l'utilisation des algues bleu-vertes, Spirulina fusiformis et Chlorella protothecoides comme source supplémentaire de protéines pour les larves de vers à soie. Chez les larves nourries avec ces algues, une amélioration significative a été observée au niveau du cocon, du poids de la chrysalide et de la coque soyeuse. Le marquage radioactif montre qu'environ 15 % des protéines de Spirulina et de Chlorella sont directement incorporés dans la protéine de la glande séricigène. Nous avons étudié le champ d'utilisation des algues dans l'alimentation du ver à soie comme supplément protéique.

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INTRODUCTION

Single cell algae such as *Spirulina fusiformis* and *Chlorella protothecoides* contain proteins as much as 62-68 g and 40-50 g/100g, respectively (Becker and Venkataraman 1978). Preliminary attempts were made to use *Spirulina* protein (Hou & Chen 1981) and *Chlorella* protein (Schmitzu 1982) as one of the components of artificial feed for silkworms. Mathavan *et al* (1984) have successfully used *Spirulina fusiformis* as additional source of proteins for *Bombyx mori*. The present paper reports the incorporation of *Spirulina* and *Chlorella* protein in the silkworm *Bombyx mori*.

MATERIALS AND METHODS

Stock culture of *Chlorella protothecoides* was obtained from the Department of Plant physiology, Madurai Kamaraj University and grown autotrophically in a nutrient medium (Sanger & Ohshima 1976). *Spirulina fusiformis* culture was obtained from Murugappa Chettiar Research Center, Tharamani, Madras and grown in Zarrouck medium (Zarrouk 1966).

Algal cells grown autotrophically were harvested at their maximum exponential growth and transferred to 50 ml of culture medium (pH 8 for *Chlorella*; pH 10 for *Spirulina*) and labelled with 100 μ Ci of ^{14} C-bicarbonate. 1 μ Ci of labelled algal cells were administered orally to the 4 day old V instar worms. The worms were maintained in separate trays and fed with mulberry leaves. After 24 hours of feeding the labelled worms were weighed and sacrificed. The body tissues, alimentary canal, silk gland, gut contents and faecal pellets (collected for 24 hours) were separated and weighed for each larvae. The samples were homogenised in 0.1 M Tris/HCl buffer pH 8. Aliquots were spotted and counted for radio activity in the liquid scintillation counter (Beckman, USA, Model 100 C). To find out the incorporation of ^{14} C label in the total protein fraction of different samples, TCA precipitation of the labelled sample was done (Mans & Novelli 1961).

^{14} C *Chlorella* hydrolysate was obtained from Bhaba Atomic Research Centre, Trombay. 1 μ Ci of ^{14} C *Chlorella* hydrolysate was administered orally to the 4 day old final instar larvae. The worms were allowed to feed on mulberry leaves for 24 hours. The samples were collected, homogenised and processed for counting as before.

RESULTS AND DISCUSSION

Incorporation of ^{14} C sodium bicarbonate labelled *Spirulina*

^{14} C labelled *Spirulina* was administered orally to silkworm larvae and the pattern of total incorporation of the same in the body tissues, silk gland, haemolymph, gut tissue, gut contents and faecal pellets is presented in Fig. 1A. About 70 % of administered materials were defecated via faeces and the remaining 30 % was absorbed by the larvae. Of this, about 14 % is incorporated in gut tissue, 7 % in the silk gland, 6 % in the body tissue and the rest in the haemolymph. Test samples were treated by hot TCA method and the incorporation of acid precipitable protein fraction was estimated. Of the total labelled samples, 35 % remained as acid precipitable protein fractions when treated with hot TCA. Assuming this 35 % as 100 %, the percentage incorporation of the labelled materials in different test fractions are presented in the Table 1. A maximum of 40 % incorporation was excreted as faecal materials. Body tissues, gut tissue, silk gland haemolymph and gut content got about 10.6, 22.6, 14.5, 3.5 and 8.9 % incorporation, respectively.

Incorporation of ^{14}C sodium bicarbonate labelled *Chlorella* :

With a view to compare the incorporation of few other SCP such as the green algal (*Chlorella*) in *Bombyx mori*, *Chlorella* labelled with ^{14}C sodium bicarbonate and *Chlorella* hydrolysate were administered orally and the results are presented in Fig. 2 & 3. The pattern of incorporation of *Chlorella* labelled with ^{14}C sodium bicarbonate was almost similar to that of *Spirulina* labelled with ^{14}C sodium bicarbonate. The percentage of labelled algal cells eliminated along with faeces was not much different in *Chlorella* (68.5 %) from that of *Spirulina* (69.54 %). Similarly the retention of algal cells in the body tissue also remained the same. There was 5.89 % incorporation of *Spirulina* and 5.7 % incorporation of *Chlorella*. However, significant increase was found in the percentage of incorporation of *Chlorella* protein in the silk gland (16.9 %) over that of *Spirulina* (7.05). In haemolymph and gut tissues, the amount of incorporation of labelled *Chlorella* was much low (0.8 % and 8.1 %) when compared to those of *Spirulina* (3.06 % and 14.45 %).

Incorporation of ^{14}C *Chlorella* hydrolysate :

Interestingly, when ^{14}C *Chlorella* hydrolysate was administered orally to silkworms, a different pattern of incorporation was observed; there was almost an uniform distribution of labelled amino acids of *Chlorella* in the various parts of the silkworm larva (Table 1).

Of the labelled algal amino acids 27.7% was eliminated along with faeces. The body tissue has retained as much as 23.1% ^{14}C *Chlorella* hydrolysate; it was 4 times more than the incorporation of *Chlorella* cells, labelled with ^{14}C sodium bicarbonate. Significant increase in the incorporation of labelled *Chlorella* amino acids was also found in the haemolymph (15.1%) and in gut tissues (17.5). However, in the silk gland the incorporation of ^{14}C *Chlorella* hydrolysate (14.5%) was not much different from that labelled with ^{14}C sodium bicarbonate (16.9%).

When hot TCA treatment was given to ^{14}C *Chlorella* hydrolysate, nearly 46 % remained as acid precipitable protein fractions. Keeping this 46 % as 100 %, the percentage of incorporation is presented in Table 1. The body tissues and gut tissues shared the maximum incorporation of 20.78 % and 28.89 % respectively. Defecation amounts to only 18.27 %. The remaining algal protein was incorporated in the silk gland (17.11 %) and haemolymph (14.95 %).

Horie et Watanabe (1983) reported that elevation of the dietary protein results in the acceleration of growth and silk production in *Bombyx mori*. Using *Spirulina* as an additional source of protein, Thomas & Raja (1980) showed the increase in the production of *Tilapia mossambica*. Mathavan et al (1984) reported for the first time that supplement of SCP (*Spirulina*) has resulted in the improvement of the economic character of the silkworm over the control animals. The pattern of incorporation of the labelled SCP observed in the present study shows that algal proteins get directly incorporated in different tissues of the silkworm *Bombyx mori*. Based on these results it can be concluded that SCP can serve as an additional source of protein to improve the yield and economic characters of the silkworm. At present, extensive field trials are being conducted with the help of the state sericulture department (Tamil Nadu) supplementing *Spirulina* along with mulberry leaves. Successful completion of the field trials will throw light on the scope for the application of SCP in sericulture.

ACKNOWLEDGEMENT

We thank the Department of Sericulture for the supply of silkworms. One of us (K.B.) acknowledge the management of Arumugam Pillai Seethai Ammal College, Thirupathur (R.D.) for encouragement. Financial assistance from CSIR to Miss Anitha Sironmani is acknowledged.

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* * *

Fig. 1. A. Incorporation of ^{14}C - sodium bicarbonate labelled spirulina in various tissues of silkworm.
 B. Incorporation of ^{14}C label in the acid precipitable protein fraction in various tissues of silkworm.

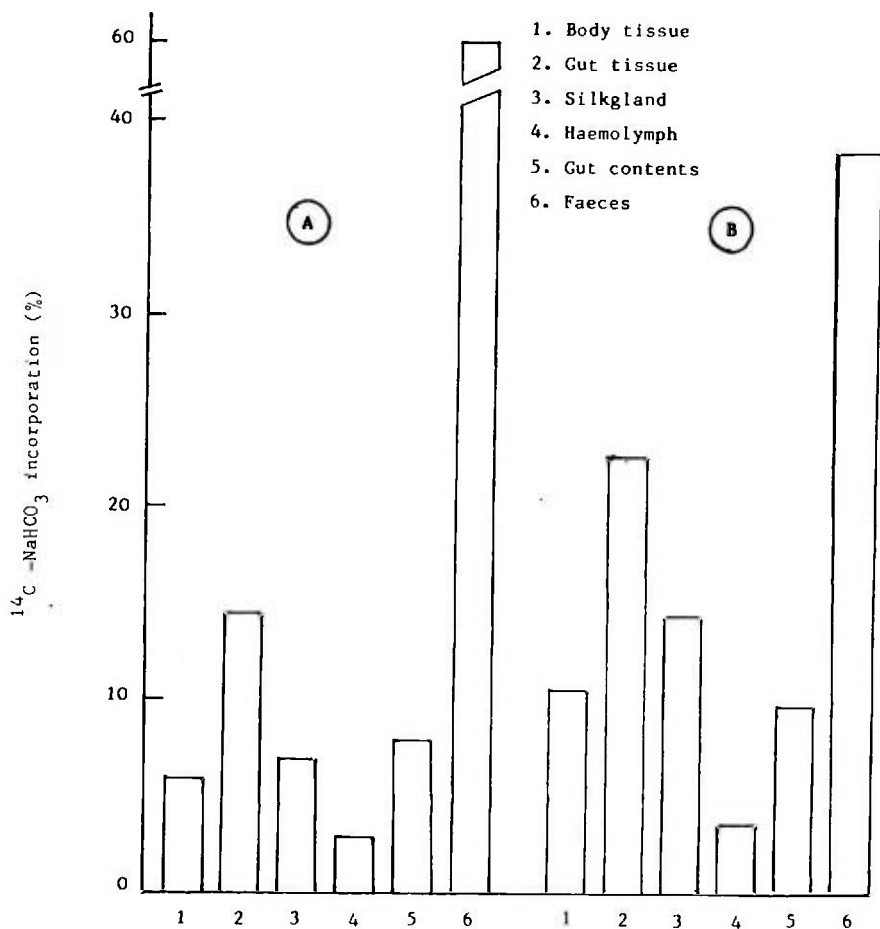


Fig. 2. Incorporation of ^{14}C - sodium bicarbonate labelled *Chlorella* in various tissues of silkworm.

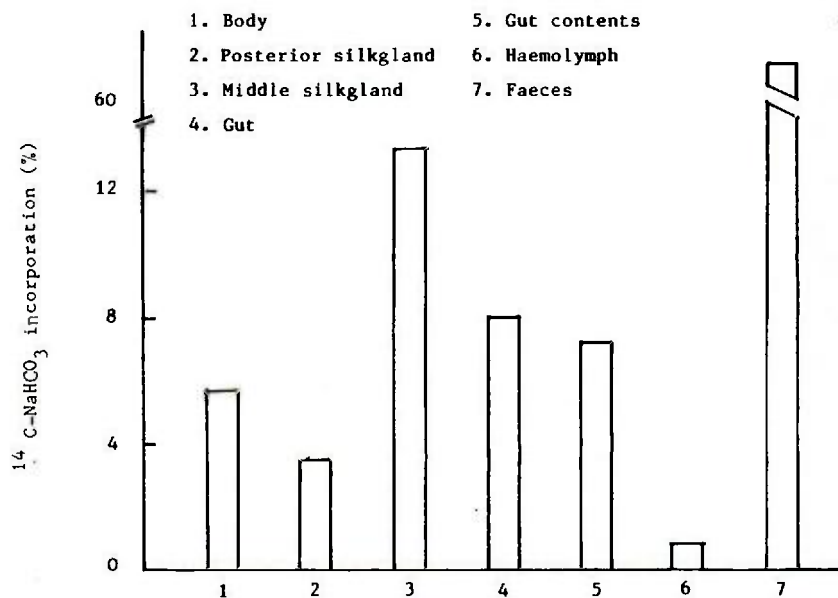
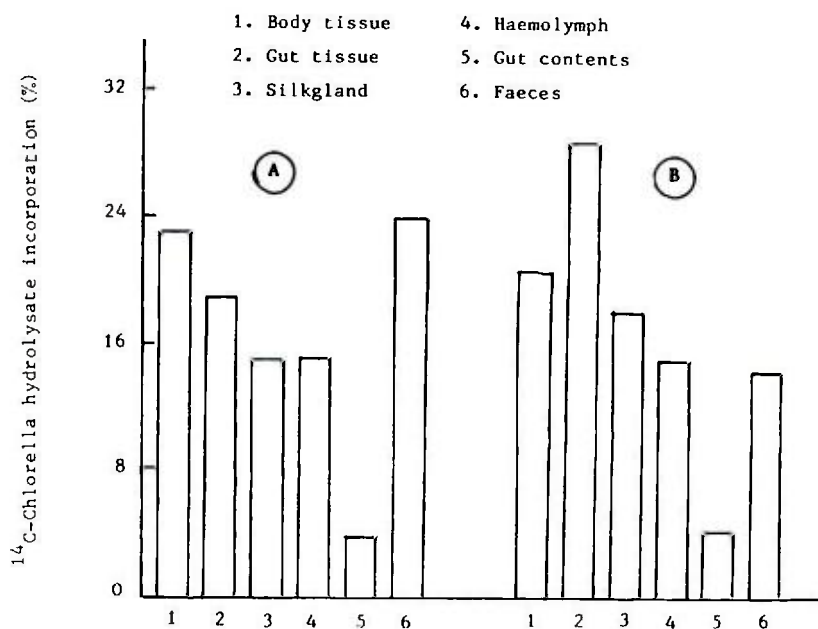


Fig. 3. A. Incorporation of ^{14}C - Chlorella hydrolysate in various tissues of silkworm.
 B. Incorporation of ^{14}C - label in the acid precipitable protein fraction in various tissues of silkworm.



EFFECTS OF PERMETHRIN ON DIETARY WATER BUDGET
IN SILKWORM BOMBYX MORI

EFFETS DE LA PERMETHRINE SUR LE BILAN DE L'EAU INGEREE
CHEZ LE VER A SOIE BOMBYX MORI

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Dietary water utilization and expenditure of water in the silkworm Bombyx mori, fed ad libitum, is reported from hatching to pupation. Water intake via food amounts to 6 345 mg. About 50% of the ingested water is absorbed into the body. About 28.9% of the absorbed water is retained in the body while 71.1% of the absorbed water is transpired. About 94.3% of water utilization occurred during the penultimate two instars. Hence, effects of Permethrin, a pyrethroid insecticide, on the water utilization during the fourth and fifth larval stages have been estimated. Insecticide, Permethrin, altered the pattern of dietary water balance, resulting in the reduced larval and pupal size, finally affecting the silk production. B. mori shows remarkable adaptive potential by altering the efficiency of water absorption when water availability was restricted by the presence of insecticide Permethrin in the food. The conclusion is significant in the light of understanding the adaptation of terrestrial insects in tropical habitats.

L'utilisation de l'eau ingérée et la consommation d'eau chez le ver à soie Bombyx mori, nourri à volonté, sont étudiées depuis l'éclosion jusqu'à la nymphose. La quantité d'eau ingérée avec la nourriture s'élève à 6 345 mg. Environ 50 % de l'eau ingérée est absorbée par le corps. Environ 28,9 % de l'eau absorbée est retenue dans le corps tandis que 71,1 % de l'eau absorbée est évacuée par la transpiration. Environ 94,3 % de l'utilisation de l'eau se réalise pendant les deux derniers âges larvaires. C'est pourquoi les effets de la Permethrine, un insecticide au pyrèthre, sur l'utilisation de l'eau ont été étudiés pendant les 4ème et 5ème âges larvaires. L'insecticide (la Permethrine) modifie l'équilibre de l'eau alimentaire, ce qui entraîne une réduction de la taille des larves et des nymphes et affecte la production de soie. Le Bombyx mori montre une faculté d'adaptation remarquable en réduisant sa capacité d'absorption d'eau

lorsque la disponibilité de celle-ci est diminuée par la présence de la Permethrine dans la nourriture. Cette conclusion aide à comprendre l'adaptation des insectes terrestres aux conditions tropicales.

INTRODUCTION

Many insects are known to drink water but others particularly the leaf feeders like *Bombyx mori* L. (Lepidoptera : Bombycidae) and pests of stored products meet most of their water requirements from their food (see Waldbauer 1968; Pandian *et al.*, 1978 Delvi 1983). water is a fundamental basis of metabolic processes and a very important item of insect diet (Ross 1956). Water equivalent to 107, 56 or 41 gms is produced for every gm of fat, protein or carbohydrate metabolised (Baldwin 1964) respectively. Such metabolic water is of great significance in large fluid feeders (Schmidt-Nielsen and Schmidt-Nielsen 1953). However, its importance is in smaller insects especially those feeding on carbohydrate rich leaves is less (Wharton and Arlian 1972). Environmental factors like temperature, food quality play an important role in understanding of the interaction between an insect and its surroundings (Delvi 1983). The environmental stress is more in insects like *Bombyx mori* which meets a major fraction of the required water from the ingested mulberry leaves *Morus alba*.

There are few papers concerning the effects of environmental factors like temperature, leaf ration (Pandian *et al.* 1978; Delvi 1983) and insecticides (Naik and Delvi 1984) on dietary water utilization in insects. The effects of environmental factors on water gain and loss during ingestion and digestion of food and the mechanisms regulating them have not been studied adequately in economically important insects like *Bombyx mori*. This paper reports on the effects of Permethrin a pyrethroid insecticide on water utilization in silkworm *Bombyx mori*.

MATERIALS AND METHODS

Egg layings of *Bombyx mori* (Pure Mysore race) were obtained from the sericulture section (World Bank Research and Development Project) of the department and transferred to plastic trays. After a period of 10 days, freshly hatched larvae were removed to separate terraria, (plastic tray size 36 x 26 x 4 cms) and maintained in the laboratory fed *ad libitum* on mulberry leaves *Morus alba* at $26 \pm 2^\circ\text{C}$, $80\% \pm 10$ RH. The test individuals were separately fed on (i) fresh mulberry leaves (ii) fresh mulberry leaves treated with Permethrin (0.04 Ppm) dissolved in acetone (Permethrin, a pyrethroid insecticide, 20% EC, supplied by Voltas (India Ltd.)). The concentration of Permethrin was calculated on the basis of active ingredient and used. It was necessary to dissolve Permethrin in acetone to obtain uniform distribution of insecticide on mulberry leaf. However, acetone itself had considerable effect on the water utilization in *B. mori*. Hence, the test individuals were separately fed on *M. alba* treated with acetone only.

Fresh leaves of *M. alba* were cut into two halves, one half was weighed and offered to larvae and the other was used as control to find the initial water content (Delvi and Pandian 1972). Larvae began to feed as soon as the leaf was offered. Since they fed throughout the day, the fresh leaves were offered four times a day to minimise the amount of water evaporated from the leaves. Amount of daily food intake was calculated following standard gravimetric method described by Waldbauer (1968). The total amount of water intake was estimated by knowing the water content of leaves

offered (Delvi 1983). Water intake (C) is expressed in terms of mg of water per insect per instar. Rate of water intake is expressed in mg of water per mg live weight of insect per day. Water loss via defaecation (F) was calculated from freshly defaecated pellets of randomly selected larvae on glass dish, weighed and dried at 90°C to weight constancy. Such estimations were repeated at different times of the day and on all days of the experiments. Using the values of the total dry faeces weight, the total water loss via defaecation was determined. Water loss through defaecation is expressed in terms of mg of water lost per insect per instar and rate of water loss through faeces is expressed in mg of water lost per mg live weight of insect per day. To estimate the amount of water retained in the body (P), few larvae were dried just before commencing the respective feeding experiments and their water content was assumed to represent the initial water content of the larvae chosen for the respective experiments (Sacrifice method of Maynard and Loosli 1962). The amount of water retained by larvae during a particular instar was estimated by subtracting the initial water content of the larvae of that particular instar from that of terminal larvae.

Some insects are known to obtain atmosphere water through the integument (Bodine 1921; Ludwig 1937; Beament 1964) and in a few cases via cloacal ends (Beament 1961) or via the spiracles (Buxten 1932). *B. mori* should have exchanged a considerable amount of water with ambient air; however, this was not estimated (see also Uvarov 1966, Delvi 1983). The absorption of atmospheric water and transpiration of body water through integument have been dealt in detail by previous workers (Wigglesworth 1957; Edney 1957, 1967; Eberling 1964; Cloudsley-Thompson 1962; Beament 1964). Perhaps *B. mori* meets almost complete required water from the ingested leaf of *M. alba*.

Water loss through transpiration (R) could be calculated by subtracting the water retained in the body (P) from water absorbed (C-F). Water absorbed is expressed in mg of water per insect per instar and absorption rate is expressed in mg of water per mg live weight of insect per day. Water absorption efficiency was calculated in percent relating (C-F) to amount of water intake (C). Transpiration is expressed in mg of water per insect per instar and rate of transpiration is expressed in mg of water per mg live weight of insect per day (see also Delvi 1983).

RESULTS AND DISCUSSION

Water budget of *Bombyx mori* fed *ad libitum* on *Morus alba* is presented in Fig. 1. It can be seen that about 98% of water is utilized during the penultimate two instars of the larvae (See also Naik and Delvi 1984; Delvi 1983), hence the last two instars of the larvae were chosen to know the effects of Permethrin. Moreover the larvae belonging to first 3 instars are extremely sensitive and total mortality occurs even with 0.04 Ppm of Permethrin.

Initial water content of the larvae chosen for the experiments ranged from 73% to 89% and averaged $83 \pm 4\%$. At pupation, water content of the terminal larvae exposed to Permethrin, decreased to 69%. Understandably, the larvae exposed to Permethrin exhibited lower percentage of water. However, the higher value 167 mg (of water retained) obtained for the fourth instar larvae exposed to acetone treated leaf may be due to the fact that these larvae, consumed less food, oxidised stored fat and accumulated metabolic water.

Water content of the freshly defecated pellets ranged from 68% to 75% in all the three groups. No statistically significant correlation between faecal water versus water intake through food could be obtained. Hence, water content of leaf fed and faeces egested, is considered almost equal.

No statistically significant correlation between faecal water versus consumption of either Permethrin treated leaf or acetone treated leaf could be obtained. However, water retained in the body studily decreased from 885.5 mg in the control to 630.4 mg or 391.0 mg in the acetone or Permethrin fed larvae. Similarly, since food consumption decreased in the larvae fed with Permethrin treated leaf the water intake also considerably decreased from its maximum of 5987 mg in the non treated larvae to about 4072 or 2888 mg in the larvae fed acetone treated or Permethrin treated leaf (Fig. 3.) It implies that the less water the larvae get the less they waste by defecation. Similarly in the tsetse fly (Bursell, 1961) the water defaecated depends on the amount of water intake. It is interesting to note that *B. mori* fed normal leaf, wastes as much as 3054 mg of water through defecation, whereas the value for larvae fed with acetone or Permethrin treated leaf is 2343 mg or 1108 mg (Fig. 3).

Dietary water intake, or water absorbed studily increased from a minimum of 107.4 mg or 105 mg during the first instar to maximum of 5405.3 or 2558.0 mg during the final instar of *B. mori* fed on fresh leaf of *M. alba* (Fig. 1). The total water intake (C) amounted to 6345.6 mg from hatching to pupation, out of which 50% of water is lost through faeces (F). Water absorption efficiency amounted to about 50%. Hence, 928.2 mg of water is retained in the body. Considerable amount of water is also lost through transpiration to the atmospheric air. The total water transpired amounted 2275.9 mg from hatching to pupation (Table 1).

The dietary water intake rate amounted to 4.52 mg per live weight of insect per day, whereas water absorption rate amounted to 4.27 mg per mg live weight of insect per day (Table 1). The rate of water transpiration decreased from its maximum of 17.1 mg per mg insect per day during the first instar to 0.4 mg per mg insect per day during the final instar larvae fed on fresh mulberry leaf (Fig. 2). The values obtained for *B. mori* on transpiration of water is not strictly comparable to transpiration values obtained by previous workers (Bursell, 1964; Uvarov, 1966; Edney, 1974; Jakovlev and Kruger, 1953). The values reported for *B. mori* represent only a fraction of the total transpiration i.e. only the dietary water transpired and not the total transpiration. Secondly, the life stage of the larvae studied has not been reported. Perhaps, they might have studied only penultimate instar(s) and might not have included transpiration from hatching to pupation.

Ingestion of water via food during fourth and fifth instars decreased from 5987.2 mg in larvae fed normal leaf to 4072.5 or 2888.6 mg in larvae fed acetone treated or Permethrin treated leaf (Table 1). It is interesting to note that the dietary water intake rate changed significantly and averaged 0.545 mg per mg live weight of insect per day in larvae feeding on fresh mulberry leaf or treated with acetone or Permethrin (Table 2). In *Philosamia ricini*, fed on fresh castor leaf *Ricinus communis* at $26 \pm 2^\circ\text{C}$, the dietary water intake rate from hatching to pupation averaged 0.54 mg per mg of insect per day. *P. ricini* when fed acetone treated and Permethrin treated leaf consumed water via food at the rate of 0.588 and 0.678 mg per mg of insect per day (Naik and Delvi 1984). This clearly indicates that both the quantity of water input and rate of water intake is influenced by acetone and Permethrin. External factors like temperature has influence on both the quantity and rate of water input in *P. pictus* and *D. chrysippus* (Delvi 1983). Pandian *et al* (1978) reported a significant increase in the rate of water intake at 30° and 35°C . Water lost through faeces in *B. mori* amounted to 2932.5, 2343.6 and 1108.1 mg in the fourth and fifth instar larvae when fed fresh leaf, acetone treated leaf and Permethrin treated leaf respectively. Water absorption efficiency, which was about 49% in larvae fed fresh leaf, significantly increased to 61.1% ($P>0.001$) in those fed Permethrin treated leaf. Similarly, the absorption efficiency increased to about 80% in *P. ricini* when fed Permethrin treated leaf (Naik and Delvi 1984). Delvi 1983 and Pandian *et al*. 1978 also reported a significant increase in the efficiency of water absorbed in *P. pictus* and *D. chrysippus* at higher temperatures. These observations suggest that the

lepidopteran larvae and the Orthopteran nymphs manage to get more water from the ingested food by increasing the efficiency of water absorption during stress like application of insecticide or increase in temperature. This conclusion is significant in the light of understanding the adaptation of terrestrial insects in tropical habitats.

Considerable amount of water is lost to the atmosphere perhaps mainly via transpiration (Table 1). Due to water lost via transpiration the water retention efficiency studily decreased from about 30% in the larvae fed fresh leaf to about 22% in the larvae fed Permethrin treated leaf. Baker and Lloyed (1970) and Bursell (1964) noted that the greater the loss of water sustained during the larval stage of the tsetse fly and the boll weevil, the lower is the rate of loss of water when they became pupae. It is suggested that *B. mori* managed to complete life cycle by not only increasing the water absorption efficiency but also by lowering the loss of water through decreased transpiration and thereby compensating the decreased ingestion of leaf water during the larval stages.

Insecticide treated leaf when fed to *B. mori* resulted in reduced intake of food as such restricted the availability of water for transpiration. At the time of pupation the water available was 312.8 mg in the treated larvae. Hence, the larvae had not only restricted availability of water but also had to transpire at a faster rate. Such a negative interaction led to reduced size of pupae and cocoon, as such the total silk production was considerably reduced.

The remarkable finding is that, Permethrin, present even in minute quantities (0.04 Ppm), not only alters the water utilization pattern of the larvae but greatly reduced the size of the cocoon; hence silk production was affected considerably.

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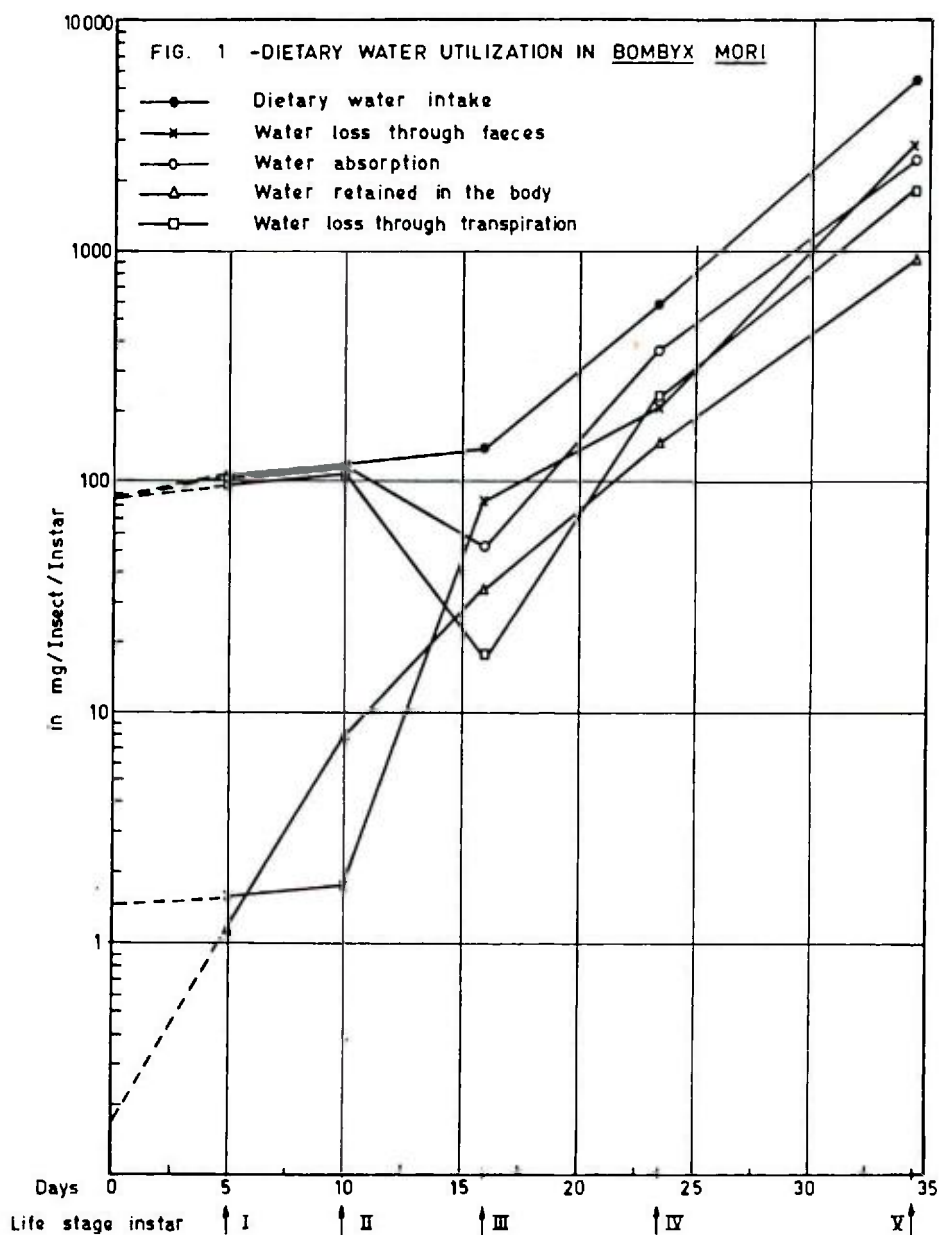
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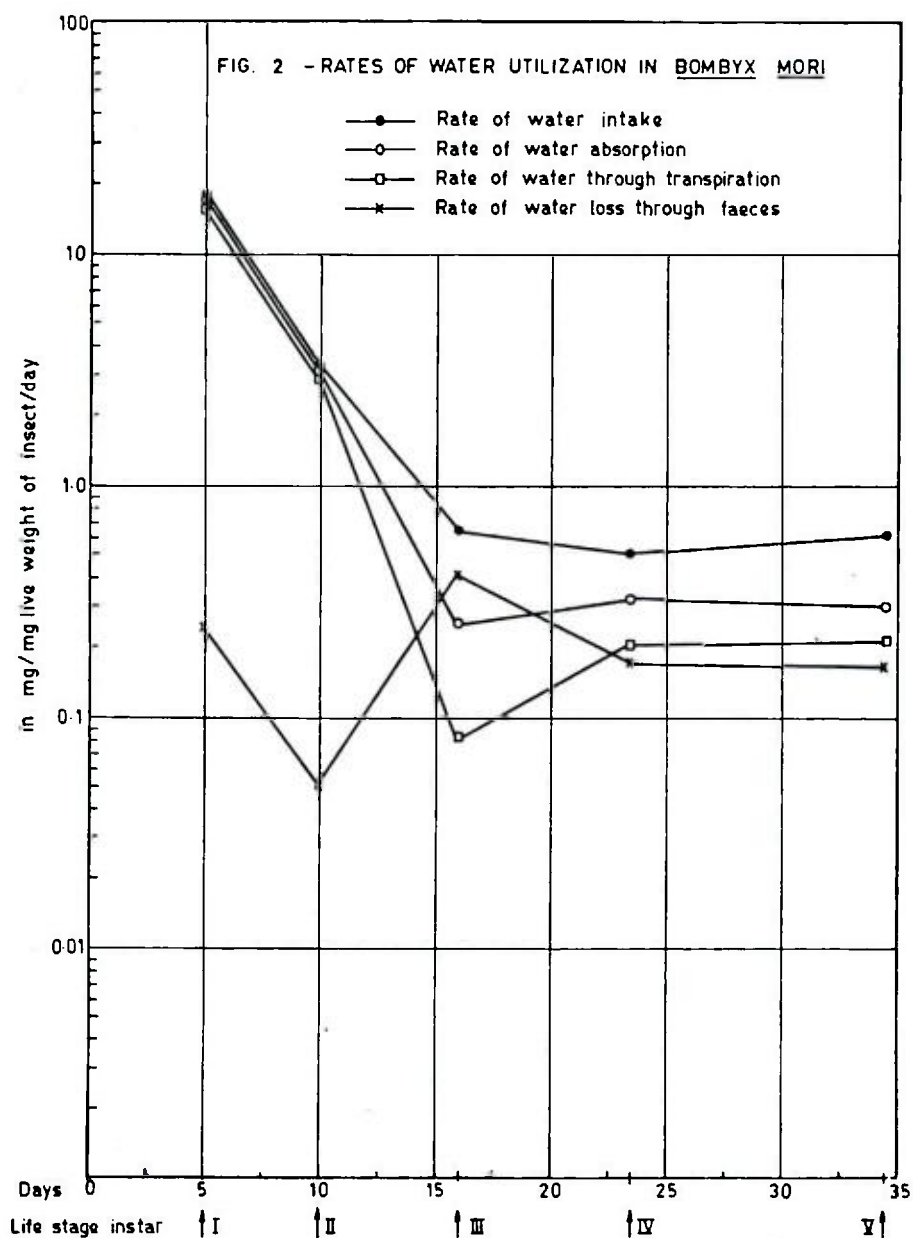
PARAMETERS	NON TREATED		ACETON TREATED	PERMETHRIN TREATED
	Hatching to pupation	Final 2 instars	Final 2 instars	Final 2 instars
Larval period days	34.5	18.5	18.5	18.5
Dietary water intake mg/insect	6345.61	5987.19	4072.49	2888.64
Water loss through faeces mg/insect	3141.15	3054.70	2343.65	1108.13
Water absorbed mg/insect	3204.15	2932.45	1728.84	1780.51
Water retained in the body mg/insect	928.24	885.57	630.42	391.03
Water loss through transpiration mg/insect	2275.91	2046.88	1098.42	1389.48
Water intake rate mg/mg live weight/day	4.5203	0.5598	0.5362	0.5184
Water loss rate through faeces mg/mg live weight/day	1.2156	0.2542	0.2047	0.0528
Water absorption rate mg/mg live weight/day	4.2793	0.4749	0.3315	0.3877
Water transpiration rate mg/mg live weight/day	4.1227	0.2041	0.2304	0.3175
Water absorption efficiency (%)	49%	-	-	61.6%

Table 1. Effects of Permethrin on water utilization in *Bombyx mori* at 26 ±2°C 80 ±10% RH fed *ad libitum* mulberry leaf *Morus alba*.

PARAMETERS Life stage (instar)	NDM TREATED		ACETONE TREATED		PERMETHRIN TREATED	
	IV	V	IV	V	IV	V
Water intake rate mg/mg live Wt/day	0.5020	0.6176	0.6126	0.4597	0.6613	0.3755
Water loss rate through faeces mg/ mg live Wt/day	0.1791	0.3292	0.1037	0.3057	0.0948	0.0108
Water absorption rate mg/mg live Wt/day	0.3228	0.3043	0.5089	0.1540	0.5660	0.2094
Transpiration rate mg/mg live Wt/day	0.2014	0.2068	0.3706	0.0901	0.4760	0.1589

Table 2. Effects of Permethrin on rates of water utilization in *Bombyx mori* at $26 \pm 2^\circ\text{C}$ fed *ad libitum* mulberry leaf *Morus alba*.





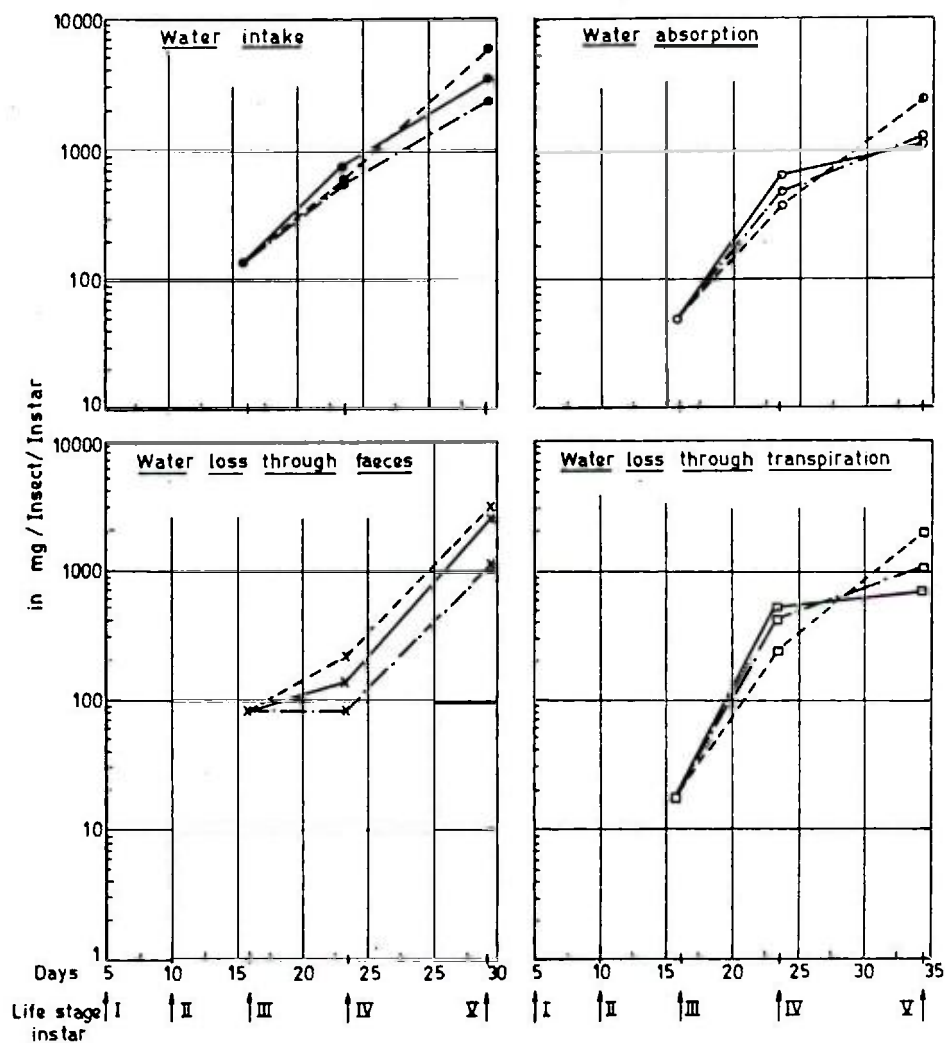


FIG. 3 -EFFECT OF PERMETHRIN ON WATER UTILIZATION IN BOMBYX MORI

--- Control
 — Acetone
 -.- Acetone + Insecticide

SUSCEPTIBILITY OF DIFFERENT RACES OF SILKWORM,
BOMBYX MORI LINNAEUS TO ASPERGILLOSIS CAUSED BY
ASPERGILLUS TAMARII KITA

SENSIBILITE DE DIFFERENTES RACES DE VERS A SOIE BOMBYX MORI
A L'ASPERGILLOSE PROVOQUEE PAR ASPERGILLUS TAMARII KITA

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Four silkworm races viz., Kalimpong A., NB₄D₂, Hosa Mysore and Pure Mysore were screened for their susceptibility against aspergillosis caused by Aspergillus tamarii Kita at Agriculture College, Dharwad. Infectivity test indicated that, all the four races were susceptible but the degree of susceptibility was varying. The Pure Mysore was less susceptible whereas Kalimpong A. was most susceptible followed by NB₄D₂ race. The Hosa Mysore race did not differ significantly in its susceptibility from Pure Mysore race.

La sensibilité à l'aspergillose causée par Aspergillus tamarii Kita a été étudiée chez quatre races de ver à soie (Kalimpong A, NB₄D₂, Hosa Mysore et Pure Mysore) au Collège d'Agriculture de Dharwad. Le test d'infectivité indique que les quatre races sont sensibles mais que le degré de sensibilité est variable. La race Pure Mysore est la moins sensible, tandis que la race Kalimpong A est la plus sensible, suivie par NB₄D₂. La race Hosa Mysore n'est pas très différente de la race Pure Mysore en ce qui concerne la sensibilité.

Acquired immunity in silkworm to the fungi, *Metarrhizium anisopliae* (Metch) Sor. and *Beauveria bassiana* (Bals) Vuilli. has been reported (Glaser, 1925; Paillot, 1930) Individual silkworm showed very little immunity against the infection of *B. bassiana* (Steinhaus, 1949). Koidsumi (1957) demonstrated the presence of immunity in silkworm due to its cuticular lipids.

Kawakami (1975) studied the difference in susceptibility of three varieties of Japanese races (J 124, J 131 and J 134) and two varieties of Chinese races (124 and C 135) of the silkworm *B. mori* to *Aspergillus flavus* link infection. The result of infectivity test showed that, Chinese races were more resistant than Japanese ones

to the infection. The loss due to different diseases of silkworm was maximum in bivoltine races followed by the cross breeds and minimum in multivoltine races (Anonymous 1975). Venkataramana Reddy (1978) reported that, Pure Mysore race was comparatively less susceptible and J 122 race was most susceptible to white muscardine. In order to find out resistant races to aspergillosis among the popularly reared silkworm races in Karnataka, the study was undertaken.

MATERIAL AND METHODS

The following four pure silkworm races were used to test their difference in susceptibility to *A. tamarit*.

1. Pure Mysore (P.M.)
2. Hosa Mysore (H.M.)
3. Kalimpong A. (K.A.)
4. NB₄D₂

Experiment was conducted on two instars viz., second and third instars of all the four pure races separately. Larvae emerged at the same time from eggs of these races were reared separately in mass beds. When the larvae attained second and third instars, larvae were separated from the bed, replicated three times each with 25 larvae and infected with one ml. of spores suspension containing 27,50,000 spores. Spore count was made by using 'Neubauer' haemocytometer (Improved double ruling). Spore suspension prepared from seven to eight day old culture was sprayed on larvae in clean sterile petridishes using a 'Amber glass Brovon Midget inhaler' with an atomiser. Race susceptibility was determined on the basis of larval mortality, per cent larval mortality was subjected to angular transformation and analysed statistically.

EXPERIMENTAL RESULTS AND DISCUSSION

The mean total larval mortality due to second instar infection, third instar infection and cumulative larval mortality were analysed and presented (Table 1).

The cumulative mean percentage of larval mortality on the first and second instar indicated that, the race Pure Mysore was less susceptible and Kalimpong A. was highly susceptible. The highest percentage of cumulative mean larval mortality was observed in Kalimpong A. (72.94) followed by NB₄D₂ (51.32) and Hosa Mysore (38.74). The lowest larval mortality 29.20 per cent was recorded in Pure Mysore race. The Pure Mysore and Hosa Mysore races did not differ significantly with each other in their susceptibility.

The second instar infection was significantly (59.38%) more to the third instar infection (37.02%).

The average total larval mortality resulted from second instar infection was the highest (25.46%) in Kalimpong A. and the lowest (9.79%) in Pure Mysore race. There was no significant difference in larval mortality among Hosa Mysore (18.55%), NB₄D₂ (20.30%) and Kalimpong A. (25.46%). A maximum of 39.70 per cent larval mortality was recorded at the third instar and minimum (1.92%) at the fifth instar. The larval mortality occurred at second instar and fourth instar did not differ significantly.

The highest 23.62 per cent mean larval mortality was observed in kalimpong A. and lowest (10.99%) in Pure Mysore race when infection was made at the third instar. Pure Mysore and Hosa Mysore did not vary significantly in their susceptibility and a similar behaviour was observed between NB₄D₂ and kalimpong A. races was significantly superior over Pure Mysore and Hosa Mysore races. The larval mortality occurred at fourth and fifth instar registered a significant difference between themselves.

Varied degree of susceptibility was observed among four races of silkworm screened against aspergillosis. The Pure Mysore race was significantly less susceptible compared to NB₄D₂ and kalimpong A. but it did not differ with Hosa Mysore. The results are in agreement with Venkataramana Reddy (1978) who reported that the Pure Mysore race was less susceptible to white muscardine.

Kawakami (1975) reported that the Chinese races were more resistant than Japanese ones to aspergillosis. In the present study the two bivoltine races NB₄D₂ and K.A. showed greater degree of susceptibility to infection of *A. tamariz* than Hosa Mysore and Pure Mysore races. Findings are in close conformity with Anonymous (1975) who reported that loss due to various diseases was maximum in bivoltine races and minimum in multivoltine races.

All the four races of the silkworm, screened against aspergillosis were susceptible to the fungus, *A. tamariz*. The Pure Mysore race was less susceptible while the bivoltine race kalimpong A. was most susceptible followed by another bivoltine race NB₄D₂.

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Treatment	State of infection	Mean per cent larval mortality					Cumulative mean (Second and third instar infection)
		Second	Third	Fourth	Fifth	Mean	
Pure Mysore	Second instar	16.71 (12.00)*	22.44 (16.00)	-	-	9.79 (7.00)	29.20 24.00
	Third instar	-	16.08 .8.00	20.09 (12.00)	-	12.06 (5.67)	
Hosa Mysore	Second instar	17.18 (9.33)	39.84 (41.35)	17.18 (9.33)	-	18.55 (15.00)	
	Third instar	-	10.95 (5.33)	22.01 (14.67)	-	10.99 (6.67)	38.74 (40.00)
Kalimpong A.	Second instar	10.60 (5.33)	49.61 (57.33)	33.92 (33.33)	7.69 (4.00)	25.46 (25.00)	
	Third instar	-	30.15 (25.33)	40.72 (42.67)	-	23.62 (21.78)	72.94 84.00
NB ₄ O ₂	Second instar	18.99 (10.67)	46.94 (53.33)	15.25 (10.66)	-	20.30 (18.67)	
	Third instar	-	19.81 (12.00)	29.12 (24.00)	9.32 (4.00)	19.41 (13.33)	51.32 (57.33)
Mean	Second instar	15.87 (9.33)	39.70 (41.75)	16.59 (13.33)	1.92 (1.00)	18.02 (16.83)	59.38 (65.67)
	Third instar	-	19.25 (12.65)	27.99 (23.34)	2.33 (1.00)	16.02 (12.11)	37.02 (37.00)

- indicates no larvae : - indicates no mortality : * Actual values; NS indicates not significant

Mean larval mortality when second instar infected		Race	Instar	Race x Instar
	S.E.M. $\pm$	2.67	2.67	5.35
	C.D. at 5%	7.67	7.67	15.37
Mean larval mortality when third instar infected				
	S.E. $\pm$	1.79	1.55	3.10
	C.D. at 5%	5.21	4.51	9.02
Cumulative mean larval mortality				
	S.E	3.73	2.63	5.27
	C.D. at 5%	11.15	7.86	NS

Table 1. Larval mortality in different races of *Bombyx mori* when infected with *A. tamarit*.

CYTOLOGICAL STUDIES OF THE UZIFLY,
TRICHOLYGA BOMBYCIS BECK (DIPTERA: TACHINIDAE)

ETUDES CYTOLOGIQUES DE LA MOUCHE OUZY,
TRICHOLYGA BOMBYCIS BECK (DIPTERA : TACHINIDAE)

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Tricholyga bombycis Beck, the uzifly is a wide known endoparasite of mulberry silkworm, Bombyx mori. Preliminary studies including the description of the karyotype and polytene chromosomes of the uzifly are presented here.

The karyotype of Tricholyga bombycis Beck has a diploid chromosome number of 12. Of the six chromosome pairs, five are autosomes with submetacentric centromeres. The sixth dot-like sex pair is heteromorphic in male (XY) and homomorphic in female (XX). The mean total lengths of the chromosomes in the complement at oogonial mitotic metaphase, meiotic metaphase I and somatic metaphase are 30.80 μm , 30.59 μm and 41.45 μm respectively. The banded giant polytene chromosomes from the malpighian tubules of the maggot and endometaphase contraction of the primary polytene chromosomes from the adult ovarian nurse cells have been observed.

The importance of the detailed cytological and genetical studies of the uzifly has been discussed.

La mouche ouzy, Tricholyga bombycis Beck, est un endoparasite bien connu du ver à soie du mûrier, Bombyx mori. Des études préliminaires comprenant la description du caryotype et des chromosomes polytènes de la mouche ouzy, sont présentées ici.

Le caryotype de Tricholyga bombycis B. a un nombre diploïde de chromosomes de 12. Des 6 paires de chromosomes, 5 sont des autosomes avec des centromères submétacentriques. La 6e paire sexuelle ponctiforme est hétéromorphe chez le mâle (XY) et homomorphe chez la femelle (XX). La longueur moyenne des chromosomes dans

leur totalité est respectivement de 30,80 μ m, 30,59 μ m et 41,45 μ m à la métaphase mitotique oogoniale, la métaphase méiotique I et la métaphase somatique. Nous avons observé les chromosomes polytènes géants bandés provenant des tubes de Malpighi du ver à soie et la contraction à l'endométaphase des chromosomes polytènes primaires provenant des cellules nourricières de l'ovaire de l'adulte.

Nous discutons l'importance des études cytologiques et génétiques de la mouche effectuées de manière détaillée.

INTRODUCTION

Tricholyga bombycis Beck (= *Exorista sorbillans* Wiedmann), the Uzifly is known as a serious endoparasite of the silkworm *Bombyx mori* (Lepidoptera : Bombycidae). Some information is available on the life history (Datta and Mukherjee, 1978); modes of infection (Krishnaswamy *et al.* 1964); rearing and reproduction (Sriharan *et al.* 1971) of the Uzifly. Barring some preliminary report on the chromosome number by Shanthamurthy and Aswath (1982), detailed cytological studies of the Uzifly have suffered much neglect. This paper presents a description of the karyotype of the Uzifly and a preliminary survey of the polytene type chromosomes from its various tissues which can be used for eventual evaluation in the genetic control method of this species.

MATERIALS AND METHODS

The full grown parasitic maggots were collected soon after emergence from the infected silkworm cocoon at the cocoon market of Channapatna (Karnataka State). Some of these maggots were immediately dissected out to obtain tissues like imaginal disks, salivary glands, malpighian tubules, mid and hind gut. Some pupae of both sexes aged about 3-4 days old were also dissected and gonadal tissues were obtained for cytological studies. The remaining pupae were allowed to metamorphose into adults in the mosquito netted cages. One to two days old adult flies (8% cotton dipped glucose fed) of both sexes were also utilized for dissection and the gonadal tissues of the same were used for chromosome preparations.

The dissections were carried out in 1% sodium citrate solution and different tissues obtained were fixed in Carnoy (1 part acetic acid and 3 part absolute alcohol) for 3 to 30 minutes depending on the type of tissue used. Temporary squash preparations were made using 2% Lacto aceto orcein stain. Except the testis of the adult fly which contained mostly mature sperms, other tissues yielded good chromosome stages. The Zeiss phase contrast system was used for both observation and photography. The measurements of somatic as well as gonadal metaphase chromosomes were taken with the help of a Calibra ocular reticulae. All the chromosomes from a karyotype have been individually numbered based on their length and decreasing order of size.

RESULTS AND DISCUSSION

The karyotype of *Tricholyga bombycis* Berk (Fig. 1) consists of six pairs of chromosomes (2n=12) including five pairs of autosomes which are more or less submetacentric and one pair of small dot like sex chromosomes. The autosomal pairs have

been numbered 1-5 in gradation of length commencing from the longest pair. The sixth sex chromosome pair is heteromorphic in male (XY), Y being smaller than the X, as it is clearly revealed in male meiotic metaphase I (Fig. 2) and homomorphic in female (XX). However, it is difficult to determine the position of the centromere in the case of the X and Y chromosomes in view of its small size and dot like appearance.

The mean lengths of arms of all the chromosomes based on a sample of 20 metaphase complements obtained from pupal testis (Primary spermatocyte), adult ovary (oogonial cell) and maggot imaginal disk have been measured (Table 1). The average chromosome length of the primary spermatocyte complement is 30.59 μm and of the oogonial metaphase complement is 30.80 μm .

The somatic metaphase chromosomes from the imaginal disks (Fig. 3) have been examined. The average chromosome length of the somatic metaphase complement is 41.36 μm . Interestingly, the somatic metaphase autosomal pairs exhibit pairing while each chromosome has split up into two chromatids lying parallel to each other which are held together in the region of the centromere.

Chiasmata have been observed in females (Fig. 4) which are conspicuously absent in males (Fig. 5) with the appearance of synapsis in both sexes. The apparent lack of crossing over in males should facilitate the development of a genetic sexing system as reported in the case of the stable fly, *Stomoxys calcitrans* (Joslyn *et al.*, 1979).

Polytene chromosomes have been observed in the salivary glands, malpighian tubules, mid and hind gut of the maggots, and in the adult ovarian nurse cells. The polytene chromosomes from the malpighian tubules (Fig. 6) appear to be more suitable to analyse some of the structural and functional properties of the chromosomes. The ovarian nurse cells of the adult female offer a special type of contracted primary polytene chromosomes (Figs. 7-8), representing an extreme form of endometaphase. These chromosomes appear similar to those of *Calliphora erythrocephala* (Bier 1957, 1960). Detailed cytological and genetical studies of the Uzi fly are under investigation which may throw some light on evolving a longstanding and effective method for its control.

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Chromosome number	Tissue	Length in microns			
		Arm a	Arm b	Total	Ratio (a/b)
1	Ovary	4.34	3.04	7.38	1.42
	Testis	4.34	3.26	7.60	1.33
	Imaginal disk	6.08	4.34	10.42	1.40
2	Ovary	3.91	2.60	6.51	1.50
	Testis	3.47	2.60	6.07	1.33
	Imaginal disk	4.78	3.47	8.25	1.37
3	Ovary	3.47	2.60	6.07	1.33
	Testis	3.26	2.60	5.86	1.25
	Imaginal disk	4.78	3.04	7.82	1.57
4	Ovary	3.04	2.17	5.21	1.40
	Testis	3.04	2.17	5.21	1.40
	Imaginal disk	4.34	2.60	6.94	1.66
5	Ovary	3.04	1.73	4.77	1.75
	Testis	2.60	2.17	4.77	1.19
	Imaginal disk	4.30	2.55	6.85	1.68
6	Ovary	-	-	0.87	-
	Testis	X	-	1.08	-
		Y	-	0.65	-
	Imaginal disk	X	-	1.08	-
		Y	-	0.54	-

The chromosomes are numbered from longest to shortest. About 20 well spread of each Oogonial, Primary spermatocyte and Imaginal disk metaphase were examined and average of these was taken as chromosome length.

Table 1. Measurements of oogonial (ovary), primary spermatocyte (testis) and imaginal disk cell metaphase chromosomes of *Tricholyga Bombycis* Beck.

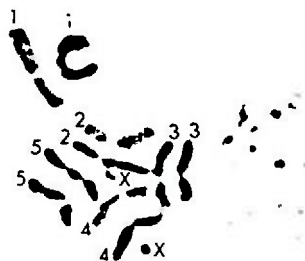


FIG. 1



FIG. 2



FIG. 3



FIG. 4



FIG. 5

Figure 1. Metaphase complement from an oogonia cell of adult fly. (4826x)

Figure 2. Metaphase complement from a primary spermatocyte of pupa. (4826x)

Figure 3. Metaphase complement from an imaginal disk cell of maggot. (4826x)

Figure 4. Chiasmata in the primary oocyte of adult fly. (4826x)

Figure 5. No chiasmata in the primary spermatocyte of pupa. (4826x)



FIG. 6



FIG. 7



FIG. 8

Figure 6. Polytene chromosome complement from a malpighian tubule cell of maggot. (3000x)

Figures 7 & 8. Endometaphase contraction of the primary polytene chromosomes from adult ovarian nurse cells. (4826x)

OSCILLATORY FREQUENCIES IN BOMBYX MORI, LR(PM) x NB₄D₂

FREQUENCES OSCILLATOIRES CHEZ BOMBYX MORI,
LR(PM) x NB₄D₂

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Overt (hatching, moulting and settling for pupation) and biochemical (determined in silk gland of fifth instar alone) (total ninhydrin positive substances (TNPS), carbohydrates and proteins) components fluctuated cyclically in Bombyx mori, LR x NB₄D₂ under LD 12:12 (natural day) and imposed 11:13 (short day) regimens of 24 hr day ($\omega = 360^\circ$). Hatching to settling for pupation varied from diurnal to nocturnal expressions while TNPS and total carbohydrates documented diurnal predominance. These were circadian in nature. Total proteins recorded additive curve properties as also body and silk gland weights. However, the rhythm is medial spread over the duration of the fifth age.

Exposure to short day (LD 11:13) schedule accomplished though, larger levels in the biochemical constituents, the fluctuations followed LD pattern. Economic characters were bettered under LD 11:13 conditions.

Certaines manifestations comme l'éclosion, la mue, l'encabannage pour la nymphose et certains composants biochimiques déterminés seulement dans la glande séricigène de larves du 5ème âge (substances totales colorables à la ninhydrine, les hydrates de carbone et les protéines) varient de manière cyclique chez le ver à soie Bombyx mori, LR x NB₄D₂, selon qu'il est soumis à un

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régime jour/nuit de 12:12 (durée de jour normale) ou à un régime jour/nuit imposé de 11:13 (durée réduite) par 24 h ($\omega = 360^\circ$). La période de l'éclosion à la montée varie de l'expression diurne à l'expression nocturne tandis que les TNPS et les hydrates de carbone totaux marquent une prédominance en période diurne. Ces variations sont par nature circadiennes. Les protéines totales enregistrent une courbe cumulative de même type que le poids du corps et le poids de la glande séricigène. Cependant, le rythme est étalé sur toute la période du 5ème âge.

L'exposition à une durée du jour court (LD : 11:13) conduit à une élévation du niveau des constituants biochimiques, les fluctuations suivant le rythme jour-nuit. Les caractères économiques sont améliorés dans les conditions jour/nuit 11:13.

INTRODUCTION

Light in nature (Bünning, 1973) and it as one of the zeitgebers phases chronobiologic events in animals (see Sollberger, 1965; Bünning, 1973; Saunders, 1978; Beck 1980). Under light-dark schedules of natural solar day (≈ 24 h; see Saunders, 1978) animals exhibit rhythmic fluctuations (see Sollberger, 1965; Bünning, 1973; Saunders, 1978; Beck, 1980; Pavan Kumar *et al.*, 1981; Subramanyam *et al.*, 1981; & C). Embryonic diapause in response to photoperiods characterizing short-day nature, (Kogure, 1933), growth rates (Hirasaka *et al.*, 1969; Hirasaka and Koyama, 1970, 1972) and various other phenomena showing cyclical fluctuations in *Bombyx mori*: location of biological clock for diapause in the brain of *Antheraea pernyi* (Williams, 1969), gated rhythm of larval ecdysis in *A. pernyi* and *Manduca sexta*, bimodal metabolic (oxygen) rhythm in *A. pernyi* (Ushatinskaya, 1969), etc. were reported for silkworms. *Bombyx mori* was reported as a short day insect (Kogure, 1933; De Wilde, 1962; Danilevskii, 1965; Lees 1968; Saunders, 1978; Beck, 1980), while Shimizu (1982) showed that artificial diet resolved long day characteristics for diapause in the next generation.

Though various oscillatory phenomena in silkworms have been thus elucidated (also see Koyama *et al.*, 1974; Yamaoka *et al.*, 1976; Ananthanarayana *et al.*, 1978), biochemical constituents received cursory attention. The present investigation resolves oscillatory functions under LD 12 : 12 natural solar day and LD 11 : 13 short day schedules.

MATERIAL AND METHODS

Procurement of experimental material

Disease free silkworm eggs (dfIs) of LR (PM) x NB4D2 cross breed variety were purchased, third day after oviposition, for the study from Government Silk Farm, Palamaner, A.P. The dfIs were transported to S.V. University, Tirupati (13.45 N and 79.30 E), during evening cool hours, where the studies were carried out. At the work spot, dfIs were immediately spread in the rearing trays.

Light regimens

The same day (when dfIs were brought) dfIs were introduced till spinning in two batches into the following Light-dark schedules :

LD 12:12 of natural solar day (control)

LD 11:13 of imposed short day (L = 06 to 17 h and D = 17 to 06 h)

The rearing was cellular at temperature $26 \pm 2^\circ\text{C}$ and Rh 80 ± 5 all through.

Rearing methods

The method followed for rearing was as per Krishnaswami (1978). However, Chawki rearing was not conducted. Hatched larvae collected into disinfected rearing trays were daily fed five times (06 h, 10 h, 14 h, 18 h and 22 h) on fresh mulberry (M5 variety) leaves. For early stages, the larvae were fed on tender leaves. While cleaning the spent leaves once in a day from III age, only healthy worms were collected into trays. The larvae under moult were not disturbed.

Parameters studied

a) Overt phenomena

Precise timings in hours for hatching, moultings and settling for pupation (pharate larvae) (by random sampling) were determined.

b) Biochemical estimates

i) Isolation of silk gland and experimental procedure.

For estimations, the 24 h day ($\omega = 360^\circ$) was divided into six 4 h periods, 02, 06, 10, 14, 18 and 22 h. All through the length (8 days) of fifth instar, the biochemical constituents were determined from the silk gland at the said hours every day during January-December 1980. At the above mentioned six periods of 24 h solar day, the silk gland (anterior, middle and posterior portions) was isolated from a single worm. The isolated silk gland was wiped off body fluids and stored in prechilled glass tubes until shortly analysed. For each condition, two replications were run.

ii) Estimates

- Estimates of total carbohydrates (TC) content.

Total carbohydrate content in the silk gland, homogenized in 10% TCA, was determined as per the method of Carroll *et al.*, (1956). To a known quantity of clear TCA supernatant (centrifuged at 3000 rpm for 15 minutes), 5.0 ml of anthrone reagent was added and the combination boiled for 25 minutes in a water bath. The color measured at 620 m μ was analysed for mg/g wet weight of the tissue.

- Estimate of TNPS.

As above, TNPS in silk gland was determined (λ 570) following the method of Moore and Stein (1954) and expressed in mg/g wet weight of tissue.

- Estimate of total protein.

Total protein content was estimated (λ 600) following the method of Lowry *et al.*, (1951). Protein content was expressed as mg/g wet weight of tissue.

- Determination of weights.

Accurate weights of body and silk gland during fifth instar were determined for individual worms each day from a random sample of 20 worms from each bed.

- Economic characters.

Accurate weights of single cocoon and shell were determined on electrical sartorius balance. Length of reelable silk thread was preliminarily determined against the measured length of single rotation of kymograph drum. Larval duration was determined from hatching to mounting (active feeding period).

Analyses of data

The biochemical constituents and as well the quantitative characters of silk-gland and body weights were analysed by single Cosinor method of Koukkari *et al.*, (1974) to characterise rhythmic patterns. Amplitude values were calculated through trigonometric functions and their significance determined through F values obtained. Significance or lack of it at a given level (here p at 2,3 = 0.05) is determined based on whether the calculated F value is greater (significant) or less (not significant) than a corresponding value from the F table. Where macroscopic data was less than corresponding F value, biological noise (Halberg, 1960) was eliminated by the method of Sasa and Tanaka (1974) and significance tested.

Symbols used

In conformity with the International Society of Chronobiology, the following symbols are used in the text :

- = Dark hour of schedule imposed during 24 hr day.
- = light hour of schedule imposed during 24 h day.
- L = Light phase of LD cycle.
- D = Dark phase of LD cycle.
- LD = Light dark cycle.
- M = Mesor.
- A = Amplitude.
- W = angular frequency.
- HO = Null hypothesis = $HO = A = 0$ - accepted (not significant).
 $HO = A \neq 0$ - rejected (significant)
- F = Estimated p value for significance.
- $\Delta\phi$ = Phase shift.
- $+\Delta\phi$ = Advance phase shift.
- $-\Delta\phi$ = Delay phase shift.

Reference time = 02 h of local time at 13.45 N and 79.30 E (Tirupati, India).

- ϕ = Computed acrophase = the lag from reference local time of the peak in the best fitting cosine function to approximate the rhythm in the data = phase angle of biological oscillation.
- SD = Standard deviation.
- SE = Standard error
- PM = Pure Mysore, multivoltine race (local race, LR).
- NB4D2 = Bivoltine race.
- LRxNB4D2 = Cross breed.

- TC = Total carbohydrate (reducing + non reducing sugars).
 TNPS = Total ninhydrin positive substances.
 TP = Total protein content.
 g = gram.
 mg = milligram.
 </> = less greater than.
 </> = less greater than or equivalent to
 h = hour.
 m = minutes.
 N = North.
 E = East.
 H = Hatching.
 I - IV M = I - IV moults.
 SP = Settling for pupation - pharate larva formation.

RESULTS

Patterns under LD 12 : 12 regime

Hatching was predominantly diurnal with about 87 % of hatching observed at early hours of the light part of natural day LD cycle (Fig. 1a). First moult recorded interestingly 180° inversion with crest in moulting (50%) occurring at 20th during the dark phase of LD cycle (Fig. 1b), thus showing nocturnal expression. Successive moults crested gradually through light phase of LD cycle with fourth moult cresting at 08 h as also settling for pupation (pharate larvae) (Figs. 1c to 1f and 7). Thus hatching to pharate larvae, the developmental stages of LR x NB4D2 *Bombyx mori* exhibited a "cycle" with diurnal and nocturnal expressions (Fig. 7).

Oscillatory patterns for total carbohydrates (TC) and ninhydrin positive substances (TNPS) were diurnal with crests at 10 h of LD 12 : 12 cycle (Figs. 2 and 3). The amplitude (A) values of the visual chronograms recorded gradually larger magnitudes for both the constituents till seventh day, hence the amplitude suddenly dropped (Figs. 2 and 3). The averages, for each day, of Mesor (M) and amplitude recorded the highest values on 6th day for TNPS and 7th day for TC (Tables 1 and 2). In both the cases, null hypothesis (H0) was rejected ($H0 = A \neq 0$) at $F(2,3)(0.05) > 9.6$. Computed acrophase (ϕ) ranging from -140° (09 h 20) to -155° (10 h 20) for TC and -142° (09 h 28) to -151° (10 h 04) for TNPS synchronized with visual chronogram crests (tables 1 & 2). The daily rhythm was circadian with periods around 24 h as determined from the ranges of ϕ values.

Total proteins (TP) offered a different situation. A clear cut circadian pattern was not observed for total proteins (Fig. 4). Hour to hour and day to day values for the constituent from silk gland of V instar were additively curvi-linear upto 7th day. On 8th day, the values recorded progressively downward trend (Fig. 4). Day to day averages for TP values, when resolved for the whole length of V instar graphically, sine wave curve with a crest on 7th day was obtained (Fig. 4). Macroscopic data analysed microscopically resolved no significance since $H0 = A = 0$ at $F(2,5) (0.05) < 5.79$

Biological noise eliminated through $Y = 100 + a \cdot \cos 45 (h-k)$ (Sasa and Tanaka, 1974). (ω for TP = $360^\circ / 8 \text{ days} = 45^\circ/\text{day}$) the total proteins resolved sine wave curve and $H_0 = A \pm 0$. φ value of -310° , converted to time 6.89 days, synchronized visual chronograms. Parallelism prevailed for silk gland and body weights (Figs. 5 & 6) with the largest values for body and silk gland weights observed on 7th day. φ values synchronized chronograms.

Patterns under LD 11 : 13 (imposed short day LD) schedule.

The observed LD 12 : 12 patterns persisted for hatching, moulting and settling for pupation (Figs. 1a to f, 7). Hatching to III moult phase advanced ($+\Delta \emptyset$) LD pattern while IV moult and pharate larva formation phase delayed ($-\Delta \emptyset$) (Fig. 7). Under the short day conditions, 1st moult to pharate larva formation finished predominantly diurnal (Fig. 7).

For TNPS, TC and TP also the curve properties persisted LD patterns (Figs. 2, 3 & 4). While TC and TNPS resolved no remarkable differences in M and A values, day to day averages for total protein documented 10.44% and 7th day and 24.5% on 8th day increment in the average values over LD 12:12 regimen. Mesor values for body weight under LD 11:13 condition recorded 40.7% larger values over LD 12:12 condition, followed by higher amplitude values (798.24 under LD 11:13 of 727.18 under LD 12:12 conditions). Silk gland weight was 18.8% large over LD 12:12 values and amplitude finished greater.

Other comparisons

In synchrony with prevalence of medial frequency oscillations for silk gland and body weights and total proteins in the silk gland, Mesor and amplitude values resolved for TC and TNPS, besides a clear cut daily oscillatory pattern, medial frequency fluctuations spread all through the 5th age (Figs. 2 to 6).

Upon elimination of biological noise, the magnitude of difference in values for total proteins was of the order of -1° (0.02 days or 32 minutes) under LD 12:12 and -16° (0.36 days or 518.4 minutes) under LD 11:13 conditions (table 3). Despite such differences, the phase of the rhythm, viz. expression of crest value on 7th day in the chronogram compared with calculated crest (φ) (around 7 days) did not differ (Table 3).

The larval duration from hatching to mounting finished less by 1 day 00h 25 m (24.41h) ($p < 0.001$) under LD 11:13 regime (Table 4). Averages of single cocoon weight by 19.94% (1.429 gm vs 1.714 gm) (699 vs 583 cocoons per kg) ($P < 0.001$) and shell weight by 72.4% (0.19 g vs 0.34 g) ($P < 0.001$) were bettered under LD 11:13 conditions. The silk percentage was 43.77% large ($P < 0.001$) under 11:13 condition which also recorded 41.7% ($P < 0.001$) more of reelable silk thread (Table 4).

DISCUSSION

Eversince the report of Koyure (1933), the silkworm was considered as a short-day insect (De Wilde, 1962; Danilevskii, 1965; Lees, 1968; Saunders, 1978; Beck, 1980) However, Shimizu (1982) showed that with artificial diet embryonic diapause in the next generation characterized long-day photoperiodic regime.

The observed pattern of rhythmic larval ecdysis under LD 12:12 conditions and the persistence of the LD 12:12 patterns under 11:13 conditions suggest endogenously free-running nature. Such phenomena were shown under different photoperiodic sche-

dules for various insects (Beck, 1980; Subramanyan *et al.* 1981). In the diurnal phenomenon of hatching resulting in nocturnal expression for 1 moult, as shown for *Antheraea pernyi* and *Manduca sexta*, a gated rhythm of hormone release that occurs in the scotophase (Truman, 1972; also see Beck, 1980) responsible for rhythmic larval ecdysis, might be the reason. However, the observed pattern might be due to (i) a fixed genetic imprint, (ii) a modelled oscillator reactive to an unidentified zeitgeber as nutrition, population density, light-dark schedule, temperature as in *Aedes taeniorhynchus* (Nayar, 1967) or humidity as generalized for insects (Aschoff, 1964), or as in *Gonyaulax polyhedra* synergistic actions of light and temperature (Njus *et al.*, 1977). As shown by Krishnaswami (1978), temperature and humidity hold significance for *B. mori*.

As per Hirasaka *et al.* (1969) and Hirasaka and Koyama (1970, 1972) the growth rates for *Bombyx mori* under short-day schedules were relatively high over a long-day schedule. In the present study, 11:13 LD regimen resolved larger weights for body and silk gland. This shows that short-day photoperiodic schedules are perhaps best suited for *Bombyx mori*. In conformity with the assumption the larger mesor and amplitude values for total protein in the silk gland clearly indicate enhanced synthesis and thus accumulation of total proteins (Tazima, 1978). This is supported by the observed patterns for carbohydrates, that might be used for energy purposes (Aschoff, 1964; Pavankumar *et al.*, 1981), and TNPS, that reflects amino acid pool (Pavan Kumar, 1983). Besides, the additive pattern for total protein till 7th day followed by growth rates in the silk gland while confirms accumulation of synthesized proteins, the downward trend on 8th day suggests utilisation of proteins for cocoon formation.

Clearcut daily rhythmic pattern for TNPS and TC reflects oscillatory phenomena within 24 h day. The period being around 24 h (cf ranges of values), the frequency of the oscillation is circadian. Lacking a circadian pattern, TP, silk gland and body weights showed a sine wave curve over the length of 5th age. This reflects medial frequency oscillation of the range of around 7 days. In addition to the circadian pattern, TC and TNPS also exhibited superimposing medial frequency. This is perhaps adaptive biological value for oscillatory phenomena (Aschoff, 1964), which for *Bombyx mori* is finally for cocoon formation. Perhaps *Bombyx mori* is the only example of the combination of two types of frequency oscillations that hold an adaptive biological significance.

Biological noise operative under normal circumstances, when macroscopic data is generally collected, mask on underlying true phenomenon (Halberg, 1960). In the present study, despite elimination of biological noise of the magnitude of -1° (32 minutes) and -16° (518.4 minutes) for LD 12:12 and 11:13 conditions respectively it did not affect phase of the rhythm, as also reported for *Laevicaulis alte* (Pavan Kumar, 1983). The larger magnitude of biological noise under 11:13 conditions reflects adjustment to imposed schedule, adaptation to which is accomplished with varying rates (Bünning, 1960).

Thus, on a overview, the short-day schedule resolved optimisation of biological functions in *Bombyx mori* bettering the economic characters. The present study offers a chronobiologic approach for a greater return of the commercial product.

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Age in days of V instar silkworm	RHYTHM CHARACTERISTICS WITH SE/RANGE					
	LD 12:12 ($\leq$ or $\geq$ 24 h) = solar day			LD 11:13 (short photophil = 24 hr (06 to 17 h = photophil 17 to 06 hr = scotophil))		
	M	A	H0	M	A	H0
I day	2.23 $\pm$ 0.04	0.97 $\pm$ 0.09	-143° (-140° to -152°)	2.32 $\pm$ 0.05	1.08 $\pm$ 0.03	-140° (-135° to -141°)
II day	2.54 $\pm$ 0.13	1.05 $\pm$ 0.06	-140° (-128° to -149°)	2.54 $\pm$ 0.13	1.03 $\pm$ 0.06	-141° (-128° to -149°)
III day	3.90 $\pm$ 0.41	2.03 $\pm$ 0.27	-153° (-141° to -162°)	3.58 $\pm$ 0.46	1.79 $\pm$ 0.26	-148° (-141° to -153°)
IV day	4.36 $\pm$ 0.11	1.74 $\pm$ 0.18	-151° (-146° to 159°)	4.38 $\pm$ 0.12	1.81 $\pm$ 0.18	-148° (-146° to -153°)
V day	4.66 $\pm$ 0.18	2.26 $\pm$ 0.15	-155° (-149° to -163°)	4.60 $\pm$ 0.13	2.00 $\pm$ 0.15	-150° (-146° to -155°)
VI day	6.50 $\pm$ 0.06	3.27 $\pm$ 0.23	-140° (-141° to -148°)	6.53 $\pm$ 0.06	3.32 $\pm$ 0.22	-145° (-140° to -148°)
VII day	8.93 $\pm$ 0.08	6.57 $\pm$ 0.12	-151° (-150° to -155°)	8.87 $\pm$ 0.06	6.63 $\pm$ 0.12	-152° (-150° to -155°)
VIII day	2.32 $\pm$ 0.05	1.11 $\pm$ 0.05	-140° (-135° to -142°)	8.23 $\pm$ 0.04	0.97 $\pm$ 0.09	-143° (-140° to -152°)

Table 1. Rhythm characteristics for total carbohydrates in the silk gland of
V instar larvae of silkworm, *Bombyx mori* (PM x NB4D2) (13.45 N and 79.30 E)

Age in days of V instar silkworm	RHYTHM CHARACTERISTICS WITH SE/RANGE					
	LD 12:12 ($\leq$ or $\geq$ 24 h) = solar day			LD 11:13 (short photophil = 24 hr (06 to 17 h = photophil 17 to 06 hr = scotophil)		
	M	A	HO	M	A	HO
I day	66.79 ± 1.32	30.72 ± 1.16	-144° (-140° to -154°)	65.32 ± 0.77	29.45 ± 1.14	-143° (-140° to -154°)
II day	78.56 ± 10.87	33.04 ± 1.16	-142° (-135° to -150°)	76.16 ± 1.82	32.94 ± 1.16	-140° (-135° to -150°)
III day	90.04 ± 15.01	47.19 ± 1.29	-144° (-132° to -162°)	88.01 ± 0.65	51.47 ± 0.72	-150° (-144° to -155°)
IV day	98.57 ± 4.42	42.71 ± 1.19	-149° (-138° to -158°)	89.34 ± 1.27	59.73 ± 2.91	-154° (-153° to -157°)
V day	93.89 ± 2.51	61.63 ± 3.30	-148° (-141° to -158°)	95.27 ± 1.30	65.97 ± 1.79	-150° (-147° to -158°)
VI day	96.38 ± 2.61	68.71 ± 0.56	-148° (-145° to -156°)	94.47 ± 1.57	69.28 ± 1.33	-152° (-151° to -156°)
VII day	89.32 ± 0.81	65.77 ± 1.24	-151° (-150° to -155°)	92.33 ± 0.98	72.08 ± 1.75	-154° (-150° to -159°)
VIII day	65.61 ± 1.00	34.66 ± 3.22	-144° (-140° to -148°)	65.36 ± 0.50	34.12 ± 1.60	-154° (-140° to -159°)

Table 2. Rhythm characteristics for total ninhydrin positive substances (TNPS) in the silk gland of V instar larvae of silkworm, *Bombyx mori* (PM x NB4D2) (13.45 N and 79.30 E

Rhythm characteristics	LD 12:12		LD 11:13		
	Macroscopic analyses	Microscopic analyses	Macroscopic analyses	Microscopic analyses	
M	159.52	100	179.96	100	
A	153.00	112.38	167.85	115.37	
φ	-310° (6.89 days)	-311° (6.91 days)	-318° (7.06 days)	-334° (7.42 days)	
H0	A = 0	A $\neq$ 0	A = 0	A $\neq$ 0	
[F(2,5){0.05}<5.79] F(2,5){0.05}>5.79] F(2,5){0.05}<5.79] F(2,5){0.05}>5.79]					
Comparative evaluation of φ values of LD 12:12 vs LD 11:13 regimens					
LD 12:12			LD 11:13		
Macroscopic data	Differences		Macroscopic data	Differences	Microscopic data
φ days	φ days	hrs.	φ days	φ days	hrs.
-310°	6.89	-1.0° +0.02° +0.53 (32m)	-311° 6.91	-318° 7.06	+16° +0.36 +8.64 (518m)
					-334° 7.42

Table 3. Rhythm characteristics for total proteins in the silk gland of V instar larvae of the silkworm, *Bombyx mori* (PM x NB4D2) (13.45 N and 7.30 E).

Table 4. Comparison of the economic characters in the silkworm, *Bombyx mori* (PM x NB4D2) with reference to LD 12:12 versus LD 11:13 regimens

A. LARVAL DURATION

S.No.	Condition	duration
1	LD 12:12	538.83 h (= 22 days 10 h 50 m) 12.28 h (= 00 days 12 h 17 m)
2	LD 11:13	514.42 h (= 21 days 10 h 25 m) 11.48 h (= 00 days 11 h 29 m)
11:13 cf 12:12		1 day 00 h 25 m less

B. SINGLE COCOON WEIGHT

S.No.	Condition	Weight (mg) $\pm$ SD
1	LD 12:12	1429 $\pm$ 126.67
2	LD 11:13	1714 $\pm$ 152.48
		t = p < 0.001

19.94 %

72.4 %

C. SINGLE SHELL WEIGHT

S.No.	Condition	Weight (mg) $\pm$ SD
1	LD 12:12	197.25 $\pm$ 82.62
2	LD 11:13	340.00 $\pm$ 72.41
		t = p < 0.001

D. SILK PERCENTAGE

S.No.	Condition	Silk % $\pm$ SD
1	LD 12:12	13.80 $\pm$ 1.99
2	LD 11:13	19.84 $\pm$ 1.63
		t = p < 0.001

43.7 %

42.2 %

E. LENGTH OF REELABLE SILK THREAD

S.No.	Condition	Length in meters $\pm$ SD
1	LD 12:12	564.50 $\pm$ 87.63
2	LD 11:13	800.00 $\pm$ 48.59
		t = p < 0.001

Averages of 12 cellular rearings from January to December 1980

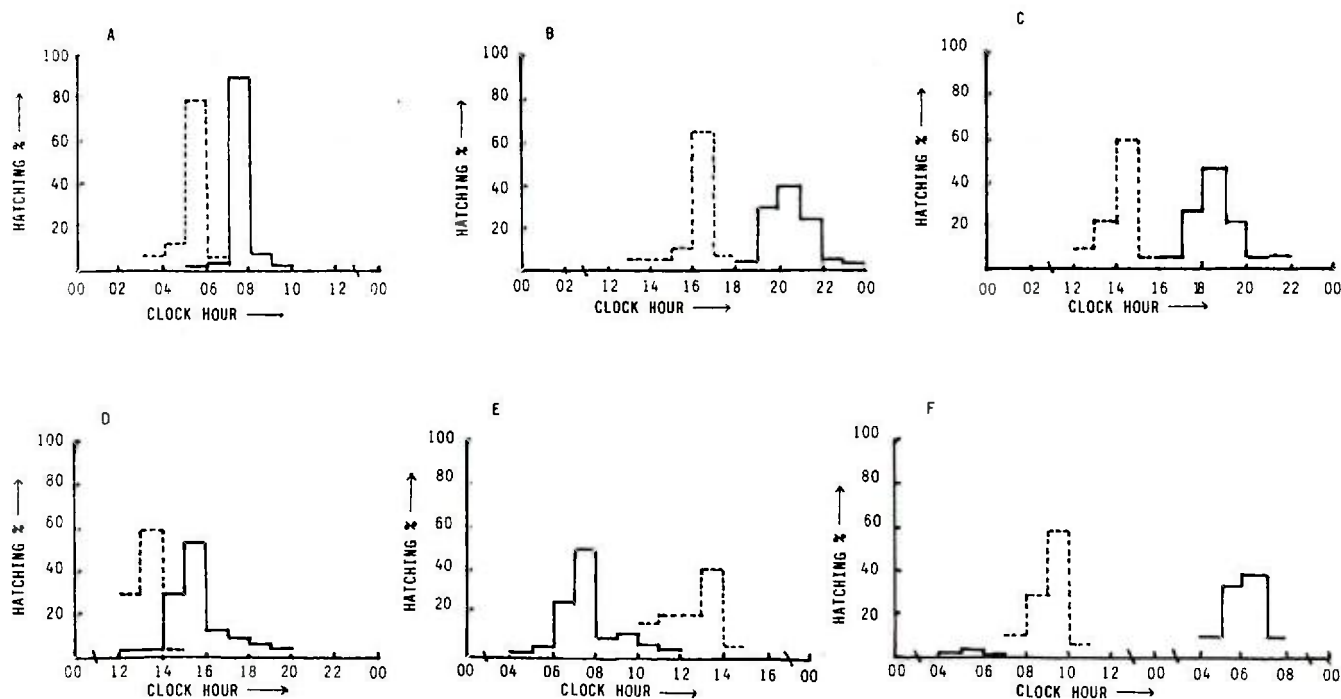
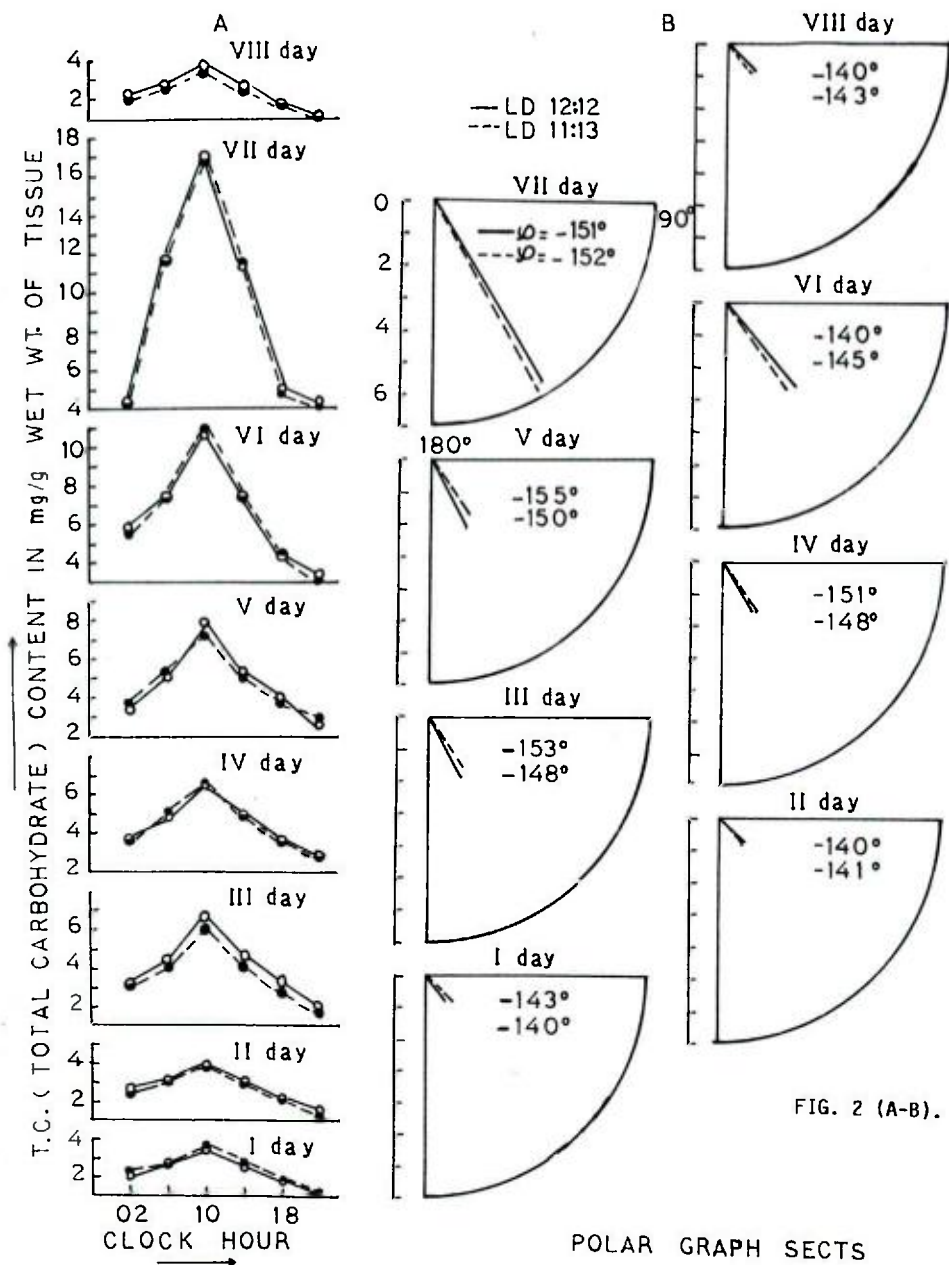


Figure 1.

Oscillatory patterns of hatching (1-A), I to IV moult (B to E) and settling for pupation (1-F) under LD 12:12 (—) and LD 11:13 (---) condition of 24 h day in the silkworm *Bombyx mori* (PM x NB4D2). Note diurnal pattern in hatching, 180° inversion for I moult and reversion to diurnal pattern during the other developmental stages under 12:12 conditions. Observe prevalence of diurnal pattern under 11:13 condition.



C

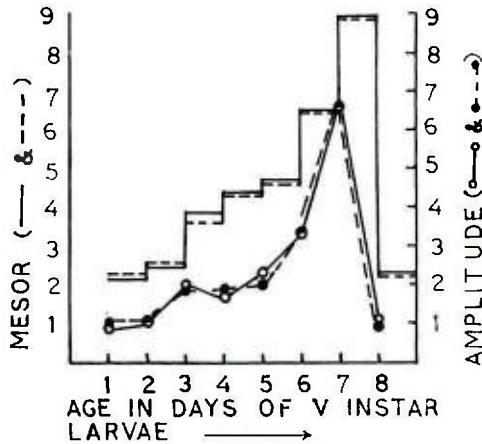


FIG. 2 (C).

Figure 2.

Circadian rhythm of total carbohydrate content from I to VIII day in the silk gland of 5th instar larvae of *Bombyx mori* (PM x NB4D2) under LD 12:12 (O-O) and 11:13 (●-●) conditions (1-A). Note increasing order of magnitude of the curves upto VII day and sudden decrement on VIII day. Level of total carbohydrate was expressed as mg/g wet weight of tissue.

Polar graphs for total carbohydrates under LD 12:12 (—) and 11:13 (---) conditions for I to VIII day (1-B).

Daily averages of mesor (histogram) and amplitude (curve) for total carbohydrates under LD 12:12 (—) and 11:13 (---) conditions from I to VIII day in the silk gland of *Bombyx mori* (PM x NB4D2). Note medial frequency of the patterns with crests on VII day.

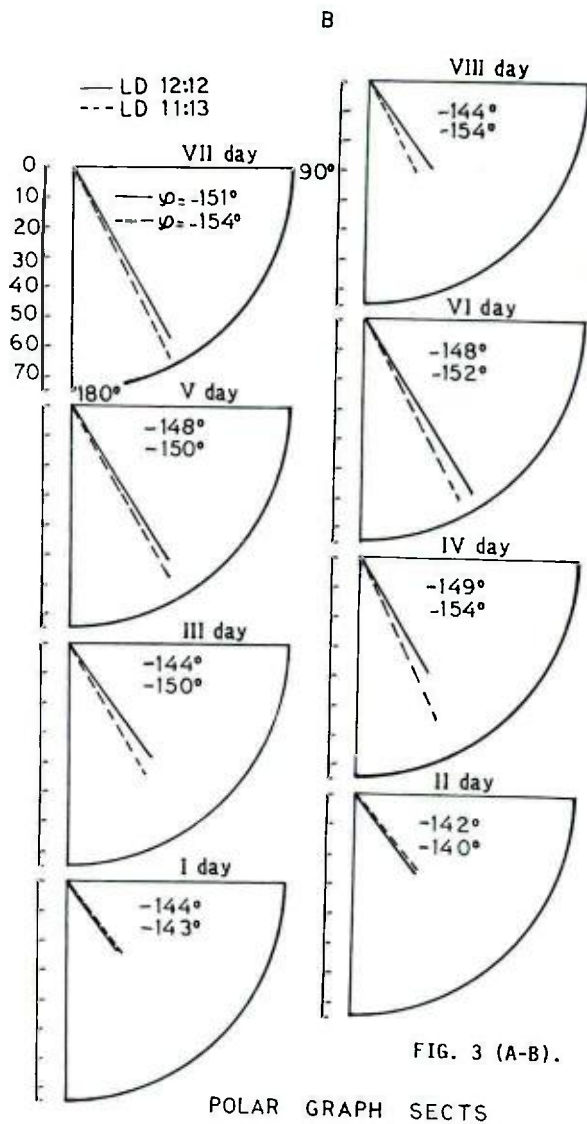
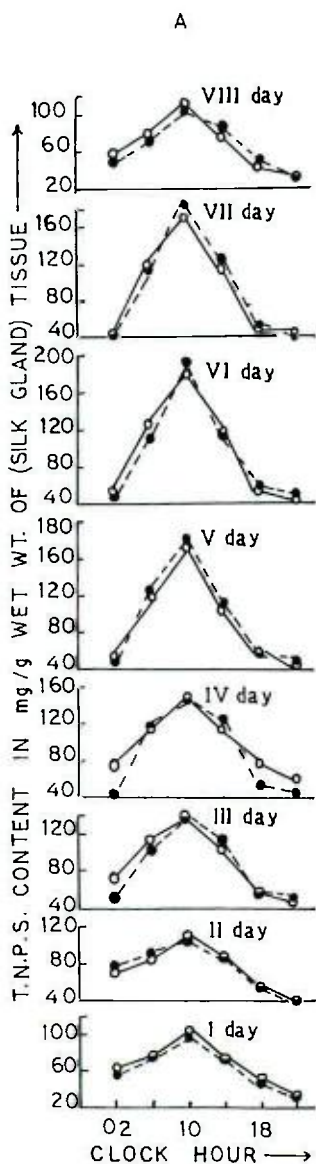


FIG. 3 (A-B).

C

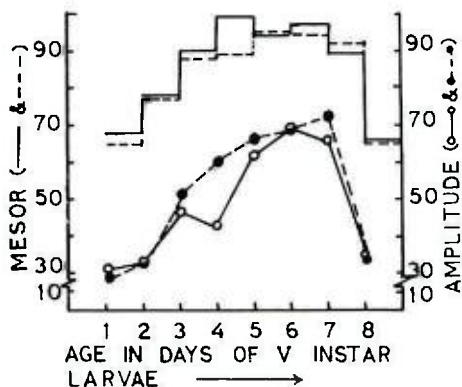


FIG. 3 (C).

Figure 3.

Circadian rhythm of total ninhydrin positive substances from I to VIII day in the silk gland of 5th instar larvae of *Bombyx mori* (PM X NB4D2) under LD 12:12 (o—o) and 11:13 (o---o) conditions (1-A). Note increasing order of magnitude of the curves upto VII day and sudden decrement on VIII day. Level of total ninhydrin positive substances was expressed as mg/g wet weight of tissue.

Polar graphs for total ninhydrin positive substances under LD 12:12 (—) and 11:13 (---) conditions for I to VIII day (1-B).

Daily averages of mesor (histograms) and amplitude (curve) for total ninhydrin positive substances LD 12:12 (—) and 11:13 (---) conditions from I to VIII day in the silk gland of *Bombyx mori* (PM X NB4D2). Note medial frequency of the pattern with crests on VII day.

Figure 4.

Daily patterns of total proteins in the silk gland of 5th age larvae under LD 12:12 (○—○) and 11:13 (●—●) conditions of the silkworm *Bombyx mori* (PM X NB4D2). Note lack of sine wave curve properties and the additive nature in the levels of TP upto VII day and progressive downward trend on VIII day (4.A). Level of TP is expressed as mg/g wet weight of tissue.

Daily averages of the constituent when resolved into a graph (4.B) a rhythmic pattern of medial frequency with crest on seventh day was obtained under LD 12:12 (○—○) and 11:13 (●—●) conditions.

Figures 5 and 6.

Silk gland (Fig. 5) and body (Fig. 6) weights from I to VIII day of 5th instar larvae of *Bombyx mori* (PM X NB4D2) under LD 12:12 (○—○) and 11:13 (●—●) conditions. Weights of silk gland in mg and body in g when resolved graphically for the duration of 5th age medial frequency rhythm was obtained.

Figure 7.

Schematic representation of the expression of crests during the developmental stage, from hatching to pharate larvae formation, under LD 12:12 and 11:13 conditions of the silkworm *Bombyx mori* (PM X NB4D2). The developmental stages followed a "cycle" in both the conditions. 180° inversion resolved nocturnal expression for I moult while rest of the stages prevailed diurnal pattern under LD 12:12 condition. Under 11:13 condition these expressions were diurnal. Note shorter duration from hatching to I moult under 11:13 conditions.

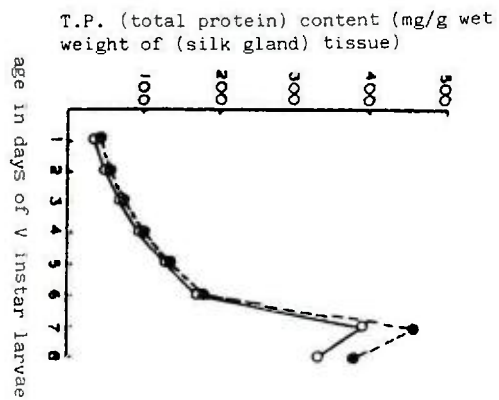
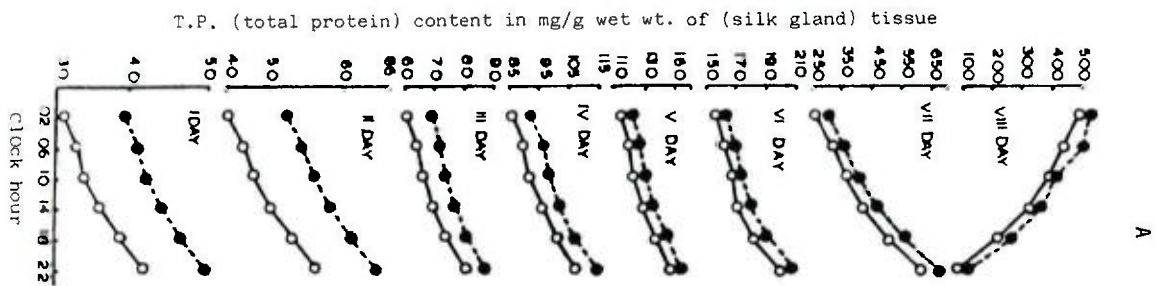


FIG. 4.

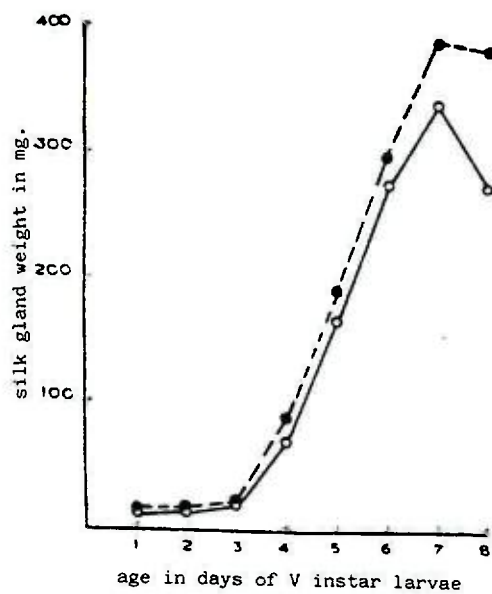


FIG. 5.

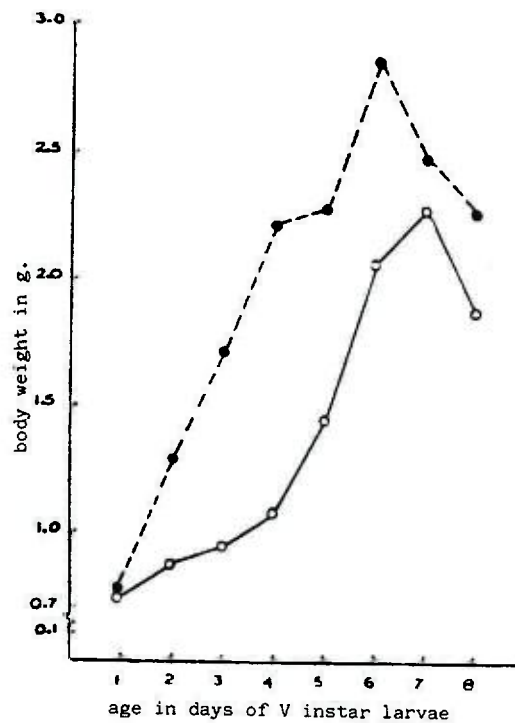


FIG. 6.

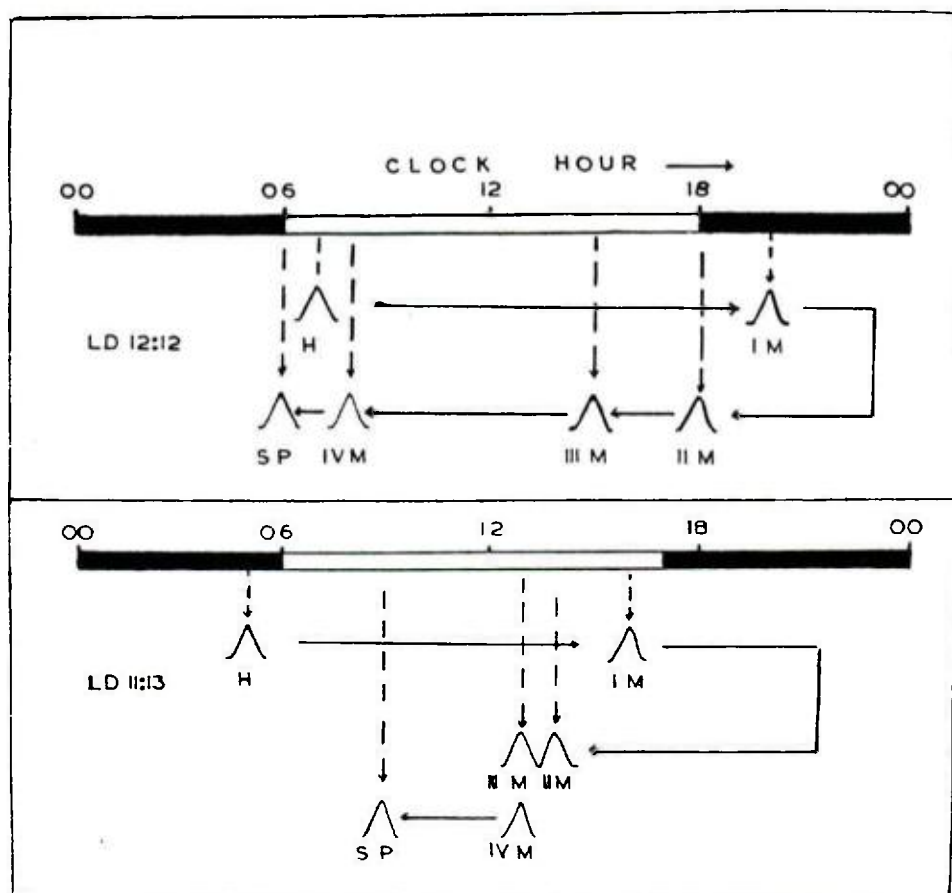


FIG. 7.

EFFECT OF BIOSTIMULATOR AND JUVENILE HORMONES
ANALOGUE TREATMENTS ON PROTEIN BIOSYNTHESIS
IN THE SILK GLAND OF BOMBYX MORI (L)

EFFET DU TRAITEMENT PAR DES ANALOGUES DES HORMONES
JUVENILES ET BIOSTIMULANTS SUR LA BIOSYNTHESE
DES PROTEINES DE LA SOIE DE LA GLANDE SERICIGENE
CHEZ BOMBYX MORI L.

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ABSTRACT

A practical procedure has been assessed for protein biosynthesis in *Bombyx mori*. Larvae were treated with biostimulators and juvenile hormones analogue showing a proteic anabolising effect. A conditioned emulsion has been sprayed on larvae of 5th instar in small, medium and large doses, eliciting the active substance to pass the tegumental permeability and "per os" as well.

On an electronomicroscopical examination during the ontogenetic development cells of the silk gland fibroinic region have shown an obvious enhancement of basal membrane permeability, an increased activity of rugous endoplasmic reticulum, of mitochondria, and of cellular nucleus, associated with an extended biosynthesis of primary fibroin granules. The fibroin granules release into the glands lumen started 24 hr. following the stimulative treatment with medium doses in the experimental group and lasted high until of cocoon shell, as compared to controls. High doses treatment resulted in an early exhaustion of fibroinic secretory cells, in the emergence of autophagolysosomes, the cellular cytoplasm becoming rapidly non functional.

The cocoons obtained were processed in a spinning mill. The medium doses treated group evidenced improved mechanical technological and production parameters of the filament and of the silk thread. A reduction of specific consumption by spinning was also obtained.

RESUME

Un procédé pratique de stimulation de la biosynthèse des protéines de la soie a été appliqué chez *Bombyx mori* par un traitement des larves par des analogues synthétiques des hormones juvéniles et des oïostimulants à effet anabolisant protéique. L'émulsion conditionnée a été pulvérisée sur des larves du 5ème âge à des doses faibles, moyennes et fortes, les substances actives pénétrant tant par la perméabilité tégumentaire que "per os".

L'aspect des cellules en microscopie électronique de la partie postérieure de la glande séricigène au cours du développement a mis en évidence une nette stimulation de la perméabilité de la membrane basale, une extension de l'activité du reticulum endoplasmatique rugueux, des mitochondries et du noyau cellulaire, accompagnées par une biosynthèse accrue des granules de fibroïne. Le déclenchement de l'excrétion des granules de fibroïne dans le lumen de la glande se produit chez le groupe traité à dose moyenne, 24 heures après le traitement stimulatif, en se maintenant élevé jusqu'au filage du cocon, comparé aux témoins. Le traitement à dose forte conduit à un épuisement prématuré de la cellule sécrétoire fibroïnique, à l'apparition des autophagolysosomes, le cytoplasme de la cellule devenant rapidement non-fonctionnel.

Les cocons filés à partir des groupes traités à doses moyennes montrent une amélioration des paramètres mécano-technologiques et productifs du filament et du fil de soie, en même temps qu'une réduction de la consommation spécifique en filature.

TWO HYPOTHESES FOR THE PREDICTION OF PUPAL DATE FROM THE
ADJUSTMENT OF CONSUMPTION RATES AFTER REACHING THE MAXIMA
IN THE FINAL INSTAR OF ERI SILKWORM PHILOSAMIA RICINI

DEUX HYPOTHESES POUR LA PREDICTION DE LA PUPAISON A PARTIR
DE L'AJUSTEMENT DES TAUX D'ACCUMULATION ENREGISTRES APRES LES
MAXIMA DU DERNIER STADE LARVAIRE CHEZ LE VER A SOIE
PHILOSAMIA RICINI

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India.

Eri silkworm, a herbivore on the second trophic level faced allelopathic ingestion barrier from four castor var/cult leaves on which ten replicates, each containing ten larvae were reared. The allelopathic hinderance from the different castors in tender, medium and mature leaves was caused by differences in water content, dry matter and caloric density. The struggle in the larvae in the various instars was judged from the CR (Consumption Rate), which fluctuated on account of allelopathy. The moultings up to the second instar were synchronous, unaffected by castor var. because of the CR being in minute quantities. In the third instar, fed on leaves of medium growth, the CR increased five-fold. Bilara castor was ingested the least and the larva was obliged to extend the third moult by an extra day of feeding. In the fourth instar, fed on mature leaves, the larva on Assam castor also ingested less amounts, while larva on Bilara continued on low CR. Thus larva feeding on Assam castor as well as that on Bilara, both extended the fourth moult for a day of additional feeding. The fifth instar, remarkable for gluttonous ingestion of 85% of total consumption within five days on each castor is characterized by larvae overcoming allelopathy and reaching the maxima of CR on different days. The point of maximum consumption marked the coming pupation date three days later in every case. The next two days were devoted to pruning and sprucing up any superfluous matter by adjusting the RCR and bringing it down to a narrow range of 185 to 187 mg. After this, feeding ceases and a day later pupation begins. Two hypotheses are being suggested for the prediction of pupation date from the adjustment of consumption rate after the maxima in the final instar.

Le ver à soie eri, herbivore du second degré trophique, affronte la barrière allélopathique de l'ingestion à partir de feuilles de quatre variétés cultivées de ricin, sur lesquelles dix échantillons contenant chacun dix larves, sont élevés. L'obstacle allélopathique rencontré chez les différents ricins dans les feuilles tendres, moyennement tendres et matures, est causé par les différences de teneur en eau, matière sèche et densité calorique. La lutte menée par les larves aux différents âges est estimée à partir du taux d'accumulation (CR) qui varie en fonction de l'allélopathie. Les mues jusqu'au 2ème âge sont synchrones et ne sont pas affectées par la variété de ricin en raison de la valeur infime du CR. Au 3ème âge, les larves étant alimentées avec des feuilles moyennement grosses, le CR est multiplié par 5. Le ricin Bilara est la variété la moins bien ingérée et les larves sont obligées de retarder leur mue d'un jour. Au 4ème âge, les larves sont alimentées avec des feuilles matures. Les larves nourries sur le ricin Assam diminuent leurs quantités ingérées et les larves nourries sur le ricin Bilara ont toujours un faible CR. Il en résulte que les larves sur Assam, comme les larves sur Bilara ont leur 4ème mue retardée d'un jour. Le 5ème âge, remarquable pour l'alimentation gloutonne qui s'élève en cinq jours à 85% de la consommation totale, sur chaque variété de ricin, se caractérise par le fait que les larves surmontent l'allélopathie et atteignent les CR maximums à des jours différents. Le point de consommation maximale précède la pupaison de trois jours dans chaque cas. Les deux jours suivants sont employés à faire le plein de nourriture puis à éliminer toute matière superflue en ajustant le taux de consommation relatif et en le ramenant au niveau de 185 à 187 mg. Après cela, l'alimentation cesse et la pupaison commence un jour après. Deux hypothèses sont émises pour prédire la date de pupaison à partir de l'ajustement des taux d'accumulation, calculés après les maxima du dernier âge larvaire.

INTRODUCTION

The purpose of consumption of food in all animals and so also in Eri silkworm (*Philosamia ricini*), from hatching to the mature final instar, serves to provide the energy required for metabolism and growth as also for building up enough stores to ensure the success of successively great events in those stages which do not have any provision for feeding. Each instar is obliged to accumulate, by consumption rates (CR), some kind of a quota in order to ensure almost geometric progression, before moulting can occur.

Since plants are the only reservoir of biologically available energy which is stored in different concentrations in various parts of their body, they also have to protect this reservoir by physical and chemical allelopathic barriers from being plundered by the herbivores. Only those herbivores can get access to these locked up stores which have developed resources and have come to possess a key to open the lock to overcome the plant defences at one more sites. Since leaves form the largest above-ground area, some herbivores develop expertise as leaf-eaters, others attack flowers or the root or the stem itself.

The first and the immediate hurdle a herbivore has to face is the ingestion barrier, consisting of high fibre, low water and low energy content. A combination of all these defence items vary not only in the different parts of the body, but also in the same part in different seasons as part of the plant growth programme. Thus, the very young tender leaves are differently endowed than the leaves of medium growth and these again differ from the mature leaves. The herbivores, which are at the second trophic level, match this situation by timing their own life cycle with the growth stage of the leaves before the allelopathic defence becomes operative (Feeny, 1970). Particular herbivores develop sensory repertoire to scout and eagerly seek in the plant the allelopathic substance as a letter addressed to them, but which is deterrent to other general herbivores who do not possess means to overcome the deterrent.

Synchronization between the growth stage of the foliage and the growth stage of the herbivore has been taken advantage of in developing the rearing technique of mulberry and non-mulberry silkworms. Thus, the first two instars are offered tender leaves, the third instar are given leaves of medium growth, while the mature leaves are fed to the fourth and fifth instar larvae. Once the food has crossed the ingestion barrier, the unusable part in it is sifted out by excretion so that the digested food becomes the raw material which is further processed by metabolism to convert it into part of the herbivore body. The herbivore, thus faces three barriers from the plant it eats :

1. the ingestion barrier,
2. the digestion barrier, and
3. the maintenance barrier.

MATERIAL AND METHODS

The present study is based on a laboratory culture of Eri silkworm, maintained at the Experimental Field Station of Zoology Department, Jodhpur University, Jodhpur, during the monsoon season as a project of the Indian council of Agriculture Research. The larvae were raised from disease-free-layings of females which deposited their eggs on bamboo '*Kharikas*'. After hatching from the eggs in petri dishes, the larvae were 'brushed' and used in the experiments under natural conditions of general prevailing temperature in the laboratory ranging from 28°C to 30°C and the R.H. from 80% to 90% read from hygrometer. The experiment contained ten replicates, each containing ten larvae, reared on leaves of four var/cultivars (hereafter referred to as varieties for simplicity). A parallel batch of larvae, under identical conditions as the experimental rearings, was maintained for making replacements for the dead and for use in computing biomass of oven-dried larvae in different instars and for computing relative consumption rates of the larvae.

The food consumption, in direct and absolute terms, is computed as wts in mg of oven-dried material. Its energy equivalents, by using oxygen bomb calorimeter, have been expressed as kcal/ml dry wt leaves. For greater accuracy in comparisons of daily consumption, the rate value of the leaf-matter has been tied up with the body wt and equivalent calories thereof, which is affected by food matter. This Relative Rate of Consumption (RCR) and its energy equivalent (RCRe) represents food ingested (mg/kcal/100 mg dry body wt/day) and is a derived but useful statistics.

The developmental period takes 17 to 19 days from hatching to the end of the feeding stage in the fifth instar. After an incubation period of 7 days during monsoon, 11 days during the winter and 10 days during the summer (Dookia, 1984), the first two moults were found to occur regularly respectively after 2 1/2 and 2 days or within 5 days. The varietal differences in the castor feed made no impact, because

the amounts taken by the larvae in these instars were very little. An average of 0.18% of the total feed during the first instar and 0.49% in the second is obviously a very small amount. In the third instar the leaves were changed from tender to those of medium growth and the feed taken was 5 times that taken in the second instar. In the fourth and fifth instars, given mature leaves, the consumption in the fourth instar was again almost five times that of the second. The fifth instar was the most voracious stage when consumption was 85% of the entire feed which was more than 7 times that taken in the fourth instar.

THE INGESTION BARRIER

a) In the third instar

The data of rearings of eri silkworm in our laboratory experiments, during the monsoon season has shown that the daily consumption (CR) and the days of moulting are the same on all castor diets upto the second moult because of very small ingestion of food. The criterion of maximum numbers of larvae crossing a moult synchronously can, therefore, be a measure of standardized physical conditions in rearing and can be used as a guide in testing idealized laboratory rearing technique. The factor would operate when the CR increases to 2.6% in the third instar, 11.6% in the fourth and 85% in the final instar (Misra and Srivastava, 1983).

The third instar gets its importance because it is in this instar that the first change in quality of leaves is made and allelopathy shows up as the first plant-resistance. Comparing the CR of Bilara (37.8 mg) with those of Udaipur (59.8 mg), Assam (58.6 mg) and Aruna (56.9 mg), the CR on Bilara castor is remarkably the lowest right from the start in third instar which began in all cases on the sixth day, giving evidence of the allelopathic struggle. While the larvae feeding on Udaipur, Assam and Aruna consumed 166, 163 and 158 mg respectively, and are ready to moult, the larva on Bilara castor could consume only 129 mg during the same interval because of the leaves of this castor containing lesser amount of water, and higher percentage of dry matter. The larva, feeding on this castor, therefore, is obliged to delay moulting by an extra day of feeding to bring the consumption in line with that of the larvae on the other three castors. Since the consumption on the various castors is directly proportional to the water content of the leaves, this observation stresses the need to keep the foliage in the grainage properly wrapped up in moist cloth to keep their quality - succulence - for the larvae.

b) In the fourth instar

This is the instar in which second change in quality of leaves is made - from those of medium growth to mature leaves - and the larval feeding increases five times that of the second instar, making the allelopathic struggle more determined. Because of a larger difference in water content and substantial difference in dry matter percentage in the four castors, the larvae feeding on them face different degrees of allelopathy. On the basis of succulence and dry matter content and caloric density, these castors can be graded as Udaipur (77.02% water, 4.210 cal, most succulent), Assam (74.99%, 4.345 cal), Aruna (74.94%, 4.654 cal) and Bilara (74.02%, 4.863 cal, being the least succulent and having maximum dry matter and caloric density). Of the less succulent three castors, although the Assam castor is richer than Aruna by a mere 0.05%, the CR on the former is most of the time slower than on the latter and the consumption, by the time Udaipur and Aruna-fed larvae are crossing the fourth moult, is far behind them (684 mg against 740 and 700 mg). The struggling, slow-eating Assam-fed larva, therefore has to feed 44.9 mg more on the 12th day to reach the total consumption (729 mg) comparable with that of the others before it could cross the fourth moult on the 13th day. From this it would appear that Aruna castor is more nutritious than the Assam variety. The Bilara-fed larva, which had started

facing lack-water resistance in the food all through the earlier instar - for that reason, already a day behind in the third instar - again starts the first day in the fourth instar badly, consuming 173 mg, which is 54% of the average of the three put together. In an effort to complete the quota of fuel, inspite of the continued resistance from the plant, the CR on Bilara is picked up fast in the next two days. The consumption is still much behind that of Aruna (which is 0.92% richer in water content than Bilara) and even the extra day of feeding on the 13th day barely enables the larva to qualify crossing the fourth moult with the fuel consumption of 690 mg dry wt.

c) In the fifth instar

The fifth instar is the most important stage - the last and the longest stage - when the gluttonous larva is gobbling 85% of its total feed within five days, whatever the food. The mad rush seems intended to reach the maxima of CR within a time-bound programme which is to fuel several important events, e.g., trading the digestive function of salivary glands to production of proteinous silk from silk glands for making cocoon (in which man has commercial interest), transformation inside the pupa in the cocoon, production of eggs and fueling reproductive activities of the short-lived adult which is denied feeding. The exacting demand in all these transactions is that no superfluous matter must remain during this last feeding stage to upset the balance sheet. The final adjustment, therefore is made by stoppage of feeding and defecation. The allelopathic ingestion barrier in the mature leaves, forming the food of larva is in water content - 2.03% lesser in Assam than in Udaipur; from Assam to Aruna it is 0.05% less. The deficiency from Aruna to Bilara is, however 0.92% and the caloric density in dry matter is also increasingly more - 0.135, 0.309, 0.209 cal/mg dry wt.

Now an interesting phenomenon is observed. Overcoming the allelopathic watery hurdle, the effort of the larva on the various castor varieties is directed to reaching the *maxima* of CR precisely on a particular day, only two days earlier than the call for halting feeding is given. This is the precise timing or punctuation to which the *maxima* of CR, based on relative nutritional merit of the food must take the larva, by topping up, at this time, any fuel reserve deficiency, which would enable all the important transformations inside the cocoon and all activities in the non-feeding short adult life to be completed. This phenomenon is very clearly seen in the RCR values. Thus the *maxima* of CR and RCR on Udaipur and Aruna reached on the 14th day, those of Assam castor on the 15th day and of Bilara on the 16th day. The absolute amounts (CR) and their relative derived values (RCR) differ in each castor, indicating the nutritional differences, but the timing and punctuation for pupation is unmistakably clear. After two days of adjustments from this point (*maxima*), the last meal in the nature of a topping up operation settles down to a narrow margin of 185 to 187 mg/100 mg body wt. Feeding stops after this meal and on the third day the larva goes into pupation, quickly spinning the cocoon after emptying the alimentary canal.

Two hypotheses may be suggested here to predict and explain the pupation date as follows :

1. Whatever may be the differences in nutritional value of the food or allelopathic resistance, if the larva is able to complete its development, the pupation occurs on the third day after the largest meal in the final (fifth) instar.

2. The next two days after the maximum CR (more precisely RCR), are used by the larva to shed superfluous matter in body wt by adjusting its last meal (around 186 mg dry wt), as a topping up operation within a narrow range (RCR around 186 mg dry wt), representing the nutritional quality of the food as illustrated in the table below :

Table 1. Consumption Rate (CR, mg dry wt) of third instar Eri silkworm in the face of allelopathic resistance given by castor foliage of four castor var/cultivars.

N.B. Leaf quality in this instar was changed from tender to those of medium growth.

Castor matter (mg dry wt) Energy (cal/mg d. wt)	6th day	7th day	8th day	9th day	10th day	Total cons.	Water % and cal density
UDAIPUR							
Matter	59.8	64.7	41.5	Moult	---	166.0	81.58
Energy	249.4	269.9	173.1			692.4	4.2
ASSAM							
Matter	58.6	63.4	40.7	Moult	---	162.7	80.74
Energy	246.2	266.3	171.0			683.4	4.2
ARUNA							
Matter	56.9	61.6	39.5	Moult	---	158.0	80.28
Energy	256.2	277.4	177.9			711.5	4.5
BILARA							
Matter	37.8	53.5	37.4	28.7	Moult	157.4	79.52
Energy	182.5	258.3	180.6	138.6		760.1	4.8
Matter		128.7					
Energy		621.4					

Table 2. Consumption Rate (CR, mg dry wt.) of fourth instar Eri silkworm in the face of allelopathic resistance given by foliage of four castor var/cultivars.

N.B. Leaf quality in this instar was changed from medium growth to mature leaves.

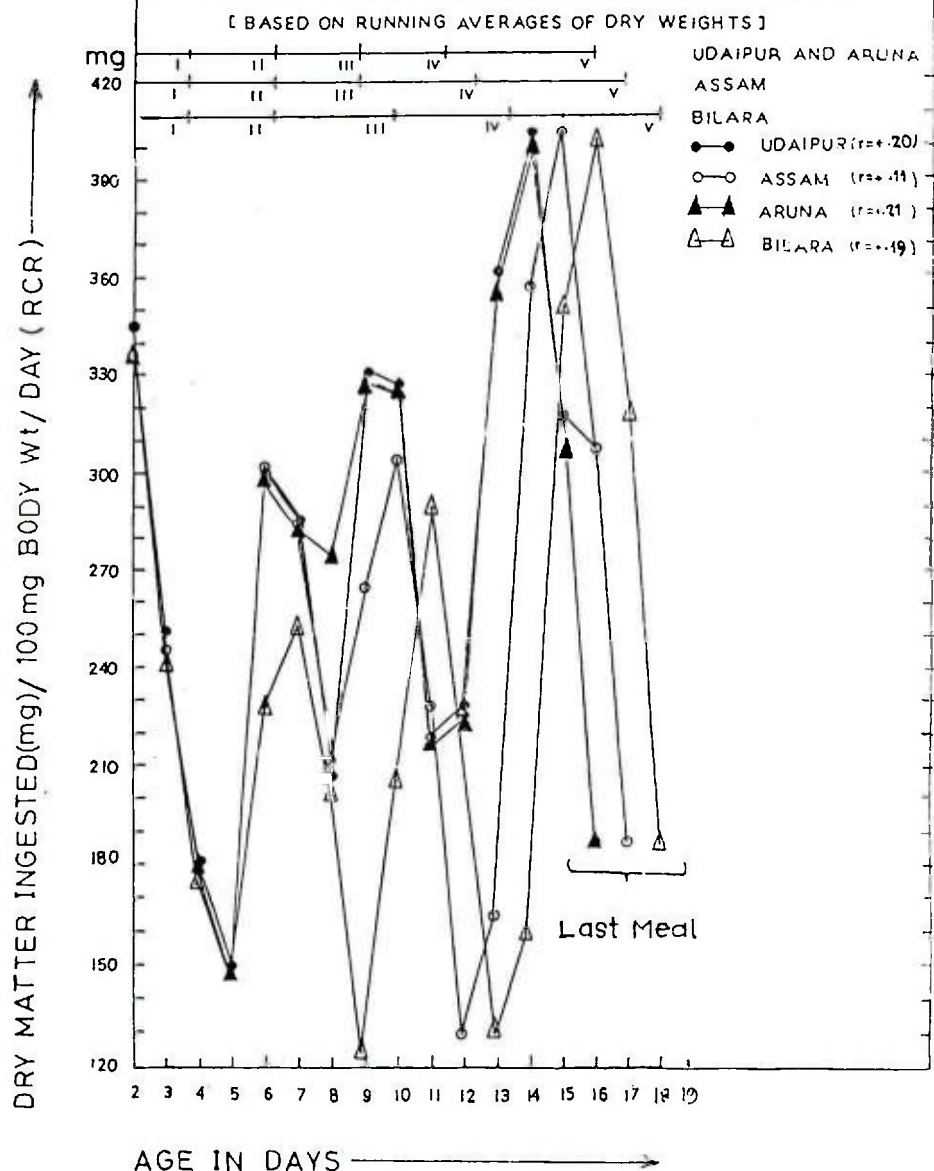
Castor matter (mg dry wt.) Energy (cal/mg d wt.)	9th day	10th day	11th day	12th day	13th day	14th day	Total cons.	Water % and cal density
UDAIPUR								
Matter	266.8	340.5	133.0	Moult	...	...	740.3	77.02
Energy	1123.2	1433.5	559.9				3116.7	4.2
ASSAM								
Matter	182.3	290.6	211.4	44.9	Moult	...	729.2	74.99
Energy	792.1	1262.7	918.5	195.1	...	...	3168.4	4.2
Matter		684.3						
Energy		2973.3						
ARUNA								
Matter	252.0	322.0	126.0	Moult	...	...	700.0	74.94
Energy	1172.8	1498.6	586.4		...	...	3258.8	4.7
BILARA								
Matter	-	172.5	276.1	199.6	42.0	Moult	690.2	74.02
Energy	-	838.9	1342.7	970.7	204.3	...	3356.3	4.9
Matter			648.2					
Energy			3152.3					

Table 3. Consumption Rate (CR, ingestion mg dry wt. or energy equivalent cal/day) and Relative Consumption Rate (RCR, ingestion (mg)/100 mg dry body wt/day) of fifth instar Eri silkmoth larva in face of allelopathic resistance given by foliage of four castor var/ cultivar.

N.B. The same quality of leaves (mature) were offered for feeding as in the fourth instar.

Castor Matter Energy RCR	12th day	13th day	14th day	15th day	16th day	17th day	18th day	19th day	Total consump- tion	Water % and cal density
UDAIPUR										
CR	553	1548	1659	1272	498	PUPA	...	...	5530	77.02
Energy	2527	6518	6984	5354	2096				23479	4.2
RCR	345	361	404	317	186					
ASSAM										
CR	-	542	1517	1625	1246	487			5417	74.99
Energy	-	2355	6591	7062	5414	2117			23538	4.4
RCR	-	163	357	405	317	187				
ARUNA										
CR	512	1436	1539	1180	463	PUPA	...	...	5130	74.94
Energy	2382	6683	7163	5489	2153				23870	4.7
RCR	336	355	402	317	185				1595	
BILARA										
CR	-	-	493	1381	1482	1137	448	PUPA	4941	74.02
Energy	-	-	2398	6716	7208	5527	2181		24030	4.9
RCR	-	-	158	350	402	318	185			

FIGURE 1. RELATIVE CONSUMPTION RATE (RCR) OF ER: SILKMOTH LARVA ON FOUR CASTOR DIETS



DIAPAUSE RELATED CHANGES IN PYRUVATE LEVEL AND LACTIC
DEHYDROGENASE ACTIVITY IN SILKWORMS EGGS

VARIATIONS DU NIVEAU DE PYRUVATE ET DE L'ACTIVITE DE LA
DEHYDROGENASE LACTIQUE LIEES A LA DIAPAUSE CHEZ LES OEUFS
DU VER A SOIE

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Quantitative changes in the level of pyruvate and complementary changes in LDH activity were studied during the process of diapause and in non-diapause eggs of the silkworm *Bombyx mori*. Pyruvate level was found to be significantly high during the initiation of diapause which decreased gradually thereafter. In case of non-diapause eggs, a slight increase in the level of pyruvate was observed. The LDH activity slowly increased to reach the maximum level around the fifth day of diapause, which was maintained upto 10th day, decreasing significantly to a very low level thereafter. But in case of non-diapause eggs, the level of LDH activity remained unchanged throughout at a very low level, the results are discussed in the light of increased anaerobic condition and accumulation of carbohydrate pools during diapause stage.

Les changements quantitatifs du niveau de pyruvate et les changements complémentaires de l'activité LDH sont étudiés pendant la diapause et chez des oeufs non-diapausants du ver à soie *Bombyx mori*. Le niveau du pyruvate est remarquablement élevé au début de la diapause et diminue ensuite graduellement. Dans le cas des oeufs non-diapausants, on observe une légère augmentation du niveau du pyruvate. L'activité LDH augmente lentement pour atteindre son niveau maximum vers le 5ème jour de la diapause, ce niveau est maintenu jusqu'au 10ème jour et diminue ensuite fortement jusqu'à un niveau très faible. Mais dans le cas des oeufs non-diapausants, l'activité LDH reste constamment à un très faible niveau. Les résultats sont discutés en prenant en compte les conditions d'anaérobiose croissante et l'accumulation des pools d'hydrates de carbone pendant la diapause.

INTRODUCTION

Extensive studies during the last two decades have shown that very interesting changes occur in the carbohydrate metabolism associated with embryonic diapause in the silkworm *Bombyx mori*. It is known that glycogen is rapidly converted into sorbitol and glycerol with the onset of diapause and that it is resynthesized when the diapause terminates (Chino, 1957, 1958). This formation of polyols is advantageous since polyols decrease the freezing and super cooling points of diapausing embryos and also confer freezing tolerance at high concentrations (Chippendale, 1978). In addition to polyols, other metabolites such as lactate have also been shown to accumulate during embryonic diapause (Kageyama & Ohnishi, 1973, Kageyama, 1976). However, the accumulation of lactate is very less compared to that of polyols (Kageyama & Ohnishi, 1973; Kageyama, 1976). Though Chino (1960) has demonstrated that the activity of lactic dehydrogenase is very low (1:20) compared to that of glycerol phosphate dehydrogenase, it is not clearly known whether these differences are responsible for the relatively lesser accumulation of lactate. It is therefore interesting to study the pyruvate level as well as lactic dehydrogenase activity during diapause. In the present study, the pyruvate level and lactic dehydrogenase activity in diapause eggs have been studied and compared with those in non-diapause as well as HCl-treated artificial non-diapause eggs.

MATERIALS AND METHODS

Materials :

Bivoltine (NB4D2) and multivoltine (Pure Mysore) silkworm races were maintained under standard conditions. Diapause eggs of the bivoltines and non-diapause eggs of multivoltines were used at different intervals for the experiments. Loose eggs were prepared on polythene sheets and kept at 25°C with a relative humidity of 75%.

HCl-treated diapause eggs :

For breaking diapause, 20 hr old diapause eggs were treated with HCl solution (sp. gr. 1.075) at 46.1°C for 3-4 minutes, washed thoroughly with water and dried. The eggs were then kept at 25 ± 2°C.

Enzyme preparations :

A 10% (w/v) homogenate of the eggs was prepared in ice-cold distilled water with a glass homogenizer fitted with teflon pestle. The homogenate was filtered through a cotton pad and centrifuged at 7,000 rpm for 15 min at 0°C. The supernatant was filtered through Whatman No.1 filter paper and the resultant filtrate was used as enzyme source.

Determination of enzyme activity :

The activity of lactic dehydrogenase was assayed according to the procedure of Bergmeyer *et al.* (1965) with suitable modifications.

1ml of the reaction mixture contained 0.95 ml of 3l mM Sodium pyruvate in 0.05 M Phosphate Buffer pH 7.5; and 0.02 ml of 6.65 mM NADH. Reaction was initiated by adding 0.03 ml of the enzyme solution and decrease in the absorbance at 340 nm was measured using Beckmann Du-2 spectrophotometer. Protein concentration was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin standards.

The enzyme activity was expressed as μ mole of NADH oxidized/minute/mg protein.

Estimation of Pyruvate :

The amount of pyruvate was estimated according to the procedure of Friedemann and Heugen (1943).

RESULTS

Changes in Pyruvate level :

The Pyruvate level was very low in non-diapause eggs soon after oviposition and later increased slightly as the embryo developed reaching a maximum around the eighth day after oviposition (Fig. 1). In case of diapause eggs, the initial level of pyruvate was twice that in non-diapause eggs and the level further increased soon after oviposition, reaching a maximum of 10 % of the initial level around the third day. However, the pyruvate level decreased to about 5 % of the maximum attained around the ninth day and remained more or less constant thereafter.

The initial level of pyruvate in HCl-treated artificial non-diapause eggs, was high as in diapause eggs. But unlike in diapause eggs there was very small increase in the pyruvate level comparable to that in non-diapause eggs. At any given time, the pyruvate level in HCl-treated artificial non-diapause eggs was found to be slightly higher than that in non-diapause eggs.

Changes in lactic dehydrogenase activity :

The lactic dehydrogenase activity remained unchanged at a very low level throughout the embryonic development in non-diapause eggs (Fig. 2). The level of lactic dehydrogenase activity in HCl-treated artificial non-diapause eggs closely paralleled that in non-diapause eggs throughout the period of embryonic development.

The lactic dehydrogenase activity in diapause eggs was interestingly low as in the other two types of eggs studied, during the first two days after oviposition. However, the activity started increasing around the third day reaching a peak around the fifth day after oviposition. This high level of enzyme activity was maintained throughout a period of ten days after which it decreased to a level comparable to that immediately after oviposition.

DISCUSSION

It is evident from the present study that the pyruvate level in diapause eggs at the time of oviposition is twice that in non-diapause eggs showing that the two already differ from each other in carbohydrate metabolism. Following oviposition, there is some increase in the pyruvate level in non-diapause eggs suggesting that there is increased breakdown of glycogen as the development proceeds, to meet the increased energy requirement. In diapause eggs on the other hand, the pyruvate level increases considerably after oviposition. In HCl-treated artificial non-diapause eggs though the initial level is high as in diapause eggs, the pyruvate level subsequently parallels that in non-diapause eggs showing that the increase in pyruvate concentration is correlated with the phenomenon of diapause. This may be an outcome of the increased breakdown of glycogen in diapause eggs.

While the pyruvate level increased to two times the initial level on the first day and to nearly ten times on the third day, the lactic dehydrogenase activity started increasing significantly only after the 3rd day. Thus the peak pyruvate level preceeded the peak in lactic dehydrogenase activity (Fig. 3). This shows that during the first three days there is very little lactate fermentation and the energy required during this period may be supplied by oxidative phosphorylation as discussed by Sato and Takesue (1975).

Earlier Studies on oxygen consumption in the diapause eggs of the silkworm *Bombyx mori* by Chino (1958) have shown that the amount of oxygen consumed nearly doubles on the first day and decreases to 15 % of that on the first day from the third day onwards. These studies also support the idea of the occurrence of aerobic metabolism during early steps of diapause. It is interesting that the time of increase in the lactic dehydrogenase activity corresponds to the period of establishment of decreased oxygen supply. This increased lactate fermentation appears to supplement the energy requirement since the energy supply by oxidative phosphorylation is now much lower and probably insufficient as shown by Chino (1958). The fact that lactic dehydrogenase activity remains low in HCl-treated artificial non-diapause eggs as in non-diapause eggs also shows that the increase in the activity of this enzyme is also correlated with diapause.

From the present studies it is clear that lactic dehydrogenase is in fact very active at least during the early phase of diapause. During the rest of the period of diapause, the activity is maintained at a level more or less comparable to that during embryonic development in non-diapause eggs which is higher than the level at the time of oviposition in both types of eggs. Hence, the lactic dehydrogenase activity may not be rate limiting in the formation of lactate as also suggested by Chino (1960).

ACKNOWLEDGEMENT

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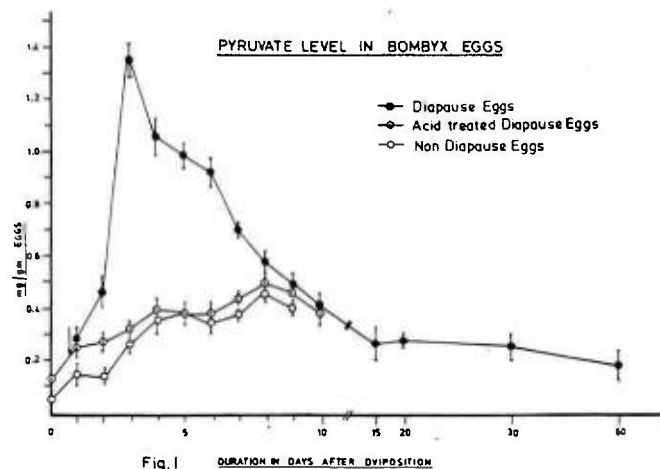


Fig. 1 Changes in pyruvate levels in the eggs. The values represent the mean of three different experiments and the vertical bars represent standard deviation. The values at "0 day" represent pyruvate levels two hours after the initiation of oviposition.

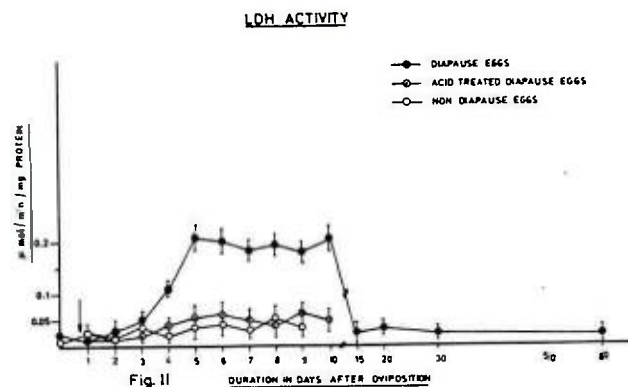


Fig. 2 Changes in lactic dehydrogenase activity in the eggs. The values represent the mean of three different experiments and the vertical bars represent standard deviation. The values at "0 day" represent the enzyme activity two hours after the initiation of oviposition.

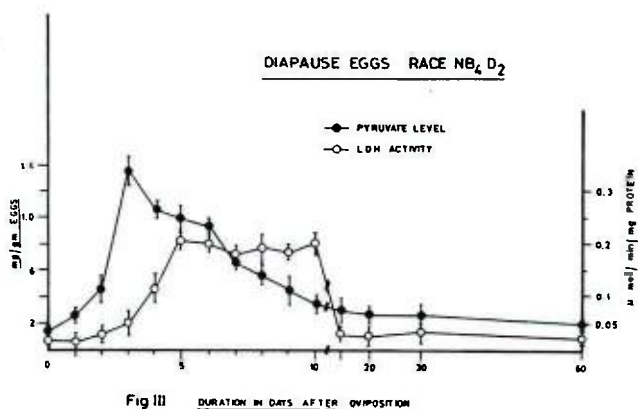


Fig. 3 Changes in pyruvate level and lactic dehydrogenase activity in diapause eggs. See figure I and II for details.

LE VER A SOIE, UN SYSTEME MODELE TRES UTILE POUR LA
RECHERCHE FONDAMENTALE EN BIOLOGIE MOLECULAIRE

THE SILKWORM, A VERY USEFUL MODEL SYSTEM FOR BASIC
MOLECULAR BIOLOGY

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Le ver à soie a été élevé et sélectionné pendant 40 siècles afin d'accroître la quantité et la qualité de la soie produite. *Bombyx mori*, le seul insecte entièrement domestique à ce jour, est devenu un animal de laboratoire et un modèle biologique pour la recherche fondamentale en physiologie, endocrinologie, génétique, etc.

Plus récemment, dans les années soixante, le ver à soie est apparu comme l'un des modèles biologiques les plus adéquats pour l'étude, au niveau moléculaire, de l'expression des gènes au cours du développement et de la différenciation. Parmi plusieurs types de travaux, j'en choisirai trois qui illustrent l'utilité du ver à soie dans ce domaine.

La formation de la coque de l'oeuf est un système très attrayant pour étudier le développement et l'évolution moléculaire. Plusieurs travaux (Kafatos, 1983; Regier Kafatos, 1983; Bock, Tiemeier, Mester, Goldsmith, 1983) effectués sur différentes espèces de ver à soie (*A. polyphorus*, *A. pernyi*, *B. mori*) ont montré que les protéines du chorion sont les produits de familles multigènes. La caractérisation des gènes du chorion, grâce à l'utilisation de "bibliothèques" d'ADN génomique, a permis d'établir que le programme de synthèse des protéines du chorion reflète la production séquentielle des ARN messagers (précoces, moyens et tardifs). Les membres des diverses familles de gènes sont regroupés dans le génôme dans un locus unique de 3,7 centimorgans. L'unité fondamentale est constituée d'une paire de gènes contigus et dont l'expression est coordonnée, voisinant des paires de gènes proches sur le plan structural et développemental. Elles forment des alignements qui se développent en un grand nombre de gènes. La comparaison avec d'autres types de familles multigé-

niques (protéines du chorion de la Drosophile, les Immunoglobulins) au niveau structural, accroît l'intérêt du système en particulier en ce qui concerne la séquence et l'organisation des promoteurs et la modulation de ces gènes.

Les protéines de la soie sont produites dans deux parties distinctes de la glande séricigène et leur synthèse se déroule à un taux élevé de manière cyclique lors du développement; la glande séricigène demeure l'un des modèles les plus appropriés pour la compréhension de la différenciation cellulaire et de la régulation de l'expression des gènes.

Depuis l'isolement historique de l'ARN messager de la fibroïne (Suzuki, Brown, 1972), de nombreux travaux ont porté sur la structure et l'expression du gène de la fibroïne (cf. Maekawa, Suzuki, 1980; Tsuda, Suzuki, 1983). De plus, l'analyse des ARN totaux poly(A) de la glande séricigène (Couble, Garel, Prudhomme, 1981) par hybridation avec leur ADN complémentaire a montré l'existence de différentes classes d'abondance pendant la quatrième mue et le cinquième âge larvaire. La transition entre ces deux stades de développement conduit à diverses modifications dans la distribution des ARN messagers des différentes classes. Tous les ARN messagers présents au cinquième âge sont déjà présents pendant la mue mais certains décroissent (nombre de molécules/génome), d'autres sont stables et un petit nombre augmente. Parmi ces derniers, deux ARNm croissent d'une manière importante et parallèle (environ 50 000 fois) : l'ARNm de la fibroïne et l'ARNm de P25 qui codent respectivement pour la fibroïne et pour une protéine de petit poids moléculaire (25 kd). Leur accumulation pendant le cinquième âge est déclenchée par l'alimentation et s'effectue de telle manière qu'un rapport moléculaire constant de 1 pour 1 est maintenu pendant la période de sécrétion de la soie (Couble, Moine, Garel, Prudhomme, 1983). La séquence nucléotidique du gène P25 et sa comparaison avec d'autres gènes du ver à soie montre des homologies dans les séquences flanquantes en 5', séquences qui pourraient jouer un rôle dans la coordination de l'expression des gènes (Couble, Chevillard, Moine, Prudhomme, 1984).

L'exemple dans lequel le ver à soie est apparu comme un modèle des plus concluants concerne l'étude de la population des ARN de transfert de la glande séricigène. Les ARNt sont les molécules servant à décoder l'ARNm et à le traduire en protéine. Dans la glande séricigène les ARNt sont quantitativement adaptés à la fréquence des codons dans l'ARNm de la fibroïne (Garel, 1974; Chavancy, Daillie, Garel, 1971). Cette adaptation prend place au cours du développement et suggère que l'expression des gènes d'ARNt est coordonnée à celle du gène de la fibroïne. Ce phénomène découvert grâce au ver à soie, a été retrouvé chez plusieurs autres systèmes cellulaires (Chavancy, Chevallier, Fournier, Garel, 1979) et est maintenant décrit en détail pour les cellules procaryotes (Ikemura, Ozeki, 1982; Gouy, Gautier, 1982).

Cette adaptation des ARNt semble contrôler la vitesse et la fidélité de la traduction (Chavancy, Garel, 1981) et explique l'allongement discontinu des chaînes de fibroïne pendant la traduction de l'ARNm de fibroïne (Chavancy, Marbaix, Huez, Cleuter, 1981; Lizardi, Mahdavi, Shields, Candelas, 1979). La traduction discontinue observée dans d'autres systèmes animaux ou viraux et qui était précédemment expliquée par d'autres modèles doit être maintenant reconsidérée à la lumière des résultats obtenus avec les ARNt de la glande séricigène et l'ARNm de fibroïne. Ce modèle du contrôle de la vitesse de traduction et l'existence de pauses pendant la traduction dues à l'adaptation de l'ARNt est confirmé par des travaux récents effectués chez les bactéries (Pedersen, 1982) et l'araignée (Candelas G., Candelas F., Ortiz, Rodriguez, 1983).

Des études ultérieures portant sur la traduction des ARNm de la fibroïne et de P25 qui sont présents en grande quantité dans les cellules séricigènes seront de ce point de vue d'un grand intérêt puisque ces deux ARN ont une composition en codons très différente et sont cependant traduits par la même population d'ARNt.

The silkworm has been reared and selected for 40 centuries to increasing efficiency for silk production and quality. *Bombyx mori*, the only one insect domesticated today, became a laboratory animal and a biological model for basic research in physiology, endocrinology, genetics, reproduction etc.

More recently, during the sixties, it appeared as one of the most convenient model for studying gene expression during development and differentiation at the molecular level. Among several works, I will choose three types of such studies which illustrate the usefulness of the silkworm.

The formation of eggshell is a very attractive system for studying development and molecular evolution. Several works (for review : Kafatos, 1983; Regier, Kafatos, 1983; Bock, Tiemeier, Mester, Goldsmith, 1983) using different silkworms (*A. polyphorus*, *A. pernyi*, *B. mori*) showed that chorion proteins are products of multigene families. The characterization of chorion genes via DNA libraries allowed to establish that chorion protein synthetic program reflects the mRNA production with developmental, early, middle or late sequences. The members of the various multigene families are clustered in the genome in a single giant locus of 3.7 centimorgans. The fundamental unit is a compact and coordinately expressed pair of genes, neighboring pairs tending to be similar in sequence and developmental control. They form arrays that evolve into a large number of new genes. The comparison with other types of multigene families (chorion proteins of *Drosophila*, Immunoglobulins) at the structural level opens a new field of interest particularly in regard with the sequence and organisation of the promoters and modulations of these genes.

Silk proteins are produced in two different parts of the silk gland and their synthesis occurs at high rates cyclically along the development; thus, the silk gland system remains one of the most appropriate model for understanding cellular differentiation and regulation of gene expression.

Since the historical isolation of fibroin mRNA (Suzuki, Brown, 1972), a lot of works have contributed to the understanding of the structure and expression of the fibroin gene (for recent results see Maekawa, Suzuki, 1980; Tsuda, Suzuki, 1983). On the other hand analysis of the whole poly(A) containing RNA in the silk gland (Couble, Garel, Prudhomme, 1981) by hybridization with their complementary DNA showed different abundance classes during the fourth molt and the fifth instar. The transition between these two developmental stages lead to several modifications in the distribution of mRNA in the different classes. All mRNA present in the fifth instar are already present during moult but some of them decrease (number of molecules/genome) others remain constant and few of them increase. Among these, two mRNA show a dramatic and parallel growth (about $\times 50\,000$) : fibroin mRNA and P25 mRNA which encode respectively for fibroin and for a low molecular weight protein (25 Kd). Their accumulation during the fifth intermolt is triggered by food uptake and proceeds in such a way that a constant 1 : 1 molar ratio is maintained during the period of silk secretion (Couble, Moine, Garel, Prudhomme, 1983). The nucleotide sequence of P25 gene and its comparison with other silkworm genes allow to show up some striking homologies in their 5' flanking sequences, which could be relevant to the coordination of gene expression (Couble, Chevillard, Moine, Prudhomme, 1984).

The field in which the silkworm has appeared as a most conclusive model concerns the study of tRNA population from the silk gland. Transfer RNA are the decoding molecules responsible for the mRNA translation into proteins. tRNA are quantitatively adapted to fibroin mRNA codon frequency (Garel, 1974; Chavancy, Daillie, Garel, 1971). This adaptation takes place during the development and suggest a coordinate expression of fibroin and tRNA genes. This phenomenon discovered thanks to the silkworm was found in other cellular systems (for review see Chavancy, Chevallier, Fournier,

Garel, 1979) and is now described in details for procaryotic cells (Ikemura, Ozeki, 1982; Gouy, Gautier, 1982).

This tRNA adaptation seems to control the translation rate and accuracy (Chavancy, Garel 1981) and explains the discontinuous elongation during the fibroin mRNA translation (Chavancy, Marbaix, Huez, Cleuter, 1981; Lizardi, Mahdavi, Shields, Candelas, 1979). Discontinuous translation in other animal or viral systems which was previously explained by other models must be now seen in the light of the results obtained with the silk gland tRNA and fibroin mRNA. This model of a translation rate control and occurrence of translation pauses due to tRNA adaptation is confirmed by recent works on bacteria (Pedersen, 1982) and a spider (Candelas G., Candelas T., Ortiz, Rodriguez, 1983).

Further studies on the translation of fibroin and P25 mRNA which are present in large quantity in the silk gland cells will be of great interest since, these two mRNA have a very different codon constitution and are translated by the same tRNA population.

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EFFECT OF SUNLIGHT ON THE ACTIVITY OF KENCHU VIRUS
OF SILKWORM BOMBYX MORI L. *

EFFET DE LA LUMIERE NATURELLE SUR L'ACTIVITE DU VIRUS
KENCHU CHEZ LE VER A SOIE BOMBYX MORI L. *

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Investigations were conducted on the effect of direct exposure of Kenchu virus to sunlight under dry as well as humid conditions, on the infectivity and virulence. Exposure periods varied from 5 to 30 hours and corresponding indoor exposures were also tested. The parameters used for assessing the virulence were a) instar and moulting durations, b) larval weight, and c) LT₅₀. The results showed inactivation of the virus after 10 and 20 hours of exposure to sunlight under dry and moist environment respectively. Stability of the virus was unimpaired under indoor conditions of diffused light, upto 30 hours, the maximum period tested in the present study.

Des recherches ont été conduites quant à l'effet de l'exposition directe du virus Kenchu à la lumière naturelle, sous condition humide et sèche, sur son infectivité et sa virulence. Les périodes d'exposition varient de 5 à 30 heures, et des périodes d'exposition correspondantes sont aussi effectuées en intérieur. Les paramètres utilisés pour mesurer la virulence sont a) la durée de l'âge et de la mue, b) le poids larvaire et c) le LT₅₀. Les résultats indiquent une inactivation du virus après 10 heures d'exposition à la lumière naturelle sous condition sèche et 20 heures sous condition humide. La stabilité du virus reste intacte en intérieur, avec lumière diffuse jusqu'à 30 heures, période maximale testée dans la présente étude.

* This work forms a part of the thesis submitted by the first author in partial fulfilment of M.Sc. (Ag.).

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EFFECT OF STORAGE AT DIFFERENT HUMIDITIES ON THE VIRULENCE
OF KENCHU VIRUS OF SILKWORM, BOMBYX MORI L. *

EFFET DE LA CONSERVATION A DIFFERENTS TAUX D'HUMIDITE SUR LA
VIRULENCE DU VIRUS KENCHU CHEZ LE VER A SOIE BOMBYX MORI L. *

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The virulence of Kenchu virus after storage at different humidity levels over a six month period at 30°C was estimated in silkworm Bombyx mori L. by using growth inhibition, incubation period and LT₅₀ as criteria. Kenchu virus was found to retain its infectivity to silkworms after a six month preservation period although there was a gradual decrease in the virulence. Storage of the virus at 10, 20, 50, 80 and 90 per cent humidities indicated a greater loss of virulence at 10 and 20 per cent levels as compared to the loss at the higher humidity levels.

La virulence du virus Kenchu après conservation à différents degrés d'humidité est étudiée sur une période de 6 mois à 30°C chez le ver à soie Bombyx mori L. en prenant comme critères l'inhibition de la croissance, la période d'incubation et le LT₅₀. Le virus Kenchu garde son infectivité pour les vers à soie après une période de 6 mois de conservation bien que sa virulence diminue graduellement. La conservation du virus à 10, 20, 50, 80 et 90 % d'humidité montre que la perte de virulence est plus grande dans le cas des taux de 10 et 20 % d'humidité que dans le cas des taux élevés d'humidité.

* This work forms a part of the thesis submitted by the first author in partial fulfilment of M.Sc. (Ag.).

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AGE SPECIFIC ACTION OF BENLATE IN INDUCING DOMINANT
LETHALS IN SILKWORM BOMBYX MORI L.

INDUCTION D'UNE LETALITE DOMINANTE CHEZ LE VER A SOIE
BOMBYX MORI L. PAR ACTION DU BENLATE A DES PERIODES PRECISES

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Benlate, a methyl-1N (1-butyl carbamyl) 2-benzimidazole is a widely used fungicide in agriculture including moriculture, was tested for its efficacy in inducing dominant lethals at different age groups of pupae in silkworm Bombyx mori. Two polyvoltine races such as pure Mysore and Hosa Mysore were used and the mode of administration employed was pupal injection technique. 0.1 ml of 400 ppm and 750 ppm of the chemical was injected to 5th and 9th day old pupae. The results have revealed that both the concentrations tested could not induce significant dominant lethals in 9th day old pupae. However, in the 5th day old pupae significant dominant lethals were induced compared to the control and 9th day old pupae. The age specific action of the Chemical and its adverse effects are discussed.

Le Benlate, un méthyl-1N (1-butyl carbamyl) 2-benzimidazole, est un fongicide très utilisé en agriculture y compris en moriculture. Son efficacité à induire une létalité dominante est étudiée chez des chrysalides de Bombyx mori réparties en groupes d'âges différents. Deux races polyvoltines sont utilisées : Pure Mysore et Hosa Mysore, et le mode d'administration employé est l'injection dans la chrysalide. 0,1 ml de 400 ppm et 750 ppm du produit chimique est injecté aux nymphes âgées de 5 et 9 jours. Les résultats montrent que les deux concentrations testées n'entraînent pas une létalité dominante significative chez la chrysalide de 9 jours. Cependant, chez la nymphe de 5 jours, une létalité dominante est induite de façon significative par rapport aux témoins et aux chrysalides de 9 jours. L'action temporelle et les effets contradictoires du produit sont discutés.

CHANCES OF EXTRA-OVARIAN INFECTION OF SILKWORM BOMBYX MORI BY
PEBRINE (NOSEMA BOMBYCIS N. SPORES)

POSSIBILITE D'INFECTION EXTRA-OVARIENNE DU VER A SOIE BOMBYX MORI
PAR LA PEBRINE (SPORES DE NOSEMA BOMBYCIS N.)

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* * *

HYPERPARASITISM OF THE UZIFLY (EXORISTA SORBILLANS WIED.
DIPTERA : TACHINIDAE)

HYPERPARASITISME DE LA MOUCHE OUZY (EXORISTA SORBILLANS WIED.
DIPTERA TACHINIDAE)

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* * *

BACOLOGY - SOME ASPECTS OF PHYSIOLOGY OF ABSORPTION AND TRANSPORT
AND PATHOLOGY

BACOLOGIE - QUELQUES ASPECTS DE LA PHYSIOLOGIE DE L'ABSORPTION
ET DU TRANSPORT ET DE LA PATHOLOGIE

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(Abstracts non received yet)
(Résumés non parvenus)

REVUE SIGNALÉTIQUE

DESCRIPTIVE REVIEWS

LIST OF LANGUAGE SYMBOLS USED

(Bg) Bulgare
Bulgarian

(C) Chinois
Chinese

(D) Allemand
German

(E) Anglais
English

(F) Français
French

(J) Japonais
Japanese

(K) Coréen
Korean

(Pl) Polonais
Polish

(Pt) Portugais
Portuguese

(R) Russe
Russian

(Ro) Roumain
Rumanian

(S) Espagnol
Spanish

The corresponding small letters are used for indicating the language of the summary.

R : We have a copy of this article.

LISTE DES INDICATIFS DE LANGUES UTILISÉS

Les lettres minuscules correspondantes sont utilisées pour indiquer la langue du résumé.

R : Nous possédons un exemplaire de cet article.

SERICICULTURE GENERALE / GENERAL SERICULTURE

An.

Situation et perspectives de la sériciculture. (Situation and prospects of sericulture.)

Informations Techniques du C.E.M.A.G.R.E.F., 1983, 51(3), p. 1-4. (F) R

An.

Rapid strides of Tamil Nadu sericulture. (Progrès rapides de la sériciculture au Tamil Nadu.)

Indian Silk, 1984, 22(10-11), p. 3-13. (E) R

ALVAREZ V.B., VILLANUEVA E.P.

Profil of the sericulture industry. (Aperçu de l'industrie séricicole.)

Philippine Textile Journal, 1984, special issue, p. 27. (E) R

BINGKUN S., et al.

An investigation of mulberry resource in Xizang. (Enquête sur la culture du mûrier au Xizang.)

Canye Kexue, 1983, 9(2), p. 65. (C) R

CHOE B.H., et al.

Survey studies on serviceable sericultural communities in Korea. (Enquête sur les services séricicoles en Corée.)

Ser. J. of Korea, 25(1), p. 34. (K,e) R

HIRATSUKA E., MASAGO Y., YAMADA A.

Some notes on the Nippon-Sanhinshu-Jitsuyokeifu, historical review of silkworm varieties in Japan. (Notes sur le Nippon-Sanhinshu-Jitsuyokeifu, revue historique des variétés de vers à soie au Japon.)

Rep. of the Silk Sci. Res. Inst., 1983, 31, p. 103. (J) R

JUSI-CRUZ S.

Marketing socio-economics. A look at our budding silk industry and its economic potentials. (Etude socio-économique du marché. Regard sur notre industrie séricicole débutante et ses possibilités économiques.)

Philippine Textile Journal, 1984, special issue, p. 29. (E) R

KUANGMING Z.

On historical experience in selecting and breeding the mulberry silkworm races in China. (Expérience historique de sélection et d'élevage des races chinoises de ver à soie du mûrier.)

Canye Kexue, 1983, 9(4), p. 240. (C) R

SHENKANG W., et al.

Consultation system in silkworm breeding. (Système de consultation pour l'élevage du ver à soie.)

Canye Kexue, 1983, 9(3), p. 143. (C) R

TAKAHASHI R., KRONKA R.N., CURTARELLI S.M., MIYADA V.S.

Silkworm (*Bombyx mori* L.) crisalys meal in growing and finishing swine rations. (Farine de chrysalides de ver à soie (*Bombyx mori* L.) dans l'alimentation des porcs, en croissance et en engraissement.)

O Solo, 1982, 74(1-2), p. 36-42. (Pt,pt,e) R

YOUYUN D.

Sericulture had its beginning in middle reaches of the Yellow River where the ancestors of Chinese people inhabited. (La sériciculture a débuté sur la rive droite du cours moyen du Fleuve Jaune, là où habitaient les ancêtres du peuple chinois.)

Canye Kexue, 1983, 9(1), p. 50. (C) R

ZHIYUAN L., et al.

On the effects of climate on the qualities of cocoons and silk. (Effets du climat sur la qualité des cocons et de la soie.)

Canye Kexue, 1983, 9(1), p. 7. (C) R

MURIER / MULBERRY

BAE K.S., KIM Y.S.

The effects of cadmium on the growth of mulberry tree and metabolic activities in the leaves. (Effets du cadmium sur la croissance du mûrier et les activités métaboliques dans les feuilles.)

Ser. J. of Korea, 1983, 25(1), p. 27. (K,e) R

BENMING Z., et al.

The preliminary report on the influence of oxytetracycline on the Mlo preparation from yellow dwarf diseased mulberry. (Rapport préliminaire sur l'influence de l'oxytétracycline sur la préparation Mlo provenant de mûrier atteint de nanisme jaune.)

Canye Kexue, 1983, 9(3), p. 184. (C) R

DEJI R., et al.

A study on the measurements of transpiration rate of mulberry tree. (Etudes sur les mesures du taux de transpiration du mûrier.)

Canye Kexue, 1983, 9(3), p. 134. (C) R

DEQUAN Y., et al.

The influence of mulching the mulberry field with coloured polyethylene films on the growth and yield of mulberry. (Influence du paillage des plantations de mûriers avec des films de polyéthylène de couleur sur la croissance et le rendement des mûriers.)

Canye Kexue, 1983, 9(4), p. 197. (C) R

ERTIAN H.

Preliminary study on the biology and control of the mulberry tussock moth *Euproctis similis* fuessly. (Etude préliminaire sur la biologie et le contrôle de l'orgye du mûrier *Euproctis similis*.)

Canye Kexue, 1983, 9(3), p. 138. (C) R

FENGTONG M., et al.

Studies on winter bud culture of grown up mulberry (11). (Etudes de la culture du mûrier adulte avec floraison hivernale.)

Canye Kexue, 1983, 9(2), p. 123. (C) R

FUKAI T., HANO Y., IKUTA H., FUJIMOTO T., et al.

Structures of new phenolic constituents of *Morus* root barks. (Structure de nouveaux composants phénoliques de l'écorce de la racine du mûrier.)

Tennen Yuki Kagobutsu, Toronkai koen Yoshishu, 1983, 26, p. 150-157. (J)

FUKAI T., HANO Y., HIRAKURA K., NOMURA T., UZAWA J.

Structure of mulberrofuran H, a novel 2-aryl benzofuran derivative from the cultivated mulberry tree (*Morus lhou* (ser) Koidz). (Structure du mulberrofuran H, un dérivé du 2-aryl benzofuran provenant du mûrier cultivé (*Morus lhou* (ser) Koidz).)

Chem. Pharm. Bull. Tokyo, 1984, 32, p. 808.

GUOFU N., et al.

Electrothermal installation for supplying heat within hotbed of mulberry cuttings for obtaining mulberry saplings. (Installation électrothermique pour amener de la chaleur à l'intérieur de la couche de bouturage et obtenir des plançons de mûrier.)

Canye Kexue, 1983, 9(4), p. 247. (C) R

HUIJIU W., et al.

Survey of mulberry planting in the region of salinized soil, bordering the sea in Jiangsu Province: On soil improvement in high-yield mulberry plantations in Santsang Brigade. (Enquête sur les plantations de mûriers dans les régions à sol salin en bordure de mer dans la Province de Jiangsu: Amélioration des sols dans les plantations de mûrier à haut rendement de la Brigade de Santsang.)

Canye Kexue, 1983, 9(4), p. 191. (C) R

ITO D.

Theoretical analysis of light-intercepting characteristics and inclination of shoots of mulberry tree. (Analyse théorique des caractères d'interception de la lumière et de l'inclinaison des branches du mûrier.)

J. Sericult. Sci. Japan, 1984, 53(1), p. 14-20. (J,j,e)

In order to clarify the ideal form of mulberry tree for light-intercepting the author made five model mulberry trees which differ to each other in the inclination of their shoots, calculated the light-intercepting characteristics of their populations by using computers, and compared them to each other. The result under diffuse radiation was almost the same as that under direct radiation. That is, when length of shoots was 50 cm, 100 cm or 150 cm, light-intercepting rate was always better in the model which has inclined shoots. This tendency was especially so in the case of 100 cm and 150 cm, because there existed in middle and upper layer a sharp difference among the models in light-intercepting efficiency. But in lower layer, on the contrary, light-intercepting efficiency was better in the model which had upright shoots under certain condition. When length of shoots reached 200 cm, it was difficult to judge which model was better because every model population became possible to intercept most of the light that came into the foliage.

Afin d'établir quelle est la forme idéale pour le mûrier pour intercepter la lumière, l'auteur a réalisé cinq arbres modèles qui diffèrent les uns des autres par l'inclinaison de leurs branches, a calculé les caractéristiques d'interception de la lumière de leur population par ordinateur, et les a comparés les uns aux autres. On obtient pratiquement les mêmes résultats sous radiation diffuse que sous radiation directe : lorsque la longueur des branches est de 50 cm, 100 cm ou 150 cm, le taux d'interception de la lumière est toujours meilleur chez l'arbre qui a des branches inclinées. Cette tendance est particulièrement forte dans les cas de 100 et 150 cm, parce qu'il existe une grande différence entre les plants quand il s'agit des parties médianes et supérieures pour l'interception de la lumière. Mais dans les parties basses, au contraire, la capacité d'interception de la lumière est meilleure chez

l'arbre qui a des branches montantes et droites sous certaines conditions. Lorsque la longueur des branches atteint 200 cm il est difficile d'estimer quel modèle est le meilleur parce que chaque population modèle est alors en mesure d'intercepter la majeure partie de la lumière qui vient dans le feuillage.

Sericultural Experiment Station, Yatabe, Ibaraki 306.

JINHO Y., et al.

Internal factors affecting the rooting and effect of accelerators on mulberry cutting. (Facteurs internes affectant l'enracinement et effet des accélérateurs sur le bouturage des mûriers.)

Canye Kexue, 1983, 9(3), p. 127. (C) R

KATAGIRI K., HIRANO H., HIRAI A., ICHIKAWA H.

Isolation of chloroplast DNA in mulberry. (Isolement du DNA de chloroplaste chez le mûrier.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 83. (E) R

KUNO K.

Effects of cultivating densities on growth of mulberry and contents of minerals in mulberry leaves after summer pruning. (Effets de la densité de plantation sur la croissance du mûrier et le contenu minéral des feuilles après la taille de printemps)

J. Sericult. Sci. Japan, 1983, 52(6), p. 484-490. (J,j,e)

In three plots whose cultivating density differed, namely S plot (833 plants/10a) D₁ plot (3996 plants/10a) and D₂ plot (6666 plants/10a), changes of growth and contents of minerals in various organs during varying periods, that is, in stage I (from March 25th to July 10th), in stage II (from July 10th to August 5th), in stage III (from August 5th to September 5th) and in stage IV (from September 5th to October 5th), were investigated. In dense plots, leaf number, dry weight of leaves and leaf area per plant were less than one-third, and sum length and dry weight of branches were less than half of those in sparse plot. The rate of dry weight increase of mulberry leaves was maximum in stage III but that of branches became larger as the stage was later. In each plot, the amount of minerals in various organs was maximum in stage III. In the leaves larger amounts of nitrogen, phosphorus and potassium were found in stages II and III. In dense plots the amounts of minerals in branches and leaves were 4/7 to 1/3 (in D₁ plot) or 1/3 to 1/8 (in D₂ plot) of those in sparse plot. Especially, accumulation of nitrogen in leaves was prevented in stages III and IV, and that of phosphorous was prevented in stages II and III.

Dans trois terrains ayant une densité de plantation différente, désignés comme le terrain S (833 plants/10 ares), le terrain D₁ (3 996 plants/10 ares) et le terrain D₂ (6 666 plants/10 ares), les changements intervenant dans la croissance et le contenu en minéraux des différents organes sont étudiés à différents stades, c'est à dire au stade I (du 25 mars au 10 juillet), au stade II (du 10 juillet au 25 août), au stade III (du 5 août au 5 septembre) et au stade IV (du 5 août au 5 septembre) et au stade IV (du 5 septembre au 5 octobre). En plantation dense, le nombre de feuilles, le poids sec des feuilles et la surface de feuille par plant sont moins importants d'un tiers par rapport aux parcelles en plantation espacée et la longueur totale ajoutée des branches et le poids sec des branches sont deux fois moins importantes que dans les terrains en plantation espacée. Le pourcentage d'augmentation du poids sec des feuilles de mûrier est à son maximum au stade III mais celui des branches s'accroît avec le temps. Dans chaque parcelle, le contenu en minéraux dans

Mulberry

les différents organes est à son maximum au stade III. Dans les feuilles, on trouve les plus fortes concentrations en azote, phosphore et potassium aux stades II et III. Dans les plantations denses, le contenu en minéraux dans les branches et dans les feuilles sont de 4/7 à 1/3 (parcelle D₁) ou de 1/3 à 1/8 (parcelle S₂) par rapport à celui des parcelles en plantation espacée. L'accumulation d'azote dans les feuilles est réduite pendant les stades III et IV, et celle du phosphore est réduite pendant les stades II et III.

Tokyo University of Agriculture and Technology, Tokyo, Fuchu 183.

KUNO K.

Effect of heavy metals on dry weight growth of mulberry seedling and development of root meristems. (Effets des métaux lourds sur la croissance en poids sec des jeunes plants de mûrier et sur le développement du méristème des racines).

J. Sericult. Sci. Japan, 1983, 52(6), p. 472-478. (J,j,e)

The effects of heavy metals on dry weight growth of mulberry seedling and development of root meristems were studied. Copper and cadmium inhibited root growth intensely according to the increase of their concentration. Copper showed inhibitory effects on cell division in epidermis, exodermis, cortex and stele. Cadmium inhibited cell division in epidermis and cortex slightly but inhibited division of stelar cells strongly. Zinc inhibited increment of epidermal cells slightly and inhibited that of stelar cells in higher concentration markedly. All of the heavy metals employed inhibited differentiation of proto sieve tubes in higher concentration. Furthermore, malformations of roots, due to unbalance growth of tissues, disorder of histogenesis or abnormal formation of lateral roots, were observed when the treatment period was prolonged.

Nous avons étudié les effets des métaux lourds sur la croissance en poids sec des jeunes plants de mûriers et sur le développement des méristèmes des racines. Le cuivre et le cadmium inhibent la croissance des racines de manière intensive et en rapport avec l'augmentation de leur concentration. Le cuivre montre des effets inhibiteurs sur la division cellulaire dans l'épiderme, l'exoderme, le cortex et la stèle. Le cadmium inhibe légèrement la division cellulaire dans l'épiderme et le cortex, et, en forte concentration, inhibe la division cellulaire dans la stèle de manière très marquée. Tous les métaux lourds employés en forte concentration inhibent la différenciation des proto-cibles. De plus, des malformations des racines, dues à la croissance déséquilibrée des tissus, une histogenèse anarchique ou la formation anormale de racines latérales, sont observées lorsque la période de traitement est prolongée.

LIANZHANG X., et al.

Studies on the infected portion of mulberry bacterial wilt by electron scanning microscopy. (Etude au microscope électronique de mûriers atteints de dépérissement bactérien.)

Canye Kexue, 1983, 9(1), p. 58. (C) R

MACHII H. YANO Y.

On the non-dormancy of three mulberry strains (M 28, M 32, M 38) selected by natural crossing. (Absence de dormance de trois souches de mûrier (M 28, M 32, M 38) sélectionnées par croisement naturel).

J. Sericult. Sci. Japan, 52(6), p. 469-471. (J,j,e)

In order to get information on the selection of mulberry varieties fitting for cultivation in subtropical regions such as the South West Islands, the sprouting percentage of winter buds, kept at 30°C for 15 days, was investigated from September to February, using one strain of Shimaguwa (*Morus acidosa* Griff.) and three strains, M 28, M 32 and M 38, which had been selected from naturally-crossed seedlings of Shimaguwa. The Shimaguwa strain gave high percentage of bud sprouting, showing that it had no dormancy. M 28, M 32 and M 38 showed almost the same tendency as Shimaguwa strain, suggesting that these three strains had no dormancy as well. Accordingly, it was ascertained that the three strains had no resting period and that their buds are ready to sprout whenever the temperature became favourable. Therefore, these three strains are regarded as the desirable strains for future selection of mulberry varieties suitable for the South West Island.

Pour obtenir des informations sur la sélection de variétés de mûrier adaptées aux régions subtropicales telles que les Iles du Sud-Ouest, nous avons étudié, de septembre à février, le taux de bourgeonnement des boutons d'hiver conservés pendant 15 jours à une température de 30°C, en utilisant une souche de Shimaguwa (*Morus acidosa* Griff.) et 3 souches, M 28, M 32 et M 38, sélectionnées à partir de croisements naturels de Shimaguwa. La souche Shimaguwa donne un taux de bourgeonnement élevé indiquant ainsi qu'il n'y a pas de dormance. M 28, M 32 et M 38 montrent à peu près la même tendance que la souche Shimaguwa, ce qui indique également que ces souches n'ont pas de dormance. De même, on constate que les 3 souches n'ont pas de période de repos et que les boutons sont prêts à bourgeonner dès que la température est favorable. Aussi, peut-on considérer que ces 3 souches conviennent pour la sélection future des variétés de mûrier pouvant être cultivés dans les Iles du Sud-Ouest.

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MIAOCHUEN Y., et al.

Studies on Zhejiang 4FS-6003(5) mulberry shoot pruning machine. (Etudes sur les machines à récolter les rameaux de mûrier.)

Canye Kexue, 1983, 9(2), p. 114. (C) R

OKABE T.

Relationship between spray of growth regulator to the mulberry seedlings and flowering-fruiting. (Relation entre la vaporisation d'un régulateur de croissance sur les jeunes plants de mûrier et la floraison-fructification.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 21-26. (J,j,e)

As a result of preliminary experiments in which various growth regulators (B9, ethrel, CCC and BA) were sprayed on the seedlings in autumn of the year they were sown, it was known that B9 and CCC were effective in flowering of the seedlings. The seedlings, which were cultivated by sand culture in various sizes of pots, were sprayed two or three times from August to September with various concentrations of B9 or CCC. Although no flowered individual appeared in non-sprayed seedlings, the percentages of flowered and fruited individuals obtained by spraying with 500-3,000 ppm of B9 or 100-500 ppm of CCC were 18-50 or 18-83, respectively. The longer the shoots of the seedlings, the higher the ratios of the flowering plants become. The seeds obtained germinated and grew normally. On the other hand, the inhibition of shoot growth, shortening of the internodes and increased lignification were observed in the seedlings sprayed with B9 or CCC. These phenomena suggest that the carbohydrate contents in the plants increased by such treatments and consequently, the flowering may be promoted.

Les résultats d'expériences préliminaires au cours desquelles divers régulateurs de croissance (B9, éthrel, CCC et BA) furent appliqués sur des jeunes plants à l'automne de l'année de leur semis, ont montré que B9 et CCC sont efficaces pour la floraison des plants. Les plants, qui sont cultivés sur sable, dans des pots de différentes tailles, sont vaporisés deux ou trois fois d'août à septembre avec différentes concentrations de B9 ou CCC. Alors qu'aucun des plants non vaporisés n'a fleuri, le pourcentage de sujets fleuris ou portant des fruits chez les plants traités avec 500-3 000 ppm de B9 ou 100-500 ppm de CCC est respectivement de 18 à 50 et de 18 à 83. Plus la tige du plant est longue, plus le pourcentage de floraison est élevé. Les graines obtenues ont germé et poussé normalement. D'autre part, on observe chez les plants traités avec B9 ou CCC, une inhibition de la croissance de la tige, un raccourcissement des internoeuds et une lignification plus importante. Ces phénomènes laissent penser que le contenu en hydrate de carbone est augmenté par de tels traitements, et favorise ainsi la floraison.

Sericultural Experiment Station, Yatabe, Ibaraki 305, Japan.

OKABE T.

Relationship between girdling or strapping of the shoots in autumn of sown year and flowering on mulberry seedlings. (Corrélation entre le cerclage ou le bandage automnal des rameaux obtenus l'année du semis et la floraison des plants de mûrier.)

J. Seric. Sci. Jpn., 1983, 52(6), p. 505-510. (J,j,e)

After the mulberry hybrid seeds were sown in April, the seedlings were cultivated by soil or sand culture in the pots of a greenhouse until August, and half of the seedlings were girdled or strapped in late August or early September. The growth of the seedlings and the contents of carbohydrates and nitrogen or C/N value in the shoots were measured in August, September and October. Furthermore, the number of inflorescences in the developing buds of seedling was counted in following February. No inhibition of the growth in treated seedlings was observed, but their leaves yellowed, the contents of carbohydrates and C/N value in the shoots of girdled seedlings were clearly higher than those of the non-treated seedlings. Half of girdled or strapped seedlings flowered. However, no flowered plant was observed in non-treated seedlings. From these results it is assumed that the translocation of the assimilates may be blocked by the girdling or the strapping treatment so that they accumulate in the shoots and promote flowering.

Après le semis de mûriers hybrides en avril, les plants sont cultivés sous serre, en terre ou sur sable jusqu'en août et la moitié des plants est cerclée ou bandée à la fin du mois d'août ou au début du mois de septembre. La croissance des plants ainsi que leur teneur en hydrates de carbone et azote, ou la valeur C/N des rameaux sont mesurées en août, septembre et octobre. De plus, le nombre d'inflorescences dans les boutons en développement est compté au mois de février suivant. On n'observe pas d'inhibition de la croissance chez les plants traités, mais leurs feuilles jaunissent, la teneur en hydrates de carbone et la valeur C/N des plants cerclés sont nettement plus élevées que chez les plants non traités. La moitié des plants cerclés ou bandés a fleuri. Cependant, aucune floraison n'est constatée chez les plants non traités. Ces résultats nous permettent de penser que la translocation des assimilés peut être bloquée par le cerclage ou le bandage, ce qui entraîne leur accumulation dans les rameaux et favorise la floraison.

OKABE T.

Relationship between the sowing period and flowering-fruiting on mulberry seedlings.
(Relation entre la période du semis et la floraison-fructification des plants de mûrier.)

J. Seric. Sci. Jpn., 1983, 52(6), p. 511-516. (J,j,e)

Some kinds of mulberry hybrid seeds were sown three times at 45-day intervals from March 10 or at 20-day intervals from April 20. After these mulberry seedlings were grown by water culture or by sand culture followed by strapping in autumn, their growth and the number of the inflorescence in the developing bud in the following February to March were measured. Furthermore, the minimum duration of growth required for flowering and the height of seedlings at the time were examined. The seedlings sown before May 10 flowered and fruited in the range of 2 to 100%, however, no fruited individual was observed in the seedlings sown after June 1. It is assumed that about four months duration of growth after sowing and above 80 cm of main shoot height may be required for flowering and fruiting in the terms of water culture or sand culture followed by strapping.

Certaines variétés de graines de mûriers hybrides ont été semées à trois reprises à 45 jours d'intervalle à partir du 10 mars ou à 20 jours d'intervalle à partir du 20 avril. Après une culture des graines hors-sol ou sur sable, les mûriers sont bandés en automne. De février à mars, nous avons étudié leur croissance et le nombre d'inflorescences dans les boutons en développement. De plus nous avons examiné la durée minimale de croissance requise pour la floraison et la hauteur des plants. Les plants semés avant le 10 mai ont fleuri et ont donné des fruits dans une proportion de 2 à 100 %, cependant les plants semés après le 1er juin n'ont pas donné de fruits. On estime qu'une période d'environ 4 mois de croissance après le semis et une hauteur d'environ 80 cm du rameau principal sont nécessaires pour que les fleurs et les fruits apparaissent lorsque la culture se fait hors-sol ou sur sable et en pratiquant le bandage.

PARK K.J., NAM H.W., LEE J.T.

The characteristics of the mulberry variety, Jeon Weon N°1. (Caractéristiques de la variété de mûrier Jeon Weon N°1.)

Ser. J. of Korea, 1983, 25(1), p. 21-26. (K,e) R

QINGHONG G., et al.

On propagating Hu-sang, a group of mulberry varieties, by means of cuttings. 2) Experiments on using charred rice bran, put in plastic pots, for cuttings to strike roots in. (Multiplication par bouturage des Hu-sang, un groupe de variétés de mûrier. 2) Essai de cendres de son de riz, dans des pots plastiques pour l'enracinement des boutures.)

Canye Kexue, 1983, 9(2), p. 71. (C) R

SEOL G.Y., LEE S.P.

Utilization of the frosted mulberry leaves as a source of artificial diet for silkworm. (Utilisation des feuilles de mûrier congelées comme source d'aliment artificiel du ver à soie.)

Ser. J. of Korea, 1983, 25(1), p. 51. (K) R

Mulberry

TAKAHASHI K.

Recent researches of viruses, mycoplasma and bacteria pathogenic to mulberry trees. (Recherches récentes sur les virus, les mycoplasmes et les bactéries pathogènes pour le mûrier.)

J. Seric. Sci. Jpn., 1983, 52(6), p. 461. (J) R

UEDA S., MATSUMOTO J., NOMURA T.

Four new natural Diels-Alder type adducts, Mulberrofuron & Kuwanon Q, R and V from callus culture of *Morus alba*. (Quatre nouveaux adduits de Diels-Alder, Mulberrofuron et Kuwanon Q, R et V, extraits des cultures de tissus de mûrier blanc.)

Chem. Pharm. Bull., 32, p. 350.

WANG Y., et al.

Preliminary investigation of the relationship between the endogenous plant growth substancein and the sprouting of Hu-mulberry (*Morus latifolia*) during spring. (Recherche préliminaire sur la relation entre la substance endogène de croissance et le bourgeonnement du mûrier Hu (*Morus latifolia*) au printemps.)

Canye Kexue, 1983, 9(3), p. 185. (C) R

XIQUN Y., et al.

Preliminary report on identifying the yields and qualities of some mulberry varieties. (Rapport préliminaire sur la productivité et la qualité de quelques variétés de mûrier.)

Canye Kexue, 1983, 9(3), p. 12. (C) R

YOSHII T., YOKOI N.

The damage on the mulberry shoot caused by the brown-marmorated stink bug, *Hyalomorpha mista* Uhler. (Dommages causés aux pousses de mûrier par la punaise brune, *Hyalomorpha mista* Uhler.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 81. (J) R

YUEJI Z.

Studies on the mulberry mosaic dwarf disease. (Etudes sur la maladie du nanisme du mûrier.)

Canye Kexue, 1983, 9(2), p. 74. (C) R

BOMBYX ELEVAGE / BOMBYX REARING

DAHUAN Z., et al.

Studies on the method of feeding the late-age silkworm larvae according to the index of their body weight. (Etudes sur le régime alimentaire des larves du ver à soie au dernier âge en rapport avec le poids corporel.)

Canye Kexue, 1983, 9(4), p. 203. (C) R

GUORUI H.

The influence of the humidity and air current during cocooning on the quality of cocoons. (Influence de l'humidité et des courants d'air pendant le coconnage sur la qualité des cocons.)

Canye Kexue, 1983, 9(2), p. 90. (C) R

MINGGUAN Y.

A self-made automatic silkworm egg counter. (Un compteur automatique d'oeufs de ver à soie.)

Canye Kexue, 1983, 9(2), p. 125. (C) R

PETKOV N., NACHEVA Y., MLADENOV G., AKIMENKO L., et al.

Rating the combinative ability of some promising Bulgarian and Ukrainian silkworm breeds (*Bombyx mori* L.). 1) On the basis of diallel crossbreds. (Evaluation des possibilités de combinaison de quelques souches de ver à soie (*Bombyx mori* L.) bulgares et ukrainiennes. 1) Sur la base d'un croisement diallèlique.)

Animal Science, 1984, 21(3), p. 100-107. (Bg,bg,e) R

QI L., et al.

A preliminary investigation on the effects of different mounting temperatures on the weight of fibroin remained within the silkglands of *Bombyx mori* L. after spinning. (Recherche préliminaire sur les effets de la température au moment de la montée, sur le poids de la fibroïne restant dans la glande séricigène de *Bombyx mori* après filage du cocon.)

Canye Kexue, 1983, 9(2), p. 85. (C) R

SEKI T.*, ABE K.*, TSUTSUI R.***, WATANABE T.***

Purification and properties of toxic protein from crystal of *Bacillus thuringiensis*. (Purification et propriétés d'une protéine toxique provenant du cristal de *Bacillus thuringiensis*.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 41-47. (J,j,e)

The crystal of *Bacillus thuringiensis* subsp. *dendrolimus* strain T 84 A1 was dissolved by the purified protease in the silkworm digestive juice. The soluble toxin was unstable in 0.2M Tris-HCl buffer or 1/15M phosphate buffer, but stabilized by adding 1mM EDTA, 0.1M glycine and 0.1-2M urea in 0.2M Tris-HCl buffer (pH 8.5). Also, the toxic protein was stable in 0.2M glycine-NaOH buffer (pH 8.5-9.0). The toxic protein was purified by means of Sephadex G-75 gel filtration and DEAE-cellulose chro-

matography. The LD₅₀ of the soluble toxin was 0.29 µg/g. The LD₅₀ of the purified toxin by gel filtration or by DEAE-cellulose chromatography was 0.14 µg/g. The toxic protein was purified 2.2 fold. The toxic protein gave a single band in slab gel electrophoresis of sodium dodecylsulfate-polyacrylamide. Molecular weight of the toxic protein was estimated to be 66,000 by SDS-polyacrylamide slab gel electrophoresis.

Le cristal de *Bacillus thuringiensis* sous-espèce *dendrolimus* souche T 84 A1 est dissout par la protéase purifiée du suc digestif de ver à soie. La toxine soluble est instable dans un tampon Tris-HCl 0,2 M ou un tampon phosphate 1/15 M, mais stabilisée lorsqu'on ajoute 1 mM EDTA, 0,1 M de glycine et 0,1-2 M d'urée dans un tampon Tris-HCl à 0,2 M (pH 8,5). De plus, la protéine toxique est stable dans un tampon glycine 0,2 M - NaOH (pH 8,5-9,0). La protéine toxique est purifiée par filtration sur gel Sephadex G-75 et chromatographie sur DEAE-cellulose. Le LD₅₀ de la toxine soluble est de 0,29 µg/g. Le LD₅₀ de la toxine purifiée par filtration sur gel ou chromatographie sur DEAE-cellulose est de 0,14 µg/g. La protéine toxique a été purifiée 2,2 fois. Elle apparaît sous forme d'une bande unique après électrophorèse sur gel de polyacrylamide en plaque en présence de dodécylsulfate de sodium. Le poids moléculaire de la protéine toxique est évalué à 66 000.

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TANAKA Y.

A study on the application of the antimold chemical (microguard M-101) to artificial diets of silkworms. (Une étude sur l'application d'un produit chimique contre les vers (M-101) dans les aliments artificiels pour ver à soie.)

Rep. of the Silk Sci. Res. Inst., 1983, 31, p. 21. (J) R

YICANG S.

Research on the compounding use of "402", a silkworm body and rearing-seat disinfectant. (Recherche sur l'utilisation de produits à base de "402", un désinfectant des vers à soie et des litières d'élevage.)

Canye Kexue, 1983, 9(3), p. 172. (C) R

YIREN L., et al.

Stage suitable for identifying the resistance of the races of silkworm, *Bombyx mori* L., being reared in summer-autumn to high temperature. (Age favorable pour estimer la résistance des races de ver à soie, *Bombyx mori* L., élevées en automne à des températures élevées.)

Canye Kexue, 1983, 9(1), p. 23. (C) R

YONGNIAN J., et al.

On heating the silkworm rearing room by a hot air generator. (Chauffage des magnaneries par générateur d'air chaud.)

Canye Kexue, 1983, 9(1), p. 44. (C) R

ZIGANG H., et al.

Studies on JCC-82 type autocontrolled box for incubating silkworm eggs. (Etudes sur la couveuse, type JCC-82, pour l'incubation des oeufs de ver à soie.)

Canye Kexue, 1983, 9(3), p. 177. (C) R

BOMBYX BIOLOGIE / BOMBYX BIOLOGY

COLL.

Studies on the breeding and heredity of some resistant strains to cytoplasmic polyhedrosis virus in the silkworm, *Bombyx mori* L. (Études sur la sélection et l'hérédité de certaines souches résistantes au virus de la polyédrose cytoplasmique chez le ver à soie *Bombyx mori* L.).

Canye Kexue, 1983, 9(4), p. 214. (C) R

AKAI H.

Ultrastructure of the cuticular intima of the posterior silk gland in *Bombyx mori*. (Structure fine de l'intima cuticulaire de la glande séricigène postérieure chez *Bombyx mori*.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 72-76. (E,e)

The silk gland is an ectodermal organ, and a thick cuticular intima lines the inner surface of anterior silk gland as is true for the other organs that arise as ectodermal invaginations in insects. However, it has been claimed that there are no cuticular coverings on apical surfaces of the cells of the middle and posterior silk gland. The author found several thin cuticular structures in the silk layer of the posterior silk gland. The silk layer contains horizontal cuticular membranes and vertical cuticular supports. Scattered fibroin passes through the spaces within this mechanical skeleton. At the molting stages this cytoskeleton is completely renewed, as is the case for the other ectodermal organs. Such specializations of the intima in the posterior silk gland cells indicate that the intima has evolved into a functional structure facilitating silk secretion.

La glande séricigène est un organe ectodermique, et une intima cuticulaire épaisse parcourt la surface interne de la partie antérieure de la glande séricigène comme c'est le cas pour les autres organes et se présente sous forme d'invaginations ectodermiques chez les insectes. Cependant, il est précisé qu'il n'existe pas de couches cuticulaires sur les surfaces apicales des cellules des parties médiane et postérieure de la glande séricigène. L'auteur a découvert plusieurs structures cuticulaires fines dans la couche de soie de la partie postérieure de la glande. La couche de soie présente des membranes cuticulaires horizontales et des supports cuticulaires verticaux. La fibroïne est disposée à travers cette ossature mécanique. Lors des mues, ce cytosquelette est complètement renouvelé, de même que pour les autres organes ectodermiques. De telles spécialisations de l'intima chez les cellules de la partie postérieure de la glande séricigène indiquent que l'intima a développé des structures fonctionnelles facilitant la sécrétion de la soie.

Sericultural Experiment Station, Yatabe, Ibaraki 305, Japan.

AKAI H., SHIBUKAWA A.

The jumbo cocoons obtained by administration of juvenoids in domesticated silkworm, *Bombyx mori*. (Les cocons géants obtenus par administration de juvénoïdes chez le ver à soie domestiqué *Bombyx mori*.)

J. Sericult. Sci. Japan, 1984, 53(1), p. 77-80. (J,j,e)

The test silkworms of HN 90 x HC 74 is one of the crossings which produce the heaviest cocoons. Two types of juvenoids "Manta" and "Moore Silk", were treated for the HN 90 x HC 74, and they produced not only heavy cocoons but also respond to the

hormone more strongly than other races. In treated animals, the average weights were 3.75 g in cocoon weight, and 951 mg in cocoon shell weight. The highest cocoon shell weight observed was 1190 mg among the treated females, and 1120 mg among the males. This large increase of silk production seems to be resulted from a combination of the optimal administration of juvenoid and the superior genotypes of the hybrid larvae that respond to the hormone.

Le croisement de ver à soie HN 90 x HC 74 est l'un de ceux qui produit les cocons les plus lourds. Deux types de juvénosides "Manta" et "Moore Silk" ont été traités pour le croisement HN 90 x HC 74 ; ils produisent non seulement des cocons plus lourds mais répondent à l'hormone d'une manière plus importante que les autres races. Chez les vers traités, le poids moyen du cocon est de 3,75 g, celui de la coque soyeuse est de 951 mg. Le poids de la coque soyeuse le plus élevé est de 1190 mg chez les femelles et de 1120 mg chez les mâles. Cette importante augmentation de la production de soie semble être le résultat d'une combinaison de l'administration optimale de juvénoside et des génotypes des larves hybrides qui répondent à l'hormone.

Sericultural Experiment Station, Yatabe, Ibaraki 305, Japan.

CAO G., LI W., GU X., ZHU L., QI G., WANG D.

Nucleotide sequence of 5 S rRNA from the posterior silk gland of the silkworm *Philosamia cynthia ricini*. (Séquence des nucléotides du rRNA 5 S provenant de la glande séricigène postérieure du ver à soie *Philosamia cynthia ricini*).

Shengwu Huaxue Yu, Shengwu Wuli Xuebao, 1983, 15(5), p. 405-413. (C)

COUBLE P., BLAES N., PRUDHOMME J.C.

Actin microfilaments and fibroin secretion in the silk gland cells of *Bombyx mori*. Effects of cytochalasin B. (Microfilaments d'actine et sécrétion de la fibroïne dans les cellules de la glande séricigène chez *Bombyx mori*. Les effets de la cytochalasine B.)

Experimental Cell Research, 1984, 151, p. 322-331. (E,e)

Bombyx mori posterior silk gland cells exhibit an impressive microfilament apparatus located at the cellular apex. It consists of bundles of packed, long microfilaments of 50-70 Å diameter running along circumferences delimiting the lumen of the gland, perpendicularly to the flow of luminal silk. Microfilaments are closely associated with microtubules of the cytoplasmic 'radial microtubule system'. Immunolabeling with purified antihuman actin antibodies was used to demonstrate their actin-like nature. Apical microfilaments are sensitive to cytochalasin B (CB) which selectively inhibits the secretion of fibroin. Following the removal of the drug, microfilaments recover their normal morphology and secretion resumes. The possible implication of contraction of microfilaments in the process of secretion is discussed.

Les cellules de la partie postérieure de la glande séricigène de *Bombyx mori* ont un appareil microfilamenteux impressionnant situé à l'apex cellulaire. Il se compose de faisceaux de longs microfilaments de 50 à 70 Å de diamètre disposés le long des circonférences délimitant le lumen de la glande et perpendiculairement à l'écoulement de la soie dans le lumen. Les microfilaments sont étroitement associés avec les microtubules du "système microtubulaire radial" cytoplasmique. Le marquage immunologique avec des anticorps antihumains d'actine purifiés a été utilisé pour démontrer leur similitude avec l'actine. Les microfilaments sont sensibles à la cytochalasine B (CB) qui inhibe d'une manière sélective la sécrétion de la fibroïne. Après

retrait de la drogue, les microfilaments retrouvent leur morphologie normale et la sécrétion reprend. Il semble possible que la contraction des microfilaments soit impliquée dans le processus de sécrétion de la soie.

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DIGNAM S.S., DIGNAM J.D.

Glycyl- and alanyl-tRNA synthetases from *Bombyx mori*. Purification and properties. (Glycyl- et alanyl-tRNA synthétases de *Bombyx mori*. Purification and propriétés).

The Journal of Biol. Chem., 1984, 259(7), p. 4043-4048. (E,e)

Glycyl- and alanyl-tRNA synthetases have been purified from an extract of *Bombyx mori* posterior silk glands by a procedure that allows for the simultaneous isolation of both enzymes. Glycyl-tRNA synthetase is a dimer of $M_r = 160,000$ consisting of similar or identical subunits, whereas alanyl-tRNA synthetase is a monomer of $M_r = 110,000$ to $115,000$. The abundance of these two enzymes in the posterior silk gland is consistent with the observed adaptation of this organ to the production of the silk protein, fibroin. The two enzymes are similar in oligomeric structure to the corresponding enzymes in *Saccharomyces cerevisiae*, but dissimilar from counterparts in *Escherichia coli*.

Glycyl- et alanyl-tRNA synthétases purifiées à partir d'un extrait de glandes séricigènes postérieures de *Bombyx mori* par un procédé qui permet l'isolement simultané des deux enzymes. La glycyl-tRNA synthétase est un dimère de $M_r = 160\ 000$ se composant de sous-unités similaires ou identiques, tandis que l'alanyl-tRNA synthétase est un monomère de $M_r = 110\ 000$ à $115\ 000$. L'abondance de ces deux enzymes dans la glande séricigène postérieure est compatible avec l'adaptation observée de cet organe à la production de la protéine de la soie, la fibroïne. Les deux enzymes sont similaires par leur structure oligomérique aux enzymes correspondantes chez *Saccharomyces cerevisiae*, mais ne sont pas similaires à leurs équivalents chez *Escherichia coli*.

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EGUCHI M., UEDA K., YAMASHITA M.

Comparison of the electrophoretic patterns of hemolymph protease inhibitors among silkworm races. (Comparaison des électrophorégrammes des inhibiteurs de protéase de l'hémolymphe de différentes races de ver à soie.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 27-32. (J,j,e)

The electrophoretic patterns of hemolymph protease inhibitors were compared using 126 silkworm strains. A number of electrophoretic variants were found; total 6 inhibitors against fungal protease and 8 inhibitors against α -chymotrypsin were discernible. Fungal protease inhibitor, bands A and E were observed in all strains, but band B was detected in 2 strains of Chinese race and in 1 strain of European race. Chymotrypsin inhibitor, band c was seen in only 4 strains of Chinese race. Band b was not found in Japanese race, whereas this inhibitor distributed in a half of European race. There was a great difference in distribution of band f among silkworm races. Comparison of the electrophoretic patterns of F_1 hybrid and parents showed that the hemolymph of offspring from single pair mating between different types produced inhibitors with both parental bands. Results obtained hitherto in the F_2 generation suggest that the expression of each band is controlled by a pair of alleles

which correspond to the strong and null bands respectively. Considering results the physiological functions of inhibitors are discussed focusing strain and race differences.

Les électrophorégrammes des inhibiteurs de protéase de l'hémolymphe sont comparés chez 126 souches de ver à soie. Un certain nombre de variantes ont été trouvées ; 6 inhibiteurs de la protéase fongique et 8 inhibiteurs de l' α -chymotrypsine sont discernables. Pour l'inhibiteur de la protéase fongique, les bandes A et E sont présentes chez toutes les souches, mais la bande B est détectée chez 2 souches de race chinoise et chez 1 souche de race européenne. Pour l'inhibiteur de la chymotrypsine, la bande e n'a été observée que chez 4 souches de race chinoise. La bande b n'apparaît pas chez la race japonaise, alors qu'elle est présente chez la moitié des souches européennes. Une grande différence existe dans la distribution de la bande f selon les races. La comparaison des électrophorégrammes de l'hybride F_1 et des parents montrent que l'hémolymphe des descendants d'un accouplement d'une paire simple entre des types différents produit des inhibiteurs comprenant les bandes des deux parents. Les résultats obtenus jusqu'ici chez la génération F_2 suggèrent que l'expression de chaque bande est contrôlée par une paire d'allèles qui correspondent respectivement à la bande majeure et à la bande nulle. Les fonctions physiologiques des inhibiteurs sont discutées en fonction de ces résultats et en mettant l'accent sur les différences existant entre les races et les souches.

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FU H., et al.

Influence of high temperature on physiological data and quantitative characters of 5th-instar larvae of the silkworm *Bombyx mori* L. (Influence des hautes températures sur les données physiologiques et les caractères quantitatifs des larves au 5ème âge du ver à soie *Bombyx mori* L.).

Canye Kexue, 1983, 9(2), p. 80. (C) R

FUGO H., NAGASAWA H., SUZUKI A.

Induction of precocious adult eclosion by the eclosion hormone extracted from the heads of *Bombyx mori* and *Samia cynthia ricini*. (Induction de l'émergence précoce de l'adulte au moyen de l'hormone d'émergence extraite de la tête de *Bombyx mori* et de *Samia cynthia ricini*).

Appl. Entomol. Zool., 1983, 18(4), p. 540-544.

FUJII H., KAWAGUCHI Y.

Changes in haemolymph proteins with special reference to the major protein 5 in the fifth larvae, pupae and adults of *Bombyx mori*. (Changements dans les protéines de l'hémolymphe particulièrement la protéine 5 chez les larves du 5ème âge, les nymphes et les adultes de *Bombyx mori*).

J. Sericult. Sci. Japan, 1983, 52(6), p. 529-536. (J,j,e)

Qualitative and quantitative studies of haemolymph proteins and the major protein 5 (MP 5, the positively migrating fifth band) during developmental stage from the fifth instar larvae to adult were carried out. The concentration of total haemolymph protein increased maximum quantity at the end of spinning, and after pupation decreased gradually throughout pupal development in male, but rapidly in female. The concentration of the MP 5 increased markedly from the day 2 to 9 of the fifth instar and its increasing tendency was similar to the case of total haemolymph protein. The

concentration of the MP 5 in female was always greater than in male till the day 4 at pupal stage, thereafter smaller in female than in male. Nevertheless, the concentration ratio of the MP 5 to total haemolymph protein did not change during larval development within both sexes. On the other hand, after pupation, the ratio in female declined progressively, while it remained constantly in male. The marked and rapid decrease of the ratio in female at pupal stage may result from the utilisation of MP 5 for the egg formation. Consequently the MP5 might be synthesized in fat body, released into haemolymph and taken up from haemolymph into ovaries.

Nous avons effectué des études quantitatives et qualitatives des protéines de l'hémolymphe et la protéine majeure 5 (MP 5, la 5ème bande migrant vers le positif) au cours du développement de la larve au 5ème âge chez l'adulte. La concentration totale des protéines de l'hémolymphe augmente à la fin du filage et diminue après la pupaison, de manière régulière au cours du développement nymphal chez le mâle, mais rapidement chez la femelle. La concentration de la MP 5 augmente de façon marquée du 2ème au 9ème jour du 5ème âge et cette tendance est semblable à celle observée pour les protéines de l'hémolymphe. La concentration de la MP 5 est toujours plus importante chez la femelle que chez le mâle jusqu'au 4ème jour de la nymphose, elle est ensuite plus faible chez la femelle que chez le mâle. Néanmoins, la concentration de la MP 5 par rapport aux protéines totales de l'hémolymphe ne change pas pendant le développement larvaire, pour les deux sexes. D'autre part, après la nymphose, le taux diminue progressivement chez la femelle tandis qu'il reste constant chez le mâle. La diminution rapide et forte du taux chez la femelle au stade nymphal peut provenir de l'utilisation de la MP 5 pour la formation de l'oeuf. En conséquence, la MP 5 pourrait être synthétisée dans le corps gras, libérée dans l'hémolymphe et passer de l'hémolymphe aux ovaires.

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FUKUDA T.

An importance of the studies on the digestibility of the silkworm (advocacy). (Importance des études sur la digestibilité du ver à soie (plaidoyer)).

Report of the Silk Sci. Res. Inst., 1983, 31, p. 1. (J) R

FUKUDA T., SUDO M., SOH Y.

Studies on the digestibility of the silkworm *Bombyx mori* L. (Etudes sur la digestibilité du ver à soie *Bombyx mori* L.)

Report of the Silk Sci. Res. Inst., 1983, 31, p. 17 (J) R

GENSITSKII I.P., TERENT'EV A.G.

Previously unknown organic acid in the haemolymph of the silkworm *Bombyx mori* (2-keto-3-deoxy-5-O-carbamoylribonic acid). (Acide organique (jusqu'ici inconnu) dans l'hémolymphe du ver à soie *Bombyx mori* (acide 2-keto-3-deoxy-5-O-carbamoylribonique)).

Zh. Evol. Biokhim. Fiziol., 1983, 19(6), p. 534-538. (R)

HAMADA N., NAKAMURA O., KOGA K., HAYASHI K.

Effects of ecdysteroid on protein synthesis of *Bombyx* ovary. (Effets des ecdystéroïdes sur la synthèse des protéines de l'ovaire de *Bombyx*).

Agr. & Biol. Chem., 1984, 48, p. 277.

HIROSE S., TAKEUCHI K., HORI H., HIROSE T., INAYAMA S., SUZUKI Y.

Contact points between transcription machinery and the fibroin gene promoter deduced by functional tests of single-base substitution mutants. (Points de contact entre la "machinerie" de transcription et le promoteur du gène de la fibroïne déduits des tests fonctionnels des mutants de substitution de base simple).

PNAS, 1984, 81, p. 1394.

IWASHITA Y., YISUN C.

The development of a densovirus isolated from silkworm larvae, *Bombyx mori*, of China. (Développement d'un virus de la densovirose isolé à partir d'une larve de ver à soie, *Bombyx mori*, de Chine).

Canye Kexue, 1983, 9(4), p. 219. (C) R

JIALU H. et al.

The blood esterase map in the silkworm varieties. (Carte de l'estérase de l'hémolymphe chez les différentes variétés de vers à soie).

Canye Kexue, 1983, 9(2), p. 103. (C) R

KATSUNO S.

Sizes of polygonal patterns of the egg-shell and facets of the compound eye in the tetraploid silkworm, *Bombyx mori*. (Tailles des empreintes polygonales de la coque de l'oeuf et des facettes de l'oeil composé chez le ver à soie *Bombyx mori*).

J. Seric. Sci. Japan, 53(1), p. 1-6. (J,j,e)

The size of polygonal patterns of the eggs laid by tetraploid female adults and that of facets of the compound eye of tetraploid male adults, both obtained by super-cooling eggs at early developmental stage, were compared with those of the normal diploids. When the individuals which hatched from treated eggs became adults, in some of them the size of polygonal patterns in the lateral, central part of the laid eggs were extremely large as compared with that of the diploid adults. It was clarified that these adults were 4n from the egg-color segregation by mating and from the number of somatic chromosomes of embryo in laid eggs. In the same manner, the male adults in which the size of facets of the compound eye was extremely large as compared to adults of diploid male were ascertained to be 4n. These outstanding features of the size of polygonal patterns of facets could be utilized for the distinction of tetraploid, and distinction could readily and accurately be made as compared to the usual methods.

La taille des empreintes polygonales des oeufs pondus par les adultes femelles tétraploïdes et celle des facettes de l'oeil composé des adultes mâles tétraploïdes, obtenus tous les deux en réfrigérant les oeufs au début du développement, sont comparées avec celles des diploïdes normaux. Quand les individus provenant d'oeufs tétraploïdes deviennent adultes, ils pondent des oeufs et, chez certains d'entre eux, la taille des empreintes polygonales, dans les parties latérales et centrale de l'oeuf pondue, est extrêmement large par rapport à celle observée chez les oeufs des adultes diploïdes. On a établi que ces adultes ont quatre chromosomes grâce à la ségrégation de la couleur de l'oeuf par l'accouplement et par la mesure du nombre de chromosomes somatiques de l'embryon. De la même manière, on a établi que les mâles adultes chez lesquels la taille des facettes de l'oeil composé est extrêmement grande par rapport à celle des adultes diploïdes, sont de type 4n.

Ces caractères étonnants de la taille des empreintes polygonales et des facettes peuvent être utilisés pour distinguer les tétraploïdes, et cette distinction peut être faite rapidement et instantanément, contrairement aux méthodes habituelles.

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KAWAGUCHI Y., FUJII H., DOIRA H.

Changes in haemolymph protein during larval development of the dimolting mutant in *Bombyx mori*. (Variations des protéines de l'hémolymphe pendant le développement larvaire du mutant à deux mues chez *Bombyx mori*).

J. Sericult. Sci. Japan, 1983, 52(6), p. 517-523. (J,j,e)

Changes in haemolymph proteins during larval development of *Bombyx mori* were investigated electrophoretically and quantitatively. As materials, normal tetramolters and *mod* mutant segregated in a single batch of B₅F₁ generation of the cross *Ze. W; K+/- mod ♀ x + mod/+ mod ♂* were used. Larvae homozygous for *mod* involved three different molting types, dimolters (*mod II*), trimolters (*mod III*) and early-maturing trimolters (*mod III'*). Results in normal tetramolters showed the characteristic sequence of changes to each larval instar, i.e., in younger instars features of nutritional growth, and in the final instar features of reproductive growth as represented by the appearance of female-specific protein which took place prior to the apparent morphological changes in metamorphosis. Compared to this result, *mod II* showed omission of the fourth and the fifth instar of the tetramolters with a slight increase of female-specific protein. Early-maturing *mod III'* was shown to be a variation of *mod II* ecdysed supernumerary, whereas *mod III* showed omission of only the fourth instar.

Les changements intervenant dans les protéines de l'hémolymphe pendant le développement larvaire de *Bombyx mori* sont étudiés par électrophorèse et de manière quantitative. Les sujets employés sont des tétramues normaux et un mutant *mod* obtenu à partir d'une seule ponte de la génération B₅F₁ du croisement *Ze. W; K+/- mod ♀ x + mod/+ mod ♂*. Les larves, homozygotes pour le *mod*, sont de trois types différents pour la mue : les larves à deux mues (*mod II*), les larves à trois mues (*mod III*) et les larves à trois mues rapidement matures (*mod III'*). Les résultats obtenus chez les tétramoltins normaux montrent la séquence caractéristique des changements à chaque âge; c'est à dire, dans les premiers âges, les composantes de la croissance nutritionnelle, et dans les derniers âges, les composantes de la reproduction comme le montre l'apparition de la protéine spécifique à la femelle qui se produit avant les changements morphologiques apparents de la métamorphose. Comparativement à ce résultat, le *mod III* montre la suppression du 4^{ème} et du 5^{ème} âge des tétramoltins avec une légère augmentation de la protéine spécifique à la femelle. Le *mod III'* rapidement mature apparaît comme étant une variation du surnombre en ecdysis du *mod II*, tandis que le *mod III* montre la suppression du 4^{ème} âge seulement.

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KAWASAKI H., IWASHITA Y.

DNA synthesis and mitosis after pupation in the wing disc of the silkworm, *Bombyx mori*. (Synthèse d'ADN et mitose après la nymphose dans le disque alaire chez le ver à soie *Bombyx mori*.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 53-58. (J,j,e)

The differentiating wing discs during the early pupal stage were studied by means of histological and autoradiographic observations. The number of mitosis in the wing was the largest 28 hours after pupation. The mitosis of scale stem cell-1 lasted for 28 to 44 hours after pupation, and that of scale stem cell-2 was detected for 32 to 44 hours after pupation. Scale forming was observed 60 hours after pupation. The rate of ^3H -thymidine incorporation became lower in prepupae, but about 10% of the cells incorporated ^3H -thymidine during 1st and 2nd days after pupation. On the 3rd day ^3H -thymidine was specifically incorporated into scale forming cells. The present study suggests that scale stem cell-1 is formed after pupation, and then mitosis takes place, followed by scale forming and polyploidization of scale forming cells.

Les disques alaires en formation sont étudiés au début de la nymphose par observations histologiques et autoradiographiques. Le nombre de mitoses apparaissant dans l'aile est maximal 28 heures après la nymphose. La mitose de la cellule souche 1 du pédoncule de l'écaille dure de la 28ème heure à la 44ème heure après la nymphose, et celle de la cellule 2 est détectée de la 32ème à la 44ème heure après la nymphose. La formation des écailles est observée 60 heures après la nymphose. Le taux d'incorporation de thymidine ^3H décroît chez les pré-nymphes mais environ 10 % des cellules incorporent la thymidine ^3H pendant le 1er et 2ème jour après la nymphose. Le 3ème jour, la thymidine ^3H est incorporée de manière spécifique par les cellules formant l'écaille. La présente étude suggère que la cellule 1 du pédoncule de l'écaille se forme après la nymphose et qu'ensuite la mitose a lieu, suivie de la formation de l'écaille et de la polyploïdisation des cellules formant l'écaille.

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KAWASE S., BANOO H., CAI Y.M.

Purification of a densovirus from silkworm feces. (Isolement d'un virus de la densovirose à partir des excréments de ver à soie)

J. Sericult. Sci. Japan, 1983, 52(6), p. 547. (J) short report R

KEIL T.A.

Very tight contact of tormogen cell membrane and sensillum cuticle : ultrastructural basis for high electrical resistance between receptor lymph and subcuticular spaces in silkworm olfactory hairs. (Contact très étroit entre la membrane des cellules tormogènes et la cuticule du sensillum : bases ultrastructurales pour une résistance électrique très grande entre les récepteurs lymphatiques et les espaces subcuticulaires chez les poils olfactifs du papillon.)

Tissue & Cell, 1984, 16, p. 131.

KEWEI H. et al.

The relationship between the existence of the pathogen and the occurrence of cytoplasmic polyhedrosis of the silkworm, *Bombyx mori* L. (Relation entre l'existence de l'agent pathogène et l'apparition de la polyédrose cytoplasmique du ver à soie, *Bombyx mori* L.).

Canye Kexue, 1983, 9(4), p. 223. (C) R

KIM J.I., LEE J.Y., KIM C.S., PARK K.E.

Purification and biological activity of ecdysterone from Korean *Achyranthes radix*. (Purification et activité biologique d'une ecdystérone extraite de la racine de l'*Achyranthes* de Corée).

Ser. J. of Korea, 1983, 25(1), p. 1-20. (K,e) R

KURIHARA Y., WATANABE H.*, MAEDA S., SHIMIZU T.

Chemical characteristics of a previously undescribed densovirus isolated from the silkworm, *Bombyx mori*. (Caractères chimiques d'un nouveau virus de la denso-nucléose isolé à partir du ver à soie *Bombyx mori*.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 33-40. (E,e)

A small spherical virus of 23-24 nm in diameter which multiplied in the nuclei of columnar cells of the midgut epithelium was isolated from diseased larvae of the silkworm, *Bombyx mori*, and the properties of the viral proteins and nucleic acid were characterized. By sodium dodecyl sulphate-polyacrylamide gel electrophoresis, four structural proteins (P1, P2, P3, and P4) were detected and their molecular weights were estimated from relative mobilities as 121,000, 116,000, 53,000, and 50,000 daltons, respectively. Peptide mapping revealed that the P1 was similar to P2, and P3 to P4. The virus contained single stranded (ss) DNA. When the DNA was extracted in a low-salt buffer, it showed properties of a single-stranded molecule, while a double stranded (ds) molecule was extracted under a high-salt condition. The ss DNA molecule had a molecular weight of about 1.99×10^6 by agarose gel electrophoresis. These data suggested that the newly isolated virus belonged to the *Densovirus* group of Parvoviridae. The newly isolated virus differed in molecular weights of structural proteins and DNA from the previously described densovirus from *Bombyx mori* and the two viruses were not serologically related.

Un petit virus sphérique de 23 à 24 nm de diamètre qui se multiplie dans les noyaux des cellules en colonne de l'épithélium de l'intestin moyen a été isolé chez des larves malades du ver à soie *Bombyx mori* et les propriétés des protéines virales et de l'acide nucléique ont été déterminées. Par électrophorèse sur gel de polyacrylamide en présence de SDS, quatre protéines structurales (P1, P2, P3 et P4) sont détectées et leurs poids moléculaires sont évalués par leur mobilité relative à 121 000, 116 000, 53 000 et 50 000 daltons. La carte peptidique révèle que P1 est semblable à P2, et P3 à P4. Le virus contient un ADN monocaténaire. Lorsque l'ADN est extrait dans un tampon à faible concentration saline, il montre les propriétés d'une molécule monocaténaire alors qu'on extrait une molécule bicaténaire à forte concentration saline. La molécule d'ADN monocaténaire a un poids moléculaire d'environ $1,99 \times 10^6$ d'après l'électrophorèse sur gel d'agarose. Ces résultats suggèrent que ce virus récemment isolé appartient au groupe *Densovirus* des Parvoviridae. Ce virus diffère par les poids moléculaires des protéines structurales et par l'ADN du densovirus précédemment décrit chez *Bombyx mori* et les deux virus ne sont pas liés du point de vue sérologique.

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LECANIDOU R., EICKBUSH T.H., RODAKIS G.C., KAFATOS F.C.

Novel B family sequence from an early chorion cDNA library of *Bombyx mori*. (Nouvelle séquence de la famille B provenant d'une bibliothèque de cDNA de chorion jeune de *Bombyx mori*).

Proc. Natl. Acad. Sci. USA. 1983, 80, p. 1955-1959. (E,e)

A cDNA library has been constructed from transcripts present during the early stages of choriogenesis in the silkworm, *Bombyx mori*. Clones representing novel sequences were selected by screening the library with (i) homotypic cDNA, (ii) rDNA and typical chorion probes of the A and B multigene families and (iii) selected clones from the library, which were positive in step (i) but negative in step (ii); this third step identified clones that hybridized to distinct subsets of the library. The sequence of one such clone was determined and shown to represent a novel subfamily of the B chorion multigene family, exclusively expressed during the early choriogenic period.

Une "bibliothèque" de cDNA a été établie à partir des transcrits apparus pendant les premiers stades de la choriogénèse chez le ver à soie *Bombyx mori*. Les clones représentant les nouvelles séquences ont été sélectionnés par criblage de la "bibliothèque" avec (i) le cDNA homotypique, (ii) le DNA ribosomique et les sondes typiques au chorion des familles de multigènes A et B et (iii) les clones sélectionnés à partir de la "bibliothèque", qui sont positifs dans le cas (i) mais négatifs dans le cas (ii); ce troisième cas a permis d'identifier des clones qui s'hybrident avec deux sous-parties distinctes de la bibliothèque. La séquence d'un de ces clones a été déterminée et l'on montre qu'elle représente une nouvelle sous-famille de la famille de multigène B du chorion, exprimée exclusivement au début de la choriogénèse.

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MIKE A.*, OHWAKI M.*, FUKUDA T.*, MIYAJIMA S.**

Preparation of monoclonal antibodies to the *Bombyx mori* cytoplasmic polyhedrosis virus. (Préparation d'anticorps monoclonaux du virus de la polyédrose cytoplasmique de *Bombyx mori*).

J. Sericult. Sci. Japan. 1984. 53(1), p. 59-63. (J.j.e)

Monoclonal antibodies were produced to the *Bombyx mori* cytoplasmic polyhedrosis virus which forms polyhedra of tetragonal shape (CPV-T). Three hybridoma antibodies, termed S 11 (IgG1), S 21 (IgG1), S 91 (IgG2a), were reactive with two strains of CPV, CPV-T and CPV-II which forms polyhedra of hexagonal shape, but unreactive with *Bombyx mori* nuclear polyhedrosis virus (NPV) by indirect enzyme-linked immunoabsorbent assay (ELISA). Both S 11 and S 21 could neutralize CPV-T but not NPV.

Nous avons préparé des anticorps monoclonaux contre le virus de la polyédrose cytoplasmique du *Bombyx mori* qui forme des polyèdres tétraédriques (CPV-T). Trois anticorps d'hybridomes, appelés S 11 (IgG1), S 21 (IgG1), S 91 (IgG2a) réagissent avec deux souches de CPV, le CPV-T et le CPV-II qui forment des polyèdres hexagonaux mais ne sont pas réactifs avec le virus de la polyédrose nucléaire de *Bombyx mori* (NPV) lors du dosage par immunoabsorbant lié à l'enzyme (ELISA). Le S 11 et le S 21 peuvent tous les deux neutraliser le CPV-T mais pas le NPV.

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MING Z., TANG S.

Studies on the genetic materials from the posterior silk gland of *Bombyx mori*. (Etude du matériel génétique extrait de la partie postérieure de la glande séricigène de *Bombyx mori*.)

Yichuan, 1983, 5(6), p. 5-8. (C)

NAGASAWA H.

Isolation and characterization of prothoracicotropic hormone from silkworms *Bombyx mori*. (Caractère d'une hormone prothoracotrope isolée des vers à soie *Bombyx mori*).

Nippon Nogei Kagaku Kaishi, 1983, 57(10), p. 1049-1053. (J)

OHASHI M.¹, TSUSUE M.¹, YOSHITAKE N.², SAKATE S.³, KIGUCHI K.³

Epidermal pigments affecting the larval colouration of the silkworm, *Bombyx mori*. (Pigments épidermiques affectant la coloration de la larve du ver à soie *Bombyx mori*.)

J. Sericult. Sci. Japan, 1983, 52(6), p. 498-504. (E,e,j)

The larval epidermis of the silkworm, *Bombyx mori*, contains the pigments xanthommatin and sepialumazine. Their combined colours along with variations of larval markings give rise to race-specific larval colouration in the insect. In order to show the relationship between the visible colour and the actual pigment contents in the epidermis, these pigments were extracted from the larval integuments of more than 30 silkworm strains and assayed by use of high pressure liquid chromatography (HPLC). Large racial differences in the levels of integument xanthommatin and sepialumazine were found. In general, the pale white epidermis (most Chinese and some Japanese strains) contains only low levels of xanthommatin and sepialumazine. The pale yellow or faintly reddish epidermis (most European and some Japanese strains) have comparatively large amounts of xanthommatin and/or sepialumazine. An extremely high concentration of xanthommatin (1,000 µg/g fresh weight) was detected in the epidermis of a *quail* mutant whose larval epidermis is pink.

L'épiderme de la larve du ver à soie *Bombyx mori* contient la xanthommatine et la sépialumazine. Leurs couleurs associées ainsi que les variations des marquages larvaires donnent lieu à une coloration spécifique de la larve chez cet insecte. Afin de mettre en évidence la relation existant entre la couleur visible et la teneur réelle en pigment dans l'épiderme, ces pigments sont extraits des téguments larvaires de plus de 30 souches différentes de ver à soie et dosés en utilisant la chromatographie liquide à haute pression (HPLC). De grandes différences apparaissent entre les races dans le niveau en xanthommatine et sépialumazine du tégument. En général, l'épiderme d'un blanc pâle (principalement, les souches chinoises et japonaises) contient un faible niveau de xanthommatine et sépialumazine. Les épidermes jaune pâle ou légèrement rougeâtres (principalement les souches européennes et quelques souches japonaises) possèdent comparativement de grandes quantités de xanthommatine et/ou de sépialumazine. Une concentration extrêmement forte de xanthommatine (1000 µg/g de poids frais) a été détectée dans l'épiderme d'un mutant *caille* dont l'épiderme larval est rose.

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OLBERG R.M.

Interneurons sensitive to female pheromone in the dento cerebrum of the female silkworm moth, *Bombyx mori*. (Des interneurons sensibles à la phéromone femelle chez le dento cérébrum du papillon mâle de *Bombyx mori*).

Physiol. Entomol., 1983, 8(4), p. 419-428. (E)

OSANAI M., YONEZAWA Y.

Age-related changes in amino acid pool sizes in the adult silkworm, *Bombyx mori*, reared at low and high temperature; a biochemical examination of the rate-of-living theory and urea accumulation when reared at high temperature. (Variations de la taille des pools d'acides aminés en fonction de l'âge chez le papillon *Bombyx mori* élevé à basses et hautes températures ; examen biochimique de la théorie du taux de vie et de l'accumulation d'urée lors des élevages à hautes températures.)

Experimental Gerontology, 1984, 19, p. 37-51. (E,e)

To examine the rate-of-living theory, age-related changes in amino acid pool sizes were investigated in the adult silkworm, *Bombyx mori*, reared at low and high temperature. At either temperature concentrations of free amino acids contained in silkworms revealed a great sexual difference. Those in females were generally much higher than in males and the former changed much more dynamically than the latter. Major amino acids or ninhydrin-positive compounds inclusive of some essential amino acids such as Leu, Ile, Val, Thr, Arg, Phe, Met, Ala, Tyr, Gln, Aspn, Lan, Cysta, GABA and PEA accumulated in 40°C-moths. However, the levels of these amino acids in 35°C-moths were higher than those in normal 25°C-moths. Other amino acid levels changed irregularly with advanced age. Inhibition of protein synthesis may occur generally at low temperature, while protein degradation may be promoted at high temperature. Higher concentrations of MSO and Tau in the moths reared at high temperature than in the normal moths suggested also catabolism of amino acids proceeding together with protein degradation at high temperature. Amino acid metabolism seems to be complicated under various temperature conditions.

When reared at the optimal temperature of 25°C, urea is not present in the body of the silkworm except for a slight amount in the secreted meconium. In silkworms reared at the higher temperature of 35°C, however, an extraordinary accumulation of urea occurs accompanied by a reduction in lifespan by one half. Undoubtedly, urea is produced in this terrestrial insect, although the accumulation mechanism is not clear: in silkworms reared at various temperatures, arginase is found, but urease is not detected. Arginase activity was found to be higher in male moths than in female moths regardless of the rearing temperature. High temperature rearing also did not induce activity and female activity never exceeded that in males at either 25°C or 35°C rearing. Protein degradation accelerated by rearing at high temperatures may result in increased amounts of free arginine, which could cause the active production of urea. This possibility would be a counter-argument to the rate of living theory relating to longevity and temperature. However, at least the above facts signify that an extrinsic factor influences the longevity of an animal by altering its intrinsic aging process.

Pour étudier la théorie "du taux de vie", nous avons examiné chez le papillon adulte *Bombyx mori* élevé à température faible et élevée, les changements de taille des pools d'acides aminés en fonction de l'âge. Il existe une importante différence sexuelle dans la concentration des acides aminés libres chez le papillon quelle que soit la température. Les concentrations chez la femelle sont généralement beaucoup plus élevées que chez le mâle et les variations sont plus dynamiques chez la femelle que chez le mâle. Les acides aminés principaux ou les composés réagissant de manière positive à la ninhydrine comprenant certains acides aminés essentiels tels que Leu, Ile, Val, Thr, Arg, Phe, Met, Ala, Tyr, Gln, Aspn, Lan, Cysta, GABA et PEA, s'accumulent chez les papillons maintenus à une température de 40°C. Cependant, le taux d'acides aminés est plus élevé chez les papillons soumis à une température de 35°C que chez ceux soumis à une température de 25°C. Le taux d'autres acides aminés change d'une manière irrégulière selon l'âge. L'inhibition de la synthèse des protéines peut survenir généralement à basse température alors que la dégradation des protéines peut s'accroître à forte température. Les concentrations de MSO et Tau sont plus importantes chez les papillons soumis à une température élevée que chez les autres papil-

lons, ce qui implique également un catabolisme des acides aminés et une dégradation des protéines à haute température. Le métabolisme des acides aminés semble se compliquer selon les diverses conditions de température.

Lorsque les papillons sont soumis à la température optimale de 25°C, on ne trouve pas d'urée dans le corps du papillon sauf une infime quantité dans le méconium sécrété. Cependant, chez les papillons soumis à une température de 35°C, une accumulation extraordinaire d'urée se produit qui est accompagnée d'une réduction de moitié de la durée de vie. Sans aucun doute, cet insecte produit de l'urée, bien que le mécanisme d'accumulation ne soit pas clair : chez les papillons soumis aux diverses températures, on trouve de l'arginase mais pas d'uréase. L'activité de l'arginase est plus forte chez les papillons mâles que chez les papillons femelles quelle que soit la température. La température élevée n'induit pas une activité et l'activité femelle n'excède jamais celle du mâle, que l'élevage s'effectue à une température de 25°C ou de 35°C. La dégradation des protéines accélérée par l'élevage à une température élevée peut entraîner une augmentation de l'arginine libre, et ainsi provoquer une production active d'urée. Cette possibilité constituerait un argument à l'encontre de la théorie du taux de vie se rapportant à la longévité et la température. Cependant, les faits décrits ci-dessus signifient qu'un facteur extrinsèque a une influence sur la longévité d'un animal par l'altération de son processus intrinsèque de vieillissement.

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SASAKI T.

Amino acid sequence of a novel kunitz-type chymotrypsin inhibitor from haemolymph of silkworm larvae, *Bombyx mori*. (Séquence d'acides aminés d'un nouvel inhibiteur de chymotrypsine de type Kunitz contenu dans l'hémolymphe de ver à soie *Bombyx mori*).

Febs Letters, 1984, 168, p. 227.

SASAKI T., KOBAYASHI K.

Isolation of two novel proteinase inhibitors from haemolymph of silkworm larva, *Bombyx mori*, comparison with human serum proteinase inhibitors. (Isolement de deux nouveaux inhibiteurs de protéases à partir de l'hémolymphe de larves de ver à soie *Bombyx mori*. Comparaison avec les inhibiteurs de protéases sériques humains).

J. of Biochem., 1984, 95, p. 1009.

SEKI H.

The serological properties and infectivity of Yamanashi isolate of the silkworm denonucleosis virus. (Propriétés sérologiques et infectivité de l'isolat de Yamanashi du virus de la denonucleose du ver à soie.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 69-71. (J,j,e)

The Yamanashi isolate of the silkworm denonucleosis virus did not react with the antisera against the Ina isolate and the small flacherie virus in immunodiffusion tests. These viruses did not appear to share any antigen with the Yamanashi isolate. The virus did not infect larvae of N 124 but did infect larvae of seven other varieties. Accordingly, the Yamanashi isolate differed from the Ina isolate in the infectivity to silkworm varieties.

L'isolat de Yamanashi du virus de la denso-nucléose du ver à soie ne réagit pas aux antisérums employés pour l'isolat d'Ina et les petits virus de la flacherie, lors des tests d'immunodiffusion. Ces virus ne semblent posséder aucun antigène de l'isolat de Yamanashi. Le virus n'attaque pas les larves de la souche N 124 mais affecte les larves de sept autres souches. L'isolat de Yamanashi se distingue de l'isolat d'Ina par l'inféctivité des souches de ver à soie.

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SHIMIZU I., KATO M.

Carotenoid functions in photoperiodic induction in the silkworm *Bombyx mori*. (Fonctions des caroténoïdes dans l'induction photopériodique chez le ver à soie *Bombyx mori*).

Photobiochemistry & Photobiophysics, 1984, 7, p. 47.

SHUNLIN L.

Histochemical observations on the function of different portions and different developmental stages of midgut of the silkworm larvae *Bombyx mori* L. (Observations histochimiques des différentes portions de l'intestin du ver à soie *Bombyx mori* au cours des différents stades du développement larvaire).

Canye Kexue, 1983, 9(4), p. 227. (C) R

SUGITA H., HORIE Y.

Site of sugar absorption in the midgut of the silkworm, *Bombyx mori*. (Site d'absorption du sucre dans l'intestin du ver à soie *Bombyx mori*).

J. Sericult. Sci. Japan, 1983, 52(6), p. 545. (J) Short report R

TAKAHASHI S.Y.

Phosphorylation of vitellin of the silkworm, *Bombyx mori*: identification of vitellin phosphorylation and protein kinase in the ovary. (Phosphorylation de la vitelline du ver à soie *Bombyx mori* : identification de la phosphorylation de la vitelline et de la protéine kinase dans l'ovaire.)

International Journal of Invertebrate Reproduction, 1983, 6, p. 65-76. (E,e)

Silkworm vitellin was radioactively labelled both in vivo upon injection of [^{32} P]phosphate and in vitro upon incubation of tissue extracts with [γ - ^{32} P]ATP. Phosphorylation of vitellin was also demonstrated in the cultured ovary. [^{32} P]phosphate groups were added to serine residues of the peptide, indicating that silkworm vitellin is a phosphoprotein. SDS slab gel electrophoresis revealed three major labelled peptides in case of in vitro and in vivo labelling methods. Two of them were identified, with specific anti-vitellin serum, as vitellin subunits, whereas another labelled peptide has not yet been characterized. The latter protein was not found in the hemolymph and fat body, suggesting that it is specifically localized in the ovary but immunologically different from vitellin.

Protein kinase activities capable of phosphorylating vitellogenin and vitellin were identified in various tissues of the silkworm and the kinases from the ovary were partially characterized.

La vitelline du ver à soie est marquée à la radioactivité *in vivo* par injection de phosphate ^{32}P et *in vitro* par incubation des tissus en présence d'ATP γ - ^{32}P . La phosphorylation de la vitelline est également démontrée dans les ovaires en culture. Les groupes phosphate ^{32}P sont ajoutés aux sérines du peptide et montrent que la vitelline du ver à soie est une phosphoprotéine. L'électrophorèse sur gel de polyacrylamide en plaque en présence de SDS fait apparaître trois peptides radioactifs principaux après marquage *in vivo* et *in vitro*. Deux d'entre eux sont identifiés, au moyen d'un sérum anti-vitelline spécifique, comme des sous-unités de la vitelline, alors que l'autre peptide marqué n'a pas encore été caractérisé. Cette dernière protéine n'apparaît pas dans l'hémolymphe, ni dans le corps adipeux et ceci suggère qu'elle est localisée de manière spécifique dans l'ovaire mais différente de la vitelline du point de vue immunologique.

Les activités protéine kinase capables de phosphoryler la vitellogénine et la vitelline ont été identifiées dans divers tissus du ver à soie et les kinases de l'ovaire ont été en partie caractérisées.

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TOKUNAGA K., HIROSE S., SUZUKI Y.

In monkey COS cells only the TATA box and the cap site region are required for faithful and efficient initiation of the fibroin gene transcription. (Dans les cellules COS de singe, la TATA box et la région du site "cap" sont suffisantes pour le démarrage fidèle et efficace de la transcription du gène de la fibroïne).

Nucleic Acids Research, 1984, 12(3), p. 1543-1558. (E,e)

The DNA sequences necessary for faithful and efficient initiation of transcription of *Bombyx mori* fibroin gene have been studied *in vivo* using monkey COS cells and a SV40 origin vector. Transcriptional analysis of 5' deletion genes and of exact substitution genes including a series of single base substitution mutants indicates that the TATA box and the cap region are required for faithful and efficient initiation of the fibroin gene transcription *in vivo*, whereas far upstream sequences and the CAAT-like sequences are not required. In addition, single base substitution genes of the cap site region have clearly shown that there exists a strict sequence requirement of the cap region of the fibroin gene. These results define the basic promoter of the fibroin gene to the region spanning the TATA box and the cap site region.

Les séquences ADN nécessaires pour débiter de façon fidèle et efficace la transcription du gène de la fibroïne de *Bombyx mori* sont étudiées *in vivo* en utilisant des cellules COS de singe et un vecteur d'origine SV40. L'analyse transcriptionnelle de gènes délétés en 5' et de gènes de substitution exacte, y compris une série de mutants ayant une seule base substituée, indique que la TATA box et la région du "cap" sont nécessaires pour débiter fidèlement et efficacement la transcription *in vivo* du gène de la fibroïne, tandis que les séquences loin en amont et les séquences de type CAAT ne sont pas nécessaires. De plus, les gènes de substitution de base unique du site "cap" montrent clairement qu'il existe une nécessité stricte de séquence de la région "cap" du gène de la fibroïne. Ces résultats définissent le promoteur de base du gène de la fibroïne comme la région s'étendant de la TATA box au site "cap".

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TSUDA M., SUZUKI Y.

Transcription modulation *in vitro* of the fibroin gene exerted by a 200-base pair region upstream from the "TATA" box. (Modulation de transcription *in vitro* du gène de la fibroïne exercée par une région de 200 paires de bases se situant en amont de la "TATA box".)

Proc. Natl. Acad. Sci. USA, 1983, 80, p. 7442-7446. (E,e)

We have previously reported that the 5'-flanking sequence upstream from the "TATA" box modulates the faithful transcription initiation of the fibroin gene in a homologous whole cell extract prepared from the silk glands, whereas such a modulating effect is not observed in a HeLa cell extract. Subsequently, we have determined that major signals responsible for the modulating effect are located within a 200-base-pair region upstream from the TATA box, mainly in a distal region between nucleotide positions -238 and -116 in a proximal one between -73 and -53. Inversion of the sequence element -234 to -66 did not alter its modulating effect. A similar modulating effect by the upstream region of the sericin gene was also observed in the silk gland extract but not in the HeLa cell extract. In contrast, a modulating effect by the upstream region of the adenovirus 2 major late gene was observed in the HeLa cell extract but not in the silk gland extract. Thus, the modulating effect by the upstream regions of these genes is exerted only in their own homologous extracts.

Nous avons précédemment décrit que la séquence flanquante 5' en amont de la "TATA box" module le début de la transcription fidèle du gène de la fibroïne dans un extrait total de cellules homologues préparé à partir de glandes séricigènes, alors qu'un tel effet de modulation n'est pas observé dans un extrait de cellules HeLa. En conséquence, nous avons déterminé que les signaux majeurs responsables de l'effet modulateur sont situés à l'intérieur d'une région de 200 paires de bases située en amont de la TATA box, principalement dans une région distale entre les positions de nucléotides -238 et -116 et dans une région proximale entre -73 et -53. L'inversion de l'élément de la séquence -234 à -66 n'altère pas son effet modulateur. Un effet modulateur similaire de la région en amont du gène de la séricine est également observé dans l'extrait de glande séricigène, mais pas dans l'extrait de cellule HeLa. Au contraire, un effet modulateur de la région en amont du gène tardif majeur de l'adénovirus 2 est observé dans l'extrait de cellule HeLa mais pas dans l'extrait de la glande séricigène. Donc, l'effet modulateur des régions en amont de ces gènes est seulement exercé dans les extraits qui sont homologues.

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UCHIDA Y., KAWAMOTO F., HIMENO M., HAYASHIYA K.

A virus inactivating protein isolated from the digestive juice of the silkworm *Bombyx mori*. (Une protéine inactivant les virus, isolée à partir du suc intestinal du ver à soie *Bombyx mori* L.)

J. Invert. Pathol., 1984, 43, p. 182.

UTSUMI S.

Effect of food condition on the anti-*Streptococcus* activity in the digestive juice of the silkworm larva, *Bombyx mori*. (Effet de l'alimentation sur l'activité anti-*Streptococcus* dans le suc digestif de la larve du ver à soie *Bombyx mori*).

J. Sericult. Sci. Japan, 1983, 52(6), p. 537-544. (J,j,e)

To clarify the anti-bacterial action as a defense mechanism in the digestive juice of the silkworm, the properties of anti-*Streptococcus* protein (ASP) and the effect of dietary conditions on the anti-bacterial activity of ASP were investigated. ASP was found in the precipitates by ammonium sulfate at the concentrations of 0-30% and 30-70%, but not 70-100%. A single peak of ASP in both precipitates was obtained by Sephadex G-75 column chromatography, whereas two active peaks were obtained by DEAE-cellulose chromatography. ASP purified by Sephadex G-75 chromatography was highest in larvae reared on mulberry leaves in spring; in the 175-time dilution the sample showed the activity to inhibit bacterial growth completely. In larvae reared on the leaves in later autumn, the 59-time dilution was effective. However, in larvae, which were reared on artificial diet by the end of the 4th instar and no leaves thereafter, only 18-time dilution was effective. When reared on the leaves stored for a long period, no dilution was effective. Thus, the presence of two forms is at least suggested and their production is seemed to be controlled by food condition.

Pour déterminer l'action antibactérienne comme mécanisme de défense dans le suc digestif du ver à soie nous avons étudié les propriétés anti-*Streptococcus* (ASP) ainsi que les effets du régime alimentaire sur l'activité antibactérienne de l'ASP. L'ASP est présente dans les précipitats obtenus par le sulfate d'ammonium à des concentrations de 0-30% et 30-70% mais pas au delà de 70-100%. Un seul pic d'ASP dans les deux précipitats a été obtenu par chromatographie sur colonne de Sephadex G-75, tandis que deux pics actifs ont été obtenus par chromatographie sur DEAE-cellulose. L'ASP purifiée par chromatographie sur Sephadex G-75 est la plus abondante chez les larves de printemps élevées sur feuilles de mûrier. Dilué 175 fois, l'échantillon montre une activité d'inhibition complète de la croissance bactérienne. Chez les larves de fin d'automne élevées sur feuilles, la dilution de 59 fois est efficace. Cependant, chez les larves élevées exclusivement sur aliment artificiel à partir de la fin du 5ème âge, seule la dilution de 18 fois est efficace. Lorsque les larves sont élevées sur des feuilles de mûrier stockées pendant une longue période, aucune dilution n'est efficace. Ainsi, la présence de deux formes au moins est probable et leur production semble être contrôlée par les conditions d'alimentation.

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WASHIDA S.

Modification of the scotophase-induced larval moult of the silkworm, *Bombyx mori* by a juvenile hormone analogue. (Modification par un analogue de l'hormone juvénile de la mue larvaire du ver à soie *Bombyx mori* induite par l'obscurité).

J. Sericult. Sci. Japan, 1983, 52(6), p. 479-483. (E,e,j.)

Effect of a juvenile hormone analogue (JHA) on the larval apolysis and ecdysis induced by scotophase stimulus were investigated employing fourth instar larvae of the silkworm, *Bombyx mori*. When the lights were switched-off at 48 hr after the third ecdysis, JHA applied by 6 hr after the lights-off delayed the larval apolysis and ecdysis by about one day as compared with those in the larvae receiving no JHA treatments. This effect was not observed when JHA was applied at 12 hr after the lights-off. The critical period for JHA action which could disrupt the scotophase signals was determined to be up to 48 to 54 hr after the third ecdysis, irrespective of whether the lights-off was performed at 36, 48 or 60 hr after the ecdysis, suggesting that there were no direct relationship between scotophase signal and JHA.

L'effet d'un analogue de l'hormone juvénile (JHA) sur l'apolyse et l'ecdysis larvaires induites par l'obscurité est étudié sur des larves du ver à soie *Bombyx mori* au 4ème âge. Lorsque la lumière est éteinte 48 h après la 3ème mue, le JHA étant administré 6 h après l'extinction des lumières retarde l'apolyse et l'ecdysis lar-

vaires d'environ un jour par rapport aux larves non-traitées. Cet effet n'est pas observé lorsque le JHA est administré 12 h après l'extinction des lumières. La période critique pendant laquelle l'action du JHA pourrait briser les signaux de scotophase, se situe 48 h à 54 h après la 3ème mue, que l'on éteigne indifféremment la lumière à 36, 48 ou 60 h après l'ecdysis. Ceci suggère qu'il n'y a pas de relation directe entre le signal d'obscurité et l'hormone juvénile.

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WATANABE K., OHNISHI E.

The mode of ecdysteroid accumulation in ovaries of *Bombyx mori* during the pupal and pharate adult period. (Le mode d'accumulation des ecdystéroïdes dans les ovaires de *Bombyx mori* pendant la période nymphale et pré-imaginale.)

Zoological science, 1984, 1, p. 114-119. (E,e)

By combining liquid chromatography with radioimmunoassay, a procedure was developed to estimate the following ecdysteroids separately and simultaneously; ecdysone, 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone and 2,22-dideoxy-20-hydroxyecdysone. By use of this procedure, mode of accumulation of these ecdysteroids in ovaries of the silkworm, *Bombyx mori*, was studied during the pupal and pharate adult period. At the early stage, ecdysone and 20-hydroxyecdysone appeared, and then 3 ecdysteroids of 2-deoxy type accumulated. The results are discussed in relation to the possible origins of these ecdysteroids.

En combinant la chromatographie liquide avec les dosages radioimmunologiques, un procédé est mis au point pour évaluer séparément et simultanément les ecdystéroïdes suivants : ecdysone, 20-hydroxyecdysone, 2-déoxy-20-hydroxyecdysone, 2-déoxyecdysone et 2,22-didéoxy-20-hydroxyecdysone. En utilisant ce procédé, on a étudié le mode d'accumulation de ces ecdystéroïdes dans les ovaires du ver à soie *Bombyx mori* pendant la période nymphale et pré-imaginale. Au début de la période apparaissent l'ecdysone et le 20-hydroxyecdysone, et ensuite 3 ecdystéroïdes de type 2-déoxy s'accumulent. Les résultats sont discutés en faisant la relation avec les origines possibles de ces ecdystéroïdes.

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XIA R., TAO S., MA Y.

Study on the extraction of aminoacids from silkworm pupae. (Etude de l'extraction des acides aminés des chrysalides de ver à soie).

Zhongyao Tongbao, 1983, 8(5), p. 28-31. (C)

XIUYAN L. et al.

Studies on the mechanism of photoperiod on the silkworm (*Bombyx mori* L.). 1) Effect of the photoperiodism on the development and cocoon quality. (Etudes sur le mécanisme du photopériodisme chez le ver à soie (*Bombyx mori*). 1) Effet du photopériodisme sur le développement et la qualité des cocons).

Ganyu Kexue, 1983, 9(1), p. 17. (C) R

XIUYAN L. et al.

Silk increasing mechanism of silkworm by administering ^{15}N -urea. (Etude du mécanisme d'augmentation de soie chez le ver à soie par administration d'urée ^{15}N).

Ganyu Kexue, 1983, 9(4), p. 209. (C) R

XUEFANG H. et al.

Identification of the properties of silkworm flacherie virus, being collected in some provinces of China. (Propriétés du virus de la flacherie du ver à soie identifié dans diverses provinces de Chine).

Canye Kexue, 1983, 9(3), p. 156. (C) R

YAMAMOTO T., ENOKIJIMA M.

On the heritability of the number of eggs laid in F2 population of the silkworm, *Bombyx mori*. (De l'héritabilité du nombre d'oeufs pondus dans une population F2 de vers à soie *Bombyx mori*).

J. Sericult. Sci. Japan, 1984, 53(1), p. 85. (J) Short report. R

YAMAOKA R., TANIGUCHI Y., HAYASHIYA K.

Bombykol biosynthesis from deuterium labeled (Z)-11-hexadecenoic acid. (Synthèse de Bombykol à partir d'acide (Z)-11-hexadécénoïque marqué au deutérium).

Experientia, 1984, 40, p. 80.

YOULIANG W.

On the resistance of the silkworm *Bombyx mori* to nuclear polyhedrosis viruses. 1) on its relation with the developmental stage of the silkworm. (Sur la résistance du ver à soie *Bombyx mori* aux virus de la polyédrose nucléaire. 1) en fonction du stade de développement du ver à soie).

Canye Kexue, 1983, 9(1), p. 29. (C) R

YOULIANG W.

Studies on resistance of *Bombyx mori* larvae infected with nuclear polyhedrosis virus. 2) Relation to rearing temperature before and after infection. (Etudes de la résistance de la larve de *Bombyx mori* infectée avec le virus de la polyédrose nucléaire. 2) en fonction de la température d'élevage avant et après l'infection).

Canye Kexue, 1983, 9(2), p. 93. (C) R

YOULIANG W.

Studies on resistance of *Bombyx mori* larvae infected with nuclear polyhedrosis virus. 3) Its relationship to the proportion of mulberry leaf powder, vitamin-C and water in artificial diet. (Etudes de la résistance de la larve de *Bombyx mori* infectée avec le virus de la polyédrose nucléaire. 3) Corrélation avec la proportion de poudre de feuille de mûrier, de vitamine C et d'eau dans l'aliment artificiel.)

Canye Kexue, 1983, 9(3), p. 167. (C) R

YUXING W. et al.

The earlier diagnostic technique of enzyme linked counter immuno electrophoresis for denonucleosis virus of the silkworm, *Bombyx mori*. (Technique de diagnostic précoce de la denonucléose du ver à soie, *Bombyx mori*, par immunoelectrophorèse et enzyme fixée.)

Canye Kexue, 1983, 9(2), p. 96. (C) R

ZHANG XIAN-YANG, TUNG LIN, LIU FENG, DU DUANG-JIE.

Chromatin from the posterior silk glands of *Bombyx mori* and *Philosamia cynthia ricini* Purification and characterization. (Chromatine de la partie postérieure des glandes séricigènes de *Bombyx mori* et *Philosamia cynthia ricini*: Purification et caractérisation).

Acta Entomol. Sinica, 1983, 26(4), p. 359-367. (C,c,e)

The purification and characterization of chromatin from the posterior silk gland of two species of silkworm - *Bombyx mori* and *Philosamia cynthia ricini* were described. These were taken from the worms on the fourth day of the fifth larval instar. The results showed that the chemical composition and ultraviolet spectra of chromatins thus obtained were similar to those of chromatins from higher eucaryotes. The electrophoretic patterns of chromatin proteins including histones from the two species of silkworm were compared. The chromatin isolated could stimulate the *in vitro* incorporation of isotopically labelled ATP when exogenous *E.coli* RNA polymerase was added. The relationship between the incorporation of labelled ATP and the DNA content of chromatin and the influence on incorporation *in vitro* of such factors as available energy regeneration systems, Mn^{++} , rifamycin and others were studied. *In vitro* incorporation time curves for the two chromatins at 25°C and 37°C were compared.

Nous décrivons la purification et la caractérisation de la chromatine du sécréteur de 2 espèces de ver à soie *Bombyx mori* et *Philosamia cynthia ricini*. Les glandes sont extraites des vers à soie le 4ème jour du 5ème âge larvaire. Les résultats montrent que la composition chimique et les spectres ultraviolets des chromatines ainsi obtenues sont similaires à ceux des chromatines d'eucaryotes supérieurs. Les profils électrophorétiques des protéines de la chromatine y compris les histones des 2 espèces de ver à soie sont comparés. La chromatine isolée peut stimuler l'incorporation *in vitro* de l'ATP marqué de manière isotopique quand l'ARN polymérase de *E. coli* est ajoutée. La relation entre l'incorporation de l'ATP marquée et la quantité d'ADN de la chromatine, et l'influence de facteurs tels que les systèmes de régénération de l'énergie disponible, Mn^{++} , rifamycine et autres, sont étudiés. Les courbes cinétiques d'incorporation *in vitro* des 2 chromatines à 25°C et 37°C sont comparées.

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ZHEN-GANG L. et al.

An observation on chromosomes of wild silkworm, *Bombyx mandarina* L. (Une observation sur les chromosomes du ver à soie sauvage, *Bombyx mandarina* L.).

Canye Kexue, 1983, 9(1), p. 57. (C) Short report. R

ZHENKUI G. et al.

Studies on serological relationship of major structural components of the nuclear polyhedrosis virus of the silkworm, *Bombyx mori* L. (Etude sur les rapports sérologiques des composants structuraux majeurs du virus de la polyédrose nucléaire du ver à soie, *Bombyx mori* L.).

Canye Kexue, 1983, 9(3), p. 160. (C) R

ZIRAN H. et al.

Detecting the pebrine sporozoon, *Nosema bombycis*, in the silkworm *Bombyx mori*, by fluorescent antibody technique. (Recherche des zoospores de la pébrine, *Nosema bombycis*, chez le ver à soie, *Bombyx mori*, par la technique de l'immunofluorescence).

Ganye Kexue, 1983, 9(1), p. 59. (C) R

ZIRAN H. et al.

Analysis of juvenile hormones from silkworm, *Bombyx mori*, by high performance liquid chromatography. (Analyse de l'hormone juvénile du ver à soie, *Bombyx mori*, par chromatographie liquide à haute performance).

Ganye kezue, 1983, 9(4), p. 248. (C) R

ZUKANG Z.

How to determine trehalose's content and trehalase activity in the blood of the silkworm (*Bombyx mori* L.). (Moyen de doser le tréhalose et l'activité tréhalase dans l'hémolymph de ver à soie *Bombyx mori* L.).

Ganye Kexue, 1983, 9(3), p. 188. (C) R

SOIE / SILK

ARIMOTO H., XIANG X.H., SAKABE H.

Influence of maximum temperature to the knots and colour of raw silk on cocoon drying. (Influence de la température maximale sur les bouchons et la couleur de la soie grège lors du séchage des cocons.)

J. Ser. Sci. Jpn., 1983, 52(6), p. 524. (J,j) R

CHOI J.S.

A discussion on the size deviation test of raw silk. (Discussion sur le test de déviation du titre de la soie grège.)

Ser. J. of Korea, 1983, 25(1), p. 44. (K,e) R

DOHMYO M.*, SHIMIZU Y.*, KIMURA M.**

Reaction mechanism of sulfatoethylsulfon dye with silk. (Mécanisme de la réaction du colorant sulfatoéthylsulfone avec la soie.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 64-68. (E,e)

In order to elucidate the reaction mechanism between sulfatoethyl-sulfon dye and silk, the reactions of sulfatoethylsulfon dye (Ester) and Vinylsulfon dye (Vinyl) of C.I. Reactive Blue 19 with silk and water were examined. At pH 5 Vinyl reacted with silk more strongly than Ester, and at pH 7 both dyes manifested the same reaction. At pH 9 the amount of Ester reacted with silk was approximately equal to that of Vinyl for the first two hours, and Ester reacted somewhat more strongly than Vinyl, thereafter. The rate of the reaction of Ester with water was almost the same as that of Vinyl. Also, Ester was converted completely to other components (that is, to Vinyl, Hydroxyl and Ether) during the short time in alkaline solution. From these results it was concluded that Ester was decomposed to Vinyl in the dyeing process, which was then reacted with silk by means of the nucleophilic addition.

Afin de déterminer le mécanisme de la réaction entre le colorant sulfatoéthyl-sulfone et la soie, nous avons étudié les réactions du colorant sulfatoéthyl sulfone (Ester) et du colorant Vinylsulfone (Vinyl) du Bleu C.I. 19 avec la soie et l'eau. A pH 5, le vinyl réagit plus fortement que l'ester avec la soie, et à pH 7 les deux colorants montrent la même réaction. A pH 9 la quantité d'ester réagissant avec la soie est à peu près égale à celle du vinyl pendant les deux premières heures, après quoi l'ester réagit plus fortement que le vinyl. La vitesse de réaction de l'ester avec l'eau est à peu près la même que celle du vinyl. De plus, l'ester se transforme en d'autres composants (c'est à dire, en vinyl, hydroxyl et éther) pendant la courte période passée en solution alcaline. Ces résultats nous amènent à conclure que l'ester se décompose en vinyl pendant le processus de teinture, lequel ensuite réagit avec la soie par addition nucléophile.

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IMAOKA H., SHIBUYA A., KAMIISHI Y.

Automatic data processing to lead the mechanical properties of fabrics. (Données concernant le procédé automatique de détermination des propriétés mécaniques des tissus.)

Bull. of Res. Inst. for Polymers and Textiles, 1984, 3(140), p. 49. (J,e) R

KATSUNO M., YAMADA A.

On the solubility of sericin at high temperature and a control with water qualities. (Solubilité de la sérécine à haute température, influence de la qualité de l'eau.)

Rep. of the Silk Sci. Res. Inst., 1983, 31, p. 41. (J) R

KUWAHARA A., YANAGIMORI H.

Effect of lubricating treatment on the raw silk of various sericin content. (Effet du traitement lubrifiant sur de la soie grège à teneur variable en sérécine.)

J. Seric. Sci. Jpn., 1984, 53(1), p. 48-52. (J,j,e)

The reduction of frictional resistance of the raw silk of various sericin content after lubricating treatment was investigated. Six kinds of raw silks were treated with different lubricating agents of various concentrations. The static frictional coefficient of the raw silk was decreased by the water or ethyl alcohol treatment. However, after the lubricating treatment the degree of the decrease was radical by 0.5% lauric acid and 0.25% lauramide treatments. The tenacity was increased by the low concentration treatment, and after the abrasion tests the treated raw silk was scarcely damaged. The elongation was decreased by the lubricating treatment. After the abrasion tests, however, the treated raw silk showed higher elongation than the untreated raw silk. These tendencies were observed in the six kinds of the raw silks tested. The raw silk treated with low concentration lubricating treatment (lauric acid, lauramide) was scarcely abraded by the friction.

Nous avons étudié la diminution de la résistance aux frictions de la soie grège à teneur variable en sérécine après un traitement lubrifiant. Six sortes de soies grèges sont traitées avec différents agents lubrifiants à des concentrations diverses. Le coefficient de friction statique de la soie grège diminue avec le traitement à l'eau ou à l'alcool éthylique. Cependant, après le traitement lubrifiant, l'ampleur de la diminution est accrue par le traitement à 0,5 % d'acide laurique et 0,25 % de lauramide. La ténacité est augmentée par le traitement à faible concentration, et après les tests d'abrasion la soie grège traitée est rarement endommagée. L'élongation est réduite par le traitement lubrifiant. Cependant, après les tests d'abrasion, la soie grège traitée montre une élongation plus accentuée que la soie grège non-traitée. Ces tendances se retrouvent chez les six sortes de soies testées. La soie grège soumise au traitement lubrifiant à faible concentration (acide laurique, lauramide) est rarement endommagée par les frictions.

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MAGOSHI Y.

Structure of composite silk fabric and distribution of transmitted light. (Structure du tissu de soie composite et distribution de la lumière transmise.)

J. Sericult. Sci. Japan, 1984, 53(1), p. 7-13. (J,j,e)

Various types of composite silk fabrics having the same size and number of warps and filling yarns per cm were produced from four kinds of warps and two kinds of fillings using the same weaving machine. The composite silk fabrics from wild silk yarn showed different structure from other fabrics tested, even though they had the same two-dimensional lattice and the same weight per area. This was attributable to the difference in thickness and density among these fabrics. The thickness of these composite silk fabrics varies with the apparent density when the weight per area was held to be constant. The optical density was proportional to the thickness of fabrics for the same two-dimensional lattice. Points of filament yarns were not located on the same line in the graph as those of spun yarns. From the optical densities of the threads, opening parts of the composite silk fabrics were different from each other in form and size. Information about structure of silk fabrics was obtained from the optical densities of the threads. Therefore, the optical densities of the threads were used instead of form function of threads. It was assumed that the form function of threads is applied to the structure equation of silk and other fabrics.

Divers types de tissus de soie composite ayant des fils de chaîne et des fils de trame de même taille et en nombre égal par cm, sont fabriqués à partir de 4 sortes de chaînes et de 2 sortes de trame, en utilisant le même métier à tisser. Les tissus de soie composée provenant de soie sauvage montrent une structure différente des autres tissus testés, bien qu'il aient le même tissage et le même poids par unité de surface. Ceci provient de la différence d'épaisseur et de densité entre ces tissus. L'épaisseur de ces tissus de soie composite varie de manière inverse à la densité apparente lorsque le poids par unité de surface reste constant. La densité optique est proportionnelle à l'épaisseur des tissus pour le même tissé en 2 dimensions. Les points des fils dévidés ne sont pas situés, sur le graphique, sur la même ligne que ceux des brins filés. Pour les densités optiques des fils, les parties ouvertes des tissus de soie composée sont différentes en forme et en taille. Des renseignements sur la structure des tissus de soie sont obtenus par les densités optiques des fils. Les densités optiques des fils ont donc été utilisées plutôt que la fonction forme des fils. On estime que la fonction forme des fils s'applique à l'équation de la structure de la soie et d'autres tissus.

Sericultural Experiment Station, Yatabe, Ibaraki 305, Japan.

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