GENERAL INTRODUCTION
Among the silk producing moths in India, the eri silkworm *Philosamia ricini* is considered to be the only domesticated non-mulberry variety (Sarkar D.C., 1980). Eri silk moth *Philosamia ricini* (Hutt) belongs to the class insecta and order Lepidoptera. *Philosamia ricini* is one of the most important silk producing moths of India. Sericulture is most possibly of indigenous origin. Assam—being the natural homeland of eri silk moth. Looking like cotton, soft as silk and having the texture of wool, eri silk is known as poor man's silk and is most popular in Assam. During the British period, Britishers called this silk as "Palm Christi Silk". Sericulture in India is progressing during recent years. Efforts have been made to increase the silk production either by evolving new viable and hardier silkworm races by hybridization of different breeds or by testing them with artificial growth factors, promoters and chemicals during the larval growth period. It is no doubt that sericulture is being done in several regions of India today, but it is only based on certain traditional methods and no serious thought was given until recently. Sericulture can best be taken as a secondary occupation along with the Castor (*Ricinus communis*) plantation or tapioca (*Manihot utilissime*) cultivation with proper leaf harvestation schedules, where the farmer gets eri silk in addition to oil seeds or the edible tubers. In Japan, efforts have been made to increase the silk cocoon crop of *Bombyx mori* yield by applying insect juvenile hormones and their bioanalogues (Akai and Kobayashi, 1971; Akai, 1979). Many vertebrate hormones have been found to improve the silk yield and fecundity (Mujumdar and Meddha, 1975, Bhaskar *et al*., 1983). In addition to these, many plant hormones showed certain effects on the
cocoon weight of the *Bombyx mori* (Kamada and Ito, 1984). It has been reported that B-carotene and -tocophenol are effective in increasing the number of eggs laid by *Bombyx mori* moths and also showed B-carotene has growth promoting effects. It was also found that mulberry leaves possess chlorogenic acid which possesses growth promoting effect (Yamada H. and Kato M., 1966).

Dietary supplementation of certain minerals like cobalt chloride seems to increase the larval weight, cocoon shell weight and fecundity in *Bombyx mori* (Bhattacharyya and Medda, 1981). Takahashi (1955) showed that the metal cobalt exerts favourable effects on the growth of *Bombyx mori* and it was also reported to have significant impact on the larval duration. Supplementation of potassium iodide seems to have exerted—a beneficial effects in polyvoltine silkworm *Bombyx mori* (Chakraborti and Medda, 1978; Mujumdar, 1982).

Karnataka State contributes 60 per cent in the total silk production in India. Pure Mysore race and their hybrids of *Bombyx mori* were reared for the silk production in Karnataka. There is a need for higher amount of silk production in India. Hence, in the present study an attempt has been made to study some aspects of nutritional physiology of *Eri* silkworm *Philosamia ricini* and thereby improve the *Eri* silk production by adding various chemical nutrients and components to the growing *Eri* silkworm.

In addition to this, the variation in the climatic factors like
temperature, humidity and light have been taken into consideration to study the physiological changes in the eri silkworm *Philosamia ricini*.

Sengupta and Singh (1974) reared *Philosamia ricini* on all the eight food plants and found the castor most successful host plant. An attempt has been made to see whether the foliage of tapioca might be useful for the rearing of eri silkworm (in terms of metabolites during development).

Under the nutritional studies our principal object is to study the effect of various minerals, hormones, vitamins, dried live saccharomyces (Lavie et al.) and hitherto unutilized solvents for the silk growth promoters.

The next part deals with the action of certain insecticides. As most insecticides are harmful to the crops and foliage plants, the use of insecticide is of great importance. The results revealed that the organophosphorous compound monocrotophos is much more toxic to eri silkworm than benzenehexachloride, the chlorinated hydrocarbon group.

Fat body being an important organ for the eri silkworm. The study of certain metabolites as well as their localization is of great importance.

In the next part, we have studied the effect of corpora cardiaca, corpora allata and brain extract on the lipid release and our
experiments showed that the endocrine organs have control over the lipid release in insects. Hence we thought, therefore, that it would be rewarding to investigate some aspects of physiology of eri silkworm Philosamia ricini.

The facets of the thesis are outlined below.

PART I

Comparative and seasonal studies on eri silkworm Philosamia ricini.

Section A

Comparative studies on feeding castor (Ricinus communis) and tapioca foliage (Manihot utilissime) on growth, development, changes in fuel reserves and cocoon weight of the eri silkworm Philosamia ricini.

Section B

Seasonal variation on the growth, development metabolic changes and cocoon formation in the eri silkworm Philosamia ricini.

PART II

Studies on the nutrition of the eri silkworm Philosamia ricini.

Section I

Effect of minerals on the growth, development and metabolic profile of the eri silkworm Philosamia ricini.
Section II

Effect of fat soluble vitamins and naturally occurring yeast (Laviest) on the growth, development and metabolic flux of the eri silkworm Philosamia ricini.

Chapter A

Use of fat soluble vitamins A & E on the growth, development and changes in the fuel reserves of the eri silkworm Philosamia ricini.

Chapter B

Effect of feeding dried live saccharomyces (Laviest) on the growth, development and cocoon crop of the eri silkworm Philosamia ricini.

Section III

Effect of feeding hormones on the growth and changes in the fuel reserves during the development of eri silkworm Philosamia ricini.

Section IV

Use of solvents as growth promoters during growth, development and its effects on fuel reserves of the eri silkworm Philosamia ricini.

PART III

Insecticide action on eri silkworm Philosamia ricini.

Section A

Effect of feeding insecticide malathion on the growth,
development and fuel reserves of the eri silkworm *Philosamia ricini*.

Section B

Effect of topical application of two insecticides Monocrotophos and Benzenehexachloride on the fuel reserves of the V instar fat body and haemolymph trehalose content in *Philosamia ricini*.

PART IV

Some histochemical studies on the fat body and musculature of the eri silkworm *Philosamia ricini*.

PART V

The role of endocrine secretions on the lipid release in the eri silkworm *Philosamia ricini*.
Eri silkworm _Philosamia ricini_ mainly feeds on the castor plant leaves, _Ricinus communis_. This species of silkworm can easily be reared in the laboratory. The rearing house of eri silkworm should be well ventilated and free from dust. The optimum temperature and humidity required are 26°C and 85 to 90 per cent respectively. The eggs of eri silkworm _Philosamia ricini_ were obtained from Sericulture Department, University of Agricultural Science, Dharwad. The eggs are ovoid, candid white, measuring 1.5 x 1.0 mm. The pattern of follicular imprints are very distinct from that of other species. The eggs were incubated in a B.O.D. incubator maintained at 26°C and 85 per cent humidity. The colour of the egg changes from white to blue on the last day prior to hatching. Hatching of the eggs normally takes place in the morning, between 7 a.m. to 9 a.m. The newly hatched larvae are yellow in colour with black segments and short setae distributed all over the body. The fully grown larvae are cream coloured.

The newly hatched larvae were fed with tender castor leaves. Feeding the young worms with the leaves chopped into bits of about 1 to 2 cm is ideal and gives the best results. The castor leaves should be washed thoroughly and dried with cloth prior to the feeding. During the first, second and third instars the larvae were fed four to five times a day while the fourth and fifth instar stages were fed six times a day at an interval of four hours starting from 6 a.m. in the morning. Every time fresh clean leaves were provided.
LIFE CYCLE

The life cycle of the eri silkworm Philosamia ricini consists of four stages, egg, larva, pupa encased in a cocoon and adult moth. A complete life cycle lasts about 44 days.

The freshly laid eri eggs are slightly white in colour. As the embryo develops inside the egg, the colour of the shell changes from whitish to yellowish, yellowish to ashy and ashy to blackish just before hatching. Then, a tiny worm comes out, leaving the shell once again, all white and empty. The minimum number of days from the date of oviposition to the date of hatching is about eight while the maximum may be 20 days or more. The difference is due to the degree of environmental temperature. During the entire larval period, the worms moult four times and after each moult the larva increase in size. When the moult, they stop eating, becomes motionless raises its head slightly and the body contracts. The larvae remains in this condition for about 24 to 48 hours. Towards the end of the mouling period the head and fore parts of the body are gradually lowered, and finally, the worm begins to stretch its body in an effort to break the old skin at the head. By continued undulatory movements and wriggling, the body completely breaks open the old skin and becomes free from it. The duration of the first and second instar larval period is four days each while the third instar lasts about three days and the fourth instar lasts five days and the fifth instar lasts six days.

After the completion of the fifth instar the larvae undergoes
pupation by spinning the pupal case or cocoon around its body. The pupal period lasts for about 16 to 17 days. Complete metamorphosis occurs within the cocoon and then the adult moth emerges. The male moth emerges usually earlier than female moths. The adult moths do not feed.

The adult moths, soon after emergence move towards the raised border of the tray and rest for two to three hours in vertical position till their wings are dried. The males start fluttering their wings and move in search of female moths. Then the mating begins. In an undisturbed condition the mating continues for about twelve to fourteen hours and then they detach from each other. The male, in its life span of four days, normally mates two to three times. The life span of females is five to seven days. The females start laying eggs in clusters and the oviposition continues for two to three days. The females usually lay the eggs during night time, sometimes they lay eggs during day time also. Each female moth lays about 350 to 500 eggs.

Thus the eri silkmoth *Philosamia ricini* reared in the laboratory, were used in the present investigation to study various aspects of physiology.
**MATERIAL AND METHODS**

**Animals:**

The specimens of the eri silkworm used in the present experiments were reared in our laboratory. The same specimens were used throughout this investigation. Care was taken to ensure that the same size (by weight) animals were used.

**Preparation of Tissues:**

Fat body and total carcass from the animals were collected from freshly dissected silkworms. Tissue was used almost immediately for analysis. The fat body samples were pooled from 2-3 silkworms.

The testis and ovary from the V instar larvae dissected and subjected for the determination of cholesterol.

**Collection of haemolymph:**

Haemolymph was collected from eri silkworm *Philosamia ricini* in a pre-cooled graduated centrifuge tubes. The blood collected from 2-3 silkworms was used almost immediately for analysis.

**Determination of glycogen:**

Anthrone method of Sciefter et al. (1950) was followed for the determination of glycogen content. A known quantity of tissue was digested with 2 ml 20% KOH. The glycogen was then precipitated by adding equal volume of 95 per cent ethanol to digest at room temperature.
STANDARD CURVE FOR GLYCOGEN

Optical density

Microgram glucose
overnight. The content was then centrifuged and the supernatant poured off. The washed residue was dissolved in a known volume of distilled water. A known aliquot in triplicate was analysed for glycogen content by the anthrone method. The intensity of the colour was read on "Spectronic 20" colorimeter at 620 nm. Glucose was used as reference standard. 1.11 was used as conversion factor for glycogen.

**Extraction and estimation of lipids:**

The lipid was estimated according to the method of Folch et al. (1957) using chloroform: Methanol mixture, (2:1 v/v). First of all the tissue was homogenized with appropriate volume of chloroform: methanol mixture (1:0). The homogenate was then quantitatively transferred to a 50 ml separatory funnel and then added another volume of chloroform. The two solvents were partitioned by the addition of 0.2 volume of water. After the funnel was shaken, the mixture was allowed to stand overnight; the lower chloroform layer containing lipid was drawn off, the solvent was removed completely under nitrogen atmosphere. The lipid sample was kept in a vacuum desiccator until constant weight was obtained. The lipid was estimated gravimetrically in triplicate.

**Separation and analysis of neutral and phospholipids:**

A known amount of lipid (usually 15 to 20 mg) from each tissue was subjected to silicic acid 100-200 mesh (Uricil, Mallinckrodt) column chromatography for their neutral and phospholipid separation. The neutral lipid along with free fatty acid (FFA) and phospholipid were respectively eluted with 150 ml of chloroform and 100 ml methanol.
Protein determination:

The protein content of the tissue was determined according to the method of Lowry et al. (1951). The protein content of the tissue was precipitated with 0.5 ml of 30% trichloro acetic acid (TCA) and then centrifuged for 30 minutes. The TCA was decanted and the precipitate was dissolved in 1.0 ml of 0.1 N NaOH. A known aliquot of this solution was then mixed with 4 ml of 2% sodium carbonate prepared in 0.1 N NaOH containing sodium potassium tartrate and 1% copper sulphate. After 10 minutes 0.5 ml of Folin c iocalteau's reagent was added. The contents were mixed properly on a Vortex mixture. The tubes were kept aside for 20 minutes until the colour develops and the readings were taken at 650 nm. Bovine serum albumin (BSA) fatty acid free was used as the reference standard.

Estimation of cholesterol:

For the estimation of total cholesterol from various tissues, the method of Harrow et al. (1960) was followed. The known weight of tissues were homogenized in absolute alcohol and solvent ether mixture (1:1 v/v). After centrifugation the supernatant was totally evaporated keeping the tubes in hot water bath. Liberman Buchard's reagent was prepared by adding 1 ml of concentrated sulphuric acid to 20 ml of acetic anhydride at 0°C. Chloroform 1 ml + L.B. reagent 4 ml was added in dark for 20-25 minutes. The optical density of light green colour developed to the solution due to the presence of cholesterol content, was measured in spectrophotometer at 625 nm using red filter. The readings for standard graph were taken in a similar manner adding known
STANDARD CURVE FOR PROTEIN

OPTICAL DENSITY

Microgram Protein
STANDARD CURVE FOR CHOLESTEROL

Optical density

$\mu$ Moles of Cholesterol
quantity of cholesterol solution. Total cholesterol content was calculated and presented as µg/100 mg wet weight of the tissue.

Trehalose estimation:

Trehalose was estimated according to the method of Roe (1955). Known quantity of haemolymph was collected in a stoppered tube containing 0.5 ml of 20 per cent NaOH. After shaking, the tubes were kept in boiling water for 10 minutes. Then the tubes were cooled in ice box. 5 ml of anthrone reagent (0.05% anthrone and 70% H₂SO₄) was added and then the tubes were kept again in a boiling water for 15 min for the colour development. Then the tubes were cooled to room temperature. The intensity of the colour was read on "spectronic 20" colorimeter at 620 nM. Crystalline trehalose (Sigma U.S.A.) was used as reference standard.