STUDIES ON THE QUALITY OF SILK AFFECTED BY SILK WORM *(BOMBYX MORI LINN.)* URINATION

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By

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CERTIFICATE

This is to certify that the thesis entitled "Studies on the quality of silk affected by silk worm (Bombyx mori Linn.) urination, submitted by Mr. K. MUDVEERAPPA, for the degree of MASTER OF SCIENCE (Agriculture) in SOIL SCIENCE, of the University of Agricultural Sciences, Bangalore, is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Enchanting and exuberating in appeal silk has equally a romantic history. Although references in ancient Sanskrit literature point out that India cultivated some kind of silk, it is to China the world owes discovery of mulberry silk.

In India silk culture dates to antiquity. Mulberry culture spread to India by about 140 B.C. from China through Khotan. The cultivation of silk first began in the areas flanking the rivers Brahmaputra and Ganges.

Sericulture being one of the important industries provides occupation to about five million people in rural areas. In certain parts of India, silk industry plays a minor yet a remarkable role in serving the rural economy by providing subsidiary occupation. With the merging of Kollegal taluk in Mysore in 1956, the state is now the biggest polyvoltine mulberry silk producer in India. The silk industry in the State is concentrated in the districts of Bangalore, Kolar, Mandya, Mysore and Tumkur and to a small extent in Belgaum, Bellary and Coorg. About three million people are deriving their livelihood from this natural fibre industry.

Silk is a very fine strand of fibre. It is a solidified protein secretion produced by certain caterpillars to encase themselves in the form of cocoons. Japan was the first country to use scientific methods in cultivating the silk worm. Japan has therefore, always ranked highest in the production of fine silk, although good quality silks are produced in India. In recent past Japan's silk is declining in world market and at the same time Indian silk is gaining considerable lead. Hence, it is highly necessary to give paramount attention to improve the quality. Experiments have shown that the cocoons of Bombyx mori Linn. produce the finest quality silk. About 75 per cent of the mulberry silk produced in India is from Mysore State. Also, Mysore State has its distinct name for its quality silk.

When farmers started rearing silk worms, many diseased worms and defective cocoons resulted, thereby affecting the grade of finished silk goods. However, application of science helped to find solutions to some problems such as the diseases of the silk worms and formation of defective cocoons facing the sericulture industry. Among the various problems associated with silk worm rearing, urination by matured silk worms is an important one for which satisfactory

solution is not yet known.

During inclement weather periods, especially during the cyclonic storms occurring continuously for three to seven days, the urination or liquid excretion by silk worms takes place. Staining of cocoons on account of excess urination by mature and spinning silk worms, Bombyx mori Linn. in mountages is a major problem particularly during monsoon months and in humid weather. Breakage of filaments while reeling in case of silk worm urine stained cocoons are more and the market rate of such cocoons is considerably low.

In view of the above problem of silk industry, an attempt was made to investigate the ways and means of reducing the losses due to urination. The findings of the investigation are presented in this thesis.

The main objectives of the study were:

i) the conditions which lead to urination in silk worm

ii) to study the technological aspects of minimising the extent of silk damage due to urination by suitable mounting

and reeling methods and

(iii) to study the methods for the removal of urine stains on the cocoons.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

There is not much literature available on this subject owing to the peculiarity of the problem. This problem has gathered attention of the scientists in India very recently, whereas some attempts have already been made in different parts of the world especially in Japan. Because of this reason most of the literature reviewed here are from the foreign authors and we find very few Indian authors contributed on this subject. Research work carried on the composition and effect of silk worm urine on reelability of silk worm cocoons is reviewed. Methods of controlling urination of silk worms, and removal of urine stain are also discussed.

1. Urination of silk worm (Bombyx mori Linn.): Reelability and quality of urine stained cocoons is considerably poor. This problem arises as the silk worms urinate during spinning stage. It is therefore necessary to review the environmental, physiological and technological conditions under which the urination of silk worms can be regulated.

The main function of the malpighian vessels in insects,

according to Wigglesworth (1931) is to eliminate free uric acid with a minimum loss of water, while Shimizu (1934) showed that the contents of the malpighian vessels of the silk worm larva give negative test with the Folin's uric acid reagent in any larval stage except larva which is already matured to become pre-pupa.

Kuwana (1937) found 0.01 to 0.15 mg of uric acid excreted per hour from the malpighian vessels of silk worm larva, though the quantities excreted varied according to the development of larva. He observed that four day larva of the fifth stage excreted 2.4 mg of uric acid per day. Hiratsuka (1967) confirmed that uric acid was excreted by the silk worm larva as in other insects, and it was 2.4 mg a day in the four day larva of the fifth stage.

Bankowska and Kalyniak (1968) noted an increase in the number of urine stained cocoons when the larvae of Bombyx mori Linn. were fed with frozen leaves of mulberry. They also observed that larva took more time to complete spinning, when they were fed with frozen leaves. They also noted that yellow staining of cocoon was influenced by high humidity. Kumararaj (1969) attributed high humidity coupled with moderate to some what cool temperature to

enhanced urination of silk worm Bombyx mori Linn. He further noticed that excess urination by matured and spinning silk worms in mountages was a major problem in some parts of Mysore State, particularly during monsoon months and during humid weather. Heavy urination occurred when the relative humidity was above 80 per cent and temperature ranged between 24° C to 26°C.

Madhava Rao (1969) stated that some times during rainy season the cocoons affected by silk worm urine are deeply stained. He further stated that such stained cocoons have poor reelability and make reeling uneconomical.

2. Influence of hormone on urine secretion: Many authors have revealed the fact that uric acid is secreted in silk worm as in any other insects. It is clearly known from the literature that certain hormones influence the secretion of urine by stimulating the malpighian tubules. The work done in this aspect by different authors is revieved in the following paragraphs:

Nadrell (1963) demonstrated that the diuresis in insects was caused by a hormone released into the haemolymph. According to him diuresis is promoted by some substance, presumably a neurohormone which can be extracted from the posterior neurosecretory cells.

Madrell (1964a) stated that the profuse diuresis in fifth stage larvae was caused by the release of a diuretic hormone into the haemolymph from the fused ganglionic mass, probably from hormone rich bodies assumed to be neurosecretory cells. He investigated the control of the release of the hormone.

Madrell (1964b) also noted that excretion process was very sensitive to changes in temperature; both the rate of excretion and the composition of the urine were affected.

Mills (1967) found that diuretic hormone was liberated into the blood by the terminal abdominal ganglion and promoted fluid secretion by the terminal abdominal ganglion. The hormone is known to promote fluid secretion by the malpighian tubules in insects. He also noted that temperature had profound effect on diuresis.

Madrell (1969) stated that 5 hydroxy tryptamine has stimulatory effect on secretion of malpighian tubules of insects.

3. Properties of silk: Urine staining of silk produces certain changes in the properties of silk. Knowledge regarding the various properties of silk and silk constituents

is necessary. A review covering varied aspects of properties of silk is therefore presented below.

Rastelli (1934) stated that the pH of the Fibroin in the silk cocoon is 7.2. According to him the quoted figure was true only in the aqueous extract of the fibre.

According to Potter and Cartman (1948) silk fibre may be stretched from 1/7 to 1/5 of its original length before breaking. Silk fibre can generally absorb moisture almost 11 per cent of its weight, but the range varies from 10 to as much as 30 per cent. Concentrated mineral acids dissolve silk. Alkalies will damage the silk if the concentration and temperature are high enough. Silk has very good affinity for dyes.

According to Menedez et al., (1950) Phenyl alanine is not synthesized in the first four stages of larval growth, but an increase apparently due to synthesis is observed in the fifth stage of Bombyx mori Linn.

Stamm et al., (1950) observed the synthesis of tyrosine in large amount and of tryptophan in lesser amount in the fifth developmental stage. These were not synthesised in the first four stages.

Ambrose et al., (1951) observed that neigher the amino-nitrogen proportion of lithium bromide solution of silk fibroin, nor the change of viscosity of dichloroacetic acid solution indicatedany great depolymerisation. They mentioned that even a small proportion of the beta-form makes the film insoluble. The molecule of fibroin is assumed to have 2 types of segments, with small side chains unstable in the folded state, and with large side chains, stable in the alpha form even in the drawn fibre.

Carboni (1952) stated that there is variation in degumming losses between the different layers of a cocoon.

Electron microscopic examination by Mercer (1952) of fibrillar fragments produced by enzymic disintegration of silk fibroin showed the existance of fine microfibrils about 100 A^{O} in diameter, extended parallel to the length of the fibre axis. Fibrinogen, the soluble precursor of fibrous fibroin extracted from the matured silk glands of the silk worm, spontaneously separated from dilute solutions in the form of fine microfibrils.

Goldmacher and Marika (1953) studied the detection and differentiation of sugars and polysaccharides by sensitive spot reactions on silk. Spot tests were made for

exact characterization of many carbohydrates using only 20 to 40 microgram of the substance.

Shimizu (1957) observed that the silk fibre of Bombyx mori Linn. mainly consisted of two proteins viz., fibroin (70-80 per cent) and sericin (20-30 per cent). He also observed other constituents such as waxy material (0.4 - 0.8 per cent) carbohydrates (1.2 - 1.6 per cent), colouring matter (0.3 per cent) and inorganic substances (0.7 per cent) in silk fibre. He found that fibroin formed the main core and is covered by 3 coatings of sericin. Fibroin is insoluble in water and is the true silk, while silk sericin is easily dissolved in hot water.

Bricteux-Gregor et al., (1959) measured the amount of total glutamic and aspartic acids, free glutamic acid and glutamine in the haemolymph of the developing Bombyx mori Linn. The results suggest an important uptake of glutamic acid and glutamine and a relatively less important uptake of aspartic acid by the silk glands.

Takagi et al., (1959) studied some properties of silk. According to their findings, finer the bave and greater the degumming loss of the cortex, the higher is the rigidity of the raw silk thread. The sericin of the white cocoon is

more cohesive and hard than that of yellow stained ones.

Fukuda and Hayashi (1960b) made biochemical studies on the formation of silk worm protein and the kinds of free amino acids involved in the biosynthesis of the silk protein. Fukuda et al., (1960a) also made biochemical studies on the formation of the silk protein. According to them the formation of fibroin takes place inside the silk gland during growth of the fifth instar. Each fibroin synthesized by the posterior division at different period of the fifth instar moves in an orderly fashion in the inside of the gland along the middle and anterior division during growth of the silk worm. Furthermore, fibroin present in the middle division of the silk worm just before spinning is due to the successive deposition at different periods of fifth stage.

Staurt (1960) studied the effect of acids and alkalies on silk. According to him concentrated mineral acids such as sulphuric, hydrochloric and nitric acids dissolve silk completely. Cold dilute acids, except hydrochloric acid do not injure silk. Oxalic, citric and tartaric acids do not injure silk if they are removed promptly.

Protein synthesis in silk gland was studied by Miura

et al., (1961) from silk gland incubated with C^{14} glycine and from silk worms which were given C^{14} glycine injection. Radio active C^{14} was effectively incorporated in the presence of ATP, GTP, amino acid and incorporating enzyme.

Shaw and Smith (1961) studied the composition and structure of chemically resistant fractions of silk fibroins. They obtained the residues by treatment of various silk fibroins with acid alkali and hydrogen peroxide and analysed for their constituent amino acids. Treatment with alkali or acid gave residue that had a considerably greater content of alanine than the untreated fibroins. It seems fairly certain that these residues are derived from the crystalline parts of the fibroins.

Warwicker (1961) examined the chemically resistant fractions of silk fibroin. He made an X-ray examination of the more resistant material left after chemical attack on fibroin of Bombyx mori Linn. He suggested that a more general view of the fine structure of fibroins is that of random packing of antiparallel pleated sheets of polypeptide chains. This depends on the super imposed sheets, crystalline order being achieved only where this sequence is perfectly regular.

Tanaka and Yoshimaro (1964) have stated that the acids and alkalies dissolve sericin coating from the silk filament.

An active substance affecting the pigmentation of pupal colour exists in the water fraction of the methanol extracts but not in the other fraction, suggesting that the hormone controlling the manifestation of the pupal colour is of protein or peptide composition as stated by Hashiguchi et al., (1965).

According to Manavathi (1965) a substance produced as a fluid in salivary glands of mulberry silk worm(Bombyx mori Linn.) forms the cocoon filament. He further stated that this substance consisted of two elements, silk fibroin an inner layer and sericin a kind of gum which forms the outer layer. He observed that the silk cocoon contained 25 per cent sericin and 75 per cent fibroin and that in the outside sericin, small residues of albumin, gelatin, fat, resin, mineral matter and waxy material were found. He observed that while fibroin was insoluble even in boiling water, sericin was soluble in hot water. The two different proteins of silk endow silk with a dualastic structure which is not seen in other fibres. Fibroin forms core of the fibre.

During the processing of silk sericin is removed to make silk soft and glossy.

Lucas (1966) studied the cystine content of silk fibroin. According to him intact fibroin has a cystine content of 0.23 per cent and that about 13 per cent of this cystine is destroyed during soap degumming and about 3 per cent is lost during degumming with super heated water.

Shyamala and Bhat (1966) found that the rate of absorption of amino acids from mixture in the silk worm mid-gut differed for individual amino acids. A characteristic absorption pattern was observed which was independent of the amino acid composition of the mixture used.

The silk fibre is capable of absorbing from 10 to 30 per cent moisture and it still feels comparatively dry inspite of the high moisture it has absorbed. They have given the composition of silk worm cocoon as 3.62 per cent fats and waxes, 22.28 per cent sericin, 73.59 per cent fibroin and 1.11 per cent ash or mineral matter.

Laborthe (1968) studied the action of bleaches on silk. According to him chlorine and hypochlorites cause yellowing of silk and make it more tender.

Ajishwa and Akiyoshi (1969) made physico-chemical studies on reeling quality of cocoons, the state of yellow fluorescent colour in cocoon layer and its sericin.

Cobra and John (1969) stated that the fibroin have a molecular weight of 50,000 to 60,000.

Chowdhury et al., (1969) studied the effect of cooking on the reelability of cocoons and could obtain 80 to 90 per cent of the filaments reeled from mulberry silk cocoons. These authors tried different soaking and cooking methods for improving the reeling ability. The more successful conditions for single cocoon reeling were 0.1 per cent sodium carbonate soaking for 6 hours followed by steaming at 5 inch pressure for 6 hours. In mass reeling, the reelability went down to 47 per cent. The low reelability in mass is attributed by these authors to inadequate cohesion between the silk layers which come out in loops of filaments given off from the cocoon surface.

Kuwahara et al., (1969 a and b) made electron microscopic investigation on the fibril formation from silk fibres on the formation and shape of silk fibril accompanying the treatment of dilute acids and alkalies. They noted the differences in size and structure of the fibril by means of

electron microscope observation. They observed branched fibrils which spread in various directions. Silk fibroin fibres treated with dilute acid solutions reveal many sheaves of fibrils. Silk fibroin fibres treated with dilute alkaline solution reveal rough surface due to swelling and many sheaves of fibrils.

Lamm et al., (1969) detected alanine as the N-terminal amino acid by FDNB method in silk fibroin, directly extracted from fibroin synthesizing region of the silk gland. Only trace amounts of glycine and sericin were detected.

Nagaraj and Basavanna (1969) made qualitative chromatographic study of silk protein and found the amino acids lysine, leucine, iso-leucine, methionine, phenylalanine and valine in it.

Passent and Szafranski (1969) found that soluble proteins in the silk glands bind different amino acids non-enzymatically. They stated that these soluble proteins were formed during the fifth instar and the quantity of the proteins reaches maximum before spinning. Soluble proteins showed highest binding activity for tryptophan.

Shiozaki et al., (1969) stated that the amino acids in soluble and insoluble fractions of fibroin partially

hydrolised with dilute acids and alkalies were determined by means of automatic amino acid analyzer to compare the composition of crystalline and amorphous regions. They observed that glycine and alanine were more abundant in the crystalline region than in the amorphous portion. According to them serine and tyrosine concentrations were lower in the crystalline region in the first soluble fraction and were approximately equal in the later fractions. All other amino acids in fibroin were abundant in the amorphous region although the content of valine was equal in both the regions. The molar ratios of alanine, serine and tyrosine to glycine/as were 2/3, 1/4 and 1/6 in the amorphous region, and 2/3, 1/6, and 1/12 in the crystalline region respectively.

Khan et al., (1970) studied the composition and mechanical properties of white and yellow silk fibroin. They observed that yellow variety was associated with higher mechanical constants, and it was rich in alanine but poor in tyrosine and phenylalanine.

4. Extraction and properties of sericin: Sericin is a gummy fraction of silk which is soluble in hot water and dilute alkali. Many workers have developed different methods to extract this proteinaceous fraction from silk cocoon.

Sericin being most important portion of the silk cocoon, study of its properties makes an important aspect in this study. Work of only few authors is available in this regard and it is reviewed below.

Shelton and Johnson (1925) heated raw silk with water in an autoclave at a pressure of 10 pounds per square inch and separated the sericin from the aqueous extract by precipitation with alcohol or ammonium sulphate.

Thompson (1925) reported that the boiling water removed about one-half of the sericin from raw silk in 10 hours. Although it is possible to remove the gum more or less completely from silk in boiling water, the process is extremely slow.

Alders (1927) extracted sericin from silk cocoons by boiling water or by treatment in an autoclave with water at a temperature of 116°C or more, and precipitated the sericin by addition of alcohol to a concentration of more than 50 per cent. He also stated that sericin contains approximately one per cent of cystine. Baroni (1933) observed 14.82 to 15.38 per cent of nitrogen in crude sericin and 15.62 to 16.5 per cent nitrogen in pure sericin.

Hoshor (1934) subdivided sericin of silk on the basis of

fermentation and other properties into three fractions and designated them as sericin A, B and C. Sericin C the innermost layer of the gum fraction is insoluble in most of the common solvents. It is almost completely resistant to enzyme activity.

Rastelli (1934) advocated the heating of the raw silk in solutions of soap and or mild alkalies under a pressure of 15 to 20 lb per square inch for extracting sericin, although the use of water alone at 50 lb per square inch for 15 minutes has also been advocated.

On extraction of sericin from various layers of the silk cocoon of Bombyx mori Linn. by treatment with 20 ml 0.5 per cent sodium hydroxide solution per gram for 12 hours, Lucioriandi (1955) observed that the outer layers of a cocoon contained 34 per cent of sericin, the middle layer contained less sericin and the inner layer contained 24 per cent of sericin. He proposed a method for sericin extraction where the presence of minor constituents like wax, carbohydrate etc., are ignored because of their presence in minute quantities.

Shikata and Mijikubota (1960) analysed the carbohydrates of sericin qualitatively after hydrolysis with sulphuric acid. A new component viz., sialic acid was detected.

Pentoses and uronic acid could not be found.

Bhavalkar (1962) studied the amino acid content of sericin derived from four different Indian silks. He found that the amino acid pattern of sericin was distinctly different from that of the corresponding fibroin filaments. Sericin contained all the amino acid of the fibroin, but markedly less of lysine and dicarboxylic acids.

Tantin and Pant (1962) stated that the outer coating of silk glue is called sericin which causes the fibres to adhere and which may be removed by boiling with soapy water.

Jolly and Krishnaswamy (1964) described a method for the determination of sericin content in cocoons. They kept the cocoon shell under test in a desiccator for 4 to 5 days till constant weight was obtained. The shell was then cut into 4 equal parts and put into a pre-weighed crucible which was also kept in a desiccator. 20 cc of 5 per cent of sodium hydroxide was poured into the crucible and the shell pieces were allowed to dissolve for 12 hours. Later shell pieces were washed in distilled water and boiled twice for half an hour.

Nishi et al., (1969) studied the effect of tyrosinase

on sericin. A sericin solution treated with mushroom tyrosinase turned red and showed an increase in viscosity. They also observed a general increase in absorption of light of higher wave length in the ultra-violet region.

Sonwalkar (1969) investigated degumming loss and spinning performance of pierced and cut cocoons in silk worm. The solution used by him contained 3 g of soap and 1 g of soda per litre. The ratio of material to liquor was 1.15. He loosely tied the sample material with a porous cloth and immersed in the alkaline bath and worked for one hour at boiling point. The material was taken out and washed.

5. Properties of silk worm urine. Not many people have studied the property of silk worm urine as could be seen from the very limitted published information on this subject.

As silk worm urine contains considerable amount of uric acid, it is suspected that uric acid may be responsible for the yellow staining of cocoons (Kuwana 1937).

According to Hawk et al., (1954) uric acid acts as a weak dibasic acid and forms two classes of salts, neutral and acidic. The neutral potassium and lithium urates are

the most easily soluble of the alkali salts, while ammonium urate is difficulty soluble. They have stated that uric acid is insoluble in alcohol and ether, difficulty soluble in boiling water and practically insoluble in cold water.

According to Kumararaj (1969) the silk worm urine is alkaline in nature. He found pH range of 8.6 to 9.8 in pooled samples. He also observed a slight increase in pH of urine on 48 hours exposure to atmosphere.

6. Filament breakage during reeling: Only few workers have studied on the causes of filament breakage in urine stained silk cocoons. Also, hardly any author has come to a definite conclusion regarding the exact cause and extent of breaking of filament during reeling.

According to Wanger (1932) the occurrence of many loose ends in raw silk is due to the action of the hard inelastic sericin envelop on the fibroin when the fibre is bent. He suggests that the extent of thread breaking during unwinding may be minimised by first steaming the hanks in order to soften the gum.

Kumararaj (1969) stated that when the silk worm urine comes in contact with the cocoons in drops on mountages, the alkaline nature of the urine may dissolve the sericin coating

of the filament in patches and this condition, he assumes results in breakage while reeling, as the filament is rendered weak by the removal of sericin in patches. He further studied the effect of pure uric acid solution on cocoons. He observed that pure uric acid applied to cocoons did not produce any characteristic brownish yellow stain compared to the silk worm urine applied to cocoons. He pointed out that this finding indicates that it is not the uric acid in silk worm urine that is responsible for producing the characteristic stain.

7. Control measure: There are two aspects in control measures viz., preventive and curative. In preventive measures few authors have approached from the physiological point of view by using some anti-diuretic hormones and using electric room heaters. Whereas some others have tried from technological point of view i.e., reducing the number of worms per mountage, improving the mounting techniques and above all observing strict sanitory conditions. Under curative measures only few authors have attempted to remove the stain by using certain bleaching agents.

Oxytocin has antidiuretic action in lower vertebrates (Edward C. Staurt, 1960). Oxytocin has a slight but definite antidiuretic action in addition to its own characteristic

effects (Chowh HaoLi, 1961). Berridge (1966) stated that it has been possible to induce antidiuresis in insects by certain hormones.

Manavathi (1965) stated that the bleaching of silk could be done by using hydrogen peroxide with or without soap. Silk is bleached in a solution at 40°C which is gradually raised to 90°C. Silk is kept in cooling liquor for over-night then washed and soaped.

A report from the Japan Silk Association (1967) reveals that a timely removal of the faeces and urine discharged by the matured silk worms would be much helpful to improve the quality of cocoons. It is suggested that with the completion of the mounting on turning cocooning frames, the paper or straw mat spread underneath must be replaced from time to time to get rid of the discharged faeces and urine.

Laborthe (1968) proposed a technique to remove the stain by sponging with soap and water or with a salt solution and then rinsing. Dilute ammonia alone or with hydrogen peroxide may then be applied for a minute and then rinsed off.

Department of Silk Worm Physiology at the Central Sericultural Research and Training Institute, Mysore studied the use of antidiuretic hormones to control the urination of

silk worm during 1969. Hormones like Vasopressin and Oxytocin were used in addition to the control of temperature humidity complex. They observed some yellow stained cocoons in hormone treated batches and slightly more yellow stained cocoons in the untreated batches.

The strong mammalian antidiuretic hormone like vasopressin or some of its salt solution in minute concentration could cause antidiuresis in silk worm, (Kumararaj, 1969). According to him one of the simplest way to tackle this problem in the affected areas is to use electric room heaters and reduce the humidity at the time of spinning. The other possibility suggested by him is to keep the mountages slanting or keeping two mountages slanting on an inverted 'V' at the time of spinning. This practice does avoid to some extent direct dribbling of urine on the cocoons below in the mountage. Another measure suggested is to avoid over crowding of silk worms in the mountage particularly under high humidity conditions, which reduces the incidence of stained cocoons as there will be sufficient gap in between spinning larvae.

Madrell (1969) found that pitressin did not stimulate malpighian tubules activity in insects. According to him

the malpighian tubules of insects are under hormonal control. Substances such as acetyl-choline, ATP and pitressin do not stimulate the tubules to secrete.

Information on the effect of urine on physico-chemical properties of silk and methods of removing urine stain on silk cocoon is meagre. Similarly there are no reports on the methods of reeling of urine stained cocoons and use of softening agents.

MATERIAL AND METHODS

CHAPTER III

MATERIAL AND METHODS

Very little systematic work has been reported in literature on the problem of silk worm urination. No well defined methods and techniques are available for ready adoption. Therefore investigation was planned and carried out exclusively keeping in view the main objectives. In view of the peculiar nature of the investigation, part of the work was done at the Central Sericultural Research and Training Institute, Mysore and the remaining part was completed in the Department of Chemistry and Soils of the University of Agricultural Sciences, Bangalore. The experiments on reeling, collection of silk worm urine for analysis, recording of temperature and humidity and collection of weather data at the time of urination etc., were carried out at the said Institute in Mysore. The remaining part of the work which mainly consisted of detailed analysis including amino acid analysis of the cocoons was done in the laboratories of the University at Hebbal, Bangalore.

1. Study on the effect of humidity and number of silk worms per mountage on urination

This study was carried out during rainy season of 1970.

Silk worms of pure Mysore x NS 6 breed were used in the study. The silk worms were reared at the Sericultural Section of Central Sericultural Research and Training Institute, Mysore. Standard size mountages (locally known as 'Chandrike') were used for mounting the silk worms. Since the population of silk worm forming the cocoons at the time of spinning will not be the same as the number of worms put on the mountage, different number of worms were placed on the mountage and then allowed to spin and form cocoons. At the time of spinning actual number of cocoons formed on the mountages were counted. Out of these, number of cocoons stained by urination were separately counted and recorded. From this percentage of stained cocoons per mountage was calculated.

Rearing of the silk worms was carried out at the prevailing climatic conditions. However, weather data such as maximum and minimum relative humidity were recorded on the day of spinning stage of the larvae during which period urination by silk worm occurs and the cocoons become stained.

2. Collection and analysis of urine

During the period of September to October 1970, urine was collected from the spinning stage larvae from the silk worms reared at the Central Sericultural Research and Training

Institute, Mysore. The urine sample was preserved in a clean stoppered bottle by keeping it in refrigerator. Later on it was used for detailed chemical analysis. The urine was analysed for the following constituents:

i) pH
ii) Electrical conductivity
iii) Total solids
iv) Uric acid
v) Total nitrogen
vi) Ammoniacal nitrogen
vii) Potassium, sodium and calcium
viii) Total sulphate
ix) Total phosphate and
x) Total chloride

Methods used for analysis of urine are briefly given below:

i) pH: Silk worm urine reaction was determined on a Toshniwal model C-14 pH meter using combined glass electrode.

ii) Electrical conductivity: Electrical conductivity of the urine sample was determined by using the conductivity bridge, having an electronic eye null point indicator and the temperature compensation device. A dip type platinised

conductivity cell was employed for the purpose.

iii) Total solids: By following the method given by Shockoll (1904) the total solids in silk worm urine were estimated. Five ml of the urine sample was taken in a tared shallow dish, slightly acidified with acetic acid and evaporated in vacuo in the presence of sulphuric acid till constant weight was obtained. The residue left was weighed and percentage of total solids in urine sample was calculated.

iv) Uric acid: This was estimated by following the method given by Benedict and Franke (1922). After diluting the urine sample twenty times and mixing well, an aliquot of ten ml was quantitatively transferred in a 50 ml volumetric flask. To this was added 5 ml of 5 per cent sodium cyanide solution and 1 ml arsinophosphotungstic acid reagent. (25 g pure sodium tungstate dissolved in 150 ml of water + 12.5 g of pure arsenic acid + 6.25 ml of 85 per cent phosphoric acid + 5 ml of concentrated HCl) and the volume was then made up with distilled water and mixed well. The intensity of blue colour of the solution was measured. Optical transmittance of the blue coloured solution was measured with a photo electric colorimeter using red filter. From the standard curve simultaneously prepared using pure uric acid similarly treated, concentration of uric acid in the silk

worm urine sample was calculated.

vi) Total nitrogen: Total nitrogen content of the silk worm urine sample was estimated by micro-kjeldahl method as described by Hawk et al., (1965). 1 ml of the silk worm urine was taken in a pyrex glass test tube, to this was added 1 ml concentrated sulphuric acid, 1 g potassium sulphate, and one drop of 5 per cent copper sulphate solution. Few glass beads were added to this mixture in order to minimise bumping. The mixture was boiled over a microburner for about 6 minutes. After cooling to room temperature, 6 ml distilled water was added to prevent formation of solid crust in the flask. The digested material was transferred into a distillation apparatus, by repeatedly rinsing the micro-kjeldahl flask with distilled water and transferring the washings quantitatively to the distillation flask. Sufficient quality of 40 per cent sodium hydroxide was added to the digest in the distillation flask so as to make the contents distinctly alkaline. Distillate was collected in two per cent boric acid solution in a receiving flask to which few drops of bromocresol green indicator were added. After completing the distillation ammonia received in the boric acid was titrated against 0.02 N sulphuric acid. Number of ml of 0.02 N sulphuric acid required in the titration when multiplied

by factor 0.28 gave the milligram of nitrogen in 1 ml sample of silk worm urine.

vi) Ammoniacal nitrogen: By following the method given by Folin and Bell (1917) ammoniacal nitrogen in the silk worm urine sample was estimated. To an aliquot of 25 ml of urine sample taken in a 150 ml earlenmeyer flask, 5 g of finely pulverised potassium oxalate was added followed by few drops of phenolphthalein indicator. The contents of the flask were titrated to a faint but permanent pink colour against 0.1 N sodium hydroxide. To this titration mixture 10 ml of neutral formalin solution was added. After mixing well the whole mixture was again titrated against 0.1 N sodium hydroxide to a permanent pink colour. Number of ml of 0.1 N sodium hydroxide required for completing the titration after the addition of formalin multiplied by the factor 1.70 gave milligram ammonia present in the quantity of silk worm urine sample taken for the estimation.

vii) Potassium, sodium and calcium: An aliquot portion of the urine sample was diluted with distilled water and after mixing well, it was used for determination of potassium, sodium and calcium. These three metal ions were estimated using a flame photometer without any prior treatment of the diluted sample in 'EEL' flame photometer by using

appropriate filters for potassium, sodium and calcium. Standard solutions of potassium chloride, calcium chloride and sodium chloride prepared from A.R. quality chemicals were run previously on the instrument to obtain the standard curves for these elements. By refering to the standard curves the instrument readings recorded for potassium, calcium and sodium, concentrations of these in the silk worm urine sample were calculated.

viii) Total sulphates: Total sulphate content in the silk worm urine sample was determined by following the method discribed by Benedict (1909). In a clean 100 ml porcelain dish, 10 ml of the silk worm urine sample was transferred. To this was added 5 ml of Benedict's reagent. (Benedicts reagent consists of copper nitrate 25 g + sodium chloride 25 g + ammonium nitrate 10 g + distilled water to make up 100 ml). The dish was kept on a hot water bath and the contents were evaporated to dryness. The residue was heated on the water bath till it was blackened. The residue was then heated on a naked flame for 10 minutes to destroy the organic matter. After cooling the dish to room temperature about 15 ml of dilute hydrochloric acid (1:4) was added and the contents were warmed gently on a hot water bath till the residue was completely dissolved. After diluting with distilled

water to about 100 ml, 10 ml of 10 per cent barium chloride solution was then added drop by drop. The precipitate formed was allowed to settle. It was then filtered through a previously weighed gooch crucible. The precipitate was washed free of chlorides. It was dried and weighed. From the weight of the precipitate total sulphates present in the silk worm urine sample was calculated.

ix) Total phosphorus: This was estimated by following the method given by Fiske and Subba Rao (1925). To a large sized pyrex test tube 1 ml of silk worm urine sample was transferred and 10 ml of 5 N sulphuric acid was added. The contents were heated over a microburner carefully until the water portion was driven out and a dark brown fluid remained in the test tube. To this 30 per cent hydrogen peroxide was added drop by drop so as to completely oxidise the organic matter. After cooling to room temperature 2-3 ml of distilled water was added and again boiled for 2-3 minutes. Then 10 ml of 2.5 per cent solution of ammonium molybdate in water was added mixed well and 4 ml of the aminonaphthol sulphonic acid reagent was added and the mixture was made to 100 ml volume in a volumetric flask and mixed well. Percentage transmittance of the blue colour obtained was read on a photo-electric colorimeter at 640

millimicron wave length using a red filter. Standard solutions prepared from AR quality potassium hydrogen phosphate were also similarly treated and their transmittance readings were recorded. From the standard curves concentration of phosphate present in the sample of silk worm urine was calculated.

x) Total chlorides: Chlorides were determined in silk worm urine sample by Volhard - Arnold (1910) method. 10 ml of urine sample was taken in a 100 ml volumetric flask, about 25 drops of nitric acid and 2 ml of cold saturated solution of ferric alum were added. Then 20 ml of standard silver nitrate solution was added which precipitated the chlorides. After allowing the precipitate to settle for 10-15 minutes in the flask the volume was made upto 100 ml mark with distilled water. The contents were filtered and washed free of acid and the filtrate along with washings were quantitatively collected in a beaker and titrated against standard ammonium thiocyannte solution, till a permanent tinge of red colour was obtained. Number of ml of standard silver nitrate solution actually required for precipitating the chlorides when multiplied by a factor 0.01 gave the amount of chlorides in g present in the sample of silk worm urine taken for analysis.

3. Analysis of silk worm cocoons:

Experimental sample of silk cocoons were analysed for the following:

i) Total protein content

ii) Sericin and Fibroin contents

iii) Amino acid composition

iv) Total carbohydrates

v) Mineral composition

Methods used for the above estimations are briefly given below:

i) Total protein content: The cocoon samples were cut into small pieces and powdered after they were completely dried. Total protein content of the samples was determined by adopting micro-kjeldahl method.

An aliquot portion of the powdered sample was weighed accurately (100 milligram) into a clean and dried micro-kjeldahl flask. 3 ml concentrated sulphuric acid and a pinch of digestion mixture wereadded to the flask. The contents were digested on a micro-kjeldahl digestion unit till the digest became clear with an apple green colour. After cooling the contents to room temperature they were diluted with distilled water and carefully transferred

quantitatively to a micro-kjeldahl distillation unit. Forty per cent sodium hydroxide was added till the contents became alkaline. Then the ammonia liberated was steam distilled and collected in 20 ml of 2 per cent boric acid to which was added few drops of mixed indicator namely bromocresol green and methyl red. After the completion of the distillation, ammonia collected in the boric acid was titrated against 0.02 N sulphuric acid. From the titre value nitrogen per cent was calculated. To get per cent protein, nitrogen per cent was multiplied by the factor 6.25.

Similar method was used for the determination of total protein content in fibroin and sericin fractions of silk cocoons.

ii) Estimation of sericin and fibroin: Cocoon samples were cut into small pieces and dried thoroughly in a desiccator. An aliquot portion of the dried cocoon pieces was taken in a pre-weighed dish to which 50 ml of 0.2 per cent sodium hydroxide was added and allowed to stand for 12 hours. By this treatment sericin dissolves into sodium hydroxide. The contents were later filtered through gooch crucible and the residue was washed free of alkali. The filtrate along with the washings was carefully

collected in pre-weighed dish and was kept on a hot water bath for complete evaporation. The dish was then transferred to an oven. After drying for 1½ hours, it was cooled to room temperature in a desiccator and weighed. In another dish, 50 ml of 0.2 per cent sodium hydroxide was taken, evaporated, dried thoroughly in an oven, cooled to room temperature in a desiccator and weighed. The weight of sodium hydroxide obtained from 50 ml of 0.2 per cent solution was deducted from the previous weight to obtain the weight of sericin fraction in the silk cocoon.

The residue left in the gooch crucible from the above was carefully dried at 60°C without allowing it to char, cooled to room temperature in a desiccator and weighed. This was the fibroin fraction of the silk cocoon. The percentage composition of sericin and fibroin were thus determined in the samples of silk cocoons. The determinations were made in duplicate.

iii) Amino acid composition: Two dimensional paper chromatography was used for the analysis of silk cocoon samples for their amino acid composition.

Since all the amino acids could not be analysed by the same method, two different techniques were used which are described below:

(a) Acid hydrolysis of silk protein for amino-acids other than tryptophan: Samples of silk cocoons were cut into small pieces and throughly dried. An aliquot portion of 100 mg of the sample was weighed and transferred to a hard glass hydrolysis tube to which 6 ml of 6 N HCl (AR quality) was added carefully. Contents of the tube were mixed and heated slowly on a bunsen burner until the hydrochloric acid fumes came out. Care was taken so that there was no spurting. The tube was vacuated and sealed. The sealed tube was labelled and incubated in an oven at 110°C for 24 hours. After the hydrolysis, the tube was unsealed by breaking open the tip and the contents were filtered through whatman No.1 filter paper into a 50 ml beaker. The tube was rinsed several times and the washings were filtered using glass distilled water. The filtrate and the washings collected in the beaker were then kept on a hot water bath for complete evaporation. To the dry residue 2 ml of glass distilled water was added and the contents were dissolved and evaporated again. This process was repeated for several times till no acidic vapours were evolved which was confirmed by litmus paper test.

One ml of double glass distilled water was added to the residue. A chromatography filter paper (Whatman number one)

was washed with N/100 hydrochloric acid and later it was washed with distilled water till it was free from acid and then dried. 100 microliters of the hydrolysate prepared as above was spotted on the filter paper. Two dimensional chromatography was run using butanol-acetic acid-water system (9:1:4) followed by phenol saturated with water (4:1). The chromatogram was allowed to develop for 16 hours in the first solvent and 12 hours in the second solvent. The descending chromatogram technique was used for separating the amino acids. The chromatograms were then air dried, sprayed with nynhydrin solution and were allowed to dry for two hours. The amino acid spots separated were identified by comparing the 'Rf' values found out in a similar way by spotting chromatographically pure known amino acids.

The quantitative estimates of the separated amino acids was done by carefully cutting out each spot and eluting in 5 ml alcohol-copper sulphate solution for 2 hours. The intensity of the colour of the eluted solution was measured in a Klett-Summerson photo-electric colorimeter using a green filter (wave length 520 to 560 milli-micron). Earlier, standard curves were prepared separately for 20 amino acids using pure compounds. By refering to the standard curves the quantity of each of the amino acids in the silk proteins were

calculated. The results were expressed as microgram of each amino acid in 100 ug of the sample. The method described above is essentially the one recommended by Dematriate (1956).

(b) Alkaline hydrolysis of silk protein for determination of tryptophan: 100 milligram of the dried cocoon pieces were weighed and transferred into a hard glass hydrolysis tube to which eight ml of 5 N barium hydroxide was added. The tube was vacuated and sealed as above and incubated at 110°C in an electric oven for 24 hours. After the hydrolysis, the tube was unsealed, filtered and dried by following the procedure described above under acid hydrolysis. 25 microlitre of the alkaline hydrolysate solution was spotted on acid washed Whatman No.1 chromatograph paper and chromatogram was run in one direction only. Butanol-acetic acid-water system was used for the separation. The chromatogram was run for 16 hours. Development of the spot, identification and quantitative estimation of tryptophan were carried out in a similar way as detailed above for other amino acids in the acid hydrolysate. Identification and quantitative estimation of amino acids in fibroin and sericin fractions of the silk cocoons was done by the procedure described under acid hydrolysis above.

(iv) Total carbohydrates: Carbohydrates in the sample of silk cocoons were estimated by adopting the colorimetric method of Somogyi (1952). 100 mg of the dried sample was used for the estimation of total carbohydrates. The sample after carefully weighing was transferred into a test tube fitted with cork and 1 ml of 2.5 N HCl was added. The tube was stoppered and kept in hot water bath for two hours. After hydrolysis was completed the contents were filtered through a dry filter paper and filterate was collected in a beaker to which a pinch of sodium carbonate was added to neutralise the acidity and later the solution was clarified with 1 ml of cadmium sulphate solution and 1 ml of 0.50 N sodium hydroxide solution. The clarified extract was made to 100 ml volume. It was mixed well. 10 ml of the diluted hydrolysate was taken in a test tube and 1 ml of copper reagent was added and heated for 10 minutes in boiling water bath. The tube was taken out cooled to room temperature and 1 ml of Nelson's arsenomolybdate reagent was added to dissolve cuprous oxide. The mixture was made upto 50 ml and the intensity of the blue colour formed was measured at 640 millimicron wave length in Spectronic - 20 spectrophoto-colorimeter. A reagent blank was run by following all the steps described above. Standard

curve was drawn for various concentrations of pure glucose solution. By refering to the standard curve the instrument readings for the samples were converted to corresponding concentration of carbohydrate, from which percentage composition of the carbohydrates in the cocoon sample was calculated.

The above method was also used for the determination of carbohydrates in fibroin and sericin fractions of silk protein.

(v) Estimation of minerals in silk cocoons: Small pieces of cocoon samples were dried and an aliquot portion of them was weighed into a crucible. After charring on low flame the contents were ignited in a muffle furnace at about 400°C. The residue was cooled and treated with 2 ml of sodium nitrate HCl and 20 ml of distilled water. The solution was evaporated to dryness first on a water bath and on sand bath. After cooling to room temperature the residue obtained was dissolved in double glass distilled water and the volume was made upto 50 ml. After shaking well, calcium, sodium and potassium were determined using :: EEL flame photometer with appropriate filters. Earlier for each of these three determinations standard solutions of known concentrations prepared from their respective pure salts were run on the flame photometer to prepare standard curves from which the

concentrations of sodium and potassium in the samples were calculated.

A similar procedure was used for the determination of mineral constituents like calcium, sodium and potassium in fibroin and sericin fractions of silk cocoons.

Analysis of different portions of silk cocoons: Silk cocoons are differentiated into three main portions - (i) Outer cover (ii) Middle portion and (iii) Inner core. These portions were removed from the silk cocoon samples by carefully peeling each of them. This was done for both normal as well as urine stained silk cocoons. The separated portions were then analysed for sericin and fibroin contents as well as for their mineral composition by following the methods described above.

4. Measurement of the extent of penetration of urine in cocoons:

In order to find out the extent of damage caused by urination it is necessary to know to what depth the urine penetrated the cocoons. For this purpose silk cocoons were picked up at random from lots of both urinated and normal cocoons, and they were carefully reeled for 100 meters. Further reeling of the cocoons was continued so as to get the second,

third and fourth hundred meter lengths. These lengths were separately cut into small pieces and dried. Aliquot portions of the same were weighed for the determination of uric acid content by following the method already described above under the estimation of uric acid in silk worm urine.

The amount of uric acid present in the first, second, third and fourth hundred meter lengths of the reeled silk gave an estimate of the extent of penetration of urine in silk cocoons. The normal or good cocoons were also reeled and uric acid in different lengths of the silk gave an indication of the extent of contamination of urine when several silk worms are reared on the same mountage. Reeling of the silk from cocoon was done by the following method:

Method of reeling silk cocoons: As a pre-treatment for reeling silk cocoons, the cocoons were boiled in water containing sodium carbonate to make the water slightly alkaline. The cocoons were cooked for a period of 5 minutes. This pretreatment is necessary to improve the reelability as well as to remove the floss on the cocoons. Thus, cooked cocoons were reeled using an 'Approvate'.

5. Reeling experiment:

Reeling experiment to study the effect of urination and

number of days of contact of urine with silk on the reelability of cocoons was conducted. Stifled cocoons were procured from C.S.R. & T.I., Mysore and three drops of silk worm urine were added on each silk cocoon in order to bring about artificial urination. The cocoons so treated were incubated for 1, 2, 3, 6, 8, 15 and 30 days. One set of the treated cocoons was removed on the days specified above on seven occasions. After the cocoons were steamed for 2, 4, 6, 8 and 10 minutes, these cocoons were subjected to sub-treatment such as cooking for two different period of times viz., 4 and 6 minutes before reeling the cocoons. For each treatment there were 5 replications.

The cocoons were cooked in water and made slightly alkaline with sodium carbonate (0.1.1). 5 cocoons were used for each of the said treatments. The cocoons so treated were subjected to reeling by following the method described above. During the experiment the total length and number of cuts per cocoon were recorded for each reeling.

In order to study wheather the stifling of urine stained cocoons had any adverse effect on reelability of the same, silk worms were reared during heavy rainy days at Central Sericultural Research and Training Institute, Mysore. Immediately after the completion of the cocoon formation,

5 cocoons that were stained were picked at random and used for the experimental purpose. The urine stained cocoons were subjected to the following treatments:

a) Hot air stifling and reeling

b) Steam stifling and reeling

c) Reeling without any stifling

These treatments were given 2, 4, 6 and 8 days after the formation of the cocoons was complete. The cocoons were cooked and reeled as described earlier. Here also 5 cocoons were reeled in each treatment. Another set of normal cocoons (not stained) were similarly treated as above and reeled. This served as control for comparision. The observations were recorded for total length of silk thread reeled and number of cuts per cocoon while reeling.

6. Examination of silk under microscope:

In order to understand the nature of damage caused due to urination on silk fiber, a single strand of thread reeled out as above from the cocoons was examined under a microscope, a drop of glycerine was added and after putting a cover slip the fiber was examined under microscope. For comparsion purpose normal silk fiber not affected by urination was also examined in the same way. Micro-photographs of silk fibers were taken.

7. Study of the effect of urination on the loss of sericin during cooking of silk cocoons:

Certain amount of sericin is lost during the process of cooking of the silk cocoons. This helps in unwinding of the silk fibre and thus facilitates reeling. The urination of cocoons however reduces the loss of sericin and the silk fibre is not easily unwound thereby reeling would be difficult. The quantitative measurement of the reduction in the loss of sericin during cooking was therefore made.

Total sericin content of silk cocoons was determined in the normal and urine stained cocoons following the method given earlier. Urine stained and normal cocoons were cooked and reeled as mentioned above in opprovate. Sericin was determined in the reeled silk separately for urine stained and good cocoons. The amount of sericin lost during cooking was determined by deducting the amount of sericin present in silk thread reeled from that of total sericin present in un-cooked cocoons.

8. Effect of softening agents on the reelability of silk cocoons:

This study was made to findout the effect of treating the cocoons with chemicals that may bring about softening on the reeling property of silk cocoons especially those that are stained by urination by silk worm.

Softening agents like sodium sulfite, sodium carbonate and sodium hydroxide were employed for the purpose. Solution containing one per cent of these chemicals were prepared separately and five cocoons of each normal and urine stained were cooked in the respective softening solutions. The cocoons were then reeled in approvate. Observations regarding the total length of thread and total number of cuts per cocoon during reeling were recorded.

9. Chemical treatment of silk cocoons for removing stain caused by urination:

For this purpose several alkali solutions and detergents were tried, both in cold and hot conditions. The reagents used for the purpose were teepol, sodium hydroxide and sodium carbonate. These were tried in different consentrations 0.5, 1.0 and 1.5 per cent. In addition to these chemicals the following solvents were also tried.

Absolute alcohol

Acetic acid

Petroleum ether

Acetone

Chloroform

Methanol

Benzene

EDTA 2 per cent and 5 per cent

Formic acid

Formaldehyde

Ascorbic acid

Sodium cyanide

Hydroxy quinolin

Sodiumdithiocarbamate

Di-ethyl glyoxime

Borate buffer and

Ammonia

The urine stained cocoons were kept in the above solutions and solvents over night. They were removed and thoroughly washed with distilled water and dried in oven. Another set of stained cocoons were immersed in hydrogen peroxide and maintained at 60°C for half an hour. Then the cocoons were removed and kept in alcohol for over night, washed thoroughly with distilled water and dried in oven at low temperature.

The cocoon samples so treated as above were examined with a gloss and reflectance meter to compare the efficiency of the treatment on the removal of urine stain on silk cocoons. Method used for taking gloss and reflectance readings is described below.

Gloss and reflectance measurements of the cocoon were made with AIMIL gloss/reflectance meter. The standard gloss plate having a reading of 3 was used to compare the glossyness of the cocoon samples. Several readings on the normal and affected cocoon samples were recorded. Averages were worked out. For reflectance measurement, standard magnesium carbonate block was used for adjusting the initial reflectance to 100 of the instrument reading. Here the reflectance of the normal, affected and treated cocoons were measured. Several samples of the three categories of the cocoons were used so as to get a good average measurement of the reflectance.

EXPERIMENTAL RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

1. Effect of humidity on urination by silk worms:

Silk worms were reared under different humidity condition. Different number of worms per Chandrike were also tried in order to study the effect of spacing between spinning worms. The number of urine stained cocoons in each set were counted. The results are presented in Table 1. Correlation coefficient worked out for number of worms per Chandrike and number of urine stained cocoons is 0.731.

2. Constituents of silk worm urine:

Silk worm urine was analysed. It was found to contain 0.029 per cent total nitrogen, 0.0027 per cent ammoniacal nitrogen, 2.31 per cent total solids, 9,200 ppm potassium, 375 ppm of calcium, 95 ppm of sodium, 4.350 mg total phosphates, 8.2 mg total sulphates and 0.80 mg chlorides per 100 ml of urine. Uric acid in silk worm urine is found to be 68.927 per cent. pH and electrical conductivity of silk worm urine were 8.6 and 5 mhos per cm (Table 2) respectively.

3. Analysis of silk cocoons:

(i) Total protein content: Total nitrogen content of silk cocoons, fibroin and sericin was estimated. Total

TABLE 1

Number of silk worms per Chandrika as affected by humidity

Average relative humidity	Number of cocoons per Chandrike			Percentage of stained cocoons
	Total	Stained	Unstained	
81	425	96	329	22.5
81	240	45	195	18.7
81	460	105	355	22.8
81	500	142	358	28.4
81	360	80	280	22.2
81	430	90	344	23.2
77	321	19	302	5.9
77	270	50	220	18.5
77	427	25	402	11.2
76	346	20	326	5.8
76	165	13	152	7.9

Correlation coefficient ("r") for number of worms per Chandrika Vs. number of urine stained cocoons = 0.731.

TABLE 2

Composition of silk worm urine

Composition		Composition	
pH	8.6	Potassium ppm	9,200
Electrical Conductivity m mhos/cm	5.0	Calcium ppm	375
Total soluble solids %	2.31	Sodium ppm	95
Uric acid %	60.93	Total phosphates mg / 100 ml.	4.35
Total nitrogen %	0.028	Total sulphates mg / 100 ml	8.20
Ammoniacal nitrogen %	0.00272	Total chlorides mg / 100 ml	0.80

nitrogen in normal cocoons is 12.63 per cent and in urine stained cocoons it is 11.50 per cent. Total nitrogen present in sericin and fibroin of normal cocoons are 10.92 per cent and 13.80 per cent respectively and 9.85 per cent and 13.15 per cent respectively in urine stained cocoons. From the total nitrogen content of the cocoon and of the two fractions of silk, crude protein content was calculated using a factor of 6.25. The results are presented in Table 3.

(ii) Sericin and fibroin in silk cocoons: The two main protein constituents of silk viz., sericin and fibroin were determined in both normal as well as urine stained cocoons. The normal and urine stained cocoons contained 27.6 per cent and 26.34 per cent of sericin which is the gummy fraction of the silk cocoons. The normal and urine stained cocoons contained 60.91 per cent and 56.99 per cent respectively of fibroin which is the true silk (Table 4).

(iii) Amino acids in silk cocoons and their fractions: Amino acids present in silk cocoons, fibroin and sericin of both urine stained and normal cocoons were estimated by two-dimensional chromatography. The results are presented in Table 5. In all, 18 amino acids could be isolated and identified. The amino acid make-up of the silk cocoon and its fractions viz., sericin and fibroin are not similar.

TABLE 3

Total nitrogen, protein and carbohydrate contents in silk cocoons, fibroin and sericin

Samples		Total N per cent	Protein per cent	Carbohydrates per cent
Whole cocoon	Normal	12.63	78.937	0.0045
	Urine stained	11.50	71.875	0.0053
Fibroin	Normal	13.80	86.250	0.0040
	Urine stained	13.15	82.187	0.0026
Sericin	Normal	10.92	68.250	0.0082
	Urine stained	9.85	61.562	0.0030

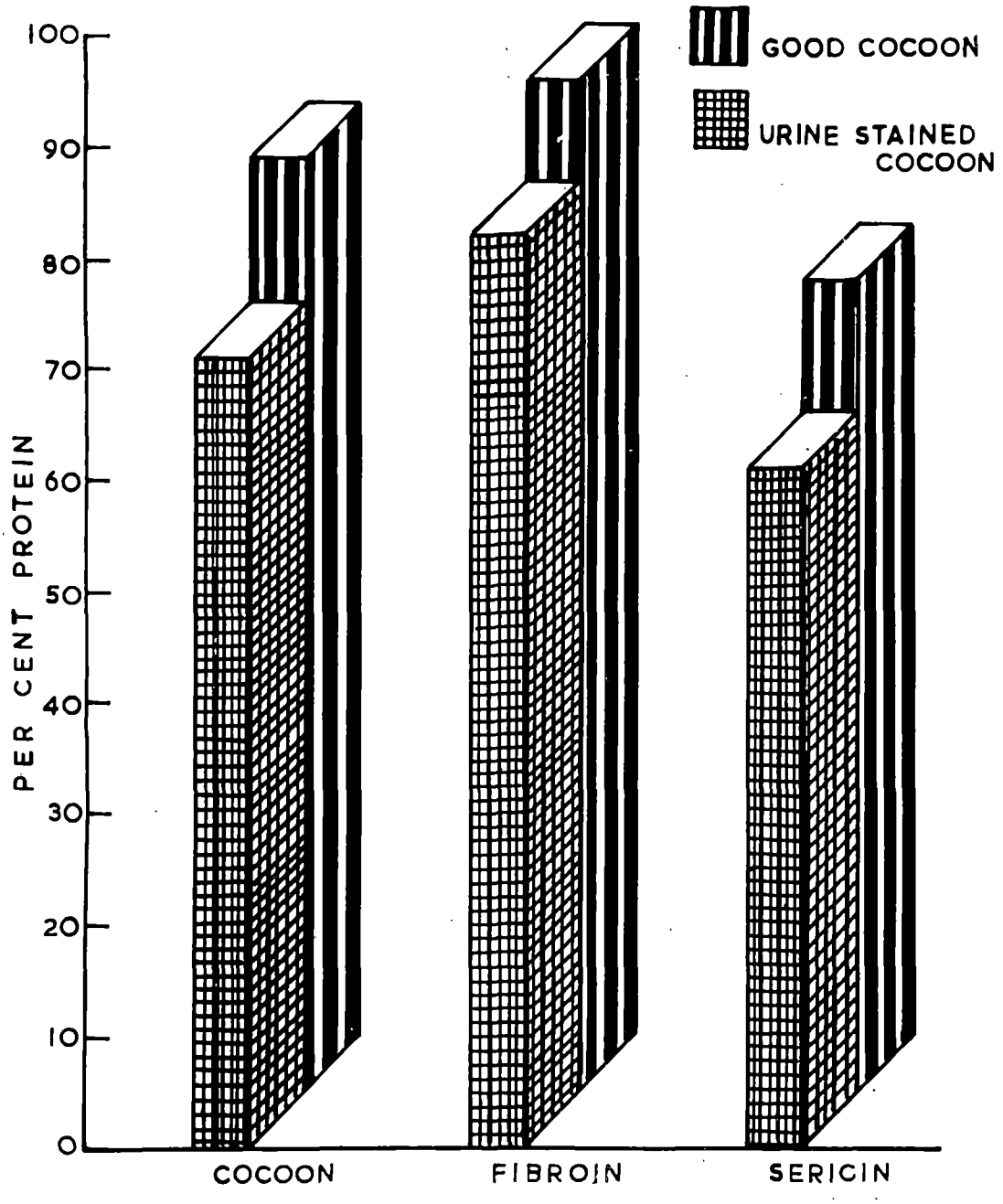


FIG.1. PERCENTAGE OF PROTEIN IN COCOON, FIBROIN AND SERICIN

TABLE 4

Composition of normal and urine stained silk cocoons

Constituents	Normal cocoons	Urine stained cocoons
Sericin %	37.60	26.24
Fibroin %	60.91	56.99
Total ash %	1.26	1.69
Calcium ppm	86.0	205.0
Potassium ppm	58.0	260.0
Sodium ppm	5.0	15.0

TABLE 5

Effect of urination on amino acid composition of fibroin, sericin and whole cocoons

Amino acids	Whole cocoons		Fibroin of cocoons		Sericin of cocoons	
	Normal	Urinated	Normal	Urinated	Normal	Urinated
	(Microgram per 100 mg of the sample)					
1. Alanine	2530	1340	1280	900	1140	230
2. Arginine	1980	1650	2100	280	2860	1130
3. Aspartic acid	1640	1570	1040	1000	1300	-
4. Cystine	2600	2100	540	360	3200	-
5. Glutamine	1300	1300	360	260	3360	-
6. Glycine	830	830	1000	60	420	-
7. Hydroxy proline	1710	1210	500	500	2280	830
8. Iso-leucine	2090	1970	2100	220	2640	1030
9. Leucine	2340	2030	2440	1360	2360	960
10. Lysine	1750	1500	560	560	1900	620
11. Methionine	2600	1450	4200	3600	4200	1950
12. Phenyl-alanine	2600	2600	2800	2560	1560	720
13. Proline	980	980	3540	2160	3000	480
14. Serine	-	-	-	-	860	-
15. Threonine	2600	2600	2300	2240	3500	950
16. Tyrosine	2600	2540	4200	1200	2600	1160
17. Valine	610	550	1000	220	420	150
18. Tryptophan	2600	2520	4200	4200	1880	870

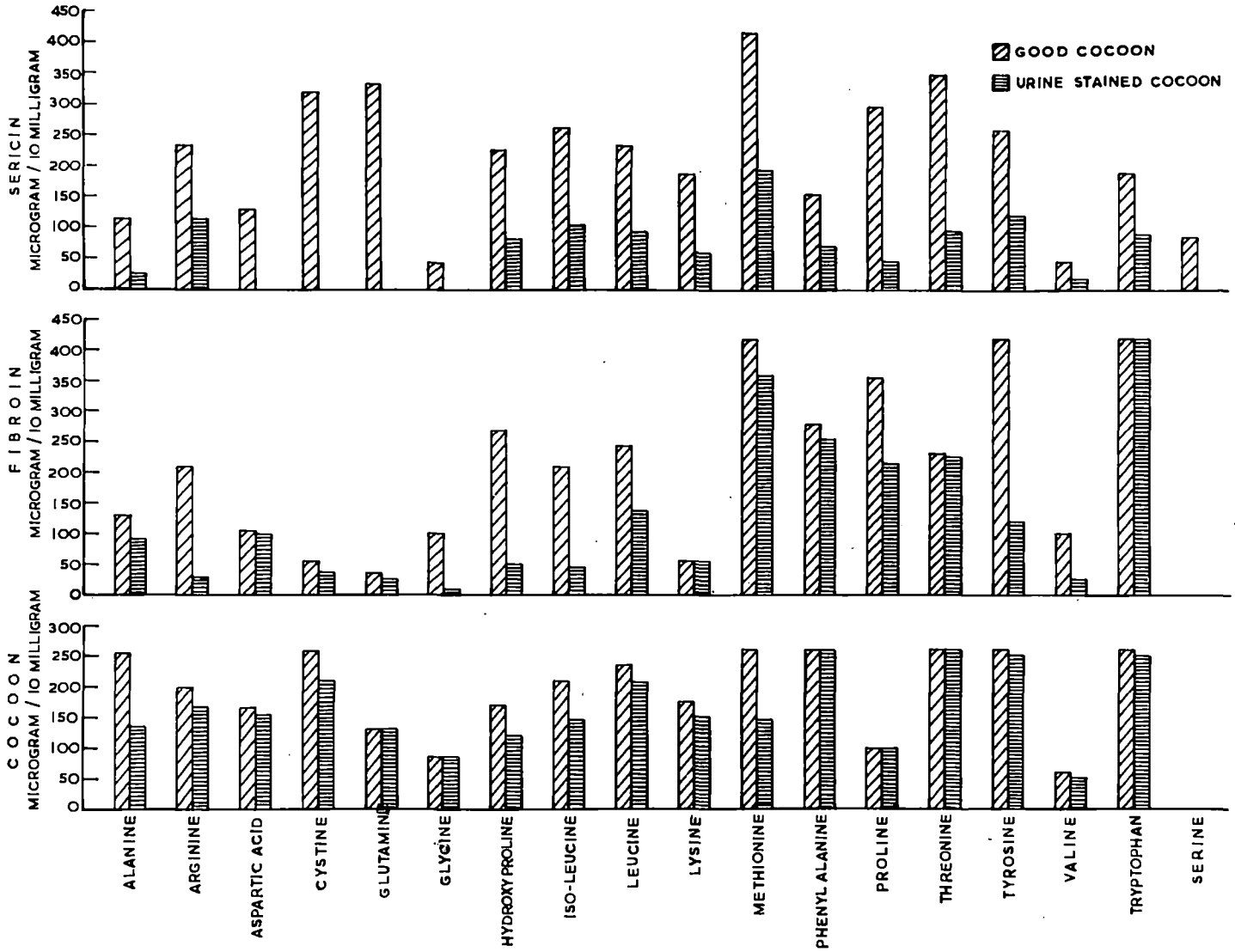


FIG. 2. AMINO ACID COMPOSITION OF SILK COCOON, FIBROIN AND SERICIN AS AFFECTED BY URINATION BY SILK WORM

In sericin fraction of the urine stained cocoon 13 amino acids were identified. However in sericin of good cocoons, 16 amino acids were spotted. 17 amino acids were detected in fibroin of urine stained and good cocoons. Serine is found to be present only in sericin of good cocoons. Quantity of some of the amino acids are less in sericin and fibroin of urine stained cocoons compared to that of sericin and fibroin of good cocoons. Cystine, methionine, phenylalanine, threonine, tyrosine, tryptophan and alanine were found to be more prominent in good cocoons. Threonine, phenylalanine, glutamin, glycine and proline are unaltered by urination of silk worms. While methionine, tyrosine, tryptophan, proline and hydroxyproline were found to be more prominent in fibroin of good cocoons, methionine, threonine, proline, glutamin and cystine were prominent in sericin of good cocoons. Amino acids in sericin appeared to have been more affected by urination than the amino acids of fibroin of cocoons as such. Similarly aspartic acid and tryptophan seem to have not altered appreciably in fibroin portion of the urinated cocoons.

(iv) Carbohydrate in different parts of silk cocoon:

The results of the total carbohydrate content of urine stained and normal cocoon, fibroin and sericin of normal as

well as urine stained cocoons are given in Table 3.

Sericin of good cocoons contained 83 ppm of carbohydrates whereas sericin of urine stained cocoons contained only 30 ppm. Fibroin of normal and affected cocoons contained less carbohydrates (40 ppm and 26 ppm). Normal whole cocoons contained 53 ppm of carbohydrate whereas urine stained whole cocoons contained 43 ppm of carbohydrate.

(v) Mineral constituents in silk cocoons, sericin and fibroin: Mineral composition of silk cocoons, good as well as urine stained ones was determined and the results are presented in Table 4. Mineral constituents are found to be more in the urine stained cocoons as compared to the normal ones.

The amounts of K, Ca, and Na, found in sericin and fibroin of normal as well as urine stained cocoons are presented in Table 6. K, and Na are found to be more in case of sericin and fibroin of urine stained cocoons as compared to those in sericin and fibroin of normal cocoons.

Total ash in normal as well as urine stained cocoons was determined. The results are presented in Table 8. Ash percentage in fibroin and sericin of urine stained cocoons is found to be more than the ash percentage in sericin and

TABLE 6

Calcium, potassium and sodium in sericin and fibroin fractions of normal and urine stained silk cocoons

Constituents	Normal cocoons		Urine stained cocoons	
	Sericin	Fibroin	Sericin	Fibroin
Calcium ppm	11.00	35.00	13.00	16.50
Potassium ppm	35.00	5.50	125.00	10.00
Sodium ppm	160.00	175.00	175.00	225.00

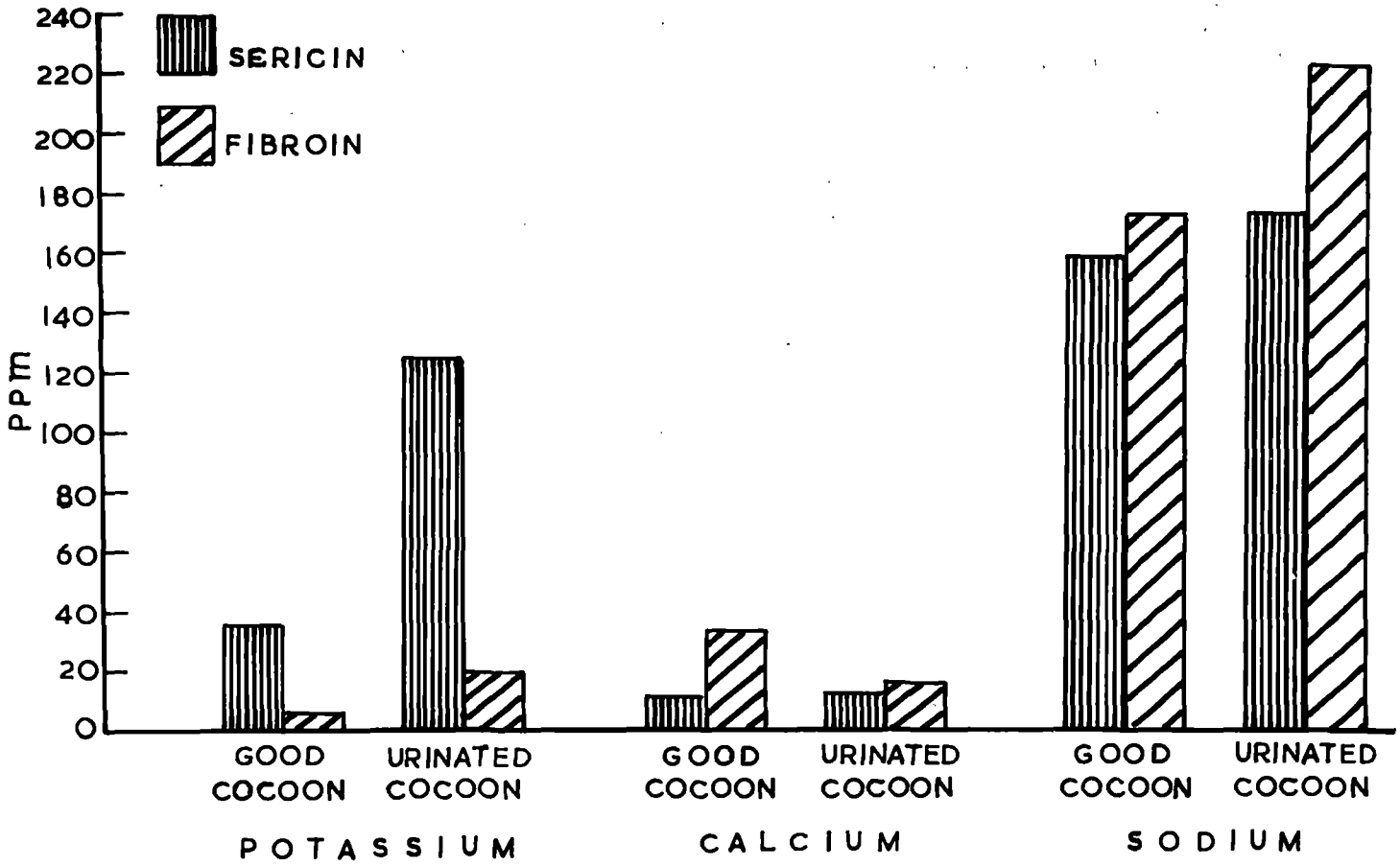


FIG. 3. POTASSIUM, CALCIUM AND SODIUM IN GOOD AND URINE STAINED COCOONS

fibroin of normal cocoons.

4. Analysis of silk from different portions of cocoon:

(i) Sericin and fibroin: The percentages of sericin and fibroin in different portions of urine stained and normal silk cocoons were estimated and the results are presented in Table 7. Sericin in both urine stained as well as good cocoons steeply decreased towards inner core. However in the middle portion of urine stained cocoons, sericin percentage was more than in the similar portion of the normal cocoons. Although fibroin was found to be slightly more in normal cocoons, its percentage was found to gradually increase towards inner core of both the normal and urine stained cocoons.

(ii) Mineral composition of different parts of the cocoons: Reference to Table 8, shows that potassium in urine stained cocoons is found to be more than that in normal cocoons in all the three portions. Calcium in sericin of urine stained cocoons was more than that of fibroin in all the three portions and gradually increased towards the inner core. Sodium in sericin of urine stained cocoons was more in/inner core than in the outer cover. In the sericin of middle portion of both normal and urine stained cocoons, sodium content was the same. Potassium in fibroin of outer

TABLE 7

Fibroin and sericin in different parts of silk cocoons

Parts of silk cocoon	Normal cocoons		Urine stained cocoons	
	Fibroin %	Sericin %	Fibroin %	Sericin %
Outer cover	53.11	44.68	51.89	44.17
Middle portion	55.16	28.57	53.98	32.52
Inner core	59.36	17.14	59.20	17.10

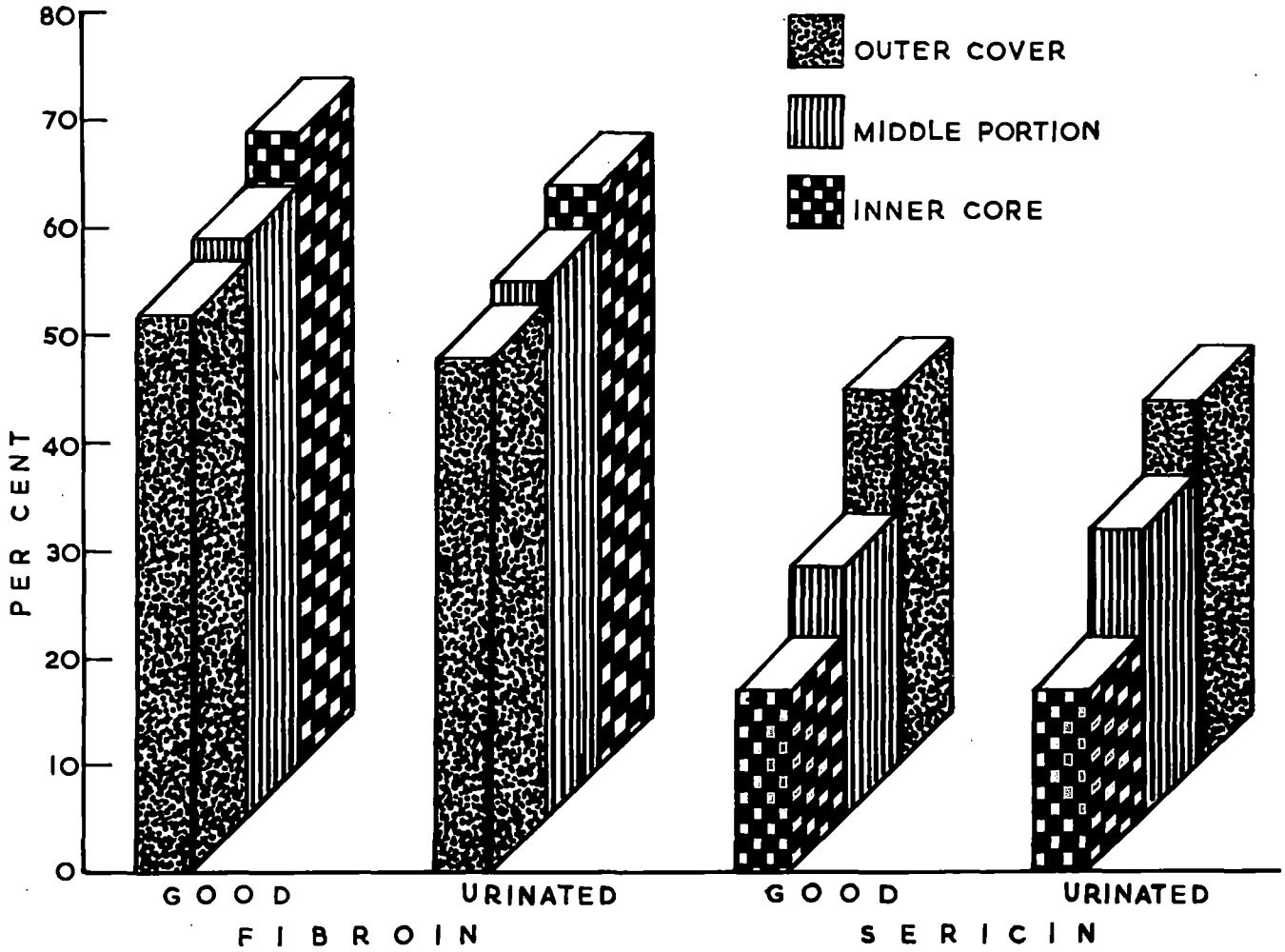


FIG. 4. PERCENTAGE OF SERICIN AND FIBROIN IN OUTER, MIDDLE AND INNER PORTIONS OF SILK COCOONS

TABLE 8

Total ash, calcium, potassium and sodium in sericin and fibroin of different parts of normal and urine stained silk cocoons

Portion of cocoon	Constituents	Normal cocoon		Urine stained cocoons	
		Sericin	Fibroin	Sericin	Fibroin
Outer cover	Total ash %	0.06	0.996	0.96	2.15
	Ca ppm	17.50	10.25	36.50	19.50
	K ppm	41.50	1.50	51.50	1.50
	Na ppm	1500.00	21.00	2400.00	64.00
Middle portion	Total ash %	0.53	0.04	0.84	2.03
	Ca ppm	21.50	9.00	38.00	18.00
	K ppm	13.00	1.49	73.00	1.50
	Na ppm	2500.00	24.00	2500.00	70.00
Inner core	Total ash %	0.50	1.20	0.97	2.43
	Ca ppm	12.00	15.50	41.00	20.00
	K ppm	25.50	1.00	42.00	2.50
	Na ppm	1500.00	23.50	2600.00	55.00

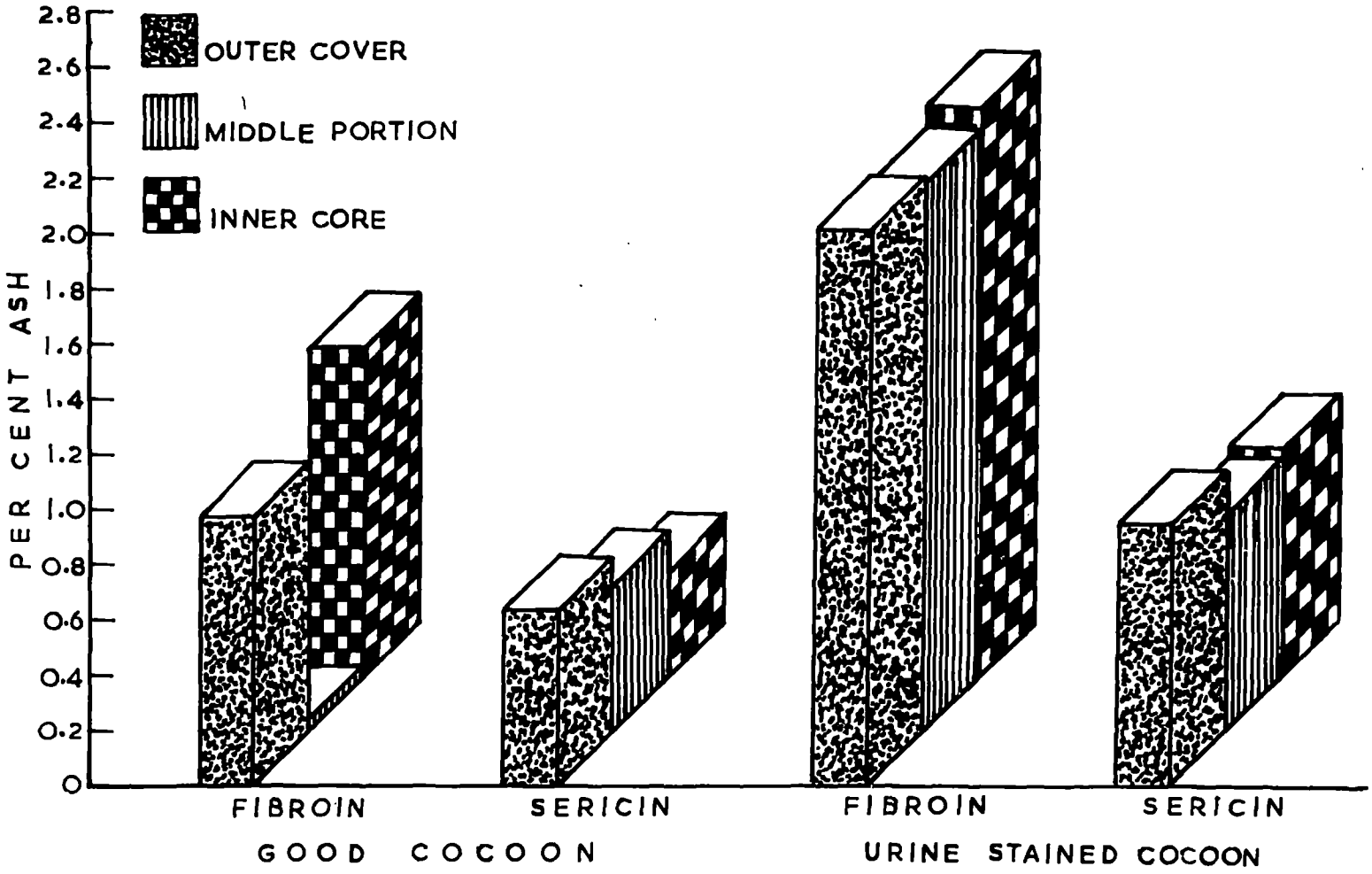


FIG. 5. PERCENTAGE OF ASH IN OUTER, MIDDLE AND INNER PORTIONS OF SERICIN AND FIBROIN OF GOOD AND URINE STAINED COCOONS

cover of good and urine stained cocoons was found to be the same but the fibroin of urine stained cocoons of other two parts contained slightly more potassium. The fibroin in all the three portions of urine stained cocoons contained more calcium and sodium than in the fibroin of good cocoons.

5. Extent of penetration of urine in silk cocoons:

To find out the extent of penetration of urine in cocoons, uric acid was estimated in each 100 meters of silk reeled and was compared with that of normal cocoons. In normal cocoons 0.021 per cent of uric acid was found in the first 100 meters length and in second, third and fourth 100 meters no uric acid could be traced. In the urine stained cocoons first, second, third and fourth 100 meter lengths contained 1.671, 1.520, 0.310 and 0.140 per cent uric acid respectively.

6. Reeling of urine stained cocoons:

One of the main problems of urine stained cocoons is poor reelability of such cocoons.

(i) Artificially urinated cocoons: Reelability experiments were conducted on cocoons sampled 1, 2, 3, 6, 8, 15 and 30 days after artificial urination. They were steamed for 2, 4, 6, 8 and 10 minutes and cooked (boiled)

for 4 and 6 minutes before carrying out the reeling trials to find out the best combination of treatments for reeling. These observations are recorded in Table 9. The observations are statistically analysed separately for total length per cocoon and number of cuts per cocoon. The design adopted was factorial experiment in R.C.B.D. design. Tables I and II in appendix show the analysis of variance pertaining to this study.

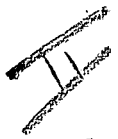
Number of cuts per cocoon was found to increase with increase in number of days after urination. Number of cuts significantly increased after second day of urination as compared to the control. No significant difference was observed between the treatments cooking and steaming. There is slight significant difference between observations of steaming and number of days interaction. The best interaction as compared to control is D_1S_4 i.e., one day after urination which was steamed for 8 minutes, whereas D_7S_5 i.e., 30 days after urination which was steamed for 10 minutes gave maximum number of cuts per cocoon.

As far as total length per cocoon is concerned the urine stained cocoons had no relation with number of days after urination. Control i.e., cocoons without urine stain were found to be significantly different from all other

TABLE 9

Effect of duration of steaming and boiling on reel

Steaming	Boiling (cooking)		1 day		2 days		3 days		
			Total length M	No.of cuts	Total length M	No.of cuts	Total length M	No.of cuts	Tot len M
2 min.	4 min.	R1	300	-	380	2	390	15	44
		R2	390	-	400	1	400	3	32
		R3	370	-	400	1	380	8	32
		R4	280	5	300	1	340	-	30
	6 min.	R1	320	1	450	2	430	10	51
		R2	450	2	400	1	380	12	43
		R3	300	1	360	2	300	9	48
		R4	400	1	420	-	360	7	39
4 min.	4 min.	R1	250	-	300	1	350	1	25
		R2	280	-	380	1	320	12	42
		R3	400	-	350	-	300	2	41
		R4	380	2	320	3	350	7	36
	6 min.	R1	290	-	250	3	350	2	30
		R2	250	2	280	1	400	9	32
		R3	300	1	320	1	320	7	41
		R4	420	-	410	-	300	11	46
6 min.	4 min.	R1	400	-	360	3	320	12	36
		R2	340	1	480	1	350	7	42
		R3	300	2	450	1	340	-	48
		R4	370	-	320	1	400	3	32
	6 min.	R1	500	1	450	-	420	4	32
		R2	390	1	300	3	450	2	32
		R3	420	-	390	2	400	3	30
		R4	350	2	400	1	380	2	41
8 min.	4 min.	R1	390	-	490	-	300	2	48
		R2	320	-	450	1	440	9	45
		R3	320	-	450	-	400	7	42
		R4	300	1	410	2	360	5	39
	6 min.	R1	380	-	480	1	390	10	36
		R2	400	-	460	-	420	2	40
		R3	450	-	380	2	490	1	46
		R4	350	2	420	1	310	7	45
10 min.	4 min.	R1	350	1	480	3	400	3	52
		R2	410	-	450	1	380	7	46
		R3	460	-	360	-	350	2	4
		R4	440	1	400	-	460	4	42
	6 min.	R1	440	-	410	-	400	5	45
		R2	390	1	490	2	440	4	41
		R3	350	-	450	1	490	2	37
		R4	400	1	390	2	400	2	48



bility of urine stained silk cocoons

6 days		8 days		15 days		30 days		Control	
l gth	No.of cuts	Total length M	No.of cuts	Total length M	No.of cuts	Total length M	No.of cuts	Total length M	No.of cuts
)	10	300	12	450	10	500	2	450	-
)	4	580	8	420	7	400	25	480	1
)	15	410	5	450	10	350	5	490	-
)	3	440	1	480	4	400	2	450	-
)	2	350	13	350	15	450	3	510	-
)	9	490	4	300	12	440	5	500	-
)	7	350	15	420	2	350	15	470	-
)	12	430	1	420	1	380	15	480	-
)	18	410	2	400	9	460	5	480	-
)	2	470	1	380	6	480	5	470	-
)	3	400	10	460	3	310	9	480	-
)	9	390	7	400	12	300	12	460	-
)	8	410	5	300	9	390	13	410	1
)	12	350	11	290	15	340	9	490	-
)	7	420	9	320	10	310	7	460	-
)	5	450	6	360	5	480	5	460	-
)	9	580	10	450	14	460	15	450	-
)	5	410	12	440	8	480	18	400	-
)	5	300	13	440	5	400	10	460	-
)	12	400	1	400	6	420	5	450	1
)	4	460	15	390	9	360	9	460	-
)	15	440	9	460	13	310	10	470	-
)	3	320	6	490	12	420	7	460	-
)	2	390	8	510	11	400	15	460	-
)	1	300	5	360	14	360	12	480	-
)	6	410	4	370	8	320	7	410	-
)	8	460	9	410	10	460	11	480	-
)	8	430	12	350	6	390	10	450	1
)	13	400	11	460	13	460	15	440	-
)	9	450	9	490	10	400	17	390	-
)	3	440	10	320	8	360	6	480	-
)	3	390	6	370	5	300	9	500	-
)	10	420	12	450	15	390	9	450	1
)	7	390	11	440	9	480	14	380	1
)	9	320	7	400	7	460	14	410	-
)	3	400	5	320	11	400	10	420	-
)	7	480	9	410	13	450	7	490	-
)	7	390	7	480	11	400	10	450	-
)	10	360	12	480	8	350	15	470	1
)	3	410	7	360	7	310	9	490	-

treatments. All the urine stained cocoons had less total length per cocoon as compared to the non-stained cocoons.

Total length of silk yarn obtained per cocoon is not affected by the number of days after urination and steaming. Slight variation observed between the different treatments are not significant. However the normal cocoon reeling values at all the durations of steaming tried are found to be significantly different from that of the urine stained cocoons.

(ii) Naturally urine stained cocoons: Reeling experiments were done with naturally urine stained cocoons, sampled 2, 4, 6 and 8 days after urination. They were given hot air stifling, steam stifling and no stifling treatments. The observations regarding the total length of silk per cocoon and number of cuts occurred while reeling are given in Table 10. Results of total length and number of cuts were statistically analysed. The statistical design applied was similar to that of split plot design. The analysis of variance tables for the two different sets of observations, are presented in Tables III and IV of appendix.

The results reveal that the total length of silk thread per cocoon is not affected by the number of days

I

TABLE 10

Total length of silk fibre and number of cuts per cocoon obtai
treated with different stifling

No.of days	Treatments	Replications R_I Total length M	R_I No.of cuts	R_{II} Total length M	R_{II} No.of cuts
2 days	Steam stifling	300	3	200	5
	Hot air stifling	350	2	430	4
	Without stifling	320	-	430	1
	Control	420	-	360	-
4 days	Steam stifling	400	8	280	15
	Hot air stifling	200	15	250	15
	Without stifling	380	5	370	1
	Control	300	1	370	1
6 days	Steam stifling	250	15	300	16
	Hot air stifling	350	7	300	14
	Without stifling	300	8	320	7
	Control	420	2	450	1
8 days	Steam stifling	290	17	350	15
	Hot air stifling	300	15	340	12
	Without stifling	410	9	360	6
	Control	450	-	480	-

ed during reeling of urine stained cocoons process

R_{III}		R_{IV}		R_V	
Total length M	No.of cuts	Total length M	No.of cuts	Total length M	No.of cuts
250	4	300	3	270	6
300	3	350	4	350	3
320	1	370	-	300	1
380	1	400	-	390	1
250	13	250	20	200	12
220	13	200	15	180	15
400	2	350	5	320	4
400	-	470	-	420	-
280	15	250	12	200	18
350	15	270	15	200	15
280	14	350	4	200	5
470	1	380	1	460	3
300	12	250	20	310	15
260	9	210	14	350	8
440	5	400	1	400	5
420	1	390	-	490	1

after urination. Different types of stifling significantly affect the total length of silk thread per cocoon. There is a significant difference between the total length of silk thread reeled from steam stifled, not stifled (un-treated) and normal cocoons. Similarly, length of thread reeled from hot air stifled batch is significantly different only with control though untreated batch of cocoons has given much better reeling than hot air stifling. However, there was no significant difference in the length of the thread reeled from cocoons subjected to steam and hot air stifling. Total length reeled out from control batch (not affected by urine) is significantly different from steam stifled and hot air stifled batches but not significantly different from that of non stifled batch. The average length in different stifling treatments are as follows.

Steam stifling 274 meters,
Hot air stifling 280 meters,
without stifling 351 meters and
control 416 meters.

Unstained cocoons gave 0.70 cuts per cocoon, stained cocoons without stifling have 4.20 cuts per cocoon while hot air stifling and steam stifling gave respectively 10.65 and 12.20 cuts per cocoon. The differences noticed are

statistically significant.

As far as number of cuts per cocoon in interaction (different treatments vs number of days after urination) is concerned, steam stifling has given more number of cuts and is significantly different from all other treatments except with hot air stifling on 2nd and 4th day after urination. Number of cuts obtained during reeling of urine stained but not stifled cocoons are comparatively less in number and the difference noticed is significant except with number of cuts obtained from unstained cocoons. The average number of cuts per cocoon under interaction are presented in Table 11.

(iii) Microscopic observation of urine stained silk: Silk threads carefully reeled from urine stained cocoons as well as from the normal cocoons were examined under a microscope. The affected silk threads were found to have irregular surfaces and non-uniform sericin coating. Photomicrographs of the normal and urine stained silk threads are shown in Figures 7 to 16.

(iv) Loss of sericin during cooking: During the cooking process 16.79 per cent sericin is lost from both the normal as well as the urine stained cocoons. Thus the

TABLE 11

Average length of silk and number of cuts per cocoon in the interaction of different stifling process and number of days after urination

No.of days after urination	Steam stifling (stained cocoons)		Hot air stifling stained cocoons		Hot stifled stained cocoons		Control normal cocoons	
	Length meters	No.of cuts	Length meters	No.of cuts	Length meters	No.of cuts	Length meters	No.of cuts
2nd day	264	4.25	356	3.25	348	0.60	390	0.40
4th day	276	13.60	210	14.60	316	3.40	392	0.40
6th day	256	15.20	294	13.25	290	7.60	436	1.60
8th day	300	15.80	292	11.60	402	5.25	446	0.40

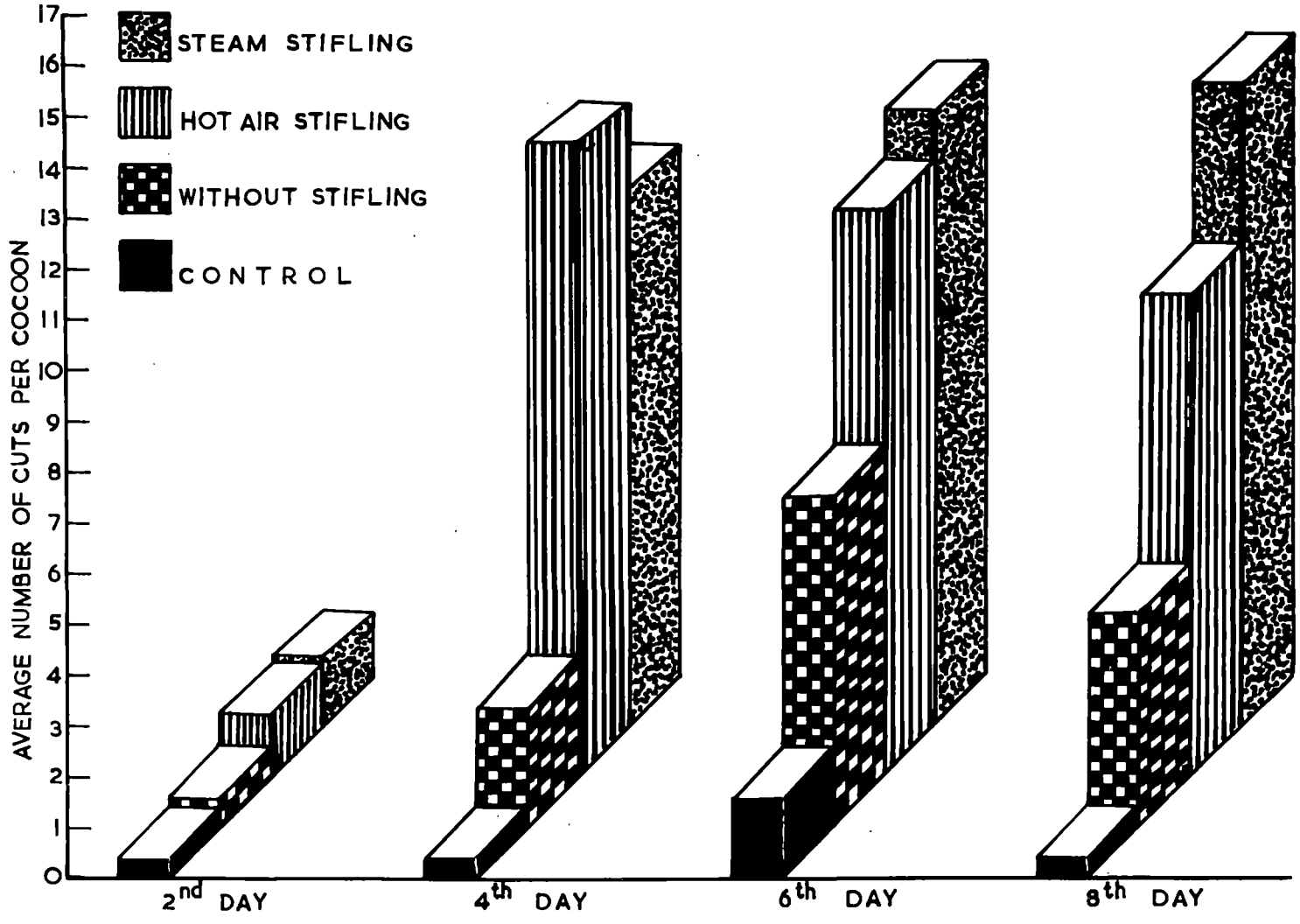


FIG.6. AVERAGE NUMBER OF CUTS PER COCOON WHILE REELING WITH INCREASING PERIODS OF CONTACT AFTER URINATION AND METHODS OF STIFLING COCOONS

Fig. 7 - Urine stained silk thread. A knot is seen and on either side bavos are apart.

Fig. 8 - Urine stained silk thread, a cut is observed.

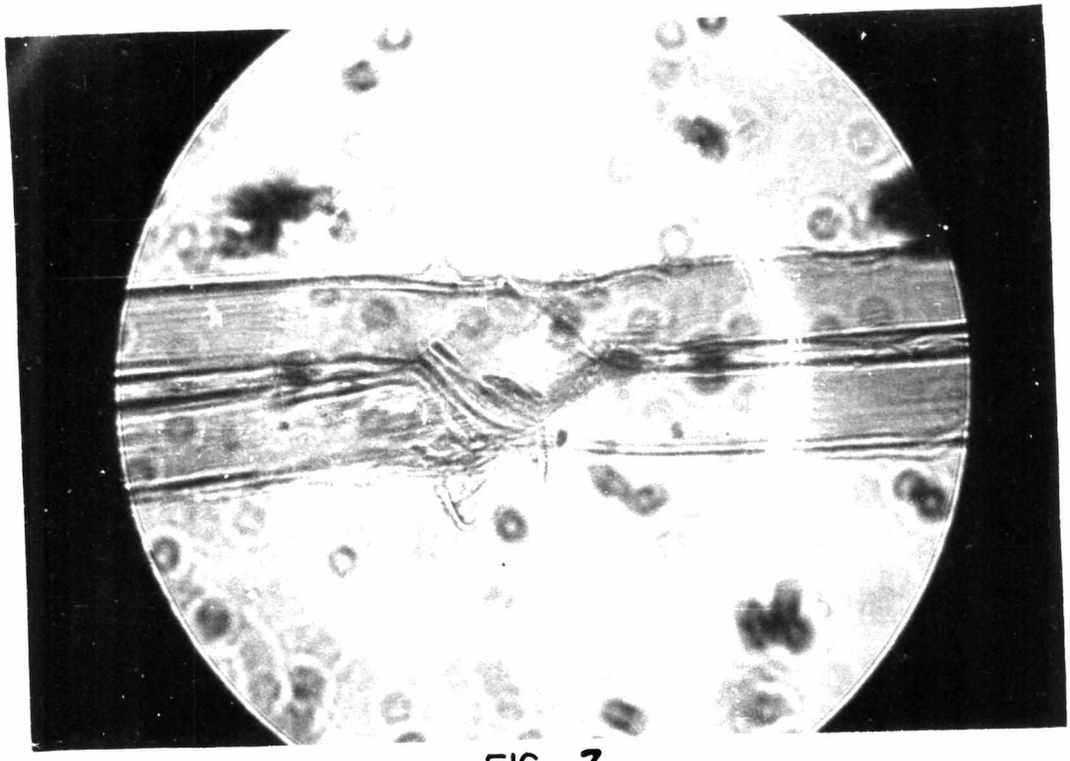


FIG. 7.

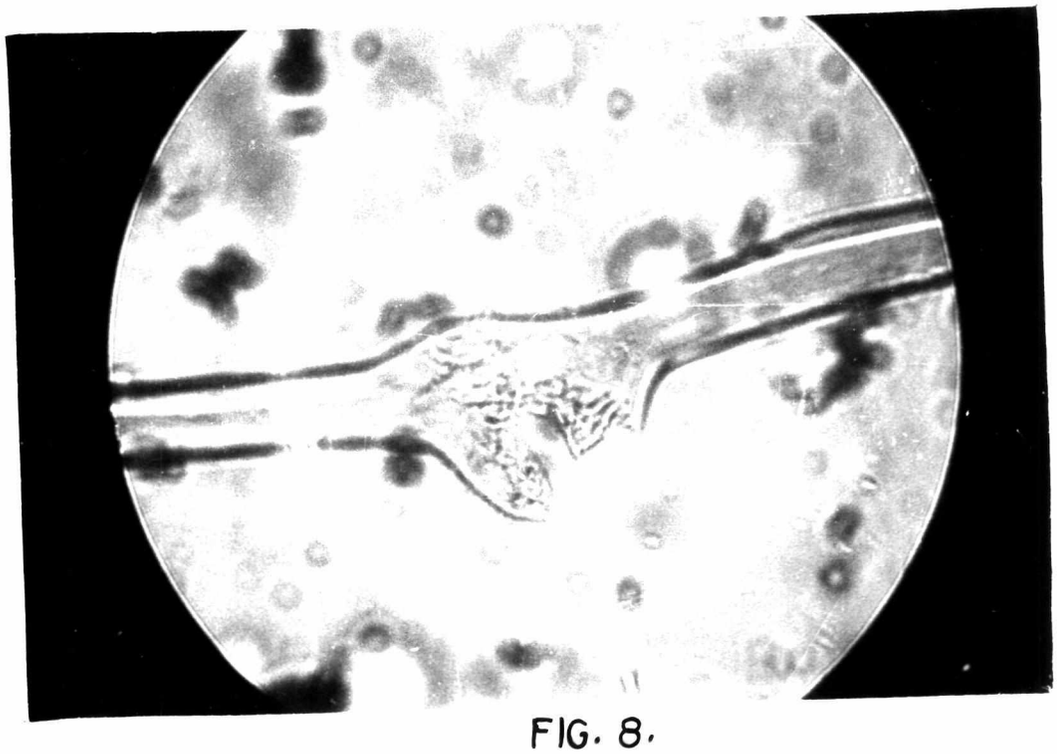


FIG. 8.

Fig. 9 - Urine stained silk thread, knot is small and the baves on either side are not uniform.

Fig. 10 - Urine stained silk thread. Baves are wide apart due to dissolution of sericin between baves.

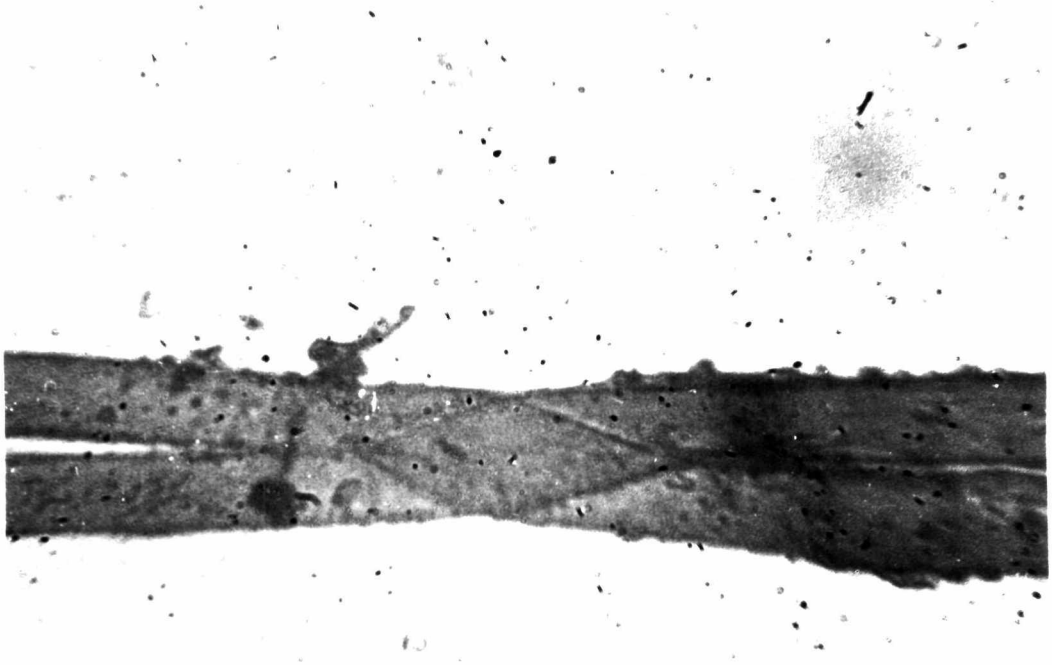


FIG. 9.

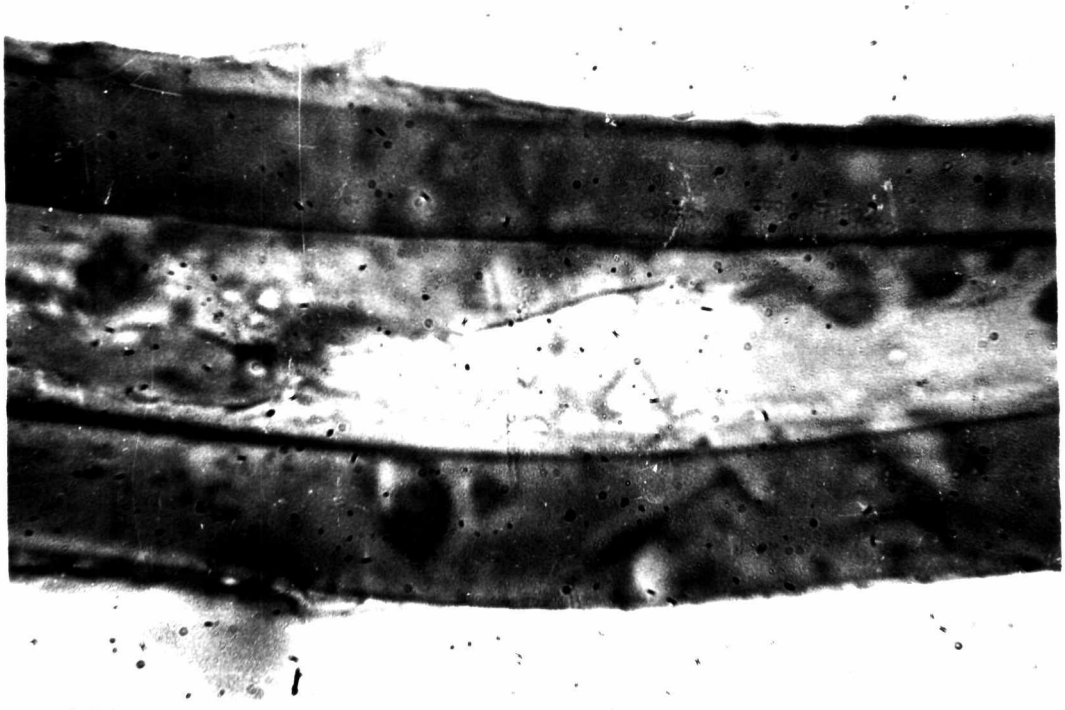


FIG. 10.

Fig. 11 - Irregular dissolution of sericin is clearly seen in urine stained silk thread.

Fig. 12 - Urine stained silk thread. Small knot found, but on either side baves are uniform.

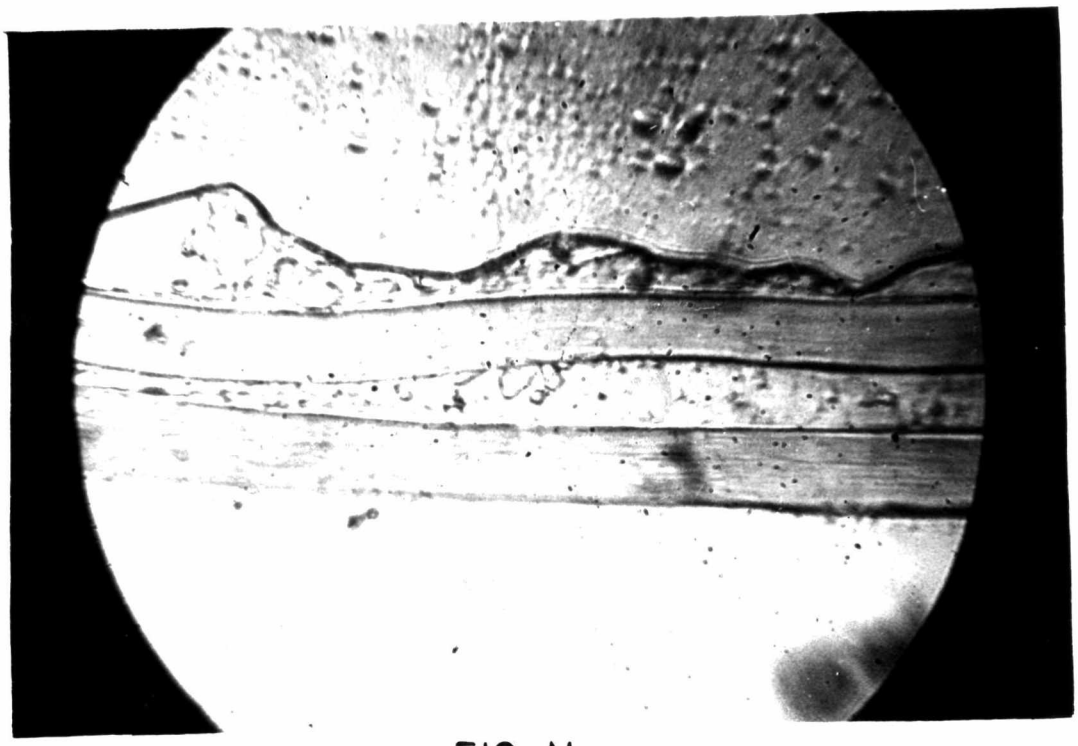


FIG. 11.

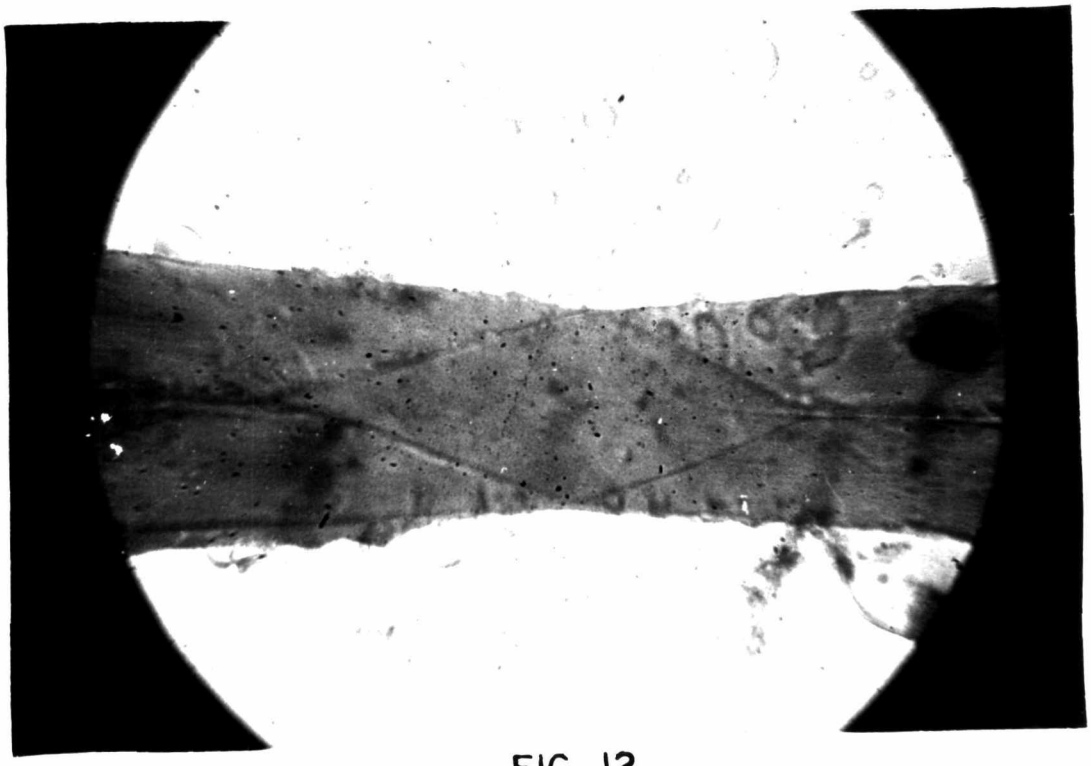


FIG. 12.

Fig. 13 - Normal silk thread. Waves found quite uniform throughout.

Fig. 14 - Urine stained silk cocoons. Shrinked and deshaped.

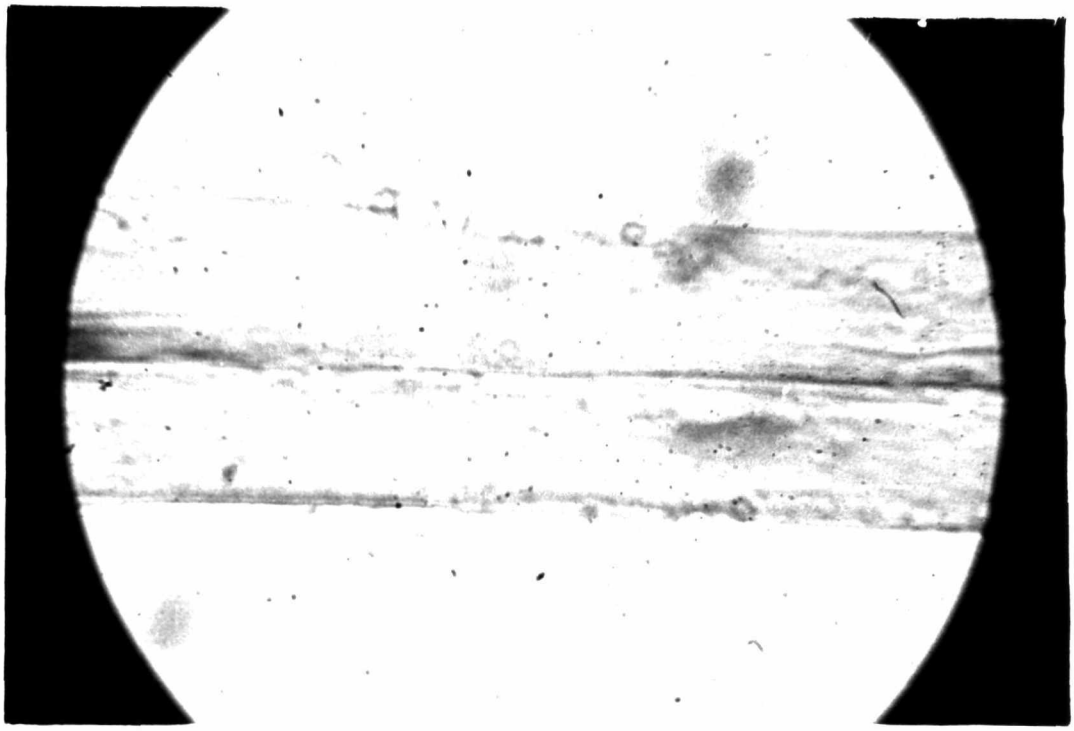


FIG. 13.

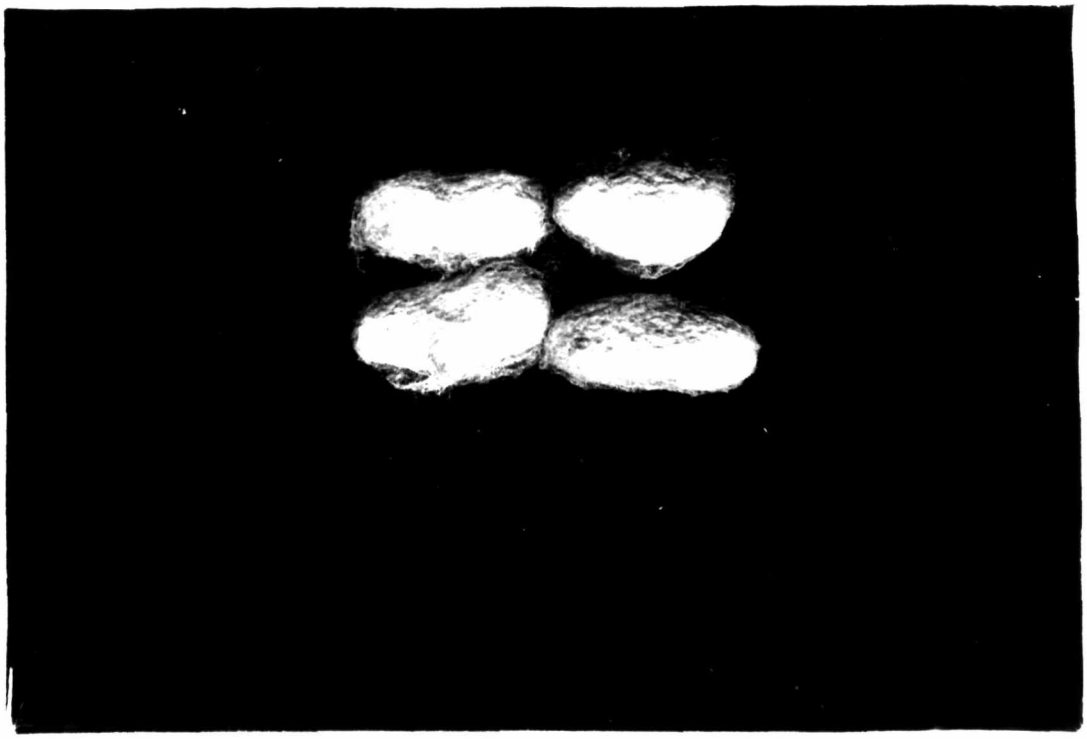


FIG. 14.

Fig. 15 - Normal silk cocoons.

Fig. 16 - Comparision of urine stained and good cocoons.
(Right hand side urine stained cocoons)

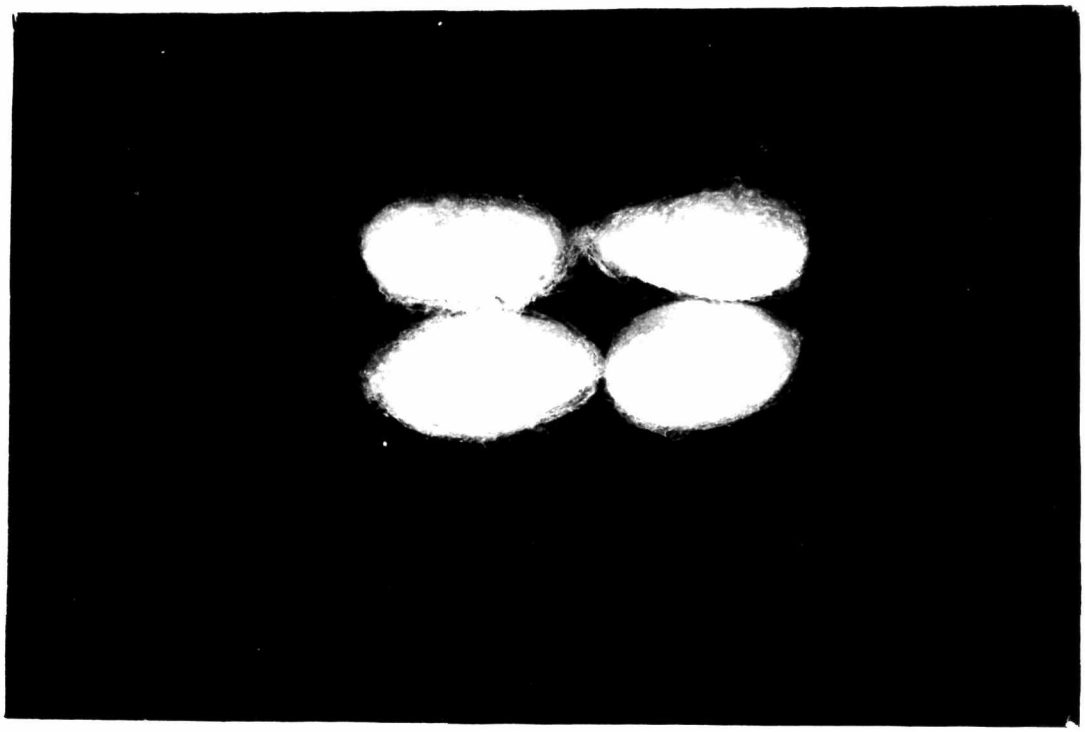


FIG. 15.

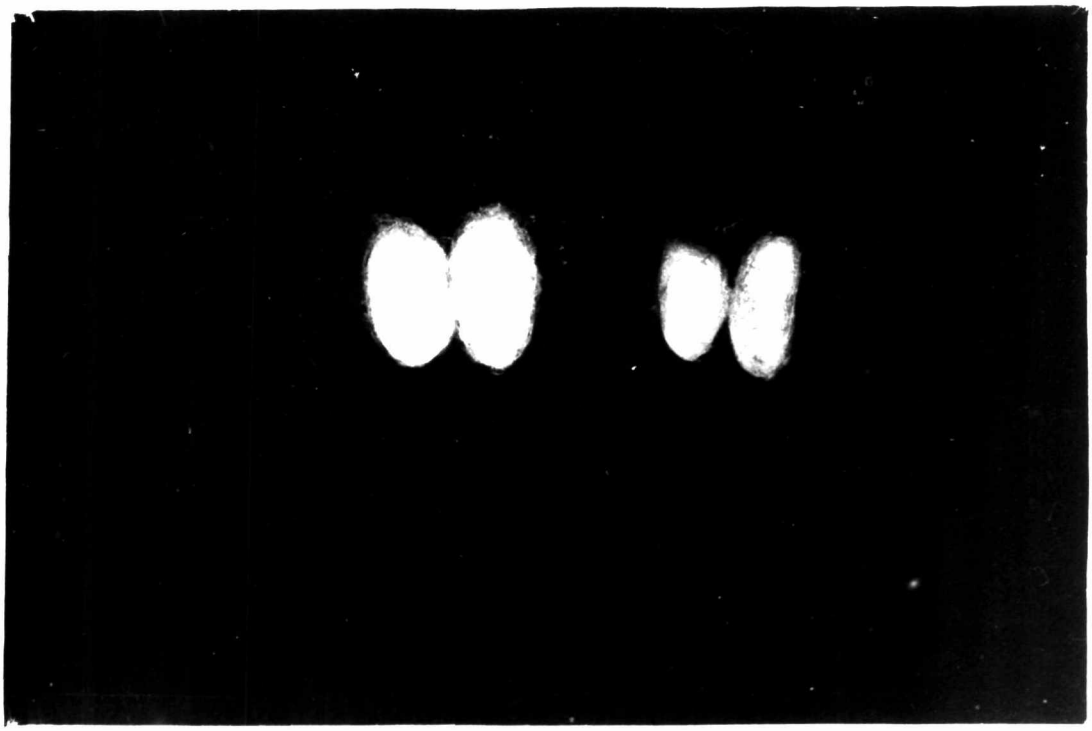


FIG. 16.

Fig. 17 - Amino acids (by two dimensional chromatography) in normal silk cocoons.

Fig. 18 - Amino acids (by two dimensional chromatography) in urine stained silk cocoons.

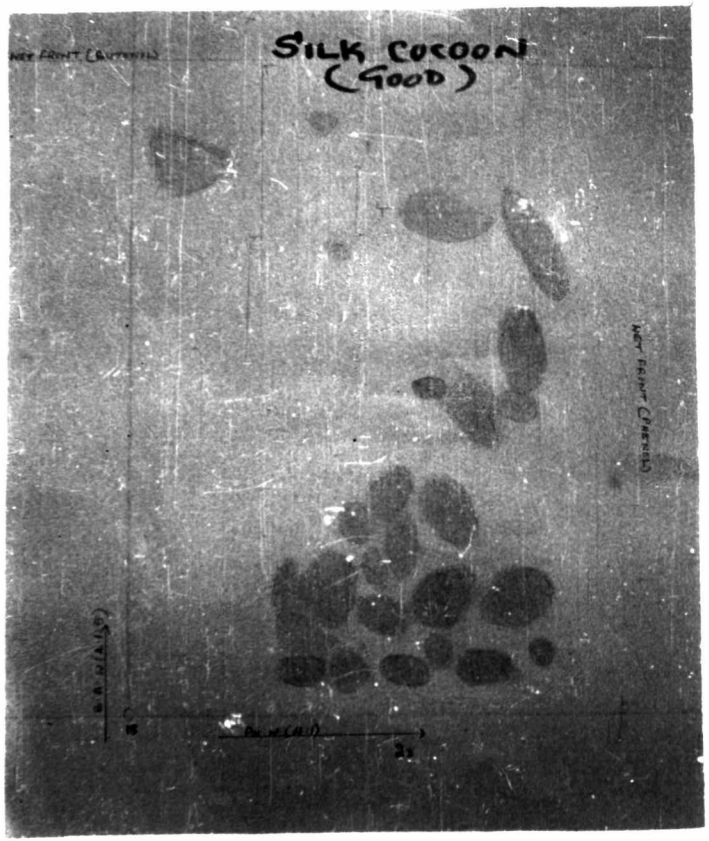


FIG. 17.

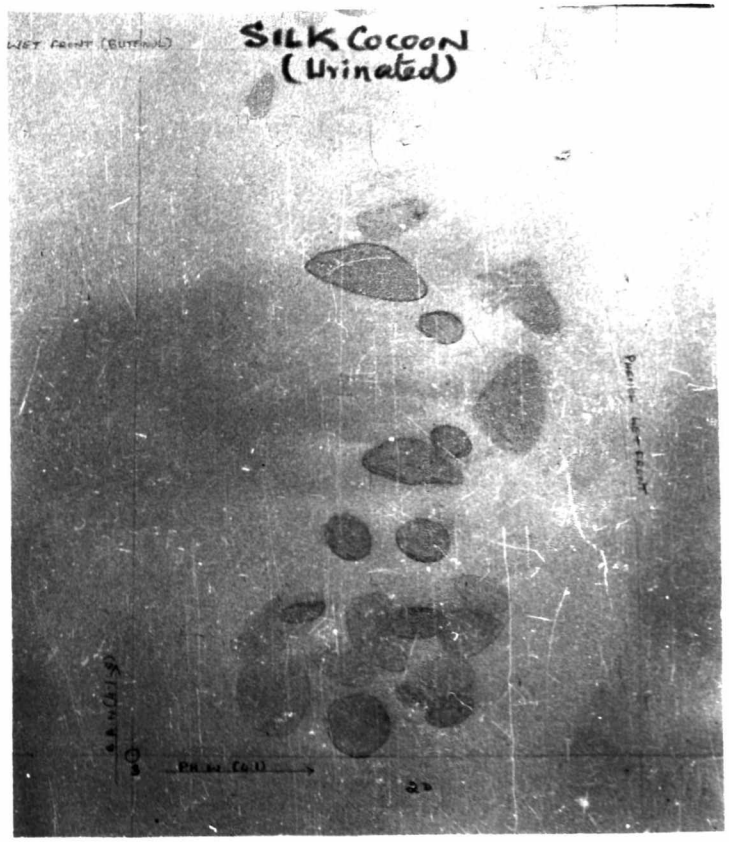


FIG. 18.

Fig. 19 - Amino acids (by two dimensional chromatography) in fibroin of normal silk cocoons.

Fig. 20 - Amino acids (by two dimensional chromatography) in fibroin of urine stained silk cocoons.

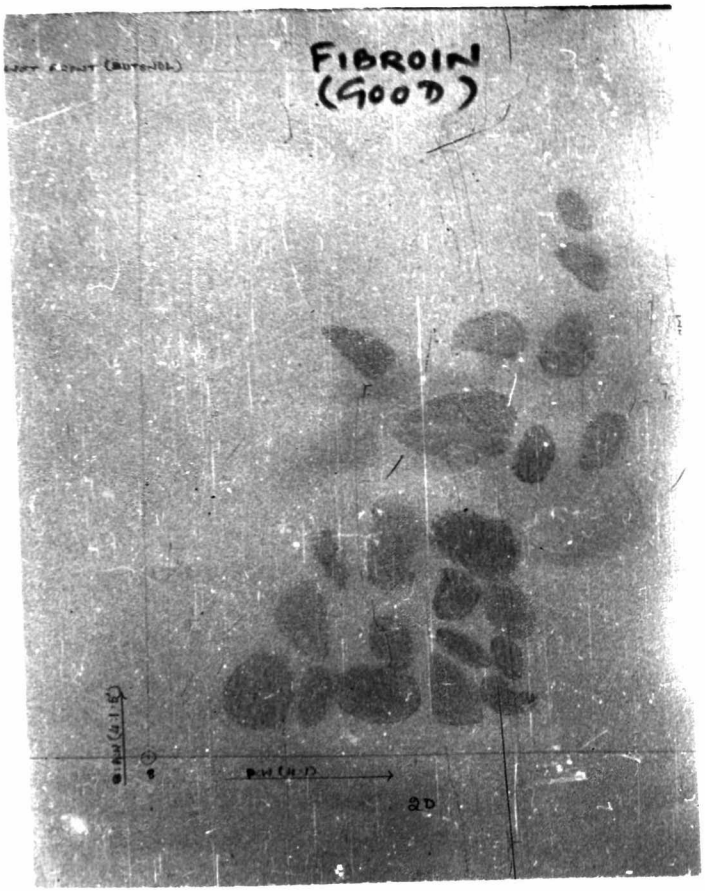


FIG. 19.

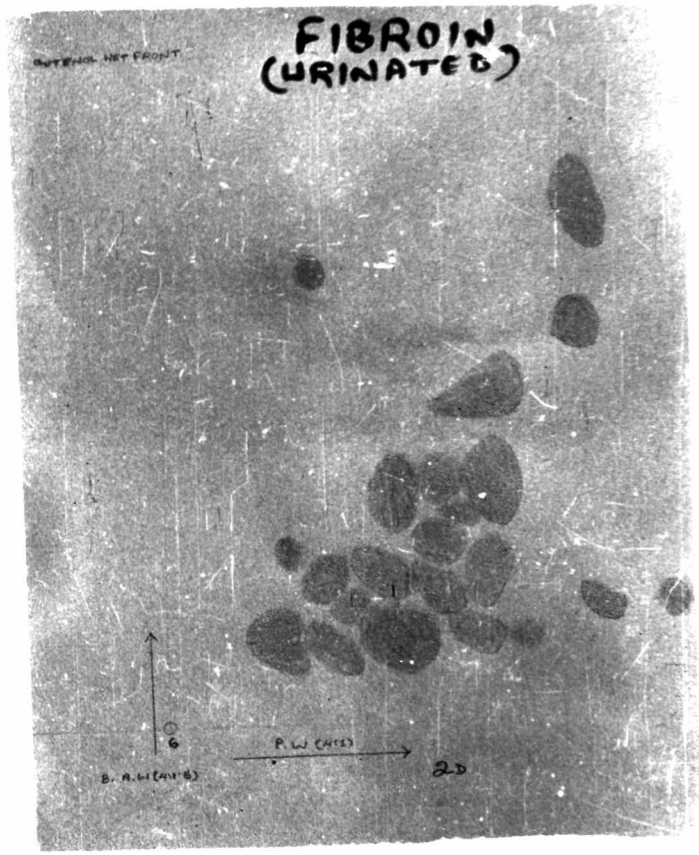


FIG. 20.

Fig. 21 - Amino acids (by two dimensional chromatography) in sericin of normal silk cocoons.

Fig. 22 - Amino acids (by two dimensional chromatography) in sericin of urine stained silk cocoons.

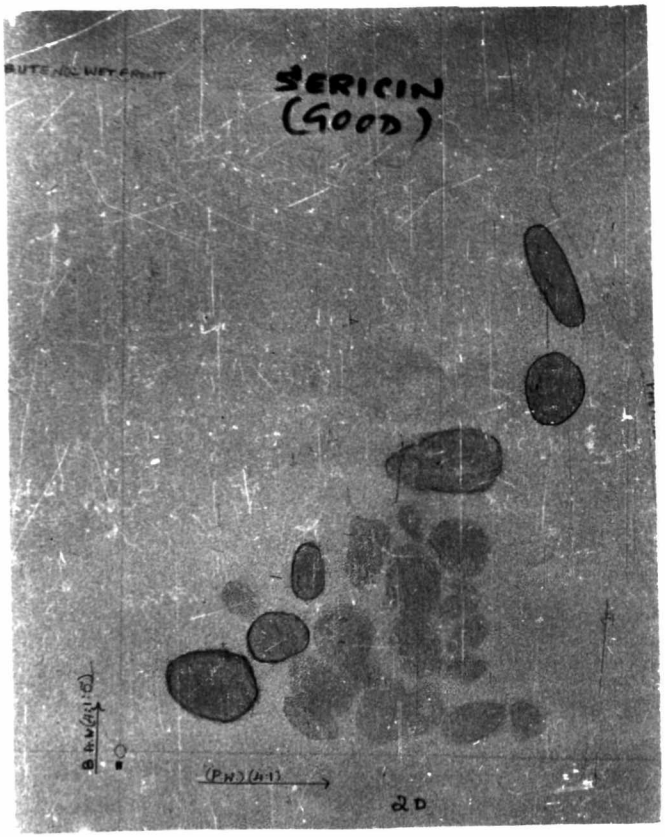


FIG. 21.

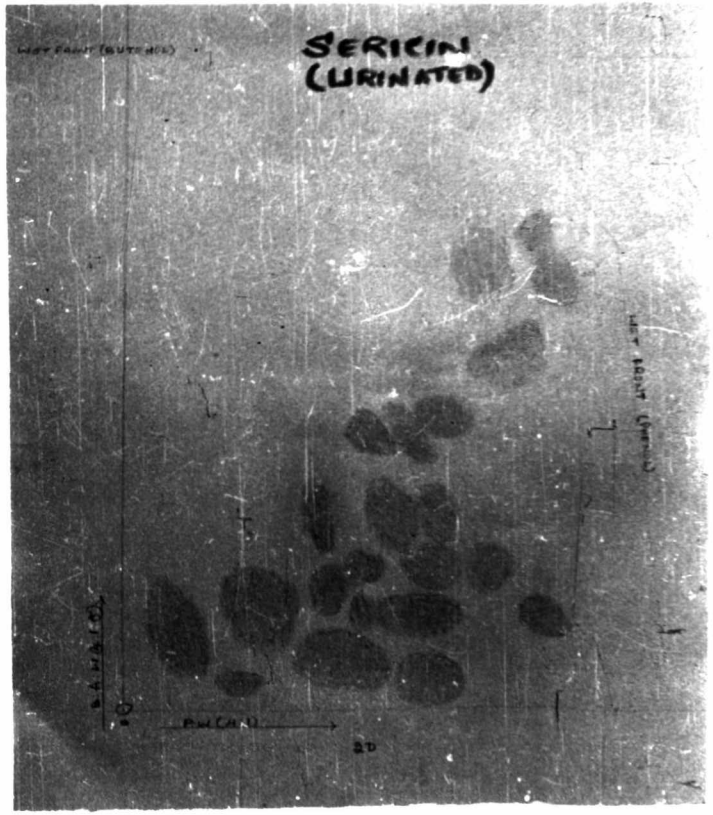


FIG. 22.

loss of sericin during cooking does not appear to be affected by urine staining of cocoons.

(v) Effect of treatment with different softening agents on urine stained cocoons: Urine stained cocoons were treated with different softening agents. They were then reeled to observe the total length of silk and number of cuts per cocoon. The results are given in Table 12. Average total length of silk reeled under different treatments were as follows:

Control urine stained (but not treated with softening agents	:	280 meters per cocoon
Sodium sulphite	:	416 meters per cocoon
Sodium carbonate	:	376 meters per cocoon
Sodium hydroxide	:	356 meters per cocoon

Average number of cuts per cocoon reeled under different treatments were as follows:

Control	:	19.00 cuts per cocoon
Sodium Sulphite	:	8.60 cuts per cocoon
Sodium carbonate	:	12.60 cuts per cocoon
Sodium hydroxide	:	13.40 cuts per cocoon

The observations were statistically analysed and the

TABLE 12

Effect of softening agents on the length of silk thread and number of cuts during reeling of the treated urine stained silk cocoons

Softening agents	R_I		R_{II}		R_{III}		R_{IV}		R_V		Average total length M	Average No. of cuts
	Total length M	No. of cuts	Total length M	No.of cuts	Total length M	No.of cuts	Total length M	No.of cuts	Total length M	No.of cuts		
Control	250	20	290	15	250	16	310	25	300	19	280	19.0
Sodium sulphate	410	6	460	9	390	7	420	12	400	9	416	8.6
Sodium carbonate	360	9	450	12	420	8	340	16	310	18	376	12.6
Sodium hydroxide	280	15	350	15	400	12	390	10	360	15	356	13.2

tables of analysis of variance for total length and number of cuts per cocoon are given in Table V and VI of the appendix. The differences observed between treatments are found to be statistically significant. Sodium sulphite, sodium carbonate and sodium hydroxide have all given more length of silk and less number of cuts per cocoon when compared with control.

7. Solvents to remove the urine stain:

Urine stained cocoons were treated with 'Teepol' a laboratory detergent and alkalies like sodium hydroxide and sodium carbonate. It was observed that urine stained cocoons kept in 'Teepol' for overnight showed slight bleaching of stain. Sodium hydroxide and sodium carbonate were tried at 0.5, 1.0 and 1.5 per cent concentrations but no improvement was observed. At higher concentrations dissolving action of alkali was observed.

The following solvents and solutions were tried in cold as well as worm conditions on urine stained cocoons to remove the stain: distilled water, absolute alcohol, acetic acid, petroleum ether, acetone, chloroform, methanol, benzene 5 per cent, EDTA 2 per cent and 5 per cent, formic acid, 5 per cent, formaldehyde 5 per cent, ascorbic acid 5 per cent, sodium cyanide 0.5 per cent, hydroxy quinoline

5 per cent, dithiocarbate 5 per cent, diethyl glyoxime 5 per cent and borate buffer. None of these solvents and solutions could effectively remove the stain on the silk cocoons caused by urination.

The treatment viz., keeping cocoons in hydrogen peroxide (6 per cent) and gradually raising the temperature upto 65°C and then keeping in alcohol overnight removed stain on the cocoon to a considerable extent. Gloss reading was 3.5 for normal cocoons, 3.0 for treated cocoons and 2.5 for urine stained cocoons. Reflectance reading was 41 for normal cocoons, 25 for the treated and 10 for urine stained cocoons. The instrument reading was previously adjusted to 100 with standard magnesium oxide block.

DISCUSSION

CHAPTER V

DISCUSSION

It is stated earlier that little work has been done on the problem of urination of silk worm and its effect on the quality and quantity of silk reeled from the urinated silk cocoons. It is therefore difficult to compare the results of the present investigation with those published elsewhere.

1. Effect of humidity and number of worms in mountages on urination by silk worms

From the results of humidity and percentage of stained cocoons recorded (Table 1), it could be observed that with the increase in humidity in the atmosphere the percentage of urine stained silk cocoons also increased. During the rainy days generally urination problem is severe because of the high humid condition coupled with fall in atmospheric temperature. Sankowska and Kalyniak (1968) found that high humidity caused more yellow staining of silk cocoons. According to Kumararaj (1969) high humidity coupled with low temperature results in heavy urination of silk worms. Madhava Rao (1969) also reported that staining of silk worm cocoons is more during rainy season.

Diuresis in vertebrates as well as in invertebrates is controlled by secretion of a hormone and this physiological process is affected by temperature and humidity. Low temperature and high humidity are found to bring about diuresis.

Madrell (1954) found that the profuse diuresis in fifth stage larvae was caused by the release of a diuretic hormone into the haemolymph. Later in 1963 Madrell demonstrated that the diuresis in insects was caused by a hormone released into the haemolymph. He also found (1964) that the urination is very sensitive to changes in temperature and that both the rate of urination and the composition of the urine are affected by changes in temperature. Mills (1967) noted that temperature had profound effect on diuresis.

From the above discussion it is evident that diuresis is both temperature and humidity controlled because the neurosecretory cells which secrete diuretic hormones are stimulated by low temperature and high humidity.

When the number of worms per Chandrike were recorded (Table 1) it was seen that less number of worms per mountage would minimise the staining of cocoons especially during heavy rainy days. The correlation co-efficient worked out between the worms per Chandrike and number of stained cocoons

is found to be positive and fairly high (0.731) revealing that more number of cocoons would be stained with increased population. In a Chandrike when the number of worms decreases, the space between cocoons increases. Hence the incidence of urine drops falling from spinning worms directly on other cocoons will be lesser than that of thickly populated mountage.

2. Constituents of silk worm urine:

When silk worm urine comes in contact with cocoons a sort of deep yellow brownish stain on cocoons is found, on drying. At the time of reeling the silk thread from stained cocoons breaks too often. This reduces not only the quantity of silk reeled but also its quality due to chemical reaction, which affects the natural colour and strength of silk.

Silk worm urine is alkaline in reaction. Its average pH is 8.6 (Table 2). This alkaline nature of the urine appears to be responsible for breaks in threads while reeling. The alkalinity of the silk worm urine dissolves the sericin coating of the filament in patches which results in breakage of silk thread while reeling (Kumararaj 1969).

Total nitrogen content of silk worm urine is only

0.028 per cent (Table 2). However the total dissolved material in urine is considerably more (2.31 per cent). This is due to high concentration of potassium, calcium and sodium (9200 ppm, 375 ppm and 95 ppm respectively) in the urine. Since all these would be in ionised state, the electrical conductivity of silk worm urine is also high (5 milimhos per cm). Phosphates, sulphates and chlorides are also found to be in fairly high amounts (4.350 mg per 100 ml, 8.2 mg per 100 ml and 0.80 mg per 100 ml of urine respectively).

The major constituent of silk worm urine is uric acid. The concentration of uric acid in the pooled sample of urine was found to be 68.927 per cent. Because of this fact, several workers thought that uric acid might be responsible for the characteristic yellow stain and filament breaking in the urine stained cocoons (Kuwana 1937 and Hiratsuka 1967). Though the silk worm urine contains about 70 per cent of uric acid, it is alkaline in reaction. According to Hawk et al., (1954), uric acid forms two classes of salts neutral and acidic. The neutral potassium and lithium urates are easily dissociated and are formed into easily soluble alkali salts while ammonium urate is more undissociated and is difficultly soluble hence the pH of the urine is alkaline in nature. However Kumararaj (1969) applied pure uric acid

on cocoons and observed that pure uric acid applied cocoons did not produce any characteristic brownish yellow stain as compared to that of silk worm urine applied cocoons. On the other hand it was observed that any alkali above 1N concentration would produce the similar brownish stain on the cocoons. This reveals that it is the alkaline nature of the silk worm urine that is responsible for the brownish yellow stain on the cocoons.

3. Composition and other properties of silk:

(a) Sericin and fibroin fractions in silk cocoons: Sericin and fibroin are the two main protein constituents of silk. Sericin, a gum like fraction forms the outer cover whereas fibroin is the true silk and forms inner most layer. Sericin in silk cocoons is found to be 27.60 per cent in good cocoons and 26.24 per cent in urine stained cocoons (Table 4). There is slight loss of sericin in urine stained cocoons. This may be due to partial solubility of sericin in silk worm urine. Fibroin in normal and urine stained silk cocoons is found to be 60.91 and 56.99 per cent respectively. Here also there is a reduction in fibroin content of cocoons. These results indicate that silk worm urine affects not only sericin but also fibroin fraction of the silk. Simizu (1957) observed that silk fibre mainly

consists of fibroin (70-80 per cent) and sericin (20-30 per cent) and Nanavathi (1965) has reported 25 per cent sericin and 75 per cent fibroin in mulberry silk. The results of the present investigation thus agree with the observations of the investigators referred above.

(b) Mineral matter in silk cocoons, sericin and fibroin: Inorganic constituents are found to be more in urine stained cocoons compared to those in the normal ones. This is expected. The analyses reveal that both sodium and potassium in sericin and fibroin of urine stained cocoons are more. This is due to the high content of inorganic substances present in silk worm urine, which will add on to the cocoons when silk worms urinate. Potassium content is very high in the silk worm urine and in urine stained cocoons. As a salt of uric acid potassium urate in the urine being alkaline in reaction reacts with the silk and causes staining and breakage of silk thread during reeling.

(c) Protein constituents of silk cocoons: According to Simizu (1957) silk fibre mainly consists of two proteins, viz., fibroin and sericin / which endow silk with a dualastic structure which is not seen in other natural and synthetic fibres (Nanavathi, 1965). Sericin fraction of silk contains

less percentage of protein than whole cocoon and fibroin fraction of silk and fibroin contains high percentage (82-86 per cent) of protein (Table 3). Urine affected sericin and fibroin fractions and whole cocoons contained comparatively less amount of protein than sericin and fibroin fractions of normal whole cocoons. This can be attributed to the denaturation of the silk proteins by alkaline nature of silk worm urine.

(d) Composition of different layers of silk cocoons: It is possible to differentiate the cocoons into three layers, viz., an outer cover, middle portion and inner core (Mosher, 1934). This differentiation is helpful in a clear understanding of the binding of sericin and fibroin on the cocoon. Sericin and fibroin were estimated in these three layers separately of the urine stained and good cocoons. The results (Table 8) show that fibroin percentage is less in outer cover and middle portion of urine stained cocoons. It is almost the same in the inner core. This reveals that fibroin in inner layer of cocoon was not affected by silk worm urine. The fibroin percentage is found to increase towards inner core whereas sericin content decreased towards inner core. Potassium, calcium and sodium in sericin of all the three layers of urine stained cocoons were more than in the good cocoons (Table 7). The inorganic constituents

are also found to be more in fibroin of all the three layers of urine stained cocoons than that in normal cocoons. The results therefore reveal that urine stained cocoons are enriched in the mineral matter as these inorganic constituents are more in silk worm urine.

(e) Extent of penetration of silk worm urine: It is important to study the damage caused by silk worm urine on different layers of cocoon by studying the extent of penetration of urine in cocoons. Uric acid was estimated in each 100 meter length filaments of both urine stained and normal cocoons. The results show that traces of uric acid could be observed only in outer 100 meter length of fibre in normal silk cocoons. Whereas in urine stained cocoons more uric acid is present in the first 100 meters of fibre and it gradually decreases towards inner layers and it is almost absent in the fourth 100 meter length of the silk filament. It may thus be seen that silk worm urine may penetrate the cocoons upto 300 meters, i.e., 3/4th of the cocoons but its concentration would be considerably more upto 200 meters, i.e., almost half of the cocoons. This implication was observed while reeling the urine stained cocoons, when upto about first 150 to 200 meters the reeling was very difficult and thereafter it was comparatively easy.

(f) Amino acids in silk: The analysis of whole cocoons and sericin and fibroin fractions of silk has indicated the presence of at least eighteen amino acids. On comparing the results of analysis of the said three fractions of silk in respect of their amino acid composition as affected by urination, it is observed that while sericin of the good cocoons contained all the eighteen amino acids, the sericin of urine stained cocoons contained only thirteen amino acids. The amino acids which are found missing in the sericin of the urine stained cocoons are aspertic acid, cystine, glutamine, glycine and serine. The amino acids present in the normal silk cocoons comparatively in larger amount are alanine, cystine, leucine, methionine, phenylalanine, threonine, tryptophan and tyrosine. Tyrosine, glycine, phenylalanine, proline, valine, tryptophan, leucine and methionine are found to be more in fibroin than in sericin fraction and whole cocoon. These findings are similar to those reported by Menedez et al., (1950), Bricteux-Gregor et al., (1959b) and Nagaraj and Basavanna (1969) as far as the amino acid composition of whole silk cocoons, fibroin and sericin fractions of silk is concerned. Glutamin, glycine, phenylalanine, proline and threonine, in whole cocoons were not affected by urination by silk worm. However, the remaining amino acid contents were

reduced by urination.

The amino acids that are found affected by urination are mostly those whose PI values are in neutral to acid range. The urine as reported earlier has pH in alkaline range. Therefore when these amino acids come in contact with the silk worm urine they behave like an anion and form salt with potassium and sodium of the urine and they get dissolved. Apart from the staining of the silk, breakage of silk thread during reeling of the urinated cocoons can be attributed to the dissolution of certain amino acids of the silk proteins in the urine.

(g) Carbohydrates in silk cocoons: In the present investigation sericin of silk cocoon is found to contain more carbohydrates than fibroin and the whole cocoons. Fibroin and sericin of urine stained cocoons contained less carbohydrates than fibroin and sericin of normal cocoons, and silk worm urine lowered the carbohydrate content of sericin to a greater extent, more than that of fibroin. While in sericin, carbohydrates are found to have been reduced by 53 ppm due to urination, in fibroin reduction in carbohydrate content is only 14 ppm. In silk fibre sericin forms the outer cover. Therefore it is first reacted by urine and loses its carbohydrates. Carbohydrates and

nitrogenous constituents of urine appear to undergo maillard type of reaction, producing brownish yellow stain on the cocoons.

4. Reeling of urine stained cocoons:

(a) Artificially urine applied cocoons: All the bad effects of urination are reflected on the reelability of the affected cocoons which lessen the marketable product of the silk industry. The results of the reeling experiments therefore reveal the extent and nature of damage caused due to urination by silk worm.

The silk thread of the urine stained cocoons do not unwind freely as in the case of the normal cocoons. There will be several cuts in the thread. The urine stained cocoons have therefore poor reelability.

Artificially urinated cocoons were steamed for five different times and also cooked for 2 different timings. Results (Table 9) show that there is no significant difference between the two times of cooking on reelability of urine stained cocoons. Loss of sericin during cooking was studied. No variation in the loss of sericin is noticed between the urine stained and normal cocoons. The urine stained cocoons were subjected to reeling test after different

number of days of urination treatment. Number of cuts per cocoon while reeling gradually increased as the number of days increased after urination treatment. However, the number of cuts remained constant from 8th day of urination onwards. The results of one day urination treatment and steaming for 8 minutes gave minimum number of cuts per cocoon during reeling. But the 30th day urination treatment and steaming for 10 minutes gave maximum number of cuts per cocoon during reeling. Silk cocoons that were in contact with urine for more number of days, naturally gave more cuts during reeling because there was more time for chemical and microbial reaction by urine on silk thread.

Observations were recorded on total length of thread per cocoon when differently treated cocoons were reeled. Although no relationship could be established between the length of thread per cocoon and number of days after urination treatment, it was observed that in general, all urine stained cocoons gave less total length of silk thread per cocoon than the unstained cocoons. Here again, the shorter length of the silk thread in the urine stained cocoons is due to the chemical and microbial action of urine on silk making it weak and thus also accounting for more number of cuts during reeling.

(b) Naturally urine stained cocoons: In addition to the reeling experiment on the artificially urinated cocoons, the reeling experiments were also conducted on naturally urine stained cocoons. Reeling of naturally stained cocoons for periods upto 8 days after urination without stifling gave better results than similarly treated cocoons but stifled by different methods. Number of cuts per cocoon during reeling increased in the following order. Normal unstained cocoons with stifling (0.7 cut per cocoon on an average), without stifling (4.20 cuts per cocoon), hot air stifling (10.67 cuts per cocoon), steam stifling (12.20 cuts per cocoon).

Stifling of urine stained cocoons therefore caused more number of cuts during reeling and it should be avoided. This is because during stifling dissolution action of the urine acts on sericin of silk and dissolves to a greater extent during stifling and results into the discontinuity of the thread. Thus the irregularly dispersed sericin may become hard on drying when stifled and this may hinder the smooth unwinding of the silk filament while reeling.

Microphotographs (Fig.7-16) of the urine stained silk filament clearly reveal the irregular distribution and dissolving action of urine on sericin.

Number of cuts per cocoon while reeling increased as the number of days after urination increased, in case of steam stifled and hot air stifled cocoons. However in case of cocoons without stifling treatment, number of cuts during reeling did not increase with the number of days after second day onward. Urine stained cocoons cooked and without stifling after one day of harvest gave reeling similar to the unstained cocoons. The results of these reeling experiments therefore indicate that it would be more convenient to reel the urine stained cocoons without stifling within 8 to 9 days after harvesting.

When urine stained cocoons are stifled, the dissolved sericin becomes dry and hard which is mainly responsible for hindering the smooth unwinding of the filament.

From the results of the above two experiments it may be stated that steaming the hot air stifled cocoons would help the reeling of urine stained cocoons. On the other hand if the cocoons are steamed without stifling, reeling will be more difficult. Either the urine stained cocoons should be stifled and then steamed before reeling or they should be reeled without stifling and without steaming before the emergence of the moth. Latter seems to be better.

(c) Effect of the treatment with softening agents on silk cocoons: It is stated above that breakage of silk filament while reeling is due to the hard inelastic nature of the irregularly dispersed sericin in the urine stained cocoons. It may be possible that softening agents would ease the reelability of such stained cocoons. Results of the study of the effect of softening agents (Table 12) have shown that sodium sulfite would give better effect than sodium carbonate and sodium hydroxide. Sodium sulfite treated cocoons reeled almost similar to that of non-urine stained cocoons. Stained cocoons treated with all the three softening agents reeled better than the non-treated urine stained cocoons. However sodium sulfite treatment in cooking water gave more length of thread and less number of cuts per cocoon. Sodium sulphite is a milder reducing agent. Its effect is therefore more gentle and better. Sodium carbonate and sodium hydroxide being more alkaline in nature would cause drastic effect on silk thread.

5. Removal of urine stain:

Deep yellow staining of cocoons due to silk worm urine on mountages is a major factor in reducing the quality of silk and subsequently also reduces the market value of the cocoons. Though stain on the cocoons has no appriciable

effect on the final dyed silk, the stained cocoons and the raw silk reeled out of such cocoons fetch less price in the market. Not many workers have reported on this aspect of the improvement of the urine stained silk cocoons. Results of the laboratory scale attempts made by the author in this regard are briefly discussed below.

Attempts were made to remove the silk worm urine stain by using several polar and non polar solvents inworm as well as cold conditions.

'Teepol' a laboratory detergent has been found to remove the stain only to a certain extent. The stain could not be removed completely by treating with 'Teepol'. Several other solvents used also did not give satisfactory results. Use of hydrogen peroxide for half an hour and then keeping the cocoons in alcohol overnight and rinsing with distilled water gave comparatively better result. The gloss and reflectance measured for normal, urine stained and treated cocoons show that the chemical treatment would appriciably improve both gloss and reflectance of the urine stained silk. These results indicate that the urine stain on the cocoon could be removed more satisfactorily by hydrogen peroxide treatment. Bleaching action of the hydrogen peroxide is due to the oxidation of the yellow pigment of the urine to a leuco compound.

SUMMARY

CHAPTER VI

SUMMARY

It is well known that yellow staining of cocoons in mountages is due to urination of silk worms during rainy days and when there is high humidity.

Problem of silk worm urine staining of cocoons is severe mostly in Mysore State, since the multivoltine races are cultivated in the State all round the year. Climatic environment during the period between the mounting and the completion of cocoon spinning of the matured silk worm especially during the first 30 hours period after mounting, has much to do with the quality of the cocoons.

According to the available literature, the problem of urination also exists in Japan and other silk producing countries. However it is not so serious in other countries as the mounting techniques are well developed there. The problem of urination is therefore concerned with proper modern mounting techniques and maintenance of good sanitation on the silk farms. In order to improve the situation, the present cocooning frames employed in Mysore State must be changed to those made of semi moisture absorbent material.

It should be similar to the rotatory mounting technique of Japan, in which some of the absorbing materials like straw mat or paper are also used. Silk worms mounted in this turning frame spin cocoons of superior quality. The other easy method to eliminate some of the spilled urine is to keep two mountages in an inverted 'V' shape. This also avoids direct dribbling of the urine on the cocoons. The only alteration needed in this case is that the mountages must be in a position to keep on the four sides giving equal interval to all the sides. Removal of the urine and faeces once or twice during spinning stage would also be of much help.

Alkaline nature of the urine is mainly responsible for the breakage while reeling. Over crowding of the silk worms in the mountages should be avoided particularly under high humidity condition. This will reduce the incidence of stained cocoons as there will be sufficient gap between spinning larvae and the cocoons formed already. Controlling the atmospheric humidity on large scale is not possible under village conditions. Though silk worm urine contains 70 per cent of uric acid, urine is alkaline in reaction.

Silk cocoon mainly consists of two proteinacious substances called sericin a gummy fraction which forms the

outer cover and fibroin a true silk. They are present in the proportion of 27.60 per cent and 60.91 per cent respectively. Minerals, amino acids, total protein and carbohydrates in silk cocoons were analysed. Silk worm urine affects not only sericin content but also fibroin content of the cocoons. Urine stained cocoons are found to be rich in mineral constituents. Protein content of silk cocoons is reduced due to urination by silk worms.

Silk cocoons can be differentiated into outer cover, middle portion and inner core. Outer layer of cocoons seems to be more affected by urine of silk worm than the middle portion and inner core. Sericin content is more in the outer cover and fibroin content is more in the inner layers. Urine is found to penetrate upto first 200 meters of silk on the cocoons.

As many as 18 amino acids are identified in silk protein. Fibroin is rich in many of the amino acids. Some of the amino acids are found to be affected by urination. This is particularly so in case of sericin protein. Sericin of silk cocoons contains more carbohydrates than fibroin or whole cocoon as such. Effect of urination by silk worms on carbohydrate content of sericin is found to be more than that of fibroin.

Reeling experiments were carried out to find a better method of reeling the urine stained cocoons. Reeling becomes difficult as number of days after urination increases. Steaming the urinated cocoons is found to have some favourable effect on their reeling. The best way to over-come the problem is to reel the urine stained cocoons without stifling before emergence of the moth. Few softening agents were tried to ease the unwinding of filaments while reeling. Use of sodium sulfite added while cooking the cocoons is found to be much useful in easing the unwinding of urine stained cocoons at the time of reeling. A correct composition of cooking water using sodium sulfite as softening agent may be worked out to get better reelability of urine stained cocoons.

Many solvents were tried to remove the urine stain. Keeping cocoons in hydrogen peroxide for about half an hour and then keeping over night in alcohol was found to remove the stain to an appreciable extent.

As a suggestion for future line of work in this regard, efforts may be made to breed the resistant races of silk worms, which can withstand high humidity without affecting the cocoons by urination. Better cocooning frames may be developed using semi-absorbent material like straw or paper,

which can be replaced once or twice during spinning stage of the worms. A suitable method for reeling the urine stained cocoons without stifling may also be developed.

REFERENCES

CHAPTER VII

REFERENCES

Anonymous, 1969, Sanitation in silk worm rearing. Bull., Sericult. Exp. Station (Sigisamuki) Jap. 169.

*Ajiswa and Akiyoshi, 1969, Physico-chemical studies on reeling quality of cocoons. J. Sericult. Sci. Jap. 38: 215-218.

Alders, M., 1927, Extraction of sericin from silk cocoons. Biochem. J. 183: 447-450.

Ambrose, E.J., Bamford, C.H., Elliott, A. and Hanby, W.E., 1951, Water soluble silk and alpha-protein. Nature (Lond). 167: 264-265.

*Bankowska Waclawa and Michal Kalyniak, 1968, Feeding silk worm caterpillars with frozen mulberry leaves. Przegl. Zool. 12: 102-105.

*Baroni, C., 1933, Protein in silk. Boll. Staz. Sper. Seta. Milano. 3: 28-29.

Benedict, S.R., 1909, Total sulphates in urine. J. Biol. Chem. 6: 363

Benedict, S.R., and Franke, E.W., 1922, Direct colorimetric method of uric acid determination. J. Biol. Chem. 52: 387.

Berridge, M.J., 1966, Hormonal control of excretion in insects. J. Exp. Biol. 44: 553-566.

Bricteux-Gregore, S.G., Duchateau, Florkin, M. and Jeuniaux, 1959(a), Contribution to silk worm bio-chemistry. Arch. Internatl. Physiol. Biochem. 67: 563-566.

Bricteux-Gregore, S.G., Verly, W.G. and Florkin, M., 1959(b), Contribution to silk worm bio-chemistry. Arch. Internatl. Physiol. Biochem. 67: 586-596.

Carboni, P., 1952, Silk biology, chemistry and technology, Chapman and Hall Ltd., London. p.248.

Cebra, L. and John, J., 1961, Some physical properties of silk. J. Immunol. 86: 190-196.

Chatterji, S.M., 1967, Degumming silk with soapless synthetic detergent. Textile Manufacturer, 93: 232-235.

Chowdhury, S.K., Jolly, M.S. and Razdan, J.L., 1969, Effect of cooking on the reelability of Tassar cocoon (Antheraea mylitta). Indian J. Sericult. 8: 38.

Dematriades, S.D., 1956, Chromatographic detection of free amino acids in normal and iron deficit plants of Hibiscus esculentus Linn. Nature. 177: 195-197.

Dhavalikar, R.S., 1962, Amino acid composition of Indian silk fibroins and sericins. I. Fibroin. J. Sci. Ind. Res. 21: 261-263.

Dhavalikar, R.S., 1962, Amino acid composition of Indian silk fibroins and sericins. II. Sericins. J. Sci. Ind. Res. 21: 303-304.

Fiske, C.K. and Subba Rao, Y., 1925, Phosphorous in urine. J. Biol. Chem. 66: 375.

Folin, O. and Bell, R.S., 1917, Ammoniacal nitrogen analysis in urine. J. Biol. Chem. 29: 329.

Fukuda, T. and Hayashi, T., 1960(a), Biochemical studies on the formation of the silk protein. J. Biochem. 48: 9-12.

*Fukuda, T. and Hayashi, T., 1960(b), The kinds of free amino acids concerned in the biosynthesis of the silk protein. J. Sericult. Sci. Jap. 29: 120-124.

Fukuda, T., Mitsumarasuto and Yoshikazunakagava, 1960, Ordered progression of fibroin in the inside of the silk gland during the fifth instar. Agric. Chem. Soc. Jap. 24: 501-505.

*Geldmacher, O.H. and Marika, 1953, Detection and differentiation of sugars and polysaccharides by sensitive spot reactions on silk. Biochem. Zeitschr. 324: 186-194.

Hasegawa, K. and Yamashita, O., 1965, Studies on the mode of action of the diapause hormone in the silk worm, Bombyx mori Linn. J. Exp. Biol. 43: 271-277.

Hashiguchi Tsutomu, Narumi Yoshitake and Nobutaka Takahashi, 1965, Hormone determining the black pupal colour in the silk worm. Bombyx mori Linn. Nature. 206: 215.

Hawk, B. and Summerson, H., 1947, Quantitative analysis of urine. Physiological chemistry, McGraw Hill Book Co., New York, p.866.

Hawk, B., Oser, L. and Summerson, H., 1954, Properties of uric acid. Practical Physiological Chemistry. 13th Ed. McGraw Hill Book Co., New York, p. 795.

Hawk, B., Oser, L., 1965, Total nitrogen in urine: Hawk's Physiological Chemistry. 14th Ed., McGraw Hill Book Co., New York, p. 1214.

*Hayashi, Y., 1961, On the uric acid formation in silk worm. Bombyx mori Linn. Jr. Sericult. Sci. Jan. 30: 89-94.

Hiratsuka, 1967, Uric acid secretion in silk worm larvae. Bull. Imp. Sericult. Exp. Sta. 2: 353.

Jolly, M.S., 1962, Biosynthesis of silk fibroin of Bombyx mori Linn. Textile Technologist, pp. 35-37.

Jolly, M.S. and Krishnaswami, S., 1964, Sericin content in cocoons of indigenous silk worm races (Bombyx mori Linn.). Indian J. Sericult. 3: 17.

*Khan, Arjamand, Ghulamnabi and Shah, S.M.A., 1970, Amino acid composition and mechanical properties of Pakistani white and yellow silk fibroin (Bombyx mori Linn.). Pak. J. Sci. Indust. Res. 13: 140-143.

Kolmer and Robinson, 1969, Clinical method of urine analysis. Approved Laboratory Techniques. Scientific Book Agency, Calcutta. p.127.

Kumararaj, S., 1969, Observations on diuresis and staining of cocoons in silk worm. Bombyx mori Linn. Indian J. Sericult. 8: 48-53.

Kuwana Zyuiti, 1937, The excretion of uric acid in the silk worm larvae. Jap. J. Zool. 7: 305-309.

* Kuwahra and Akira, 1969(a), Electron microscopic investigation on the fibril formation from silk fibres. J. Sericult. Sci. Jap. 38: 11-15.

*Kuwahra and Akira, 1969(b), Electron microscopic investigation on the fibril formation from silk fibres. II. J. Sericult. Sci. Jap. 38: 49-54.

Laborthe, 1968, Properties of silk. Silk from grub to glamour. Paramount Pub. House, Bombay. 75.

*Lamma, G.Y. and Ioffe, K.G., 1969, N-terminal amino acid of the silk fibroin of Bombyx mori Linn. Biokhimiya, 34: 908-910.

Li, C.H., 1961, Antidiuretic activity of hormones. Encyclopaedia of Science and Technology. McGraw Hill Book Co., New York.

Lucas, F., 1966, Cystine content of silk fibroin (Bombyx mori Linn.). Nature. 210: 925-953.

*Luciorlandi, 1955, The percentage of sericin in various layers of the cocoon of Bombyx mori Linn. Ann. Sper. Agrar. 8: 1881-6.

Madhava Rao, N.R., 1969, Staining of silk worm cocoons due to urination by silk worm, Bombyx mori Linn. Silk Worm Information Bulletin. 1: 100-102.

Madrell, S.H.P., 1954, Action of hormones on diuresis of insects. Nature. 194: 605.

Madrell, S.H.P., 1963, Neuro-hormone to promote the diuresis in insects. J. Exp. Biol. 40: 247-256.

Madrell, S.H.P., 1964(a), Normal course of diuresis and the effect of temperature. J. Exp. Biol. 41: 163-176.

Madrell, S.H.P., 1964(b), The control of the release of diuretic hormone. J. Exp. Biol. 41: 459-72.

Madrell, S.H.P., 1969, Stimulatory effects of 5 hydroxy tryptamine on secretion of malpighian tubules of insects. Nature. 222: 784-785.

*Menedez, M.A.D., Stamn, N., Comenge and Santos, A., 1950, The metabolism of phenyl alanine in Bombyx mori Linn. Rev. Espanola Fisiol. 6: 181-186.

Mercer, E.H., 1952, The fine structure and biosynthesis of silk fibroin. Aust. J. Res. Ser. Biol. Sci. 5:366-373.

Mills, R.R., 1967, Diuretic hormons that promotes fluid secretion by the malpighian tubules. J. Exp. Biol. 46: 39.

Miura, Yoshiaki, Hirowoito, Shiglotanaka, Kenhikomomose, Kiyoshisunga and Akikomoriyama, 1961, Studies on the protein synthesis in silk gland. J. Biochem. (Tokyo). 50: 458-466.

Mosher, H., 1934, The sericin fractions of silk. Am. Silk Rayon J. 53: 43-44.

Nagaraj, G. and Basavanna, H.M., 1969, Quantitative chromatographic study of the silk pupae. Silk worm Information Bull. (Mysore). 1: 30.

Nanavathi, M., 1965(a and b), The fibre called silk, Bleaching of silk. Silk from grub to glamour. Paramount Pub. House, Bombay. 186.

*Nishi, Hisami and Reik Tomura, 1969, Effect of tyrosinase on sericin. J. Sericult. Sci. Jap. 38: 117-122.

Passent, J. and Szafranski, P., 1969, Non-enzymatic binding amino acids from silk worm silk glands to soluble proteins from the silk glands of the silk worm, Bombyx mori Linn. J. Insect. Physiol. 16:715-724

Ramaswamy, J.A., 1952, The excretion of sodium and potassium by the malpighian tubules of insects. J. Exp. Biol. 29: 110-126.

*Rastelli, 1934, Aqueous extract of fibroin and its reaction. Kolloidzs Chr. 67: 55-59.

Shaw, J.T.B. and Smith, S.G., 1961, The composition and structure of chemically resistant fractions from some silk fibroins. Biochem. Biophys. Acta.52:305-318.

Shaw, J.T.B., 1964, Fractionation of the fibroin of Bombyx mori Linn. with trypsin. Biochem. J.93:45-54.

Shaw, J.T.B., 1964, Fractionation of the fibroin of Bombyx mori Linn. with alkali. Biochem. J. 93:54-61.

Shelton, E.M. and Johnson, T.B., 1925, Extraction of sericin from silk. J. Am. Chem. Soc. 47: 412-418.

*Shikata Shogo, and Eijikubota, 1960, Studies on the carbohydrates of sericin. Res. Rept. Fac. Textile and Sericult. Shinshu Univ. 10: 230-231.

Shimizu, M., 1934, Detection of uric acid in malpighian vessels of silk worm larvae. Bull. Imp. Sericult. Exp. Sta. 8: 525.

*Shimizu, M., 1957, Composition of natural silk. Ann. Soer. Agrar. 10: 6-18.

*Shiozaki, Hideki and Ryochimurage, 1969, On amino acid distribution in the fibroin fibre. J. Sericult. Sci. Jap. 38: 230-236.

Shyamala, M.B. and Bhat, J.B., 1966, Intestinal transport in silk worm, Bombyx mori Linn. of amino acids from mixtures. J. Insect Physiol. 12: 129-135.

Somogyi, M., 1952, Note on sugar determination. J. Biol. Chem. 2002: 145-154.

Sonwalkar, T.N., 1969, Investigation on degumming loss and spinning performance of pierced and cut cocoons in silk worm. Indian J. Sericult. 8: 43.

*Stamm, M.A., Doderes, M., Comenge and Santosruiz, 1950, The metabolism of tyrosin and tryptophan in Bombyx mori Linn. Rev. Esp. Physiol. 6: 187-193.

Staurt, E.G., 1960, Oxytocin as antidiuretic hormone in lower vertibrates. Encyclopaedia of Science and Technology, 4: 592. McGraw Hill Book Co., New York.

Stout, E.E., 1960, Silk and its properties. Introduction to textiles, John Wiley and Sons Inc., New York. 402.

Takagi, 1958, Degumming of silk under pressure. Takamine Lab. Inc. B.P. 301: 419.

Takagi, Haruo and Hisookutsukake, 1959, The rigidity of raw silk and silk fabrics. Res. Repts. Fac. Textile and Sericult. Shinshu Univ. 9: 117-126.

Tanaka and Yoshimoro, 1964, Action of alkali and acids on sericin coating of silk cocoon. Sericology, pp. 200. Central Silk Board, Bombay. (Pub.).

Teotia, T.P.S. and Pant, C.P., 1962, Sericin content of silk cocoon. Indian J. Sericult. 1: 27-31.

Thompson, J.O., 1925, Removal of sericin by boiling water. Text. Colourist. 47: 221.

*Volhard-Arnold, 1910, Quantitative analysis of chlorides in urine: Hawk's Physiological Chemistry, McGraw Hill Book Co., New York. (1965), 1218.

*Wanger, W., 1932, Action of hard inelastic sericin on reelability of silk. Seide. 37: 160-165.

Warwicker, J.O., 1961, X-ray examination of chemically resistant fractions of some silk fibroins. Biochem. Biophys. Acta, 52: 319-328.

Wigglesworth, V.B., 1931, Composition of the insect urine. J. Exp. Biol. 8: 411-427.

Zahn, H., 1967, Fractionation of the chymotryptic precipitate of Bombyx mori Linn. Silk fibroin. Biochem. J. 104: 1019-1025.

* Originals not seen

APPENDIX

APPENDIX

Table I - Analysis of variance table for different number of days after urination with different times of steaming and cooking the cocoons and reeled for number of cuts per cocoon.

Source	d.f.	S.S.	M.S.S.	F.obs.	F.o.05	Inference
Between replication	3	119.0344	39.6781	9.8652	2.60	Significant
Between treatment	79	4831.2210	61.1547	15.2050	1.00	Significant
D	7	4449.5469	635.6495	158.0431	2.015	-do-
B	1	2.2532	2.2532	1.3061	3.84	-
S	4	9.9249	2.4812	0.6169	2.37	-
DB	7	18.7218	2.6745	0.6649	2.015	-
DS	28	242.8751	8.6741	2.1567	1.00	-do-
BS	4	24.0751	6.0187	1.4964	2.37	
DBS	28	80.8249	2.8866	0.7177	1.00	
Error	237	953.2156	4.0220			
Total	319	5903.4719				

D = No.of days after urination
B = Boiling (cooking) time
S = Steaming time

(1) S.E. difference between two observations in case of No.of days after urination = 0.4484
C.D. = 0.9825

(2) S.E. difference between two observations in case of interaction between No.of days and different times of steaming is = 0.448 and C.D. = 1.9682

(3) S.E. difference between two means in case of replication is = 1.42 and C.D. is 2.783.

APPENDIX

Table II- Analysis of variance table for different number of days after urination with different times of steaming and cooking and reeled for total length in meters per cocoon.

Source	d.f.	S.S.	M.S.S.	F.obs.	F.0.05	Inter-ence
Between replication	3	7095.94	2365.31	0.555	2.60	Not significant
Between treatment	79	553142.20	7001.80	1.645*	1.00	Significant
D	7	221654.69	31664.9557	7.4389*	1.94	"
B	1	427.69	427.69	0.10047	3.84	Not significant
S	4	81615.63	20403.0075	4.7930*	2.37*	Significant
DB	7	26299.81	3757.1157	0.88264	1.94	Not significant
DS	28	124034.37	4429.7989	1.040674	1.00	Significant
BS	4	8743.17	2185.7925	0.513499	2.37	Not significant
DBS	28	90366.83	3227.3867	0.758196	1.00	"
Error	237	1008829.07	4256.662			
Total	319	1569067.20				

D = No.of days after urination
B = Boiling (cooking) time
S = Steaming time

1. S.E. for No.of days after urination = 16.3107
   C.D. for No.of days after urination = 31.9689
2. S.E. for different times of steaming= 20.6310
   C.D. for different times of steaming= 40.4367
3. S.E. for the interaction of days = 32.6215
   and steaming
   C.D. for the interaction of days = 63.9381
   and steaming

APPENDIX

Table III—Analysis of variance table for total length of thread per cocoon reeled after different number of days urination with stem stifling, hot air stifling without stifling and control

	Source	d.f.	S.S.	M.S.	F.obs.	FO.05	Inference
Main Treatment	Block	4	11407	2851.75	0.0365	3.26	Not significant
	Main treatment	3	29425	9808.33	0.1226	3.49	"
	Error (a)	12	959663	79971.91			
Sub Treatment	Sub treatment	3	254335	84778.33	6.4578	2.84*	Significant
	Interaction sub treat x main treat.	9	75155	8350.56	0.636	2.00	Not significant
	Error (b)	48	630173	13128.60			
	Total	79	1000495				

1. S.E. for different types of stifling = 36.233
   C.D. for different types of stifling = 71.01668

APPENDIX

Table IV- Analysis of variance table for number of cuts per cocoon while reeling after different number of days of urination with steam stifling, hot air stifling, without stifling and control

	Source	d.f.	S.S.	M.S.S.	F.obs.	F0.05	Inference
Main treatment	Block	4	2.13	0.532	0.00263	3.26	Not significant
	Main treatment	3	646.34	215.44	1.0673	3.49	"
	Error (a)	12	2422.22	201.852			
Sub Treatment	Sub treatment	3	1757.54	585.846	82.0628*	2.80	Significant
	Interaction	9	322.01	35.778	5.0116*	2.10	"
	Error(b)	48	342.67	7.139			
	Total	79	3070.69				

1. S.E. for different stifling treatments, viz., steam stifling, hot air stifling, without stifling and control = 0.844
   C.D. -do- = 1.65424

2. S.E. for interaction between different types of stifling and number of days after urination = 1.689
   C.D. -do- = 3.31044

APPENDIX

Table V - Analysis of variance table for total length of thread per cocoon reeled after using different softening agents

Source	d.f.	S.S.	M.S.S.	F.obs.	F0.05	Inference
Treatment	3	48860	16286.67	10.184*	3.49	Significant
Blocks	4	9170	2292.50	1.433	3.26	Not significant
Error	12	19190	1599.16			
Total	19	77220				

S.E. of difference between two means = 25.285

C.D. for treatments = 55.096

APPENDIX

Table VI- Analysis of variance table for number of cuts per cocoon while reeling the urine stained cocoons after using different softening agents

Source	d.f.	S.S.	M.S.S.	F.obs.	F0.05	Inference
Treat-ment	3	275.20	91.73	9.938*	3.49	Significance
Blocks	4	68.80	17.20	1.863	3.26	Not signifi-cant
Error	12	110.80	9.233			
Total	19	454.80				

S.E. of difference between two means = 1.921

C.D. for treatments = 4.1858