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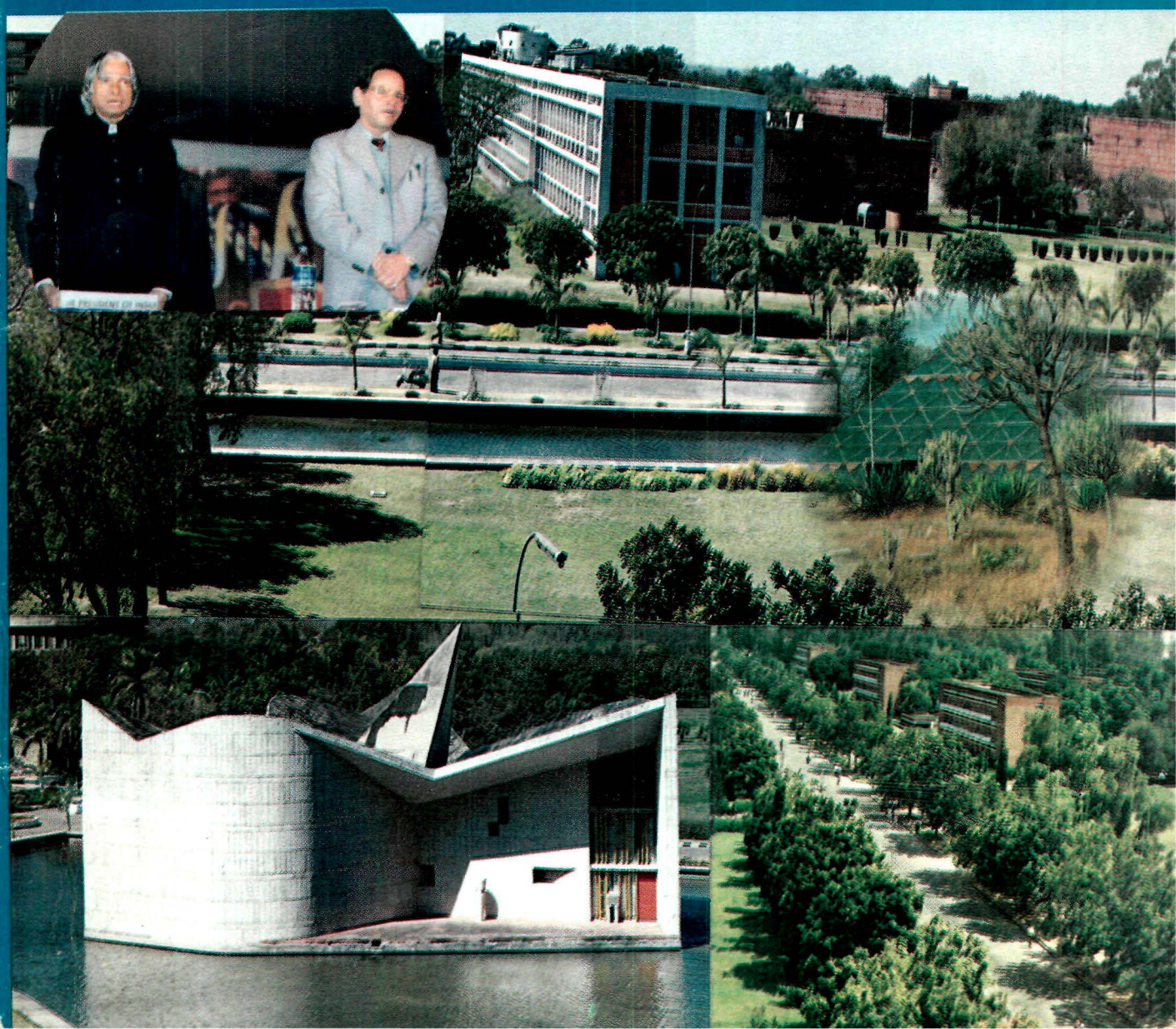

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PROMISE OF BIOTECHNOLOGY IN ADDRESSING CURRENT NUTRITIONAL PROBLEMS IN DEVELOPING COUNTRIES

Gurdev S. Khush

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Access to healthy diet is fundamental right of every human being on this planet. Yet 800 million people, mostly in the developing world, go to bed hungry everynight. Micronutrient deficiencies affect 3 billion people. Malnutrition hinders the development of human potential and nation's social and economic development.

Access to food depends on income. Currently, more than 1.3 billion of the population are absolutely poor, with income of less than 1 U.S. dollar pay per capita, while another two billion are marginally better off (World Bank, 1997). Thus, investments in employment generation are as important as investments in food production.

The malnutrition problem is further exasperated by increasing world population which is likely to reach 8 billion by 2030. Most of this increase (93%) will take place in the developing world, whose share of the global population is projected to increase from 78% in 1995 to 83% in 2020. To meet the challenge of feeding ever-increasing population and alleviation of protein-energy malnutrition, we will have to produce 50% more food grains. To meet this challenge we need crop varieties with higher yield potential and greater yield stability. Conventional plant breeding as well as biotechnology techniques will be employed to develop crop varieties with higher yields and durable resistance to diseases and insects.

Tackling the Micronutrient Malnutrition

In addition to protein-energy malnutrition, deficiencies of minerals and vitamins affect a high proportion of world's poor. Deficiencies of iron (Fe), zinc, (Zn), iodine (I) and vitamin A are most acute. Estimates are that two billion of the world's people are Fe deficient. At least 400 million have vitamin A deficiency and of that number 100 million are young children. As many as three million children die annually as a result of vitamin A deficiency (Sommer, 1990). One billion of the world's populations reside in I-deficient regions with numerous inhabitants of these areas suffering from I-deficiency disorders, including goiter, cretinism, lower intelligence and increased prenatal mortality (Hetzel, 1990). Zinc deficiency, thought to be widespread, can lead to retarded growth, depressed immune function, anorexia, dermatitis, skeletal abnormalities and mortality during childhood if

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An invited article

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it is prolonged (International Life Sciences Institute, 1990). Furthermore Zn deficiency has been linked to vitamin A under- utilization.

Even in developed countries, micronutrient deficiencies affect a significant number of populations. Taken together, micronutrient deficiencies affect a far greater number of the world's population than protein-energy malnutrition (Chandra, 1990). Intervention programs for alleviating micronutrient malnutrition include supplementation, food fortification, education and biofortification. Supplementation programs have been successful in reducing malnutrition in specific situation, *e.g.* I-supplementation through the use of iodized salt. However, for the micronutrients, Fe, Zn and vitamin A such programs are expensive, incur ongoing costs and are unlikely to reach all of those at risk. Moreover, such intervention programs have often been suspended for economic, political and logistical reasons (Gibson, 1994).

Part of the solution to micronutrient deficiencies is convincing the people to make their diets more nutritious. However, attempts to change eating behavior have been unsuccessful. It is often difficult to make dietary changes using local foods if you are poor. They require a lot of input, constant follow up and education. When they are scaled up, they rarely work, so they tend not to be sustainable. Under these limitations, biofortification is considered most effective for tackling micronutrient malnutrition. This strategy for supplying micronutrients to the poor in developing countries involves making staple foods they eat more nutritious by using conventional plant breeding and biotechnology. This strategy is low cost and sustainable, and it does not require a change in eating habits and does not impose the recurring costs that accompany food supplementation and fortification.

Increasing the Mineral and Vitamin Concentration of Staple Crops

The main question about the potential benefits of using mineral- or vitamin-dense staple crops is whether the increased concentrations will in fact result in significant increases of bioavailable minerals (or vitamins) and consequently improve the nutritional status of malnourished populations. For this to happen, vulnerable groups have to consume the improved varieties of staple crops in sufficient quantities, but even more important, the net amount of bioavailable nutrients they ingest must be increased relative to traditional crops. For example, main sources of iron in impoverished populations are staple cereals and starchy roots, tubers and legumes. Most of the iron ingested from these sources has low bioavailability. Estimates indicate that cereals contribute up to 50 percent of Fe intake among households from lower socio-economic groups (Bouis, 1996). For Zn, the contribution from plant sources can be as high as 80%. This means doubling the Fe and Zn density of food staples could increase total intake by at least 50%. The main problem, thought is that diets based on plant staples usually contain large amounts of phytic acid (Gibson, 1994) which inhibits Fe and Zn absorption. Thus, crop improvement strategies should aim at increasing the level of

micronutrients on one hand and reducing the amount of phytic acid on the other.

Improving the Amount and Bioavailability of Fe and Zn

A research project to develop improved rice varieties with high Fe and Zn content was initiated at the International Rice Research Institute (IRRI) in 1992. Considerable variation for both Fe and Zn was observed in the rice germplasm. Fe concentrations ranged from 6.3 to 24.4 mg/kg with a mean value of 12.2 mg/kg. For Zn, the range was 15.3-58.4 mg/kg (Gregorio *et al.*, 2000).

Efforts are underway to develop improved rice germplasm with elevated levels of Fe and Zn. Crosses between these traditional varieties and high yielding varieties have produced progenies with both high yield and high level of micronutrients. For example, an improved breeding line IR68144 has high concentration of Fe in grain (about 21 mg/kg) and high yield potential. Milled rice of this variety is being used in human feeding trials to determine the bioavailability of the Fe (Gregorio *et al.*, 2000).

Genetic engineering approach has been successfully applied to raise the Fe content of rice. Goto *et al.* (1999) transferred the soybean *ferritin* gene into the rice variety Kita-ake through transformation. The Fe content of the transgenic seeds was as much as threefold higher than that of untransformed seeds. Similarly, Lucca *et al.*(2001) introduced *ferritin* gene from common bean into rice and the transgemic lines had double the amount of Fe as compared to controls. To increase the bioavailability of Fe, Lucca *et al.*(2001) introduced thermo-tolerant phytase gene from a fungus into the rice endosperm which breaks down phytic acid thus improving the bioavailability of Fe in rice. Mutants of barley, maize and wheat with low amounts of phytate are available and may be employed to develop varieties of these crops with improved bio-availability of iron.

Ortiz-Monasterio (1998) found four-fold variation between the lowest and highest Fe and Zn concentrations in grains among several hundred wheat accessions. Studies at CIAT showed that certain varieties of common bean had as such as 60-80% more Zn than those of commonly grown varieties. Breeding efforts are underway to incorporate high levels of Zn into improved varieties (Beebe *et al.*, 2000).

Improving the vitamin A content of crops

Beta-carotene, a precursor of vitamin A does not occur naturally in the endosperm of rice. Therefore populations, which derive most of their calories from rice, suffer from vitamin A deficiency. In many Asian countries (Vietnam, Laos, Cambodia, Myanmar, Bangladesh, India) poor people derive more than 60% of their calories from rice. Ye *et al.* (2000) introduced 2 genes from daffodil and one gene from a bacterium (*Ervinia uredovora*) in a rice variety Taipei 309 through genetic engineering. Ten plants had a yellow endosperm (due to presence of β-carotene), had a normal vegetative phenotype and were

fully fertile. Taipei 309 was used to introduce β-carotene biosynthetic pathway, as it is easy to transform. However, it is no longer cultivated. IRRI has started a project with the aim of introducing the genes for β-carotene production into widely-grown improved varieties through transformation, as well as through conventional hybridization techniques. It is anticipated that elite rice varieties containing β-carotene will become available during next 2-3 years. Strong carotenoid pigmentation was present in older bread-wheat varieties. However, during this century market demand has driven wheat breeding to be focused on the production of wheat for white flour. The pigmented type wheats could be brought back into breeding programs, if desired. Similarly, in maize, there are high Beta-carotene types (yellow maize) that are also high yielding. However, in many cultures, consumers prefer white maize which lacks carotenoids and is nutritionally inferior. Education programs should be undertaken to popularize the use of yellow maize.

Cassava is an important staple food for 50 million poor people. Genetic variation in cassava roots for β-carotene content is high. Orange coloured roots have 9-10 times more β-carotene as compared to white roots. Thus there is obvious advantage in popularizing the use of orange colored varieties of cassava.

An action research project was recently implemented by the Kenya Agricultural Research Institute (Nairobi, Kenya) in collaboration with the International Potato Center (Lima, Peru). Orange-fleshed varieties of sweet potatoes, both high yielding and rich in β-carotene were introduced to women farmers. The result was that orange-fleshed sweet potatoes, both eaten alone and as ingredients in processed foods, were highly acceptable to both producers and consumers. Using standard method of analysis, it was demonstrated that their increased consumption did in fact contribute to the alleviation of vitamin A deficiency in case of study households (Hageniwana, 2000). In Sub-Saharan Africa, sweet potatoes are an important source of calories for poor people. Most of the sweet potato varieties grown there have white flesh and thus lack β-carotene. Introduction of orange-fleshed sweet potatoes should receive priority.

Improving the Amino Acid Balance

Human diet derived from cereal grains is deficient in some of the ten essential amino acids which are required for normal growth and development. Lysine is the most limiting amino acid. Natural variation in the maize germplasm was exploited to develop quality protein maize (QPM) at International Maize and Wheat Improvement Center (CIMMYT) in Mexico. The *opaque* 2 gene was incorporated in improved maize germplasm and it doubled the amount of lysine and tryptophan. QPM maize varieties have been released in several countries which are now grown on almost one million hectares and the area under QPM maize is increasing.

Biotechnology approaches are also being used to enhance the lysine content of rapeseed (canola) corn and soybean.

Introduction of two bacterial genes for dihydrodipicolinic acid (DHDPS) and aspartokinase (AK) enzymes encoded by *dapA* gene from *Corynebacterium* and *lysC* gene form *Escherichia coli* led to five fold increase in lysine in canola, corn and soybean (Falco *et al.*, 1995). Similarly, amino acid profile and total protein content of potato was improved through introduction of *AmAI* gene from *Amaranthus hypochondriacus* (Chakraborty *et al.*, 2000).

Above paragraphs highlight the importance of biotechnology and germplasm improvement in alleviating the problem of malnutrition which affects almost half the world population. Thus linking agriculture and nutrition to promote dietary change and improve nutritional status can generate wide economic benefits.

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MOLECULAR DESIGN OF NOVEL CHOLESTERYL LIPIDS FOR THE DELIVERY OF GENES ACROSS THE CELL MEMBRANES

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Abstract

Novel cationic cholesterol derivatives with ether and ester linkages between cationic head group and cholesterol backbone have been developed. Their gene transfection efficacies have been tested as mixtures with a helper lipid, dioleoyl phosphatidyl ethanolamine (DOPE). It has been observed that there is an improvement in gene transfection efficacies when cationic head group is linked to cholesterol via an ether type of linkage.

Key words: Genes, influx, membrane, therapy.

INTRODUCTION

Recent advances in genomics and molecular biology reveal that almost all diseases have a genetic component. In some cases, such as cystic fibrosis or hemophilia, mutations in a single gene result in a disease. In other situations, such as hypertension or hyper lipid levels, certain genetic variations may interact with environmental stimuli to cause a disease. Pathological conditions associated with aging frequently result from the alteration in gene activity in specific types of cells. Even viral or bacterial infections have a genetic component - the gene(s) of the invading pathogen.

Gene therapy is the process by which DNA sequences encoding specific genes are delivered to cells with the goal of treating or curing disease. As the genetic and molecular basis for a number of diseases is elucidated, the promise of gene therapy continues to grow. Although initial efforts in gene therapy focused on delivering a normal copy of a missing or defective gene, current programs are applying gene delivery technology across a wide spectrum of disease conditions. DNA transport through the cell membrane is an essential requirement for gene therapy, which utilizes oligonucleotides and plasmid DNA. However, membrane transport of DNA is an inefficient process, and the mechanism(s) by which this process occurs is not clear. Although viral vectors are effective in gene therapy, the immune response elicited by viral proteins poses a major problem.

Aqueous suspensions of cationic cholesterol derivatives have been shown to bring about DNA transfer in cells with greater efficiency than commercial transfection formulations

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comprising of cationic amphiphiles based on the pseudoglyceryl skeleton (Moradpour, *et al.*,1996; Krishnan-Ghosh, *et al.*, 2000). An alternative class of amphiphiles that is based on synthetic cationic cholesterol derivatives currently received a great attention because many compounds of this type are used as drugs. In addition, they are also being employed for diverse purposes besides gene therapy (Cooper *et al.*,1998; Vigneron *et al.*, 1996; Gao and Huang, 1991; Leventis and Silvius, 1990), such as enzyme inhibition (Bottega and Epand, 1992), membrane-spanning conductors (Otto *et al.*, 1999) or in medicinal applications (Kreimeyer *et al.*, 1998). Moreover, the interest in the utilization of cationic cholesterol derivatives as cytofectins (the active component of a liposomal transfection reagent, usually a cationic amphiphile is referred as a cytofectin *'cyto'* meaning 'cell' and *'fectin'* for 'transfection', that is, gene delivery and its expression, Miller, 1998) over traditional cationic glycerol-based amphiphiles stems from the fact that the 3β OH of cholesterol is easily functionalizable and this facilitates the generation of a range of derivatives. In addition, fatty acid based cytofectins are normally employed as a mixture with a helper lipid such as DOPE (dioleoyl phosphatidyl ethanolamine) which limits shelf-life and over a period of time, transfection efficiencies (TEs) of these formulations often become irreproducible as they tend to phase separate (Miller, 1998).

The emergence of this class of molecules as efficient cytofectins has prompted structural studies such as the headgroup variation and location of the positive charge on the cholesterol monomer (Fichert *et al.*, 2000). However, not much is known on the effect of the linker region, which connects the positive charge to the steroid backbone. Preliminary studies (Krishnan-Ghosh *et al.*, 2000) using COS-7 cells suggested that use of an ether linkage over the more frequently used ester or urethane based linkage offers significant advantage in effecting gene transfer. In the present study, we present the results of a detailed investigation of these cytofectins and also their utilization in transfection of various cell lines. A relation between headgroup hydration and transfection efficiency (TE) of cationic fatty acid derivatives of glycerol-based transfection reagents has been suggested (Webb *et al.*, 1993; Bloomfield, 1996, Safinya *et al.*, 1998). Hence, we also synthesized additional cationic cholesterol derivatives bearing varying lengths of oligo-oxyethylene glycol units. Herein we also include the results of the investigation where transfection abilities of three series of cationic cholesterol derivatives with different linker regions and headgroup hydration yield a correlation between molecular structure and gene transfection efficency.

MATERIALS AND METHODS

All reagents, solvents and starting materials were obtained from the best known commercial sources and were distilled, recrystallized or used without further

purification, as appropriate (Perrin *et al.*, 1990).1,2-Dioleoyl-sn-glycero-3-phosphatidy lethanloamine (DOPE) was purchased from Avanti Polar Lipids. Cholesterol was obtained from Sigma (St. Louis, MO). *N, N*-dimethyl ethanolamine was obtained from Aldrich and trimethyl amine from Merck. Commercially available transfection reagents Lipofectin® and Lipofectamine® were obtained from GIBCO-BRL. Luciferase assay kit was obtained from Promega. The syntheses of cationic cholesterol-based lipids have been reported elsewhere (Bhattacharya *et al.*, 2001; Ghosh *et al.*, 2002).

Liposome Preparation. A mixture of cationic amphiphile and DOPE in the desired mole ratio were taken in $CHCl_3$ in autoclaved Wheaton glass vials, solvent was evaporated under vacuum from the solutions to form respective thin films. This was resuspended in freshly autoclaved Milli-Q water. The final cationic cholesterol lipid/DOPE concentration was 0.2 mg/ml for all formulations. The mixture was left for hydration at room temperature for ~6 h and then vortex mixed for 5 min followed by bath sonication for 15 min. The solutions were filtered under sterile conditions through a 0.22 μm filter (Sartorius) and stored at 4°C. The diameters of the cationic liposomes prepared by this method ranged generally between 80 to 400 nm as checked by transmission electron microscopy of individual aliquots of sterile vesicular suspensions from respective cationic cholesterol derivative with DOPE suspensions.

Plasmid DNA. Plasmid PRL-SV40 vector containing the luciferase reporter gene was amplified in *Escherichia coli* (DH5α) and purified using the QIAGEN Midi Prep Plasmid Purification protocol.

Transfection Procedure. A general procedure for a typical transfection was as follows. Cells were seeded in 24 well tissue culture plates (Nunc) the day before transfection in order to be approximately 40% confluent the next day. All cell lines (COS-7, NIH3T3, T84 and HEK293) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS). Cells were routinely maintained on plastic 24 well tissue culture dishes (Nunc) at 37°C in a humidified 5% CO_2/ 95% air-containing atmosphere. Plasmid DNA (0.3 μg) and the desired cationic cholesteryl amphiphile (4 μg) were each diluted in 100 μl of DMEM without FCS. After ≈ 5 min. the two solutions were combined, gently mixed and the resulting solution was allowed to incubate for 15 min at room temperature to allow complex formation. The transfection mixture was then added (0.2 ml /well) to the cells that had been washed with serum free medium. After 8 h incubation at 37°C, the transfection mixture was removed and 0.4 ml 10% FCS was added to each well. The cells were harvested at 2 days post transfection for monitoring of the transient expression of the luciferase gene. Control transfections were

performed by using commercially available transfection reagents The lipopolyamine Lipofectamine® was used at a concentration of 4 μg/well. For transfection with the commercial grade Lipofectin® -DNA complexes were obtained using the standard conditions specified by the manufacturer.

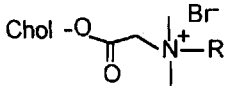
Transfections in the presence of Serum. Transfections in the presence of serum were carried out in an identical manner with the transfection mixture being overlaid on wells containing an equal volume of 10% FCS such that the wells contained the lipid-DNA complex in 5% FCS. All experiments were performed in duplicate.

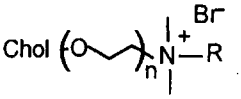
Luciferase Assay. Luciferase activities were assayed 48 hours post-transfection using a modified procedure of De Wet *et al.* (1986). This is briefly presented herein. After removal of the culture medium, the cells were washed with cold phosphate buffer - saline and lysed by incubation with 60 μl cell lysis buffer (25 mM Tris-phosphate buffer, pH = 7.8, 2 mM DTT, 2 mM CGTA, 10% glycerol, 1% Triton X 100). The lysate was clarified from insoluble material by centrifugation (for 5 sec at 12000 × g). The supernatant was transferred to a new tube and the pelleted cell debris was discarded. An aliquot (2 μl) of the cell extract at room temperature was mixed with 20 μl of Luciferin also at room temperature. Samples were placed in a photon counting luminometer (Turner Designs Model, TD 20/20 Luminometer) and the integration value of light emission was measured for 10 seconds. Protein concentrations were measured by using Bradford's method with BSA as a standard. Data for luciferase activity are expressed as Relative Light Units (RLU)/μg of cell protein.

RESULTS AND DISCUSSION

Figure 1 shows the molecular structures of the three families of cationic cholesterol based amphiphiles (*cholamphiphiles*) **1-3**. We first synthesized two families of cationic cholamphiphiles, one where the charge is linked to the cholesteryl skeleton either via an *ester* or an *ether* bond. In cholesteryl derivatives **1a-d**, the cationic headgroup is linked to 3β-cholesterol via an *ester* connection. In the derivatives **2a-d**, the cationic headgroup was linked to the hydrophobic cholesteryl backbone via an ether linkage. In view of the fact that there is a relation between headgroup hydration and transfection efficiency we chose oligo-oxyethylene residues as elements of hydration modulation at the headgroup. Hence in series **1a-d**, the positive charge is located at a fixed distance from the cholesteryl backbone and the headgroup hydration is continually modified with the progressive increase in the number of oxyethylene units on the $-NMe_2^+$ center. In addition, due to lack of information on the optimum location of the positive charge with respect to the hydrophobic backbone, **2a-d** were synthesized where cholesterol derivatives are connected to the cationic NMe_3^+ groups via varying lengths of an oligo-oxyethylene spacer units. The cationic NMe_3^+ group is therefore placed at

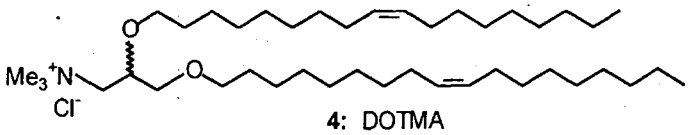
incrementally greater distance from the cholesteryl backbone with the insertion of increasing number of oxyethylene units.





1a: R = CH_3
1b: R = CH_2CH_2OH
1c: R = $(CH_2CH_2O)_2H$
1d: R = $(CH_2CH_2O)_4H$
3b: R = cis-$(CH_2)_8CH{=}CH(CH_2)_7CH_3$
3c: R = cis-$(CH_2CH_2O)_4CO.(CH_2)_7CH{=}CH(CH_2)_7CH_3$

2a: n = 1, R = CH_3
2b: n = 2, R = CH_3
2c: n = 3, R = CH_3
2d: n = 4, R = CH_3
3b: n = 1, R = cis-$(CH_2)_8CH{=}CH(CH_2)_7CH_3$
3d: n = 1, R = CH_2CH_2OH



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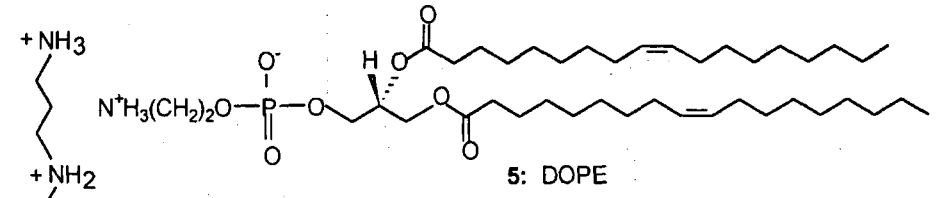

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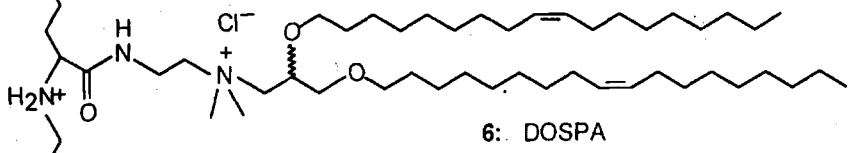

6: DOSPA

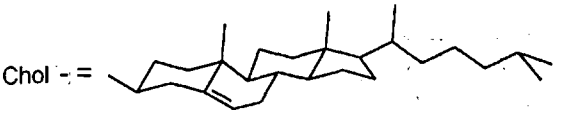

***Figure 1** : Molecular structures of the various cytofectins and helper lipid (DOPE) employed in the present investigation.*

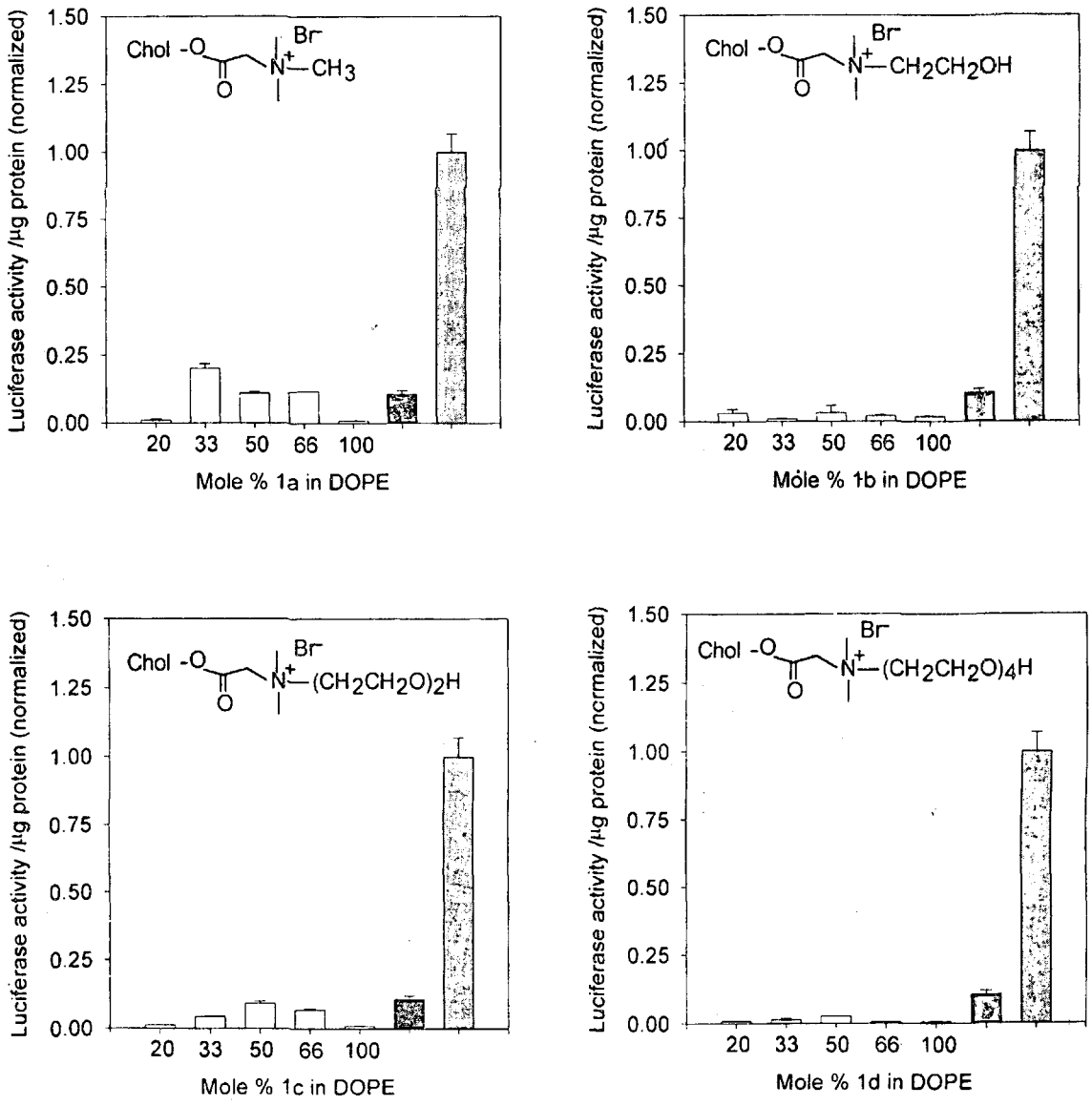

Figure 2 : *Comparison of transfection efficiencies of pGL3 control DNA using various compositions of* ***2a-d*** *in DOPE (□) with lipfectamine® (▒) and lipofectin® (▒). DNA concentration was 0.3 µg/well and lipid concentration was 4.0 µg/well. Data is expressed as RLU/µg total protein content normalized to lipofectamine.*

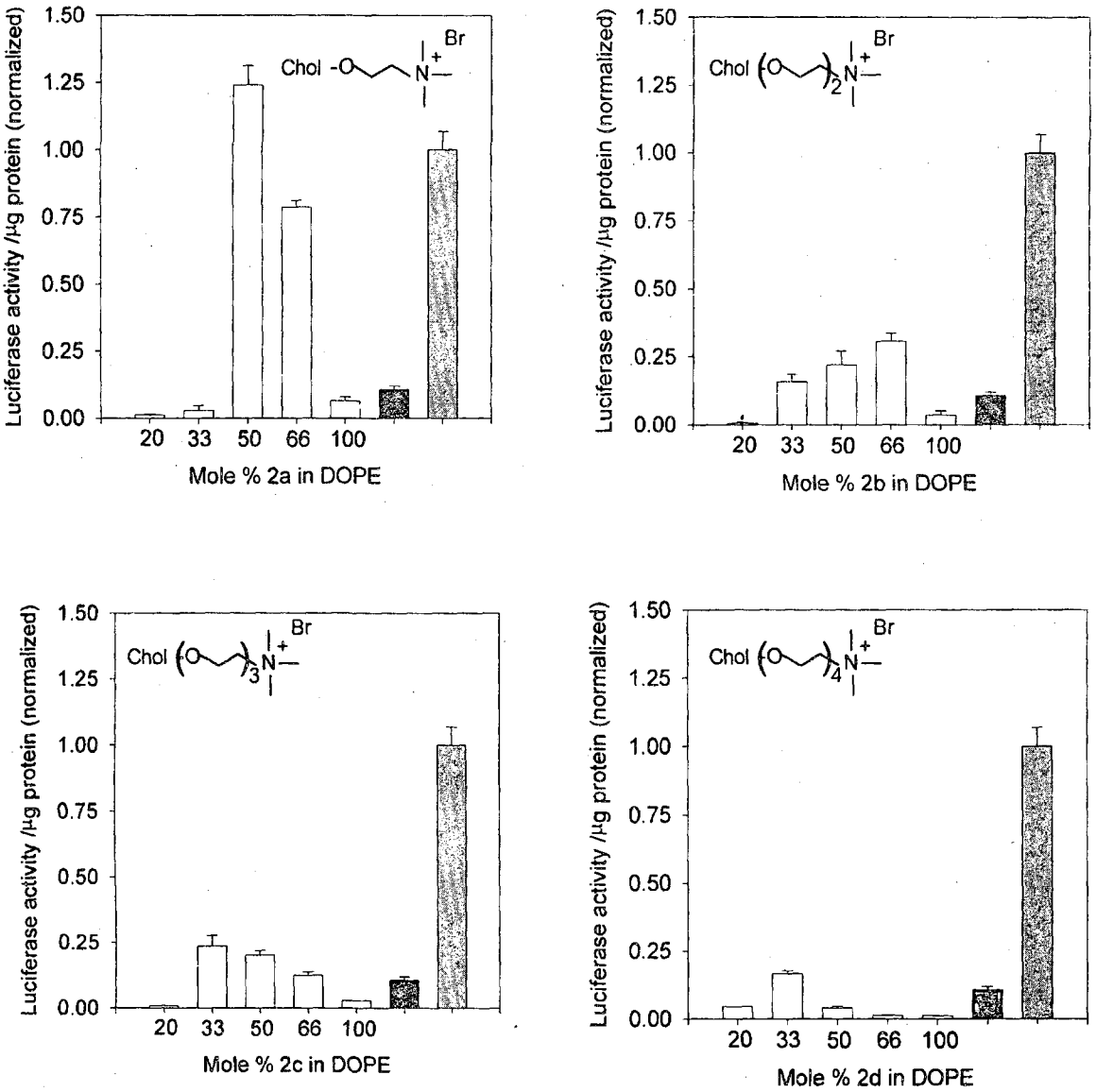

***Figure 3** : Comparison of transfection efficiencies of pGL3 control DNA using various compositions of **2a-d** in DOPE (□) with lipfectamine® (▩) and lipofectin® (□). DNA concentration was 0.3 µg/well and lipid concentration was 4.0 µg/well. Data is expressed as RLU/µg total protein content normalized to lipofectamine*

Membranes formed from the aqueous suspensions of these amphiphiles found to be highly rigid. For instance, these aggregates were found to be thermally insensitive (Bhattacharya *et al.*, 2001), as no solid-to-fluid transition was observed under differential scanning calorimetry. To examine the role of such thermally insensitive hydrophobic segments towards transfection, a known membrane 'disorder' promoting fatty acyl chain namely the oleyl chain, was appended to **1a**, **1d** and **2a**, to give **3a-c**. In addition, the ether analogue of **1b**, i.e. **3d** was also synthesized.

The potency of a given cytofectin towards DNA transfection was checked as follows. The cytofectin of interest was mixed with DOPE (**5**) in mole percentages of 20, 33, 50, 66 and 100 in separate vials and the corresponding mixed liposomes were prepared as given in the Experimental Section. Transfections were carried out with five different liposomal formulations and the luciferase assay was used to quantitatively assess the corresponding transfection efficiencies. Two control transfections, with commercially available liposomal transfection reagents i.e., Lipofectin® (which is a 1:1 mixture of 1, 2 –dioleyloxypropyl-3-trimethylammonium bromide (DOTMA) **4**/DOPE) and Lipofectamine® (a mixture of 1:1 DOSPA **6**/DOPE) were carried out every time. It was ensured that the variation of light units obtained for control transfections was not greater than 5 %. Transfection efficiency (TE) of a given formulation is expressed as Relative Light Units (RLU)/μg protein content normalized with respect to Lipofectamine®. Thus a plot of mole-% cytofectin in DOPE versus the TE gave the transfection profile of the cytofectin so investigated. The transfection profile of the cytofectin shows the amount of DOPE required by the cytofectin for optimal transfection efficiency (OTE).

The transfection profiles so obtained for the ester-based series **1a-d** are presented in Figure 2. **1a** showed the highest activity in the series, with the most effective formulation being 1:2 **1a**/DOPE (Fig 2A). This formulation was at least three times more active than that of Lipofectin®. The most active formulations for all the other compounds in this series were 1:1 formulations of cytofectin/DOPE (Fig. 2B-D). OTE for a given cytofectin was found to decrease from **1a** to **1d**. When a similar study was carried out with the ether-based series of cytofectins **2a-d**, appreciable improvement in transfection abilities was observed (Fig. 3). **2a** was found to be the most active cytofectin in this series, which showed an OTE as a 1:1 mixture with DOPE (Fig. 3A). This mixture was found to transfect COS-7 cells at least 1.2 times as efficiently as Lipofectamine® and over one order of magnitude as efficiently as Lipofectin®. **2b** was most active as a 2:1 **2b**/DOPE mixture (Fig. 3B) while 1:2 **2c**/DOPE and 1:2 **2d**/DOPE were the most active formulations for **2c** and **2d**, respectively (Fig. 3C & 3D). OTE for a given cytofectin was again found to decrease from **2a** to **2d**. A close examination of the optimal

transfection efficiencies of both the series revealed that without exception, all ether-based cytofectins in series **2a-d** showed TEs greater than 0.25, which is better than the OTE of the most efficient ester-based cytofectin formulation (1:2 **1a**/DOPE). This reflects a clear advantage which the cytofectins with ether link possess over their ester linked counterparts.

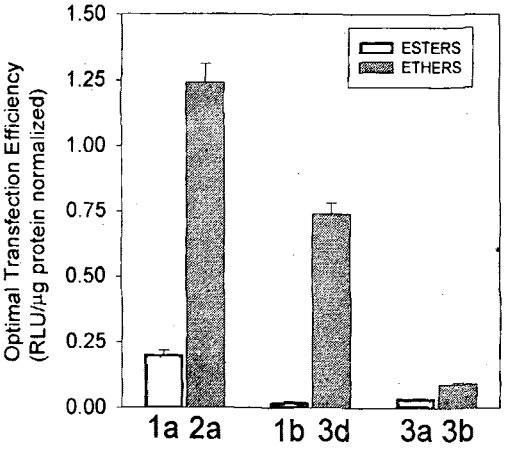

***Figure 4** : Comparison of transfection efficiencies of ether-based cationic cholesterol derivatives and the corresponding ester based analogues.*

Appending **1a** and **2a** with oleyl chains to give **3a** and **3c** resulted in a decrease in transfection efficiencies for both compounds (not shown). This suggests that introduction of disorder promoting elements reduce the activity of these cytofectins. Interestingly again in this series also, the ether-based analogue of **1b** i.e., **3d** was the most active without DOPE and showed an OTE of 0.74 ± 0.03 at 100% **3d**. Closer scrutiny of the molecular structures of **1a** and **2a**, **1b** and **3d** as well as **3a** and **3b** reveal that these pairs are identical in all respects except for the fact that the ester link in the former is replaced by an ether link in the latter. A comparison of their OTEs normalized to Lipofectamine® (Fig. 4.) shows that while **2d** transfects 600 % as efficiently as **1d**, **3d** transfects 15 times as efficiently as **1b** and **3b** transfects twice as well as **3a**. This reveals unambiguously that the ether-based lipids transfect DNA with much greater efficiency than their ester counterparts.

We then decided to study in detail, two of the most efficient cytofectin formulations, i.e., 100% **3d** and 1:1 **2a**/DOPE. Transfection efficiencies for the above formulations were optimized by varying DNA concentration from 0.76 μM to 30.3 μM while maintaining the lipid (liposomal formulation) concentration constant. Similarly the lipid concentration was varied from 3.85 μM to 77.04 μM (for 1:1 **2a**/DOPE) and up to 137 μM (for **3d**) keeping the DNA concentration constant. Transfection efficiencies for both cytofectin formulations increased with increasing concentrations of DNA till 1μg/well. With further increase in DNA concentration, transfection efficiencies decreased. This bell shaped curve has been previously reported for other cationic lipid formulations and is most likely related to the over-saturation of the cationic charges by the excess DNA (Rose *et al.*,1991). The optimum DNA concentration of both 1:1 **2a**/DOPE and **3d** was 1.0 μg/well of DNA. However, the activity profiles of both formulations showed different characteristics. **2a** exhibited a bell shaped

dependence with lipid concentration (Fig 5A). For all DNA concentrations the optimum [Lipid]/[DNA] ratio was ~4. In contrast, **3d** showed a curve that saturated at an optimum [Lipid]/[DNA] ratio of ~5 (Fig. 5B).

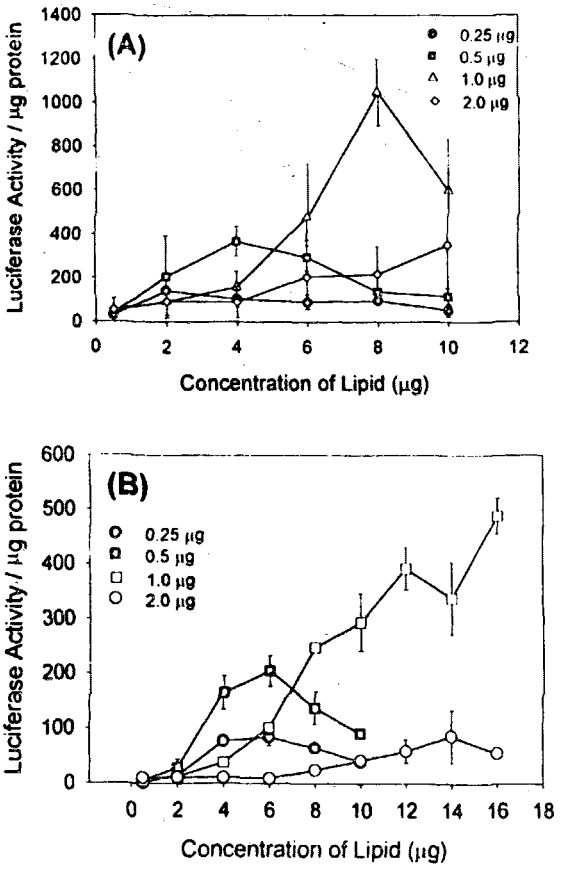

***Figure 5** : Optimization of transfection efficiency of 1:1 **2a**/DOPE (**A**) and **3d** (**B**) in DOPE. DN concentrations were 0.05, 0.25, 1.0 and 2.0 µg/well. Data is expressed as RLU/µg of total cellula protein. Experiments were performed twice and in duplicate.*

We, then probed the efficiency of transfection of 1:1 **2a**/DOPE and **3d** in the presence of serum. Serum is known to inhibit the transfection due to the presence of negatively charged sulphate groups in certain constituents of serum which competes with the phosphodiester anions of the DNA backbone for the cationic liposome This then interferes with the formation of the cationic lipid-DNA complex and affects transfection efficiencies. To examine this aspect, transfections were carried out in the presence and absence of 10 % fetal calf serum using the optimized conditions for the two transfection formulations (Fig. 6). A similar experiment was carried out with commercially available Lipofectamine® as a comparison to evaluate the performance of these cationic cholesterol ether-based formulations. The conditions of Lipofectamine® for transfection were maintained at those recommended by the manufacturers. In the presence of serum, the efficiency of **3d** was reduced to one-tenths of the activity in the absence of serum showing that serum significantly hampers the ability of **3d** to transfect DNA. Lipofectamine® activity in presence of serum was suppressed to less than one-fourth of the transfection efficiency under serum-free conditions. In comparison, the efficiency of 1:1 **2a**/DOPE in presence of serum was only reduced to ~ 50%. Thus, in the presence of serum, this formulation is about 1.4 times as efficient as Lipofectamine® under these conditions.

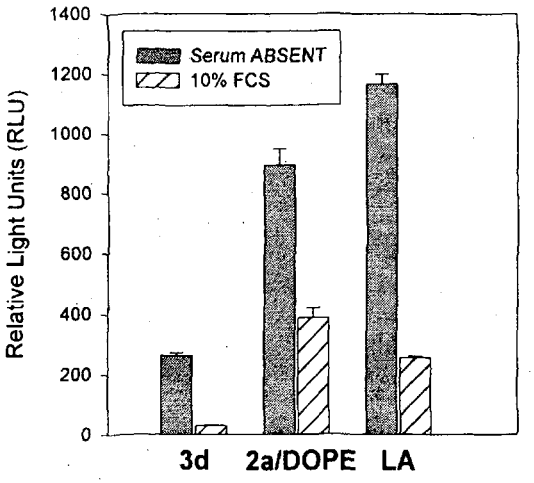

***Figure 6** : Transfection efficiencies in the presence of 10% FCS and absence of serum for **3d**, 1:1 **2a**/DOPE and LA (Lipofectamine®). Concentration of DNA = 1.0 μg/well, concentration of **3** = 10μg/well; LA = **2a**/DOPE = 4.0 μg/well. Data expressed as RLU for 10 μl of cell lysate.*

These findings suggest that **2a** induces gene transfer by a mechanism that is probably different from the one mediated by Lipofectamine®. Lipofectamine® is composed of a lipid derivative of spermine (DOSPA) which at physiological pH remains multi−protonated giving it a polycationic character. The large concentration of positive charges offered by this lipid on the membrane surface facilitates both DNA binding and fusion with the eukaryotic cell surface leading to efficient transfection. However, in the presence of serum the multicationic character of Lipofectamine® makes it compete with serum against DNA leading to significant suppression of transfection efficiency. On the other hand **2a**, possesses only one cationic center per molecule and is therefore not as drastically affected by serum.

The amount of extractable protein from treated cells was also determined (not shown) as an indication of the cytotoxicity of the treated cells. The total cellular protein for cells treated with 2 μg/well of DNA was about 80% of the untreated control. For DNA concentrations lower than 2 μg, the lowest protein content was observed for those conditions that showed the highest transfection efficiency indicating that the lipid–DNA complex is slightly toxic. However, under optimized conditions, the toxicity of 1:1 **2a**/DOPE and **3d** are at least 20% less than the toxicity of the Lipofectamine or Lipofectin-DNA complex.

Intrigued by the fact that **2a**/DOPE (1:1) induced excellent TE under various conditions in COS-7 cells, we proceeded to check its generality in conferring DNA transfection across other cell lines. Using the above determined optimized ratio of [Lipid]/[DNA], transfections using 1:1 **2a**/DOPE was used to transfect cell lines such as HEK293, NIH3T3 and T84. Table 1 shows that 1:1 **2a**/DOPE is indeed able to transfect a wide variety of cell lines. Even a cell line such as T84, which is not conducive to transfection, shows reasonably high luciferase activity. Thus, as a liposomal transfection reagent, the cationic ether-based cholesterol derivative **2a** as a 1:1 formulation in DOPE is indeed versatile and efficient.

Table 1 : Optimal Transfection efficiency of pGL3 DNA using 1:1 2a/DOPE in different cell lines.

Cell line	Luciferase Activity (RLU/mg protein)	Cell line	Luciferase Activity (RLU/mg protein)
COS-7	1.1×10^6	NIH3T3	1.79×10^5
T84	3.36×10^4	HEK293	4.41×10^5

Concentration of DNA = 1.0 µg/well; concentration of 1:1 **2a**/DOPE = 4.0 µg/well. Data expressed as RLU/mg of cellular protein content. Experiments were performed in duplicate.

In summary, we have systematically investigated the transfection abilities of a number of new cytofectins based on cholesterol that vary in terms of linkage by which the cationic charge is attached to cholesterol. In addition, within a given series of identical linkage, the headgroup hydration was also progressively modified. In the ester linked series **1a-d** it was observed that the progressive introduction of oxyethylene units on a cationic center at a fixed location reduced the transfection efficiency. Besides, the introduction of oligo-oxyethylene units as a spacer in the linker region of the lipid monomer (Figure 4b) in the ether linked series **2a-d** also resulted in a marked diminution of the transfection ability. Thus efficient transfection requires a rather "dry" positive charge located as close as possible to the steroid skeleton of the cytofectin. Introduction of a membrane 'disorder' promoting element like an oleyl chain to **1a** and **2a** resulted in considerable loss of transfection efficiency. Thus there seems to exist an optimum balance between rigidity and fluidity for this class of cytofectins. The effect of the nature of linkage was investigated in detail and the advantage of the ether linkage over the ester linkage was clearly evidenced. We are now extending these strategies to the design of other novel cytofectins.

ACKNOWLEDGMENTS

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MICROBIAL STRATEGIES IN RESPONSE TO ACID STRESS

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Abstract

Microbes are involved in a series of interactions with the hostile and diverse physiological conditions of the host where they may ultimately cause disease(s). Pathogens not only encounter potentially lethal conditions in the natural environment, but resist the host defensive mechanisms as well. The ability of bacteria to sense and respond to unscheduled changes in the environments is crucial to their survival. In order to survive, micro-organisms have to overcome many stresses mounted in the form of availability of nutrients, ions, variation in temperature, acidic conditions (inorganic and organic), changes in osmo-molarity, oxidative stress and relatively anaerobic conditions within the gut. They adapt to these stresses by coordinated expression of a number of genes/proteins, which contribute directly or indirectly to their pathogenicity. Understanding of their behaviour in different environments will enable dissection of more targeted strategies to control their infections. Their response to acidic stress is being reviewed in the present attempt.

Key words : Acid stress, microorganisms, survival strategy.

Acid stress can be defined as combined biological effect of low pH and weak acids present in an environment. For bacteria to grow within the human body or in an external environment, they need to withstand extreme changes in the external pH (Slonczewski, 2000; Foster, 2000). One of the most frequently encountered hostile conditions faced by *E.coli, Salmonella enterica* serovar Typhimurium or *Shigella flexneri,* while travelling through the gastro-intestinal tract is extreme low pH of the stomach as well as volatile fatty acids present in the intestine and feaces. Macrophage phagolysosome encountered by intracellular pathogens is another site of low pH, (Lin *et al.*, 1995; Bearson *et al.*, 1997; Baik *et al.*, 1996). Even upon exiting a host, enteric organisms may confront acid stress in the form of industrial waste, acid mine drainage or in decaying organic matters. Acidified ponds, degradative cellular organelle and host digestive systems, as well as by products of the catabolic activities of the bacteria, contribute to microbial acid stress (Bearson *et al.*, 1997; Merrel and Camilli, 2002). Acidic stress is intensified by the presence of short chain fatty acids, which are produced in high concentrations as a result of fermentation by natural microflora in the intestine (Cumminghs *et al.*, 1987). These short chain

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fatty acids are also present in food preservatives in acidic foods such as apple cider, dry fermented sausage, mayonnaise and yogurt (Cheville *et al.*, 1996; Morgan *et al.*, 1993). Thus, the ability to sense and respond to potentially lethal changes in environmental pH is crucial to the survival of the members of the family Enterobacteriaceae.

Acid Stress Response

Classically, pH homeostasis mechanisms involving a series of protein antiport systems have been viewed as critical feature for surviving acid stress among neutrophiles such as *E.coli* and *Salmonella enterica* serovar Typhimurium (Booth, 1985). However, these systems do not appear to be inducible by acid and protect the cell only within a certain pH limits (Foster and Hall, 1991). Several inducible amino acid decarboxylases appear to contribute to emergency pHi (inside pH) maintenance in *Salmonella enterica* serovar Typhimurium. One of these systems has been identified as lysine decarboxylase (CadA) working in collaboration with lysine – cadaverine antiporter (CadB) (Park *et al.*, 1996). There is also evidence for an emergency pH homeostasis system, albeit ill defined, that is not dependent on extracellular amino acids (Foster and Hall, 1991; Bearson *et al.*,1998). The impact of acidic pH on the pathogenesis of microbes is profound. The stomach acidity is a barrier that must be breached. The less severe acid pH present in the macrophage phagolysosome environment, while not threat to survival, is an important virulence signal, evoking the synthesis of numerous virulence factors (Rathman *et al.*, 1996; Cirrillo *et al.*, 1998). The importance of gastric secretions as an early line of defense against enteric pathogens has been recognized for nearly a century, with the primary bactericidal barrier being acid dependent (Peterson *et al.*, 1989). Clearly, the infectious dose (ID) of different enteric pathogens corresponds to their relative abilities to withstand acid. For example, the infectious dose for *Salmonella* species including *S. typhi* is significantly decreased if stomach acidity is bufferd (Blaser and Newman, 1982). This suggests that the better prepared an organism is to tolerate acid, the more likely it will survive passage through the stomach and cause disease.

Acid Tolerance Response

The existence of inducible acid survival mechanisms in Enterobacteriaceae has become apparent. In the recent years, the most extensively studied bacteria in terms of acid stress responses are *Salmonella, Shigella* and *E.coli.* Different terminolsogies have been used to describle acid stress response systems. Acid resistance (AR), acid tolerance response (ATR) and acid habituation (AH) are all terms used to describe survival to low pH under different conditions. (Small *et al.*, 1994; Lin *et al.*, 1995).

The enteric group of microorganisms has proven diverse in the ways that they handle acid stress. Acid survival strategies among these organisms can be classified into two general categories, based on whether a system can be induced and function in *unsupplemented minimal medium* (ATR systems) or requires some form of supplementation for either induction or function (AR) mechanisms. The results establish that *Salmonella enterica* serovar Typhimurium possesses only the ATR systems, *S. flexneri* utilizes only supplementation-dependent AR mechanism, while *E.coli* has both types of systems (Lin *et al.*, 1995). *Salmonella enterica* serovar Typhimurium has been shown to develop several low-pH-inducible acid defense systems, collectively referred to as the acid tolerance response (ATR), that push the limits of pH tolerance (Lee *et al.*, 1995; Hall *et al.*, 1995; Wilmes-Risenberg *et al.*, 1996). Cells actively growing at pHo (outside pH) 7.7 rapidly die when shifted to conditions below pH0 4. However, adapting these organisms to a mildly acidic pH (pHo 5.8) for one generation increases their tolerance to more extreme acid conditions (pHo3) (Foster and Hall, 1990). This inducible acid tolerance response (ATR) is a two-stage process involving overlapping acid protection systems triggered at different levels of acidity. To provide an explanation for *Salmonella* surviving such harsh, acidic environments within the host, Foster and Hall (1990), examined the adaptive ability of *Salmonella enterica* serovar Typhimurium to become acid tolerant. They characterized a phenomenon and termed it as the adaptive acid tolerance response (ATR), in which exposure to slight or moderate acid stress results in the synthesis of proteins which then protect the bacterium from more-severe acid challenge.

An inducible ATR has been demonstrated for the number of pathogenic microorganisms. As the pathogen that has chosen to colonize the stomach, *Helicobacter pylori* it has developed exceptional systems of acid tolerance. Even though urease plays an important role in survival of *Helicobacter* at extreme low pH, the organism also has a urease independent ATR (Karita and Blaser, 1998; Mc Gowan *et al.*, 1998). Work with exponential-phase planktonic cells of *Streptococcus mutans* has shown that a pH change from 7.5 to 5.5 resulted in the induction of acid tolerance response (Kroll and Hamilton, 2003).

Acid Shock Proteins

The ATR system was found to require protein synthesis and thus represents a newly described genetic response to acid stress (Fig.). Analysis of polypeptide profiles has revealed that 18 polypeptides change during pH 5.8 preshock exposures, of these, 12 are induced and 6 are repressed during adaptation (Foster and Hall, 1990; Goodson and Rowburry, 1989). Two-dimensional analysis of the proteome revealed that 60 acid shock proteins (ASPs) are induced during log phase ATR while 45 ASPs are induced following adaptation of

stationary phase cells. However, only 5 of those proteins overlap, indicating separate systems of acid tolerance. ASPs are important for protecting the cells against extreme acid pH (pH3) in minimal or complex medium (Bearson *et al.*, 1998; Lee *et al.*, 1995). In a recent study conducted on *E.coli*, the pH dependent proteins identified by MALDI-TOF MS or by gel positions, only three of the identified proteins (TnaA, MalE and YfiD) have been shown to regulate previously by pH (Blankehorn *et al.*, 1999; Chagneau *et al.*, 2001; Heyde *et al.*, 2000; Kirkpatrick *et al.*, 2001). The pH dependence of the 19 other identified proteins, as well as the remaining 20 proteins have recently been reported (Stanick *et al.*, 2002). However, the localization of these proteins remains to be identified. In our laboratory, we have identified a 55 kDa outer membrane protein from *S. typhi* (the causative agent of typhoid fever) which gets expressed under inorganic as well as organic acid stress (Chander *et al.*, 2003). This protein has also been found to have the potential to induce apoptosis in macrophages.

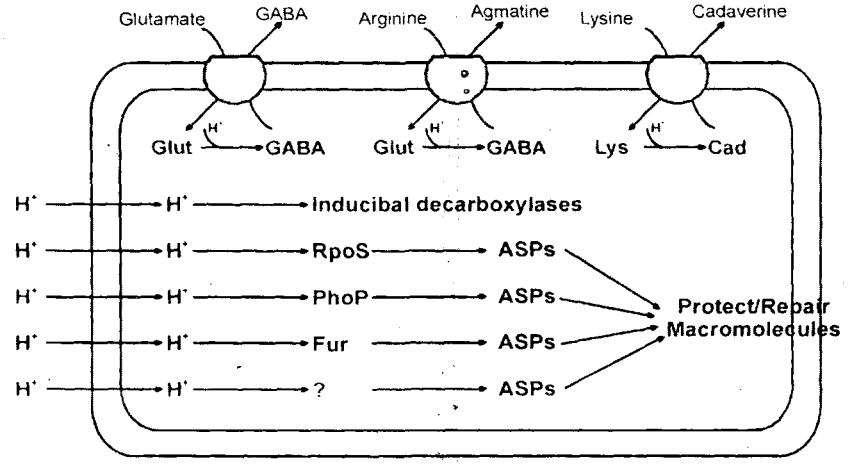

Figure : *Acid survival responses in enteric micro-organisms (Adapted from Bearson et al., 1997).*

The regulation of porin synthesis is tightly controlled by environment, and changes in the ratio of OmpC to OmpF levels had already been shown to occur in response to pH, osmolarity and temperature (Pratt and Silhavy, 1995; Todt *et al.*, 1992). Upregulation of OMPs by *Neisseria* on exposure to acid conditions has been reported (Petit *et al.*, 2001). Recently, it has been shown that cadaverine inhibition of porin may play a role

in the adaptive response to acidic conditions (Samartzidou *et al.*, 2003).

Even the other outer surface proteins such as flagella and fimbriae found to be elaborated under acidic conditions (Walker *et al.*, 1999). The X-ray crystal structure of *E.coli* stress response protein HDEA α has been determined. The single domain α-helical protein is found in the periplasmic space, where it supports an acid resistance phenotype, essential for infectivity of enteric bacterial pathogens, such as *Shigella* and *E.coli* (Gajiwala and Burley, 2000).

Organic Acid Stress

Short chain fatty acids namely acetate, propionate and butyrate are the predominant anions occurring in the large intestine of all mammals including herbivores, omnivores and even carnivores. Short chain fatty acids are produced by anaerobic fermentation from carbohydrates, the major components in human colon being plant cell wall polysaccharides such as cellulose, pectins and hemicellulose. Plant cell wall polysaccharides are composed of hexoses (glucose and galactose), pentoses (xylose and arabinose) and uronic acid monomers, which are fermented by the gut microorganisms along a variety of pathways (Prins, 1977).

Effect of SCFAs on microorganisms

The anaerobic breakdown of carbohydrates yields short chain fatty acids upto 150 mM (Macfarlane *et al.*, 1992) alongwith the production of H_2, CH_4, CO_2 and energy which bacteria require for growth and maintenance of their cellular functions (Cummings *et al.*, 1987). These organic acids have deleterious effects on the growth and viability of bacteria and are thus commonly used as food preservatives (Ricke, 2003). Organic acids can affect cell growth in at least two ways, by lowering pHi and by increasing turgor through anion accumulation. The lethal effects of SCFAs are not only concentration–dependent, but also related to the pH of the environment and the dissociation constant of the chemical. For example, benzoic acid at a given concentration is more bactericidal in an acidic environment than at neutral or alkaline pH. This property is due to the fact that the unionized (protonated) from of a weak acid is more permeable to the cell membrane than the ionized form (Cherrington *et al.*,1991). Thus, as an environment containing organic acid acidifies, the proportion of undissociated weak acid will increase, making the organic acid available to penetrate the cell. After unionized form of acid is inside the cell, the more alkaline intracellular environment (pH internal 7.5–7.8) will cause dissociation of weak acid and accumulation of dissociated weak acid proves lethal for the cell (Russell, 1992) by perturbing the cellular homeostasis (Baik *et al.*, 1996; Roe *et al.*, 1998). Though, very little information concerning the resistance of enteric organisms, to organic acids is available (Goodson and Rowbury, 1989) the inhibitory effects as reported in *E.coli* include: inhibition of macromolecular synthesis (Cherrington *et al.*, 1990), and dissolution of pH gradient across the

membrane and selective enzyme sensitivity to the lowered internal pH (Cherrington *et al.*, 1991). In *S. enterica* PT4 and *E. coli,* SCFAs, specifically formate and propionate, have been reported to increase the cell size due to the inhibition of DNA synthesis without any apparent damage to the cell membrane. Moreover, propionic acid incubated bacteria showed increased resistance to shrinkage after fixation because of altered phospholipid and fatty acid composition (Thompson and Hinton, 1996). The *in vivo* attachment of *Salmonella enterica* serovar Typhimurium in chick ceca has been reported to decrease logarithmically after selected feeding of SCFAs. *In vitro* estimation of the effect of SCFAs on the growth of *Salmonella enterica* serovar Typhimurium has revealed 50% growth inhibition and a 10% increase in SCFA concentration caused 80% inhibition (McHan and Schotts, 1993).

A system that potentially caved combat organic acid stress is the acid tolerance response (ATR) of *Salmonella enterica serovar* Typhimurium (Baik *et al.*, 1996). Studies with *Salmonella* indicate that organic acids at low concentration (below inhibitory concentrations) do not induce resistance to higher concentrations of these acids at pH 4.4 (Foster, 2000). Another study, however, indicates that short chain fatty acids (SCFAs) induce inorganic acid tolerance in exponential phase of *Salmonella* (Kwon and Ricke, 1998).

It was shown that *Vibrio cholerae* ATR consists of two branches: inorganic and organic, while some bacterial factors are required for both inorganic and organic ATR (Merrell and Camilli 2000). Approximately 60 different protein species were upregulated upon shift to adaptation conditions showing that *V.Cholerae* is able to induce the expression of a large number of organic ASPs. In addition, approximately 50 polypeptide species were down regulated (Merrell *et al.*,2001). The exposure of microorganisms to SCFAs causes the induction of ATR, i.e., resistance to extremely low pH and it has been implied in an increasing number of *Escherichia coli* mediated food borne diseases (Jarvis and Russel, 2001). Despite the characterization of the genes of *S. typhi*,(Parkhill *et al.*, 2001) the effect of SCFAs on the pathogenicity of *Salmonella enterica* serovar Typhi is still to be evaluated and studied in details.

Regulation of Acid Tolerance by Alternative Sigma Factor

The sigma factor σ^S, encoded by the *rpoS* gene was identified as being important to both stationary as well as log phase cells subjected to a brief acid shock (pH 4.5, 30min (Lee *et al.*, 1995; Ibanez-Ruiz *et al.*, 2000). Expression of *rpoS* and production of σ^S in stationary phase is regulated at all levels (transcription, translation, and protein stability). Acid shock, however, only targets translation and protein turnover (Webb *et al.*, 1999). Acid shock in the form of short chain fatty acids (e.g. acetate) increases the expression of translational but not transcriptional *rpoS-lacZ* fusions by 10 to 15 fold. A regulatory cascade that is required for

efficient translation of the *rpoS* message begins with DksA, a zinc-finger protein with no known function and a gene regulated by DksA called drgA (Webb *et al.*, 1999).

Degradation of σ^S is very rapid in log phase cells (3 minute turnover rate) but slows considerably following acid shock. Degradation requires the ClpXP protease and a chaperone called MviA (RssB in *E.coli*) (Schweder *et al.*, 1996). Bacterial two-hybrid analysis indicates that MviA cross-links σ^S and ClpX (the ATPase subunit of the protease). MviA essentially expands the substrate specificity of ClpXP to include σ^S. The reason for acid shock control of σ^S turnover remains a mystery (Moreno *et al.*, 2000).

One regulator of σ^s–independent ATR is phoP, the regulatory component of phoP/phoQ 2–component system. The phop/Q regulon is known to be important for macrophage survival, protection against antimicrobial peptides and virulence (Mahan *et al.* 1996). The Pho P/Q system has been shown to sense external Mg^{2+} (Vescovi *et al.*, 1994). However, it was shown that phoP is also an ASP used for protection against low pH (Bearson *et al.*, 1997). Another regulator of acid tolerance is the ferric uptake regulator (*fur*). In the presence of excess intracellular Fe^{2+}, this 17kDa protein represses the expression of iron-regulated genes. Surprisingly, fur also governs the expression

Table : Genes whose expression changes as a function of pH.

Gene/Protein	Process/Function	Organism
Adi	Arginine decarboxylase	*E. coli*
GroEl. DnaK,	Stress proteins	*E. coli, S. typhimurium*
lysP (cadR)	Lysine permeae	*E. coli*
OmpF, ompC	Porins	*E. coli, S. typhimurium*
pag genes	Virulence factor, macrophage survival	*S. typhimurium*
vir genes	Bacterial-host interactions	*A. tumefaciens*
ToxR regulon	Virulence factors	*V. cholerae*
MenCD	Menaquinone synthesis	*B. subtilis*
agr and *Are*-regulated genes	Regulation of exoprotein synthesis	*S. aureus*
Arginine deaminase	Arginine catabolism	*Streptococci*

(Adapted from Olson, 1993)

of several ASPs as an activator in an iron independent manner, this regulation occurs even when the iron–binding site of *Fur* is compromised leading to the hypothesis that fur senses iron and pH separately (Hall and Foster, 1996). The roles of various genes whose expression changes as a function of pH are listed in the table.

CONCLUSIONS

It is thus indicated from various studies that organisms have the ability to sense the specific environments and undergo a programmed molecular response in which stress inducible proteins are synthesized. Future studies are required to elucidate the role of ASPs in the virulence strategies of microbes. Virulence attributes of pathogens have the potential to modulate host immune response through cytokine network. Environmental stresses have a key role to play in this set up of host-parasite interactions. Following proper understanding of host-parasite interaction, it may be possible to modulate the virulence of invading bacteria which could be more susceptible to host's defensive mechanisms. Key questions that remain to be answered include the identification of key regulators of ATR, giving a more thorough understanding of bacteria that actually sense changes in pH. The essence of signal transduction to gene expression, translation machinery or other cellular responses, are the areas which deserve an in depth research. Application of molecular tools to study pathogen behaviour at different stages would enable dissection of specific bacterial regulation involved in response to acid stress. This could lead to the development of more targeted strategies to control food borne pathogens with acids stress.

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GROWTH AND NUTRITIONAL STATUS OF SCHOOL GOING PUNJABI RURAL ROPAR GIRLS

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Abstract

A cross-sectional data was collected on randomly selected 282 school going rural girls of Ropar ranging between 5 to 10 years of age. Eleven anthropometric measurements were taken on each subject. Results indicate that rural Ropar girls show better performance in growth patterns at 5 and 6 years of age in comparison to other rural Punjab girls and urban Indian girls. However, on the contrary, the girls aging between 7 to 10 years from other rural Punjab and urban India showed better performance in growth status. It has also been observed that American and well nourished Indian girls show higher values for all the anthropometric parameters than those of rural Ropar girls. The rural Ropar girls are found to be superior in their growth status as compared to the general rural Indian girls at all ages. To assess nutritional status of the rural Ropar girls, weight deficit for age, height deficit for age and upper arm circumference deficit for age have been calculated using NCHS standards and the results indicate that the Grade I and Grade II malnutrition were prevalent among the rural Ropar girls, though, the percentage of severely malnourished girls was insignificant.

Key words: Anthropometry, nutritional status, physical growth, rural Ropar girls.

INTRODUCTION

The growth and nutritional status of children is an important index of health status of a community. Several studies have been conducted on physical growth and nutritional status of children in different parts of the country as malnutrition is a common social and public health problem among children in developing countries (Begum and Choudhury,1996; Gupta and Bhandari, 1974;Jondhale *et al.*, 2001; Kumar *et al.*, 2000; Nigam and Vir, 2001; Rao. 1978).

Our country consists of diverse agro-climatic regions and ethnic multiplicities. Socio-cultural practices, life styles and eating habits vary not only among states but also among districts within a state. Updated profiles on growth and nutritional status of children especially girls are therefore, important for formulation and implementation of nutritional policy not only at the state level but also at district and tehsil levels. According to National Nutrition Monitoring Bureau (NNMB, 1989-90), only 10% children in our country are normal (Gomez classification), while 8.7% are severely malnourished, 68.7% are underweight, 65.1% are stunted and 19.9% are wasted (Kumar *et al.*, 2000). The National Family Health Survey report (1992-93) shows 46% of the children in Punjab are underweight.

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Growth and development of children is influenced by intra-uterine environment, parental size and genetic constitution, climatic factors, nutrition, disease and socio-economic factors (Bogin, 1988; Tanner, 1978). Growing children require balanced and nutritious food to meet the general physical requirements. If in this period of rapid growth, a child is not taken care of, it influences the state of his/her health not only during childhood but throughout life (Bogin,1988). Assessing physical growth and nutritional status is important in case of a girl child as the later are brought up for their imminent identity as wives and mothers especially in a rural set up. Achievement of optimum growth and nutrition especially in context of a girl child is therefore, considered to be of utmost importance for betterment of child's survival and development as well as the maintenance of maternal nutrition. Hence, assessment of physical growth and nutritional status is most essential for comprehensive care of the girls. Unfortunately, not many studies have been conducted to evaluate growth and nutritional status of school-going girls in the Ropar district of Punjab. The present study, therefore, is an attempt in this regard to evaluate the growth and nutritional status of school-going girls aged between 5 to 10 years in Tehsil and District Ropar in Punjab and also to compare the results with few other studies conducted in Punjab, other Indian regions and abroad.

MATERIALS AND METHODS

A cross-sectional sample of Punjabi speaking 282 school-going girls, aged between 5 to 10 years, was taken from Tehsil and District Ropar of Punjab. The subjects were randomly selected from Government Primary Schools in seven villages namely, Pool Khurd, Kharabad, Badi Haveli, Chhoti Haveli, Bhuda Bhora, Jallian Chhoti and Bada Mansuha. The study area located in the sub-mountain region is predominantly occupied by *Sainis* and *Jat Sikhs*, besides, other castes like Scheduled Castes, *Lohars*, *Tarkhans*, *Kumhars* etc. also inhabit the region. Farming, agricultural labourers, animal husbandry and low-salaried jobs form the major occupations of the population.

All the subjects studied were physically and mentally normal and did not suffer from any apparent illness. Date of birth of each subject was recorded and confirmed from school-admission registers and doubtful cases were excluded from the study. General information regarding their socio-economic status, caste, religion, parent's education and occupation, family size, structure and income etc. was also recorded.

The sample was grouped into 6 decimal age groups of one year interval. Eleven anthropometric measurements i.e. stature, weight, sitting height, bicondylar humerus, bicondylar femur, head circumference, chest circumference, upper arm circumference, biceps skinfold, triceps skinfold and subscapular skinfold were taken on left side of each subject with minimum possible clothing and without any footwear. The measurements were recorded with the help of an anthropometer, weighing machine, sliding caliper, steel tape and skinfold caliper

following standard procedures and landmarks defined by Weiner and Lourie (1969). All absolute and circumferential measurements were recorded to the nearest 0.1 cm except for weight, which was recorded to the nearest 0.1 Kg. However, skinfold measurements were recorded to the nearest 0.1mm with the help of Swiss made skinfold caliper. For each measurement, the data were collected by one of the authors (CK). Instruments were calibrated in the course of the study and repeated measurements were undertaken to establish intra and inter-observer variability which did not differ significantly from zero.

For assessing nutritional status of the subjects, weight deficit for age, height deficit for age and upper arm circumference deficit for age have been calculated. The subjects were divided into three levels of malnutrition besides normal i.e. grade-I, grade-II and grade-III. The classification for weight deficit for age, height deficit for age and upper arm circumference deficit for age were followed respectively from Gomez *et al.* (1956), Waterlow *et al.* (1977); Jelliffe (1996) (Table 1).

Table 1 : Classification of grades of malnutrition for weight deficit for age, height deficit for age and upper arm circumference deficit for age according to Gomez *et al.*,1956; Waterlow *et al.* 1977 and Jelliffe *et al.*, 1996 respectively.

Grades or types of Malnutrition	Normal	Grade-I (mild)	Grade-II (moderate)	Grade-III (severe)
Weight for age	Above 90%	75%-90%	60%-75%	Below 60%
Height for age	Above 95%	90%-95%	85%-90%	Below 85%
Upper arm circumference for age	Above 90%	80%-90%	70%-80%	Below 70%

Conventional statistics like mean, standard deviation, coefficient of variation, standard error etc., were employed to interpret the data.

RESULTS

Means and standard deviations for the values of height, weight, sitting height, bicondylar humerus and bicondylar femur, head circumference, chest circumference and upper arm circumference of rural Ropar girls are presented in Table 2. All the absolute and circumferential body dimensions show a gradual increase in mean values as age advances. Total gain in head circumference, chest circumference and upper arm circumference over the growth period of 5 through 10 years is 1.16cm, 5.97cm and 2.17cm respectively.

Mean values and standard deviations of triceps, biceps and subscapular skinfolds are shown in Table 2. Log transformation of mean values of skinfold measurements has been calculated by applying the formula as suggested by Edwards *et al.*, 2001. Triceps skinfold shows a varying pattern of rise and fall as the age advances from 5 through 10 years. It shows a decline from 5 years to 8 years and sudden rise from 8 to 9 years, thereafter decreases up to 10 years. Biceps skinfold exhibited a similar trend as triceps skinfold. Subscapular skinfold shows a gradual decline from 5 to 7 years where a sharp decline occurred again between 7 to 8 years, thereafter, the value rises sharply from 8 to 9 years followed by a slight decline between 9 to 10 years.

Table 3 presents the age wise distribution of malnutrition among the rural Ropar girls from 5 to 10 years of age as per weight deficit for age. Out of a total of 282 girls, only 40 girls (14.2%) were found to be normal while 105 (37.4%) were under grade-I malnutrition, 129 (45.5%) under grade-II malnutrition and only 8 (2.9%) girls were severely malnourished (grade-III malnutrition). In the age group 5 years, %age of normal girls was found to be highest and in the age groups 5, 6, and 7 years, no one was found to suffer from severe malnutrition. In the age group 6 and 7, grade-I malnutrition was prevalent and in the age groups 8, 9 and 10, the grade-II malnutrition was found to be more prevalent.

Table 4 presents the agewise distribution of malnutrition among the rural Ropar girls from 5 to 10 years of age as per height deficit for age. Out of a total of 282 girls, 120 (42.55%) suffered from grade-I malnutrition and 38 (13.5%) from grade-III malnutrition and only 4 (14%) were found to be severely malnourished. In the age group 5 and 6 years, percentage of normal girls was found to be higher and in the age group 6, 7 and 8 years, no one suffered from severe malnutrition.

Table 5 presents the extent of malnutrition as per upper arm circumference deficit for age among rural Ropar girls from 5 to 10 years of age. Out of 282 girls, 181 (64.1%) were found to be normal, 100 (35.5%) showed grade-I malnutrition and only 1 (0.37%) showed grade-II malnutrition and no one suffered from severe malnutrition.

By using these three different parameters for the assessment of nutritional status, it is apparent that maximum number of girls are either normal or suffer from only mild (grade-I) malnutrition, while grade-II malnutrition is prevalent among comparatively less number of girls. While, the prevalence of severely malnourished girls was found to be very low. Generally malnutrition was found to be more prevalent among the girls of 8, 9 and 10 years as compared to 5, 6 and 7 years of age groups.

Table 2 : Means and standard deviations of various anthropometric measurements of Ropar girls from age group 5 to 10 years.

Anthropometric Measurements	Age (years)					
	5(n=38)	6(n=40)	7(n=42)	8(n=45)	9(n=57)	10(n=60)
Height (cm)	104.46 ± 6.60	111.37 ± 4.48	113.56 ± 5.05	118.22 ± 5.34	123.20 ± 5.61	128.69 ± 4.69
Weight (Kg)	15.05 ± 2.06	16.71 ± 1.64	17.70 ± 1.75	18.22 ± 2.17	20.82 ± 2.34	22.56 ± 2.63
Sitting height (cm)	56.92 ± 2.90	60.48 ± 2.41	60.58 ± 2.52	60.89 ± 3.88	64.48 ± 2.69	66.65 ± 2.27
Bicondylar humerus (cm)	4.04 ± 0.26	4.21 ± 0.25	4.30 ± 0.22	4.37 ± 0.30	4.60 ± 0.26	4.87 ± 0.35
Biocondylar fermur (cm)	6.19 ± 0.31	6.45 ± 0.37	6.56 ± 0.35	6.56 ± 0.33	6.87 ± 0.35	7.08 ± 0.29
Head circumference (cm)	48.38 ± 1.00	49.12 ± 1.44	49.14 ± 1.09	49.27 ± 1.95	49.90 ± 2.02	49.99 ± 1.84
Chest circumference (cm)	52.58 ± 2.79	53.13 ± 2.72	54.14 ± 3.30	54.57 ± 2.74	57.25 ± 3.04	58.55 ± 2.79
Upper arm circumference (cm)	16.11 ± 1.20	16.52 ± 1.11	16.95 ± 1.01	71.00 ± 1.00	17.71 ± 1.09	18.28 ± 1.31
Triceps skinfold (mm)	165.04 ± 12.54	160.56 ± 15.93	155.82 ± 12.51	151.71 ± 14.95	159.58 ± 15.74	156.50 ± 23.69
Biceps skinfold (mm)	142.63 ± 15.85	128.12 ± 25.66	127.34 ± 17.01	118.13 ± 25.30	124.94 ± 21.27	121.73 ± 23.78
Subscapular skinfold (mm)	152.99 ± 12.57	149.35 ± 15.64	147.93 ± 12.49	139.12 ± 13.86	148.59 ± 13.89	148.38 ± 12.84

Table 3 : Level of malnutrition as per weight deficit for age among rural Ropar girls from age group 5 to 10 years.

Age Group (years)	N	Normal		Grade-I		Grade-II		Grade-III	
		No.	%age	No.	%age	No.	%age	No.	%age
5	38	16	42.1	12	31.6	10	26.3	-	-
6	42	12	28.6	27	64.2	03	07.2	-	-
7	40	07	17.5	24	60.0	09	22.5	-	-
8	45	02	4.4	13	28.9	29	64.5	1	2.2
9	57	02	3.6	21	36.8	31	54.3	3	5.3
10	60	01	1.7	08	13.3	47	78.3	4	6.7
Total	282	40	14.2	105	37.4	129	45.5	8	2.9

Table 4 : Level of malnutrition as per height deficit for age among rural Ropar girls from age group 5 to 10 years.

Age Group (years)	N	Normal		Grade-I		Grade-II		Grade-III	
		No.	%age	No.	%age	No.	%age	No.	%age
5	38	25	65.8	05	13.2	07	18.3	01	2.7
6	42	29	69.2	12	28.5	01	02.3	-	-
7	40	17	42.5	16	40.0	07	17.5	-	-
8	45	16	35.6	21	46.7	08	17.7	-	-
9	57	17	29.8	33	57.8	05	08.7	02	3.7
10	60	16	26.4	33	5.0	10	16.8	01	1.8
Total	282	120	42.55	120	42.55	38	13.5	04	1.4

Table 5 : Level of malnutrition as per upper arm circumference deficit for age among rural Ropar girls from age group 5 to 10 years.

Age Group (years)	N	Normal		Grade-I		Grade-II		Grade-III	
		No.	%age	No.	%age	No.	%age	No.	%age
5	38	29	76.3	09	23.7	-	-	-	-
6	42	31	73.8	11	26.2	-	-	-	-
7	40	32	80.0	08	20.0	-	-	-	-
8	45	22	48.9	23	51.1	-	-	-	-
9	57	34	59.6	23	40.4	-	-	-	-
10	60	33	55.0	26	43.3	01	1.7	-	-
Total	282	181	64.1	100	35.5	01	0.37	-	-

DISCUSSION

Mean values of weight, height, sitting height, chest circumference, head circumference, upper arm circumference and triceps skinfold of rural Ropar girls aging from 5 to 10 years were compared with well nourished Indian girls (Vijayaraghavan *et al.*, 1971), American girls (NCHS, 1977), Rural Punjab girls (ICMR, 1972), Average Indian girls, Urban Indian girls and Rural Indian girls (ICMR, 1989).

Table 6 and figure 1 present the comparison of mean values of weight of rural Ropar girls ranging in age from 5 to 10 years with other available data. The results indicate that the girls of the present study are lighter as compared to well-nourished Indian girls. The differences in weight between the rural Ropar girls and the well nourished Indian girls vary from 3.5 Kg to 10 Kg as the age advances, thereby suggesting progressive growth retardation. Rural Ropar girls show lower mean values of weight than those of rural Punjab girls and urban Indian girls from 7 to 10 years of age. However, at the ages 5 and 6 years, they show comparable values. The rural Ropar girls show comparatively faster rate of growth than the rural Indian girls. The average Indian girls show slower growth in weight as compared to the present study girls from 5 to 7 years of age, however, thereafter up to 10 years, the former show comparatively better performance.

Table 7 and figure 2 presents the age wise comparison of mean heights of the rural Ropar girls ranging from 5 to 10 years of age with other studies. The results indicate that the mean heights of the rural Ropar girls were far below than those of well nourished Indian and American girls of the same age. It is evident that the difference in mean heights of the girls of the present sample and well nourished Indian girls ranges from 8 to 10 cm. This gap in height is more or less uniform in magnitude throughout the age groups studied suggesting that the rate of change in linear growth of rural Ropar girls is equal to that of well-nourished Indian girls. The graph indicates that the rural Ropar girls show fast growth performance in height than the rural and average Indian girls, however, a slightly slow growth was shown by present study girls as compared to urban Indian girls except at 5 and 6 years when the former overtook the later. In comparison with the rural Punjab girls, the present study girls are shorter except at the age of 6 years where the present study girls show a slight higher value of mean height.

Table 6 : Comparison of mean values of weight (in Kgs) of rural Ropar Girls (5 -10 years) with other available studies.

Age (years)	Rural Ropar (Present Study)	Rural Punjabi (ICMR, 1972)	Average Indian (ICMR, 1989)	Urban Indian (ICMR, 1989)	Rural Indian (ICMR, 1989)	Well-Nourished Indian (Vijayaraghavan et al 1971)	American (NCHS,1977)
5	15.1	16.1	14.5	14.7	13.9	18.6	17.7
6	16.7	16.6	16.0	16.3	15.4	20.5	19.5
7	17.7	18.8	17.6	17.9	17.2	23.8	21.8
8	18.2	20.2	19.4	19.7	18.8	26.0	24.8
9	20.8	22.1	21.3	21.6	20.8	29.0	28.5
10	22.6	24.3	23.6	24.1	22.5	32.6	32.5

Table 7 : Comparison of mean values of Height (in cms.) of Rural Ropar Girls (5-10 years) with other available studies.

Age (years)	Rural Ropar (Present Study)	Rural Punjabi (ICMR, 1972)	Average Indian (ICMR, 1989)	Urban Indian (ICMR, 1989)	Rural Indian (ICMR, 1989)	Well-Nourished Indian (Vijayaraghavan et al 1971)	American (NCHS,1977)
5	104.5	105.4	101.4	102.1	99.5	112.2	108.4
6	111.4	109.1	107.4	108.0	106.3	117.7	114.6
7	113.6	115.6	112.8	113.3	111.9	122.7	120.6
8	118.2	120.2	118.2	118.7	117.2	127.2	126.4
9	123.2	125.3	122.9	123.4	122.9	133.1	132.2
10	128.7	130.4	128.4	129.1	126.7	138.9	138.3

When compared with average and urban Indians, the rural Ropar girls show fast growth in sitting height at the ages 5 and 6 years and at other age levels, the average and urban Indian girls overtook rural Ropar girls. In comparison with rural Indian girls, the rural Ropar girls were placed better at most of the ages except at 8 years where former overtook the later.

The growth trends in chest circumference when compared with rural, average and urban Indian girls, the rural Ropar girls show markedly better performance mostly at all the ages except 8 years where average and urban Indian girls showed slight higher values. Mean values of growth in head circumference were higher at most of the ages in rural Ropar girls as compared to those of average and urban Indian girls except at 10 years of age where average and urban Indian girls showed better performance.

The mean values of upper arm circumference of rural Ropar girls were compared with well nourished Indian girls and American girls and the results indicate that they show lower mean values than those of well nourished Indian girls and American girls at all age levels. Means of triceps skinfold of the rural Ropar girls were compared with well nourished Indian girls and WHO figures (Jelliffe, 1966) and the results indicate that the rural Ropar girls were having less subcutaneous fat at triceps at all age levels.

Comparative low performance in physical growth of rural Ropar girls than other Indian study groups might be attributed to the low-middle socio-economic background of the rural Ropar population and the observed growth retardation in anthropometric parameters may be more of nutritional origin although racial or genetic factors also contribute.

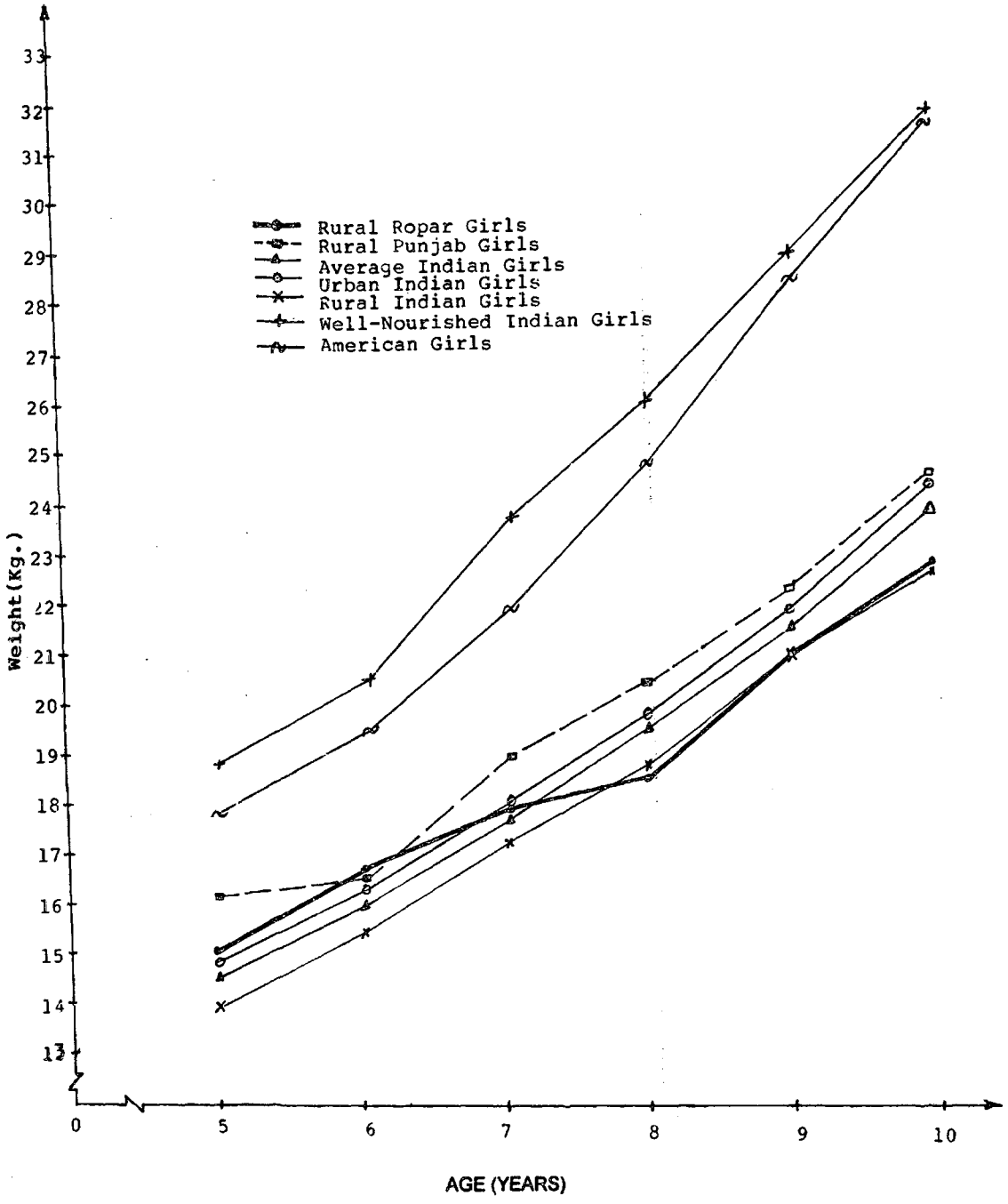

Figure 1 : *Comparision of Mean Weights (kg) of Rural Ropar Girls (5-10 years) with other studies.*

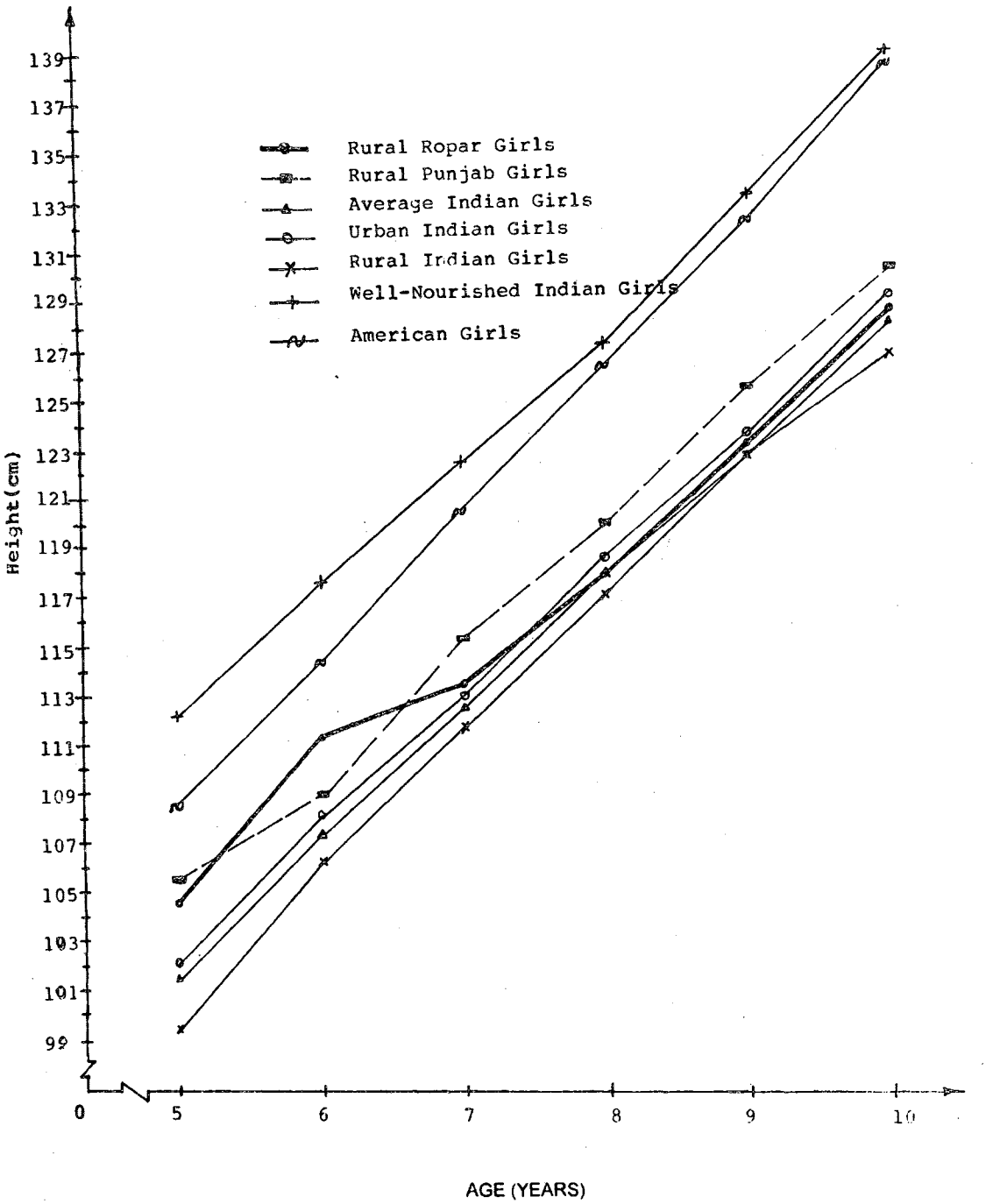

Figure 2 : *Comparision of Mean Heights (cm) of Rural Ropar Girls (5-10 years) with other studies*

In the present study, according to weight deficit for age, it was found that only 14.2% girls were normal and a large number of girls were suffering from mild to moderate malnutrition, however, only a small portion of the girls showed severe malnutrition. Body weight being easily affected by malnutrition provides the current state of nutrition of a community (Rao *et al.*,1979). Weight has been considered as a more sensitive indicator to environmental influences than height (Waterlow, *et al.*, 1977) and for the assessment of nutritional status, its validity has been well established (Jelliffe, 1966). It is therefore, stated that the current state of nutrition of a large section of population under study is not adequate. Similarly, according to height deficit for age, majority of the population showed mild to moderate malnutrition, however, it has been found that the percentage of individuals showing normal height was considerably higher i.e. 42.6%. In accordance with the upper arm circumference deficit for age, the girls were found to be either normal or suffering from only mild state of nutrition.

The present state of malnutrition in the rural Ropar girls may probably be traced due to low-middle socio-economic background of the population. Other factors responsible for their poor nutritional profile could be the educational status of the parents, family size, occupation and poor dietary intake. It was observed that the parents of most of the children were uneducated or partially educated. They were mostly farmers, agricultural labourers or having jobs with low monthly incomes. Family size was significantly large with 3-4 children in each family. Sex discrimination also contributes as male child enjoys better food and other facilities than the female child.

CONCLUSIONS

It is concluded that most of the physical growth parameters of the rural Ropar girls of 5-10 years of age show gradual and continuous increase as the age advances. Rural Ropar girls show better performance in growth as compared to rural Indian girls at all ages and mild to moderate performance when compared with other Indian and American girls. Nutritional status of rural Ropar girls assessed by three different parameters; is average to poor (mild to moderate) i.e. grade-I and grade-II level of malnutrition were prevalent, however, only a small number of girls showed severe malnutrition. According to weight evaluated against age and height evaluated against age, only 14.2% and 42.6% girls were normal respectively. Low-middle socio-economic background, uneducated or partially educated parents, large family size, inadequate diet and gender discrimination might have contributed to poor nutritional status of the rural Ropar girls.

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FOOD AND FEEDING HABIT OF DIFFERENT SIZE GROUPS OF GOLDEN MAHSEER, *Tor putitora* (HAM.) FROM PONG RESERVOIR, HIMACHAL PRADESH

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Abstract

Qualitative and quantitative analysis of the diet composition of Tor putitora (Hamilton) of Pong reservoir have revealed that the fish is cami-omnivorous in feeding habit and there is a variation in the percentage of frequency occurrence of different food items in different size groups. The frequency of feeding intensity was poor in majority of the size groups and the feeding intensity decreased with the increase in size.

Key words: Cami-omnivorous, feeding habits, *Tor putitora*.

INTRODUCTION

The study of food and feeding habits of fishes is an important aspect in fishery biology and extremely useful in pisciculture practices, conservation and environmental biology. Though, the golden mahseer, *Tor putitora* (Ham.), is an important game fish widely distributed in the middle Himalayas and is considered as indicator to determine the health of an ecosystem, but the information regarding its food and feeding is meager. Thomas (1897) studied its food of this fish during the major part of the year. Food of this fish from Kumaun (Das and Pathani, 1978) and from Garhwal (Badola and Singh, 1980; Joshi and Kumar, 1980; Nautiyal, 1990; Nautiyal and Lal, 1984; 1985) has been described earlier. Pong reservoir support substantial mahseer fishery in this region and even then in this part, no study has been undertaken relating to its food and feeding habits. Hence, the present study has been conducted with view for its rational management.

MATERIALS AND METHODS

To study the food and feeding habits of mahseer, *Tor putitora* from Pong reservoir, special request was made to the fishermen involved in commercial fishing at the Khatiar landing station of Pong reservoir (30°25'N, 75° 45'E) to supply the fish with gut during the period from 1998 to 2000. The gut was removed and preserved in 5% formalin for further analysis. The preserved gut was uncoiled, after removing the fat. The length of complete gut was recorded and relative gut length (RGL) was calculated as follows:

$$RGL = \frac{\text{Length of gut}}{\text{Total length of fish}}$$

The intestinal bulb was removed and weighed by calculating the difference in the weight of the intestinal bulb with and without food contents. The gastrosomatic index (GSI) was determined for the study of qualitative variation in the food in different

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groups and sexes using the following method:

$$GSI = \frac{\text{Weight of intestinal bulb}}{\text{Weight of fish}} \times 100$$

The feeding intensity was assessed by classifying the intestinal bulb as full, ¾ full, ½ full, ¼ full and poor and empty and assigning the points 20, 15, 10, 5, 2.5 and 0 respectively. The term 'empty' does not mean the complete absence of food.

Macroscopic and microscopic examination of the gut contents was made to identify the food items. The quantitative analysis of food items was done by volumetric (water displacement method) (Das and Moitra, 1963; Pillay, 1952; Tandon, 1960) and the frequency of occurrence-by-occurrence method (Holden and Raitt, 1974).

OBSERVATIONS AND DISCUSSION

Intensity of feeding

Intensity of feeding is compared with relative feeding activity in different size groups of the fish. Fish with full, ¾ full and ½ full intestinal bulb were considered as indicating high feeding activity, while those ¼ full, poor and empty were assumed to how poor feeding (Fig.1). Fish were grouped at 5 cm. class interval. It is concluded from the data that frequency of feeding intensity was poor in majority of the size groups, but the feeding intensity decreased with the increase in size. Thus, majority of fishes are always under fed most of the time. This may be due to the fact that all fishes were caught by gill net and kept for more than 12 hrs. before bringing them to the landing stations.

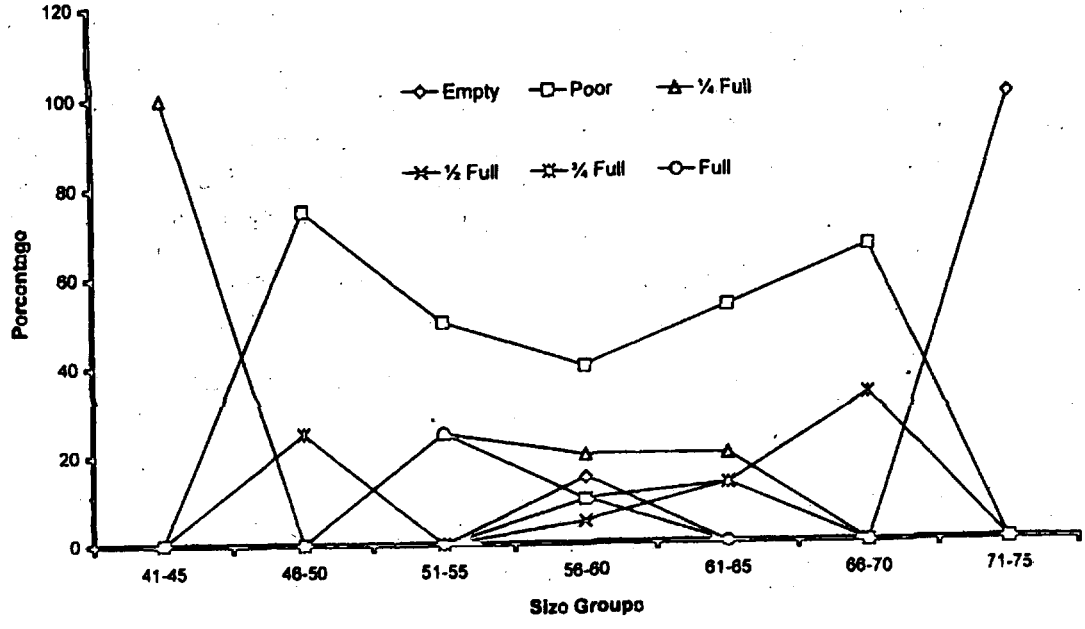

Figure 1 : Intensity of feeding in percentage in different size groups of Tor putitora on the basis of conditions of intestinal bulb.

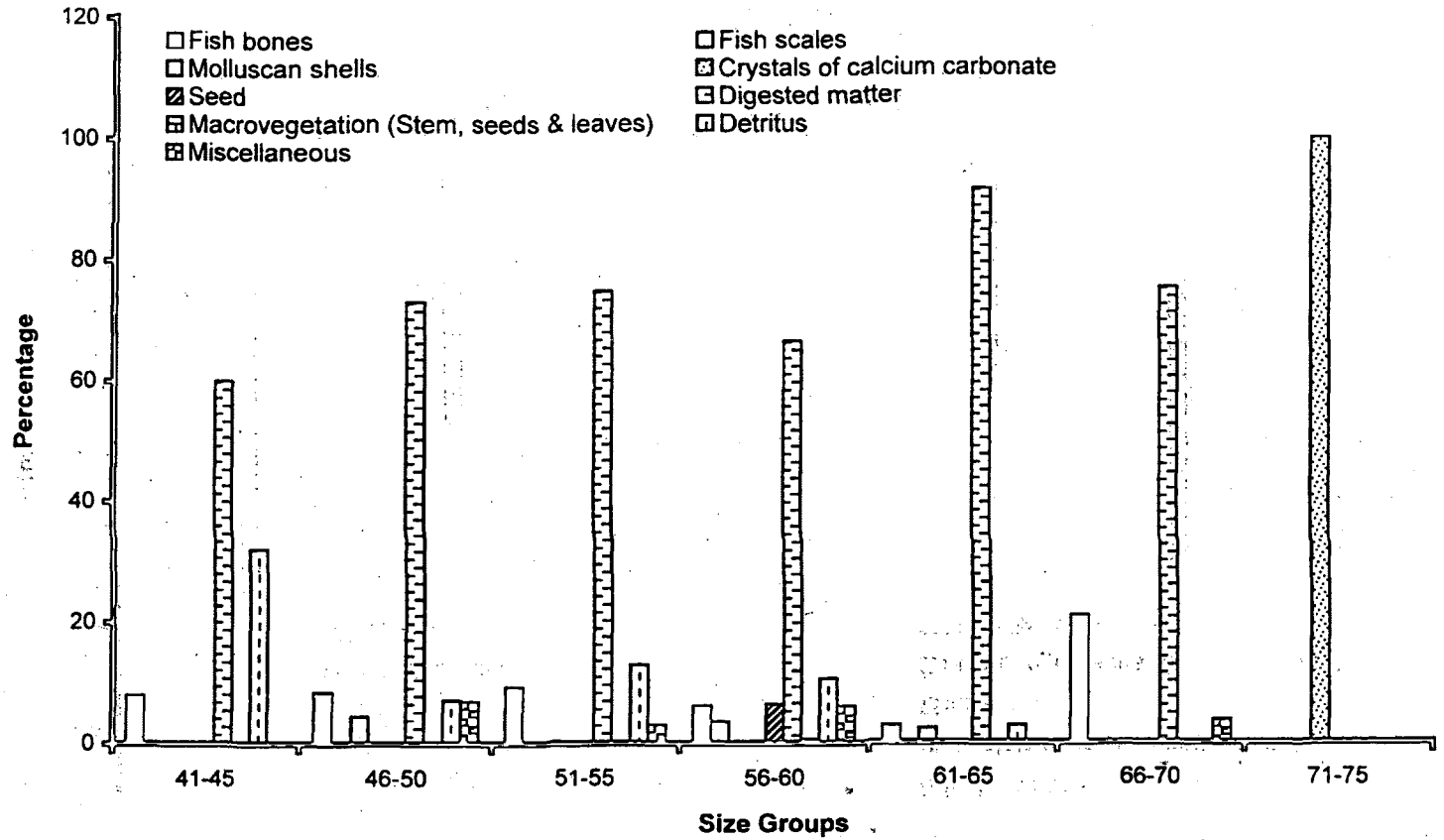

***Figure 2** : Percentage volume of different food items in foreguf of different size groups of Tor putitora from Pong reservoir.*

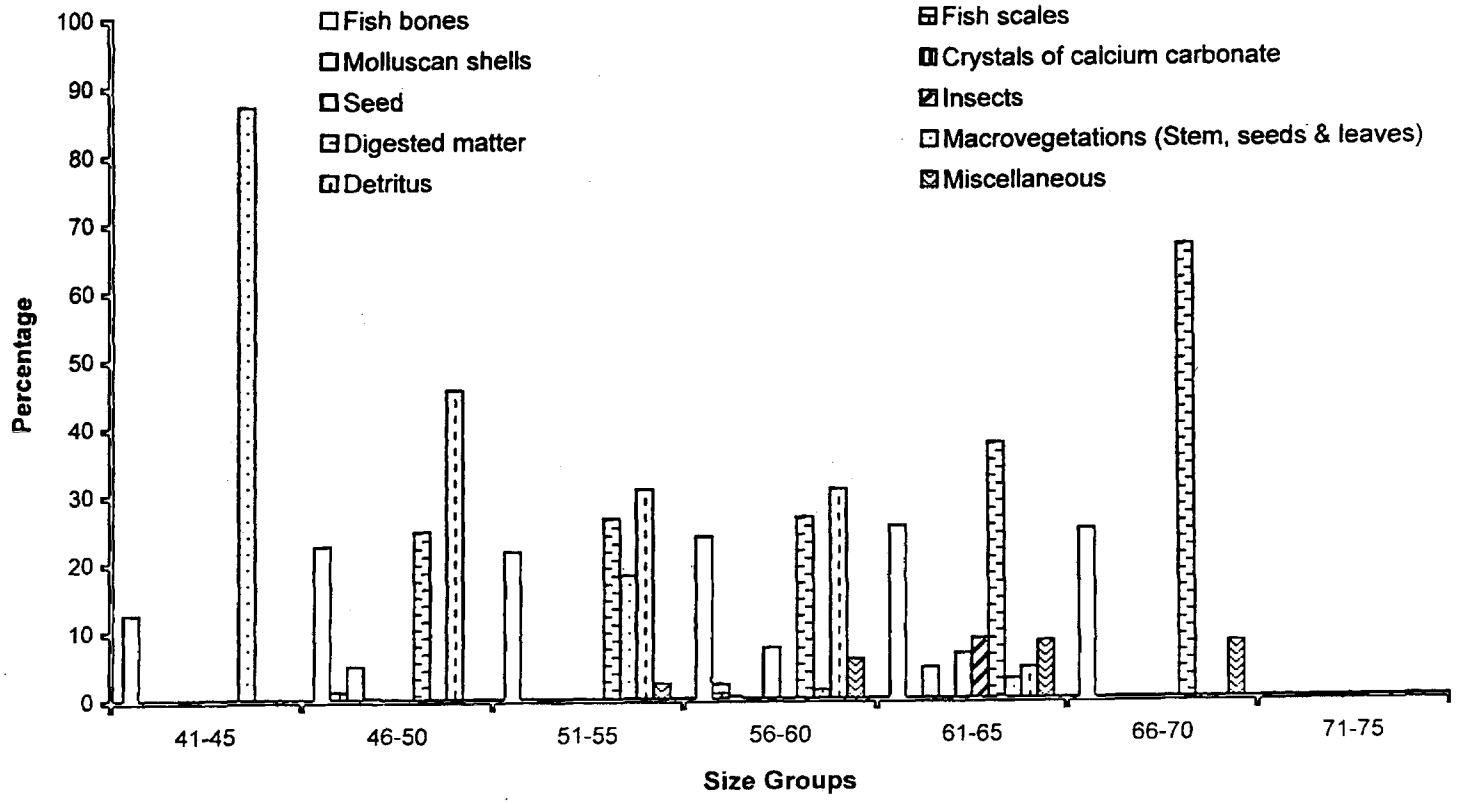

***Figure 3** : Percentage volume of different food items in midgut of different size groups of Tor putitora from Pong reservoir.*

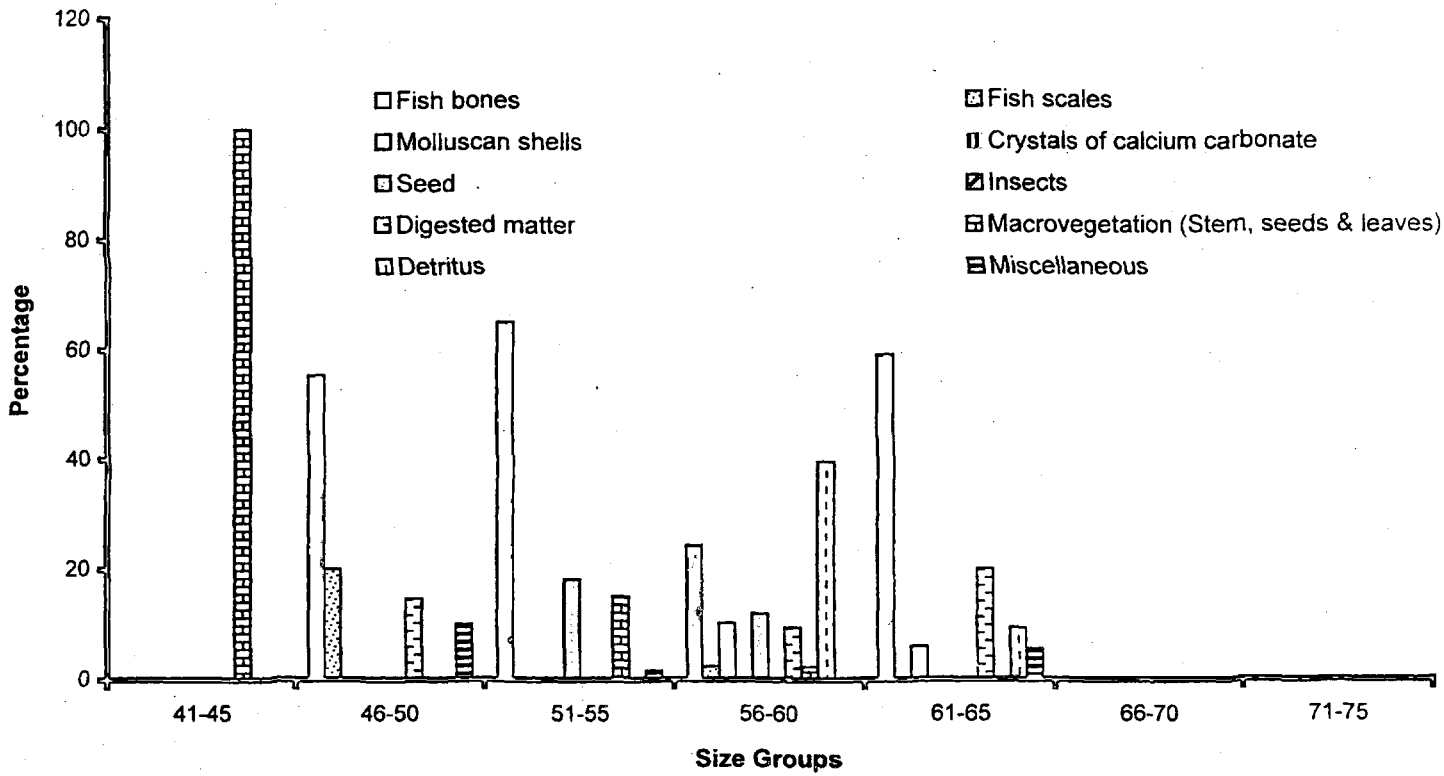

***Figure 4** : Percentage volume of different food items in hindgut of different size groups of Tor putitora from Pong reservoir.*

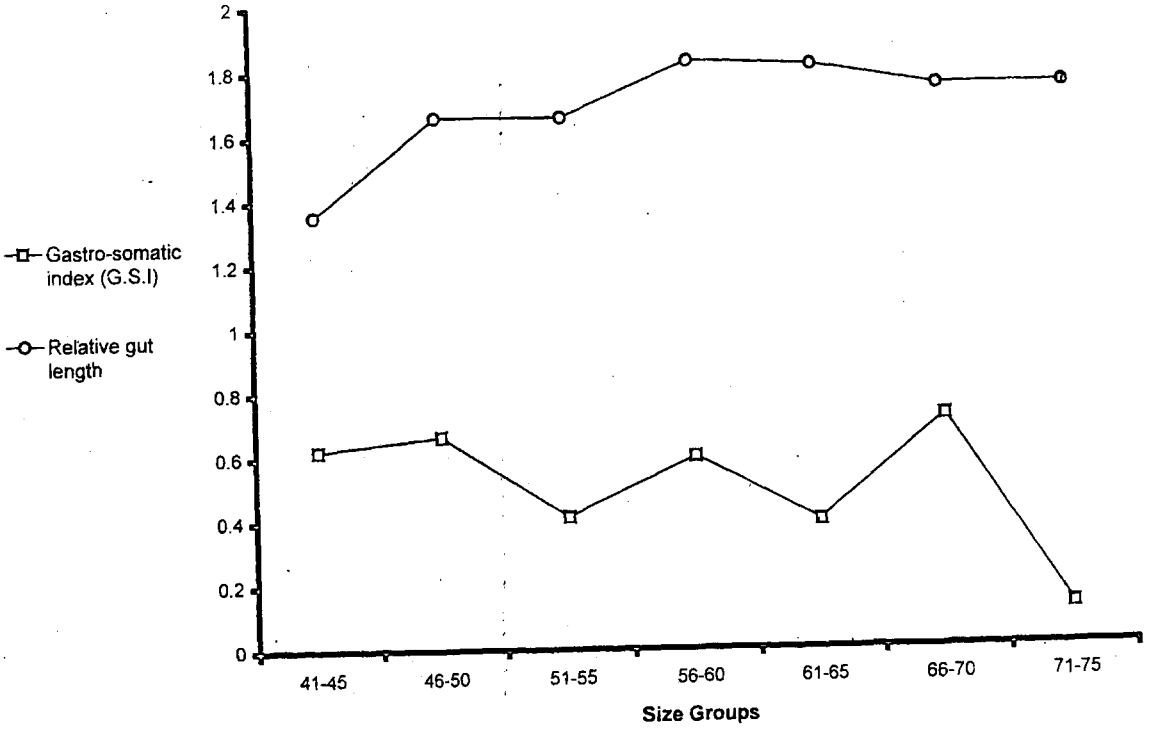

Figure 5 : Gastro-somatic index (GSI) and Relative gut length (RGL) of Tor Putitora in different size groups.

Quantitative and Qualitative Analysis of food items

The qualitative and quantitative analysis of different food items in the entire gut and in different size groups have been carried out (Figs. 2,3 & 4). It has been concluded that fish feed upon small fishes, macrovegetation, molluscan shells, insects, detritus and small amount of calcium carbonate crystals, however, there is a variation in the percentage of frequency occurrence of different food items in different size groups. As far as the percentage volume of food items in different parts of the gut is concerned, it is concluded that fish foregut contained most of the digested matter, midgut, the digested matter and fish bones and hindgut has maximum amount of undigested matter i.e. fish bones, molluscan shells etc.

Gastro-somatic index and Relative gut length

Gastro-somatic index (GSI) and Relative gut length (RGL) of different size groups of 50 specimens were taken (Fig.5). On the basis of Gastro-somatic index, active feeding intensity was recorded in size groups having the total fish length 41-45 cm., 46-50 cm. and 66-70 cm. and relative gut length varies from 1.355-1.826.

The study of food and feeding habits of fishes has been a nuclear point of discussion among global fish ecologists. Hora and Mukherji (1936) stated that *Tor mahseer* feed preferably on filamentous algae and aquatic plants. Nikolsky (1963) classified fishes on the basis of amount of variation in the type of food they consumed as euphagic, stenophagic or monophagic. Karamchandani *et al.* (1967), Znamensky (1967) considered *Tor putitora* as a piscivorous fish. Das and Pathani (1978) concluded that it is a herbiomnivore. Badola and Singh (1980) have assessed it to be a carni-omnivore. The observations of Nautiayal and Lal (1984; 1985) on food and feeding habit of fingerlings and juveniles of *Tor putitora* have indicated that this species is monophagic and column feeder. Sharma *et al.* (1992) revealed that juveniles of *Tor tor* in Jamuna waters are omnivorous. However, it is concluded that *Tor putitora* in Pong Reservoir of Himachal Pradesh is carni-omnivore, which is in conformity with the findings of Madhubala (2000).

CONCLUSIONS

It is concluded that fish is carni-omnivorous in habit throughout the year. It has revealed poor feeding intensity in majority of size groups on the basis of Gastro-somatic index and relative gut length and quantitative and qualitative analysis of food.

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MATURITY, FECUNDITY AND SEX-RATIO OF AN ENDANGERED FISH, GOLDEN MAHSEER *TOR PUTITORA* (HAM.) FROM PONG RESERVOIR

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Abstract

Maturity, fecundity and sex-ratio of mahseer, Tor putitora (Ham.) from Pong reservoir (H.P.) has been studied. It has been observed that all stages of maturity in most of the months of year, however, the peak is in the months of May and August and five maturity stages have been identified. It was also difficult to tell a definite season of maturity. Size at first maturity has been found to be 58.6-83.6 cms. total fish length. It was also observed that the Tor putitora fish from Pong reservoir spawns in the months of May and August. The fecundity of the fish recorded was 15504.872. However, due to insufficient data, no attempts have been made to find out the relationship between total length and weight of fish and fecundity. During present studies, male : female sex-ratio calculated was 1:4.64. From this data, it is clear that in Pong reservoir, female population mahseer dominate the males.

Key words: Fecundity, maturity, *Tor putitora*.

INTRODUCTION

Fish culture technique can be improved if detailed knowledge regarding the reproductive biology of the species is thoroughly investigated and seasonal rhythm is well understood, hence, the study of maturity and spawning is essential. Investigations on maturity and spawning, breeding behaviors, larval development, life history etc. of Indian fresh water fishes have been carried out extensively by various workers (Alikunhi, 1956; Bhatnagar, 1964; Hora, 1945; Jhingran, 1968; Khan, 1924; 1925; 1942; 1943). The studies on maturity and spawning, artificial breeding and development of mahseer have been carried out by different workers (Bhatnagar, 1964; Bhatt *et al.*, 1998a; David, 1953; Desai, 1972; 1973; 2000; Hora, 1939; Johal *et al.*, 2000; Pathani, 1983; 2000; Singh, 1990; Thomas, 1897). However, in *Tor putitora*, in particular from Pong reservoir, these aspects have not been worked out in detail. Thus, in the absence of any earlier detailed report from Pong reservoir, the present studies will be helpful in the management of this fish species in natural water and for cultural practices in future if possible. As this fish species has been declared as endangered, hence, the hatchery technique to produce mahseer seed for its stocking in the natural water bodies is one of prerequisite to enhance the population stocks

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of this species in the natural water. Keeping in mind the above facts, the results presented in this paper assume significance.

MATERIALS AND METHODS

Specimens of *Tor putitora* were collected from the Khatiyar landing station of Pong reservoir (Latitude 30° 25N, Longitude 75° 45E), Himachal Pradesh, India, from August 1998 to January 2000, except in June and July, employing different mesh sized gill nets. Fresh specimens were used to record maturity stages, colour, length and weight of the ovary. To study the development of ova to maturity, the ovaries were preserved in 5% formaline solution. After they attained hardness, they were kept between the folds of blotting papers to remove excess of water. Samples were taken from anterior, middle and posterior regions of both the ovaries (Clark, 1934; Desai, 1972). The diameter of 100 ova from the samples of each ovary of the fish was measured using stereoscopic microscope fitted with an ocular micrometer.

To determine the seasonal propagation of ova, fishes were studied as and when available. For the purpose of fecundity, only ripe specimens were considered. Weight of the preserved ovaries was recorded and three samples viz. anterior, middle and posterior part of one ovary were taken and mixed for homogeneity. The ova were separated by teasing and then counted. The sample was subjected to gravimetric count (Holden and Raitt, 1974) by using the following formula:

$$\text{Fecundity} = \frac{\text{Weight of ovary} \times \text{No. of ova in the sample}}{\text{Weight of the sample}}$$

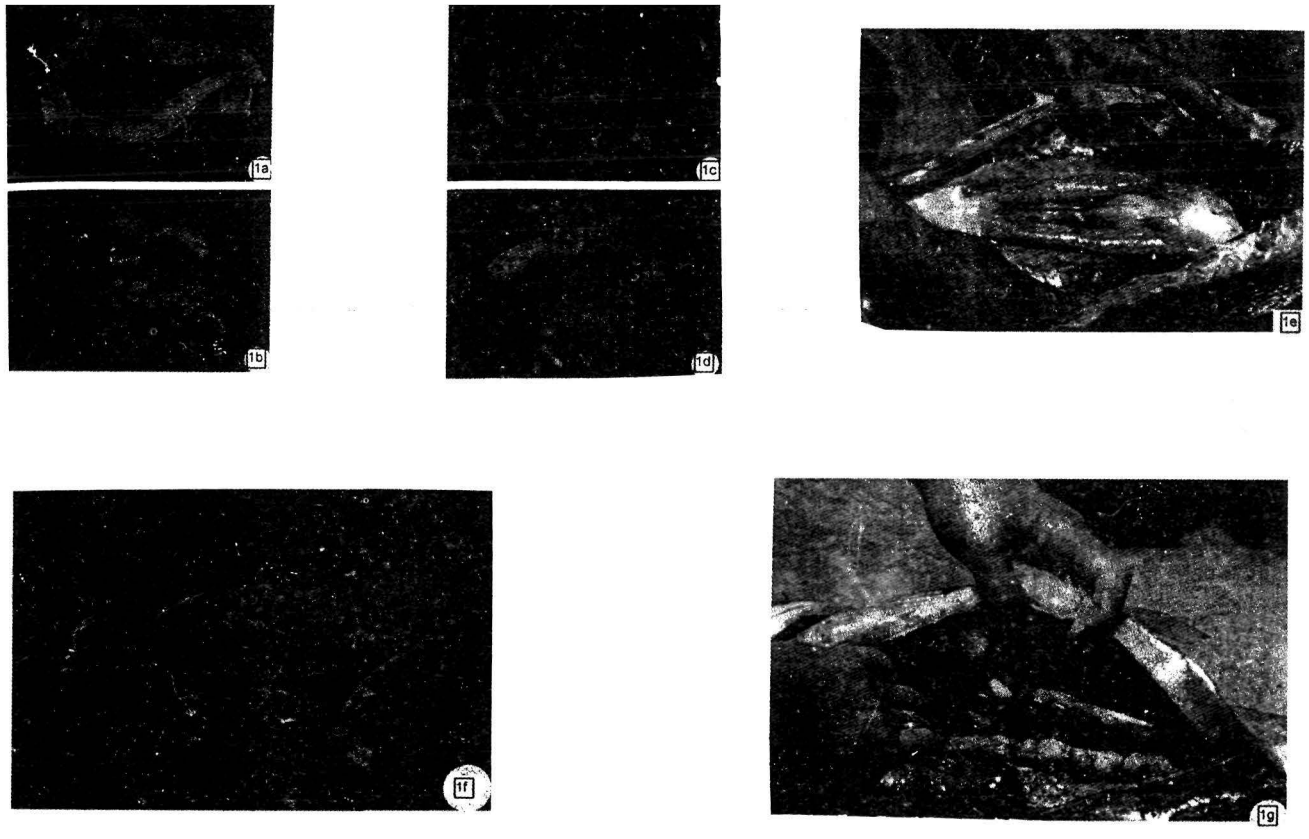
The average of two samples was recorded as mean absolute fecundity.

OBSERVATIONS AND DISCUSSION

The gonads occur as a pair of elongated, light coloured, strap-shaped bodies lying one on each side of the intestine and lodged into a groove between air bladder and abdomen. In *Tor putitora*, all stages of maturity occurred during most of the months of the year. The maturity stages were classified on the basis of colour, shape and size of gonads in relation to the body cavity and the diameter of unspawned eggs (Holden and Raitt, 1974). Following five maturity stages in females have been identified with distinct possibility of sixth stage.

Absence of spawning, spent and recovering females in the collection may be due to their up stream spawning migration.

The different stages of maturity in female i.e. seven in *Tor tor* from Udaipur lake (Chaturvedi, 1976), five in *Tor putitora* from Garhwal Himalayas (Nautiyal and Lal, 1985), three in *Tor putitora* from the foothill section of Ganga (Bhatt *et al.*, 1998a) and five from Gobindsagar (Johal *et al.* 2000) and five from Gobindsagar (Johal *et al.*, 2000) have been reported. Keeping in mind the range or variation of the maturity stages in females different species of mahseer, it can be inferred that there are several local or geographical populations having different stages of maturity. It is opined that ovarian



Figures showing maturity stages of ovaries and testis of Tor putitora. ***Fig.1a :*** *Immature ovary (Stage-I) total fish length 58.6 cm and length of ovary 9.5 cm;* ***1b :*** *Maturing ovary (Stage-II) (I), total fish length 59.1 cm and length of ovary 10.0 cm;* ***1c :*** *Maturing ovary (Stage-II (ii), total fish length 63.4 cm and length of ovary 10.2 cm;* ***1d :*** *Maturing ovary (Stage-II (iii) total fish length 68.0 and length of ovary 10.5 cm;* ***1e :*** *Fully mature ovary, length of fish 80.5 cm and length of ovary 20.0 cm;* ***1f :*** *Immature testis total fish length 54.4 cm and length of testis 8.0 cm;* ***1g :*** *Fully mature testis, total fish length 83.8 cm and length of testis 15.0 cm*

STAGE I (Immature)	Ovaries slender, thin, short, light flesh-coloured and ribbon-like and occupy one half of the body cavity. Ova minute, transparent, not visible to the naked eye. Ova diameter ranges from 0.10 to 0.43 mm (Fig.1a)
STAGE II	
Maturity (I)	Ovaries slightly enlarged, ova become yolky, opaque, diameter ranges from 0.17 to 0.77 mm (Fig.1b).
Maturity (ii)	Ovaries fleshy in colour. Ova have granulated appearance, diameter ranges from 0.43 to 0.94 mm (Fig.1c)
Maturity (iii)	Ovaries greatly enlarged with large mature eggs, diameter ranges from 0.60 to 1.46 mm (Fig. 1d)
STAGE III (mature)	Ovaries greatly enlarged with large yolky eggs, diameter ranges from 2.73 to 3.05 mm (Fig.1e)
STAGE IV (Ripe)	Not observed
STAGE V (Spent)	Not observed
STAGE VI (Recovering)	Not observed

maturity stages are greatly influenced by the local ecological conditions, hence no generalization can be made for golden mahseer, *T.putitora*.

Maturation of males

The immature testis differ much from the ovaries as they are light cream in colour and ribbon-shaped. They occupy less space in the body cavity of the fish. The mature testes are beaded and cylindrical in appearance, fringed and filled with milky fluid. During the present investigations only one immature and one mature testes have been reported during the month of May. Hence, it is not possible to assign maturity stages in case of male fish. (Figs. 1f & 1g). However, this information is useful for undertaking the breeding of golden mahseer, *Tor putitora* (Ham.) in confined waters.

Seassonal progression of ova and size at first maturity

The diameter of 100 ova from anterior, middle

and posterior regions of the ovary was recorded. In almost all the female specimens the eggs were present in three stages i.e. immature, maturing and mature. Hence it is difficult to pinpoint a definite season of maturity of *Tor putitora* in Pong reservoir.

For determining the size at first maturity of female fish, the percentages of immature, maturing and mature ovaries were calculated. According to this data, 42.5% of fish mature at 67.54 cm., 21.0% at 58.97 cm., 36.0% at 55.8 cm. Thus, *T.putitora* attains first maturity in the size range of 58.6 - 83.0 cm. Different sizes of first maturity in female i.e. 77.05 cm. (Nautiyal, 1984) and 34.0 - 38 cm.(Desai, 2000) reported from different water bodies, clearly indicates that maturity in female is influenced by the local conditions such as high temperature and high transparency of water and more availability of food especially in the month of February to April.

Spawning season

In order to determine the spawning season, ova diameter was calculated during each month and were drawn, (Figs. 2a, 2b & 2c). On the basis of these observations, there is no clear indication of the spawning season of *Tor putitora* from Pong reservoir. However, May and August peak values can be considered as the spawning seasons of *Tor putitora* (Ham.) from Pong reservoir, and require long term study.

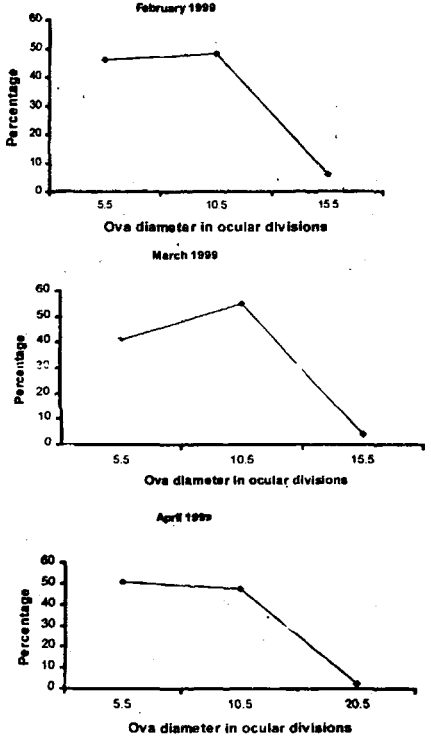
Khan (1939) observed that mahseer spawns thrice, from July to September. Desai (1972) reported three larval stages of *Tor putitora* from the river Narmada. Pathani (1983) reported May to September spawning for *Tor putitora* from Garhwal, Singh (1990) and Bhatt and Pathak (1992) reported June to Septmber spawning of *Tor putitora* from Kumaun hills.

Fecundity

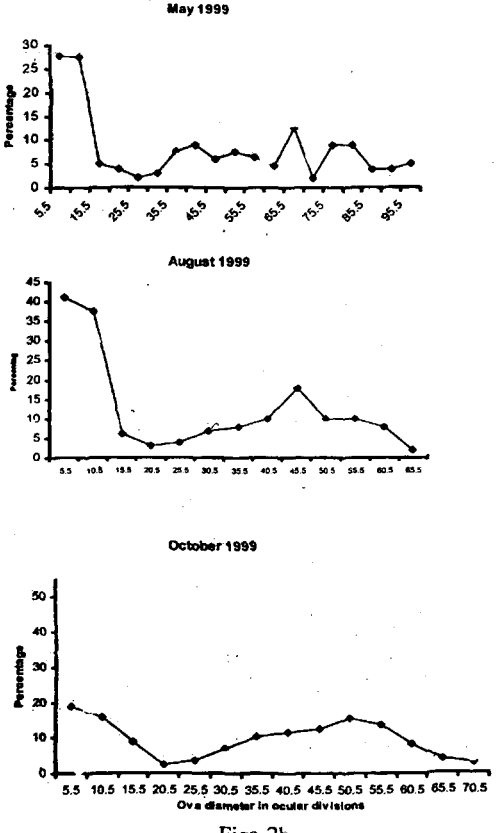
During the present study, only two mature female specimens ranging from 77.5 cm and 80.5 cm total length respectively and weighting between 4095 gms to 4495 gms could be collected from Pong reservoir. For calculating fecundity, counting of mature eggs having an ova diameter of more than 0.75 mm was considered. In the absence of sufficient number of specimens, no attempt has been made to find out the relationship between total length and weight of fish and fecundity. Considerable work has been done on the fecundity of the fishes (Aggarwal *et al.* 1988; Johal *et al.* 2000; Karamchandani *et al.* 1967; Khan, 1939; Kulkarni and Ogale, 1978; Nautiyal and Lal, 1985; Pillay, 1954; Sarojini, 1957) and it can be concluded that due to low fecundity, the autostocking in the nature is at very low level.

Sex Ratio

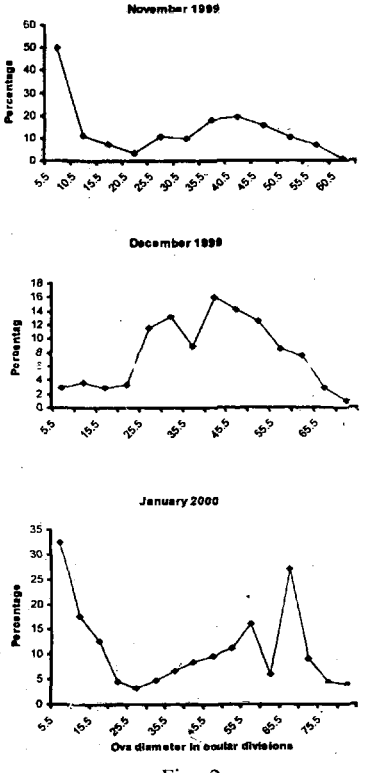
Theoretically, the expected ratio of males to females is 1:1 (Holcik *et al.*, 1988). The present studies have indicated 1:4.64 sex ratio in *Tor putitora* from the Pong reservoir and is considered as abnormal. Nautiyal (1984) reported 1:7 sex ratio of *Tor putitora* in Alaknanda and Nayar rivers during the spawning season only, Bhatt *et al.* (1998a) states that sex ratio was 0.33 : 3.0, 0.16-1.75 and 0.81-0.94 as per size-wise, month-wise and season-wise, respectively.



Figs. 2a



Figs. 2b



Figs. 2c

Figures *: **2a, 2b & 2c.** Seasonal progression of ova in different months to determine the peak spawning seasons of Tor putitora (Ham.) from Pong reservoir. H.P. Analysis of ova diameter frequency polygons indicates possibility of spawning from June-August and again during November and January.*

Pathani (2000) indicated the sex ratio of *Tor putitora* of Kumaun water as 1:1.64 and 1:1.57 during the years 1975-76 and 1979-1980. Johal *et al.* (2000) reported that in a sample male and female occurred in the ratio of 1:4.9 from Gobindsagar population.

The sex ratio show seasonality variations in the size group and its locality and fishing gear dependent, therefore, it is very difficult to give comment on this aspect at this moment. From the present observations and the earlier observations one point has emerged that the population of the golden mahseer, *Tor putitora* (Ham.), from almost all the localities is female dominated and it is intermittent breeder i.e. its spawns in batches.

The main object of the present study was to pin point to spawning season, determine the maturity stages in both the sexes in order to develop a mahseer hatchery in the vicinity of breeding grounds of this fish. The information presented in this paper can be considered as a base line data to continue the further investigations in this regard. It may be pointed here that work on the aspect like sexual maturity, fecundity and sex ratio of *Tor putitora* has been undertaken for the first time.

CONCLUSIONS

Five maturity stages in the female *Tor putitora* have been identified with the distinct possibility of sixth stage. However, peak spawning seasons were observed during months of May and August. The fecundity of fish recorded was 15504.872. The sex ratio shows that female fish was dominating population at the Pong reservior.

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EFFECT OF ZINC SUPPLEMENTATION ON THYROIDAL ^{131}I UPTAKE, RETENTION AND DOSE DELIVERED TO THYROID FROM ^{131}I IN ^{131}I-TREATED FEMALE WISTAR RATS

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Abstract

Studies were conducted to elucidate the role of zinc on thyroidal ^{131}I uptake, retention and dose delivered to the thyroids from 0.37 MBq of ^{131}I (carrier-free) administered intraperitoneally after 4-weeks of study. Thirty two female wistar rats were randomly and equally segregated in four treatment groups. Animals in group 1 were kept as normal control. Animals in groups 2 & 4, were administered a dose of 3.7MBq of ^{131}I (carrier-free) intraperitoneally. Groups 3 & 4 animals, received Zinc (as $ZnSO_4.7H_2O$) at a dose level of 227mg/L added to their drinking water for a period of 4-weeks. In conclusion, zinc has been found to increase the retention of ^{131}I in thyroid of ^{131}I-treated rats & may be used as an adjuvant to ^{131}I therapy.

Key words: Biokinetics, biological half-life, ^{131}I, radiation dose, rats, thyroid, zinc.

INTRODUCTION

Hyperthyroidism is a very common clinical condition and the lifetime risk has been estimated at up to 5% in women (Cooper, 1998). Surgery for hyperthyroidism is infrequently used in any part of the world and only a minority of patients on antithyroid drugs would remit (Nygaard *et al.*, 1996,1999; Hermus and Huysmans, 1998). Therefore, it is not surprising that majority of the patients may have to be treated with ^{131}I (Leech and Dayan, 1998). ^{131}I has become the treatment of choice, since it is effective and safe, but hypothyroidism could be an unavoidable consequence in some of the patients (Kendall-Taylor *et al.*, 1984; Ratclife *et al.*, 1986; Alexander *et al.*, 1998). So, it becomes important to evaluate the efficacy of agents, which can increase the retention of ^{131}I in the rat thyroid thereby increasing the dose to be delivered to the thyroid and in turn may reduce the radioactivity to be administered for ^{131}I therapy. Earlier in the study, lithium has been shown to increase the uptake and retention of ^{131}I in the rat thyroids (Dhawan *et al.*, 1988).

Zinc is an essential trace element and is relatively nontoxic and is vitally used in many important functions in human metabolism (Prasad, 1983). Zinc is ubiquitous in sub-cellular metabolism and, an essential component of catalytic site or sites of at least one enzyme in every enzyme classification (McCall *et al.*, 2000; Coyle *et al.*, 2002). Zn has been shown to have an antioxidant effect and stabilizes cell membranes (Kadrobova *et al.*, 1996; Dhawan and Goel, 1996). Another

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putative mechanism of Zn action is in thyroid hormone metabolism at the cellular level and it may be needed for thyroid hormone attachment to receptor (Colvard and Wilson, 1984; Ramirez *et al.*, 1991; Dhawan *et al.*, 1994). Thyroid hormone receptors require zinc ion (Licastro *et al.*, 1992; Sustrova and Strbak, 1994), which facilitate folding into active shape (Bucci *et al.*, 1999).

The present study was planned to ascertain the role of zinc with regard to the study of biokinetics of ^{131}I (carrier-free) in conditions of radiation-induced stress in thyroid.

MATERIALS AND METHODS

Animals

Female Wistar rats weighing 145-160g were used in the study. The principles of animals care as laid down by National Institute of Health (NIH publication no. 23-85, revised in 1985) were strictly followed. The animals were procured from the Central Animal House, Punjab University and were acclimatized in the departmental animal quarters for 1 week before being subjected to various treatment schedules. Thirty two female wistar rats were randomly and equally segregated into four treatment groups, viz., normal control (1), ^{131}I-treated (2), zinc treated (3), and zinc+^{131}I - treated (4) (combined treatment group). Rats in groups 2 & 4, were administered a dose of 3.7MBq of ^{131}I (carrier-free) intraperitoneally. The animals in group 3 & 4, received zinc (as $ZnSO_4.7H_2O$) at a dose level of 227mg/L added to their drinking water for a period of 4-weeks, which delivered a dose of 1-2 mg zinc /Kg to each animal (Dhawan and Goel, 1996; Goel and Dhawan, 2001).

Thyroidal radioiodine uptake measurements

Animals of all the four groups were injected intraperitoneally with 0.37MBq of ^{131}I (carrier-free) procured from (BRIT-BARC, Mumbai, India) after a treatment period of 4 weeks. ^{131}I uptake measurements over the thyroid were performed at 2h, 24h and thereafter, daily at 24-h intervals, for a total duration of 10 days by using well-type gamma-sensitive probe (ECIL, Hyderabad, India). For these measurements, the rats, under light ether anesthesia, were held over the suitably shielded gamma sensitive probe in such a way that only the neck embracing the thyroid was exposed to the probe through the hole of 1.7 cm diameter in the lead shield. The lead shield (dimensions: 18.0cm X 10.5cm X 1.5cm) with a hole in one corner was kept over the probe, which served the dual purpose of selectivity by a) exposing the thyroid to the probe and b) preventing the background body radiation from reaching the detector (Singh and Dhawan, 1999). Furthermore, a glass sheet of 0.5cm thickness was placed above this shield to avoid faecal/urinary contamination of the lead shield during the uptake measurements. This glass sheet also did not allow the neck area of the animal to be pressed into the hole of the lead shield, thus maintaining the counting geometry throughout the entire study.

During the course of recording the radioactivity, five sets of measurements/ counts over the thyroid were taken on each animal in order to minimize the statistical error (SE). The standard activity of ^{131}I (equivalent to that injected in each animal) was also measured each time, to account for the physical decay of the radioisotope and the possible instrument error, if any, during the study.

Percentage uptake values of ^{131}I by the thyroid at 2h and 24h interval were calculated by comparing the activity in the thyroid with that of the injected activity.

To determine the biological half-life (T_{biol}) in the thyroid, the percentage of ^{131}I uptake values at different time intervals from 24h onward were calculated by taking the 24 h uptake as 100%. The percent thyroidal ^{131}I values were plotted on y-axis (log scale) and the time interval on x-axis (linear scale) of the semi log paper. Further, the (T_{biol}) of ^{131}I was interpolated from the semi-log plot and was calculated by taking the difference on the x-axis of any two points, where the percentage uptake was being bisected.

The dose delivered to the thyroid following injection of 0.37MBq of ^{131}I (uptake dose) to each rat after 4-weeeks of study was calculated according to the following formula as used by Abdel-Nabi and Ortman (1983) and Dhawan *et al.*, (1988) .

$$D = 73.8\ E\ C\ T_{eff}$$

Where:

D = Total dose absorbed (1 rad = 10^{-2} gray) at the time of complete decay

E = The local energy deposited per disintegration, and is equal to 0.187 MeV for ^{131}I

C = The isotope concentration in μCi per g thyroid weight

T_{eff} = The effective half-life of ^{131}I

73.8= A constant related to the complete decay of the radioisotope

RESULTS

The results of various treatment groups were compared with their corresponding normal controls. The observations of animals given combined (Zinc + ^{131}I) treatments were also compared with the corresponding ^{131}I-treated rats. In addition, the animals receiving combined (Zinc + ^{131}I) were also compared to zinc treated animals. The statistical significance of values has been determined using analysis of variance (ANOVA) followed by Newman Keul's test and the results are represented as Mean ± S.D. Rats thyroidal ^{131}I uptake (after 2h and 24h) and biological half-lives of ^{131}I in rat thyroids are shown in Table 1.

Table 1 : Effect of Zinc on rat thyroidal ^{131}I uptake after 2 and 24h and biological half-lives (T_{biol}) of ^{131}I in rat thyroid.

		Thyroidal uptake of ^{131}I (%) after		Thyroidal biological half- life
	Groups	**2h**	**24h**	**T_{biol} (days)**
1	NORMAL CONTROL	38.51 ±6.30	54.48 ±10.2	3.31 ± 0.17
2	I-131	16.04 ±3.11	23.28 ±6.60	3.87 ± 0.30
3	ZINC CONTROL	25.85 ±4.73	69.97 ±8.92	3.70 ± 0.29
4	ZINC + I-131	18.92 ±2.90	27.83 ±2.91	4.21 ± 0.28
ANOVA				
F-Value		39.97	66.50	15.23
		P< 0.001	P < 0.001	P < 0.001
Newman- Keuls (q-values)				
1	vs 2	14.22***	11.51***	5.89***
1	vs 3	8.00***	5.71***	4.10**
1	vs 4	12.39***	9.83***	9.47***
2	vs 4	1.82	1.67	3.57*
3	vs 4	4.30**	15.54***	5.36**

***P< 0.001, **P< 0.01, *P< 0.05

Statistically significant decrease in 2h (P< 0.001; 58.3%) and 24h (P< 0.001; 57.2%) ^{131}I uptake by the thyroid was noticed in ^{131}I-treated rats. Interestingly, zn supplementation alone to normal rats showed significant elevation in 24h radioiodine uptake (P< 0.001; 28.4%). Further, combined (Zinc + ^{131}I) treatment also resulted in higher uptake values at both 2h and 24h interval, but the increase was found to be statistically insignificant.

^{131}I-treatment resulted in a statistically significant increase thyroidal biological half-life of ^{131}I (T_{biol}) (P< 0.001; 16.01%) as compared to its normal control. Interestingly, combined (Zn + ^{131}I) treatment resulted in a significant increase in (T_{biol}) (P< 0.001; 8.78%) as compared to its respective ^{131}I-treated group. Zn supplementation alone to normal rats also resulted in significant increase (P< 0.01; 11.78%) in T_{biol}.

The product of T_{eff} for ^{131}I and 24 h thyroidal ^{131}I uptake, together with the dose of ^{131}I delivered to the thyroid and wet thyroid weight in all treatment groups are shown in Table 2. ^{131}I-treatment resulted in significant decrease ($P< 0.001$; 41.42%) in wet thyroid weight. However; zinc supplementation to ^{131}I-treated rats resulted in a increase in thyroid weights but were statistically insignificant. The product of T_{eff} and 24 h of ^{131}I uptake and the dose of ^{131}I delivered to thyroid was observed to be significantly depressed (48.08%, $P< 0.001$);(19.13%, $P< 0.05$) in ^{131}I-treated rats compared to its respective normal rats. Interestingly, significant elevation in the product of T_{eff} and 24 h of ^{131}I uptake and the dose of ^{131}I delivered to thyroid ($P< 0.001$; 49.12%);($P< 0.001$; 52.27%) was observed in zinc supplemented controls. Further, an elevation in the product of T_{eff} and 24 h of ^{131}I uptake and the dose of ^{131}I delivered to thyroid was observed in combined (Zn + ^{131}I) treated animals (35.61%, $P< 0.05$);(37.38%, $P< 0.01$) as compared to its respective ^{131}I-treated animals.

Table 2 : Thyroid weight, product of T_{eff} and 24 h thyroidal ^{131}I uptake, and dose delivered to the thyroid from ^{131}I (carrier-free) at 4-week control and treated rats.

	Groups	Thyroid weight (mg)	T_{eff} x 24 h ^{131}I uptake	Radiation dose (gray)
1	Normal control	12.60 ± 0.90	131.01 ± 22.40	142.76 ± 24.03
2	^{131}I treated	7.38 ± 1.01	68.01 ± 14.71	115.45 ± 18.26
3	Zinc control	12.75 ± 0.61	195.37 ± 24.80	217.39 ± 34.98
4	^{131}I + Zinc	8.00 ± 0.58	92.23 ± 12.39	158.61 ± 21.50
ANOVA				
F-value		104.00	51.88	22.87
		P < 0.001	P < 0.001	P < 0.001
Newman-Keuls (q-values)				
	1 vs 2	18.64***	9.25***	3.03*
	1 vs 3	0.53	9.45***	8.29***
	1 vs 4	16.42***	6.24***	1.76
	2 vs 4	2.21	3.55*	4.79**
	3 vs 4	16.96***	15.14***	6.53***

***P< 0.001, **P< 0.01, *P< 0.05

DISCUSSION

Statistically significant reduction in uptake of ^{131}I in thyroid after ^{131}I-treatment has been observed, which is in agreement with earlier studies (Jeevanram *et al.*, 1986). Interestingly, Zn supplementation alone to normal rats showed significant elevation in 24h radioiodine uptake. This may be because of an increased level of TSH (thyroid-stimulating hormone), which has caused an increase in Na^+/K^+ ATPase enzyme activity. There are also reports that zn exposure alone afforded a 20% increase in Na^+/K^+ ATPase enzyme activity in cultured rat cortical cell, although the differences were statistically insignificant (Lovell *et al.*, 1999). Arreola *et al.* (1993) have also reported that oral zinc supplementation could increase plasma level of TSH in uremic patients under peritoneal dialysis. Further, combined (Zinc + ^{131}I) treatment resulted in higher uptake values than in the corresponding ^{131}I-treated animals, which were statistically insignificant.

^{131}I-treatment resulted in statistically significant increase in $T_{(biol)}$. This increased $T_{(biol)}$ of ^{131}I-treated animals could be because of damage to histo architecture thereby affecting the release of ^{131}I from thyroid (Bellabarba *et al.*, 1972). Interestingly, combined (Zn + ^{131}I) treatment resulted in significant increase in $T_{(biol)}$ as compared to ^{131}I-treated group. Thus Zn supplementation resulted in increased retention of ^{131}I in the rat thyroid.

Significant decrease in thyroid weight was observed in animals following ^{131}I-treatment; which is in agreement with earlier studies (Hegedus *et al.*, 1986; Nygaard *et al.*, 1995). In order to find out the retention of ^{131}I in thyroid, the dose of ^{131}I delivered to the thyroid has been calculated. As effective half-life of ^{131}I in thyroid as well as ^{131}I uptake increases with zinc supplementation, thus the dose delivered to the thyroid has also increased.

In conclusion, since zinc has been able to increase the retention of ^{131}I in thyroid of ^{131}I-treated rats, thus it may be used as an adjuvant to ^{131}I therapy.

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KARYOLOGICAL STUDIES ON *STICTOCHIRONOMUS* SP. (DIPTERA: CHIRONOMIDAE) FROM JAMMU REGION

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Abstract

Karyological studies were carried out on a population of Stictochironomus sp. from Gadigarh locality of Jammu region, J&K state, India. The work attempts to examine the polytene chromosomes of the species. The investigated species possesses the usual four polytene chromosomes with the arm combination AB, CD, EF and G. The karyotype of Stictochironomus sp. is being elucidated with a photomap of polytene chromosome complement (2n=8) is presented with the description of detailed banding pattern in each chromosome. The results are compared with three other worked out species of the same genus viz., Stictochironomus n. sp.1, Stictochironomus n. sp.2 and Stictochironomus marmoreus.

Key words: Chironomidae, cytophotomap, karyology, polytene chromosomes, *Stictochironomus* sp.

INTRODUCTION

Members of the genus *Stictochironomus* are poorly known in India. The present study is the first time report of *Stictochironomus* sp. from J&K. Polytene chromosomes of Jammu population of *Stictochironomus* sp. have presently been studied and cyto-photomap has been constructed for future cyto-genetic and comparative cyto-taxonomic studies of the genus. A comparison of the banding pattern in the polytene chromosomes of the present species has been made with those of three other worked out species of the genus, viz., *Stictochironomus* n. sp.1 (near *annulicrus* (Townes)), *Stictochironomus* sp.2 (near *annulicrus* (Townes)) and *Stictochironomus marmoreus* (Townes) (Martin *et al.,* 1979) from New Mexico.

Construction of polytene chromosome map is important so as to get an insight into the genetic make up of a species, since the conventional morphometric features of chironomids do not always provide suitable data for determining different species. However, a combination of morphological and cytogenetical studies has facilitated successful revision of taxonomic and phylogenetic position of a number of species (Keyl, 1962; Kinkadze *et al.,* 1991a; b; 1996; Martin, 1966; Michailova, 1989; Wulker, 1973; Wulker *et al.,* 1981). The chironomid midges are very useful for investigations into chromosome evolution because of the presence of good quality polytene chromosomes. As a result of this, the banding patterns can be compared easily and homology of the banding patterns of the chromosomes can be recognized in most species (Hilburn, 1979; Martin, 1969; 1974). A comparison of banding pattern on the

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polytene chromosomes serves as an excellent tool to study speciation and evolutionary relationships among various species of the same group and to draw phylogenetic relationships.

MATERIALS AND METHODS

For the present study, larvae and pupae of *Stictochironomus* sp. were collected from a big drain located in Gadigarh locality in Jammu region, J&K state, India. The adults emerged from the collected pupae after two-three days in the laboratory. Polytene chromosome preparations were made from the fourth instar larvae according to the conventional squash procedure, using 2% lacto-aceto stain orcein (French *et al.*, 1962). For cytophotomaps, the system of Martin *et al.* (1979) for *Stictochironomus* n. sp. 1 (Townes) has been followed. Thus the chromosomes have been divided into different regions as follows: chromosome I, regions 1-12; chromosome II, regions 13-22; chromosome III, regions 23-28 and chromosome IV, regions 29-32.

OBSERVATIONS AND DISCUSSION

The salivary gland chromosome complement of *Stictochironomus* sp. comprises four polytene chromosomes due to intimate pairing of homologous chromosomes (2n = 8). The arms of chromosome I are designated as AB, chromosome II as CD, chromosome III as EF and the right arm of chromosome IV as G. The naming of the chromosome arms of *Stictochironomus* sp. implies no homology with the chromosomes of *Chironomus*. Distinctive features of each chromosome have been illustrated in Fig.1 and have been described below.

Chromosome-I

It is the longest member of the complement with arms A and B and shows the presence of three regions of nucleolar activity in regions 4, 6 and 7 wherein a large and a small swellings comprise region 4 and the large swelling has the predominance of dotted bands, while the small one contains a dark-thick and a dark broken bands present in between a sequence of light-dotted bands. A large and a moderate sized swelling comprise region 6 having a group of dark bands sandwiched in between light-dotted bands in the large swelling while, moderate one contains a dark-thick wavy band and a broken band at the tip followed by an alternate arrangement of light-dotted and dark-thick bands. Region 7 begins with the presence of a small swelling, consisting of light-dotted and dark-thick bands alternating with one another in a sequence, followed by a large swelling showing a predominance of light-dotted bands. Typical to the chromosome are the expansions at the two ends. The other characteristic feature of the chromosome is a series of dark-thick bands in regions 1, 2, 3, 4, 6, 7, and 11.

Chromosome-II

This is designated by the arms C and D, which correspond to the right and left arms of the chromosome respectively. This chromosome can be recognized by a constriction in region 21 of arm D. An alternate arrangement of a few light bands sandwiched in between dark bands is seen in region 21.

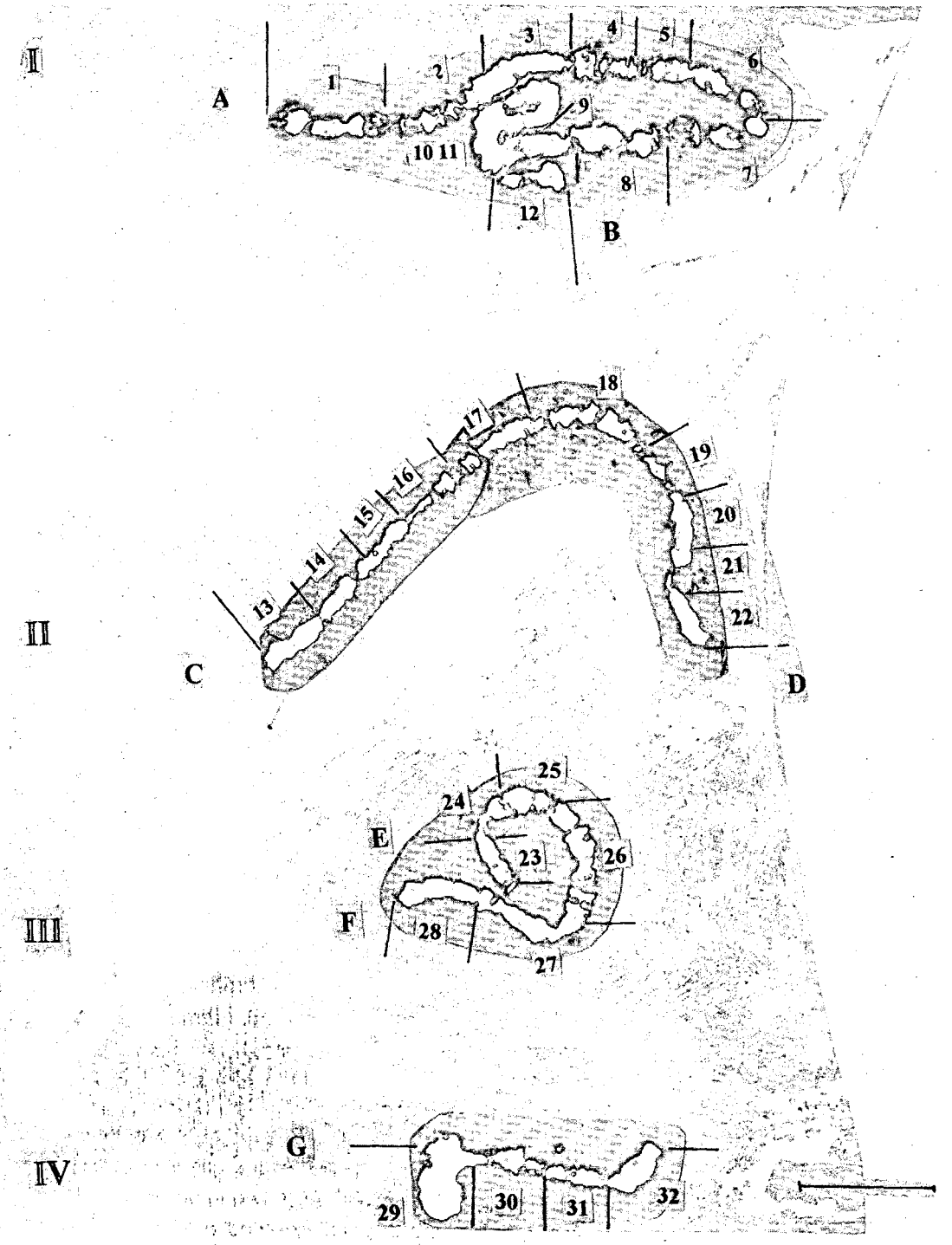

***Figure 1** : Photomap of salivary gland chromosomes of Stictochironomus sp. Scale = 0.01mm.*

Region 22 marks the end of arm D and shows alternately arranged light and dark bands in the beginning followed by a few light bands at the end. Typical to the chromosome are dark-thick bands in regions 13,15 and 16.

Chromosome-III

Chromosome III comprises of arms E and F. The dark-thick bands in regions 23, 25 and 27 serve as markers for the chromosome. The free end of arm E exhibits a series of dark-thick bands alternating with series of light-dotted bands in region 23. Region 28 marks the end of arm F and consists of an alternate arrangement of dark-thick and light-dotted bands in the beginning followed by a series of light-dotted bands with a few dark bands scattered in between.

Chromosome-IV

It is the shortest member of the complement represented by arm G where expansions at the ends are typical.

A comparison of banding pattern on the polytene chromosomes of *Stictochironomus* sp. (present study) has been done with that of *Stictochironomus* n. sp.1 (near *annulicrus* (Townes)), *Stictochironomus* sp.2 (near *annulicrus* (Townes)) and *Stictochironomus marmoreus* (Townes) (Martin *et al.*, 1979) from New Mexico. The quality of the photographic reproduction of the chromosomes of the above-mentioned species was not sufficiently clear to enable complete band comparisons. The number of polytene chromosomes in *Stictochironomus* sp., *S.* n. sp.1 and *S. marmoreus* is four i.e., 2n=8, however, *S.* n. sp. 2 has three polytene chromosomes i.e., 2n=6 due to the tandem fusion of small chromosome IV to the left end of chromosome III. *Stictochironomus* sp. shows the presence of three regions of nucleolar activity in chromosome I as compared to five regions in *S.* n.sp.1, three regions in *S.* n.sp.2 and four regions in *S. marmoreus*. Chromosome II does not show any obvious nucleolus in *Stictochironomus* sp., whereas there are five such regions in chromosome II in case of *S.* n.sp.1, two in *S.* n.sp.2 and two in *S. marmoreus*. Chromosome III is free of any prominent nucleolar activity regions in *Stictochironomus* sp., whereas it shows three such regions in *S.* n.sp.1, and a single nucleolus in *S. marmoreus*. *S.* n. sp. 2 which shows three nucleoli in chromosome III and one at the point of fusion between 3L and arm IV. Chromosome IV shows one nucleolus and two Balbiani rings in *S.* n.sp.1, and no obvious nucleolus in *S. marmoreus* and *Stictochironomus* sp. (Jammu population). However, *S. marmoreus* shows a single Balbiani ring next to band 31a in this chromosome.

No inversion polymorphism has been seen in *Stictochironomus* sp. (Jammu population) and *S.* n.sp.2. However, *S.* n.sp.1 shows two inversions, a long one covering most of the right arm of chromosome I and a shorter one in the right arm of chromosome II. *S. marmoreus* has revealed five inversions out of which two inversions occur in chromosome I, one in each arm, two in chromosome II, again one in each arm and one in the right arm of

Chromosome-III

The availability of standard chromosome map of this species from Jammu region will be helpful for future cytogenetic researches. Differences with respect to the presence of nucleoli on the chromosomes of different species of *Stictochironomus* have been observed. Changes in the number and site of nucleoli can be related to chromosome/gene mutations, which result in the differences observed in the chromosome pattern of various species (Bauer, 1945). Differences with respect to chromosomal polymorphism have been observed only in two populations viz. *S.* n. sp. 1 and *S. marmoreus*. The exact significance of these differences cannot be evaluated at this stage since only a single population from Jammu has been studied. Though it seems that diversity of environment in different areas can lead to increase in the number of ecological niches and consequently to an increase in chromosomal variability for adaptiveness. Further investigations on more species may provide a better insight into the evolutionary relationships amongst the species of this genus.

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TWO NEW SPECIES AND FIRST RECORD OF THE GENUS *HELCYSTOGRAMMA* ZELLER (LEPIDOPTERA : GELECHIIDAE) FROM INDIA

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Abstract

Besides two new species i.e., Helcystogramma clarkei and H. uedai another species i.e., H. hibisci (Stainton) is recorded for the first time from Siwaliks in North-Western India. The congeneric nature of the species has been established on the basis of various morphological characters particularly the male genitalia.

Keywords: Gelechiidae , genitalia, *helcystogramma*, lepidoptera, Siwaliks.

INTRODUCTION

While redefining the genus *Helcystogramma* Zeller through an addition of the male genitalic criterion Park and Hodges (1995) have also clarified that *Gelechia (Helcystogramma) obseratella* Zeller 1877 (=*Gelechia hibisci* Stainton, 1859) is the type-species of this genus. Keeping in view, this diagnosis, the present authors have identified three species i.e., *H. hibisci* (Stainton), *H. clarkei* sp. nov. and *H. uedai* sp. nov. (Meyrick, 1905, 1907, 1908, 1908a, 1909, 1910, 1911, 1913, 1914, 1912-1916, 1916-1923, 1923-1930, 1930-1936; Gaede, 1937; Clarke, 1969, 1969a; Park and Hodges, 1995; Ueda, 1995) collected from Siwaliks in North-West India. Besides a key to these species, the new species has been described in detail.

OSBSERVATIONS

Genus *Helcystogramma* Zeller

Helcystogramma Zeller, 1877, *Horae Soc. ent. Ross.*, **13** : 369.

Type-species : *Gelechia (Helcystogramma) obseratella* Zeller, 1877, *Horae Soc. ent. Ross.*, **13** : 371, pl. 5, fig. 127, by subsequent designation : Meyrick, 1910, *Entomologist's mon. Mag.*, **46**: 282.

Key to the species of the genus *Helcystogramma* Zeller

1. Forewing with anal margin with a dark semicircular blotch medially, extending more than half distance across wing, a similar mark beyond cell, a broad preapical pale fascia extending from 2/3rd length of anterior margin to

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Abbreviations in this text are given at the end of paper

tornus, a small black spot on cell distally; hindwing with M_2 relatively arched; male genitalia with aedeagus rather long.....***hibisci* Stainton**

– Forewing without such pattern; hindwing with M_2 straight; male genitalia with aedeagus relatively smaller.....**2**

2. Hind tibia with white and black bands alternately; hindwing with CuA_2 arising at 1/3rd of discal cell; male genitalia with coremata, valva with sacculus margin slightly concave at middle, aedeagus broader at base.....***clarkei* sp. nov.**

– Hind tibia beset with ochreous hair; hindwing with vein CuA_2 arising at middle of discal cell; male genitalia without coremata, valva with sacculus margin slightly convex, aedeagus globular at base .....***uedai* sp. nov.**

***Helcystogramma hibisci* (Stainton)**

Gelechia (?) *hibisci* Stainton, 1859. *Trans. ent. Soc. Lond.*, (2)5, p. 117.

Onebala hibisci : Meyrick, 1925, in Wytsman, *Genera Insect.*, p. 138; Gaede, 1937 *Lepid. Cat.*, p. 377.

Gelechia (Helcystogramma) obseratella Zeller, 1877, *Horae Soc. ent. Ross.*, 13, p. 371.

Croesophora eudela Turner, 1919, *Proc. Roy. Soc. Queensland*, 31, p. 160.

Male genitalia (Plate 1, Figs. A-C) : Uncus long, narrowed basally, broader apically, sparsely setose, with small hair on the inner surface; gnathos long, hook-like, heavily sclerotized; tegumen long and broad, hood-like, moderately sclerotized, with two small membranous setose lobes at base; vinculum V-shaped; saccus small, blunt apically; valvae symmetrical, elongate, basally narrowed, distally broader, costa slightly convex, sacculus almost straight, cucullus broad, bearing moderately long hair-like setae on the inner surface, convex dorsally, apex rounded, slightly convex ventrally; aedeagus broad and long, moderately sclerotized, apically pointed, slightly bend at 1/3rd basally, with a broad internal lobe, globular at base, about 2/3rd length of the genitalia; cornutus lacking in vesica.

Alar expanse : Male 14 mm.

Material examined :

Himachal Pradesh : Dist. Sirmour, Renuka Lake, 740m, 13.iv.1999, 2♂♂ : Dist. Solan, UHF Nauni, 1360m, 5.iv.2000, 3♂♂.

Uttaranchal : Dist. Dehra Dun, FRI Dehra Dun, 700m, 23.iv.1999, 1♂.

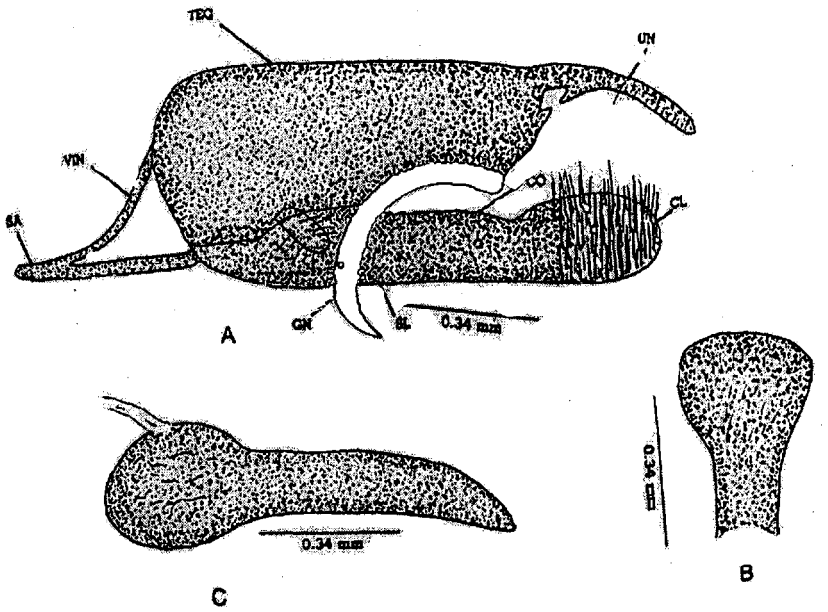
Punjab : Dist. Patiala, PUP, 250m, 19. viii.1999, 1♂.

Distribution : India (Calcutta), Southern China, Taiwan, Sri Lanka, Java and Australia (Park and Hodges, 1995).

Larval host plant : *Hibiscus esculentus* (Fletcher, 1921).

Remarks : Robinson *et al.* (1994) reported this species under the genus *Oneballa* Walker but Park and Hodges (1995) while handling this species have proposed its new combination as *Helcystogramma hibisci* (Stainton). Further, it has been observed that this species shows some population variations in the forewing, especially the dark brown semicircular blotch on the hind margin of this wing (Robinson *et al.*, 1994). Further, in the examined material, it has been seen that three specimens possess a white speck on left forewing and the same is missing on the right side wing; and four individuals have no such spot at all. In view of these variations, a series of five individuals (two from Nauni and one each from Renuka, Dehra Dun and Patiala) were dissected to confirm their conspecificity on the basis of an examination of the male genitalia. All the aforesaid localities form new distribution sites from North-West India. The species has earlier been captured from Calcutta in East India (Park and Hodges, 1995).

PLATE - 1



Helcystogramma hibisci (Statinton) : Figures. (A) Male genitalia : lateral view, (B) Uncus: ventral view, (C) Aedeagus.

***Helcystogramma clarkei* sp. nov.**

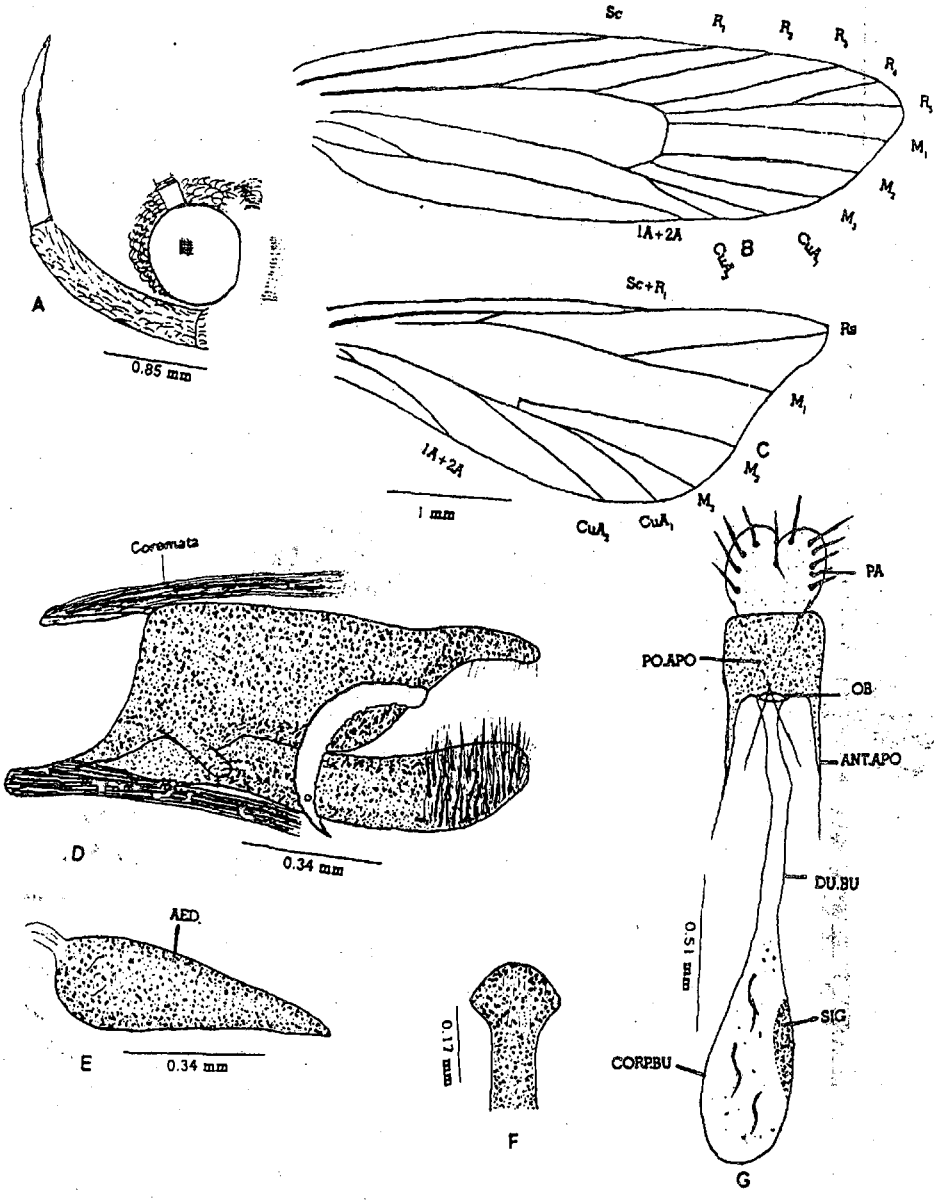
Male and female : Alar expanse : 12 mm. Vertex covered with dark fuscous scales; frons decorated with light fuscous scales; labial palpus long, sickle-shaped, second segment covered with ochreous scales, third segment long, acute, fuscous (Plate 2, Fig. A); antenna black, 3/4th as long as forewing; thorax grey; forewing black scaled, costa slightly arched, apex rounded, termen oblique, tornus convex, anal margin convex, costa with a large white spot distally near apex, anal margin ochreous 2/3rd from base, cilia on the termen dark grey; hindwing somewhat quadrate, light fuscous scaled, costal margin straight for 2/3rd then slightly curved, apex subacute, termen trapezoidal, tornus convex, anal margin convex distally then curved, anal and termen margin with cilia grey; prothoracic and mesothoracic legs fuscous, hind tibia dark fuscous, metathoracic leg fuscous, hind tibia with white and black band alternately.

Wing venation (Plate 2, Figs. B-C) : Forewing with Sc join costa at middle, R_1 arising at middle of discal cell, R_1, R_2, R_3 free, R_4+R_5 long stalked, arising at angle of cell, M_1 free, straight, M_2, M_3 free, M_1 closer to R_5 than M_2, CuA_1+CuA_2 stalked, discal cell closed; hindwing 1A+2A forked at base, ending near to CuA_2; hindwing with R_1 running into Sc beyond base of the wing, Sc+R_1 join costa at 3/4th, Rs+M_1 stalked, Rs to apex, M_1 to termen, M_2 free, nearer to M_3 than M_1, M_3 + CuA_1 stalked, CuA_2 arising at 1/3rd of discal cell, cell open, CuP vestigial, 1A+2A forked at base.

Male genitalia (Plate 2, Figs. D-F) : Uncus small, broader at apex, narrowed at base, sparsely setose, with small hair on the inner surface; socii absent; gnathos moderately long, hook-like, heavily sclerotized; tegumen small and broad, somewhat hood-like, with two membranous setose lobes at base; vinculum V-shaped, thin; saccus small, rounded apex, coremata with very long hair directed distally; valvae symmetrical, long, broader at apex, narrowed basally, costal margin almost straight, sacculus margin slightly concave at middle, cucullus broad, bearing small hair like setae on the inner surface, margin slightly convex dorsally, apex rounded, convex ventrally; aedeagus small, broader at base, narrowed distally, apex acute, about half the length of genitalia, moderately sclerotized; vesica lacking cornutus.

Female genitalia (Plate 2, Fig. G) : Papillae anales small, sparsely setose, somewhat rounded, anterior apophyses long, broader at base, posterior apophyses long and thin, almost equal to the length of anterior apophyses; ostium bursae small, centrally placed; ductus bursae comparatively long and thin, broader near ostium and 2/5th from ostium; corpus bursae ovate in shape, small; signum crescent shaped, moderately sclerotized.

PLATE - 2



***Helcystogramma clarkei sp. nov.* : Figures. (A) Labial palpus, (B) Forewing venation, (C) Hindwing venation, (D) Male genitalia : lateral view, (E) Aedeagus, (F) Uncus: ventral view, (G) Female genitalia: ventral view.**

Material examined :

Holotype : Punjab : Dist. Patiala, PUP, 250m, 23.x.1998, 1♂.

Paratypes : Punjab : Dist. Patiala, PUP, 250m, 21.vii.1998, 1♀; 15.xii.1998, 1♂; 3.x.1999, 2♂♂; 4.x.1999, 1♂; 5.x.1999, 1♂; 13.x.1999, 1♂.

Uttaranchal : Dist. Dehra Dun, FRI Dehra Dun, 700m, 23.ix.1999, 2♂♂,1♀.

Remarks : On the basis of male genitalic structures, *Helcystogramma clarkei* sp. nov. has been found to somewhat nearer to *H. hibisci* (Stainton). However, the coremata in the male genitalia are quite distinct in the former and wanting in the latter species. Besides, the forewing maculation is entirely different from *H. hibisci* (Stainton) and *H.uedai* sp. nov.

Etymology : *Helcystogramma clarkei* sp. nov. is named after Dr. J.F. Gates Clarke of USA who has done a monumental job by publising a series of catalogues of the Meyrick's type specimens.

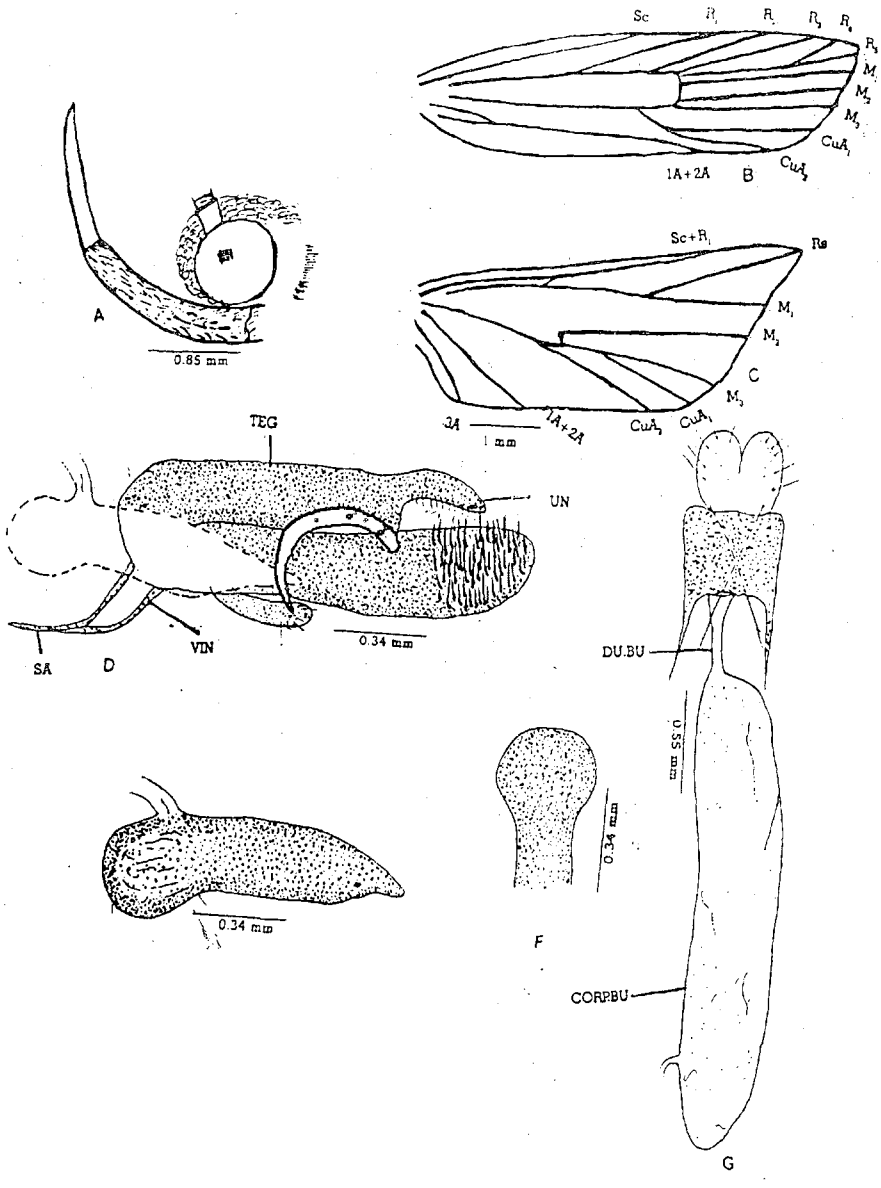
***Helcystogramma uedai* sp. nov.**

Male and female : Alar expanse : 14 mm. Vertex and frons covered with fuscous scales, labial palpus long, upturned, second segment covered with small ochreous scales, third segment long and acute, ventrally fuscous scaled, dorsally ochreous scaled (Plate 3, Fig. A); antenna long, filiform, ochreous and fuscous band alternately, approaching 3/4th length of the forewing; thorax dark fuscous; forewing elongate, fuscous scaled, costa slightly arched at base then straight, apex sub acute, termen oblique, tornus oblique, anal margin convex at base then straight, termen margin with small black spot, cilia brownish in colour, apices white; hindwing quadrate, greyish scaled, apex acute, termen trapezoidal, tornus convex, anal margin curved at base then straight, anal and termen margin with cilia grey; prothoracic and mesothoracic legs light fuscous, metathoracic leg ochreous, hind tibia with ochreous hair.

Wing venation (Plate 3, Figs. B-C) : Forewing with Sc ending at middle, R_1 arising at middle of discal cell, R_1, R_2, R_3 free, R_1 and R_2 closer at ending, R_3 arising at angle of cell, R_4+R_5 long stalked, R_4 to costa, R_5 to apex, M_1, M_2, M_3 free, parallel, M_2 closer to M_3 near base, CuA_1+CuA_2 stalked; discal cell closed, 1A+2A forked at base, ending near CuA_2; hindwing with R_1 running into Sc beyond base of Sc+R_1 join costa at 3/4th, Rs+M_1 stalked, Rs to apex, M_1 to termen, M_2 free, straight, M_3+CuA_1 stalked, CuA_2 arising middle of discal cell, discal cell open, 1A+2A straight, arising at base, 3A present.

Male genitalia (Plate 3, Figs. D-F) : Uncus small, sparsely setose, narrowed at base, broader distally, with small hair on the inner surface; socii absent; gnathos moderately long, sickle shaped, strongly

PLATE - 3



Helcystogramma uedai sp.nov. Figures. *(A) Labial palpus, (B) Forewing venation (C) Hindwing venation, (D), Male genitalia : lateral view, (E) Aedeagus, (F) Uncus: ventral view, (G) Female genitalia: ventral view.*

sclerotized, strongly curved; tegumen long, equal to the length of valvae, hood-like, with two membranous setose lobes basally, rounded apex; vinculum V-shaped, thin, weakly sclerotized; saccus small with acute apex; valvae symmetrical, elongate, narrowed at base, costal margin somewhat straight, sacculus margin slightly convex, cucullus broad, bearing small hair-like setae on the inner surface, slightly convex dorsally, apically rounded, convex ventrally; aedeagus small, moderately sclerotized at base, apex pointed, with rather broad internal lobe, globular at base, about half length of genitalia; vesica without cornutus.

Female genitalia (Plate 3, Fig. G) : Papillae anales somewhat small, rounded, sparsely setose; anterior apophyses moderately long, broader at base, posterior apophyses thin and long, almost equal to the length of anterior apophyses; ostium bursae small, open at side; ductus bursae comparatively smaller, broader near ostium bursae; corpus bursae long and broad, weakly sclerotized, ductus seminalis open near base of corpus bursae; signum absent.

Material examined :

Holotype : Himachal Pradesh : Dist. Sirmour, Renuka Lake, 740m, 11.iv.1999, 1♂.

Paratypes : Uttaranchal : Dist. Dehra Dun, FRI Dehra Dun, 700m, 3.iv.1999, 1♀; Himachal Pradesh : Dist. Sirmour, Renuka Lake, 740m, 11.iv.1999, 21.iv.2000, 2♂ : Dist. Mandi, Tanyhar 1120m, 5.vi.1999, 1♂.

Punjab : Dist. Patiala, PUP, 250m, 6.ix.1998, 1♀.

Remarks : *Helcystogramma uedai* sp. nov. is broadly allied to *H. hibisci* (Stainton) as far as structure of the uncus, tegumen and the valvae in male genitalia are concerned but both can be distinguished on the bases of the maculation of their forewings. There is no black blotch or marking in *uedai* while *hibisci* has a black blotch and markings.

Etymology : The species *Helcystogramma uedai* sp. nov. is named after a Gelechiid worker, Tatsuya Ueda of Japan.

DISCUSSION

In the present studies, it has been observed that both *Helcystogramma clarkei* and *H. uedai* have the labial palpi sickle-shaped and the ocelli are wanting and accordingly conform to the type species i.e., *Gelechia (Helcystogramma) obseratella* Zeller of the genus. The congeneric nature of the three species is also evident that the uncus is elongate, gnathos hook-like and the vinculum is rather narrower in the saccal region and bears a pair of lobes arising from its lateral arms in the male genitalia. The venation too supports that all the presently examined three species are allied to each other in having the veins R_4+R_5 and CuA_2 stalked in the forewing. The discal cell is open in the hindwing in all the three species.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

1A+2A : Vein representing fused first and second anal vein, 3A : Third anal vein, AED : Aedeagus, ANT.APO : Anterior apophyses, CL : Cucullus, CO : Costa, CORP.BU : Corpus bursae, CuA_1 : First anterior cubital vein, CuA_2 : Second anterior cubital vein, CuP : Posterior cubital vein, DU.BU : Ductus bursae, FRI : Forest Research Institute Dehra Dun, GN : Gnathos, M_1 : First median vein, M_2 : Second median vein, M_3 : Third median vein, OB : Ostium bursae, PA : Papillae anales, PO.APO : Posterior apophyses, PUP : Punjabi University, Patiala, R_1 : First radial vein, R_2 : Second radial vein, R_3 : Third radial vein, R_4 : Fourth radial vein, R_5 : Fifth radial vein, Rs : Radial sector, Sc : Subcostal vein, $Sc+R_1$: Stalk of subcostal and first radial vein, SIG : Signum, SL : Sacculus, sp. nov. : New species, TEG : Tegumen, UN : Uncus, VIN : Vinculum.

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GRASSES FOR REVEGETATING FOREST ROADSIDE SLOPES : A CASE STUDY IN MACKA ENVIRONS (TURKEY)

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Abstract

This study aims to try greening and stabilising forest roadside slopes using some grass species in Macka, the North-eastern Black Sea Region of Turkey. For this purposes, a total of 8 experiments were conducted in the area according to altitude, aspect and slope type and selected grass species (Festuca arundinaceae, F. rubra, Lolium perenne and Poa pratensis) [Family Poaceae] and Trifolium repens (Family Fabaceae) were established in these areas by seeding as trial plant materials. Species height, rooting and coverage were measured in experimental plots during the research period, which continued for 5 months after seeding in 1998. The general observations and statistical analysis showed that Festuca arundinacea, F. rubra and Lolium perenne should be used on the slopes, rather than Poa pratensis and Trifolium repens. Consequently by assessing selected species which could be adapted easily on these slopes are proposed.

Key words: Forest roads, grass species, planting, slopes, stabilisation.

INTRODUCTION

Forest roads are the most important infrastructures to supply certain services of forestry, raising economic and social profits as well as cultural ones (Erdas, 1997). According to geometrical structure of roads environment, the slopes have been considerably focused on their maintenance or stabilisation especially, after constructing the roads, these areas lose their stability by some of the factors such as run-off, wind and frost, and then result in finger erosion, wind erosion, landfill and stone-rock rolling with respect to soil type, slope angle, drainage etc. etc. (Celem,1988). In this context revegetation of road sides is a tool to enhance greening and preserving roads environment.

Waldron (1977) referred that *Medicago sativa* had a potential to control soil and to prevent slopes of a hill from erosion. Harrington (1989) and Bugg *et al.* (1997) studied some native and non-native perennial grasses to gauge their potential value in roadside vegetation management programs in USA as an alternative approach involving the establishment and maintenance of these grasses. Acar (2001) mentioned that plant species on cut-slopes generally come from those present on the upper part of the slope, but full-slopes were occupied by pioneer plants that take advantage of the medium created

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by new full-slopes. Thus, previous studies demonstrated that most species could be used or had usage potential with ecological-botanical attributes on the roadside slopes. But, studies regarding grasses for forest roadside were scanty.

In this study, we believed that grass species would raise effective habitat conditions for transplanting than other herbaceous and woody plant materials to control soil erosion if these are easily grown on the forest roadside slopes. The goal of this study was to determine growing performances of selected grass species which are commonly found for practical usage, and to select appropriate ones out of these species.

MATERIALS AND METHODS

The study was carried out in Yesiltepe and Esiroglu, Macka, located in the North Eastern Black Sea Region of Turkey. These areas are mostly mountainous and lie between latitude 40° 47' 56"-40° 54' 52" N, longitudes 39° 27' 38"-39° 43' 11" E (Anonymous, 1985).

Prior to conducting the seeding experiment, the roadside routes in this study area were surveyed and then routes of new constructed forest roadsides were selected for this purposes. A total of 8 trial plots (1-4 plots in Esiroglu, 5-8 plots in Yesiltepe) were randomly taken in 2 altitudes (0-750 m. and 751-1500 m.), 2 aspects (north and south) and 2 slope types (cutting and filling). A complete randomised block design was used to take account of the presumed moisture gradient across the north sloping site (Hitchmough *et al.*, 1996). Each trial plot for each grass species included five blocks and was 40 m^2 (2 m x 20 m). Table 1 also presents some of the habitat characteristics of the studied roadsides. As shown in the table, soils are generally sandy loam, sandy clay loam and loamy sand, pH values of these soils varied between 5.30-7.80, lime between 1.23-8.20 and organic matters between 0.37-4.43 and general assessment of the soils was that, these are not resistant to erosion.

In this study, seeds of the *Trifolium repens, Festuca rubra* sub sp. *rubra* 'Franklin', *Festuca arrundinacea* 'Apache', *Lolium perenne* 'Peramo' ve *Poa pratensis* 'Geronimo' were used in trial plots. Seeds were obtained from a commercial source (Ulusoy Tohumculuk, Ankara). Grass species were sown by hand broadcasting in two paths at right angles to each other in April, 1998. Seeding rates were about 40gr m^{-2} for the individuals species sown. Measurements were taken after germination for 8 months starting from May. Measurements were taken on the first day of each month. Species heights were measured by scale. Canopy cover/coverage percentage was visually assessed using the quadrate method described by Erac and Ekaz (1986), using a 100 cm. x 100 cm. frame. Root length has measured by portable quadrate with 5 x 5 cm. sections.

Table 1 : Some physiographic features of trial plots and analysis results of their soils.

Trial Plots	Altitude groups	Aspect groups	Slope type	Soil texture	pH	$CaCO_3$	Organic Matter (%)	Dispersion (%)	Erosion tolerability
1	751-1500 m.	North	Cutting	Sandy loam	5.30	1.64	3.69	45.69	Sensitive
2		South	Cutting	Sandy loam	6.00	1.23	4.43	46.90	Sensitive
3		South	Filling	Sandy clay loam	6.40	2.46	2.59	36.38	Sensitive
4		South	Filling	Sandy loam	5.90	1.23	3.69	60.41	Sensitive
5	0-750 m.	South	Cutting	Loamy sand	7.50	3.69	0.74	29.22	Sensitive
6		North	Filling	Loamy sand	7.60	2.46	0.74	54.19	Sensitive
7		North	Filling	Loamy sand	7.80	8.20	1.11	57.61	Sensitive
8		North	Cutting	Sandy clay loam	7.50	1.23	0.37	21.88	Sensitive

The top soil samples after sifting with 2 mm. sieve were analysed in the laboratory and interpreted according to Gulcur (1974), soil texture was determined by means of hydrometer method (Bouyoucos, 1936), dispersion rate of Middleton, pH (Orion 420 A digital pH meter) and lime.

In order to assess the data so obtained, one-way analysis of variance (ANOVA) was performed as to how some soil and physiographic factors effects on the species growth. In the event of statistically differences of samples at $p \leq 0.001$ according this ANOVA (Sokal and Rohlf, 1995), Duncan Multiply Test was conducted to show different group levels. In addition, regression analysis was made out to determine factors and effects on the height (H), growth root length (R) and coverage (C) for different grass species. Analysis were based on the measured and observed variables used for a total of 8 parameters i.e. soil type (SOT), lime (LIM), measured time-months (TIM), organic matter (ORM), slope type (SLO) altitude (ALT) pH and aspect (ASP). All observations were analysed using SPSS 7.5 for Windows.

RESULTS

Height, Root Length and Coverage of Grass Species

In this study, evaluations relating to growth data of selected species are based on the results of the five months measurements. While evaluating height, root length and coverage of the species in all the plots three factors, viz. altitude, aspect and slope type were entailed. At the end of the study, number 5, 6 and 7 showed both visually and statistically very low and spotty covering values (<30% canopy cover). Considering all species and trial plots, there were differences in performance by individual grass species and in topographical zones at $p<0.0001$ (ANOVA). As given in Figure 1, Duncan Multiply Test revealed different groups with respect to the variability among height, root length and covering.

From the point of view of the species height, Figure 1 (a1, ...,a5) showed that *Festuca arundinacea* (33.500 cm.), *Trifolium repens* (10.416 cm.), *Lolium perenne* (32.250 cm.) and *Poa pratensis* (9.250 cm.) showed best performance in height on the number 3 which is at the southern filing slope at 750-1500 m. *Festuca rubra* (19.583 cm) became higher at the northern cutting slope at 0-750 m.

The root lengths of various species measured in five months showed differences in all plots. Whereas *Festuca rubra* (17.750 cm.) and *Poa pratensis* (9.250 cm.) at the southern cutting slopes in 750-1500 m. were the best taking root, *Lolium perenne* (17.666 cm) at the northern filling in 750-1500 m had maximum rooting value. Beside these species, *Festuca arundinacea* (14.916 cm) at the northern cutting slope in 0-750 m and *Trifolium repens* (18.666 cm.) at the northern cutting slope in 0-750 m as well as the southern cutting slope in 750-1500 m had longer roots Figure 1 (b1,.....,b5).

For the final canopy cover data from the plots, results of ANOVA and Duncan Multiple Test indicated highly significant differences due to the factor species coverage rates (Figure 1 (c1,.....,c5)). Coverage findings also showed that both *Festuca rubra* (95.625%) and *Lolium perenne* (98.125%) covered more area at the northern cutting slope and the northern filling slope in 0-750 m., respectively. Similarly, *Festuca arundinacea* (92.187%) had the most covering rates at the northern cutting slope in 0-750 m and at the northern filling slope in 750-1500 m, it showed higher rates in *Trifolium repens* (90.000%) at the northern cutting slope in 0-750 m and *Poa pratensis* (89.375%) at the northern filling slope in 750-1500 m.

Factors affecting the Growth of Species

Table 2, 3 and 4 present regression results relating to some factors and models that effected the levels of growth and coverage of experimental species. These are given as follows.

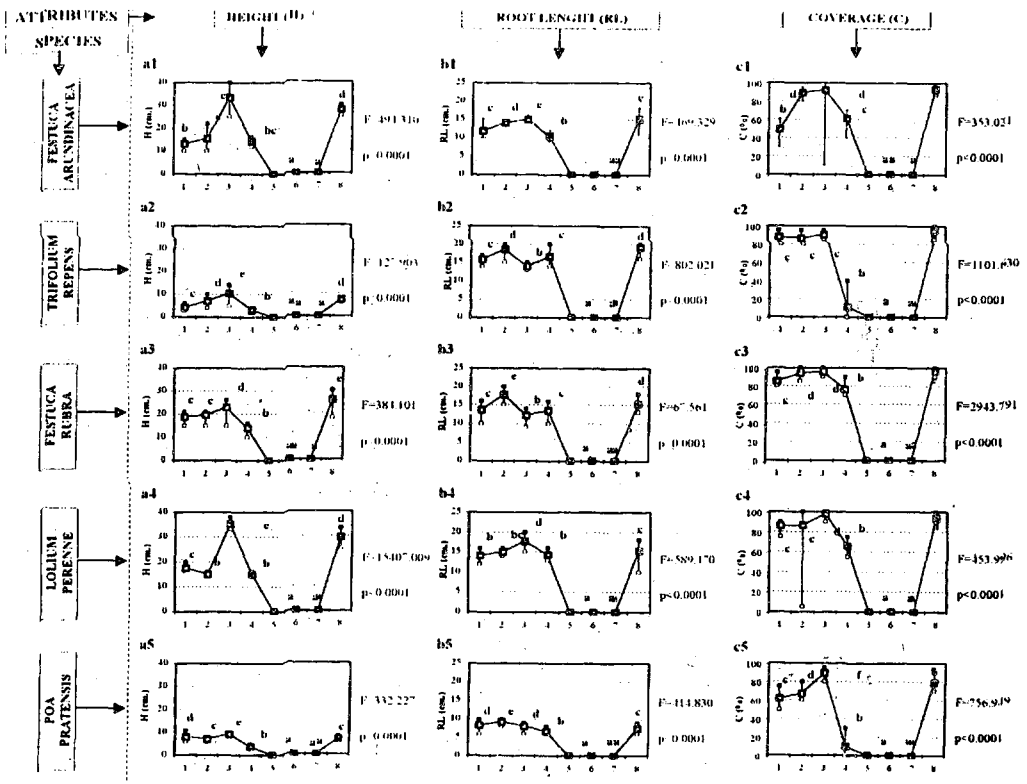

***Figure 1** : Graphics showing height (a1,....,a5), root length (b1,.....,b5) and coverage (c1,....,c5) results of five grass species. Small letters indicate different groups based on ANOVA and Duncan Multiply Test.*

Table 2 : Regression models predicting heights of selected five grass species.

Models	Variables	*T*	Probability	Overall *F*	R^2
Festuca arrundinacea					
H = -2.974+8.373* ASP-1.828* LIM+1.93* TIM	CONSTANT	-0.807	0.425	11.465	0.489
	ASP	4.180	0.000	($P \leq 0.000$)	
	LIM	-4.026	0.000		
	TIM	2.858	0.000		
Trifolium repens					
H= -34.884+3.302* ASP-0.812* LIM+0.551* TIM+7.277* ALT+3.395*pH+0.897* ORM	CONSTANT	-.3.203	0.003	12.475	0.654
	ASP	4.760	0.000	($P \leq 0.000$)	
	LIM	-4.108	0.000		
	TIM	2.349	0.025		
	ALT	3.614	0.001		
	PH	2.856	0.007		
	ORM	1.392	0.024		
Festuca rubra					
H= 3.436+4.506*ASP-2.604*SOT + 1.930*TIM	CONSTANT	0.981	0.334	8.954	0.434
	TIM	3.521	0.001	($P \leq 0.000$)	
	SOT	-2.890	0.007		
	ASP	2.861	0.007		
Lolium perenne					
H= 7.694 +10.707*ASP-2.859*LIM+1.690*TIM-5.408*SLO	CONSTANT	1.440	0.159	10.894	0.592
	ASP	5.033	0.000	($P \leq 0.000$)	
	LIM	-5.154	0.000		
	TIM	2.358	0.024		
	SLO	-2.270	0.030		
Poa pratensis					
H=2.563 + 2.308*ASP - 0.267*LIM	CONSTANT	2.375	0.023	10.281	0.357
	ASP	3.278	0.002	($P \leq 0.000$)	
	LIM	-3.923	0.000		

Table 3 : Regression models predicting root length of selected five grass species.

Models	Variables	*T*	Probability	Overall *F*	R^2
Festuca arrundinacea					
R=2.568 + 2.993*ASP - 1.102*LIM+1.027*TIM	CONSTANT	1.347	0.186	11.088	0.480
	ASP	2.890	0.006	($P \leq 0.000$)	
	LIM	-4.693	0.000		
	TIM	-2.920	0.006		
Trifolium repens					
R=16.132+3.349*ASP-7.791*SOT+1.593*TIM-1.288*ORM	CONSTANT	4.228	0.000	23.461	0.734
	ASP	3.066	0.004	($P \leq 0.000$)	
	SOT	-6.762	0.000		
	TIM	4.105	0.000		
	ORM	-2.735	0.010		
Festuca rubra					
R = 9.377 + 1.133*TIM-2.433*SOT	CONSTANT	5.688	0.000	12.899	0.417
	TIM	3.121	0.004	($P \leq 0.000$)	
	SOT	-4.140	0.000		
Lolium perenne					
R = 8.544+ 1.132*TIM - 1.066*LIM	CONSTANT	4.835	0.000	9.166	0.331
	TIM	2.409	0.021	($P \leq 0.000$)	
	LIM	-3.540	0.001		
Poa pratensis					
R= 5.544+1.773*ASP-0.586*LIM-0.983*SOT	CONSTANT	4.637	0.000	16.916	0.585
	ASP	2.918	0.006	($P \leq 0.000$)	
	LIM	-3.158	0.003		
	SOT	-2.167	0.037		

Table 4 : Regression models predicting coverage of selected five grass species.

Models	Variables	*T*	Probability	Overall *F*	R^2
Festuca arrundinacea					
C=47.858+20.579*ASP-10.388*LIM	CONSTANT	3.543	0.001	13.640	0.430
	ASP	2.335	0.025	($P \leq 0.000$)	
	LIM	*-5.199*	*0.000*		
Trifollium repens					
C=4.152+52.546*ASP-12.905*LIM	CONSTANT	0.371	0.713	45.225	0.715
	ASP	7.239	0.000	($P \leq 0.000$)	
	LIM	-7.943	0.000		
Festuca rubra					
C=117.259+25.587*ASP-4.655*LIM-7.610*ORM-32.183*SOT	CONSTANT	4.887	0.000	18.346	0.683
	ASP	3.873	0.000	($P \leq 0.000$)	
	LIM	-2.102	0.043		
	ORM	-2.599	0.014		
	SOT	-3.542	0.001		
Lolium perenne					
C=114.165+28.163*ASP-5.747*LIM-7.508*ORM-31.381*SOT	CONSTANT	4.421	0.000	18.012	0.679
	ASP	3.960	0.000	($P \leq 0.000$)	
	LIM	-2.411	0.021		
	ORM	-2.382	0.023		
	SOT	-3.209	0.003		
Poa pratensis					
C=28.908+43.533*ASP-11.188*LIM-5.523*TIM	CONSTANT	3.010	0.005	44.820	0.789
	ASP	8.342	0.000	($P \leq 0.000$)	
	LIM	-9.458	0.000		
	TIM	-3.177	0.004		

The perusal of the tables reveal that ASP, LIM and TIM had effect on height and root growth of *Festuca arundinaceae* at the rate of 48.9% and 48.0%. However, the effects of ASP and LIM on the coverage were rated at 43.0%. The factors indicating height and root growth for *Trifolium repens* were more. The height of this species was effected by ASP, LIM, pH, ORG and TIM (R^2 = 0.654), while ASP, SOT, ORG and TIM correlated with species root lengths. The coverage was based on ASP and LIM with R^2 =0.715.

The regression equations for *Festuca rubra* showed that ASP and TIM on the height, TIM and SOT on the root length and ASP, LIM, ORG and SOT on the coverage were effected to 43.4%, 41.7% and 68.3%, respectively. LIM, SLO and TIM were influenced to 59.2% the height growth of *Lolium perenne*, whereas LIM and TIM to 33.1% on the root length and, ASP, LIM, ORG and SOT with 67.9% were effected. The height, root length and coverage of *Poa pratensis* were generally correlated with ASP and LIM according to each regression models.

DISCUSSION

Recently, the landscape integration between roadside and its adjacent environment in both urban and rural areas has been getting increased attention (Anonymous, 1991). At this point, planting on the slopes and revegetation for ecological rehabilitation would encourage developments of new techniques. The usage of some grass species, rapidly growing on the forest slopes increases, speeding soil formation and preventing finger erosion in the soil surfaces exposed to some negative influences from the beginning of the process.

Present research deals with an aim of selecting grass species for forest roadside greening and determining growth of these species on some habitat features.

Generally, according to height, root length and covering, species obtained less performance in the loamy sand soils; yet, these showed best adaptation capacity in the sandy loam soils. Similarly, species attributes measured during the study were observed more at the northern cutting slopes in the second altitude class (750-1500 m) than other plots. *Festuca arundinacea, Lolium perenne* and *Festuca rubra* were found to be the most adaptable species. Although *Trifolium repens* and *Poa paratensis* germinated in their trial plots, it was found that both these species became less on the slopes or disappeared completely. It was concluded from the study that *Festuca arundinacea, F. rubra* and *Lolium perenne* should be used one by one on the forest roadside slopes. Still, using these species as mixed types such as *Festuca arundianceae-F. rubra* or *Festuca rubra-Lolium perenne* may give effective results.

Grass and some herbaceous or woody ground cover species have favourable effectiveness on soil surfaces for preventing soil erosion (Clouston, 1990). Therefore, the mixtures between grass and ground cover species have been taken into account in many rehabilitating fields as a bio-preventive measure. Once these are established, later on tree or shrubs could contribute for stabilisation of these areas. Results of this investigation decipher that in order to stabilise forest roads, such planting models which are cheaper and require less efforts should be selected. Some trials as to whether selected species or models would be harmonised with roadside environment with regional ecological characteristics should be aim at.

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DAPI BAND POLYMORPHISM IN THE POLYTENE CHROMOSOMES OF *PARASARCOPHAGA MISERA*

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Abstract

DAPI staining of polytene chromosomes shows a single fluorescent band in the region 3B of chromosome arm VR. An additional band is also found in the proximity of this band. The proximal band i.e., the band towards the centromeric end is polymorphic in the local population of P. misera.

Key words : DAPI, polymorhism, polytene chromosomes.

INTRODUCTION

Introduction of fluorescent DNA binding dyes of different specificities has made it possible to explore the heterogeneity of heterochromatic regions in different organism e.g. in *Drosophila* (Lakhotia and Mishra,1980; Zacharias and Costas, 1988) and homo sapiens (Perez-Castillo *et al.*,1987; Schweizer, 1976; Schweizer *et al.*, 1978). During past few years AT-specific fluorochromes-quinacrine dihydrochloride, Hoechst 33258 and DAPI have been successfully used to explore the nature of heterochromatin of several sarcophagid species (Gaur *et al.*, 1985; Kaul *et al.*, 1986, 1989a, 1989b; Rai, 1989, 1999,2000; Rai *et al.*, 1985; Tewari *et al.*, 1986, 1988). The present paper presents the results of DAPI staining of the polytene chromosomes of *Parasarcophaga misera*.

MATERIALS AND METHODS

P.misera was collected locally and stock culture was maintained in the laboratory. For polytene chromosome air-dried preparations, the procedure of Tewari *et al.*, (1983) was followed.

For DAPI staining the protocol of Schmid *et al.*,(1983) was adopted. Slides were stained for 10-15 minutes with 0.2µg/ml DAPI in McIlvaines buffer (pH 7.0), rinsed in McIlvaine's buffer (pH 7.0) and mounted in 1:1 solution of buffer and glycerol. DAPI stained slides were viewed and photographed in Carl Zeiss Standard 14 Fluorescence Microscope with HBO 50W high pressure mercury source fitted with MC 63 automatic photomicrographic camera using 400 ASA Kodak Tri X Pan black and white films. For DAPI filter set G 365, LP 420, FT 395 has been used.

RESULTS AND DISCUSSION

The polytene karyotype of *P. misera* comprises five long polytenized elements. These polytene elements represent the five

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autosome pairs of mitotic metaphase. However, the dot-like sex chromosomes have no counterpart either as banded element or in the form of heterochromatic granular mass. All the five polytene chromosomes are not united at a common chromocenter (Rai, 1989).

DAPI fluorescence pattern of polytene chromosomes of *P. misera* is characterized by the presence of a single fluorescent band in the region 3B of chromosome arm VR. An additional fluorescent band is also found in the proximity of this band in region 3B towards the centromeric end. The proximal band i.e., the band towards the centromeric end shows polymorphism. Some individual are homozygous for the presence of this band, some are heterozygous and some other individuals lack this band altogether (Figs. 1-3). Similar results were reported after C-,Q- and H-banding of *P. misera* (Kaul *et al.*,1989b; Tewari *et al.*, 1983).

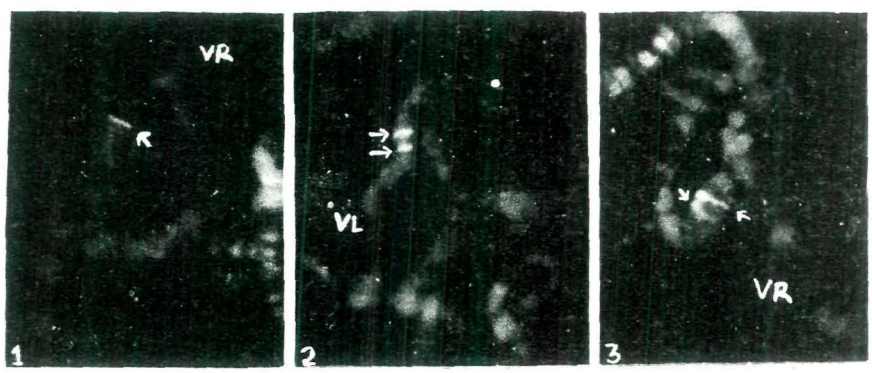

***Figure 1** : Chromosome V, absence of proximal band.*

***Figure 2** : Chromosome V, Hozozygosity for the proximal band.*

***Figure 3** : Chromosome V, heterozygosity for the proximal band.*

In cytological preparations the chromosome regions which are rich in AT-base pairs fluoresce brightly with DAPI (Leeman and Ruch, 1978; Rai *et al.*, 1985; Schweizer *et al.*, 1978). The staining property of DAPI is due to its AT- and GC-rich DNA but the enhancement by AT-rich DNA is significantly greater and DNA with high GC-or poly dG-poly dC do not quench the DAPI fuorescerce (Linn *et al.*, 1977).

The DAPI band polymorphism may have arisen due to a change within the local populations of *P. misera* in the content of AT-rich constitutive heterochromatin. In this case

of polymorphism no progeny test could be made to examine the inheritance pattern. A clear picture of the extent of polymorphism can be obtained only after an extensive survey of natural population.

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SIGNIFICANCE OF THE EXTERNAL GENITALIA IN THE FAMILY OECOPHORIDAE (LEPIDOPTERA) ALONG WITH A NEW SPECIES AND THREE NEW COMBINATIONS FROM NORTH-WEST INDIA

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Abstract

In all thirteen species i.e., Apethistis insulsa (Meyrick), A. metoeca Meyrick (Autostichinae), Cophomantella pumicata (Meyrick), C. lysimopa (Meyrick), C. myadelpha (Meyrick), C. juxticata sp. nov. (Xyloryctinae), Psorosticha zizyphi (Stainton) (Depressariinae), Stathmopoda auriferella (Walker), S. balanarcha Meyrick (Stathmopodinae), Tonica niviferana (Walker), T. zizyphi (Stainton), Periacma circumclusa Meyrick and P. continuata Meyrick (Oecophorinae) collected from North-West India have been taxonomically treated. Besides detailed description of the new species, the new combinations of the species pumicata (Meyrick), lysimopa (Meyrick) and myadelpha (Meyrick) under the genus Cophomantella Fletcher have also been justified. The male genitalia of all and the female genitalia of the species viz., Apethistis insulsa, A. metoeca, Cophomantella juxticata sp. nov., C. lysimopa, C. pumicata, Psorosticha zizyphi, Stathmopoda auriferella, S. balanarcha, Tonica zizyphi and Periacma circumclusa have been examined and described in detail. The taxonomic significance of the external genitalia at the species and genus levels is highlighted.

Key words : Genitalia, India, lepidoptera, oecophoridae.

INTRODUCTION

During the course of present studies, thirteen species i.e., *Apethistis insulsa* (Meyrick), *A. metoeca* Meyrick (Autostichinae), *Cophomantella pumicata* (Meyrick) comb. nov., *C. juxticata* sp. nov., *C. lysimopa* (Meyrick) comb. nov., *C. myadelpha* (Meyrick) comb. nov., (Xylorctinae), *Psorosticha zizyphi* (Stainton) (Depressariinae), *Stathmopoda auriferella* (Walker), *S. balanarcha* Meyrick (Stathmopodinae), *Tonica niviferana* (Walker), *T. zizyphi* (Stainton), *Periacma circumclusa* Meyrick and *P. continuata* Meyrick (Oecophorinae) referable to the respective subfamily were collected from North-West India and treated taxonomically (Meyrick, 1910, 1912-1916, 1916-1923, 1923-1930, 1930-1936; Fletcher, 1921; Gaede, 1937; Clarke, 1965, 1969; Roonwal *et al.*, 1964; Nye and Fletcher, 1991; Robinson *et al.*, 1994). The literature shows that due consideration has not been given to the external genitalia for diagnosis of species, besides delimitation of higher taxa. Accordingly, the needful is being done in the present communication, which also includes distribution, remarks and a key to the presently studied subfamilies and key to the genera represented by more than one species.

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Abbreviations in this text are given at the end of paper

OBSERVATIONS

FAMILY : OECOPHORIDAE

Oecophoridae Bruand, 1850, *Mem. Soc. Emul. Doubs,* (1) **3** (5-6) : 45 (as Aecophoridae).

Type-genus : *Oecophora* Latreille, [1796], *Précis Caractéres genériques insectes,* : 146

Key to the subfamily of the family Oecophoridae

1. Forewing with veins CuA_1 and CuA_2 stalked.....**2**

– Forewing with veins CuA_1 and CuA_2 free.....**3**

2. Forewing with CuP very weak on anal margin; abdominal tergal setae slender, sometimes deciduous....**Autostichinae**

– Forewing with CuP absent; abdomen with band of spiniform setae on posterior part of terga two to six.....**Xyloryctinae**

3. Hindwing with veins Rs and M_1 separate, fore and hindwings broad, hindwing subquadrate, discal cell usually closed.....**Depressariinae**

– Hindwing with veins Rs and M_1 stalked (rarely separate), fore- and hindwings not as above, hindwing not as above, discal cell rarely open.....**4**

4. Abdominal terga with spiniform setae on posterior margin of each segment; wall of aedeagus with a ventro-distal projection, the latter sclerotized.....**Stathmopodinae**

Abdominal terga without spiniform setae, the latter in broad band, when present; aedeagus not as above.....**Oecophorinae**

SUBFAMILY : AUTOSTICHINAE

Autostichinae Le Marchand, 1947, *Revue fr. Lepidopt.,* **11** : 153.

Type-genus : *Autosticha* Meyrick, 1886, *Trans. ent. Soc. Lond.,* **1886**: 281.

Apethistis Meyrick

Apethistis Meyrick, 1908, *J. Bombay nat. Hist. Soc.,* **18** : 459.

Type-species : *Apethistis metoeca* Meyrick, 1908, *ibidem.,* **18**:460, by original designation.

Vertex and frons dressed with semi-erect scales; labial palpus long, second segment small, scaled, third segment long and acute; antenna long, filiform, about 3/4th length of forewing; legs with erect scales on tibia; forewing with veins R_1, R_2 R_3 free, R_4+R_5 stalked, R_4 to costa, R_5 to termen, M_1 and M_2 free, CuA_1+CuA_2 stalked; hindwing with Rs+M_1 stalked, Rs to costa, M_1 to termen, M_3 and CuA_1 connate; male genitalia with uncus furcate, long, sclerotized, pointed apically; socii absent; gnathos with both arms pointed apically; saccus absent; valva small, broad, costa with a hook-like process, inner surface of cucullus densely setosed; aedeagus rounded basally, swollen; vesica without any cornutus; female genitalia with ductus bursae centrally placed; corpus bursae ovate in shape; signum present, the latter kidney-shaped.

Remarks : Meyrick (1908) proposed the genus *Apethistis* with *A. metoeca* Meyrick as its type-species from Ceylon (Maskeliya) in the family Gelechiidae, which was transferred to the family Oecophoridae by Hodges (1978). Out of fourteen species otherwise recorded in this genus, eight i.e., *Apethistis sitiens* Meyrick (South India), *A. cenchritis* Meyrick, *A. consummata* Meyrick, *A. inspersa* Meyrick (Assam), *A. officiosa* Meyrick (Madras), *A. purificata* Meyrick (Sikkim), *A. superans* Meyrick (Kumaon) and *A. insulsa* (Meyrick) (Pusa, Bihar) have been reported from the respective area from India (Gaede, 1937; Clarke, 1965). In fact, none of the species has so far been reported from the Siwaliks in North-West India, from where besides the type-species i.e., *A. metoeca* Meyrick another species i.e., *A. insulsa* (Meyrick) have been collected for the first time. Further, it has been observed on the basis of the female genitalia that the latter species conforms to the type-species in having characters such as globular corpus bursae with a signum present, the posterior apophyses being longer than the anterior apophyses and simple ostium bursae. Also, the valvae are leaf-like with a small but distinct costal process besides that the uncus and the gnathos are bifid in the male genitalia of either species. Having examined the type-species, these characters have been added to the diagnosis of the genus, under reference. It may be mentioned that though Clarke (1969) has given a photograph of the male genitalia, yet description of various constituent parts is completely lacking. Besides giving an illustrated account of the male, the female genitalia of both the species are described for the first time.

Key to the presently studied species of the genus *Apethistis* Meyrick

1. Forewing with vein Sc ending near middle of costa, vein R_4 to costa; hindwing with CuP fully developed; male genitalia with valvae large and broad, aedeagus almost straight upto 3/4th, then narrowed; female genitalia with relatively smaller ostium bursae.....***insulsa* Meyrick**

– Forewing with vein Sc ending at 2/3rd of costa, vein R_4 to costa near apex; hindwing with CuP vestigial, visible near anal margin only; male genitalia with valvae small and relatively less broader, aedeagus slightly curved near apex, basally bulbous; female genitalia with larger ostium bursae.....***metoeca* Meyrick**

***Apethistis insulsa* (Meyrick)**

Brachmia insulsa Meyrick, 1914, *J. Bombay nat. Hist. Soc.*, **22** : 774.

Male genitalia (Plate 4, Figs. A-B) : Uncus furcate, somewhat long, basally broader, pointed and well sclerotized at apex; socii absent; gnathos reduced with two arms, each with apex pointed, well sclerotized and directed towards uncus; tegumen broad and long, basally arched; vinculum V-shaped, thin; saccus absent; juxta small, membranous; valvae symmetrical, broad and large,

narrowed at base, costal margin convex, with hook-like process near cucullus, sacculus margin convex, basally narrowed, cucullus large, inner surface densely setose, convex dorso-distally, apically rounded, ventro-distally convex; aedeagus smaller than the length of valvae, otherwise almost straight upto 3/4th, then narrowed, apex pointed, ventral side with elongate sclerotization, basally rounded; vesica lacking cornutus.

Female genitalia (Plate 4, Fig. C) : Papillae anales large, somewhat rounded, sparsely setosed; anterior apophyses rather long, posterior apophyses longer than anterior apophyses; ostium bursae small, centrally placed; ductus bursae long, slightly curved near ostium and at middle, broader near corpus bursae; the latter comparatively small, subovate in shape; signum reduced, somewhat kidney-shaped.

Alar expanse : Male and female : 14-16 mm.

Material examined :

Uttaranchal : Dist. Dehra Dun, FRI Dehra Dun, 700m, 3.iv.1999, 3♂♂, 2♀♀; 22.iv.1999, 12♂♂, 2♀♀; 25.iv.1999, 1♂; 17.iv.2000, 9♂♂, 6♀♀; 18.iv.2000, 2♂♂; Haridwar, 800m, 26.iv.1999, 8♂♂, 6♀♀; 27.iv.1999, 2♂♂.(H.S. Rose & P.C. Pathania coll.)

Distribution : India (Pusa, Bihar) (Clarke, 1969).

Larval host plant : Unknown

Remarks : Though there are no apparent variations in different individuals of the species, *Apethistis insulsa* (Meyrick) which have been collected from the aforesaid two localities, yet four males and two females from Dehra Dun and three males and two females from Haridwar were dissected to ensure their conspecificity. As per present collection record, the species is quite abundant in the Doon Valley in Uttaranchal.

Apethistis metoeca Meyrick

Apethistis metoeca Meyrick, 1908, *J. Bombay nat. Hist. Soc.*, **18**: 460.

Male genitalia (Plate 4, Figs. D-E) : Uncus furcate, long, broader at base, apex pointed, well sclerotized; socii missing; gnathos represented by two small arms, well sclerotized, directed towards uncus; tegumen broad and long, basally arched; vinculum V-shaped, thin; saccus very reduced; juxta small, weakly sclerotized; valvae symmetrical, broad and large, narrowed at base, costal margin almost straight, with hook-like large process near base, sacculus strongly convex, narrowed at base, cucullus large, inner surface fringed with setae, dorso-distally straight, rounded at apex, ventro-distally convex; aedeagus smaller than the length of valvae, narrowed, bulbous at 1/4th basally, then convex upto 3/4th, slightly curved near apex, the latter somewhat pointed, a spindle-shaped sclerotization at the posterior end of the apex; vesica without cornutus.

Female genitalia (Plate 4, Fig. F) : Papillae anales large, rounded, beset with a few setae; anterior apophyses shorter than posterior apophyses; ostium bursae opening large, centrally placed; ductus bursae long, broader near corpus bursae and ostium bursae,

narrowed above middle towards ostium bursae; corpus bursae reduced and somewhat ovate; signum small, kidney-shaped.

Alar expanse : Male and female : 12-16 mm.

Material examined :

Punjab : Dist. Patiala, Punjabi University, Patiala, 250m, 23.iii.1998, 1♂; 16.iv.1998, 1♂; 11.iii.1999, 2♂♂; 15.iii.1999, 1♂; 24.iii.1999, 1♂; 25.iii.1999, 3♂♂, 2♀♀; 27.iii.1999, 3♂♂, 3♀♀; 28.iii.1999, 1♂; 29.iii.1999, 1♂; 30.iii.1999, 1♂, 1♀; 31.iii.1999, 1♀; 6.iv.1999, 1♀; 17.viii.1999, 1♂; 19.viii.1999, 1♂; 24.viii.1999, 2♂♂; 29.viii.1999, 1♀; .27.ix.1999, 1♂; 12.x.1999, 1♂; 18.x.1999, 2♂♂; 21.iii.2000, 1♂; 29.iii.2000, 2♂♂ : Dist. Ropar, Anandpur Sahib, 400m, 16.x.1999, 2♂♂ : Dist. Hoshiarpur, FRH Chohal, 400m, 20.iii.2000, 1♂.

Jammu and Kashmir : Dist. Jammu, Jammu University Campus, 350m, 9.xi.2001, 3♂♂, 1♀;. (H.S. Rose & P.C. Pathania coll.)

Distribution : Maskeliya (Ceylon) (Clarke, 1969).

Larval host plant : Unknown

Remarks : *Apethistis metoeca* Meyrick is being reported for the first time from India. Out of thirty-eight males and ten females, ten males and two females were dissected and found to be truely conspecific. The species is quite abundant within an elevation range of 250m to 400m in the North-West India.

SUBFAMILY : XYLORYCTINAE

Xyloryctinae Meyrick, 1890, *Trans. R. Soc. S. Aust.*, **13** : 23 (as Xyloryctidae).

Type-genus : *Xylorycta* Meyrick, 1890, *Trans. R. Soc. S. Aust.*, **13**: 25 (key), 57.

Cophomantella Fletcher

Cophomantella Fletcher, 1940, *Entomologist's Rec. J. Var.*, **52** : 17.

Type-species : *Onebala elaphopis* Meyrick, 1910, *J. Bombay nat. Hist. Soc.*, **20** : 459, by original designation (for *Cophomantis* Meyrick, 1925).

Vertex and frons beset with smooth scales; labial palpus long, second segment scaled, third segment long and acute; antenna filiform, longer than 3/4th length of forewing; hind leg with small hair like scales on tibia; forewing with veins R_4+R_5 stalked, R_5 to costa or apex, R_3 absent, M_2 absent, CuA_1+CuA_2 stalked; hindwing with Rs+M_1 stalked, Rs to costa, M_1 to termen, M_2 absent, M_3 +CuA_1 stalked, CuP present or vestigial; male genitalia characteristic uncus somewhat thorn-like, long or short; socii absent; gnathos long, apically pointed; saccus wanting; valvae elongatae, inner surface densely setose; female genitalia with anterior apophyses rod-like; antrum broad; ductus bursae broader in middle, sclerotized, coiled near corpus bursae; ductus seminalis departs from near coiling of ductus bursae; corpus bursae with signum present.

Remarks : The genus *Cophomantis* was

proposed with *Onebala elaphopis* Meyrick as its type-species by Meyrick (1925), who recorded its distribution in India and Borneo. The genus has either been considered under the family Gelechiidae or Lecithoceridae by Fletcher (1929) and Clarke (1955) respectively. In a later publication, Fletcher (1940) noticed that this generic name is occupied in Amphibia and, accordingly, proposed *Cophomantella* Fletcher as an objective replacement name for *Cophomantis* Meyrick. The latter named followed by Nye and Fletcher (1991) in the subfamily Xyloryctinae of the family Oecophoridae has been currently used in the present studies, as well.

After Meyrick (1925), the diagnosis of the genus has been improved by Clarke (1965), who gave an outline of the wing venation and furnished photographs of the male genitalia and the labial palpus of the lectotype of the type-species i.e., *Onebala elaphosis* Meyrick. As per diagnosis, the genus *Cophomantella* Fletcher is conspicuous due to an absence of one of the radials (R_3) in the forewing, whereas, one of the medials (M_2) is wanting in both the wings and the discal cell is about 3/4th length of the wing in either case. In view of this characterization, a sample consisting of sixty closely allied individuals representing a sibling complex has been sorted out into four species i.e., *Cophomantella pumicata* Meyrick, *C. lysimopa* Meyrick, *C. myadelpha* Meyrick and *C. juxticata* sp. nov., out of which, the former three species make new combinations in view of the non-availbility of the generic name *Cophomantis*, as explained above.

According to Gaede (1937) and Clarke (1965), the genus *Cophomantella* Fletcher is represented by fifteen species i.e., *elaphopis* Meyrick (Khasi Hills), *lysimopa* Meyrick (Bombay, Mahableshwar), *myadelpha* (Meyrick) (N. Coorg), *osphrantica* (Meyrick) (Madras, Palni Hills), *pumicata* Meyrick (Bombay, Poona), *bythota* (Meyrick) (Aburi, Goldcost), *eremota* (Meyrick) (Ceylon), *syngonarcha* Meyrick (Uganda, Masaka), *bifrenata* Meyrick (Africa), *crypsizyga* Meyrick (Nyassaland), *cubiculata* Meyrick (Seychellen), *ephanozona* Meyrick (Transvaal), *homogramma* Meyrick (Natal) and *lychnocentra* Meyrick (Australia), out of which, the former five species belong to India. In the present field surveys, three species which have already been reported are recaptured besides a new species. It may be high lightend here that not only in respect of the wing venation but also in structures such as well developed uncus, tegumen and juxta in the male genitalia of all the aforesaid species corroborate their congeneric nature and broad similarity with the type-species i.e., *Oneballa elaphopis* Meyrick, as well.

Key to the presently studied species of the genus *Cophomantella* Fletcher

1. Forewing with vein R_1 originating from middle of the discal cell; aedeagus with one of its walls produced into a fine spine, the latter curved ..... ***pumicata* Meyrick**

– Forewing with vein R_1 originating from much beyond middle of discal cell; aedeagus not as above.....**2**

2. Hindwing with veins M_3+CuA_1 long stalked; male genitalia with each

valva broadly tapering, saccular margin somewhat excurved in the basal half, then notched in middle; juxta quite conspicuous, plate-like, produced into knob like processes, the latter sparsely setosed, gnathos relatively small, beak-like.....***juxticata* sp. nov.**

– Hindwing with veins M_3+CuA_1 small stalked; male genitalia with each valva retangular in shape, saccular margin smooth, juxta not as above, gnathos much elongated and produced into fine point apically.....3

3. Alar expanse 16-20mm; hindwing with discal cell closed by arched discocellulars; male genitalia with aedeagus short and broad, apex pointed, vesica without cornutus, coecum absent, juxta well developed, long, apically pointed.....***lysimopa* Meyrick**

– Alar expanse 15mm; hindwing with cell closed by angular discocellulars; male genitalia with aedeagus rather long, apex rounded, vesica with cornutus, coecum present, juxta reduced, apically rounded.....***myadelpha* Meyrick**

***Cophomantella pumicata* (Meyrick) comb. nov.**

Cophomantis pumicata Meyrick, 1929, *Exot. Microlepid.*, **3**: 525.

Male genitalia (Plate 2, Figs. A-B) : Uncus thorn-like, moderately long, broader at base; socii absent; gnathos very long, apex pointed, broader basally; tegumen long, broader at distal end; vinculum U-shaped, thin; saccus absent; juxta broad, concave distally; valvae symmetrical, long and broad, costal margin convex, sacculus almost straight, cucullus with long, dense setae on inner surface, side with long setae, concave dorsally, apically acute, convex ventrally, slightly curved at base; aedeagus moderately sclerotized, with one of its walls reduced into a fine spine, gradually curved from 1/6th to 4/6th from base, apex rounded; vesica armed with three to five cornuti.

Female genitalia (Plate 2, Fig. C) : Papillae anales somewhat large, rounded, sparsely setosed; anterior apophyses short, rod-like, broader at base, posterior apophyses rather, otherwise long and thin, longer than anterior apophyses; eight sternite medium emargination V-shaped; ostium quite large, centrally placed; antrum broader, weakly sclerotized; ductus bursae very long, broader at middle, coiled at base, upper half from antrum gradually curved; ductus seminalis open at middle of ductus bursae; corpus bursae subovate; signum epresented by clerotizations, one sickle-shaped and other slit-like.

Alar expanse : Male and female : 20-25 mm.

Material examined :

Uttaranchal : Dist. Dehra Dun, FRI Dehra Dun, 700m, 22.ix.1999, 1♂; 23.ix.1999, 2♂♂, 2♀♀; 18.iv.2000, 3♂♂; 19. iv. 2000, 4♂♂. (H.S. Rose & P.C. Pathania coll.)

Distribution : Bombay, Poona (Clarke, 1965).

Larval host plant : Unknown

Remarks : *Cophomantella pumicata* (Meyrick) is a very common species as per present field survey undertaken in the Doon Valley (Uttaranchal). It is being reported for the first time from North-West India.

***Cophomantella juxticata* sp. nov.**

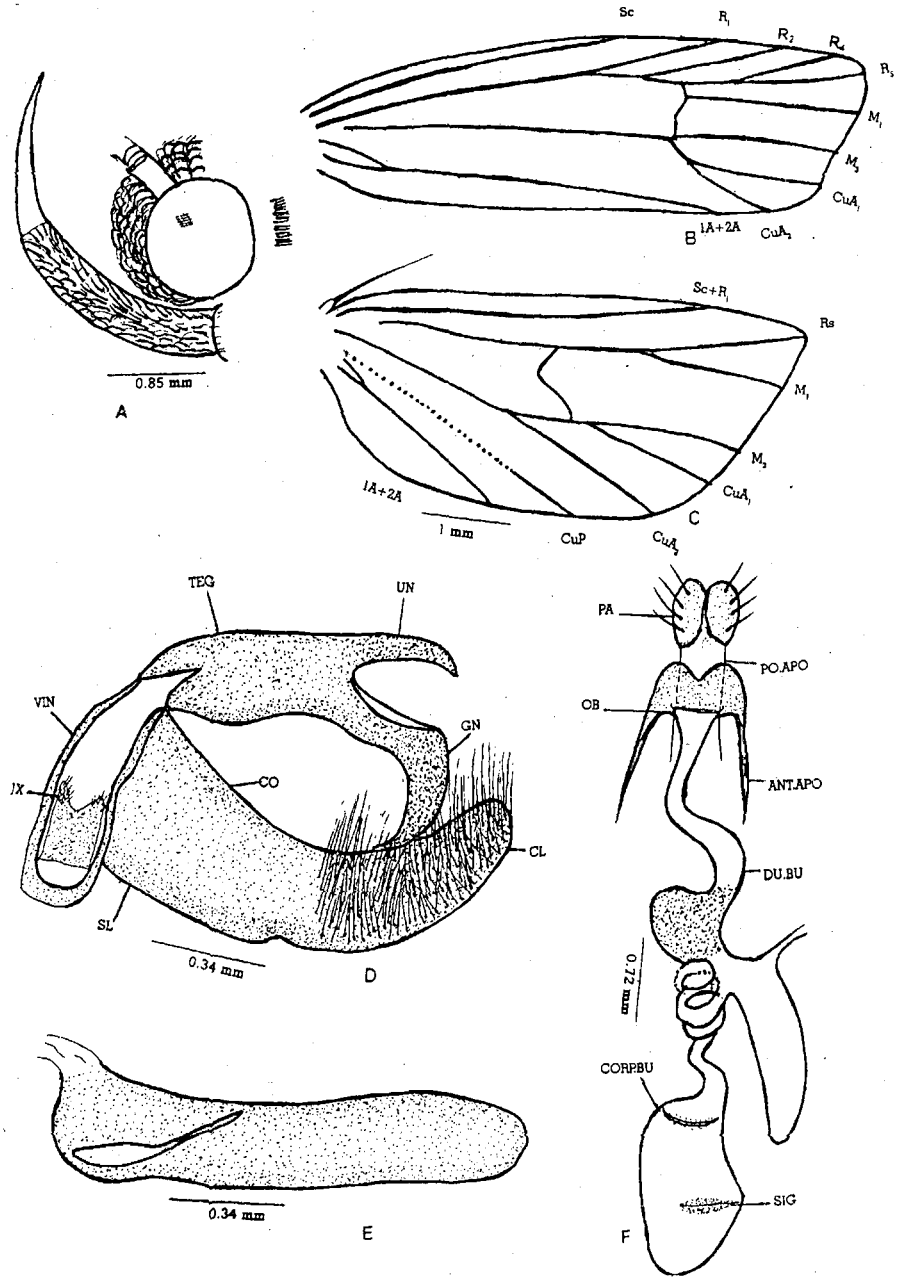
Male and female : Alar expanse : 22-23 mm. Vertex at middle covered with fuscous scales, side ochreous; frons decorated with fuscous scales; labial palpus long, upturned, second segment covered with dark fuscous scales, third segment long, acute, fuscous (Plate 1, Fig. A); antenna filiform, ochreous, longer than 3/4th length of forewing; thorax fuscous; abdomen with band of spiniform setae on posterior part of terga two to six; forewing elongate, fuscous, costal margin light ochreous, slightly arched, apex rounded, termen oblique, tornus slightly convex, anal margin convex at base then staight, termen fringed with fuscous cilia; hindwing fuscous, somewhat quadrate, costa slightly convex at base, then straight, apex rounded, termen oblique, anal margin convex, anal and termen with grey cilia; pro and meso legs fuscous, meta leg grey, hind tibia with small grey hair.

Wing venation (Plate 1, Figs. B-C) : Forewing with Sc ending at middle of costa, R_1 arising at slightly above 2/3rd of discal cell, R_1, R_2 free, R_3 absent, R_4 +R_5 short stalked, R_5 to apex, M_1, M_3 free, parallel, M_1 arising from near upper angle of cell, M_3 from lower angle of discal cell, M_1 nearer to R_5 than M_3, M_2 absent, CuA_1+CuA_2 stalked, arising at lower angle of discal cell, discal cell closed, 1A+2A forked; hindwing with Sc+R_1 ending at 4/5th of costa, Rs+M_1 stalked, Rs to apex, M_1 to termen, M_2 absent, M_3+CuA_1 short stalked, arising from lower angle of cell, CuA_2 from 3/4th of discal cell, CuP visible at anal margin, 1A+2A forked.

Male genitalia (Plate 1, Figs. D-E) : Uncus thorn-like, long, broader at base; socii absent; gnathos relatively small, beak-like, basally broader, apically pointed; tegumen long and broad, rather more broader at middle; vinculum U-shaped, saccus indistinguishable; juxta quite conspicuous, plate-like, produced with knob-like processes, the latter sparsely setosed; valvae symmetrical, elongate, broader at base, tapering and apically narrowed, costal margin concave, sacculus margin excurved in basal half, then notched in middle, cucullus with long dense setae on its inner surface, slightly concave dorsally, apically rounded, convex ventrally, with small protrusion near base; aedeagus long, longer than valvae, moderately sclerotized, apically and basally broader, apex rounded; vesica with cornutus long, spoon-like.

Female genitalia (Plate 1, Fig. F) : Papillae anales small, rounded, studded with sparsely setae; anterior apophyses short, rod-like, broader basally; eight sternite broader, sparsely setosed, emargination V-shaped; ostium bursae broad, centrally placed; antrum broad, weakly sclerotized; ductus bursae long, broader near antrum and near corpus bursae, moderately sclerotized at middle, coiled near corpus bursae and curved near antrum; ductus seminalis open near coiling; corpus bursae small and subovate; signae two, one sickle-shaped, other rod-like.

PLATE - 1



Cophomantella juxticata sp. nov. : Figures (A) *Labial palpus,* ***(B)*** *Forewing venation,* ***(C)*** *Hindwing venation,* ***(D)*** *Male genitalia : lateral view,* ***(E)*** *Aedeagus,* ***(F)*** *Female genitalia: ventral view.*

Material examined :

Holotype : Himachal Pradesh : Dist. Sirmour, Renuka Lake, 740m, 3.iv.1999, 1♂. (deposited in the Lepidoptera Lab., Department of Zoology, Punjabi University, Patiala).

Paratypes : Himachal Pradesh : Dist. Sirmour, Renuka Lake, 740m, 4.iv.1999, 3♂♂; 5.iv.1999, 2♂♂; 11.iv.1999, 2♀♀;12.iv.1999, 2♂♂, 2♀♀. (H.S. Rose & P.C. Pathania coll.)

Larval host plant : Unknown

Remarks : *Cophomantella juxticata* sp. nov. is somewhat allied to *C. eremota* (Meyrick) in respect of structure of the valvae in the male genitalia. However, both the species can be differentiated on the basis of the juxta, which is knob-like in *C. juxticata* and produced into sharply pointed process in *C. eremota* (Meyrick) (Clarke, 1965). Also, the alar expanse ranges from 22-23mm in the former and 15-17mm in latter species.

Etymology : The juxta in the male genitalia is quite conspicuous and specialized and, accordingly, the species (gender: masculine) is named as *Cophomantella juxticata* sp. nov.

***Cophomantella lysimopa* (Meyrick) comb. nov.**

Cophomantis lysimopa Meyrick, 1933, *Exot Microlepid.*, **4**: 357.

Male genitalia (Plate 2, Figs. D-E) : Uncus thorn-like, moderately long; socii wanting; gnathos very long and broader; tegumen broad and small, vinculum U-shaped, weak; saccus not developed; juxta broad, concave distally; valvae symmetrical, elongate distally, broader at base, costal margin convex, sacculus margin convex, cucullus densely setose in the inner surface, margin concave dorso-distally, apex pointed, convex ventro-distally, basally straight; aedeagus short and broad, smaller than valvae, apex pointed, well sclerotized, vesica without cornutus.

Female genitalia (Plate 2, Fig. F) : Papillae anales rounded and small, sparsely setosed; anterior apophyses small, rod-like, broader at base, posterior apophyses longer than anterior apophyses; eight sternite emargination broader, V-shaped; ostium bursae wide, centrally located; antrum somewhat large; ductus bursae very long, broader proximally, coiled near base, broader part moderately sclerotized; ductus seminalis open at middle near coiling; corpus bursae subovate in shape; two signae present, one sickle-shaped, other slit-like.

Material examined :

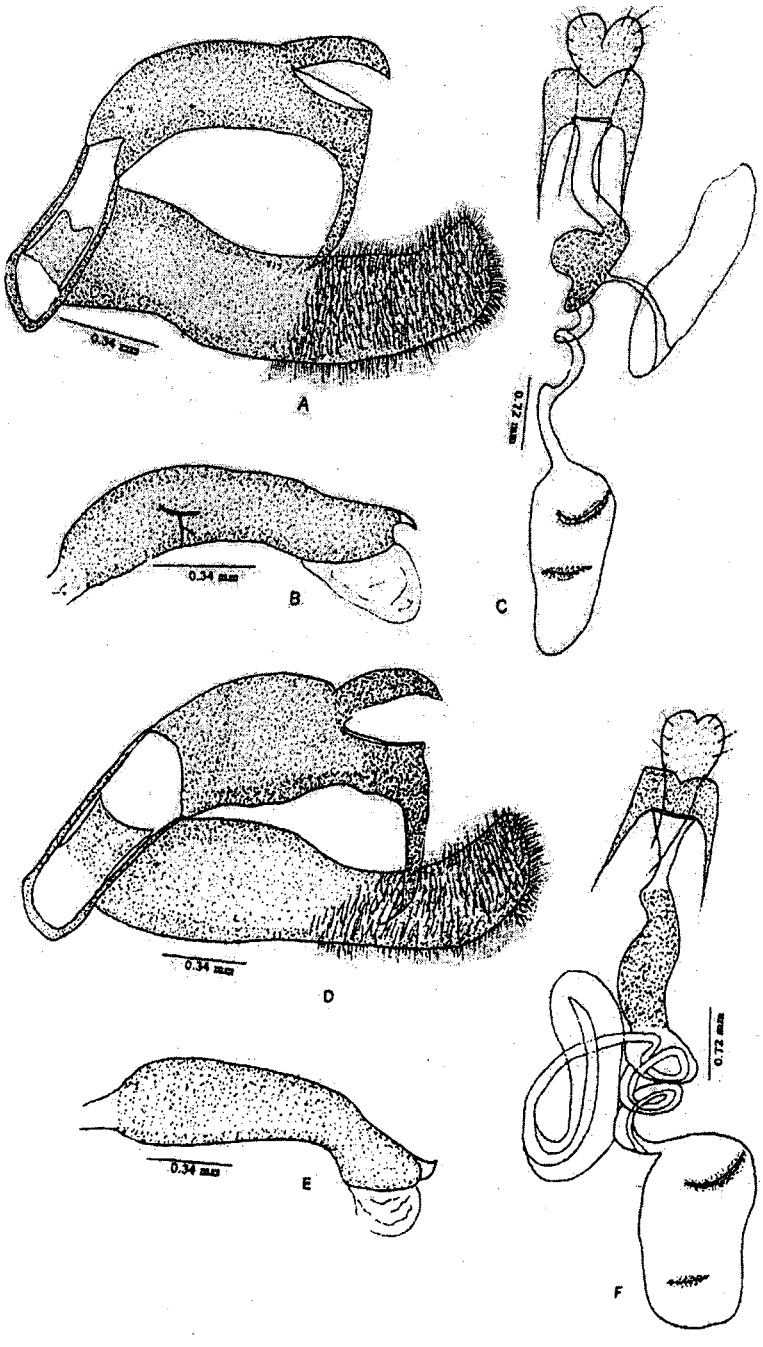
Himachal Pradesh : Dist. Solan, UHF Nauni, 1360m, 10.ix.1998, 2♂♂; 11.ix.1998, 3♂♂, 2♀♀; 11.ix.1999, 7♂♂; 12.ix.1999, 5♂♂, 2♀♀; 14.ix.1999, 1♂; Dharampur, 1500m, 16.ix.1999, 2♀♀. (H.S. Rose & P.C. Pathania coll.)

Distribution : Bombay, Mahableshwar (Clarke, 1965).

Larval host plant : Unknown

Remarks : As per present collection record from the Siwaliks in North-West India, the species, under reference, is very common.

PLATE - 2



Cophomantella pumicata (Meyrick) comb.nov. : Figures (A) *Male genitalia ;lateral view,* ***(B)*** *Aedeagus,* ***(C)*** *Female genitalia : ventral view;* ***Cophomantella lysimopa (Meyrick) comb. nov.*** *: (D) Male genitalia : lateral view, (E) Aedeagus, (F) Female genitalia: ventral view.*

The sample comprising twenty-four individuals was collected from between an elevation range of 1360m to 1500m. Further, two males from Nauni and one female each from Nauni and Dharampur in the Western Himalaya have been dissected and found conspecific in each respect. The localities from which the species has now been collected are an additional records. As such, the species is reported for the first from North-West India.

***Cophomantella myadelpha* (Meyrick) comb. nov.**

Onebala myadelpha Meyrick, 1910, *J. Bombay nat. Hist. Soc.*, **20**: 459.

Male genitalia (Plate 3, Figs. A-B) : Uncus moderately long and thorn-like; socii absent; gnathos long, beak-like, broader at base, pointed distally, curved towards costa of valvae; tegumen somewhat long, broader at base; vinculum U-shaped, broader distally; saccus not traceable; juxta large, concave distally; valvae symmetrical, long, broad, elongate distally, costal margin broader at base, sacculus slightly convex, inner surface of cucullus densely setosed, concave dorso-distally, apically somewhat rounded, convex dorso-distally; aedeagus rather long and broad, smaller than valvae, moderately sclerotized, globular at base, straight upto 4/6th, apex somewhat rounded, vesica with fifteen small and five moderately long rod-like cornuti present.

Female genitalia : Not studied.

Alar expanse : Male : 15 mm.

Material examined :

Himachal Pradesh : Dist. Mandi, Tanyhar, 1120m, 5.vii. 1999, 4♂♂; 11.vii.1999, 1♂. (H.S. Rose & P.C. Pathania coll.)

Distribution : Khasi Hills (Assam) (Meyrick, 1910).

Larval host plant : Unknown

Remarks : *Cophomantella myadelpha* (Meyrick) is being reported from Tanyhar (H.P.) in the Western Himalaya as an additional record in India (first record from North-West India).

SUBFAMILY : DEPRESSARIINAE

Depressariinae Meyrick, 1883, *Trans. ent. Soc. Lond.*, **1883** : 123 (as Depressariidae).

Type genus : *Depressaria* Haworth, 1811, *Lepid. Br.*, 505.

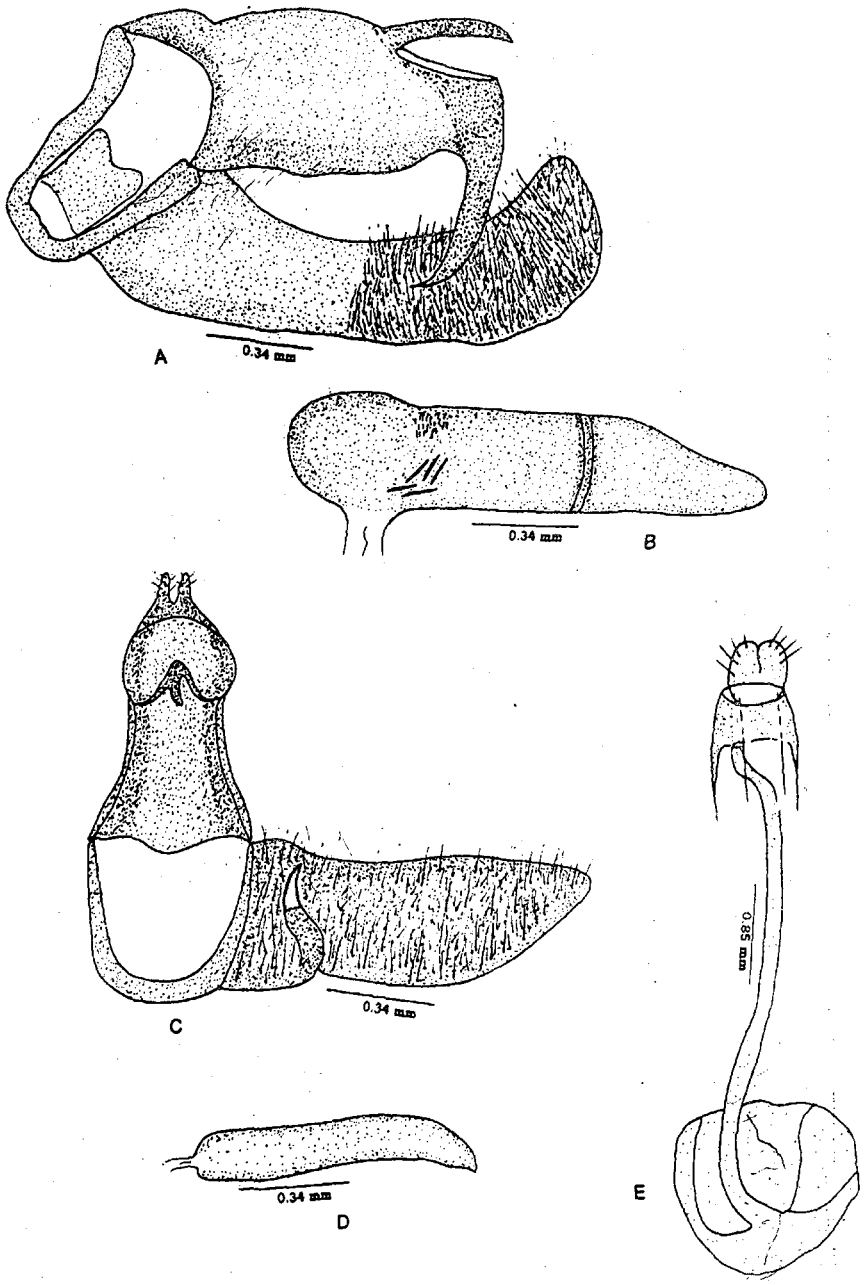
***Psorosticha* Lower**

Psorosticha Lower, 1901, *Trans. R. Soc. S. Aust.*, **25** : 91.

Type-species : *Psorosticha acrolopha* Lower, 1901, *ibidem*, **25** : 92, by monotypy.

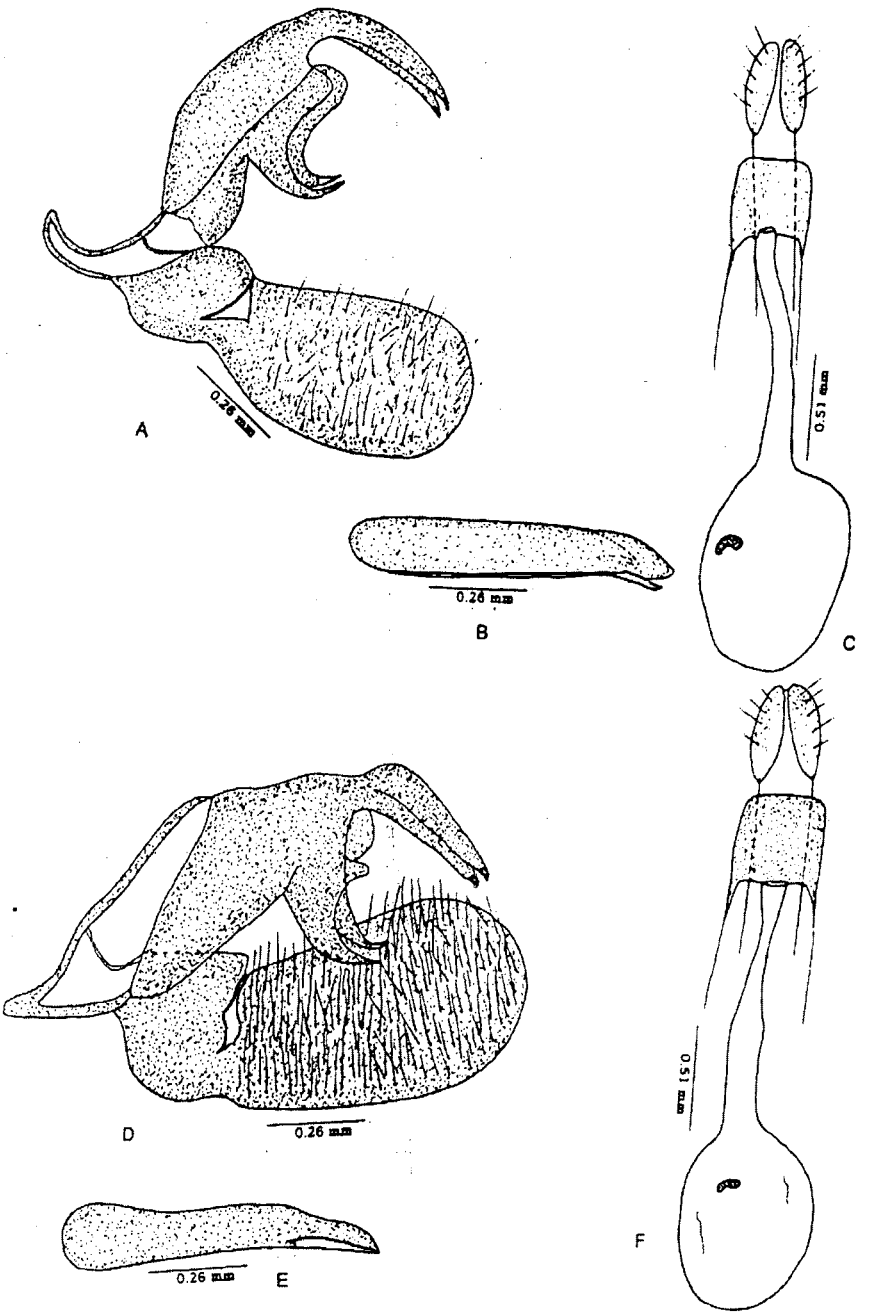
Vertex and frons decorated with erect scales; labial palpus long, recurved, second segment long, third segment small and acute; antenna long, filiform, longer than 3/4th length of forewing; metathoracic leg with erect scales on hind tibia, forewing with veins R_1, R_2, R_3 free, R_4+R_5 stalked, R_5 to costa, M_1, M_2, M_3 free, CuA_1+CuA_2 stalked; hindwing subquadrate, with vein Rs to costa, M_1 to termen, M_1, M_2 free, M_3+CuA_1 stalked; male genitalia with uncus bilobed, broader at base;

PLATE - 3



Cophomantella myadelpha (Meyrick) comb.nov. : Figures (A) *Male genitalia : lateral view,* ***(B)*** *Aedeagus;* ***Psorosticha zizyphi (Stainton) (C)*** *Male genitalia : ventral view;* ***(D)*** *Aedeagus,* ***(E)*** *Female genitalia: ventral view.*

PLATE - 4



Apethistis insulsa (Meyrick)* : Figures (A)** *Male genitalia : lateral view,* **(B)** *Aedeagus,* **(C)** *Female genitalia : ventral view;* ***Apethistis metoeca Meyrick *:* **(D)** *Male genitalia: lateral view,* **(E)** *Aedeagus,* **(F)** *Female genitaliā : ventral view.*

socii absent; gnathos turned towards inner surface; tegumen broad, sclerotized; valvae long and broad, setose, sacculus beset with a hook-like process; aedeagus broad; vesica with cornutus present; female genitalia characteristic, papillae anales small, sparsely setose; ostium bursae open at side; ductus bursae long, weakly sclerotized; corpus bursae globular in shape; signum lacking.

Psorosticha zizyphi (Stainton)

Depressaria zizyphi Stainton, 1859, *Trans. ent. Soc. Lond.*, (2) 5: 115.

Male genitalia (Plate 3, Figs. C-D) : Uncus small in size, bilobed, each lobe rounded apically, sparsely setosed, socii absent; gnathos small, turned towards inner side; tegumen small and broad, moderately sclerotized, vinculum U-shaped, reduced, basally broader; saccus absent; juxta small and thin; valvae symmetrical, long and broad, furnished with dense setae on the inner surface, narrowed distally, costa concave, sacculus almost straight, with long hook-like process curved towards costal margin near base, cucullus somewhat straight dorso-distally, apex somewhat rounded, apex well sclerotized; aedeagus, smaller than the length of valvae, slightly bent at middle, apex somewhat pointed, ductus ejaculatorius centrally placed; vesica lacking cornutus.

Female genitalia (Plate 3, Fig. E) : Papillae anales small, somewhat rounded, sparsely setosed; anterior apophyses shorter than posterior apophyses, the latter long and thin; ostium bursae small and narrowed; ductus bursae long, moderately sclerotized, broader near corpus bursae, curved near ostium, corpus bursae small, globular in shape, moderately sclerotized; signum lacking.

Alar expanse : Male and female : 13-17 mm.

Material examined :

Himachal Pradesh : Dist. Sirmour, Renuka Lake, 740m, 9.viii.1998, 1♂; 12.iv.1999, 1♂; 4.ix.1999, 2♀♀; 5.ix.1999, 1♀ : Dist. Solan, UHF Nauni, 1360m, 10.ix.1998, 1♀.

Uttaranchal : Dist. Dehra Dun, FRI Dehra Dun, 700m, 23.iv.1999, 1♂.

Punjab : Dist. Patiala, PUP, 250m, 12.viii.1999, 2♂♂ : Dist. Gurdaspur, FRH Dhar, 700m, 23.x.2001, 2♂♂. (H.S. Rose & P.C. Pathania coll.)

Distribution : India, Sri Lanka, W. Malaysia, Java, Australia (Robinson *et al.*, 1994).

Larval host plant : *Zizyphus jujuba, Citrus, Murraya koenigii* (Robinson *et al.*, 1994).

Remarks : According to Robinson *et al.* (1994), the genus *Psorosticha* Lower consists of three species including *P. zizyphi* (Stainton) from India and other two from Japan and Philippines. The presently dealtwith species i.e., *P. zizyphi* (Stainton), which has been reported from an elevation range i.e., 250m-1360m is being redescribed from India. One of the most diagnostic feature of its identification is its dark brown thorax and extreme base of the forewings. The latter is

otherwise rather pale and unicolourous but has several indistinct brown markings along the costa and a series of black dots at the bases of cilia on the termen. The alar expanse of presently collected eleven individuals varies (17 mm : Robinson *et al.*, 1994) from 13mm to 17mm. It may also be mentioned that the individuals varying in their alar expanse were dissected and found to be highly conspecific.

SUBFAMILY : STATHMOPODINAE

Stathmopodinae Janse, 1917, *Check-List S. Afr. Lepid. Heterocera* : 190 (as Stathmopodidae).

Type genus : *Stathmopoda* Harrich-Schäffer, 1853, *Syst. Bearbeitung Schmett. Eur.*, **5**(14) key, 54; 1894, *ibidem*, 6: *Microlepid*; pl.9 figs 17-22, included in Fletcher 1929 within the Schreckensteiniidae.

Stathmopoda Herrich-Schäffer

Stathmopoda Herrich-Schäffer, 1853, *Syst. Bearbeitung Schmett. Eur.*, **5** : 14 (key), 54; 1894, *ibidem*, 6 : Microlepid. pl. 9 figs. 17-22.

Type-species : *Phalaena pedella* Linnaeus, 1761, *Fauna Suecica* (Edn 2): 367, by subsequent designation by Meyrick, 1914, in Wytsman, *Genera Insect.*, **165** : 10.

Vertex and frons decorated with semi-erect scales; labial palpus long, upturned, second segment small, scaled, third segment long and acute; antenna long, filiform, longer than 3/4th length of forewing; forewing with veins R_1, R_2, R_3 free, R_4+R_5 stalked, R_5 to costa, M_1 free, straight, CuA_1 and CuA_2 free; hindwing with $Rs+M_1$ stalked, Rs to costa, M_1 to termen, M_2, M_3 free, CuA_1 and CuA_2 free, discal cell open; forewing pattern the line separating the yellow basal half of the wing from the brown distal half may be at almost any angle to costa from 90 to 45 degrees, the brown distal half of the wing may contain a more or less distinct diagonal yellow streak, divide the brown area; abdominal terga with spiniform setae on posterior margin of each segment; male genitalia with uncus long, apex pointed, socii absent; gnathos long arm, broader basally, narrowed at apex; tegumen long; vinculum U-shaped; saccus absent; valvae small and broad, cucullus semicircular, inner surface densely setose; aedeagus long and broad; vesica with cornutus; female genitalia with papillae anales small, sparsely setose; ductus bursae long; corpus bursae ovate or subovate shaped; ductus seminalis enter at middle of corpus bursae; signum present.

Remarks : The genus *Stathmopoda* Herrich-Schäffer has invariably been included in the families Schreckensteinidae, Stathmopodidae and Oecophoridae by Fletcher (1929), Gaedike (1967) and Nye and Fletcher (1991) respectively. The latter authors have kept it in the subfamily Stathmopodinae of the latter family (Robinson *et al.*, 1994) and this arrangement is currently followed in the present studies, as well. Two species i.e., *Stathmopoda balanarcha* Meyrick and *S. auriferella* (Walker) collected from the area, under reference, have been dealtwith taxonomically.

Key to the presently studied species of the genus *Stathomopoda* Herrich-Schäffer

1. Forewing with Sc ending at 1/3rd of

costa; male genitalia with sacculus rounded apically, cucullus wavy ventro-distally, aedeagus relatively broad, apically rounded, vesica beset with thin, plate-like cornutus; female genitalia with corpus bursae subovate in shape, ductus seminalis open near middle in corpus bursae.....***auriferella* Walker**

Forewing with Sc ending at middle of costa; male genitalia with sacculus pointed apically, cucullus convex ventro-distally, aedeagus narrowed, apex pointed, vesica beset with a rod-like broad cornutus; female genitalia with corpus bursae ovate in shape, ductus seminalis open in corpus bursae near ductus bursae.....***balanarcha* Meyrick**

***Stathmopoda auriferella* (Walker)**

Gelichia auriferella Walker, 1864, *List Specimens Lepid. Insects Colln Br. Mus.*, **30**: 1022.

Male genitalia (Plate 5, Figs. A-C) : Uncus long, basally broad, apex pointed, sparsely setosed; socii absent; gnathos long, tapering towards apex, the latter rounded; tegumen long and broad; vinculum U-shaped, thin; not produced into saccus anteriorly; juxta small, weak; valvae symmetrical, small and broad, inner surface densely setose, costal margin narrowed at base then convex, sacculus long, almost straight margin, apically rounded, cucullus semicircular, convex dorso-distally, apex rounded, wavy ventro-distally, basally narrowed; aedeagus long and relatively broader, longer than the length of valvae, moderately sclerotized, straight, apex rounded, ductus ejaculatorius enter midway; vesica with cornutus thin, long, plate-like.

Female genitalia (Plate 5, Fig. D) : Papillae anales small, circular, sparsely setosed; anterior apophyses small and thin, posterior apophyses very long and thin, much longer than anterior apophyses; ostium bursae reduced, centrally placed; ductus bursae narrow, broader near corpus bursae; ductus seminalis open in the corpus bursae almost at middle; corpus bursae small, subovate; signum curved, long, plate-like, moderately sclerotized.

Alar expanse : Male and female : 9-10 mm.

Material examined :

Himachal Pradesh : Dist. Solan, UHF Nauni, 1360m, 10.ix.1998, 2♂♂; Dharampur, 1500m, 16.ix.1999, 2♂♂, 2♀♀ : Dist. Mandi, Tanyhar, 1120m, 17.vii.1999, 2♂♂.

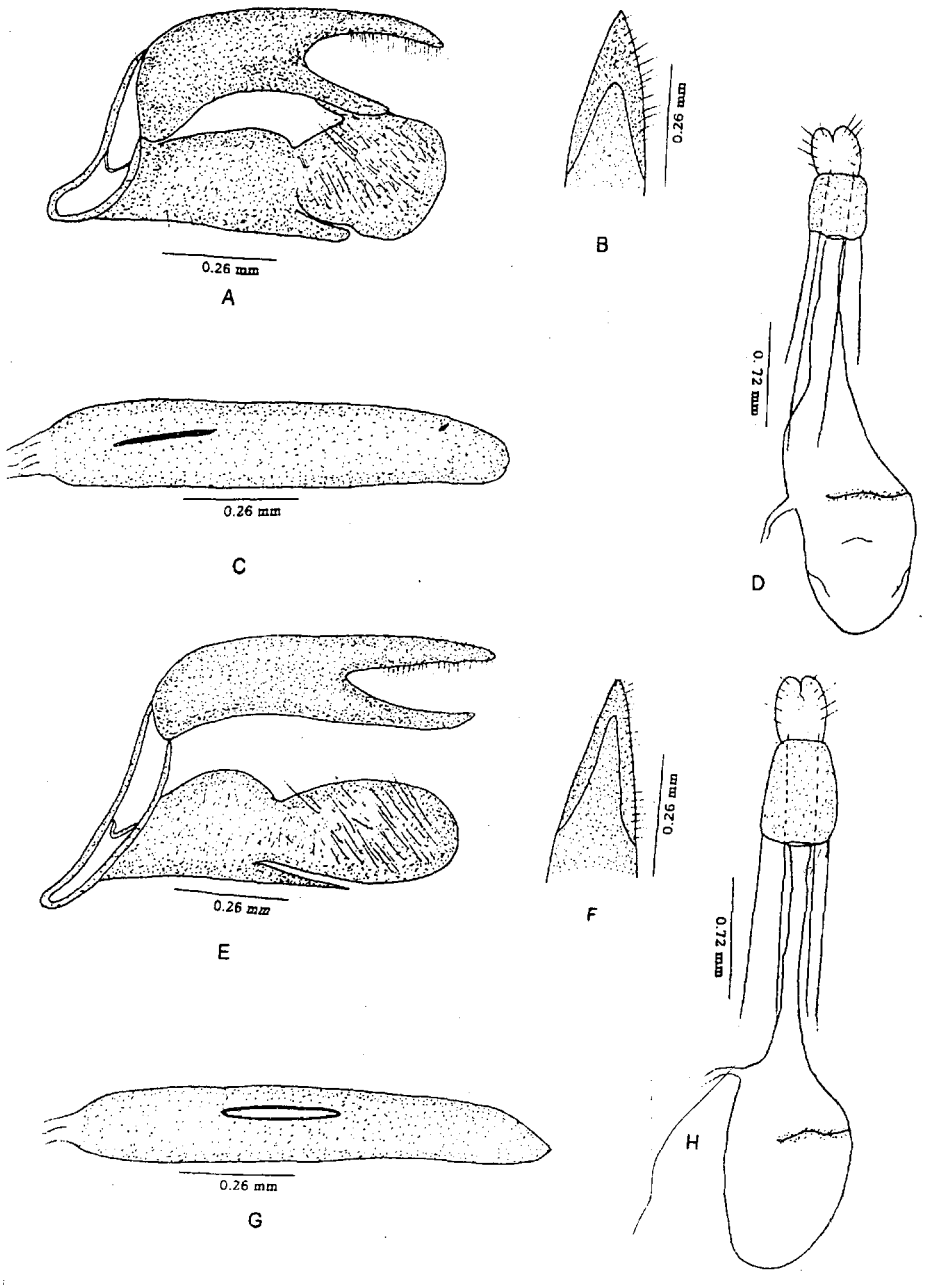
Punjab : Dist. Ropar, YH Ropar 350m, 15.vi.2000, 1♀. (H.S. Rose & P.C. Pathania coll.)

Distribution : W. Africa to Australia, Thailand, W. Malaysia, Java, Philippines (Robinson *et al.*, 1994).

Larval host plant : *Cocos nucifera, Albizia altissiunum* (Robinson *et al.*, 1994).

Remarks : A sample comprising nine individuals (six males and three females) collected between an elevation range of 350m to 1500m has been identified as *Stathmopoda auriferella* (Walker) from Robinson *et al.* (1994).

PLATE - 5



Stathmopoda auriferella (Walker) : Figures (A) *Male genitalia : lateral view,* ***(B)*** *Uncus,* ***(C)*** *Aedeagus,* ***(D)*** *Female genitalia : ventral view;* ***Stathmopoda balanarcha Meyrick : (E)*** *Male genitalia : lateral view,* ***(F)*** *Uncus,* ***(G)*** *Aedeaugs,* ***(H)*** *Female genitalia : ventral view.*

It has been observed that the forewing pattern of this species varies considerably. In fact, the line separating the yellow basal half of the wing from the brown distal half may be at any angle to costa from 90 to 45 degrees. One male specimen collected from each locality viz., Nauni, Dharampur, Tanyhar, Ropar were dissected and their genitalia examined with great precision. All these variable individuals were found to be highly conspecific genitalically.The species is being reported for the first time from India.

Stathmopoda balanarcha Meyrick

Stathmopoda balanarcha Meyrick,1916-1923, *Exot. Microlepid.*, **2**: 461.

Male genitalia (Plate 5, Figs. E-G) : Uncus long, broader proximally, pointed distally, sparsely setosed; socii absent; gnathos long, narrowed gradually to apex, the latter pointed; tegumen long and broad; vinculum U-shaped, thin; saccus lacking; juxta absent; valvae symmetrical small and broad, inner surface densely setose, costa narrowed at base, strongly convex, sacculus long with pointed apex, almost straight margin, cucullus semicircular, convex dorso-distally, apex rounded, ventro-distally convex, narrowed basally; aedeagus long and narrow, longer than the length of valvae, moderately sclerotized, almost straight, apex pointed, ductus ejaculatorius centrally placed; vesica with cornutus long, rod-like.

Female genitalia (Plate 5, Fig. H) : Papillae anales small, fringed with a few setae; anterior apophyses somewhat long and thin, shorter than posterior apophyses; ostium bursae small, centrally placed; ductus bursae somewhat long, broader near corpus bursae and near ostium bursae; corpus bursae small, ovate in shape; ductus seminalis open in corpus bursae near ductus bursae; signum long, thin, plate-like, moderately sclerotized.

Alar expanse : Male and female : 11 mm.

Material examined :

Punjab : Dist. Patiala, PUP, 250m, 24.iii.1998, 2♂♂; 2.iv.1998, 1♂; 5.ix.1998, 1♂; 15.ix.1998, 1♀; 26.ix.1999, 1♂; 5.x.1999, 1♀; 7.x.1999, 1♂; 24.x.1999, 1♀; 4.v.2000, 1♂. (H.S. Rose & P.C. Pathania coll.)

Distribution : Assam, Shillong (Meyrick, 1916-1923).

Larval host plant : Unknown

Remarks : The species is being reported for the first time from North-Western Siwaliks.

SUBFAMILY : OECOPHORINAE

Oecophorinae Bruand, 1850, *Mem. Soc. Emul. Doubs* (1) 3 (5-6): 45 (as Oecophoridae).

Type-genus: *Oecophora* Latreille [1796], *Précis Caractéres genériques Insectes* : 146.

Key to the genera of the subfamily Oecophorinae

1. Labial palpus three segmented; forewing with a sinuous costa and

scattered tufts of raised scales; male genitalia with gnathos not well developed in respect of ventral plate.....***Tonica* Walker**

Labial palpus two segmented; forewing without sinuous costa and scattered tufts of raised scales; male genitalia with gnathos well developed in respect of ventral plate.....***Periacma* Meyrick**

Tonica Walker

Tonica Walker, 1864, *List Specimens lepid. Insects Colln Br. Mus.*, **29** : 788.

Type-species : *Tonica terasella* Walker, 1864, *ibidem,* **29** : 788, by monotypy.

Vertex and frons studded with semi-erect scales; labial palpus small, upturned, second segment small, scaled; third segment long; antenna long, filiform, about 3/4th length of forewing;abdominal terga without spiniform setae; legs with erect scales on hind tibia; forewings with a sinuous costa and scattered tufts of raised scales, vein R_2 absent, R_3+R_4+R_5 stalked, arising from a common stalk, R_5 arising at the stalking of R_4+R_5, R_4 to costa, R_5 to termen, M_2 absent, M_3+CuA_1 stalked; hindwing with Rs+M_1 stalked, Rs to costa, M_1 to termen, M_2 absent, M_3+CuA_1 stalked; male genitalia characteristic, uncus long; socii absent; gnathos present; vinculum U-shaped; saccus absent; valvae small and broad; sacculus prominent, sclerotized, dorsomedially with Y-shaped process; aedeagus broad, sclerotized, vesica with cornutus; female genitalia characteristic, papillae anales large, setose; corpus bursae large, irregular in shape, sclerotized; signum present.

Remarks : During the course of present studies, two species i.e., *zizyphi* Stainton and *niviferana* Walker referable to the genus *Tonica* Walker have been identified from the Natural History Museum, London. As such, the genus is represented by twenty species on global basis, out of which 10+ have been reported from South-East Asia, including the presently dealtwith species (Robinson *et al.*, 1994).

Key to the presently studied species of the genus *Tonica* Walker

1. Forewing with a black spot near base, one black streak and a small triangular spot present near middle of costa, vein Sc join by a bar at 3/4th with discal cell, CuP visible at anal margin; male genitalia with gnathos small, sacculus with pointed apex exceeding beyond each valva, coecum absent.....***niviferana* Walker**

– Forewing maculation not as above, with only one black spot present near base of costa, vein Sc join by a bar at 4/5th with discal cell, CuP obscure; male genitalia with gnathos large, sacculus with apex not exceeding beyond each valva, coecum present.....***zizyphi* Stainton**

Tonica niviferana (Walker)

Binsitta niviferana Walker, 1864, *List Specimens Lepid. Insects Colln Br. Mus.*, **29** : 832.

Male genitalia (Plate 6, Figs. A-B) : Uncus long, moderately sclerotized, narrowed basally, broader at middle, apex concave; socii absent; gnathos reduced, weakly sclerotized, ventral plate not developed; tegumen small, broad, moderately sclerotized; vinculum small, U-shaped, narrowed; saccus absent; valvae symmetrical, large and broad, costal margin concave, sacculus distinct, strongly sclerotized, long, apically pointed, apex exceeding upto cucullus distally, wavy dorsally, dorso-medially produced into a Y-shaped process, one apex pointed join costa, convex ventrally, cucullus inner surface densely setosed, convex dorso-distally, apex rounded; aedeagus small and broad, smaller than the length of valvae, sclerotized, broader distally and basally, narrowed at middle, ductus ejaculatorius centrally placed; vesica beset with a rod-like cornutus.

Female genitalia : Not studied.

Alar expanse : Male : 26 mm.

Material examined :

Himachal Pradesh : Dist. Kangra, NRHH Andhretta, 900m, 26.vi.1999, 4♂♂. (H.S. Rose & P.C. Pathania coll.)

Distribution : Dehra Dun, Pusa (Roonwal *et al.*,1964).

Larval host plant : *Bombax malabaricum* (Fletcher, 1921).

Remarks : *Tonica niviferana* (Walker) is being reported from the Western Himalaya for the second time from Kangra as it has earlier been reported from Dehra Dun (Roonwal *et al.*, 1964).

Tonica zizyphi (Stainton)

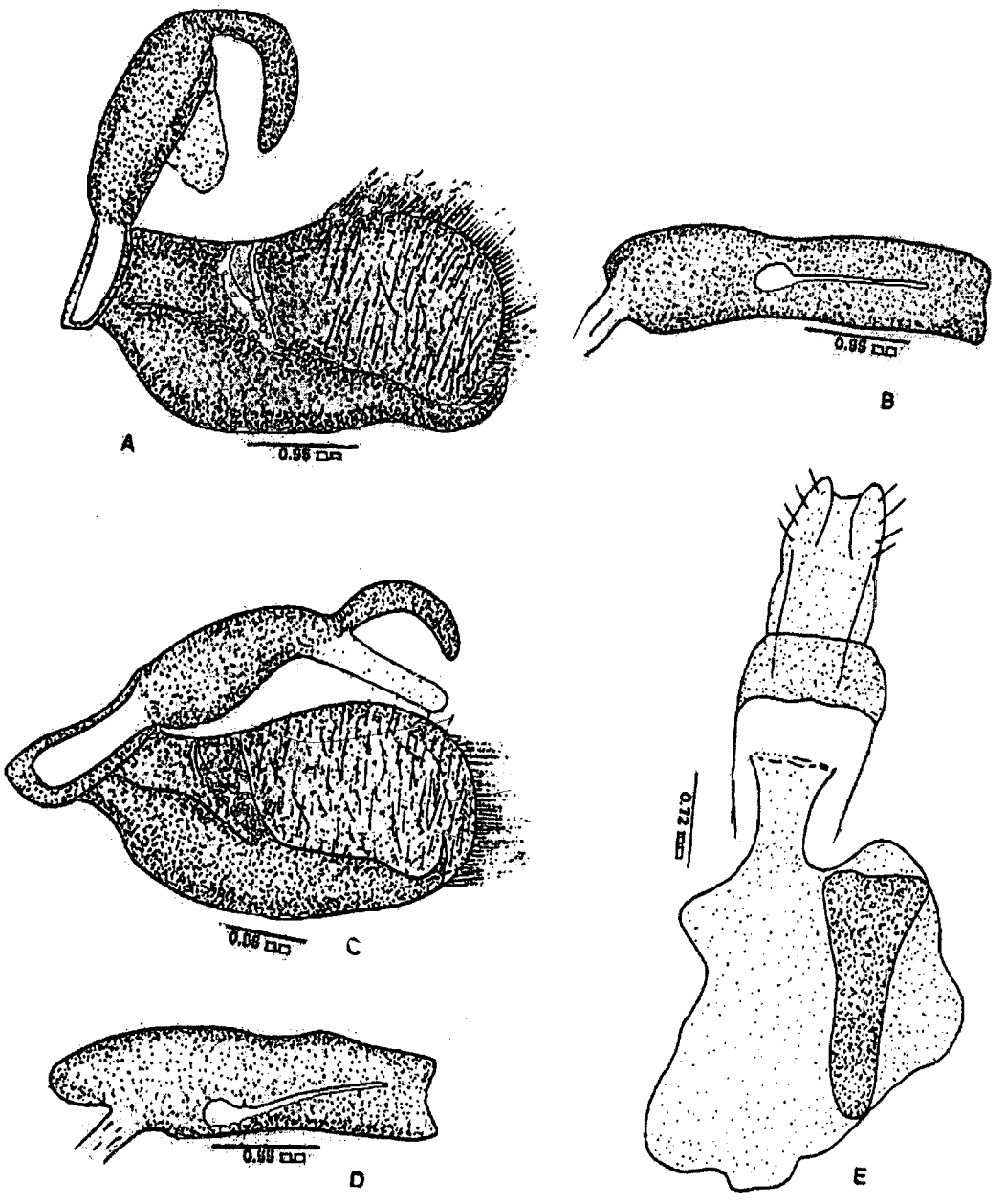
Depressaria zizyphi Stainton, 1859, *Trans. ent. Soc. Lond.*, (2) **5**: 115.

Male genitalia (Plate 6, Figs. C-D) : Uncus as in *niviferana* Walker, socii not developed, gnathos broad and large, apically rounded; tegumen small, distally narrowed, slightly better sclerotized; vinculum U-shaped, small, narrowed, thin; saccus absent; valvae symmetrical, somewhat large and broad, costal margin slightly concave, sacculus very conspicuous, strongly sclerotized, long, apically narrowed, not exceeding valva, cucullus inner margin densely setosed, dorso-distally convex, apex rounded, slightly concave dorso-medially, with a Y-shaped process joining costa; aedeagus small broad, moderately sclerotized, broader at base and distally, narrowed at middle, ductus ejaculatorius enter sideways, coecum small, rounded apically; vesica lacking cornutus.

Female genitalia (Plate 6, Fig. E) : Papillae anales large, broad, somewhat rounded, sparsely setosed; anterior and posterior apophyses small, almost equal in length; ostium bursae broad, centrally located; ductus bursae very reduced, broad, weakly sclerotized; corpus bursae large, irregular, heavily sclerotized; signum very large, broader at base, narrowed gradually.

Alar expanse : Male and female : 27-30 mm.

PLATE - 6



***Tonica niviferana (Walker) : Figures (A)** Male genitalia : lateral view, **(B)** Aedeagus; **Tonica zizyphi (Stainton) : (C)** Male genitalia : lateral view, **(D)** Aedeagus, **(E)** Female genitalia : ventral view.*

Material examined :

Himachal Pradesh : Dist. Sirmour, Renuka Lake, 740m, 4.ix.1999, 2♂♂, 4♀♀; 4.x.1999, 2♂♂; 6.x.1999, 2♂♂; 4.xii.1999, 1♀: Dist. Solan, UHF Nauni, 1360m, 12.ix.1999, 2♂♂: Dist. Kangra, Dharamshala, 1200m, 17.vi.1998, 1♀.

Punjab : Dist. Ropar, YH Ropar 350m, 21.x.1999, 1♀. (H.S. Rose & P.C. Pathania coll.)

Distribution : India, Ceylon (Fletcher, 1921).

Larval host plant : *Citrus, Zizyphus* and *Murraya* (Fletcher, 1921).

Remarks : *Tonica zizyphi* (Stainton) has been reported from India and Ceylon without any precise distribution record about the localities (Fletcher, 1921). Under these circumstances, it is not possible to say anything as to whether the present collection from the area is an additional or new record.

Periacma Meyrick

Periacma Meyrick, 1894, *Trans. ent. Soc. Lond.*, : 21.

Type-species : *Periacma ferialis* Meyrick, 1894, *ibidem*, : 21, by original designation.

Vertex and frons decorated with semi-erect scales; labial palpus two segmented, upturned, second segment exceptionally long and acute; antenna long, filiform, longer than 3/4th length of forewing; legs with semi-erect scales on tibia; forewing with veins R_4+R_5 stalked, R_5 to termen or apex, M_1 and M_2 free, CuA_1 and CuA_2 free; hindwing with Rs to costa, M_1 and M_2 free, M_3 and CuA_1 connate, 1A+2A forked at base; male genitalia with uncus large, broader apically, narrowed basally, sclerotized; socii absent; gnathos with well developed ventral plate, with minute spines; tegumen small or broad, saccus absent; valvae with costa distinct, sclerotized, sacculus prominent with processes distally, cucullus with long, dense setae; aedeagus small, with spine apically, coecum present, vesica without cornutus; female genitalia characteristic, papillae anales large, setose; ostium bursae broad, centrally placed; ductus bursae small; corpus bursae large; signum present.

Remarks : The Oecophorid genus, *Periacma* Meyrick proposed on the basis of the type-species *P. ferialis* Meyrick by Meyrick (1894) is unique and exceptionally one in having two segmented labial palpus in the male and it is mainly distributed in the Oriental region (Moriuti *et al.*, 1985; Ueda and Moriuti, 1996). The latter workers, while reporting three new species i.e., *Periacma harutai, P. nepalensis* and *P. himalayensis* have stated that it contains, as many as, thirty-nine species from different localities of the Oriental region. In India, seven species i.e., *Periacma selenota* (Nilgiri Hills), *P. teraturga* Meyrick (N. Coorg., Nilgiri Hills), *P. plumbea* Meyrick (Kumaon), *P. continuata* Meyrick (Palni), *P. eparphna* Meyrick (Assam), *P.deltophanta* Meyrick (Madras) and *P. circumclusa* Meyrick (Assam) have been reported in this genus from the respective locality/ies mentioned in brackets (Meyrick, 1912-1916, 1916-1923, 1923-1930,

1930-1936). In view of this distributional record it becomes obvious that none of the species has, so far, been recorded from the Siwaliks in North-West India, except one i.e., *P. plumbea* Meyrick from Kumaon in the Western Himalaya. The presently collected phenon comprising closely allied individuals has been sorted out into two species i.e., *P. circumclusa* Meyrick and *P. continuata* Meyrick. Like bisegmented palpus, it has been observed that the gnathos is also quite conspicuous in having a well developed ventral plate beset with minute spines in the male genitalia. Also, the valvae are distinctly differentiated into three regions i.e., the costa, the sacculus and the cucullus. In this regard, the presently treated species broadly conform to the male genitalia of some other species such as *Periacma harutai, P. napalensis* and *P. himalayaensis* which have been reported as new from Nepal by Ueda and Moriuti (1996). While reporting this genus as new to the fauna of Nepal, the latter authors could not get sufficient material as the latter species has been named and described on the basis of a lone male specimen.In the current work, though the congeneric nature of the presently studied two species could not be made out on the basis of their female genitalia, as the same has been examined only for one species i.e., *P. circumclusa* Meyrick, yet it can be safely stated that it broadly conforms to that of *P. himalayanesis* Ueda and Moriuti (Ueda and Moriuti, 1996). Both the species i.e., *P. himalayanesis and P. continuata* are closer to each other in view of structures such as the lamella antevaginalis, the ductus bursae and the ductus seminalis which originates 1/3rd posteriorly from the ductus bursae in the female genitalia.

Key to the presently studied species of the genus *Periacma* Meyrick

1. Forewing with dark fuscous band beyond half of costa join tornus, vein R_1 arising at middle of discal cell; hindwing with vein CuP visible near anal margin; male genitalia with valva with saccular margin broad, with two processes apically, costa concave, aedeagus apex with one spine-like process distally, coecum long..... ***circumclusa* Meyrick**

– Forewing with dark fuscous band near half of costa join tornus, vein R_1 arising at 1/3rd of discal cell; hindwing with vein CuP visible in distal half; male genitalia with valva with saccular margin narrow, beset with three processes, costa slightly convex, aedeagus apex with two spine-like processes distally, one another near middle, coecum relatively smaller..... ***continuata* Meyrick**

***Periacma circumclusa* Meyrick**

Periacma circumclusa Meyrick, 1916-1923, *Exot. Microlepid.*, **2**: 546.

Male genitalia (Plate 7, Figs. C-D) : Uncus large, broader and rounded apically, narrowed at base, moderately sclerotized; socii absent; gnathos with a well developed ventral plate, beset with many minutes spines; tegumen broad and small, with two sparsely setosed, long lobe at base, each lobe rounded distally;

vinculum circular, thin; saccus indistinguishable; juxta broad, plate-like; valvae symmetrical, long and broad, costa distinct, elongate, moderately sclerotized, slightly concave, sacculus prominent, well sclerotized, dorso-proximally convex, dorso-distally with two processes, one with rounded and other pointed apex, convex ventro-proximally, cucullus with long dense setae on inner surface, almost straight dorso-distally, rounded at apex, ventro-distally convex; aedeagus small, thin, moderately sclerotized, stout, slightly curved at 3/4th from base, with small thumb-like structure near ductus ejaculatorius, apex with two processes, the latter with one rounded and other pointed apex, coecum well developed, long; vesica lacking cornutus.

Female genitalia (Plate 7, Fig. E) : Papillae anales large, somewhat rounded, sparsely setosed; anterior apophyses small and thin, posterior apophyses long and thin, longer than anterior apophyses; ostium bursae broad, centrally placed; ductus bursae comparatively smaller, broad, weakly sclerotized; corpus bursae large, narrowed near ductus bursae, broader at base; signum somewhat hook-like, broader at base, pointed apex.

Alar expanse : Male and female : 16-18 mm.

Material examined :

Himachal Pradesh : Dist. Mandi, Tanyhar, 1120m, 16.vii.1999, 8♂♂.

Punjab : Dist. Gurdaspur, FRH Dhar, 700m, 23.x.2001, 4♀♀. (H.S. Rose & P.C. Pathania coll.)

Distribution : Assam, Sadiya (Meyrick, 1916-1923).

Larval host plant : Unknown

Remarks: The species *Periacma circumclusa* Meyrick is being reported for the first time from North-West India.

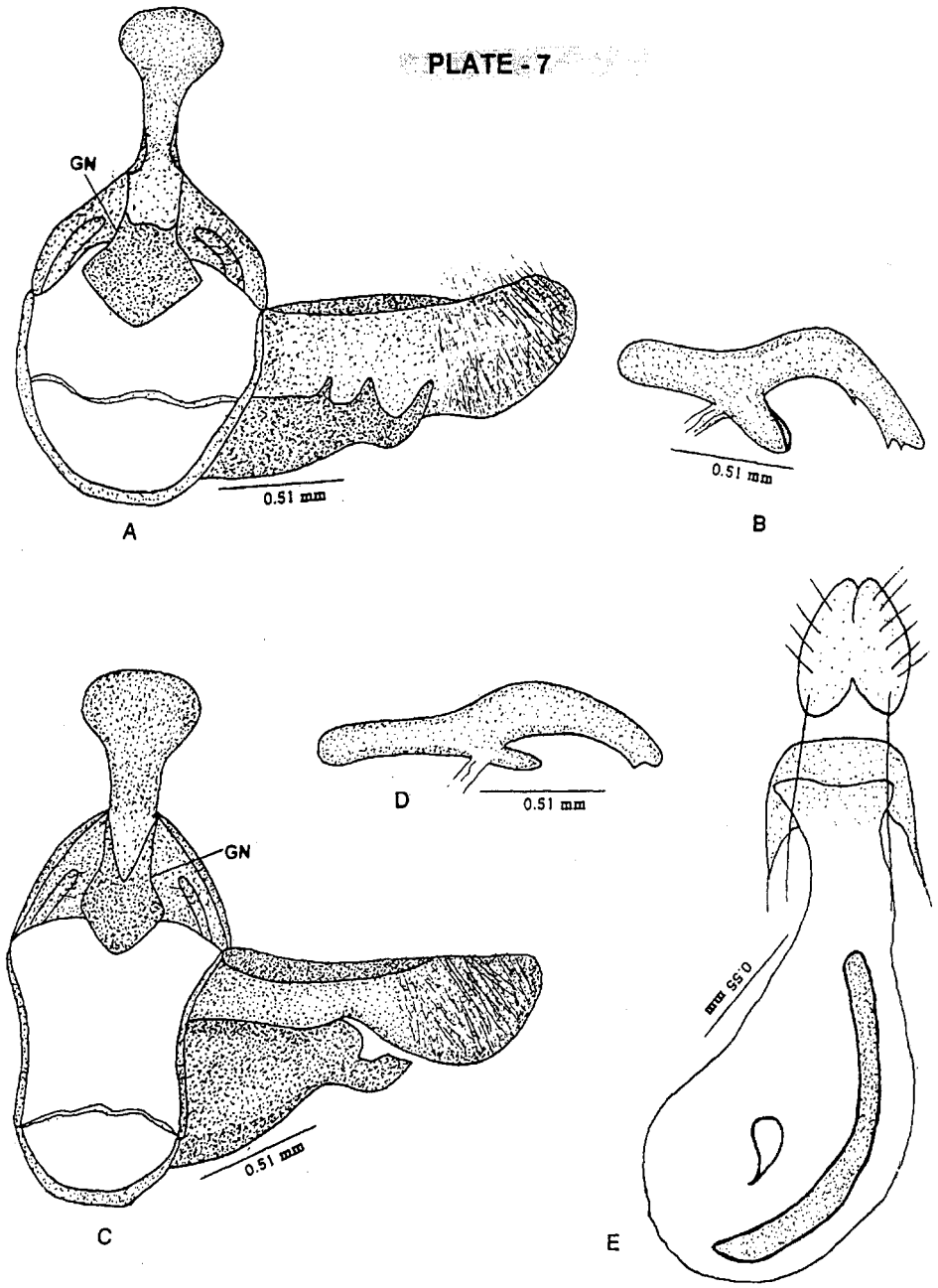
***Periacma continuata* Meyrick**

Periacma continuata Meyrick, 1916-1923, *Exot. Microlepid.*, **2** : 546.

Male genitalia (Plate 7, Figs. A-B) : Uncus large, broader distally, narrowed at base, rounded distally, moderately sclerotized; socii absent; gnathos with a well-developed ventral plate, with many minute spines; tegumen broad and small, with two elongate, sparsely setose lobe at base, rounded distally; vinculum rounded, thin; saccus absent; juxta broad, plate-like; valvae symmetrical, long and broad, costa distinct, elongate, straight, weakly sclerotized; sacculus prominent, dorso-proximally convex, with three processes distally, two small equal, pointed apex, triangular in shape, third rounded apex, long, ventro proximally convex, cucullus inner surface densely setose, slightly concave dorso-distally, apex rounded, ventro-distally convex; aedeagus small, smaller than the length of valvae, thin, stout, slightly bent at 3/4th from base, with large thumb-like structure near ductus ejaculatorius, apex with three processes, one rounded and two with pointed apices, coecum developed, small, apex rounded; vesica without cornutus.

Female genitalia : Not studied.

Alar expanse : Male : 16-17 mm.



Periacma continuata Meyrick : Figures (A) *Male genitalia : ventral view,* ***(B)*** *Aedeagus;* ***Periacma circumclusa Meyrick : (C)*** *Male genitalia : ventral view,* ***(D)*** *Aedeagus,* ***(E)*** *Female genitalia : ventral view.*

Material examined :

Himachal Pradesh : Dist. Kangra, KV Palampur, 700m, 24.vi.1999, 2♂♂; 27.v.2001, 2♂♂; NRHH Andhretta, 900m, 27.vi.1999, 2♂♂. (H.S. Rose & P.C. Pathania coll.)

Distribution : South India, Palni Hills (Meyrick, 1916-1923).

Larval host plant : Unknown

Remarks : The present distributional record of *Periacma continuata* Meyrick from Palampur (700m) and Andhretta (900m) in the Western Himalaya in Himachal Pradesh is an additional one, as it has only been reported from Palni Hills in South India from the Western Ghats (Meyrick, 1916-1923). The dissection of two and one males from the respective locality prove that they are conspecific to each other.

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ABBREVIATIONS

1A+2A : Vein representing fused first and second anal veins, 3A : Third anal vein, AED : Aedeagus, ANT.APO : Anterior apophyses, CL : Cucullus, CO : Costa, Comb. nov. : New combination, CORP.BU : Corpus bursae, CuA_1 : First anterior cubital vein, CuA_2 : Second anterior cubital vein, CuP : Posterior cubital vein, DU.BU : Ductus bursae, FRH : Forest Rest House, FRI : Forest Research Institute, Dehra Dun, KV : Krishi Vishavidyalya, M_1 : First median vein, M_2 : Second median vein, M_3 : Third median vein, NRHH : Norha Richard Holiday Home, OB : Ostium bursae, PA : Papillae anales, PO.APO : Posterior apophyses, PUP : Punjabi University, Patiala, R_1 : First radial vein, R_2 : Second radial vein, R_3 : Third radial vein, R_4 : Fourth radial vein, R_5 : Fifth radial vein, Rs : Radial sector, Sc : Subcostal vein, $Sc+R_1$: Stalk of subcostal and first radial vein, SIG : Signum, SL : Sacculus, sp. nov. : New species, TEG : Tegumen, UHF : University of Horticulture and Forestry, UN : Uncus, YH : Youth Hostel.

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FIRST EVIDENCE OF AUTHENTICITY OF PECTORAL FIN RAY SECTIONS FOR AGE DETERMINATION OF EXOTIC CARP, *HYPOPHTHALMICHTHYS MOLITRIX* (VAL.1844)

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Abstract

For the first time the authenticity of annual marks on the sections of pectoral fin ray of silver carp, Hypophthalmichthys molitrix (Val. 1844) from Gobindsagar, Himachal Pradesh, India has been ascertained for age determination and back-calculated lengths to calculate the various growth parameters. The methodology for the preparation of sections of pectoral fin ray has been described. It is opined that this hard part of this exotic carp should be tried in other commercial carps too as its removal will not lower the market value of the fish.

Key words: Age determination, back-calculated lengths, *Hypophthalmichthys molitrix*, pectoral fin ray.

INTRODUCTION

The determination of age and various growth parameters occupy key position in fish biology of commercial fish species (Tandon and Johal, 1996). These investigations are useful for the determination of growth rate of same fish species from the different types of water bodies and different fish species from the same water body and provide information on subdaily, daily and annual growth rates, size at first maturity, harvestable size, mortality and survival rates, spawning season(s), calculation of back-calculated lengths in different age groups, migration, onset of old age, taxonomic position below species level, pollution, discriminating between hatchery reared and wild populations and to ascertain the past living conditions of fish through elemental analysis (Sawhney and Johal, 1999; Tandon and Johal, 1996).

Several hard parts such as scales, cleithra, post-cleithrum, urohyal bones, opercular bones, otoliths are in vogue for age determination in various fish species (Johal *et al.*, 2000; 2000; 2001; Tandon and Johal, 1996) The use of sections of pelvic and pectoral fin rays for age determination is common in cat fishes (Ashley and Garling, 1980; Beamish, 1981; Clay, 1982; Ezenwa and Ikusemiju, 1981; Pantulu, 1961), cartilaginous fishes, cod (Beamish and Chilton, 1977; Westrheim and Shaw, 1982), eel , salmons (Shirvell, 1981), pike fishes , walleye (Belanger and Hogler, 1982), sturgeon ,tuna ,ground fish and roach (Cass

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and Beamish, 1983) but not in commercial carps. It is opined that in some fishes, especially the cat fishes, the sections of fin rays are considered to be superior than other hard structures for age determination (Ashley and Garling, 1980; Cass and Beamish, 1983; Ezenwa and Ikusemiju, 1981; Beamish and Chilton, 1977; Belanger and Hogler, 1982; Clay, 1982; Gulland, 1958; Johal *et al.*, 2000; 2000; 2001; Pantulu, 1961; 1962; Quinn and Ross, 1982; Rao, 1993; Westrheim and Shaw, 1982).

For the first time an attempt has been made to use the sections of pectoral fin rays of silver carp, *Hypophthalmichthys molitrix* (Val. 1844) from Gobindsagar for the age determination and for the calculations of back-calculated lengths. The present paper deals with the methodology regarding the processing of pectoral fin rays for age determination and the measurements of the annual marks for ageing and back-calculations.

MATERIALS AND METHODS

The specimens of silver carp *Hypophthalmichthys molitrix* (Val. 1844) varying between the size range of 235-1000 mm total fish length were collected monthly from the commercial catches during August 1998-May 2000 (except close season during the months of June and July each year). The specimens were brought to the laboratory for further studies. In the laboratory, the first pectoral fin ray of each specimen was removed from the base with the help scissor. In some cases the entire pectoral fin was cut from the base, dipped in warm water (60-70°C) for five minutes to remove the muscles and the extraneous tissue present between the first and second pectoral fin rays. The cleaned and dried first pectoral fin was stored in ordinary envelope in such a way that they should remain erect with base downward. On the envelope the relevant data e.g., total length, standard length, weight, date of collection and sex were recorded so that after age determination the back-calculated lengths and various growth parameters could be calculated.

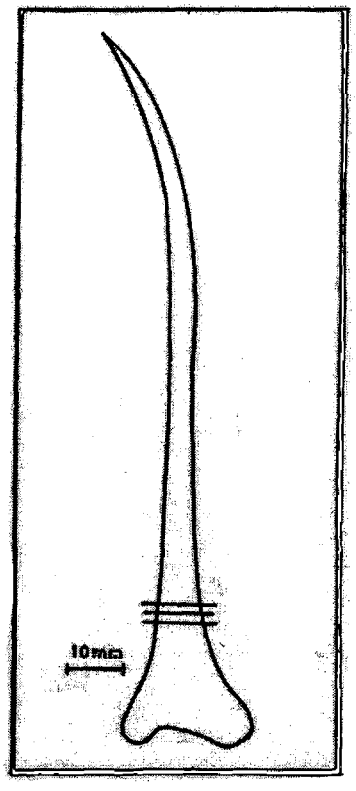

Figure 1 : *Schematic diagram of the first pectoral fin ray of silver carp, Hypophthalmichthys molitrix showing the position from where the sections were cut for age determination.*

Transverse sections having thickness varying between 0.3 – 0.5 mm starting from the base of the first pectoral fin ray (Fig. 1) were cut using jeweler's saw. Each section was grinded and polished using carborundum stone and fine ground glass plate of 12mm thickness using water or liquid paraffin as lubricant. The grinded and polished sections having the thickness of 0.2 – 0.3 mm were mounted on glass slides in DPX and observed under transmission light using Carl Zeiss DL 5.3 VEB Dokumator or Getner Stereobinocular Microscope at varying magnifications. For photography the sections mounted on the microslides were used as negatives.

RESULTS AND DISCUSSION

The first pectoral fin ray of silver carp consists of two closely jointed rays. Outwardly, they appear to be single ray because of their jointed heads. Study of thin sequential cross-sections obtained from near the base of these rays (Figs.2) under transmitted light showed that growth bands are distinct in the form of translucent and opaque zones. Translucent light (light) assumed to be indicative of slow growth and are separated by opaque zones (dark) which represent fast growth. A single translucent band and associated opaque band represent one year of growth of this fish and the single translucent band can be considered as an annulus. Sometime two translucent zones appear together. In this case only the complete or continuous one should be *considered as true annulus and the other one* is false. The false annulus is usually is less distinct than the true annulus and often is located irregularly. On observing the large number of sections of fin rays, it is easy to distinguish the true and false annuli.

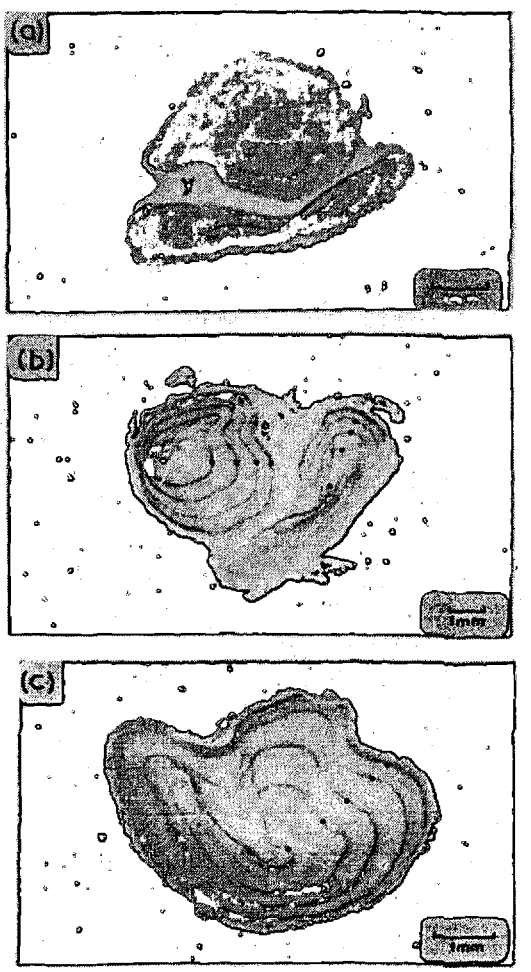

***Figure 2** : Transverse sections of first fin ray of silver carp, Hypophthalmichthys molitrix **(a)** total length - 568 mm; age - 3 years **(b)** total length - 765 mm; age - 5 years. (c) total fish length 824 mm, age – 5 years. The dot indicates the year or annular mark.*

The formation of true annulus is attributed to several factors such as low feeding intensity, spawning stress and various hydro-biological changes. As all these factors occur periodically, therefore, the formations of true annulus are also periodic. The false annulus is formed due to the cessation of growth as a result of disease, overpopulation and adverse environmental factors, which can occur at any time in a year, hence, the formation of false annulus is irregular. Before determining the age a clear concept regarding the true annulus must be made, otherwise, the age of the specimen may result in over or under estimation of age and incorrect back-calculated lengths will be made.

There are some advantages with the use of fin rays for age determination and growth studies. One obvious reason is that the use of fin ray does not require the sacrificing of the fish and their removal does not lower the market value of the fish. Moreover, removing, cleaning and storage of fin rays are much easier than the other hard parts such as cleithra, otoliths, opercular bones and urohyal bones.

The back-calculation from the section of the pectoral fin rays of silver carp, *H.molitrix* can be made using the following formula:-

$$Ln = \frac{An - An}{C1 - C1} \quad L$$

Where:-

C1 –C1 = Maximum fin ray width and A1 –A1, A2 – A2, A3 –A3 *and* An-An (Fig.3) is the maximum distance between the respective annuli and L is the total length at the time of capture and the Ln is the back-calculated length at the time of formation of particular annulus.

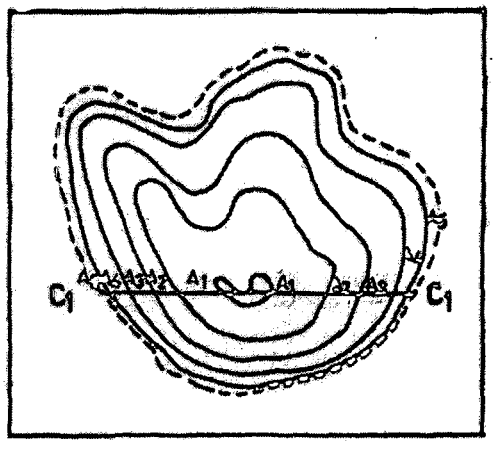

***Figure 3** : Schematic sketch of the first transverse section of the first pectoral fin ray of Hypophthalmichthys molitrix showing the annuli and method of measuring them.*

For the back-calculations, it is opined that only the measurements from the fin ray sections having the maximum width should be considered. For the purpose of confirmation of back-calculated length, it is suggested that these lengths should be confirmed with back-calculated lengths using scale or opercular or vertebral methods.

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RHYTHM IN EMERGENCE OF CERCARIAE OF *ISOPARORCHIS HYPSELOBAGRI* FROM THE SNAIL *INDOPLANORBIS EXUSTUS*

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Abstract

A diumal rhythmicity in the emergence of cercariae of Isoparorchis hypselobagri from its first intermediate host is determined. The variation was in the range of 02 to 90 per hour and exhibited variation with the host. Light and temperature were found to have a positive impact on a phenomenon. The cercarial generation, maturation and emergence were opined to began outcome of host-parasite compatibility.

Key words: Cercaria, *I.hypselobagri*, light, rhythmicity, temperature.

INTRODUCTION

The cercarial production is one of the determinants of the rate of infection of the snail host with the flukes. A few of the earlier authors (Erasmus, 1972; Theron, 1984) have reviewed this aspect in a number of digenean parasites. The physico-chemical and biological factors have been correlated with the cercarial output (Asch, 1972; Chu and Dawood, 1970; Coles, 1973; Combes and Theron, 1977; Das and Manna, 1993; Pages and Theron, 1990; Raymond and Probert, 1987; Shameem and Radhika, 2001; Theron, 1984; Touassem and Theron, 1989) Besides, the rate of release of cercariae and its periodicity in some other trematodes have also been a center of investigation (Ahn *et al.*,1989; Chen *et al.*, 1990; De Souza *et al.*, 1985; Kechemir and Theron, 1989; Loker, 1983; Matsumura *et al.*,1984; Mohandas, 1974; Niemann and Lewis, 1990; Shostak and Esch, 1990; Ward *et al.*, 1988; Xie *et al.*, 1990). But the species of the digenetic trematode parasite specifies the natality and rythmic pattern of emergence of cercariae. The present communication determines the diurnal and cercadian rhythm of cercarial birth in *I. Hypselobagri* (Billet 1898) which has not been demonstrated earlier and attempts a comparative account.

MATERIALS AND METHODS

The ten infected snails, *Indoplanorbis exustus*, were incubated at room temperature 27±1°C with light intensity 25±5 lux, individually in separate test tubes of 2.6 cm diameter, each with 20 ml of distilled water at pH 6.5. At an interval of one hr up to 24th hr of a day, each snail was transferred to a separate test tube in similar fresh condition. The cercariae which emerged from it after every hr were counted, fixed in 5% neutral formalin and preserved in 70% ethyl alcohol.

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The daily cercarial productivity from five snails of a separate group was accounted up to five days. The results were analysed statistically by 't' test at P 0.05 level.

RESULTS

Of ten infected snails studied, the respective range of cercariae released in every hr. of a day, their average values and the total number in all the hrs together (Fig. 1,2) were 19-86, 36.583± 3.508 and 878 in the 1st, 02-47, 11.583±1.940 and 278 in the 2nd, 01-45, 22.416±2.168 and 538 in the 3rd, 08-39, 19.375±1.556 and 465 in the 4th, 19-55, 32.58±2.125 and 782 in the 5th, 07-30, 20.166±1.558 and 484 in the 6th, 02-14, 7.416±0.650 and 178 in the 7th, 02-07, 3.583±0.365 and 86 in the 8th, 3-11, 6.958±0.431 and 167 in the 9th and 20-91 54.416±5.148 and 1306 in the 10th. The total number of cercariae released from 1st to 10th snails in 12 hrs light (6th-17th hr) and 12 hrs dark period (18th-5th hr) of a day were 437 and 441, 177 and 101, 311 and 277, 227 and 238, 412 and 370, 264 and 220, 88 and 90, 51 and 35, 98 and 69 and 661 and 645 respectively. Thus the average values of cercarial release per snail per hr was 21.466±1.230 and per snail per day was 516.200±114.360. In the 18th, 6th, 15th, 5th, 12th, 11th, 12th, 11th and 17th hrs of the day the maximum values of cercarial production were recorded from the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 9th and 10th snail respectively, while the 8th snail exhibited two peaks, one in the 6th and the other in the 17th hrs. The lowest values of cercarial production from 3rd, 4th, 6th 7th, 9th and 10th snails were obtained in 13th, 15th, 24th and 22nd hrs of a day respectively.

Each of the 1st, 2nd and 5th snails showed two periods of minimum cercarial output – 14th and 15th in the first two and 11th and 21st in the 3rd, whereas the minimum number of cercariae are released by the 8th snail in each of the six periods, 2nd, 12th, 18th, 19th 20th and 22nd hrs of the day. No linear relationship between the release of cercariae and the hrs of the day was exhibited.

The 't' value, for hourly emergence of cercariae from each of the ten infected snails, being higher than its corresponding table value estabilishes significant variation in the rate of cercarial production from hr to hr in a day. The 't' value, 17.452 for average number of cercariae released per hr from individual snails is higher than its corresponding table value, 1.960 as well as then any of its values in hourly cercarial emergence from ten individual snails. Thus the variation in the rate of release of cercariae in the hrs of the day depends more on the snail hosts than on the hrs of its production. The total number of cercariae produced in a day is also determined through 't' test to be related significantly with the individual host.

In the study of day to day cercarial emergence from five infected snails, of species, *I. exustus*, for 5 days, the results obtained were, 2280-835-1250-646-245 in the 1st, 192-344-33-162-26 in the 2nd, 2040-588-372-300-120 in the 3rd, 138-145-102-42-30 in the 4th and 70-70-18-18-03 in the 5th snail (Fig. 3). In the first snail, the number of obtained from the 1st day of testing was

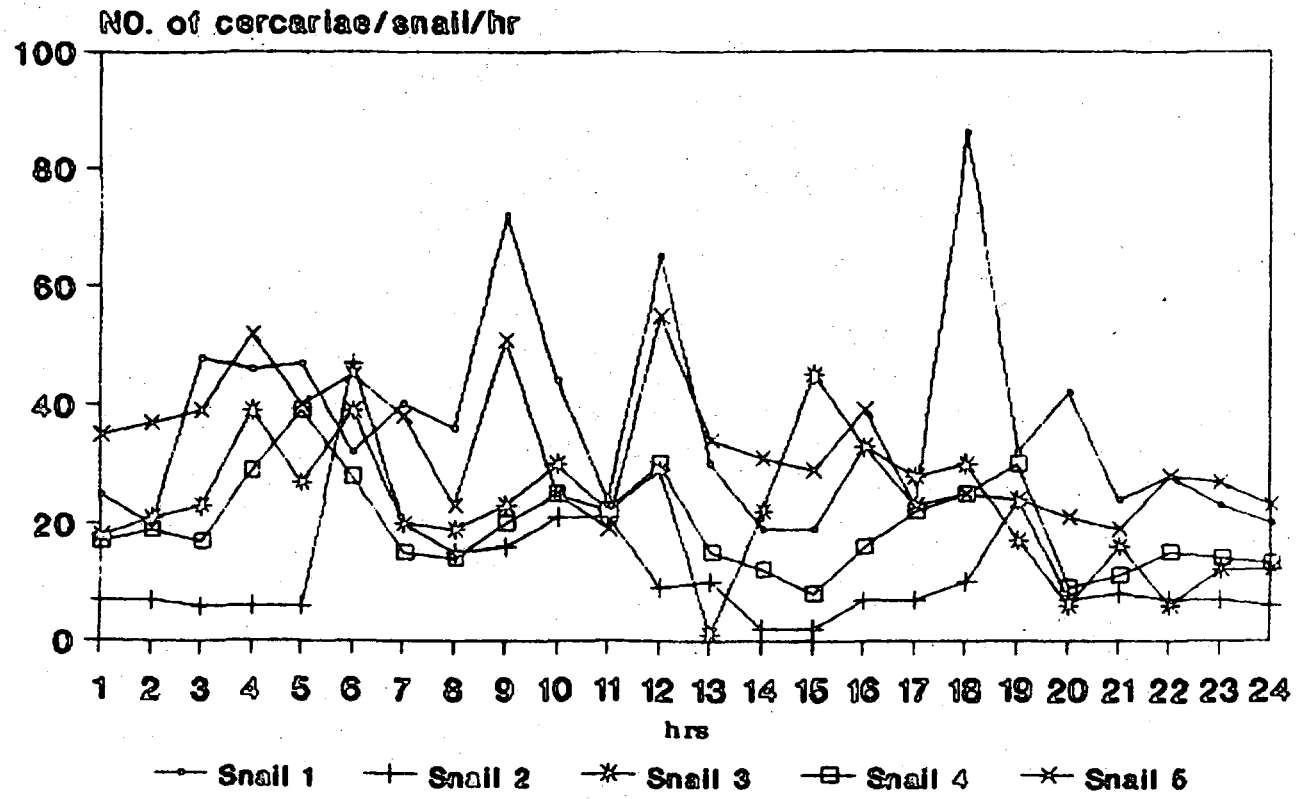

Figure 1 : Diurnal rhythm of cercarial emergence from I. exustus from the field in 1-5 snails.

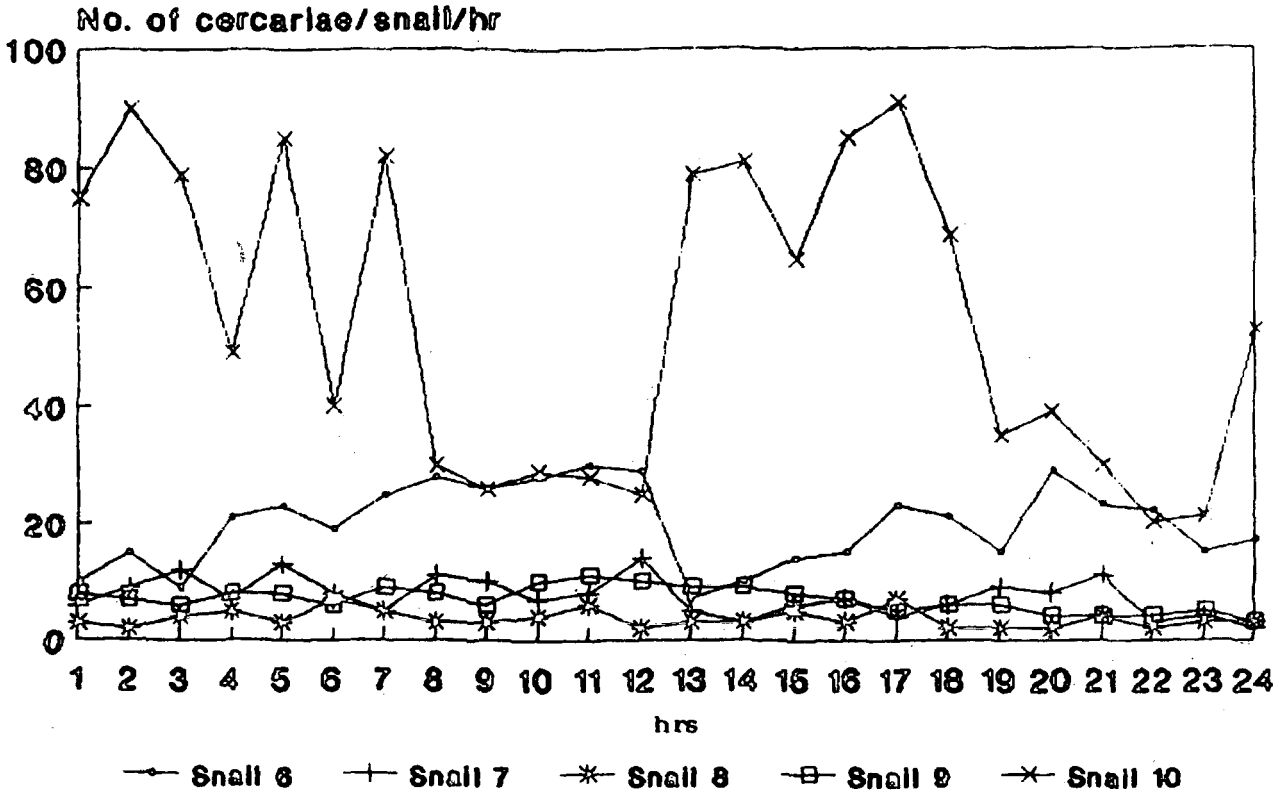

Figure 2 : *Diurnal rhythm of cercarial emergence from I. exustus from the field in 6-10 snails.*

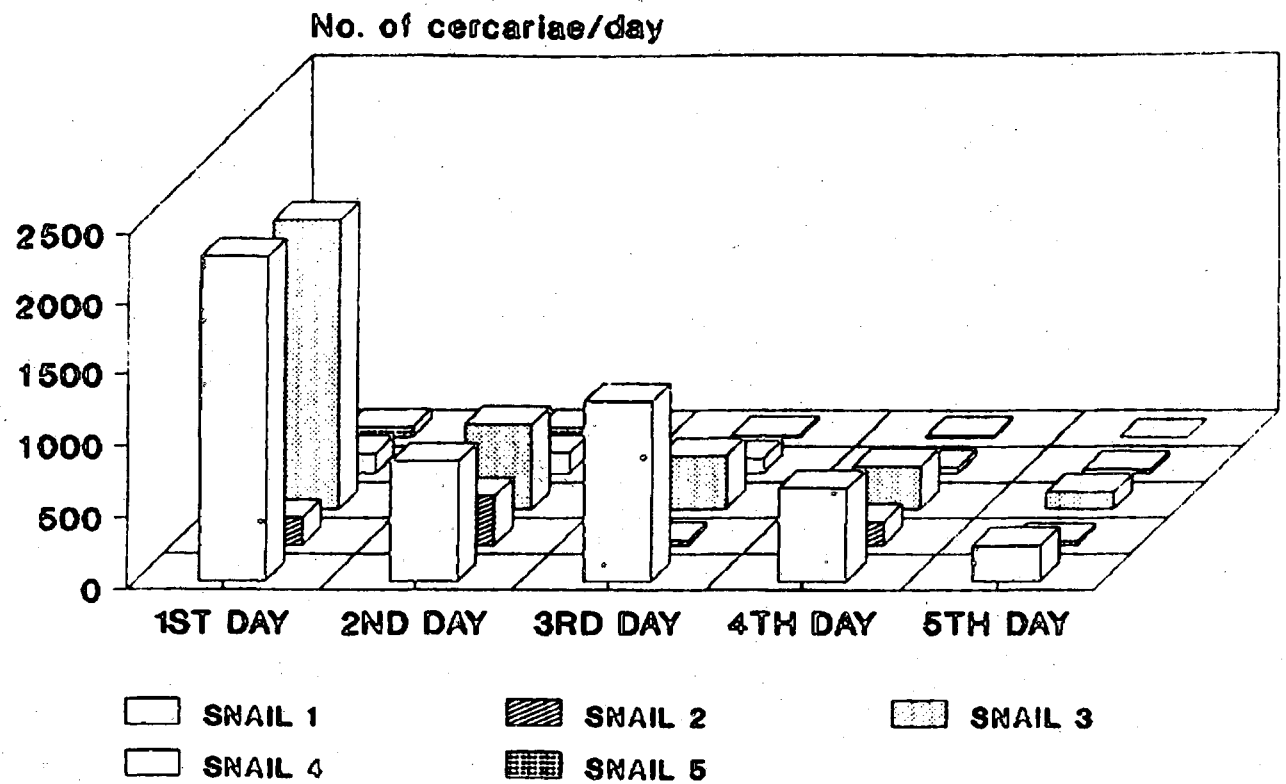

Figure 3: *Day to day cercarial emergence from I. exustus from the field.*

maximum, showed a sudden increase in the 3rd day from the 2nd and declined gradually. The cercarial production fluctuated with the alternate lower and higher values in the 2nd snail and comes down gradually in the 3rd. In the 4th snail, the number of maximum in the 2nd day and decreased thereafter. The 5th snail shows a steady decline in the cercarial output with the same values in the 1st and 2nd and 3rd and 4th days. The average numbers of cercariae released per day were 1051.200 ± 310.516, 151.400±52.384, 684.000±310.547, 91.400±21.324 and 35.800±12.726 from the 1st, 2nd, 3rd, 4th and 5th snail respectively. Thus the average cercarial production per day per snail was 402.760±119.177. From the respective 't' values, 3.385, 2.890, 4.286 and 2.813, each of which was higher than its corresponding table value, 2.776, the fluctuation in the day to day cercarial output from the 1st, 2nd, 4th and 5th snail was determined to be statistically significant. The 't' value for average number of cercariae released per day per snail is 3.379 which is higher than its corresponding table value, 2.064 and values for day to day cercarial production from most of the snails, 2nd, 3rd and 5th . Thus the influence of the host in the fluctuation in day to day cercarial emergence is evidenced to be more significant than that of the incubation period.

DISCUSSION

In the study of 10 infected snails, daily cercarial emergence per snail (ranging between 86 and 1306), varied significantly with the snail host. In another study of five infected snails during five days' incubation most of the snails showed a significant fluctuation in daily cercarial production. The average daily cercarial emergence in this case also varied significantly from host to host. The data suggest the dependence of the cercarial production on the host parasite compatibility. The daily release of cercariae of *Isoparorchis hypselobagri* from the infected *Indoplanorbis exustus*, ranging between 86 and 1306 differed considerably from those reported earlier for *Echinostoma* sp., *Halipegus eccentricus* and *Clinostomum marginatum*. *Biomphalaria glabrata* is considered to be able to produce 2 million cercariae per week with a mean shedding of 3,500 per snail in *Schistosoma mansoni* infection (De Souza *et al.*, 1985). In general the output of a single snail rarely exceeds 1500 cercariae per day for *S.mansoni* and 2000 cercariae per day for *S. haematobium* infection (Ward *et al.*, 1988), 6311 in *S. incognitum* and 5071 in *S. spindale* (Shameem and Radhika, 2001). In a weekly exposure of *Bulinus truncatus* infected with *S. haematobium*, the average cercarial shedding is 109 at 25° C, 8 at 18°C and 62 at 32°C and the absolute maxima of cercariae shed by one snail during 5 hrs stimulation is 2150 at 25°C, 48 at 18°C and 529 at 32°C (Pfluger *et al.*, 1984). Experimentally infected *Hippeutis* snail started shedding of cercariae from 25th day of exposure and continued upto 7th to 9th day with a total number of 684 cercariae per snail (Ahn *et al.*, 1989), while from *B. truncatus* experimentally infected with *S. haematobium* an average 120 cercariae per day per snail was obtained (Kechemir and Theron, 1989). The average rate of cercaria

production from experimentally infected *Oncomelania* snails is 673 per snail in its whole life time (Xie *et al.*, 1990). From monomiracidial and plurimiracidial infection of *S. rodhaini* in *B. glabrata,* an average of 507 and 508 cercariae per day per snail was obtained (Touassem and Theron, 1989).

Considerable differences in the emergence of cercariae, in the present work, from that of other species of trematodes and from other species of snail hosts in different geographical regions is mostly due to the variations in (i) many biotic and abiotic factors in the experimental conditions for cercariae release (Webbe,1965), (ii) the water temperature (Foster, 1964; Roushdy, 1984), (iii) water pH (Chu *et al.*, 1966; Sturrock and Sturrock, 1970) , (iv) the food given to the snail and possible crowding effects (Coles, 1973), (v) the size of the snails (Chu and Dawood, 1970) and the host parasite compatibility (Das and Manna, 1993) and lastly (vi) the species of the host and parasite. However, the cercarial productivity is considered to be independent of the miracidial dose (Chu *et al.*, 1966; Sturrock and Sturrock, 1970).

The snails, in the present investigations, produce cercariae in each hour of the day, though with varied intensity. Significant variation in the rhythmic hourly emergence of cercariae with the hosts indicates the cercarial productivity in every hour of its diurnal cycle to be a subject of host parasite compatibility. The peak period of cercarial production also varies from snail to snail.The peak period, 18th hr, for the 1st snail, though is literally under the dark is actually the marginal hr between the light and the dark periods. Besides, from the majority of the snails, more numbers of cercariae emerge in day time than that at the night and the variations are significant.

The appraisal of data suggest a definite and positive impact of light on cercarial emergence. Similarly, some other cercariae are reported to emerge in maximum during day time (Dutt and Srivastava, 1926 a,b; Giovanolia, 1936; Oliver, 1951). A diurnal periodicity of emergence of the cercariae of *S. haematobium* from *B. globosus* in a 12 hr light or dark cycle shows a peak emission at 11.00 hr with a smaller peak at 20.00 hr (Raymond and Probert, 1987) .

The emergence of *S. mansoni* cercariae exhibits daily periodicity (Asch, 1972) and a peak between 10-15 hr, 11-17 hr and 15-30 hr in different areas (Combes and Theron, 1977). A comparative analysis of the cercarial shedding of two *S. mansoni* populations originating from the same endemic area (Guadeloupe, Germany) allows to distinguish an early peak of emergence at 11.00 hr and late peak at 16.00 hr (Theron, 1984). In experimental infection of *S. intercalatum* the peak emergence time for cameroon strain is between 13.00 hr and 14.00 hr with the mean emergence time 13.59 hr and for the Zaire strain it is between 12.00 hr and 13.00 hr with the mean emergence time 12.13 hr (Pages and Theron, 1990) , 2-6 a.m. for *S. incognitum* and 8-10 a.m. in *S. spindale* (Shameem and Radhika, 2001). But still other authors view in a different way.Some cercariae are observed to release predominantly in the dark. The

cercarial emergence is also considered not to occur continuously but in spurts, separated by irregular interval of times (Erasmus, 1972). In *S. japonicum* the process is stopped at certain periods and started again (Pesigan *et. al.*, 1958).

Thus the cercarial emergence from snails, its diurnal rhythmicity and relationship with light and darkness is a function of the host-parasite compatibility which is subjected to the variation of the species of the host and the parasite.

The significant variation in the day to day cercarial production in most of the snails, 4 out of 5, in the present study, is due to the variations in the production level of cercariae according to the species of the parasites and the molluscan hosts and the time cercariae need for its maturation prior to emergence. In some cases, the production of large number of cercariae is followed by the periods of greatly reduced emergence and after continuation of this low level for a few days it increased gradually until a higher level (Erasmus, 1972). As to the present observation, Erasmus (1972) suggest of a phased cercarial production, within the rediae or sporocysts, need for cercarial maturity before emergence and collection of pools of cercariae occurring within the molluscan tissue, as the causes of day to day variation in cercarial output is most relevant.

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RHEOLOGICAL BEHAVIOUR OF WATERMELON JUICE CONCENTRATE

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Abstract

Flow behaviour of concentrated watermelon juice was studied over a wide range of temperatures (30-70°C) and concentration (30-70°Brix). Results indicated that the power law model described well the shear stress–shear rate relationship. The concentrates exhibited shear-thinning behaviour. Variation of flow behaviour index (n) was not consistent and ranged between 0.199 and 0.342. Both consistency index (K) and apparent viscosity (η) decreased with increase in temperature and the activation energies of flow were found to be in the range of 7.61 to 22.99 kJ/mol.

Keywords : Activation energy, power law model, rheology, watermelon juice concentrate.

INTRODUCTION

Watermelon (*Citrusllus vulgaris* schard.) belongs to the family *cucurbitaceae*. It is known to have originated in Tropical Africa and is cultivated throughout the warmer parts of the world. The fruit is generally consumed fresh. Although watermelon contains approximately 50% juice, it has not received adequate attention from processing viewpoint. Concentration of juice can considerably enhance the shelf-life. One of the appreciable changes during concentration of juice is in its rheological behaviour.

Knowledge of rheological properties of liquid foods is essential for the product development, design and evaluation of food processing equipment, such as pumps, pipings, heat exchangers, sterilizers and mixers. Several studies have been conducted on the rheological properties of fruit and vegetable products (Rao, 1977; Tanglertpaibul and Rao, 1987; Truong and Walter, 1994; Godfrey *et al.*, 1995; Ahmed *et al.*, 2000; Ahmed *et al.*, 2002). Factors affecting the rheological behavior of juice concentrates include particle size, total soluble solids (TSS), and temperature.

Manohar *et al.* (1991) studied the flow behavior of tamarind juice concentrate over a concentration range of 7-32° Brix and a temperature range of 25-70°C. The concentrates followed the power law relationship at concentrations above 19° Brix. The flow behaviour index (n) of the concentrates (26-62° Brix) was in the range of 0.625 to 0.747 over the entire temperature

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range. The activation energy did not vary with concentration and equaled 18.65 kJ/mol. During the study of rheological behaviour of kiwi fruit juice concentrate between 22.5-63.0° Brix and temperature range 4-70°C, the power law model fitted well below 55.7° Brix. At higher concentrations, Herschel-Bulkley model fitted well (Ibarz *et al.*, 1995) and the concentrates showed yield stress which varied linearly with temperature.

This paper reports results on rheological characteristics of watermelon juice concentrate (WJC) at selected processing temperatures and concentrations.

MATERIALS AND METHODS

Fresh watermelon were procured locally and washed thoroughly under running tap water. The fruits were cut and rind was separated using a stainless steel knife. The separated pulp was passed through a pulper (Narang Scientific Corporation, New Delhi) to obtain juice. Juice (5.8° Brix) was immediately transferred to a refrigerator (5±1°C). Juice was concentrated in a batch type vacuum concentrator at 30 kPa and 70°C to 30, 40, 50, 60, 65 and 70° Brix respectively.

Rheological characteristics (shear stress, shear rate and apparent viscosity) of WJC were measured at 30, 40, 50, 60 and 70°C using a Brookfield DV-II digital viscometer (Brookfield Engineering Instruments, USA). Approximately 500-mL WJC was placed in a 600-mL graduated glass beaker with flat bottom. Constant temperature water bath (±0.5% accuracy) was used to maintain the desired temperature. The viscometer was operated between 10 and 100 rpm, and shear stress, shear rate and apparent viscosity were recorded. The S#18 spindle was selected for the measurement and was used without the spindle guard of the instrument.

Rheological Model

The power law has been widely used to relate shear stress and rate of shear for liquid foods (Rao, 1977)

$$\tau = K (\gamma)^n \quad (1)$$

The temperature dependence of apparent viscosity (η) at 100 rpm and consistency index (K) can be described by the Arrhenius model:

$$\eta = A_\eta \exp (E_\eta / RT) \quad (2)$$

$$K = A_k \exp (E_K / RT) \quad (3)$$

where,

τ - shear stress (Pa)

γ - shear rate (s^{-1})

K- consistency index ($Pa.s^n$)

n - flow behaviour index (dimensionless)

η - apparent viscosity (Pa.s)

A_η - frequency factor for apparent viscosity at 100 rpm (Pa.s)

A_k - frequency factor for consistency index ($Pa.s^n$)

E_η - activation energy for apparent viscosity at 100 rpm (kJ/mol)

E_K - activation energy for consistency index (kJ/mol)

Each experiment was replicated twice and the average values were used in the analysis.

RESULTS AND DISCUSSION

Linear regression of the power law model (equation 1) was performed and the coefficients were computed. The power law described well the rheological behaviour of WJC over the entire range of temperature (30-70°C) and concentration (30-70° Brix) (Figures 1 and 2). The R^2 ranged from 0.884 to 0.999 while the standard error values were less than 0.05. The flow behaviour index (n) was less than unity signifying pseudoplastic nature of the WJC. Variation of n with TSS and temperature was not systematic and ranged between 0.199 and 0.342. Harper and El Sahrigi (1965), Saravacos (1970) and Ahmed *et al.* (2000) have reported similar observations on variation of n with temperature.

Effect of Temperature and Concentration on Consistency Index and Apparent Viscosity.

The apparent viscosity (η) and consistency index (K) decreased with an increase in temperature of WJC at all concentrations. The Arrhenius model (equations 2 and 3) described well the dependence of the apparent viscosity at a shear rate of 100 s^{-1} and the consistency index of power law model on temperature (Figures 3 and 4). The coefficients of the equations (2) and (3) were computed using the least square technique and are reported in Table 1. The flow activation energies ranged between 7.61 and 22.99 kJ/mol. This behaviour is in agreement with the observation of Vitali and Rao (1984) for orange juice concentrate. These activation energy values are consistent with those reported in literature for pureed foods and concentrates (Vitali and Rao,1982; Manohar *et al.*, 1991).

Table 1 : Coefficients of equations (2) and (3)

TSS	Eq. (2)			Eq. (3)		
	E_η	A_η	R^2	E_K	A_K	R^2
30	11.06	0.574	0.939	11.03	0.249	0.946
40	15.57	0.123	0.917	8.57	0.617	0.964
50	13.79	0.279	0.920	11.15	0.268	0.949
60	15.50	0.204	0.949	7.606	0.119	0.905
65	15.49	0.204	0.949	16.13	0.053	0.963
70	22.99	1.12	0.948	16.49	0.156	0.907

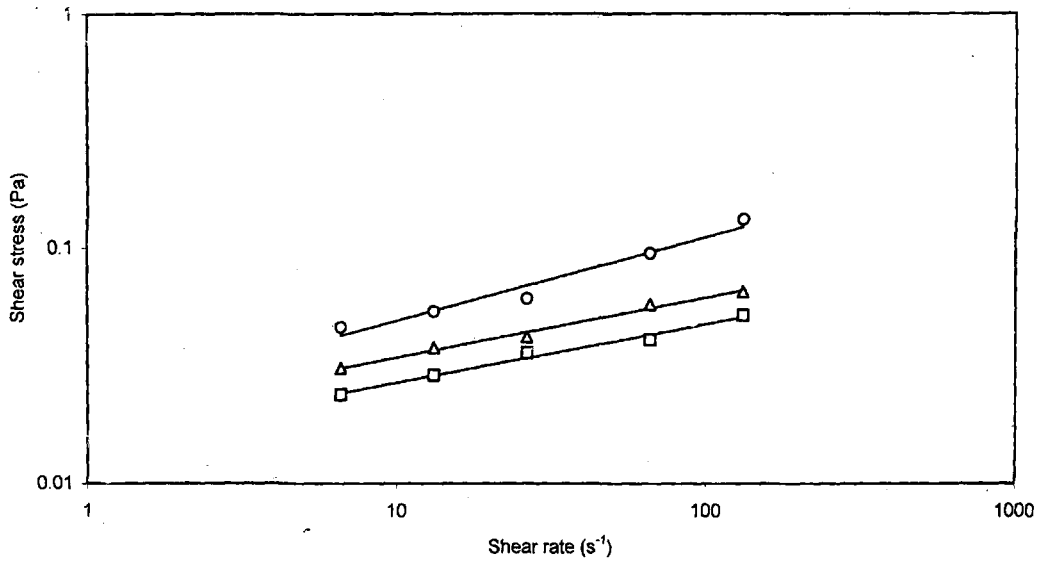

Figure 1 : *Applicability of the power law model to watermelon juice concentrate at 60° brix at selected temperature (O: 30°C; Δ: 50°C; □:70°C; - equation 1).*

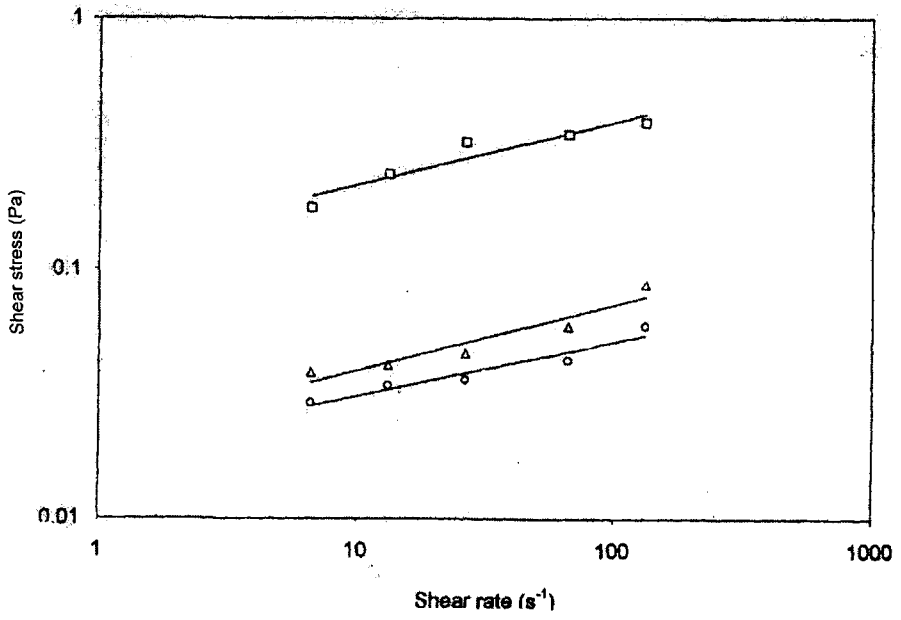

Figure 2 : *Applicability of the power law model to watermelon juice concentrate at 30° at selected concentration (O: 30°Brix; Δ: 50°Brix; □:70°Brix; - equation 1).*

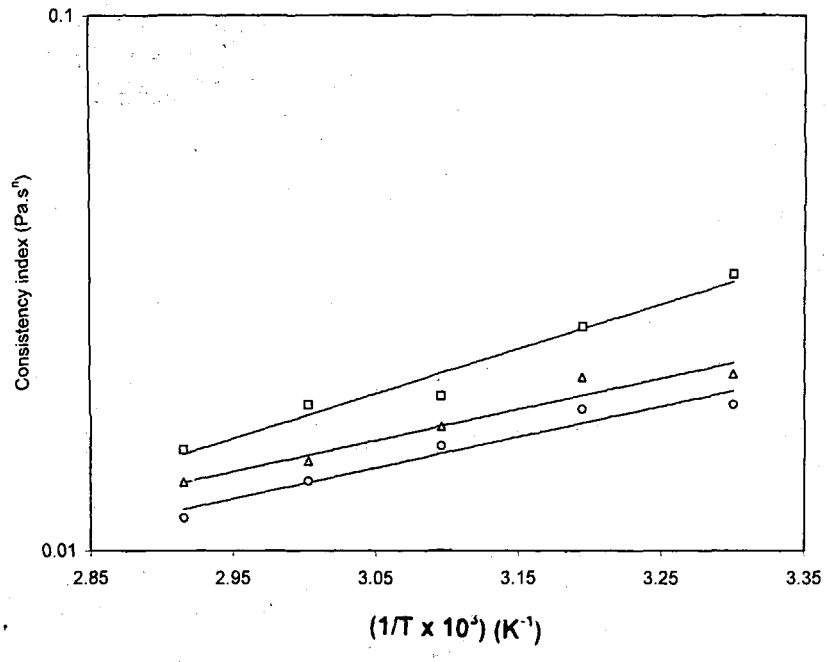

Figure 3 : Dependence of consistency index on process temperature (O: 30°Brix; Δ: 50°Brix; 65°Brix; - equation 3).

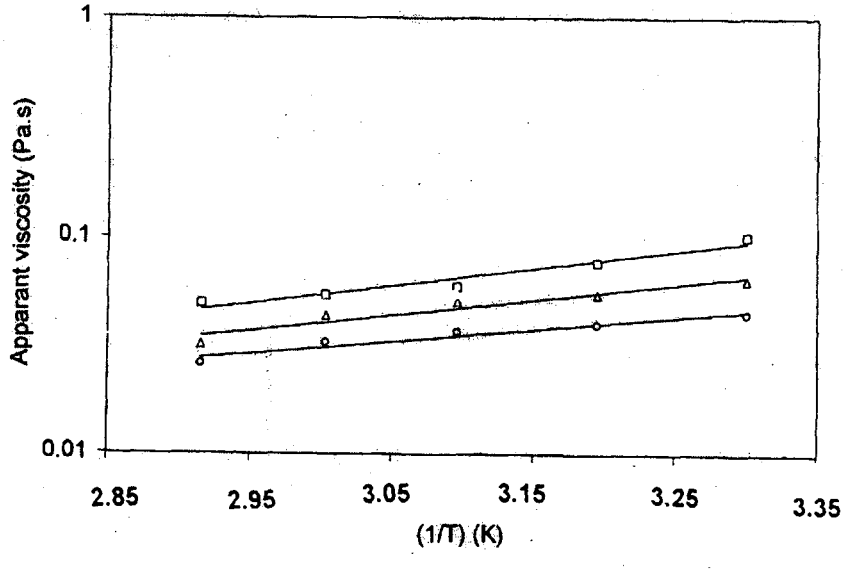

Figure 4 : Dependence of apparent viscosity on process temperature (O: 30°Brix; Δ: 50°Brix; 65°Brix; -equation 2).

The apparent viscosity and consistency index increased with the concentration of WJC at all temperatures (Figs. 3 and 4). However, variation of flow activation energies with concentration was not systematic (Table 1). Similar behaviour of effect of concentration on activation energy has been reported by several researchers (Vitali and Rao, 1982; Vitali and Rao, 1984).

CONCLUSIONS

Water melon juice concentrate behaved as a shear-thinning fluid and the power law model described well the shear stress-shear rate relationship. Both consistency index and apparent viscosity increased with concentration but decreased with increase in temperature. Variation of both consistency index and apparent viscosity with the temperature followed the Arrhenius relationship.

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STUDY OF FACTORS AFFECTING THE PRODUCTION OF BACTERIOCIN BY *LACTOBACILLUS CASEI*

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Abstract

The effect of production medium, initial pH of the medium, incubation temperature and incubation period on the production of bacteriocin by L. casei (isolated from infant stool sample) against S. aureus was studied. Among the three media (MRS, TGE and Elliker broth) tested, maximum amount of bacteriocin was produced in MRS medium. Enhanced production of bacteriocin was achieved by growing the cells at optimum pH and incubation temperature. Maximum bacteriocin titre (640 AU/ml) was obtained in MRS medium, having initial pH 6.5 when cells were incubated at 37° C for 24 hours.

Key words : Bacteriocin, incubation period, incubation temperature, initial pH, *L. casei*, media.

INTRODUCTION

Current consumer interest for commercially processed foods that are not preserved with non-food preservatives has provided an incentive to search for safe and food grade preservatives of biological origin (Miller, 1993). Bacteriocins produced by many strains of LAB, have generated interest as potential food biopreservatives (Daeschel, 1990; Ray, 1992). Bacteriocins are bacterial proteins that are bactericidal to closely related species as well as several other species of bacteria including food borne pathogens (Klaenhammer, 1988).

To our knowledge, very few reports on bacteriocin from *L. casei* have been published. Casecin 80 is produced by *L. casei* B80 and it has a narrow spectrum bacteriocin (Rammelsberg *et al.*, 1990). Another group of workers reported Lactocin 705, a bacteriocin produced by *L. casei* CRL 705 and studied the influence of growth conditions on its production (Yignolo *et al.*, 1995). Identification and nucleotide sequence of genes involved in the synthesis of lactocin 705, a two-peptide bacteriocin from *Lactobacillus casei* CRL 705 was done (Cuozzo *et al.*, 2000). Recently, a bacteriocin produced from *Lactobacillus casei* spp. *rhamnosus* (SN 11) is reported (Kumthavee *et al.*, 2000).

The determination of optimum parameters (media composition, temperature, pH and incubation period) for production of bacteriocins is amongst the prerequisites for their use in food, veterinary and

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Abbreviations in the text are given at the end of paper

pharmaceutical industries. The purpose of this study was to evaluate the effect of various media and culture conditions on bacteriocin production by *L. casei* isolate and to look for a potential biopreservative.

MATERIALS AND METHODS

Bacterial cultures and maintenance

Bacteriocin producing *Lactobacillus casei* isolate (previously identified and isolated from infant stool sample) was maintained in litmus milk with calcium carbonate and activated before use by successive transfers into MRS broth (de-Man *et al.*, 1960) (Hi-media) at 37°C for 24 hours. The clinical isolate of *Staphylococcus aureus* which served as indicator organism was procured from National Collection of Dairy Cultures (NCDC), NDRI, Karnal (India). The pathogenic organism was preserved on Nutrient agar slants and activated by repeated sub culturing into BHI (Hi-Media) broth.

Optimization of cultural conditions for bacteriocin production

The growth conditions for maximum bacteriocin production from *L. casei* isolate were optimized with respect to production medium, initial pH of the production medium, incubation temperature and incubation period.

Production medium- MRS, Elliker *et al.*, (1956) and TGE broths (Hi-media) were inoculated with the active culture (OD 0.42 at 600 nm) with shaking at the level of 1% and incubated at 37°C.

Initial pH – MRS broths adjusted to various pH values (5.0, 5.5, 6.0, 6.5 and 7.0) were inoculated and incubated at 37°C.

Incubation temperature- MRS broth (pH 6.5) was inoculated and incubated at 0°, 25°, 30°, 37° and 45°C.

In all the above experiments, samples were drawn after 12 and 24 hour of incubation period and assayed for bacteriocin activity units (AU/ml).

Incubation period- MRS broth (pH 6.5) was inoculated with the active culture at the level of 1% and incubated at 37°C. Samples were drawn at regular intervals and were assayed for bacteriocin activity units (AU/ml).

Assay for bacteriocin activity

The isolate was grown in MRS broth at 37°C for 24 hours. The culture supernatant was obtained by centrifugation of culture broth at 6000 rpm for 20 minutes. Bacteriocin activity of *L. casei* isolate against *S. aureus* was determined by agar well assay method (Barefoot and Klaenhammer, 1983). Nutrient agar (Hi-media) was used for well assay.

Measurement of activity units

The bacteriocin titre was defined as the reciprocal of the highest dilution showing definite inhibition of the indicator lawn and was expressed in activity units per millilitre (AU/ml). One hundred microlitres of two-fold serial dilution of the culture supernatant was used to determine the highest dilution in the agar well assay (Barefoot and Klaenhammer, 1983).

The activity units (AU/ml) were calculated using the formula

Bacteriocin titre (AU/ml) = 2^n X 1000/V

where, n is the number of last dilution showing inhibition. V is the volume of the supernatant (μl).

RESULTS AND DISCUSSION

The effect of various factors such as culture medium, initial pH of the medium, incubation temperature and period of incubation on production of bacteriocin by *L. casei* isolate was studied.

Effect of culture media

MRS, Elliker and TGE media were used for the study. Maximum bacteriocin production occurred in MRS broth followed by Elliker broth, which showed very less bacteriocin production, while no bacteriocin activity was observed in TGE broth even after 24 hours of incubation period (Fig. 1). The bacteriocin activity of 640 and 40 AU/ml was observed in MRS and Elliker broth, respectively after 24 hours of incubation period. The titre in Elliker broth was calculated to be 6.25 % of the highest titre in MRS broth during the same period.

MRS broth was found to be most suitable medium for maximum production by *L. casei* isolate as compared to Elliker. It may be attributed to the lack of certain essential nutrients such as $MgSO_4$, $MnSO_4$ etc in the Elliker medium and the selective agents such as citrate and acetate in TGE broth. Similar findings were reported by another group of workers that the most effective medium for the production of lactocin 705 from *L. casei* was MRS broth when compared with Elliker, BHI and M-17 broth medium (Yignolo *et al.*, 1995).

On the basis of maximum bacteriocin production MRS medium was found to be the most suitable medium and hence was used as an indicator strain for further studies.

Effect of initial pH of the medium

Bacteriocin production was found to be highly dependent upon the initial pH of the growth medium (Fig. 2). MRS broth with initial pH of 5.0 to 5.5 resulted in negligible production of bacteriocin as compared to MRS broth with other values of initial pH after 24 hours of incubation period. MRS broth with an initial pH of 6.0 supported only 12.5 % of the maximum activity.

The maximum bacteriocin production was observed in case of MRS broth having initial pH of 6.5 after 24 hours of growth in comparison to other initial pH values employed in the study. The pH 6.5 was also observed optimum for maximum bacteriocin production by *L. acidophilus* LF-221 (Bogovic-Matijasic and Rogelj, 1998). The effect of pH on the cell is due to the induction of cell membrane enzymes for maximal adsorbtion of nutrients from the medium. The decreased titre of bacteriocin at low initial pH could be due to reduced cell growth, which in turn, is due to lesser adsorption of nutrients at low pH.

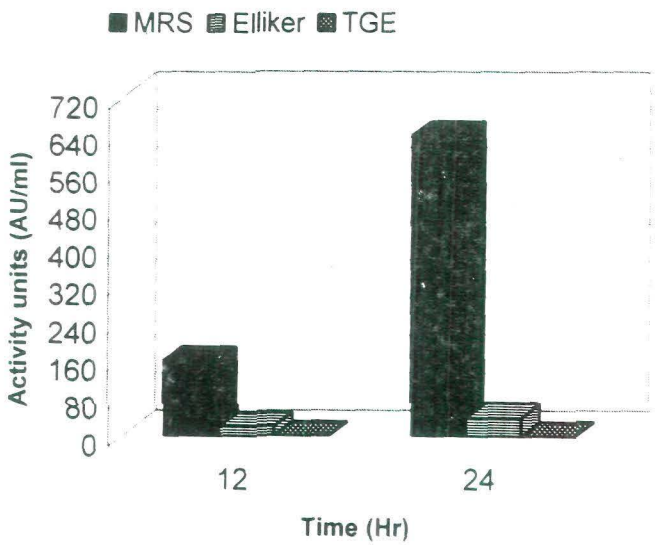

Figure 1 : Effect of culture medium on bacteriocin production by L. casei isolate.

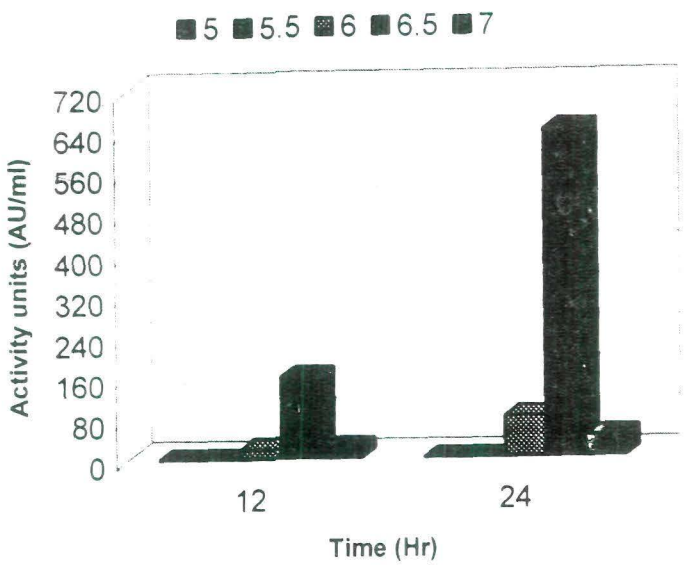

Figure 2 : Effect of initial pH of MRS broth on bacteriocin production by L. casei isolate.

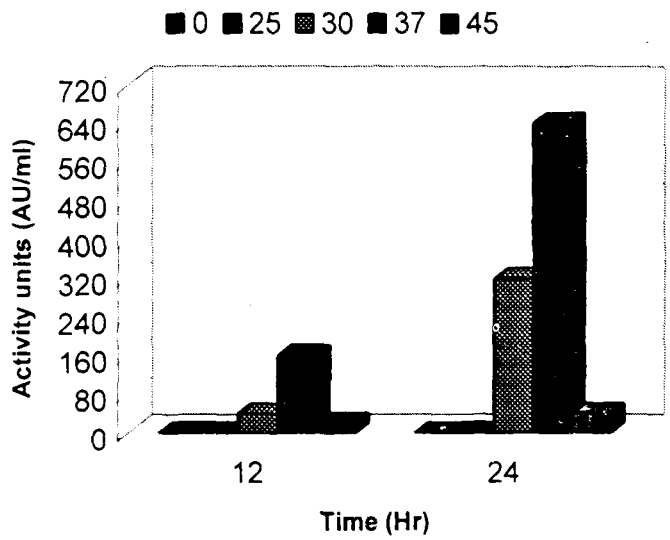

Figure 3 : Effect of incubation temperature (°C) on bacteriocin production by L. casei isolate.

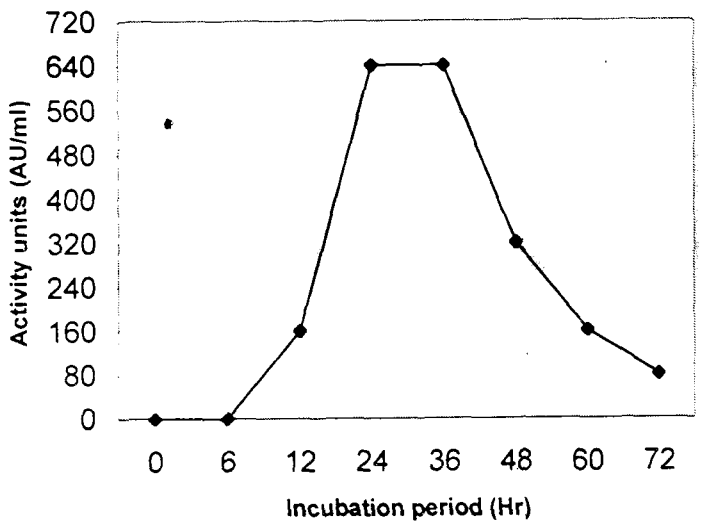

Figure 4 : Effect of incubation period on bacteriocin production by L. casei isolate.

Effect of incubation temperature

The result of bacteriocin production at different degrees of temperatures are presented in Fig. 3. It was observed that maximum bacteriocin production occurred at 37°C after 24 hours followed by 30°C and 45°C as is clear from the titres obtained at these temperatures (640, 320 and 40 AU/ml respectively). However, no bacteriocin was produced at 25°C.

Maximum bacteriocin production has been reported to occur at optimum growth temperature. Incubation temperature of 37°C, which is also optimum growth temperature for lactobacilli, has been reported optimum for maximum production of bacteriocin (Jack *et al.*, 1995).

Effect of incubation period

It is clear from the Fig. 4 that culture started producing bacteriocin in the growth medium after 6 hour of incubation. Thereafter, bacteriocin production continued and reached the highest value of 640 AU/ml after 24 hours of incubation period. The bacteriocin titre remained at the highest up to 36 hours. Thereafter, activity started declining. 12.5 per cent of maximum activity was observed at 72 hour of incubation.

It has also been reported by some workers that the activity declined when the incubation period was extended. Dave and Shah (1997) reported that activity of acidophilicin LA-1 declined when the culture entered death phase. The decrease in the bacteriocin activity is believed to be on account of cell bound proteases and peptidases released due to lysis of cell during death phase.

CONCLUSIONS

The results of the present study have shown that bacteriocin production by *L. casei* isolate was greatly influenced by the composition of growth media, initial pH of the medium, incubation temperature and incubation period. The maximum bacteriocin production by *L. casei* isolate occurred in MRS broth having intial pH 6.5 during 24-36 hours when the culture was grown at 37° C. Optimization of culture conditions may prove beneficial for producing large amount of bacteriocin and adds for its use as biopreservative.

Further work in this area can be done for purification and characterization of the bacteriocin produced.

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ABBREVIATIONS

MRS (de-Man Rogosa and Sharpe medium), TGE (Tryptone glucose yeast extract agar) AU/ml (activity units per milliliter).

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ORDER-LEVEL LOT-SIZE MODEL FOR DETERIORATING ITEMS WITH EXPONENTIALLY INCREASING DEMAND

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Abstract

A lot-size inventory model for deteriorating items is derived with exponentially increasing demand. The model starts with an instantaneous replenishment and ends with no shortages in last cycle. The total relevant cost for this model is a convex function of the replenishment number. Sensitivity analysis of the optimal solution with respect to the parameters of the system is carried out with the help of a numerical example.

Key words: Deterioration, inventory, lot-sizing, shortages.

INTRODUCTION

A cost-minimization inventory model in which demand rate is constant is the classical EOQ model. It is simple and easy to apply. Now only in the maturity stage of a product life cycle, the demand rate remains constant and it will cause varying magnitudes of error by using the EOQ formulation in stages other than the maturity stage. The demand rate can be well approximated using a linear form in the growth stage of a product life cycle. An algorithm to determine both the optimal number and the optimal timing of replenishment is established by Donaldson (1977) and Resh *et al* (1976). The solutions for different demand models are also generalized by Barbosa and Friedman (1978). Further, the demand to any log-concave demand function is extended by Henery (1979).

The comparison of an inventory policy which allows shortages with an inventory policy which does not allows shortages, suggests that an inventory policy which allows shortages is always less expensive to operate than a policy without shortages. An exact replenishment policy for an inventory model is developed by Donaldson (1977) and Dave (1989) in which shortages are allowed. In general the replenishment does not start with shortages, Goyal et al (1992) gave an alternative that starts with shortages in every cycle.

The products namely medicines, volatile liquids, blood banks etc. are deteriorating continuously in real life situations. Dave and Patel (1981) developed an inventory model for deteriorating items in which shortages were not permitted. Schan (1984) extended the same model developed by Dave and Patel (1981) with shortages. For the problem with increasing demand, an exact solution is

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presented by Benkherouf and Mahmoud (1996). Also, Hariga (1993) developed an optimal inventory demand lot-sizing model for deteriorating items under the replenishment policies.

In the present paper, we have developed a deterministic inventory model for deteriorating items with exponentially increasing demand. Shortages are allowed. The sensitivity analysis is carried out with the help of a numerical example. The paper concludes with a conclusion section.

ASSUMPTIONS AND NOTATIONS:

The mathematical model of the continuous-in-time inventory replenishment problems is based on the following assumptions:

1. Lead time is zero.
2. Shortages are permitted.
3. The replenishment rate is infinite.
4. The initial inventory level is zero.
5. H is time horizon under consideration.
6. $f(t) = ae^{bt}$ is demand rate at time t where $f(t)$ is positive in (0, H], and continuous in [0, H].
7. c is purchasing cost per unit.
8. h is inventory holding cost per unit per unit time.
9. s is shortage cost per unit per unit time.
10. o is fixed cost of replenishment per order.
11. θ $(0 \le \theta \le 1)$ is rate of deterioration on which is a constant fraction of on-hand inventory. The deteriorated units can neither be repaired nor replaced during the planning horizon under consideration.
12. m is total number of replenishments and a decision variable.
13. t_i is the i^{th} replenishment time; i = 1, 2, ..., m.
14. S_i is time at which the inventory level in the i^{th} cycle $[t_i, t_i+1)$ reaches zero, i = 1, 2, ..., m-1 or m (see fig. 1) and decision variable.

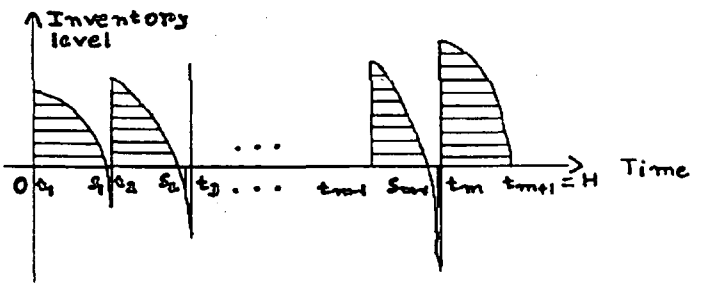

Figure 1 : Graphical representation of Inventory Model

MATHEMATICAL FORMULATION:

The cumulative inventory during the i^{th} cycle is given by

$$I_i = \frac{1}{\theta}\int_{t_i}^{S_i}\left[e^{\theta(u-t_i)}-1\right]f(u)du$$

$$= \frac{1}{\theta}\int_{t_i}^{S_i}\left[e^{\theta(u-t_i)}-1\right]ae^{bu}du$$

$$= \frac{a}{\theta(\theta+b)}e^{-\theta t_i}\left[e^{(\theta+b)S_i}-e^{(\theta+b)t_i}\right]-\frac{a}{\theta b}\left[e^{bS_i}-e^{bt_i}\right] \quad \ldots\ldots(1)$$

The cumulative shortage during the i^{th} cycle is given by

$$S_i = \int_{S_i}^{t_{i+1}}(t_{i+1}-u)f(u)du$$

$$= \int_{S_i}^{t_{i+1}}(t_{i+1}-u)ae^{bu}du$$

$$= \frac{a}{b}S_i e^{bS_i}+\frac{a}{b^2}e^{bt_{i+1}}-\frac{a}{b^2}e^{bS_i}-\frac{at_{i+1}}{b}e^{bS_i} \quad \ldots\ldots(2)$$

Therefore, by using (1) & (2) the corresponding total relevant costs during the planning horizon H for this model is as follows:

$$C_1(m,\{S_i\},\{t_i\}) = mo+\sum_{i=1}^{m}(h+\theta c)I_i+\sum_{i=1}^{m-1}s.S_i \quad \ldots\ldots(3)$$

$$\text{with } t_1 = 0; \quad t_{m+1} = S_m = H$$

C_1 is to be minimized for any fixed m, the first order conditions for finding the optimal $\{t_i^*\}$ and $\{S_i^*\}$ are given as follows:

$$\frac{\partial C_1}{\partial S_i} = 0, \text{ for } i = 1, 2, \ldots\ldots, m\text{-}1 \text{ or } m.$$

which means to solve

$$X_1 S_i^{\,2} + X_2 S_i + X_3 = 0, \quad i = 1,2,\ldots\ldots,m-1 \text{ or } m.$$

where

$$X_1 = \sum_{i=1}^{m}(h+\theta c)\frac{a}{\theta}e^{-\theta t_i}(\theta+b)^2 - \sum_{i=1}^{m}(h+\theta c)\frac{ab^2}{\theta} + \sum_{i=1}^{m-1} s.ab - \sum_{i=1}^{m-1} s.a.b^2.t_{i+1}$$

$$X_2 = \sum_{i=1}^{m}(h+\theta c)\frac{a}{\theta}e^{-\theta t_i}(\theta+b) - \sum_{i=1}^{m}(h+\theta c)\frac{ab}{\theta} + \sum_{i=1}^{m-1} s.a - \sum_{i=1}^{m-1} s.a.b.t_{i+1}$$

$$X_3 = \sum_{i=1}^{m}(h+\theta c)\frac{a}{\theta}e^{-\theta t_i} - \sum_{i=1}^{m}\frac{a}{\theta}(h+\theta c) - \sum_{i=1}^{m-1} s.t_{i+1}.a$$

and $\dfrac{\partial C_1}{\partial t_i} = 0$, for $i = 1, 2, \ldots\ldots, m$.

which means to solve

$$X_4 t_i^{\,2} + X_5 t_i + X_6 = 0, \quad i = 1,2,\ldots\ldots,m.$$

where

$$X_4 = \sum_{i=1}^{m}(h+\theta c)\frac{(-a)}{(\theta+b)}\theta^2 e^{(\theta+b)S_i} + \sum_{i=1}^{m}(h+\theta c)b^3 + \sum_{i=1}^{m}(h+\theta c)\frac{ab^2}{\theta}$$

$$X_5 = \sum_{i=1}^{m}(h+\theta c)\frac{a}{(\theta+b)}\theta e^{(\theta+b)S_i} + \sum_{i=1}^{m}(h+\theta c)b^2 + \sum_{i=1}^{m}(h+\theta c)\frac{ab}{\theta}$$

$$X_6 = \sum_{i=1}^{m}(h+\theta c)\frac{(-a)}{(\theta+b)}e^{(\theta+b)S_i} + \sum_{i=1}^{m}(h+\theta c)b + \sum_{i=1}^{m}(h+\theta c)\frac{a}{\theta}$$

Hence,

$$S_i = \frac{-X_2 \pm \sqrt{X_2^{\,2} - 4X_1X_3}}{2X_1}, \quad i = 1,2,3,\ldots\ldots,m-1 \text{ or } m.$$

$$t_i = \frac{-X_5 \pm \sqrt{X_5^{\,2} - 4X_4X_6}}{2X_4}, \quad i = 1,2,3,\ldots\ldots,m$$

NUMERICAL EXAMPLE

Consider the parameters of the inventory system as

$o = 1, h = 0.25, \theta = 0.25, c = 1.5, s = 2.5, a = 10, b = 0.1.$

It is observed that C_1 = 105516.66 becomes optimum for $m^* = 26$.

SENSITIVITY ANALYSIS

		m	C_1			*m*	C_1
h	0.21	39	107336.88	*s*	2.1	24	59388.50
	0.23	34	106123.75		2.3	25	73579.24
	0.25	26	105516.66		2.5	26	105516.66
	0.27	25	82276.07		2.7	31	107632.27
	0.29	24	74997.76		2.9	32	109884.87
θ	0.250	26	105516.66	*b*	0.092	42	110700.15
	0.252	34	107273.28		0.094	40	108088.75
	0.254	38	108467.01		0.096	38	107131.84
	0.256	40	109531.74		0.098	34	106295.63
	0.258	41	109617.20		0.100	26	105516.66
c	1.5	26	105516.66				
	1.7	25	85554.16				
	1.9	24	80570.08				
	2.1	23	79094.77				
	2.3	22	78020.39				

It is observed that

1. Increase in inventory holding cost, reduces number of orders to be placed and total cost of an inventory system.
2. Increase in deterioration rate increases number of orders to be placed and hence C_1, significantly.
3 Increase in purchase cost, reduces number of orders to be placed over a planning horizon and hence C_1.
4. Increase in s, shortage cost increases number of replenishment orders and hence C_1.
5. Increase in b has status as that of inventory holding cost.

CONCLUDING REMARK

The realistic features of items, which are subject to deterioration, are incorporated in the study of inventory system in this paper. The model deals with exponentially increasing trended demand. The occurrence of shortages is allowed in the present model. Different products are deteriorating continuously in real life situations. This model is a lot-size inventory model with shortages and deterioration, so it is useful in such a situations. Since almost all items undergo either direct spoilage or physical decay in the due course of time, deterioration factor has its important role to play in an inventory system.

The analytical model is developed based on the above stated concepts. The sensitivity of the solution to changes in the values of different parameters has been discussed.

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FORMS OVER SEMI-LOCAL RINGS WITH INVOLUTION

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Abstract

A theory of anti-hermitian form over a commutative ring with identity having an involution is being proposed. The work is based on the observations of E. Witt who observed equivalence classes of anisotropic quadratic forms may be viewed as the quotient ring of the integral group ring Z[G] by an ideal $\mathfrak{I}$, where $G \cong F^*/(F^*)^2$ and $\mathfrak{I}$ is the ideal generated by elements of the form $(g_1 + g_2 - g_3 - g_4)$ and $(1+g_5)$ with $g_i \in G$. The results hold for λ- hermitian forms where λ is such that $\lambda.\lambda^* = 1$.

Key words: Antihermitian form, idempotent, involution, witt-ring.

INTRODUCTION

In this paper we develop a theory of anti-hermitian form over a commutative ring with identity having an involution. Witt (1937) observed in Satz 7 that the ring **W(F)** of equivalence classes of anisotropic quadratic forms may be viewed as the quotient ring of the Integral group ring **Z[G]** by an ideal $\mathfrak{I}$, where $G \cong F^*/(F^*)^2$ and $\mathfrak{I}$ is the ideal generated by elements of the form $(g_1 + g_2 - g_3 - g_4)$ and $(1+g_5)$ with $g_i \in G$. This gave us the inspiration to look at the corresponding situation for anti-hermitian forms over rings with involution. The only information we need about the ideal $\mathfrak{I}$ is that any homomorphism of **Z[G]** to **Z** sends $\mathfrak{I}$ to **0** or an ideal of the form $2^n Z$. Such principal ideals of the ring **Z** of integers will be called admissible ideals. Our main concern in this note is the generalization of **W(F)** and the Witt-Grothendieck ring **GW(F)** to the Witt ring **W(C,*)** and the Witt - Grothendieck ring **GW(C,*)** of equivalence classes of anti-hermitian forms over a connected semi local ring (see below for definitions) **C** with involution *. In fact these results hold for λ-Hermitian forms where λ is such that $\lambda.\lambda^* = 1$.

Definition 1. Let **(C, J)** be a commutative ring with identity endowed with an involution **J** with **J(a) = a*.** A finitely generated projective left **C**-module **M** is called an anti-hermitian space over **(C , J),** if there is a mapping φ from $M \times M \to C$ satisfying the following properties.

(i) $\varphi(c.m, n) = c.\varphi(m, n)$,

(ii) $\varphi(m, n) = -\varphi(n, m)^*$,

(iii) $\varphi(m, d.n) = \varphi(m, n).d^*$.

If we set $M^* = Hom._C(M, C)$ then we can view it as a left **C** module by stipulating that $(c.f)(m) = f(m).c^*$ where f is in M^* and c is in **C**. We have a **C**-linear map d_φ from **M** to

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M^* given by $d_\varphi(n)(m) = \varphi(m, n)$ for each n in M. In case d_φ is bijective we say that (M, φ) is non-degenerate. In case M is a free C- module we call (M, φ) a free anti-hermitian space. Suppose (M, φ) is free space with $B = \{x_1, x_2, \ldots x_n\}$ as a basis. Then (M, φ) is a non degenerate if and only if the matrix $[|\varphi(x_i, x_j)|], 1 \le i, j \le n$, is non singular where $|\varphi(x_i, x_j)|^2 = \varphi(x_i, x_j).\ \varphi(x_i, x_j)^*$.

A sub module N of the anti-hermitian space (M, φ) is said to be a subspace if N is a direct summand of M as a module and the restriction of φ to N is an anti-hermitian form on it. Given a subspace N of (M, φ), we set $N^\perp = \{ x \in N \mid \varphi(x, N) = 0 \}$. We call (M, φ) an orthogonal sum of the subspaces N_1 and N_2 if $\varphi(x, y) = 0$, $\forall$ x in N_1 and y in N_2 and $M = N_1 \oplus N_2$ as a module (and written as $M = N_1 \perp N_2$). Suppose (M, φ) is a free anti-hermitian space over $(C, *)$ and $B = \{x_1, x_2, \ldots x_n\}$ is a free basis of M. We say B is an orthogonal basis if $M = C.x_1 \perp C.x_2 \perp \ldots \perp C.x_n$ If (M, φ) is a non-degenerate space then $\varphi(x_i, x_i)$ are all units in C.

Lemma 1. Let (M, φ) be a non-degenerate anti-hermitian space and N be a subspace of M. If N is non degenerate then so is $N^\perp$ and $M = N \perp N^\perp$. Moreover $N = (N^\perp)^\perp$.

Let (L, φ) be an anti-hermitian space over (C, J) and $M(L) = L \oplus L^*$. On this $M(L)$ which is a C- module we define an anti-hermitian form ψ as follows:

$$\psi(x + f, y + g) = \varphi(x, y) + g(x) - f(y)^*$$

where $x, y \in L$ and $f, g \in L^*$.

Any anti-hermitian space (M, φ) which is isomorphic to $(M(L), \psi)$ is called a metabolic space and these metabolic spaces can be verified to be non-degenerate.

The following lemma characterizes metabolic spaces.

Lemma 2. A non degenerate anti-hermitian space (M, φ) is metabolic if and only if M has a non zero subspace N such that $N = N^\perp$.

Proof. Suppose (M, φ) is metabolic. Then $(M, \varphi) \cong (M(L), \psi)$ where (L, φ) is an anti-hermitian space. If we set $N = L^*$, then

$N^\perp = \{x \in M \mid \varphi(x, N) = 0\} = \{x = u + f$ in M and $\varphi(u + f, g) = 0$ for all g in $L^*\} = \varphi(u, o) + g(u) - f(0)^* = 0$ for all g in $L^* \Rightarrow u = 0$ as φ is non-degenerate.

Therefore $N^\perp = \{x$ in $L^*\} = N$.

Conversely, if $M = U \oplus V$ where $V = V^\perp$ is non-degenrate, then the mapping from V to $U^* = \mathrm{Hom}_C(U, C)$ given by $v \to d_\varphi(v)$ restricted to U gives an isomorphism and we have an isometry from $M = U \oplus V \to U \oplus U^* = M(U)$, where $M(U)$ is provided with the anti-hermitian form ψ given by

$$\psi(u_1 + d_\varphi(v_1) \mid U, u_2 + d_\varphi(v_2) \mid U)$$

$$= \varphi(u_1, u_2) + d_\varphi(v_2)(u_1) - d_\varphi(v_1)(u_2)^*$$

$$= \varphi(u_1, u_2) + \varphi(u_1, v_2) - \varphi(u_2, v_1)^*$$

$$= \varphi(u_1, u_2) + \varphi(u_1, v_2) + \varphi(v_1, u_2)$$

$$= \varphi(u_1 + v_1, u_2 + v_2) = \varphi(x, y) \text{ as}$$

$x = u_1 + v_1$ and $y = u_2 + v_2$.

If $\mathbf{M(U)}$ *is a metabolic space associated with* an anti-hermitian form $(\mathbf{U}, \varphi)$ and φ is the zero form on $\mathbf{U}$, then we call the space $\mathbf{M(U)}$ a hyperbolic space and this we denote by $\mathbf{H(U)}$. Given an anti-hermitiasn space $(\mathbf{M}, \varphi) = \mathbf{M}$ we denote by $-\mathbf{M}$ the space $(\mathbf{M}, -\varphi)$.

Lemma 3. Let $(\mathbf{U}, \varphi)$ be an anti-hermitian space over $(\mathbf{C}, \mathbf{J})$. Then we have

(i) $\mathbf{M(U)} \perp \mathbf{M(-U)} \cong \mathbf{H(U)} \perp \mathbf{M(-U)}$.

(ii) $\mathbf{U} \perp -\mathbf{U} \cong \mathbf{M(U)}$ in case $\mathbf{U}$ is non-degenerate.

(iii) $\mathbf{M(U_1)} \perp \mathbf{M(U_2)} \cong \mathbf{M(U_1 \oplus U_2)}$ in case $\mathbf{U}$ is the orthogonal sum of $\mathbf{U_1}$ and $\mathbf{U_2}$.

Proof. (i) Let $\mathbf{W} = \{ u \perp (u + f) \mid u \in \mathbf{U}, f \in \mathbf{U^*}\} \subset \mathbf{M(U)} \perp \mathbf{M(-U)}$. Then $H(\mathbf{U})$ to $\mathbf{W}$ given by $u + f \to u \perp (u + f)$ is an isometry. Now $\psi(u + f, v+ g) = \varphi(u, v) + g(u) - f(v)^* = g(u) - f(v)^*$ and $\psi(u \perp (u + f), v \perp v+ g) = \varphi(u, v) + g(u) - f(v)^* = g(u) - f(v)^*$ as φ is zero on $\mathbf{U}$

So we have the desired isometry. Now

$\mathbf{W}^{\perp} = \{u + f \perp -u - d\varphi(u) : u \text{ in } \mathbf{U}, f \text{ in } \mathbf{U^*}\}$

(ii) Since $\mathbf{U}$ is non-degenerate $d\varphi : \mathbf{U} \to \mathbf{U^*}$ is an isomorphism, so every element x in $\mathbf{M(U)}$ can be written uniquely as $x = u + d\varphi(v)$. Then

$\mathbf{M(U)} \to \mathbf{U} \perp \mathbf{(-U)}$

$u + d\varphi(v) \to (u + v) \perp v$

$\psi(u_1 + d\varphi(v_1), u_2 + d\varphi(v_2)) = \varphi(u_1, u_2) + \varphi(u_1, v_2) + \varphi(v_1, u_2)$

$\varphi(u_1 + v_1 \perp v_1, u_2 + v_2 \perp v_2) = \varphi(u_1 + v_1, u_2 + v_2) - \varphi(v_1, v_2) = \varphi(u_1, u_2) + \varphi(u_1, v_2) + \varphi(v_1, u_2)$

So this gives that $\mathbf{M(U)}$ is isometric to $\mathbf{U} \perp \mathbf{(-U)}$

(iii) Suppose $\mathbf{U} = \mathbf{U_1} \perp \mathbf{U_2}$ as subspaces, then

$\mathbf{M(U)} = \mathbf{(U_1 \oplus U_2 \oplus U_1^* \oplus U_2^*, \psi)}$, where

$\psi(u_1+u_2 + f_1+ f_2, v_1+ v_2 + g_1+ g_2) = \varphi(u_1+ u_2, v_1+ v_2)+ g_1(u_1)+ g_2(u_2) - f_1(v_1)^* - f_2(v_2)^*$.

Define a mapping $\varphi : \mathbf{U_2} \to \mathbf{U_1^*}$ by $\varphi(u_2)(u_1) = \varphi(u_1, u_2)$

The mapping from $\mathbf{M(U_1)} \perp \mathbf{M(U_2)}$ to $\mathbf{M(U)}$

Given by $(u_1+ f_1) \perp (u_2 + f_2) \to u_1+ u_2 + (f_1 - \varphi(u_2))+ f_2$ gives an isometry.

The isometry classes of non-degenerate anti-hermitian spaces over $(\mathbf{C}, \mathbf{J})$ form a semigroup of $\mathbf{S(C, J)}$ and the metabolic spaces form a sub-semigroup of $\mathbf{S(C, J)}$ and we shall denote these metabolic spaces by $\mathbf{M(C, J)}$.

We say two classes $[\mathbf{M}]$ and $[\mathbf{N}]$ are equal if there exists a non-degenerate space $\mathbf{M'}$ with $\mathbf{M} \perp \mathbf{M'} \cong \mathbf{N} \perp \mathbf{M'}$. We can construct the Grothendieck group of $\mathbf{S(C, J)}$ and $\mathbf{M(C, J)}$ which we shall denote by $\mathbf{GS(C, J)}$ and $\mathbf{GM(C, J)}$. If $\mathbf{[M(U)] - [M(V)]=0}$ in $\mathbf{GS(C, J)}$ then it can be seen without difficulty that $\mathbf{[M(U)]-[M(V)]=0}$ in $\mathbf{GM(C, J)}$ as well. Thus the natural map from $\mathbf{GM(C, J)}$ to $\mathbf{GS(C, J)}$ is injective. That is to say, we can view the group of metabolic spaces as a subgroup of the group of all anti-hermitian spaces. Moreover, for any space $\mathbf{U}$, $\mathbf{[M(U)]=[H(U)]}$.

Consequently, we observe that **GM (C, J)** is generated by the hyperbolic spaces. The quotient group **G(S(C, J) / GM (C, J)=W (C, J)** will be called the Witt group of **(C, J).**

Let **E** and **F** be two non-degenerate anti-hermitian spaces over **(C, J)**. If there exist metabolic spaces **M** and **N** such that **E** $\perp$ **M** is isometric to **F** $\perp$ **N**, then we say that **E** and **F** are equivalent and denote it by **E ~ F**. The quotient **S(C, J)/~** is a group(for by lemma 3(ii), we have **E** $\perp$ **(- E) ~ 0)** and the natural map **S(C, J)** $\rightarrow$ **W (C, J)** factors through this equivalence. Moreover, **S(C, J)/~** $\cong$ **W (C, J)** since – **[E]** and **[- E]** of **GS(C, J)** have the same image and that it is surjective. From the universal property of **GS(C, J).** We have a canonical homomorphism from **GS(C, J) to S(C, J)/~** and it vanishes exactly on **GM(C, J).** Thus **W(C, J)** $\rightarrow$ **S(C, J)/~** is an isomorphism.

Example : Let **A** be a commutative ring and **C = A x A** with component wise operations. Let J $(a_1, a_2) = (a_2, a_1)$. Suppose (M, φ) is any non-degenerate anti-hermitian space over (C, J).If $e_1=(1,0)$ and $e_2=(0,1)$ then we can write $M= e_1M \oplus e_2 M$ so that each $e_i M$ is a projective A module. Now φ $(e_1M, e_1M) = \varphi (e_2 M, e_2 M) = e_2\varphi (M, M)e_2^* = 0$. Defined : $e_2 M \rightarrow (e_1M)^* = Hom_A(e_1M , A)$ by $d(e_2 y)(e_1x)=\varphi (e_1x, e_2 y)= e_1\varphi(x, y)e_2 = e_1\varphi (x, y)e_1= e_1e_1\varphi (x, y)= e_1\varphi (x, y)$ (as $e_1 =e_2^*$ and e_1 belong to the cenre of C). Thus the non-degeneracy of φ => d is an isomorphism. Therefore, we have an isomorphism T: M $\rightarrow$ e_1M x $(e_1M)^*$ given by $T(e_1x + e_2 y) = (e_1x, d(e_2 y))$. Here C acts on e_1M x $(e_1M)^*$ co-ordinatewise. The map ψ on e_1M x $(e_1M)^*$ x e_1M x $(e_1M)^*$ given by ψ ((x, f), (y, g)) = (g(x), - f(y)) is an anti-hermitian form. For, ψ ((y, g),(x, f))=(f(y), –g(x))=(–g(x),f(y))* = – ψ ((x, f),(y, g))*. Thus, T is an isometry with respsct to φ and ψ. Thus we see that up to isometry every non-degenerate anti-hermitian space over (C, J) can be put in the form (UxU*, ψ) where U is a projective A module and $U^* = Hom_A(U, A)$, where A= {a in C : J(a)=a }. Especially, if (M, φ) is any non-degenerate anti-hermitian space over (C, J), then M is isometric to $H(e_1M)$. Then W(C, J) = 0. If P(A) is the semi group of isomorphism classes of projective A modules then GP(A) $\cong$ G(C, J).

BASE CHANGE

Let (C, J) and (C', J') be two rings with involutions and **f : C** $\rightarrow$ **C'** be a ring homomorphism such that J' . f = f. J. If (M, φ) is an anti-hermitian space over (C, J) then we get an anti-hermitian space over C' by taking M' = M $\otimes$ C' with the anti-hermitian form φ' given by

φ '(c' $\otimes$ x, d' $\otimes$ y) = c'φ (x, y)J'(d') for x, y in M and c', d' in C'.

One can verify that (M', φ') is an anti-hermitian space. Further (M', φ') is non-degenerate (Metabolic) if (M, φ) is non-degenerate (Metabolic). Thus we get homomorphisms,

G (f) : G(C, J) $\rightarrow$ **G (C', J')** and **W (f) : W (C, J)** $\rightarrow$ **W(C', J') .**

Let **(C, J)** be a ring with an involution and A

in C we see that c is a root of $x^2 - (c + c^*) x + cc^* = 0$. Clearly $c + c^*$ and cc^* are in A. Thus we find that C is an integral quadratic extension of A. We call cc^* the norm of c. If (M, φ) is an anti-hermitian space over (C, J) and for x in M, then set $|x|^2 = \varphi(x, x).\ \varphi(x, x)^*$. Not that $|x|^2$ is in A for all x in M.

We say that (C, J) is connected if C has no factorisation as the product of two rings such that $C = C_1 \times C_2$ and $J = J_1 \times J_2$ where J_i is an involution on C for i = 1,2.

Lemma 4 . If (C, J) is connected then, A = {c in C:J(c) = c} has no non-trivial idempotents. Secondly, C has no non-trivial idempotents or (C, J) is isomorphic to (A x A, J') where $J'(a_1, a_2) = (a_2, a_1)$.

Proof : If $e \neq 0$, 1 is an idempotent of C , then $e \cdot e^* \neq 1$ is an idempotent of A.

$e^2 = e$ and $e^{*2} = e^*$, since $(e.\ e^*)^2 = e^2.\ e^{*2} = e \cdot e^* \Rightarrow e \cdot e^* = 0$. Thus $(e + e^*)^2 = e^2 + e^{*2} + e \cdot e^* + e^* \cdot e = e + e^*$, since $e.\ e^* = 0$ and $e + e^* \neq 0 \Rightarrow e + e^* = 1$ Therefore, $C = C.e + C.e^*$. Define a ring homomorphism φ: A x A $\rightarrow$ C, by $\varphi(a_1, a_2) = a_1.e + a_2.e^*$. Then φ is an isomorphism. Because if $a_1.e + a_2.e^* = 0$, then $a_1.e = - a_2e^*$ and $a_1 = a_1(e + e^*) = a_1.e + a_1.e^* = - a_2.e^* + a_1.e^* = (a_1 - a_2)e^*$, $a_2 = a_2(e + e^*) = a_2.e - a_1.e = (a_2 - a_1)e = 0$. Since $a_1.e = a_1.e^2 = - a_2.e^*.e = 0$.

Similarly $a_2.e = 0$. So φ : Ax A $\rightarrow$ C is an isomorphism.

If J': A x A $\rightarrow$ A x A defined by $(a_1, a_2) \rightarrow (a_2, a_1)$ then $J \circ \varphi = \varphi \circ J'$. $J(\varphi(a_1, a_2)) = J(a_1e + a_2.J(e)) = a_1.J(e) + a_2.e = a_2.e + a_1.J(e) = \varphi(a_2, a_1) = \varphi(J'(a_1, a_2))$, since C is commutative under addition. Thus $J \circ \varphi = \varphi \circ J'$.

From now on we shall assume that C is a semilocal ring which is commutative.

Lemma 5: Suppose $(C, J) = (C_1, J_1) \times (C_2, J_2) \times \ldots\ldots \times (C_t, J_t)$, where (C_i, J_i) is connected for i = 1,2,.....t. The factorisation induces an isomorphism of the semi-group (S, J), the Grothendieck group GS (C, J) and the Witt group W (C, J) respectively with the direct sum of $S(C_i, J_i)$, $GS(C_i, J_i)$ and $W(C_i, J_i)$.

Proof: Suppose C has only s-maximal ideals, any factorisation of C into components must have length $t \leq s$. Therefore, there exists a factorisation of the given type which can not be refined further and each component is connected. Let e_i be the identity of C_i for $1 \leq i \leq t$. If (M,φ) is any anti-hermitian space over (C, J), then writing $M = \oplus e_i M$ and $\varphi_i = \varphi / e_iM$ and observing $\varphi(e_ix, e_jy) = \delta_{i,j}.e_i.\varphi(x,y)$, we see that (e_iM, φ_i) is an anti-hermitian space over (C_i, J_i) where $J_i = J/C_i$. Further $(e_iM, \varphi_i) \perp (e_jM, \varphi_j)$ whenever $i \neq j$. Moreover (M, φ) is metabolic if and only if all (e_iM, φ_i) are so. Consequently $S(C, J) \cong \Pi_i\ S(C_i, J_i)$ and $GM(C, J) \cong \Pi_i\ G\ M(C_i, J_i)$. These along with the universal properties of Grothendieck groups gives the desired isomorphisms.

In case C is connected, we have the following.

Lemma 6: If (C, J) is connected, then every non-degenerate anti-hermitian space over (C, J) is free.

Proof: As (C, J) is connected, it has no non-trivial idempotents. Consquently every projective C module is free. Supposing (C, J) = A x A with J/A = identity and J (a, a') = (a', a), we see that if (M, φ) is a non-degenerate space over (C, J), then M $\cong$ U x U*, where U is a projective A-module and U* = Hom$_A$ (U, A). Now A has no non-trivial idempotents. Therefore U is free over A and rank.U = rank.U*. Thus M $\cong$ U x U* is a free C = A x A module.

Lemma 7: Let C be an arbitrary commutative ring with an involution J and (M, φ) be a non-degenerate space over (C, J). If Rad. C is the Jacobson radical of C then

(i) ($M/(Rad.C)M$, $\bar{\varphi}$) is an anti-hermitian space over C/Rad. C with φ defined by $\bar{\varphi}(\bar{x}, \bar{y}) =$

$\pi\varphi(x, y)$, where $\bar{x}$ denotes the image of x in M/(Rad.C)M and π is the natural projection of C on C/Rad.C

(ii) If ($M/(Rad.C).M$, $\bar{\varphi}$) has an orthogonal basis $y_1, y_2 \ldots\ldots y_n$ then there exists an orthogonal basis $x_1, x_2 \ldots\ldots x_n$ for (M, φ) with $y_i = \bar{x}_i$ for $1 \leq i \leq n$.

Proof :

(i) The proof is omitted as it is straight forward.

Let x_1 in M be such that $\bar{x}_1 = y_1$. Then $\varphi(x_1, x_1)$ is mapped onto a unit in C/ Rad.C because $\bar{\varphi}(y_1, y_1)$ is a unit. Consequently $\varphi(x_1, x_1)$ is itself a unit. Hence any x in M can be expressed as

$x = x - \{\varphi(x, x_1) / \varphi(x_1, x_1)\}.x_1 + \{\varphi(x, x_1)/\varphi(x_1, x_1)\}.x_1$, where $x - \{\varphi(x, x_1)/\varphi(x_1, x_1)\}.x_1$ belongs to the orthogonal compliment of x_1. Thus $M = C\,x_1 \perp (C\,x_1)^{\perp}$. Applying the projection map π from M to M / (Rad.C) M to this decomposition we get $M/(Rad.C)\,M = (C/Rad.C).y_1 \perp \pi(C\,y_1)^{\perp}$. Clearly $(\pi C.y_1)^{\perp} = (C/Rad.C).y_2 \perp \ldots\ldots\ldots \perp (C/Rad.C).y_n$ Now $(\pi C y_1)^{\perp}$ is non-degenerate. By induction the result follows.

Definition 2: A non-degenerate space **(M, φ)** over **(C, J)** is called proper if φ **(x, x)** is a unit in **A** for some x in **M.**

Now the previous lemma enables us to consider the case when Rad.C = 0 and the decomposition of (C, J) is as a direct sum of fields and ring of the form A_i x A_i, where A_i is a field and J_i (a, b) = (b, a). If (M,φ) is a proper anti-hermitian space over (C,J) with Rad.C = 0, then we have (M , φ) = Π_i (M_i , φ_i) where (M_i , φ_i) is a non-degenerate space over (C_i, J_i) and that each (C_i ,J_i) is proper. In particular if C_i is a field, φ_i is not alternating. If $C_i = A_i$ x A_i where A_i is a field and J_i (a,b) = (b ,a) then for these (M_i,φ_i) has the form (U x U*, ψ_i) with U as a vector space over A_i, U* = Hom$_{Ai}$ (U, A_i) and ψ ((u, f) , (v, g)) = (g(u), – f(v)). If $u_1, u_2, \ldots\ldots u_r$ is any basis of U and $u_1^*, u_2^* \ldots\ldots u_r^*$ is the dual basis of U*, then for $j \neq k$, $\psi((u_j, u_j^*), (u_k, u_k^*)) = (u_k^*(u_j), -u_j^*(u_k)) = (0, 0)$, so that ($u_j$,u_j^*) is an orthogonal basis for (M_i, φ_i) , from these considerations and the orthogonal decomposition Π_i (M_i, φ_i), we get an orthogonal basis for (M, φ).

Suppose (C, J) is semilocal ring with involution and (M, φ) is an anti-hermitian free space over

(C, J), B and B' are two orthogonal basis for (M, φ). We say that B and B' are n-connectable if there can be found orthogonal basis $B = B_1, B_2, \ldots\ldots, B_k = B'$ such that B_i differs from B_{i+1} in atmost n-places. This is just a generalization of adjacent basis. The following lemma shows that lifting of basis to M from M / Rad.C.M is two connectable. More precisely we have.

Lemma 8: Let (C, J) be a commutative ring with involution and (M , φ) be a free space over

(C, J). Let $B=\{y_1,y_2 \ldots\ldots,y_n\}$ and $B' = \{y_1', y_2' \ldots\ldots y_n'\}$ be two basis of (M, φ). If $\pi(y_i) = \pi(y_i')$ for $1 \leq i \leq n$, where π is a natural homomorphism from M to M/RadC.M then B is 2-connectable to B'.

Proof : We use induction on n and show that B is 2-connectable to an orthogonal basis $(y_1', z_2, \ldots\ldots z_n)$ with $\pi(y_i) = \pi(z_i)$ for $i = 2,3, \ldots n$ and $\pi : C \rightarrow C/Rad.C$, then $(z_2, \ldots\ldots z_n)$ and $(y_2, \ldots\ldots y_n)$ will be orthogonal basis for $\{Cy_1'\}^{\perp}$. By renumbering if need be, write $y_1' = y_1 + \Sigma_{i=2} r_i y_i$ where r_i in Rad.C and all $r_i \neq 0$ for $2 \leq i \leq t \leq n$. Use induction on t. For, if $y_1' = (1 + r_1)y_1$ then since $1+r_1$ is a unit in C, $\{y_1', y_2 \ldots\ldots y_n\}$ is an orthogonal basis of M which is 1-connectable to B and lemma is proved. If $t \geq 2$, set $y_1'' = (1+ r_1)y_1 + r_t y_t$ then $\varphi(y_1'', y_1'') = (1+ r_1)(1+ r_1^*)\varphi(y_1,y_1) + r_t\varphi(y_t,y_t)r_t^* \equiv \varphi(y_1,y_1) \pmod{Rad.C}$. Hence $\varphi(y_1'',y_1'')$ is a unit of C . Therefore, $Cy_1 \perp Cy_t = Cy_1'' \perp Cy_t''$ where $y_t'' = y_t - \{\varphi(y_t,y_1'') / \varphi(y_1'',y_1'')\}.y_1''$.

Now the orthogonal basis $B'' = \{y_1'', y_2, \ldots\ldots y_{t-1}, y_t'', \ldots y_n\}$ is clearly 2-connectable to B. Moreover, $\pi(B) = \pi(B'')$ and $y_1' = y_1'' + \Sigma_{i=2} r_i y_i'$. This completes the proof.

Lemma 9 : Assume (C, J) is connected with Rad.C = 0 and (M,φ) is a non-degenerate anti-hermitian space with an orthogonal basis $B=\{x_1,x_2,\ldots\ldots, x_n\}$. Let $y_1 = \sum_{i=1}^{t} c_i x_i$ with all $c_i \neq 0$ be an element of M that can be augmented to an orthogonal basis of M.

1. If $(C, A) \neq (F_4, F_2)$ or (F_2, F_2) then there exists an orthogonal basis $\{y_1, z_2, \ldots\ldots, z_n\}$ which is 2-connectable to B. Here, by F_q we denote a field with q-elements.

2. If $C = F_4$ and $A = F_2$ then there is an orthogonal basis $\{y_1, z_2, \ldots\ldots, z_n\}$ which is 3-connectable to B.

3. If $C = F_2$ then there is an orthogonal basis $\{y_1, z_2, \ldots\ldots\ldots, z_n\}$ which is 4-connectable to B.

Proof: As Rad.C = 0, we have A = {a in C: J(a) = a } is a field so that every element in A is a unit. The proof of the lemma now proceeds by induction on t.

When n =1, the proof is evident. Suppose $n > 1$ and there exists integers i, j with $1 \leq i \leq j \leq n$ and $\varphi(c_ix_i + c_jx_j , c_ix_i + c_jx_j) \neq 0$. Then we can extend $x_i' = c_ix_i + c_jx_j$ to an orthogonal base $\{x_i', x_j'\}$ of the space $Cx_i \perp Cx_j$ and we

have an orthogonal basis $B_1 = \{x_1, \ldots x_i', x_j', \ldots x_n\}$ which is 2-connectable to B, since y_1 is a C-linear combination of the $t - 1$ = elements of B_1. We get the proof by induction.

Suppose $a.J(a) = 1$ for all a not in A. If $b \neq 0$, b in A and a not in A, then

$1 = \varphi(b + a, b + a) = \varphi(a, a) + (a + J(a)).b + b^2 = 1 + (a + J(a)).b + b^2$. Thus $b(b + (a + J(a)) = 0$ so that $a + J(a) = b$ as charact.C = 2. This yeilds $b = a + J(a)$ for every $b \neq 0$ in A and we are reduced to the case when $A = F_2$ and $C = F_4$. In this case we take C = A (v) with $v^2 + v + 1 = 0$. As $t \geq 3$. By defining $x_1' = x_1 + x_2 + x_3$, $x_2' = x_1 + vx_2 + v^*x_3$, $x_3' = x_1 + v^*x_2 + vx_3$. $\{x_1', x_2', x_3'\}$ is an orthogonal basis for $Cx_1 \perp Cx_2 \perp Cx_3$. Now B is 3-connectable to B' = $\{x_1', x_2', x_3', x_4, \ldots\ldots, x_n\}$ and y_1 is a sum of $t - 2$ elements of B'. So induction again completes the proof.

3.C is a field and J is the identity on C.

We first of all see that $t < n$ = rank(M). Since y can be augmented to an orthogonal basis of M. There exists an element x in M with $n(x) \neq 0$ and $\varphi(y, x) = 0$.

Let $x = \Sigma_i a_i x_i$. Then $\varphi(y, x) = \varphi(y, \Sigma_i a_i x_i) = (\Sigma_i a_i x_i)\alpha$. Since the char.C = 2,

$0 = (\varphi(y, x))^2 = (\Sigma_i a_i^2 \alpha)\alpha$, so that $\Sigma_i a_i^2 \alpha = 0$ Now if $t = n$, $0 \neq \varphi(x, x) = \Sigma_i a_i^2 \alpha$, a contradiction.

3(a). $C \neq F_2$ and J = identity

Let $\alpha_n = n(x_n) \neq 0$. If $\alpha + c^2\alpha_n = 0$ for all $c \neq 0$ in C, then $\alpha_n = -\alpha$ and $-\alpha_n + c^2\alpha_n = -\alpha_n(1 - c^2) = 0 \Rightarrow c = \pm 1$ and hence $C = F_2$. Thus there exists c in C with $\alpha + c^2\alpha_n \neq 0$. Now set

$x_1' = (\alpha / \alpha + c^2\alpha_n).x_1 + (\alpha c / \alpha + c^2\alpha_n).x_n$

$x_n' = (c^2\alpha_n / \alpha + c^2\alpha_n).x_1 + (c\alpha / \alpha + c^2\alpha_n).x_n$

Then $\varphi(x_1', x_n') = (\alpha c^2\alpha_n / (\alpha + c^2\alpha_n)^2).\alpha + (c^2\alpha^2 / (\alpha + c^2\alpha_n)^2).\alpha_n$

$= 2c^2\alpha^2\alpha_n / (\alpha + c^2\alpha_n)^2 = 0$.

and $\{x_1', x_n'\}$ is an orthogonal basis for $Cx_1 \perp Cx_n$. Thus $x_1 = x_1' + x_n'$ and $n(x_n') = (\alpha c^2\alpha_n / \alpha + c^2\alpha_n) \neq \alpha$. The basis $B_1 = \{x_1', x_2, \ldots\ldots, x_n'\}$ is 2-connectable to B and in terms of B, $y = x_1' + x_2 + \ldots\ldots + x_t + x_n'$, which is a longer expression, but has $n(x_n') \neq \alpha$. Now set $x_2'' = x_2 + x_n'$ with $n(x_2 + x_n') = \varphi(x_2, x_2) + \varphi(x_n', x_n') \neq 0$, therefore there exists x_n'' such that $\{x_2'', x_n''\}$ forms an orthogonal basis for $Cx_2 \perp Cx_n'$. Thus basis B_1 is 2-connectable to $B_2 = \{x_1', x_2'', x_3, \ldots\ldots x_{n-1}, x_n''\}$ and we have $y = x_1' + x_2'' + \ldots\ldots + x_t$. Once again set $x_2''' = x_2'' + x_3$ with $n(x_2'' + x_3) = n(x_2 + x_n' + x_3) = n(x_n') \neq 0$. Therefore, there exists an element x_3''' such that $\{x_2''', x_3'''\}$ is an orthogonal basis of $Cx_2'' \perp Cx_3$. Now B_2 is 2-connectable to $B_3 = \{x_1', x_2''', x_3''', x_4, \ldots\ldots, x_{n-1}, x_n''\}$ and y is a linear combination of $t - 1$ elements of B_3. Which completes the proof in this case.

3(b). $C = F_2$.

We wish to show that B is 4-connectable to an orthogonal basis of M containing y. So

suppose

$n > 4$. We still have $t < n$. Set $M' = C\,x_1 \perp C\,x_2 \perp C\,x_3 \perp C\,x_4$ and set $x_1' = x_1 + x_2 + x_3$. Since $\alpha = 1$, we have $n(x_1') = 1$ and hence by lemma 1, $M' = C\,x_1' \perp (C\,x_1')^{\perp}$. Since x_4 is in $(Cx_1')^{\perp}$ and $n(x_n)$ is a unit of C, the space $(C\,x_1')^{\perp}$ is proper and so by previous lemma has an orthogonal basis $\{x_2', x_3', \ldots x_n'\}$. Then $B_1 = \{x_1', x_2', x_3', x_4, \ldots x_n'\}$ is 4-connectable to B and $y = x_1' + x_4 \ldots + x_t$. Induction completes the proof.

Theorem : Let C be a semi-local ring with involution J. $(\tilde{E}, \varphi)$ be a proper free space over (C, J) and $B = \{x_1, x_2, \ldots, x_n\}, B' = \{y_1, \ldots, y_n\}$ be two orthogonal basis of E.

1. B and B' are 4-connectable.
2. If C has no maximal m with J(m) = m and $C / m = F_2$, then B and B' are 3-connectable .
3. If in addition C has no maximal ideal m with J(m) = m and $C/m = F_4$, $A/A \cap m = F_2$, then B and B' are 2-connectable.

Proof : We can suppose by changing the (renumbering) x_i's that $y_1 = \Sigma_i c_i x_i$ with $c_i \neq 0$.If Rad. C= 0 and (C, J) is connected, then lemma 9 shows that B is n-connected to an orthogonal basis $\{y_1, z_2, \ldots z_m\}$. Here n = 4 if $C = F_2$, n = 3 if $A = F_2$, $C = F_4$ and n = 2 otherwise. Since $\{y_2, \ldots y_m\}$, $\{z_2, \ldots z_m\}$are orthogonal basis of the proper free space $(Cy_1)^{\perp}$. Induction on m finishes the proof in this case.

Next, assume Rad. C = 0, then by Lemma 5 $(C, J) = (C_1, J_1) \times (C_2, J_2) \times \ldots \ldots \times (C_t, J_t)$, where

(C_i, J_i) is connected for i = 1,2,.....t and $Rad.C_i = 0$. Hence $E = E_1 \times E_2 \times \ldots \ldots \times E_t$ with E_i a proper free space over (C_i, J_i).Let e_i denotes the identity element of E_i . Then $\{e_i x_1, e_i x_2, \ldots\ldots, e_i x_n\}$ and $\{e_i y_1, e_i y_2, \ldots\ldots, e_i y_n\}$ are orthogonal basis of E_i. By lemma 9, these are n-connectable with n = 2 or 3 or 4. By adding the connecting chains of basis, the theorem is proved in the case Rad.C = 0. Finally, let $\pi : E \to E/(Rad.C)E$ be the natural map . Then the orthogonal basis $\pi(\mathbf{B})$, $\pi(B')$ of $\pi(E)$ are n-connectable for n =2 or 3 or 4.Let $\tilde{B}_i$ and $\tilde{B}_{i+1}$be two adjacent basis for $\pi(E)$ occurring in the chain between $\pi(\mathbf{B})$ and $\pi(B')$ and let $\tilde{E}_o$ be the C/Rad.C sub-module of $\pi(E)$ generated by those elements of $\tilde{B}_i$ which are not in $\tilde{B}_{i+1}$. Thus $\tilde{E}_0$ has an orthogonal basis u_h , h = 1,2 or h = 1,2,3 or h = 1,2,3,4. Further $\pi(E) = \tilde{E}_0 \perp \tilde{E}_1$.Where $\tilde{E}_1$ has its orthogonal basis $\tilde{B}_i \cap \tilde{B}_{i+1} = \{\tilde{W}_j\}$.By lemma 7, E has an orthogonal basis $B_i = \{u_h, w_j\}$ with $\pi(u_h) = \tilde{u}_h$ and $\pi(w_j) = \tilde{w}_j$ Now the elements of $\tilde{B}_{i+1}$ which are not in $\tilde{B}_i$ must span $(\tilde{E}_1)^{\perp} = \tilde{E}_0$. Thus let $\tilde{v}_h$,h = 1,2 or h = 1,2,3 or h =1,2,3,4 be the basis of $\tilde{E}_0$ belonging to $\tilde{B}_{i+1}$ and the corresponding basis of $B_{i+1} = \{v_h, w_j\}$ with $\pi(v_h) = \tilde{v}_h$ and $\pi(w_j) = \tilde{w}_j$ .Thus it follows that B is n-connectable with n = 2, or 3, or 4 to an orthogonal basis $B'' = \{y''_1, y''_2, \ldots y''_m\}$ with $\pi(y''_i) = \pi(y_i)$. By lemma 8, B'' is 2-connectable to B' It follows that B is n-connectable to B' with n =2 or 3 or 4. Now from lemma 9, B and B' are 2-connectable unless $(C/Rad.C, \tilde{J})$ contains as a direct

summand F_2 or a direct summand F_4 with J not identity on it . In the later case there is a maximal ideal m of C with J(m) = m and C/m = F_4. If J = identity on C / m = F_4 then it is clear that c^{-2} lies in A/A $\cap$m for every c in C/m Since A/m $\cap$ A is finite, forces that A/m $\cap$A = C/m. Thus the last hypothesis implies that J does not induce identity on C/m = F_4, which proves the theorem.

Remark : In case (C,J) is not necessarily connected it can be shown that Im. φ is the Grothendieck ring **KF(C, J)** of the semi-ring **SF(C, J)** of isometry classes of non-degenerate spaces over (C,J). If **WF(C, J)** is defined as **KF(C, J)/Z(H)** and ψ is still the natural composite, it can be seen that Ker. φ and Ker. ψ have the same descriptions as before.

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ABSTRACTS

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THE SOCIO-CULTURAL IMPACT OF DRUG ADDICTION ON THAI YOUTH: AN ANTHROPOLOGICAL STUDY

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Drug addiction in the recent decades has become a concern of every individual, community and nation. Mankind no doubt has been using and abusing drugs right form very beginning, but in the present century drug addiction has emerged as a cancer and has brought within its fold almost all the segments of the society. The technological revolution has brought about sweeping changes through out the world and has reduced the entire world to a global village. Knowledge explosion and technological revolution, on one hand, have brought an overall development and bestowed on humanity all the comforts, but on the other hand it has engulfed mankind in confusion and chaos and has made youth to resort to drugs.

Objectives of the study:

The study deals with the problems of narcotic drug on rural Thai Youth in Sisaket province, North-eastern region of Thailand. The aims and the objectives of this research work are as follows:

1. To find out the major socio cultural and economic determinants affecting the drug addict youth.
2. To find out the impact of drug addict youth on their family life.
3. To assess the attitude, beliefs and Thai values on the drug addict youth.
4. To attempt at some possible remedies to check and control the problem of drug addiction.

RESEARCH HYPOTHESES:

1. Youth making demands on their parents and family members are at great risk of getting drug addicted.
2. The incidence of drug addiction among youth is high among the higher socio-economic group of Thais. Drugs and substance smelling which are cheap (cost-wise) would have more clientele in rural set up.
3. There is a direct relationship between drug addiction and the family of the drug addict. Both cause and the remedy of drug abuse lies within the family itself, parents are the primary agent of de-addiction.

4. The incidence of drug addiction is higher in broken families. Broken families and peer group pressure are the two primary agents of youth drug addiction.
5. Drug addiction, by and large, is a male activity. Takers of opium, heroin and morphine are exclusively males.
6. Though religion plays a very important role in the way of living in a Thai Society, its significance in the cause of remedy of drug addiction is negligible or limited.
7. Drug addiction leads to crime and other illegal activities.

Findings of the research work:

The findings of this piece of research work, based on the formulated hypotheses are discussed as under:

1. Youth making demands on their parents and family member are at great risk of getting drug addicted. The stated hypothesis is invalidated in this study.
2. The incidence of drug addiction among youth is high among the higher socio-economic group of Thai. Drugs and substance smelling which are cheap (cost-wise) would have more clientele in rural set up. The first part of the hypothesis is partially validated whereas the second part is totally validated.
3. There is a direct relationship between drug addiction and the family of the drug addict. Both cause and the remedy of drug abuse lies within the family itself, parents are the primary agent of de-addiction. The hypothesis is validated.
4. The incidence of drug addiction is higher in broken families. Broken families and peer group pressure are the two primary agents of youth drug addiction and is higher in broken families could not be substantiated in absolute terms but peer group pressure is a strong agent of addiction is, by and large, validated.
5. Drug addiction, by and large, is a male activity. Takers of opium, heroin and morphine are exclusively males. The hypothesis stands validated.
6. Though religion plays a very important role in the way of living in a Thai society, its significance in the cause of remedy of drug addiction is negligible or limited. Religion certainly is not a cause for drug addiction but its role in remedial measures is valued, though in a limited way.
7. Drug addiction leads to crime and other illegal activities. The hypothesis stands validated.

The research findings reveal that the rural Thai Youth, both males and females, is passing through a critical stage where illiteracy, poverty and other social maladies are rampant childhood, youth and later-life socialization is in a flux owing to the influence of western standards and values. The tradition is slowly being eroded on account of urbanization and modernization. Drug addiction, as an epidemic wave invaded the West, is slowly and gradually making societies in the third world weak and crippled. It has been observed that in spite of the best intensions of the welfare-oriented government, the results, as intended, are not forthcoming. A sustained and concerted effort on the part of the government and people is required to tide our this period of crisis. We must admit that the problem of drug addiction is not unique to any particular country but the magnitude of this problem taking a monumental proportion in Thailand. Hence, social, economic, psychological dimensions must be considered to be important to count the menace of drug abuse.

UTILISATION OF AYURVEDIC SYSTEM OF MEDICINE AND THE HEALTH CARE SERVICES: AN ANTHROPOLOGICAL STUDY OF AN URBAN MILIEU

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Human health has been such a wide and complex subject that it is difficult to find simple solutions to every problems of the society regarding its well-being, as remaining healthy is the cumulative effect of social, economic and political factors on the behaviour of an individual. The goal of 'health for all' by 2000, accepted by Indian policy makers in 1978 at Alma Ata could not be achieved.

Objectives of the study:

The main aim of the present study is to find out the scope of Ayurvedic system of medicine in the field of health care services and people's perceptions towards the utilization of these services. The study is based upon the findings from Ayurvedic practitioners, the patients undergoing treatment and other persons or employees who are associated with this system of treatment. The objectives of the study are:

1. To probe into the particular type of health needs for which the services of Ayurvedic medicine have been sought by the patients.
2. To find out the various kinds of health services that are being received from different health centres.
3. Also are the services provided by the Ayurvedic centres (the quality of treatment and its scope) perform satisfactorily by really helping the patients?
4. Regarding the myths and beliefs of the people towards the Ayurvedic system of medicine, do people have faith in the system, or do they simply treat it as an alternative way of cure?
5. The factors that influence the people's thinking in approaching the system.
6. The scope of improvement of the existing health services.

Hypotheses:

The present study is based upon the following assumptions. These hypotheses have paced the way and guided the present study:

1. The popular perception for the traditional systems of medicine is that they have a limited scope of cure. The health services in Ayurvedic are not as widespread and advanced as in the case of biomedicine.
2. There is a significant difference in the dispensation of services in Ayurvedic system as compared to the biomedicine.
3. Majority of health services providers in Ayurvedic system are in the private sector. They are traditional medical practitioners like Vaids who have been practicing Ayurveda for generations.
4. In order to utilize the services available in field of health care, Ayurvedic system of medicine has been considered as the second choice. The first option always been biomedicine. This is possible because of some misconceptions about the validity of Ayurvedic system.

Findings of the Research:

The role of traditional medicines in providing the health care to the population in the developing countries in general and India in particular, needs to be investigated, understood and the findings should be incorporated while formulating the future health care policies. As the Indian government has reported that for 65% of its population, traditional medicine is the only available source of health care (WHO traditional Medicine strategy 2002-2005). The traditional nature of Ayurveda has greatly played a role in shaping the perceptions of people about this system, whether positive or negative. It has been found in this study that majority of the people consider this system as reliable, because of the use of plant based medicines. They feel that Ayurvedic therapies are closer to the nature and thus harmless to the body. Until now, popular perception was that the traditional medicines are cheaper and easily available, but this study has presented a rather different picture. Ayurvedic treatment when followed in its entirely takes longer time period and proves expensive to the major proportion of the population. This factor has even led some of the patients to abandon the treatment in between as they found it difficult to bear the burden of slow paced and expensive treatment. With the passage of time, the general practice of Ayurveda has also shown certain changes, particularly in the diagnostic techniques and in the field of dispensation of medicine. Today the practitioners of Ayurveda try to present themselves with modern looks, which is more of less similar to that in the biomedicine.

In view of the huge working structure and manpower in the country, adequate acceptability of Indian medicines and the enormous difficulty in making biomedicine available to the common man, modern health programmes should incorporate studies of ancient lore and empirical data which have survived scrutiny for thousands of years. The most necessary requirement today is to make the Ayurvedic system affordable and within the reach of common man.

LIMB BILATERAL ASYMMETRY AND FOOTPRINTS OF MALE ADULT GUJJAR POPULATION IN PARTS OF PUNJAB AND HARYANA

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As the title suggests, the study deals with bilateral asymmetry of the limbs and the investigation of footprints both in biological/physical and forensic anthropological context. The present study was conducted on adult male Gujjars inhabiting the Siwalik hills and adjoining plains in parts of Punjab and Haryana in the north east of Chandigarh city. The Gujjar population under study is sedentary and agriculturist having animal husbandry as a secondary occupation.

Objectives of the study:

(a) evaluate bilateral difference (left-right) in the most commonly measured dimensions for anthropological and other studies,

(b) study the significance of bilateral variation with respect to age changes,

(c) compare the various human body parts with respect to the magnitude of bilateral asymmetry

(d) study the influence of working handedness on the degree of bilateral asymmetry of the limbs.

(e) take geometrical and angular measurements of the footprint and foot outlines and anthropometry of the foot with respect to various standard landmarks.

(f) correlate stature with various length-breadth measurements of foot, footprint and foot outline.

(g) study the shape, size, alignment of toes, humps, crease etc. of foot so as to draw conclusions for individualizing characteristics.

(h) see the correlation of weight with footprints by subjecting 50 individuals to additional weights ranging from 5 to 20 kg.

This cross-sectional study is based on a sample of 1040 adult male Hindu Gujjars ranging in age from 18 to 30 years. The data were collected from a total of 16 villages; 14 in district Ropar of Punjab and 02 in district Panchkula of Haryana. Out of total 1040 subjects, 967 were right-handed and 73 were non-right handed. For the study of bilateral asymmetry, nineteen

measurements were taken independently on left and right sides of the each subject. The measurements- height acromion, height radiale, height stylion, height dactylion, height ilio-cristale, height tibiale, height sphyrion, hand breadth, wrist breadth, humerus bi-epicondylar breadth, femur bicondylar breadth, ankle breadth, biceps girth, calf girth, biceps skinfold, triceps skinfold, subscapular skinfold, supra-iliac skinfold, calf skinfold and body weight were taken on each subject with standard procedures and methods. From the first seven anthropometric measurements, six anthropometric dimensions viz. upper extremity length, upper arm length, forearm length, hand length, lower extremity length and lower leg length were derived. The total sample (N=1040) was divided into three subsamples (Younger right-handed subsample, 18 to 25 years, n=624; Older right-handed sub-sample, 26 to 30 years, n= 343; Nonright-handed subsample, 18 to 30 years, n=73) based on age and stated hand preference.

For the study of footprints, a total of 2080 footprints were obtained from left and right feet of 1040 subjects. Besides stature and body weight, measurements were taken independently on left and right feet of each individual. The fleshed foot, footprint and foot outline measurements include the maximum lengths of the foot from all the toes and breadths of the foot at ball and at heel, big toe pad length, big toe pad breadth, toe 1-5 angle of declination. All the measurements were taken according to the standard landmarks and procedures. Bilateral asymmetry (left-right) between the measurements on left and right side was calculated within each individual and for each of the measurement taken on fleshed foot, footprint and foot outline. Stature was estimated from various measurements of the fleshed foot, footprint and foot outline using two different methods i.e. division factor method and regression method. The reliability of estimating stature from these two methods was compared. Correlation coefficients between various fleshed foot measurements and footprint measurements; footprint measurements and foot outline measurements and fleshed foot measurements and foot outline measurements were obtained. Similarly, the correlation coefficients between these measurements with stature were obtained separately in each case. Correlation coefficients of the body weight with the measurements of these different kinds of footprints (normal weight footprints; footprints taken by subjecting the individual with 5 kg. weight; and then with 20 kg. weight.) were also obtained to see the relationship of body weight with these measurements (N=50; total 300 footprints of left and right sides). Body weight was estimated using the regression method from these measurements. The morphological features of the footprints were studied and information about their frequency was also recorded.

Research Findings:

The salient findings of the present study are that statistically significant bilateral asymmetry exists in upper and lower limbs of the human body in Gujjar population- an agricultural community. The degree of asymmetry not only differs from one part of the body to the other but also between various body segments. Most of the upper limb and lower limb dimensions are significantly asymmetric and larger on the right and left side respectively. It is further concluded that

handedness can contribute significantly to the development of bilateral asymmetries in the upper and lower limbs. The statistically significant and higher degree of bilateral asymmetry in the limbs of Gujjar population may be attributed to the involvement in the hard physical work. The age and body weight have no significant effect on the bilateral asymmetry of the limbs and do not show any definite trend.

It has also been concluded that footprints are of immense importance in estimating the stature and body weight of the person and ultimately leading to the identity of the person by studying various individualizing characteristics of the footprints. Division factor method and regression method employed for the stature estimation were compared for their reliability of accuracy and concluded that the reliability of prediction by the latter method is better. Although, a close relationship exists between the measurements of left and right side of the fleshed foot, footprint and foot outline, but in view of the significant bilateral asymmetry occurring in some of these measurements in Gujjar population, one should be careful in using the formula and the use of appropriate formula for the appropriate side is recommended. A close relationship between the body weight and various measurements of the footprints has also been established. It has also been observed that in a footprint taken from the individual who is subjected to 20 kg. additional weight, some of its dimensions are altered significantly. The formulae for estimating body weight from the measurements on normal weight footprints and the footprints taken when the individuals are subjected to 20 kg. additional weight, are provided. Studying the individualizing characteristics in detail confirms the fact that in establishing personal identity, the analyst may be as precise as in case of dermatoglyphic features.

BIOMEDICAL ANTHROPOLOGICAL INVESTIGATIONS ON EPILEPTICS

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The study of human diseases has been traditionally a major focus of interest of biological anthropologists, because diseases have played a major role as agents of natural selection to channelise human evolution and group differentiation in man. Epilepsy is the disease of brain in which excessive electrical impulses are produced in the brain for a short period of time resulting in tremors of seizures. It is very heterogenous neurological disease and prevalence of epilepsy is not uniform in various human populations. Keeping in view, biomedical anthropological investigations on epileptics were done with the following major aims and objectives:

1. To study genetic variation for *PTC*, *ABO* blood groups, *ABH* secretions, and dermatoglyphic patterns of palms and finger balls in epileptic patients in comparison with controls.
2. To investigate the role of genetic markers in understanding the aetiology of disease and to investigate the heterogeneity of disease, if any.
3. To investigate socio-cultural and clinical correlates of epilepsy.

The data for the study were collected on a sample of 200 idiopathic, 200 Symptomatic epileptics and 100 controls from North-West India.

SALIENT FINDINGS

No rural-urban differences were observed. 39% of the patients were married. Twenty seven (17.3%) married epileptic women became pregnant and delivered normal children and eight (5.12%) did not become pregnant due to fear of having epileptic child. Three delivered epileptic children (1.92%). In 81.75% cases, a doctor was consulted within a week on occurrence of a fit. A large number of respondents (95.75%) had not taken any specific precautions. Practices like putting water in the mouth (8.75%), restraining of movements during fits (4%), forcing patients to smell a leather shoe (1.25%), massaging palm/soles (21%), putting a key/spoon in the mouth (1.25%) and religious measures (4.75%) were observed. 6.25% families had concealed the illness. School teachers were informed about the disease by 20.25% parents of epileptic children. 31.75% had informed neighbours. 7.5% had informed their employers. 2.5% families of married epileptics had informed their in-laws before marriage. Traditional healers were

consulted by 16% families.

Among epileptic patients, majority of the patients were non vegetarians (idiopathic – 54%, symptomatic – 64%). Familial occurrence of epilepsy was seen in 14% of idiopathic and 17.5% of symptomatic epileptic patients. The probands had first degree (idiopathic-9.5%, symptomatic – 12.5%) and second degree (idiopathic – 3.5%, symptomatic – 5%) affected relatives. Of the precipitating factors, unknown factors constituted the major group (idiopathic – 52.5%, symptomatic – 54.5%). Seizure seem to have serious impact on the scholastic and vocational abilities of the patients (idiopathic – 66.5%, symptomatic – 62.5%). Loss of memory was observed in 43.5% of idiopathic epileptics and 46% of symptomatic epileptics followed by poor performance (idiopathic – 23%, symptomatic – 16.5%). Psychiatric disorders (idiopathic – 3%, symptomatic – 3%) and behaviour problems (idiopathic – 4%, symptomatic – 4%) were also observed. Neurological investigations reveal that *EEG* was normal in 66.5% of idiopathic epileptics and 68.5% of symptomatic epileptics. Computed tomography (CT) scan was normal in all idiopathic epileptics and 8% of symptomatic epileptics. Magnetic resonance imaging (MRI) was abnormal in equal number of patients, i.e., 8.5% each.

GENETIC MARKERS

ABO blood groups

The results of *ABO* blood group system revealed a relatively high frequency of the blood *B* followed by *O, A* and *AB* respectively in both idiopathic and symptomatic epileptics. The control group also showed the similar result. The relative incidence among idiopathic epileptics was higher in *O* versus *AB* (2.44%) phenotypes followed by *A* versus *AB* (2.3%) phenotypes and the lowest was for *A* versus *B* (0.4%) combination. Symptomatic epileptics also show similar results, though statistically insignificant.

ABH secretions

30% of the idiopathic epileptics were non-secretors, while 18% of the symptomatic were non-secretors. The frequency of non-secretors in control group was 37%. The gene frequency in idiopathic epileptics is : *Se* (0.45), and se (0.55). The gene frequency in symptomatic epileptics is: *Se* (0.82) and se (0.18). The gene frequency in control is : *Se* (0.39), and *se* (0.61). The relative incidence values of *ABH* secretions for idiopathic epileptics (secretor versus non-secretor) was 1.3703, and for symptomatic epileptics it was (secretor versus non-secretor) + 2.6754. Chi-square test showed significant differences for *ABH* secretions in idiopathic versus symptomatic ($p \leq 0.01$) and symptomatic versus controls ($p \leq 0.01$) comparisons.

PTC taste sensitivity

In the present study, there was no sex differences in the incidence of tasters and non-secretors. In idiopathic 35.5% individuals with epilepsy were non-tasters, while in the symptomatic group

32.5% individuals were non-tasters. Chi-square test showed significant differences in the frequency distribution for taste thresholds between idiopathic epileptics and controls (p_0.01) and between symptomatic epileptics and controls (p_0.05). The relative incidence values of *PTC* tasting ability for idiopathic epileptics (taster versus non-taster) was 0.3921 and for symptomatic epileptics (taster versus non-taster) it was 0.5192.

Dermatoglyphics

Axial triradius was primarily placed (*t* position) in higher frequency in epileptics as compared to controls. Means of inter-digital counts (*a-b, b-c, c-d*) of the epileptic patients were significantly lower than that of controls. The main line *D* showed the lowest tendency to terminate at point 9 in epileptics (28.25%) than controls (39.5%). *D* line termination at position 11 showed a greater frequency in symptomatic epileptics than idiopathic epileptics and controls. Dankmeijer's index was lower in epileptics as compared to controls indicating a low frequency of arches in epileptic patients. Pattern intensity index was higher in epileptics as compared to controls indicating the number of triradii to be higher in epileptic patients.

The discriminant function analysis of the finger ball pattern scores and ridge counts was performed to study the traits which could discriminate the three groups (idiopathic, symptomatic and controls). The plotting of group centroids of the three groups for discriminant functions reveal supportive group centroids. The results of the present study, clearly revealed that computer aided diagnosis of idiopathic and symptomatic epileptics is possible with the help of a large battery of dermatoglyphic variables. Hence, dermatoglyphics can become an important tool in the field of medicine to diagnose various types of epileptic patients.

ULTRASONIC VELOCITY AND TRANSPORT STUDIES OF SOME ELECTROLYTES IN ACETONITRILE AND N, N-DIMETHYLFORMAMIDE MIXTURES

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The study of solvation behaviour of electrolytes in mixed solvents has been an important subject of solution chemistry during the last few years. Since in mixed solvents, the dielectric constant and viscosity of the medium can be varied to desired value, therefore, the solvation behaviour can be better understood in mixed solvents than in pure solvents. The studies of electrolytic solutions provide significant information about ion-ion interaction, ion-solvent interaction, structure making and structure breaking capacities of ions, specific interactions and preferential solvation of ions in solutions. With the advancement of technology and increasing applications of solution chemistry in research, industry and other fields, this field has been gaining importance decade by decade. These studies are becoming focus of attention from both the theoretical and practical points of view. Such studies have main applications in hydrometallurgy, energy generation in electrochemical cells and synthetic organic chemistry.

Acetonitrile (AN)and N, N-Dimethylformamide (DMF) have nearly the same dielectric constant (AN = 36.0 and DMF = 37.6) but both these solvents differ considerably in their solvating capacities. DMF solvates the cations to the large extent while AN strongly solvates both cations as well as anions. Binary mixtures of AN with DMF are therefore found to be good solvent systems for a comparative study of solvation behaviour of Cu^+ and Na^+ ions which are the ions of almost same ionic radius (Cu^+ = 0.09 nm, Na^+ = 0.095 nm) and are solvated by different mechanisms. The work in this dissertation presents a comparative study of the solvation behaviour of Cu^+ and Na^+ ions in AN + DMF mixtures containing 0, 10, 20, 40, 60, 75, 80, 90 and 100 mol % DMF using compressibility, viscosity and molar conductance measurements. There are four main aspects of the work.

1. **Solvent properties of acetonitrile + N, N-dimethylformamide (AN+DMF) and structural effects.**

   Physical parameters like density (p), viscosity (η), dielectric constant (ε) ultrasonic velocity (u) and isentropic compressibility (K_o) for AN + DMF mixtures containing 10, 20, 40, 60, 75, 80, 90 and 100 mol % DMF, have been measured at 298.15 K. The variation of these physical parameters has been examined as a function of mol % DMF in the

mixtures. The variation of excess functions $\Delta\varepsilon$, $\Delta\eta$, Δu and ΔK_o as a function of DMF mol % in all cases has been found non-linear and pass through small maximum or minimum which indicates some interaction between AN and DMF molecules. The magnitude of these interactions between 30-50 mol % DMF is stronger than at all the other compositions.

2. **Ultrasonic velocity and density measurements of salts in AN + DMF mixtures and evaluation of isentropic compressibility (K_s), apparent molal isentropic compressibility ($K_{s,\phi}$) and shear relaxation times (τ)**

Ultrasonic velocities (u) and densities (p) for Bu_4NBPh_4, Bu_4NClO_4, $CuClO_4.4CH_3CN$, $NaClO_4$ and $NaBPh_4$ have been measured in AN + DMF mixtures containing 0, 10, 20, 40, 60, 75, 80, 90 and 100 mol % DMF at 298.15 K. Using ultrasonic velocity and density data, isentropic compressibility (K_s,) for various salts in AN + DMF mixtures have been calculated using the equation

$$K_s = \frac{1.}{\mu^2 \rho} \qquad \text{.....(1)}$$

The shear relaxation time (τ) have been calculated by using the relationship proposed by Kinsler and Frey

$$\tau = \frac{4}{3} K_s \eta \qquad \text{.....(2)}$$

The K_s values are highest at lowest DMF mol % in the mixtures and these values decrease linearly with the increase in salt concentration in all the cases. Since the plots are almost non-linear, therefore the formation of ion-pairs is absent in all cases. This result is in agreement with the fact that in solvent systems having relatively high dielectric constant (ε), which remains nearly the same (between 36 and 37.6) at all the compositions of the mixtures, the ion pairing of the salts is not expected. The τ values for Bu_4NBPh_4, Bu_4NClO_4, $CuClO_4.4CH_3CN$, $NaClO_4$ and $NaBPh_4$ linearly increase with the increase of salt concentration. The order of relaxation time at a fixed concentration in AN + DMF mixtures is: 100 mol % DMF> 90 mol % DMF > 80 mol % DMF > 75 mol % DMF > 60 mol % DMF > 40 mol % DMF > 20 mol % DMF > 10 mol % DMF > 0 mol % DMF The results show that relatively stronger structural effects are present at higher salt concentration and at higher DMF composition in AN + DMF mixtures.

For getting quantitative information, the partial molal volume (V_ϕ) and the apparent molal isentropic compressibility ($K_{s,\phi}$) of the electrolytes have been calculated using equations

$$V_\phi = \frac{M}{\rho} - \frac{10^3[\rho - \rho_o]}{m\rho\rho_o} \qquad \ldots\ldots(3)$$

$$K_{s,\phi} = V_\phi K_s + \frac{10^3[K_s - K_o]}{m\rho_o} \qquad \ldots\ldots(4)$$

where p is the density of the electrolyte solution and p_o is that of the solvent mixture, K_s and K_o are the corresponding isentropic compressibilities, M is the molar mass of the salt and m is the molal concentration.

The limiting apparent molal isentropic compressibilities $K^o_{s,\phi}$ were obtained by extrapolation from the linear plots of $K_{s,\phi}$ vs $m^{1/2}$ by the least square method using the equation

$$K_{s,\phi} = K^o_{s,\phi} + A_{s,\phi} m^{1/2} \qquad \ldots\ldots(5)$$

where $K_{s,\phi}$ is the apparent molal isentropic compressibility, $K^o_{s,\phi}$ (limiting apparent molal isentropic compressibility) is the intercept, $A_{s,\phi}$ is the slope. $K^o_{s,\phi}$ values for $NaClO_4$, $CuClO_4$ and $NaBPh_4$ are negative at all the compositions of the solvent mixtures. For Bu_4NBPh_4 and Bu_4NClO_4, $K^o_{s,\phi}$ values, however, are positive at all the compositions of the mixtures. Negative or less positive $K^o_{s,\phi}$ values indicate strong structural or solvation effects. The limiting ionic compressibilities for various ions have been calculated. The $(K^o_{s,\phi})_\pm$ values of Na^+ are negative and larger in magnitude as compared to that for Cu^+ at higher DMF compositions. The values become more negaive for Na^+ with the increase of DMF composition, reach to a maximum value at 75 mol % DMF and then decreases in magnitude in pure DMF. The magnitude of $(K^o_{s,\phi})_\pm$ value for Na^+ is greater in DMF than in AN. On the other hand, $(K^o_{s,\phi})_\pm$ for Cu^+ is also negative and greater in magnitude in AN as compared to that in DMF. The magnitude of $(K^o_{s,\phi})_\pm$ values for Cu^+ is greater at the intermediate compositions between 40-75 mol % DMF than in pure AN or DMF. For Bu_4N^+ and Ph_4B^- $(K^o_{s,\phi})_\pm$ values are positive in all solvent systems and become more positive with the increase of DMF in the mixture. The $(K^o_{s,\phi})_\pm$ values for ClO_4^- are small but negative at all compositions. The negative $(K^o_{s,\phi})_\pm$ values for ClO_4^- indicate that this ion has some solvation in AN + DMF mixtures and the extent of solvation is more in the AN-rich region of the mixtures.

3. **Viscosity measurements and calculation of viscosity B-coefficients (B_E) using extended viscosity equation.**

Viscosities of Bu_4NBPh_4, Bu_4NClO_4, $CuClO_4$, $NaClO_4$ and $NaBPh_4$ have been

measured in the concentration range 0.02 – 0.6 mol dm^{-3} at 298.15 K in AN + DMF mixtures, containing 0, 10, 20, 40, 60, 75, 80, 90 and 100 mol % DMF. The viscosity data in all the cases have been first analyzed by using Jones-Dole equation.

$$\frac{\eta}{\eta_0} = 1 + AC^{1/2} + BC \qquad \cdots\cdots(6)$$

where C is the molar concentration of the electrolyte and A and B are constants. Since we made viscosity measurements at higher concentrations, therefore, an extended equation in the form

$$\frac{\eta}{\eta_0} = 1 + AC^{1/2} + BC + DC^2 \qquad \cdots\cdots(7)$$

was also used to analyze the viscosity data in the present case, where A, B and D are constants. The B values obtained using Jones-Dole equation, i.e. equation (6), denoted as B_J and obtained by using the extended equation, i.e. equation (7), denoted as B_E, are compared with each other. The B_J values in most cases are larger than the B_E values. These values in some cases differ from each other by reasonably good extent. This difference is due to formation of ion-pairs in several of these salts at higher concentrations in AN + DMF mixtures. The B_E coefficients in all cases pass through a maximum at around 60 mol % DMF in the mixtures. The B_E values for Bu_4NBPh_4 and $NaBPh_4$ are the largest and for $NaClO_4$, the smallest.

The ionic $(B_+)_E$ values for Na^+ and Cu^+ are larger than the $(B_-)_E$ values for ClO_4^-. This shows that Cu^+ and Na^+ are more strongly solvated than the ClO_4^- ion. Na^+ has stronger solvation than Cu^+ in AN + DMF mixtures. In both the cases (i.e. for Cu^+ and Na^+ ions) the $(B_+)_E$ value passes through a maximum between 40 to 60 mol % DMF in AN + DMF mixtures. Both these ions have thus maximum solvation between 40 to 60 mol % DMF. ClO_4^- also shows a maximum at about 60 mol % DMF.

4. **Conductance measurements**

Molar conductances of Bu_4NClO_4 and $NaClO_4$ in AN + DMF mixtures containing 0, 10, 20, 40, 60, 75, 80, 90 and 100 mol % DMF and for Bu_4NBPh_4, $CuClO_4$ and $NaBPh_4$ in AN + DMF mixtures containing 20, 60, 75 and 80 mol % DMF have been measured in the concentration range (4.0 – 65.0) x 10^{-4} mol dm^{-3} at 298.15 K. The conductance data in all cases have been analyzed for obtaining Λ_o and K_A values of the electrolytes by using Shedlovsky's equation. The Λ_o values in all cases decrease non-linearly as the mol % DMF in the mixture increases. The Λ_o values for Bu_4NBPh_4 $CuClO_4$ and $NaBPh_4$ in some AN + DMF mixtures taken from literature, also fall in good agreement with the values

measured at other compositions of AN + DMF mixtures in the present work. The limiting ionic conductances ($\lambda^{o}_{\pm}$) for various ions have been calculated by using Bu_4NBPh_4 assumption. The solvated radii (r_i) for various ions in AN + DMF mixtures have beer calculated by using the equation

$$r_1 = \frac{|z|F^2}{6\pi\eta N\lambda^0_{\pm}} + 0.0103\varepsilon + r_y \quad \text{.....(8)}$$

where r_i is the actual solvated radius of the ion and r_y is an adjustable parameter equal to 0.085 nm for dipolar aprotic solvents. In cases of Cu^+ and Na^+ the r_i values vary non-linearly with solvent composition and pass through a maximum in the AN + DMF mixtures. Cu^+ and Na^+ are strongly solvated and the solvation passes through a maximum and then decreases. Cu^+ shows stronger solvation in AN-rich region and relatively lesser solvation in the DMF-rich region of the AN + DMF mixtures. Na^+ on the other side is less solvated in An-rich region and relatively more strongly solvated in DMF-rich region of AN + DMF mixtures. ClO_4^- is very weakly solvated in AN-rich region of the AN + DMF mixtures.

USE OF UNCONVENTIONAL METHODOLOGIES IN THE SYNTHESIS OF ORGANIC COMPOUNDS

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The dissertation report work focused on the use of 'green' methodologies namely microwave energy, ultrasound and solid imported reaction for the synthesis of reactive inter-mediates.

Chapter-I deals with the speedy and regioselective 1,2-reduction of conjugated α,β-unsaturated aldehydes/ketones to the corresponding allylic alcohols and iodine in THF.

Chapter-II reports the solvent free oxidation of olefin using quinolinium-I_2 on microwave irradiation to furnish chloroiodoalkanes as the major product and iodohydrins a the minor product.

Chapter-III records the solvent free monobromination of diols/cyclic ethers and lactones to corresponding bromoalcohols and bromoacids in excellent yields.

In Chapter-IV, microwave promoted conversion of mesylates to iodides has been reported using anhydrous NaI object on neutral alumina within 3 to 4 min.

Chapter-V deals with selective oxidation of benzylic and allylic alcohols to corresponding aldehydes under microwave irradiation using quinolinium fluorochromate.

In Chapter VI, preparation of Schiff bases has been reported using may a mixture of aldehydes and amines on exposure to microwave radiation.

Part-I of Chapter VII deals with the first total synthesis of pentadecyl 6-hydroxydodecanoate , a component of a medicinal plant Artaborys odoratissimus.

Which part II embodies the first synthesis of aliphatic ester, undecyl hexadecanoate and a the long chain alcohol 12-hydroxyhentriacontane components of the plant ziziphous mauritiana.

Chapter VIII incorporates the first novel synthesis of two fatty acids 16-methyl-8(Z)-heptadecenoic acid and 16-methyl-6(Z)-heptadecenoic acid, isolated as the major fatty

acid components of the bacterium, Micrococus.

Chapter-IX deals with the use of solid support ultrasound and microwave energies for the synthesis of three phenyl heptanes namely 1-phenylheptane-1,5-dione, 7-phenylheptan-3-one and 7-phenylheptan-3-ol.

DESIGN SYNTHESIS AND CHARACTERIZATION OF 2-PICOLYL AND METHYL SUBSTITUTED 2-PYRIDYL CHALCOGEN (Se, Te) COMPOUNDS AND THEIR DERIVATIVES

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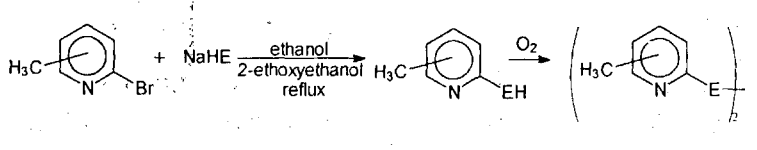
Chemistry of alkyl, aryl and mixed alkyl aryl diselenides/selenides and -ditellurides/tellurides is well documented and has lately been the subject of keen interest. Very little is known about the corresponding chemistry of pyridyl and picolyl derivatives because of the lack of convenient synthesis of these compounds. The primary objective of the present work was aimed at the design, synthesis and characterization of novel pyridyl andj picolylchalcogen compounds by convenient, economically viable and eco-friendly synthetic routes.

The work presented has been apportioned into five chapters. Chapter-1 gives a brief review of the literature of organoselenium/tellurium compounds. Chapter-2 deals with the detailed experiments carried out during these investigations. Chapter-3 describes the preparation and characterization of a number of methyl substituted 2,2'-dipyridyl diselenides/ditellurides, 2,2'-dipyridyl selenides/tellurides and 2-plyridylselenenyl halides. The number of methods employed for the preparation of various methyl substituted 2,2'-dipyridyl diselenides/ditellurides are being described below –

1. Sodium borohydride as a reducing agent

Sodium borohydride reduces elemental selenium/tellurium to NaHE or Na_2E_2 depending upon the molar ratio of the material used. 2-Pyridylselenols/tellurols were prepared by the reaction of sodium hydrogen selenide/telluride with the corresponding 2-bromopyridine in refluxing ethanol or 2-ethoxyethanol. These were subjected to aerial oxidation for the preparation methyl substituted 2,2'-dipyridyl diselenides/ditellurides.

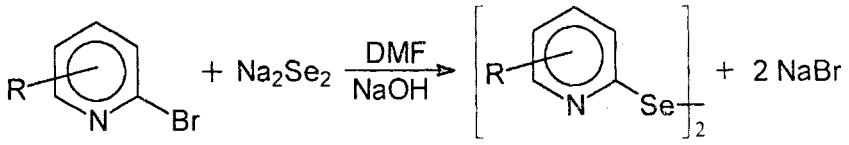


(E = Se, Te)

2. Hydrazine hydrate as reducing agent

Addition of 1.0 equivalent of hydrazine hydrate to 2.0 equivalent of elemental selenium in the presence of sodium hydroxide suspended in DMF under nitrogen atmosphere resulted in the formation of diselenide Se_2^{2-} anion. This solution was made to react with various 2-bromopyridines resulting in the formation of corresponding 2,2' –dipyridyl diselenide in good to excellent yields.

$$4\,Se + N_2H_2 + 4\,NaOH \longrightarrow 2\,Na_2Se_2 + 4\,H_2O + N_2$$



(R = H, CH_3)

3. Synthesis of diselenides and ditellurides under cryogenic conditions

Lithiation of pyridine using BF_3 was attempted for the synthesis of 2,2'-dipyridyl diselenide/ditelluride. The deprotonation of BF_3-complexed pyridine was accomplished at the 2-position with the highly hindered base lithium tetramethyl piperidine (LTMP) in ether at -78°C. Elemental selenium/tellurium was added to this solution of BF_3-complexed 2-lithiopyridine to give 2-pyridylselenolate/tellurolate anion which was converted to the corresponding diselenide and ditelluride on hydrolysis in the presence of oxygen.

4. Use of tributylmagnesiate complex, *n*-Bu_3MgLi for the synthesis of diselenides and ditellutides

n-Bu_3MgLi was utilized for bromine-magnesium exchange of various 2-bromo pyridine in toluene at -10°C. Addition of three equivalents of elemental selenium or tellurium followed by aerial oxidation gave nearly a quantitative yield of corresponding diselenides.

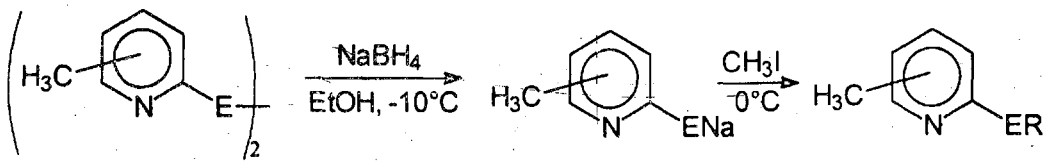
Single crystals of several compounds were grown and the structure of 6,6'-, 4,4'- and 3,3'-dimethyl-2,2'-dipyridyldiselenides, 5,5'-, 3,3'-dimethyl-2,2'-dipyridyl ditelluride and *tris* (2-pyridylseleno) methane have been solved by the X-ray crystallography.

Various methyl substituted 2,2'-dipyridyl diselenides were found to react readily with SO_2Cl_2 and elemental bromine under mild conditions to afford excellent yields of the corresponding 2-pyridylselenenyl chlorides and bromides.

The Chapter-4 deals with the preparation and characterization of unsymmetrical 2-pyridylchalcogenides and *bis-/tris* (2-pyridylseleno) methanes. Reductive cleavage of E-E

(E=Se/Te) bond in the already prepared 2,2'-dipyridyl diselenides/ditellurides was carried out using.

Sodium borohydride as a reducing agent in ethanol and subsequent alkylation with various electrophiles.



E = Se, Te

Hydrazine hydrate (with or without phase transfer catalyst) as the reducing agents followed by alkylation to afford *bis*- and *tris* (2-pyridylseleno) methanes.

Chapter-5 deals with the preparation and characterization of benzylic counterpart of 2-picolyl chalcogen compounds. These compounds were prepared by carrying out the lithiation of cheap and easily available 2-picoline with hindered base, diisopropylamide in THF at -78°C, followed by insertion of elemental selenium.

The use of 2-pyridyltellurides in organic synthesis was explored by carrying out the tellurium-metal exchange in 2-*(n)*-butyltelluro pyridines with *n*-Bu_3MgCl/*n*-BuMgCl at room temperature. A number of mercurials Hg $(SePy)_2$ were prepared by stirring mercury with a solution of methyl substituted 2,2'-dipyridyl diselenide in diethyl ether.

EXPERIMENTS TOWARDS THE SYNTHESIS OF NATURAL PRODUCTS AND RELATED COMPOUNDS

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The dissertation reports the synthesis some natural product and related compounds using non conventional methodologies such as microwave/ultrasonic energies and solid supports in combination to the conventional techniques.

Chapter-I deals with the synthesis of two sesquiterpene homologs using modified Wittig reaction as the step. Part I describes the synthesis of 10-oxo-3,7,11-trimethyldodecanoic acid isolated from marine green algal species caulerpa recemosa. Part-II describes an attempt to synthesize methyl 10-oxo-3,7,11-trimethyldodeca-(2E,6E)-dienoate, isolated from the stem bark of W. Central African tree cleritopholis Glauca.

Chapter-2 describes the synthesis of 3 insect pheromones using zinc-copper catalyzed conjugate addition of α,β-unsaturated carbonyl compound with an alkyl halide under aqueous sonochemical conditions. Part-I gives the synthesis of 1-hydroxynonan-5-one the male sex pheromone component of Bactrocera cacuminate. Part-II reports the synthesis of l-methylnonan-5-ol or Ferrugineol the major component of the aggregation pheromone produced by red palm weevil, Rhynchophorus ferrugineus. Part-III describes the synthesis of 2-methyloctan-5-ol, the aggregation pheromone component of Metamasius hamipterus.

Part-I of Chapter-III, includes studies towards the cyclisation of phenyl ester of α,β-unsaturated esters on microwave exposure. Part-II describes an attempt to cyclize different substituted cinnamanilides on solid supported media.

The fourth chapter reports microwave assisted one-pot synthesis of flavanones under aqueous conditions.

SYNTHESIS AND PHYSICO-CHEMICAL STUDIES OF SOME POLYMERIC COATING MATERIALS

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The physical and chemical properties of the resins or polymers determine the role which they will play in protective coatings. A knowledge of their origin, chemical composition, various structural aspects and physico-chemical properties goes a long way towards their proper utilization. There are many polymeric materials which can be used as coatings, but a few of them like alkyds, phenolics, amino and epoxies are widely used for different types of coatings due to their superiority over others in many aspects. Thus the following *synthetic resin* samples were synthesized and characterized by ultrasonic, dielectric and X-ray scattering studies :

1. Linseed oil modified alkyd resin
2. Styrentated alkyd resin
3. Resin modified phenolic resin (estenfied with pentaerythritol)
4. Rosin modified phenolic resin (esterified with glycerol)
5. Butylated urea-formaldehyde resin
6. Butylated melamine-formaldehyde resin
7. Bisphenol based epoxy resin (epoxy A, with Mol.Wt. 340)
8. Bisphenol based epoxy resin (epoxyl B, with Mol. Wt. 908)
9. Glycerol based epoxy resin (epoxy C, Mol.Wt.324)

The resins prepared were characterized by IR spectroscopy and other physico chemical parameter like acid value, epoxies equivalent, viscosity etc.

Cellulosics, especially cellulose esters and ethers have an important place in surface coating technology due to their superior physico-chemical properties and lower cost but the main drawback is their high flammability. The phosphorus containing cellulose esters (phosphates and thiophosphates) however are difficult to ignite, then a few (as given below) were synthesized and characterized by thermal, morphological and spectral studies.

1. Cellulose Phosphates:

a) Cellulose-6-(1-pyrrolidinyl)phosphate (CPP), samples prepared at 80, 90, 100, 110 and

120°C for 6 hr.

b) Cellulose diisoamylaminophosphate (CDSP) prepared at 80,85,90 and110°C for 8 hr.

2. Cellulose thiophosphates:

a) Cellulose phenylthiophosphate (CDSP) prepared at 80,85,90 & 110°C for 6 hr.

b) i) Cellulose diethylaminothiophosphate (CDSP) prepared at 100°C for 10, 20, 30 & 40 hr.

ii) Cr(II), Mn(II), Co(II), Zn(II) and Cd(II) complexes of CDSP (100°, 20hr.).

SYNTHESIS AND BIOLOGICAL INVESTIGATIONS OF A NEW SERIES OF β-ADRENERGIC BLOCKING AGENTS

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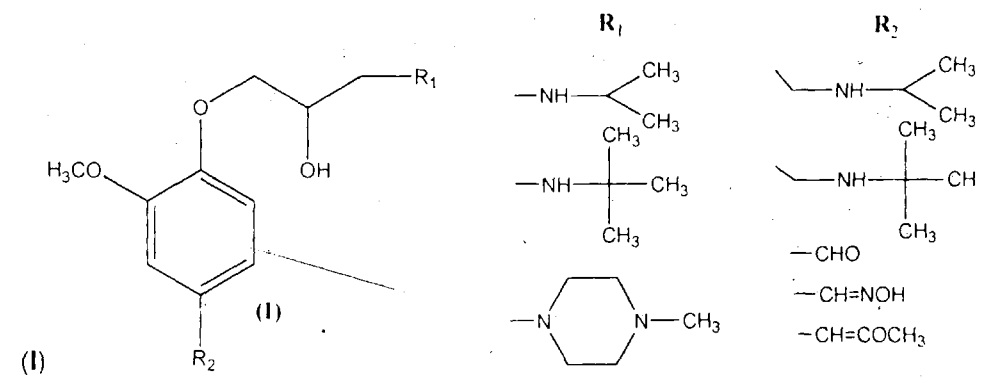
Hypertension has long been know to be a major factor in the development of coronary artery disease and cerebrovascular diseases. Use of β-adrenergic blocking agents for the treatment of hypertension has been well established for the past 30 years and recommended by US guidelines as one of the first line therapeutic agents for hypertension. Despite the availability of various classes of drugs, cardiovascular diseases are still the leading cause of death, and hence there is need to improvise upon the existing antihypertensive therapies. In view of this we have undertaken a research programme to find new compounds with β-adrenergic blocking activity.

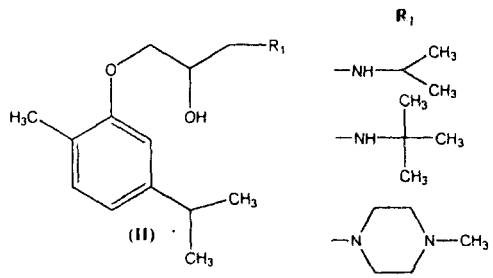
The aim of this study was to synthesize aryloxypropanolamine class of compounds using Vanillin and Thymol as starting material and test them for their β-adrenergic blocking activity. Also aryloxypropanolamines with para-amidic substituents were planned to be synthesized starting from 4-Aminophenol and 5-Amino-1-naphthol, so as to get cardioselective β-adrenergic blocking agents.

Vanillin and Thymol derived aryloxypropanolamines:

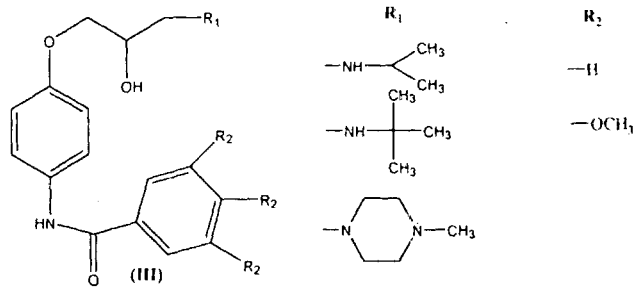
Vanillin (4-hydroxy-3-methoxybenzaldehyde) is converted to aryloxypropanalmine derivatives



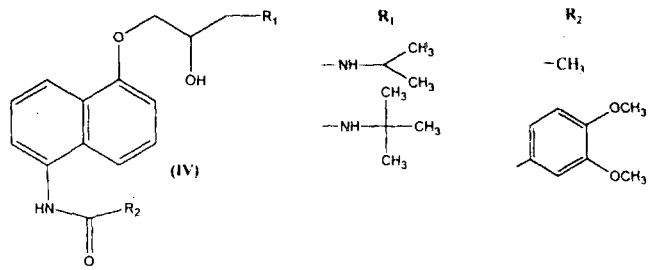
by standard method involving *O*-alkylation with epichlorohydrin, followed by epoxy ring opening with various amines such as isopropylamine, *tert*-butylamine and *N*-methylpiperazine. Similarly Thymol (2-isopropyl-5-methylphenol) was used to develop another series of compounds (II)



4-Aminophenol and 5-Amino-1-naphthol derived aryloxypropanolamines: In both the series the amino functionality is first converted to the required amidic functionality, followed by



transformation of phenolic group to oxypropanolamine side chain through *O*-alkylation with



epichlorohydrin, followed by epoxy ring opening with various amines gave 4-Aminophenol derived (**III**) and 5-Amino-1-naphthol derived (**IV**) aryloxypropanolamines.

Pharmacological assay: The oxalate salts of the aryloxypropanolamines were used for the assay. It was found that the thymol derivatives (**II**) had non-selective β-adrenergic receptor binding affinity similar to that of Propranolol, while the 4-Aminophenol derived (**III**) and 5-Amino-1-naphthol derived (**IV**) aryloxypropanolamines had selectivity towards β_1-adrenergic receptors similar to that of Atenolol (cardioselective β-adrenergic blocker). The log P values of the synthesized compounds were determined by shake flask method and correlated with the β-adrenergic receptor binding affinity, and found to have no correlation between them. The most active compounds, Thymol derivatives (**II**) were tested in animal models and found to posses β-adrenergic blocking activity. The findings of this work are published in reputed international journals.

PREPARATION AND EVALUATION OF CONTROLLED RELEASE ORAL DOSAGE FORMS OF GLIPIZIDE AND NIFEDIPINE

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The World Health Organization (WHO) had estimated that by the year 2025, the overall number of people with diabetes will go up to 300 million. Thus diabetes is growing into one of the most costly diseases on a global basis in both human and economic term. It is difficult to achieve the tightest level of glycemic control (to minimize microvascular and other complications) while at the same time avoiding even a slight degree of hypoglycemia (to avoid central nervous system tolerance to subnormal glucose levels). Studies have proved that chronic use of extended-release glipizide does not enhance the hypoglycemic effect of fasting plus mild exercise for people with NIDDM. The controlled release preparations of glipizide have shown better efficacy than immediate release dosage forms. In the present investigation, it was proposed to prepare sustained release dosage forms of glipizide.

Polyethylene oxide (PEO) is a non-ionic homopolymer of ethylene oxide. It has good water solubility, low toxicity, high swelling capacity, good compressibility and bioadhesion. Upon hydration it forms a hydrogel that can control the release of drugs via diffusion and, or erosion mechanism. The strength of the hydrogel is molecular weight dependent and greatly governs the primary drug release mechanism. In the present investigation, it was intended to exploit matrix system to achieve controlled release of glipizide using PEO. This study aimed at examining the range of concentration of polymers/combinations for preparation of glipizide tablets with parameters such as hardness or compressibility of the tablets. Of particular interest to the formulator is the comparative evaluation study of the drug release characteristics from such matrices and to quantify the differential release behaviour and specific formulation requirement.

Infact varying the polymer (PEO) content was found to be the most efficient way to control the release of the drug from the matrices. At extremely low polymer content, the hydrophobic effect of dicalcium phosphate resulted in low swelling tablets and the system behaved like an erosion system. Due to the hydrophobic nature of dicalcium phosphate, the entry of the penetrant into the matrix was relatively slower, thereby resulting in a low swelling. However, at a particular concentration, the hydrophilic effect of the polymer was high enough to form a gel that acted as a diffusion barrier. After that concentration the release of the drug decreased with increase in polymer content. But when lactose was used as a diluent, the release rate decreased with an

increase in polymer content due to the formation of an effective gel layer. Increasing the relative amount of drug in the formulations led to a decrease in the percent drug release due to increase in the water insoluble fraction of the matrix. When β-CD and PEG was incorporated into the formulations, it led to increase in release rate either due to diffusion of β-CD into the dissolution medium leading to reduction in tortuosity of the matrix or by increasing the solubility of the drug.

Nifedipine, a calcium channel blocker with peripheral and coronary vasodilator properties, is used in the treatment of angina and hypertension. Sustained release nifedipine formulations are generally better tolerated than their conventionally formulated counterparts, particularly with regard to reflex tachycardia. The present investigation, therefore also aimed at exploiting the potential of microsphere delivery system for controlled release of nifedipine.

Nifedipine loaded chitosan microspheres with relatively high yields and minimum aggregations were prepared by emulsification phase separation technique. The surface morphology of unloaded and nifedipine loaded microspheres was examined by SEM. Small imperfections such as roughness, cracks and drug particles embedded in the outer surface which can act as major routes for solute diffusion, were visualized by SEM. Incorporation of drug into the microspheres imparted roughness to the surface as was confirmed by SEM. In the present investigation, a simple technique was also employed to obtain cross-sections of unloaded and nifedipine loaded chitosan microspheres. Unloaded microspheres exhibited a uniform dense matrix structure while crystals of nifedipine were clearly visible in the drug-loaded microspheres. However, at 1.84% drug loading, no crystals could be seen in the microspheres indicating that either the drug was molecularly dispersed or dissolved in the matrix at this concentration. The physical state of nifedipine in chitosan microspheres was also confirmed by DSC and XRD studies. Nifedipine was found to be in crystalline state at all the loadings except at 1.84 % where no peak due to crystalline nifedipine was observed in DSC and XRD. At high Span 85 concentration (1.5% w/v), the external surface of the microspheres collapsed, but the internal structure remained dense. When the drug was dispersed in the chitosan solution with stirring during preparation, the entrapment was good and the shape of the crystals was changed. The internal structure of the microspheres following dissolution exhibited the presence of pores. The drug content in the microspheres had a marked effect on the size distribution. There was a considerable increase in statistical diameters of the drug loaded microspheres as compared to unloaded microspheres. The microspheres had good mucoadhesive properties. The amount of mucin adsorbed on the microspheres was inversely propotional to the loading of the drug. The microspheres showed good stability and swelling properties at high pH. The entrapment efficiency and percentage equilibrium swelling were found to increase with an increase in polymer content. The release of nifedipine from the microspheres was dependent upon the loading, the release was fast at low payloads and slow at high loadings.

THE SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW HETEROSTEROIDS OF MEDICINAL INTEREST

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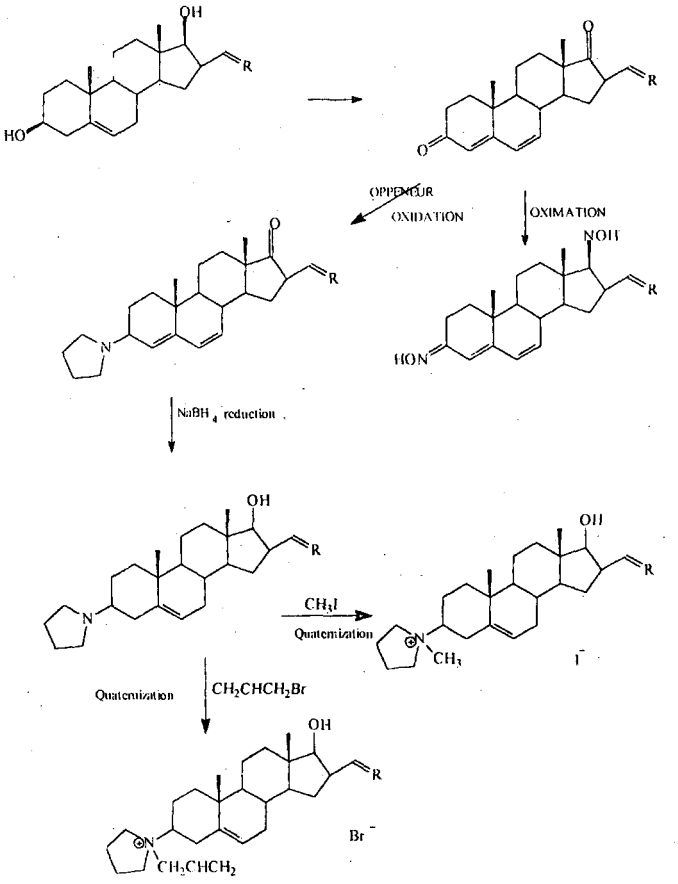
The drug designing encompasses the alteration of chemical and physical properties leading to the discovery of new chemical entities possessing the potential to be deemed as drugs. The synthesis of hetrosterods is based on empirical grounds and/or on rational drug design, which involves: i) study of the physiological mechanism, ii) pathophysiology of disease, iii) chemical exploration and its biological counter part and iv) heteromodification of basic steroid nucleus. A large number of heterosteroids have been synthesized with diverse activities like adrenocorticoidal, antimineralocorticoidal, androgen antagonist and biosynthesis



inhibitory, antiprogestational and progestational, estrogenic and antiestogenic, antifertility, antilipaemic, antimicrobial, cardiac, catatoxic, local anaesthetic, neuromuscular blocking, antineoplastic, etc. This work embodies the synthesis and study biological activities of some heterosteroids in epiandrosterone and dehydroepiandrosterone series related to neuromuscular blocking and antineoplastic activities. The rationale for this work is based on the difference in the interonium distances from the ideal distance of $10.4A^0$, as in the case of non-steroidal atracurium and steroidal pipecuronium.

The different aldehydes- nicotinaldehyde, piconaldehyde, p-methoxy benzaldehyde, 3,4 dimethoxybenzaldehyde, p-isopropylbenzaldehyde, p-nitrobenzaldehyde, p-cyanobenzaldehyde,4-(2-dimethylaminoethoxy)-3-methoxybezaldehyde, 3-methoxy-4-(2-mopholin-4-yl-ethoxy)benzaldehyde where attached to position 16 of steroidal nucleus by aldol condensation. Further modifications were performed at position 3 and 17 of steroidal nucleus using oximation, acetylation, oppenuer oxidation-followed by pyridine addition reduction and then quaternization. All the intermediates and final compounds were subjected to antineoplastic activity testing at National Cancer Institute, Bethesda, USA, against three cell lines- breast, brain and lungs-using one primary dose *invitro* bioassay. The neuromuscular blocking activity of synthesized quaternary compounds was tested at Strathclyde University, Glasgow, U.K.

Of all the selected compounds tested at NCI, Bethesda, many were found to be potentially active as antineoplastic agent when tested in *invitro* using three cell lines. The quaternary ammonium compounds that were tested for nicotinic receptor affinity in *invitro* were found to posses more affinity as compared to d-tubocurarine. The detail results of the testing are still awaited.

STUDY OF TRANSITION METAL DOPING IN CHALCOGENIDE SEMICONDUCTORS

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Among the amorphous semiconductors, chalcogenide glasses are one of the most widely known families of amorphous materials which have been extensively studied for several decades. In the middle of twentieth century, revolutionary work started upon chalcogenide glasses, and now at the beginning of twenty first century, although, we have obtained a fair amount of experimental data, several hypothesis and a few basic ideas, but a perfect theoretical model has not yet been suggested. Some models have been anticipated but, apart from one or two, none could every time explain the behaviour of amorphous semiconductors.

Common applications of chalcogenides semiconductors are switching and memory devices, optical mass memories, phases contrast holograms, infrared lenses, submicron optical lithography etc., but these materials have obtained a considerable importance in the field of solar energy conversion, image processing, fibre optics and integrated electronics etc. In spite of almost fifty years of technological advances, the field of amorphous semiconductors is still relatively new, and it is not surprising that our understanding of the physics of these important materials is not yet comparable with the level reached in crystalline semiconductors, therefore, the devices manufactured using these materials, are likely to take longer time to come to a level of industrial production.

The entire work reported in this thesis, refers to some chalcogenide semiconductors and study of dc/ac conductivity of these semiconducting vitreous alloys and the effect of some transition metal doping on their electrical properties. On the other hand an attempt has been made to explain the experimental results on the basis of existing models. This thesis has been divided into four chapters.

Chapter 1. This chapter essentially contains a brief introduction to amorphous semiconductors and their Classification. Conductivity in chalcogenide semiconductors and different possible mechanisms for ac conduction and some well established models are described briefly in this chapter. Correlated Barrier Hopping model (CBH model) which has been extensively used by author is discussed in detail.

Chapter 2 deals with the description of experimental procedure used for the preparation of materials and also preparation of samples for measurements. Different experimental set up and

equipments used for this work have been discussed. The chapter also involves the techniques used for measurements of the electrical properties of different chalcogenides.

Chapter 3. Effect of impurities in amorphous semiconductors in general and effect of transition metals in Chalcogenide glasses and effect on dc/ac conductivity in particular have been discussed. Generally, amorphous semiconductors are less sensitive to doping as compared to their crystalline counterparts. In this regards, Mott's explanation is well accepted. But, in some cases, when amorphous semiconductors are doped with transition metals, significant modifications in their properties take place even by diffusion of metals under external stimuli. This behaviour has several important applications in image transfer and processing, submicron lithography, memory devices, switching etc.

The chapter reports some new measurements on temperature and frequency dependent behaviour of dc and ac conductivity of silver and Iron doped As_2S_3. Author studied the changes occurring in the electrical properties of chalcogenide semiconductor As_2S_3 when doped with transition metal impurities (Silver and Iron). It has been found that ac conductivity decreases several times when Silver diffuses to As_2S_3. The ac conductivity of pure as well as Silver doped As_2S_3 obeys a well known relationship : $\sigma_{ac} \propto \omega^s$. Frequency exponents "s" was found to decrease with increasing temperature. The author successfully used CBH model to explain the entire behaviour of ac conductivity with respect to temperature and frequency in case of Silver doped As_2S_3.

It has been observed that Iron doping in As_2S_3 leads to the change in behaviour from p-type conductivity to n-type conductivity. The decrease in the value of density of states is also predicted by the experimental results. Best fits have been obtained using Correlated Barrier Hopping model (CBH) to explain the temperature and frequency dependence of ac conductivity.

Chapter 4, Ge-Se system exhibits properties which are unusual, and possibly unique, relative to other semiconductors. These unusual characters have obviously added much flavour to stimulated study.

The fourth chapter offers an account of the work done by the author on the study of electrical properties of chalcogenide semiconductor Ge_xSe_{100-x}. It may be mentioned that, in general, the conductivity in chalcogenide glasses at higher temperatures is dominated by band conduction (dc conduction). But, at lower temperatures, hopping conduction dominates over band conduction. A study at lower temperature can, eventually, provide useful information about the conduction mechanism and the defect states in the material. Therefore, the author chose to study electrical properties of Ge_xSe_{100-x} in the lower temperature region (room temperature). Temperature and frequency dependence of Silver doped Ge_xSe_{100-x} (x = 15, 20 and 25) has been studied over different range of temperatures and frequencies.

An agreement between experimental and theoretical results suggested that the behaviour of Germanium Selenium system (Ge_xSe_{100-x}) has been successfully explained by Correlated Barrier Hopping (CBH) model. Calculations show that the effective correlation energy U_{eff} is much more for Ge-Se system as compared to As_2S_3. This value increases with increase in Ge contents in the system. The results clearly indicate that the increase in the value of U_{eff} leads to the dominance of bipolaron hopping over single polaron hopping.

Mechanism of Silver dissolution to Ge-Se system has also been studied. It has been found out that, if the number of D° centres in the host material is less, then the ac conductivity increases after dissolution of Silver.

An attempt has been made to explain the results on the basis of Correlated Barrier Hopping model only.

IMPLICATIONS OF CHIRAL QUARK MODEL WITH CONFIGURATION MIXING FOR LOW ENERGY HADRONIC MATRIX ELEMENTS

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Chiral quark model with configuration mixing (χQM_{config}) is formulated by considering the baryon octet wavefunction to be of the form

$$|B\rangle_{config} = \cos\phi\ |56,0^+\rangle_{N=0} + \sin\phi|\ 70,\ 0^+\rangle_{N=2}$$

which arises due to the spin-spin forces among the constituent quarks. In the context of the **"proton spin problem",** a detailed analysis of spin polarization functions, quark flavour distribution functions as well as the hyperon β - decay parameters, has been carried out in χQM_{config}. The chiral quark model parameters have been fixed by using the well known sum rules having weak Q^2 dependence, for example, Bjorken sum rule, Ellis-Jaffe sum rule and Gottfried sum rule, whereas the mixing angle ϕ is fixed by fitting neutron charge radius. Using the latest data, we are not only able to improve the predictions of chiral quark model with broken SU(3)xU (1) symmetry but are also able to get a striking agreement in the case of Δ_3, Δ_8, F, D, f_s and f_3/f_8. The results corresponding to E866, NMC and HERMES data show good overlap, however, the agreement with data is best in the case of E866.

The above analysis is extended to the case of baryon octet and decuplet magnetic moments without taking any of the magnetic moments as input. In the case of octet magnetic moments, apart from configuration mixing and the *generalized Cheng- Li mechanism,* when the "mass adjustments" due to confinement are also included we are able to get an almost perfect fit for the case of $\mu(p)$, $\mu(\Sigma^+)$, $\mu(\Xi^0)$, $\mu(\Sigma|\Lambda)$ and the violation of Coleman-Glashow sum rule for the octet magnetic moments, whereas in all the other cases the results are within 5% of the data. In the case of decuplet magnetic moments, we obtain a good overlap for $\mu(\Delta^{++})$, $\mu(\Omega^-)$ and $\mu(\Delta|N)$, cases for which data are available.

To estimate the intrinsic charm content of the nucleon in χQM_{config}, the formalism considered above is extended to broken SU (4)xU (1) symmetry and its implications have been studied for the spin polarization functions and the quark flavor distribution functions. The predicted phenomenological quantities regarding the charm spin polarization functions, for example,

$\Delta c, \frac{\Delta c}{\Delta\Sigma}, \frac{\Delta c}{c}$ as well as the charm quark distribution functions, for example, $\bar{c}, \frac{2\bar{c}}{(\bar{u}+\bar{d})}, \frac{2\bar{c}}{(u+d)}$ and $\frac{(c+\bar{c})}{\Sigma(q+\bar{q})}$ are in agreement with other similar calculations. Specifically, we find $\Delta c = -0.009, \frac{\Delta c}{\Delta\Sigma} = -0.02,\ \bar{c} = 0.03$ and $\frac{(c+\bar{c})}{\Sigma(q+\bar{q})} = 0.02$.

Our analysis in χQM_{config} also reveals several interesting aspects about the partitioning of the nucleon spin among its constituents. For example, the gluon polarization (Δg) when calculated by considering $\Delta\Sigma\ (Q^2) = \Delta\Sigma - \frac{3a_s(Q^2)}{2\pi}\ \Delta g(Q^2)$, leads to $\Delta g = 2.33$ which is not only in good agreement with certain recent measurements as well as theoretical estimates but also leads to an almost perfect fit for spin polarization functions. When the implications of Δg are investigated in the case of octet magnetic moments, we find further improvements in our earlier estimates, for example, in the case of μ (p), $\mu(\Sigma^-)$, $\mu(\Xi^0)$, $\mu(\Lambda)$ and $\mu\ (\Sigma\Lambda)$, we get an almost perfect fit whereas in the case of $\mu(\Xi^-)$, a difficult case for most of the models, we find a considerable improvement. In case, we attempt to explain the "angular momentum sum rule"

$$\frac{1}{2} = \frac{1}{2}\Delta\Sigma + \Delta L_q + \Delta g + \Delta L_g,$$

we find the gluons not only contributing to spin polarizations but also having considerable amount of orbital angular momentum.

The present analysis strongly suggests that, at leading order, constituent quarks and the weakly interacting Goldstone bosons constitute the appropriate degrees of freedom with the Cheng-Li mechanism providing the dominant dynamics in the region between confinement scale Λ_{QCD} and the chiral symmetry breaking $\Lambda_{\chi SB}$, the weakly interacting gluons (*a la* Manohar and Georgi) contributing at the non-leading order.

STRAIN FIELD DUE TO IMPURITIES IN CONDUCTING MATERIALS

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The main objective of the current work is to study the behavior of impurities at the substitutional, and interstitial sites in transition metals (TMs). The present work is presented as thesis entitled, "Strain field due to impurities in transition metals". When impurity is introduced in a perfect crystal lattice, the crystal gets perturbed due to difference in the size of host and impurity atoms, as well as in the valence electrons. The difference in the size makes the lattice strained and the difference in the valency changes the electronic structure which further causes the strain. These two interrelated effects are considered to calculate strain field in transition metal dilute alloys due to transition metal impurities.

The present work is carried out considering the discrete nature of the lattice using Kanzaki lattice static method based on Bon-Von Karman theory. The method has been extended to the substitutional, interstitial and self-interstitial defects in *fcc* and *bcc* transitional metals. The dynamical matrix for *fcc* and *bcc* lattice are calculated considering few nearest neighbors, since screening is quite large in transition metals. The interionic interactions are considered through Wills and Harrison potential, Embedded atom method (EAM) and the Density functional theory (DFT).

The entire work in this thesis is presented in five chapters and important details of Wills and Harrison potential are given in the Appendix-A. The Chapter-1, contains introduction to the topic, starting with brief background and recent theoretical and experimental developments in transition metal alloys, the importance of defects in metals and their influence on the physical properties of the alloys are discussed.

In Chapter-2, Kanzaki lattice static method in harmonic approximation is described in detail and the relation between impurity induced forces and change in potential due to impurity is established. The relevant expressions for the forces and force constants are derived. The description of Wills and Harrison potential for the perfect and alloy systems is also given. The calculational procedure and the relevant approximations are described in detail. The calculations are carried for the Cr (V, Mn, Fe, Ni, Nb, Mo, Ta and W), V (Ti, Cr, Mn, Fe, Nb, Mo, Ta and W), Fe (Cr, Mn, Ni, Cu, Nb, Mo, W and Pt), Nb (V, Cr, Mn, Fe, Zr, Mo, Ta and W) and Mo (V, Cr, Mn, Fe, Zr, Nb, Ta and W) dilute alloys. In Cr, V, and Fe dilute alloys, the strain filed due to 3d impurities is found to be less as compared to 4d and 5d impurities. In Cr dilute alloy, the strain

field is minimum for V(3d) impurity and maximum for W(5d) impurity. In V dilute alloys, the strain field in minimum for Fe and Mn (3d) impurities and maximum for W(5d) impurity. In Fe dilute alloys, the strain field is minimum due to 3d impurities Cr and Mn, and is maximum for 4d impurity Nb.

In Nb and Mo dilute alloys, the strain field is found to be minimum for 4d and 5d impurities and maximum for 3d impurities. In Nb dilute alloy, the strain field is minimum due to Ta(5d) and Zr(4d) impurities and maximum for Mn and Fe(3d) impurities. In Mo dilute alloy, the strain field is maximum due to Mn and Fe(3d) impurities and minimum for W(5d) impurity. The calculated results are found in agreement with experimental values for VTi, FeCr, FeMn, FeCu, and NbZr dilute alloys. The calculated relaxation energies show that 3d impurities may be easily dissolved in Cr, V, Fe, where as 4d impurities in Nb and 5d impurities in Mo.

The formulation of Chapter-2 is extended to *fcc* metals in Chapter-3 to study the strain field in Ni, Cu, and Pd *fcc* transition metal hosts due to 3d, 4d and 5d transition metal impurities. The explicit calculations are carried out for Ni (Fe, Co, Cu, Nb, Mo, Pd, Pt and Au), Cu (Co, Ni, Pd, Ag, Pt and Au) and Pd (Fe, Co, Cu, Ni, Nb, Mo, Pt and Au) dilute alloys. The Wills and Harrison potential is used to evaluate the interionic interactions.

In Ni and Pd alloys, the maximum displacements of 4.6% and 3.8% of first nearest neighbor distance (1NN) are found for NiNb and PdNb respectively, while the minimum displacements of 0.63% and 0.23% of 1NN distance are found for NiFe and PdFe alloys. Except for Cu impurity, the atomic displacements are found to be proportional to the core radii and d-state radius. The relaxation energies for 3d impurities are found to be less than those for 4d and 5d impurities. In Cu dilute alloys, the lattice shows expansion due to Co, Pd, Ag, Pt and Au impurities and contraction due to Ni impurities. The maximum displacement of 2.3% of 1NN distance is found for CuAu alloy while the minimum displacement 0.43% of 1NN distance is found for CuNi alloys. The relaxation energies for Ni and Pd impurities are found less to be than Co, Ag, Pt and Au impurities. The calculated atomic displacements are found to be in good agreement with the experimental data for NiFe, NiPd, NiPt, NiAu, CuCo, CuNi, CuPd, CuAg, CuPt, CuAu, PdFe, PdCo, PdFe, PdNi, PdCu, PdPt and PdAu dilute alloys.

In Chapter-4, the strain field is calculated due to self-interstitial impurity in Ni. The formalism described for *fcc* metals, is used to find strain field due to interstitial defects. The EAM, used to study the interatomic interactions is also described there. The atomic displacements are calculated up to 20NN's of the self-interstitial impurity. The displacements are maximum at the 1NN's. The behavior of the atomic displacements is found to be oscillatory in nature and decreases in magnitude with increasing distance. The self-interstitial formation energy and volume change are also calculated from atomic displacements and are found in accordance with earlier studies.

We have also used EAM to calculate the strain field due to Cu, Pd, Pt and Au substitutional impurities. The maximum atomic displacement of 3.6% of 1NN distance is found for NiAu, while minimum displacement of 0.78% of 1NN distance is found for NiCu alloy. These results are compared with the results obtained using Wills and Harrison potential and it is found that results obtained using EAM are more close to the experimental values than those from Wills and Harrison potential.

In Chapter-5, the strain field is calculated due to interstitial hydrogen in Cu and Pd. The density functional theory is used to calculate interatomic forces between interstitial hydrogen and transition metal hosts (Cu and Pd). The norm-conserving pseudopotentials for H, Cu, and Pd are generated self-consistently in density functional formalism. The formalism described in the Chapter-4, is used to calculate the atomic displacements due to interstitial hydrogen at octahedral position in Cu and Pd lattice. The atomic displacements show maxima at the 1NN's of hydrogen in Cu as well as in Pd, however the magnitude of the lattice expansion is more in Cu lattice than in Pd lattice. The relaxation energy is less in Pd than Cu, therefore, hydrogen is more easily solvable in Pd than in Cu.

It is emphasized that the atomic displacements calculated due to 3d, 4d, and 5d impurities in *bcc* and *fcc* transition metals will be quite useful to investigate heat of solution, electric field gradients, asymmetry parameter, wipe out number, Knight shift, and other properties of defect lattice where the impurity induced displaced positions of the host atoms are needed. This will help in the basic understanding of the alloy formation. The *ab initio* study of self-interstitial in Ni and hydrogen in Cu and Pd is very promising. The use of density functional theory to calculate the strain field due to H in Cu and Pd confirms the lattice expansion of the hosts and excessive solubility of hydrogen in Pd.

ENERGY DENSITY FORMALISM FOR FUSION REACTIONS

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We have analyzed the heavy-ion collisions at low energies i.e. E (c.m.) < 20 MeV/nucl., within the framework of the Energy Density Formalism (EDF) using density dependent Skyrme forces. Our primary aim was to look for the effect of different model ingredients as well as to present an unified parameterization of potential and fusion barriers. We have applied EDF to alpha cluster transfer reactions in colliding s-d shell alpha and non-alpha nuclei. We have found that α-clustering effects exist in spin density part of heavy-ion potential only for colliding (N=Z, A=4n) α–nuclei if due to an α–particle transfer, atleast, one of the product nucleus becomes a closed j-shell nucleus for both protons and neutrons. Closed shells are also obtained for two-nucleons transfer products in the colliding α-nuclei, but then it is either a proton closed j-shell or a neutron closed j-shell and the same closed j-shell for both protons and neutrons. For non-alpha colliding nuclei, however, the discontinuity is found to occur also at the transfer of 2-nucleons irrespective to the shell closure effects. We have solved the stationary Schrodinger equation in mass asymmetry to get the mass transfer yields. We have calculated the particle strength and number of valance particles for each pair of the alpha-particle transfer. We have noticed that the variation of particle strength and valance particles is same with respect to mass asymmetry parameter 'η'.

We have studied the effect of different values of diffuseness on nucleus-nucleus interaction potential. We have compared the calculated fusion cross sections using our theoretical potential based on SEDF and the empirical potentials for symmetric and asymmetric nuclear reactions. Based on this finding, a new density parameters are also suggested which is in good agreement with other reported results.

We have done a detailed comparison of two earlier reported parameterization formulations by calculating the fusion cross sections and comparing with experimental values (see list of publications –no.-2). Next, we have analyzed more than 50 nuclear reactions and suggest a new parameterization for fusion barrier and heights. Our new parameterization can reproduce the barrier within few percentage.

At last, we have tried to parameterize the universal functions for different Skyrme forces within the framework of EDF. To find the analytical expression of the universal functions, large number of reactions were considered. The parameterized form of universal functions for different Skyrme forces is in good agreement with other theoretical potentials for the spin independent part. We

could also correlate the different constant values used in the parameterized form of the universal function with the Skyrme density parameters. Such a unified parameterization is valid for any even-even colliding nuclei.

In my thesis work, our main focus was to find the parameterization for interaction potentials and barriers apart from the study of different model ingredients. We still need detailed calculations with our new parameterization and their comparison with experimental data.

SOME INFERENCES UNDER ORDER RESTRICTIONS AND MULTIPLE COMPARISON PROCEDURES

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The work in this dissertation, presented in five Chapters, addresses the problems of testing the homogeneity of location (scale) parameters of $k(k\geq 2)$ members of a family of distributions against simple ordered alternative along with some related simultaneous confidence intervals for the parametric functions of the underlying distributions. The following practical situations, in which there is prior information about the inequality among the parameters of the k members of a family of distributions, form the basis of motivation of this work:

(i) the probability of a particular response (in dose-response experiments) may increase with the treatment (dose) level;

(ii) a regression function may be non-decreasing or convex or both;

(iii) the failure rate of a component increases as it ages;

(iv) the variability of the quality product may decrease due to renovations and improvements in successive production runs.

To answer the problems of testing the homogeneity of location (scale) parameters of the k members of a family of distributions against the simple ordered alternative, in this dissertation, we continue the studies due to:

(i) Govindarajulu and Gupta (1978), Rao (1982), Kochar and Gupta (1986), Kochar and Amita (1989), and Kusum and Bagai (1988) for testing the homogeneity of scale parameters against simple ordered alternative by taking $k\ (k \geq 2)$ members of symmetric location – scale family of distributions having common unknown point of symmetry but possibly differing in their scale parameters (Chapter-II).

(ii) Jonckheere (1954), Tryon and Hettmansperger (1973), Gobindarajulu and Haller (1977), Deshpande (1980), Shanubhogue (1988), and Amita and Kochar (1989) by taking $k\ (k \geq 2)$ members of a family indexed by location parameters (Chapter-III).

(iii) Hayter (1990) by taking $k\ (k \geq 2)$ exponential distributions having same scale parameter and possibly differing in location parameters (Chapter-IV). In Chapter–V, again restricting to the Hayter's (1990) approach, we addressed the problem of testing homogeneity of

scale parameters against simple ordered alternative by taking k ($k \geq 3$) members of location-scale family. An advantage of the test procedure due to Hayter is that it can simply be inverted to obtain 100 (1 - α) percent simultaneous one-sided confidence intervals for ordered pair-wise deviations of parameters. We have also shown that the constants of test procedures of Chapter-IV and Chapter-V maintain probability of Type – III error at level α.

In Chapter-II of this dissertation we have assumed that an observation from population π_i follows a distribution, which is a member of symmetric location-scale family, with cumulative distribution function (cdf).

$$F_i(x) = F((x-\mu)/\theta_i)$$

indexed by an unknown common point of symmetry $\mu \in R$ and unknown scale parameter $\theta_i \in R_+$, $i = 1,...,k$. Here $F(.)$ is any absolutely continuous cdf such that $F(x)+F(-x)=1$, i.e., $F(.)$ is symmetric about origin with probability density function (pdf) $f(.)$. For testing the null hypothesis $H_0:\theta_1=...=\theta_k$ against simple ordered alternative $H_1:\theta_1 \leq ... \leq \theta_k$, with at least one strict inequality, we have proposed a class of distribution free tests in which the test statistic is the weighted linear combination of consecutive two-sample statistics based on Hodges-Lehmann (1963) type of estimators $T_{i,i+1}$ of ratio of scale parameters θ_{i+1}/θ_i, $i = 1,...,k-1$. The distributions of test statistic under H_0 and for a sequence of local alternatives is discussed. The optimal members in this class of tests, obtained by finding the weighting coefficients which maximize the efficacy of the test in the proposed class, when the scale parameters are assumed to be equally spaced and sample sizes are all equal, are identified. Finally the proposed class of tests is compared with the other known tests in the Pitman asymptotic relative efficiency (ARE) sense and a numerical example, using real life data, is also given.

In Chapter-III, we assumed that the distribution associated with the data from population π_i is a member of location family with cdf $F_i(x) = F(x-\mu_i)$, where $\mu_i \in R$ and F(.) is any absolutely continuous cdf with associated pdf f(.) , i = 1, ..., k . For testing null hypothesis $H_0: \mu_1= ...= \mu_k$ against simple ordered alternative $H_1:\mu_1 \leq ... \leq \mu_k$, (with at least one strict inequality), we have proposed a class of distribution free tests in which the test statistic is the weighted linear combination of consecutive two sample U-Statistics derived from a kernal based on sub-sample medians. The test procedure and the results of this Chapter have been derived on the lines of Chapter-II for the balanced case, i.e,, by taking equal samples from all the populations. Pitman AREs of the optimal members of the proposed class for the sequence of equally spaced location parameters relative to some existing tests are obtained, with interesting results. We have used the approach of Govindarajulu and Haller (1977) or Kocher and Gupta (1986), by taking suitable

two sample statistics, to propose and derive the results of test procedures discussed in Chapters II and III.

In Chapter–IV, we considered $k(k\geq 3)$ independent exponential populations such that an observation from the ith population has probability density function (pdf) $f(x|\mu_i\theta_i) = 1/\theta_i \exp\{-(x-\mu_i)/\theta_i\} I_{(\mu_i,\infty)}(x)$, where $\mu_i > 0$, $\theta_i > 0$ and I(.) is the indicator function, $i = 1,...,k$. Test procedures for testing the null hypothesis $H_0 : \mu_1 \ldots \mu_k$ against simple ordered alternative $H_1:\mu_1 \leq \ldots \leq \mu_k$, with at least one strict inequality, are proposed in two situations: (i) $\theta_1 = \ldots = \theta_k = \theta$ (unknown) and (ii) all θ's equal to unity. For some significance levels $\alpha \in (0,1)$, exact critical points of each test procedure are tabulated for k = 3 , ..., 9 by solving two or three dimensional integral equations. Simultaneous one-sided confidence intervals for all ordered pair wise differences $\mu_j - \mu_i$ $(1 \leq i < j \leq k)$ and all nonnegative contrasts of μ_s , obtained by simple inversion of these test procedures, are discussed using these critical points. A multiple three-decision procedure, which maintains the probability of Type-III error at level α, is also proposed with the help of critical points of the test procedure.

Chapter-V addresses the extention of Hayter's (1990) approach to the scale parameter case under location-scale probability model. Consider k $(k \geq 3)$ treatments or competing firms such that an observation from the ith treatment or firm follows a distribution with cumulative distribution function (cdf) $F_i(x)=F[x-\mu_i)/\theta_i]$, where F(.) is any absolutely continuous cdf, i =1,...,k. We propose a test to test the null hypothesis $H_0:\theta_1=\ldots=\theta_k$ against the simple ordered alternative $H_1:\theta_1\leq\ldots\leq\theta_k$, with at least one strict inequality, using the data X_{ij}, $i = 1,...,k$; $j = 1,...,n_i$. Two methods to compute the critical points, at level α, of the proposed test have been demonstrated by taking k two parameter exponential distributions. The test procedure also allows us to construct simultaneous one sided confidence intervals (SOCIs) for the ordered pair-wise ratios of scale parameters θ_i/θ_j, $1\leq i<j\leq k$. The critical points of the test procedure are also used to propose a three-decision procedure for the two-sided pair-wise comparisons of the populations in terms of their scale parameters and it has been shown that the type-III error of such procedure is controlled at level α. We carried out statistical simulation, which revealed that: (i) actual sizes of the critical points are almost conservative and (ii) power of the proposed test relative to some existing tests is higher.

Remark: The contents of Chapters II to V of this dissertation have been published in various international journals (refer Gill and Dhawan (1996, 1999) Kumar *et al.* 1994., Dhawan and Gill (1997)).

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