INTRODUCTION
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Man has been using vegetable fibres for thousands of years, but the first reference to respiratory disease among textile workers (flax and hemp) was made by Bernardino Ramazzini, the Father of Occupational Medicine, in 1705. The disease, now known as byssinosis, was described in flax workers in the 18th century and in cotton industry workers in the 19th century. Byssinosis is an occupational disease of respiratory system induced after long term exposure to dust generated during the processing, cleaning and spinning of cotton, flax, soft hemp and sisal.

In 1831, Kay, a Lancashire physician, was the first in Britain to describe a respiratory disease in cotton workers. He termed this respiratory disease, which differed from chronic bronchitis, as "Spinner's Phthisis". The first British investigator Greenhow, in 1860, described the characteristic symptoms of both mill fever (the effect of cotton, flax or soft hemp dusts on the people at their first exposure) and byssinosis. By the second half of the 19th century it became evident that the cotton workers suffered from respiratory disease called "Byssinosis", the term which was first employed by Sir Thomas Oliver (1902) in Great Britain.

Epidemiological surveys of byssinosis among workers in cotton industry were started about 30 years ago, and it was first considered a negligible problem in many countries until its true prevalence rate was established. First this disorder was thought to be mostly confined to Lancashire cotton workers (Schilling, 1956) and to flax workers in...
Northern Ireland (Smiley, 1951), but then it was subsequently reported to occur from the countries like Scotland (Smith et al, 1962), Germany (Ehrhardt, 1964), Egypt (El Batawi, 1962), Greece (Dimoiliopoulos, 1963), Sweden (Belin et al, 1965), United States (McKerrow et al, 1958), India (Gupta, 1958), Taiwan (Tsai, 1964), Spain (Bouhuys et al, 1967), Belgium (Tuypens, 1961), Australia (Gandevia and Milne, 1965) and Israel (Chwat and Mordish, 1963). Thus the occurrence of byssinosis amongst the textile workers has been reported from almost all the countries, and it is now recognised as a world wide problem, not only in the developed countries, but also in the third world.

Cotton textile industry is the single largest organised industry having largest number of employment with the risk of exposure to cotton dust leading to respiratory disease, "Byssinosis". This industry accounts for roughly 20% of India's total industrial production and is one of the leading foreign exchange earners (International Textile Review, 1979). There are 661 textile mills in India, of which 370 are spinning and 291 are composite mills. Gujarat alone registers 112 mills (21 spinning and 91 composite mills). Out of the 112 mills, 66 mills are situated in Ahmedabad City itself and 46 are in the rest part of Gujarat. Total number of installed spindles and looms in India are 2,06,81,000 and 2,06,445, respectively (Times of India Directory, 1982). India occupies the second position in terms of spindles after China and fourth in terms of cotton consumption. There are 11,47,000 cotton textile workers in India (Pocket Book of Labour Statistics, 1982). India has the largest area in the world under cultivation of cotton, which is about one-fourth of the total world area for cotton cultivation. India can be divided into three major zones as regards the area
and production of cotton. They are: Central Zone, comprising of the States of Madhya Pradesh, Maharashtra and Gujarat; the Southern Zone, comprising of Karnataka, Andhra Pradesh and Tamil Nadu; and the Northern Zone, comprising of Punjab, Haryana, Rajasthan and Uttar Pradesh.

In India different varieties of cotton are cultivated. Three types of seeds are generally used in cultivation viz., Indian seeds, American seeds and Egyptian seeds. Varieties cultivated from Indian seeds are AK, 235/277, 197/3, Y-1, A-51/9, V-797, Karungani (Jayadhar and Suyodhar), CJ-73, Hampi, Kalagin Wagad, Bangal Deshi, Digvijay, Vijay, CJ-1, G-6 and SRT, of short to medium staple. Varieties cultivated from American seeds are Laxmi, Cambodia, C02/170, MCU 1 to 5, H4/H5, S4/H4, Niskar, L-147/1007, 320-F, H-14, J-34, Punjab American and Ac-122, of higher medium to long staple; while varieties cultivated from Egyptian seeds are Varalaxmi, Suvin, Sea Island Andrews, CBS-156, NH-12, Sujata, ISC-67/Guj-67, of extra long staple. Distribution of Indian cotton varieties is given in Fig.1.

The processes used in the manufacture of cotton yarn and cloth are ginning, baling, bale breaking, bale opening, scutching, carding, spinning, weaving and finishing. The functions of the processes in the cotton mill are as follows:

i) to divide up and clean the compressed cotton from the bale,

ii) to form the fibres into a rope or sliver which can be attenuated until it is thin enough to form threads,

iii) to comb out the fibres and set them parallel by drawing them between carding cylinders,
Fig: 1

Distribution of Indian Cotton Varieties
Note: Attempt is made only to show main varieties of cotton grown in particular area

Fig. 1
iv) to insert sufficient twist into the material to make a firm thread,
v) to finish and prepare the yarn,
vi) to divide the warp or horizontal threads of the fabric so that the weft or cross threads may be interwoven between them,
vii) to insert the weft or cross threads,
viii) to beat up the last inserted weft thread to the fabric proper, and
ix) to finish the cloth by smoothing, stiffening and glazing.

Byssinosis appears to occur mainly amongst workers in preparatory areas of the mill, where the dust is liberated in huge quantity and the exposure is very high. The preparatory areas of the mills include cotton chamber, the blow room and the card room. Raw cotton in a bale is tightly compressed and contains significant numbers of weeds, grasses, and parts of cotton plants, like bract, leaf, vein material, petiole, capsule, cotyledon, burr, pericarp (exocarp, mesocarp and endocarp), seed coat fragments, notes, lint fragments, stalk, sticks, stems, wood, et al. (Morey, 1976; 1979). All these parts, most often, are heavily contaminated with micro-organisms (Fischer, 1979).

National Institute for Occupational Safety and Health has defined "cotton dust as dust which generates into the atmosphere as a result of processing of cotton fibres combined with any naturally occurring materials such as stems, leaves, bracts, inorganic matter (soil, sand), fungi, bacteria and materials from other plants (weeds and grasses) which may
have accumulated on the cotton fibres during growing or harvesting periods" (NIOSH, 1974). Cotton and other vegetable dusts have been conveniently classified into three grades depending on the size, viz., 'Coarse' - greater than 2 mm, 'Medium' - 2 mm down to 7 μm, and 'Respirable' - less than 7 μm (Gilson et al, 1962). The composition of cotton dust is varied and complex. Dried plant materials are made up of host of organic chemicals, which include carbohydrates, lignins, tannins, proteins, lipids, amines, phenolic pigments and porphyrins. Thus, byssinosis seems to be attributed to the effect of various components of cotton dust on respiratory passages.

A number of studies have been carried out on the prevalence of byssinosis, but the identification of main causal agent or agents still remains obscure. The main symptoms of byssinosis are shortness of breath and/or chest tightness and cough occurring on Monday or the first day, after a break, back to work. For this reason, byssinosis is commonly known as 'Monday Sickness'. As a result of long-term exposure, the symptoms extend to other days of the week and eventually permanent incapacity ensues from shortness of breath, irrespective of dust exposure. Clinical grading of severity of symptoms ranging from 1/2 to 3 based on the regularity of chest tightness and cough on Mondays and progression of these symptoms thereafter, was introduced by Schilling et al, (1963); while Bouhuijs (1970) described a functional grading system which was based on measurement of the forced expiratory volume in one second (FEV₁₀₂). Clinical grades (Schilling, 1963) are as follows:

Grade C 1/2 Occasional chest tightness or respiratory irritation on the first day of the working week.
Grade C 1 Chest tightness and/or shortness of breath on every day of the working week (Mondays in Europe and Saturdays in Arab countries).

Grade C 2 Chest tightness and/or shortness of breath on the first and other days of the working week.

Grade C 3 Grade C 2 symptoms accompanied by evidence of permanent incapacity from diminished effort in tolerance and/or reduced ventilatory capacity.

Functional grades (Bouhuys, 1970) are categorised as follows:

Grade FO No demonstrable acute effect of the dust on ventilatory capacity; no evidence of chronic ventilatory impairment.

Grade F 1/2 Slight acute effect of dust on ventilatory capacity; no evidence of chronic ventilatory impairment.

Grade F 1 Moderate to acute reduction of ventilatory impairment.

Grade F 2 Evidence of slight to moderate irreversible impairment of ventilatory capacity.

Grade F 3 Evidence of moderate to severe irreversible impairment of ventilatory capacity.

In the present context of knowledge the investigations regarding elucidation of mechanism of pathogenesis of byssinosis are centred around mainly in three disciplines: (i) Pharmacological, (ii) Immunological and (iii) Microbiological.
Pharmacological studies have shown that the aqueous extracts of cotton dust could cause contraction of smooth muscle preparations (Davenport and Paton, 1962; Nicholls, 1962; Llyod and Nicholls, 1964). Studies with inhibitor suggested that this effect might be due to histamine, 5-hydroxytryptamine, or some other unidentified substance. There are informations that cotton mill dust and extracts of cotton plant bract could cause histamine release (Hitchcock, 1974; Elissalde and Greenblatt, 1979; Elissalde et al, 1980). It is not clear whether a direct active substance is involved in byssinosis or not, but a histamine releasing factor appears to be more important. Various mechanisms for histamine release in byssinosis have been described but actual mechanism is still unclear. It was also suggested that more than one substance may mediate histamine release and the mechanism may be dependent on cell metabolism (Evans and Nicholls, 1974).

Immunological studies have so far been inconclusive. Skin testing with a variety of textile dust extracts has been found to be non-specific for the disease (Cayton et al, 1952; Gavrilescu et al, 1969). Serum antibodies to the components of cotton dust were also found in control subjects who never worked in textile mill (Massoud and Taylor, 1964; Bouhuya, 1968; Popa et al, 1969).

Studies with bacterial endotoxins have been found to elicit a variety of responses, including chemotaxis, complement activation and an increase in immunoglobulin titres. Several observations suggest that bacterial contamination of cotton is important in causation of byssinosis (Pernis et al, 1961; Linskotai et al, 1977; Linskotai and Whitaker, Cooke, 1979; Fischer et al, 1980; 1978; Rylander et al, 1979; Haglind et al, 1981). The major microbial cause suggested for etiology of byssinosis involves Gram
negative organisms and their endotoxins. It has been also suggested that proteases could play a role in the pathogenesis of byssinosis and these proteases may be of fungal origin (Braun et al, 1973b).

Rylander et al (1975) showed that aqueous extracts of cotton dust, when left under conditions conducive to bacterial growth, caused an increased recruitment of leucocytes to airways of guineapigs when exposed to such an extract. They also found that the toxicity of aqueous extract did not correlate with the total number of bacteria, but correlated with number of certain individual bacterial species, e.g. Escherichia coli and Enterobacter spp. Heat treated dust produced the same response. So the effect could not be related to living bacteria. Indeed, treating the dust at 80°C to 100°C reduced the viable bacterial count, but increased the toxic effect indicating that the effect was due to the bacterial endotoxins.

Endotoxins are distinct cellular components of the Gram negative bacteria and are not released into the medium in any quantity except upon the death and lysis of the bacteria. These cell associated toxins "aggressin" represent a small heterogenous group within wide range of bacterial surface components that are known to contribute to pathogenesis (Smith, 1977). Gram negative bacteria contain a substance with particular toxic properties - lipopolysaccharide (LPS, endotoxin) in the outer portion of bacterial cell wall. LPS contains a hydrophilic and a lipid rich part. The hydrophilic part consists of a polysaccharide (R antigen). The lipid portion A, containing phosphate groups, diglucosamine and fatty acids - is generally responsible for several toxic effects (Bradley, 1979). The toxic effects of endotoxins include abortion, pyrogenecity, the Schwartzman reaction, hypertension,
shock and lethality. Interaction of endotoxin with granulocytes is regarded as being of prime importance.

Rylander and Snella (1976) showed that the increase in number of pulmonary alveolar macrophages and polymorphonuclear leucocytes in guineapigs exposed to extracts of cotton dust correlated with the number of Gram negative bacteria present in the dust.

Rylander and Lundholm (1978a) showed that aerosols of Gram negative bacteria increased the number of pulmonary alveolar macrophages and polymorphonuclear leucocytes in lungs of guineapig; whereas exposure to aerosols of Gram positive Bacillus failed to increase the number. According to Mergenhagen et al (1973) endotoxin could activate the alternate pathway of complement fixation. Activated fragments of complement C3a and C5a have chemotactic and anaphylatoxic properties, which cause the granulocyte recruitment and histamine release respectively. This might be the first mode of action of endotoxins.

Recruitment of polymorph leucocytes to the surface of airways of hamsters and guineapigs has been demonstrated following the inhalation of cotton mill trash dust extracts and different strains of Gram negative bacteria cultured from cotton (Kilburn et al, 1973; Rylander and Lundholm, 1978a). Polymorph leucycytosis also occur in human being after the subjects were exposed to cotton dust (Pernis et al, 1961; Merchant et al, 1975). Therefore, it is found that cotton extracts which contain endotoxins have the property to activate alternate pathway of complement in fresh normal human serum and can consume complement proteins in a dose-response manner. As the half life of factor B (C3PA) of alternative pathway is apparently 75 hours, synthesis and replenishment
of complement protein levels might be expected to occur in workers when they are away from exposure to dust at the week ends. Thus it explains why the greatest severity of the asthmatic symptoms is found at the first day of the working week (Wilson et al, 1976; Wilson et al, 1980).

Rylander and Mattsby (1979) found that more than 50% of the rats showed increased level of IgA titre after inhalation of 0.3 μg of Escherichia coli endotoxin for 10 days. Cavagna et al (1969) also found antibodies against E. coli endotoxin. Increased immunoglobulin titres in animals exposed to endotoxin suggested that an antigen/antibody reaction took place. Thus complement might be activated by classical pathway, and so chemotaxis might occur followed by the release of anaphylotoxins. This might be the second possible mode of action of endotoxin.

Rylander and Snella (1980) described the third possible mode of action of endotoxin. According to them, the macrophages were the main mediators in response to endotoxin. They suggested that the macrophages activated by a stimulus such as endotoxin, might secrete leucotactic substances which could explain the accumulation of granulocytes in airways and that the eventual drop in polymorphonuclear leucocytes reflected the development of an immune response. Rylander (1981) suggested that potential mechanism for non-immunological release of histamine were:

1) an influence of macrophages on platelets
2) disruption of neutrophiles or
3) an effect on mast cells.
Thus endotoxins could play a major role in the causation of byssinosis, as they are known to release histamine and serotonin via complement activation (Cavagna et al, 1969; Hinshaw et al, 1961) and they also could cause an increased synthesis of histamine (Schayer, 1960).

On the contrary, little work has been carried out on the role of proteolytic enzymes in relation to byssinosis. Tuma et al (1973); Chinn et al (1976) and Cinkotai et al (1977) have found a relation between airborne protease concentration and prevalence of byssinosis. The source of these enzymes is not clear. Braun et al (1973b) suggested that fungi including *Aspergillus* and *Streptomyces* could be the source of these airborne proteases. Studies of de-Treville (1971) showed that proteases were present in various parts of the cotton plant. Braun et al (1973b) also showed that subcutaneous injection of the protease derived from the culture of *Aspergillus oryzae* could cause an increase in the histamine release level in various guinea pig tissues including lungs. Battigelli et al (1977) demonstrated that when the spore suspensions of fungal isolates were incubated with minced pig's lung, histamine release was reproducible.

Over the various agents proposed during the last few years as causative agents of byssinosis, Gram negative bacteria (GNB) and endotoxins (lipopolysaccharide-LPS) have been shown to produce effects that closely relate to clinical effects observed in man (Rylander, 1981). This has been evident from the experimental studies on animal and human exposures.

There are few studies from India on prevalence of byssinosis.
including dust measurements (Gupta, 1958; Vishwanathan et al, 1963; Siddhu et al, 1966; Thiruvengadam et al, 1970; Kamat et al, 1971; 1977; 1981). There are only two studies on microflora of Indian cotton fibres used in textile mill and from working environment of textile mill (Ghosh et al, 1977; and Thakker et al, 1978). Many recent reports indicate that the bacteria coming from raw cotton fibre and bract fragments into the air of mill and the endotoxins may play important role in the causation of byssinosis (Cinkotai et al, 1977; Cinkotai and Whitaker, 1978; Rylander et al, 1979). Hence the present investigation has been directed towards determining:

1) microflora of Indian cotton fibres and synthetic fibres with special reference to the identification of GNB and fungi;

2) microflora of cotton textile mill and synthetic mill environment (card room and blow room); and

3) endotoxin concentration (card room, blow room and research laboratory of cotton textile mill and card room of synthetic textile mill).

Thus this study is mainly designed to find out whether different types of GNB are present in large number in Indian cotton fibres and in cotton textile mill environment or not, and does the dust sample of card room and blow room contain endotoxin or not. Attempts have been made to draw a possible correlation between the microflora or endotoxins and byssinosis.