

105

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ABSTRACT

Fungi isolated from sputum of labourers in Quose sugar factory were described. *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Mucor*, *Penicillium*, and *Rhizopus* were isolated from different departments. The culture of bagasse revealed presence in some of them fungi and Thermophilic actinomycetes. Precipitins in serum were found to *Aspergillus flavus* in 22 workers out of 33 volunteered in this study. Also precipitins to *Micropolyspora faeni* were found in 6 workers. It is interesting to note that 3 of them had also precipitin to *Aspergillus flavus* as well. From the sputum of one labourer *saccharo-monospora Viridis* was isolated. *Aspergillus niger*, *Candida albicans* and *Aspergillus flavus* were the commonest fungi isolated from sputum of labourers investigated.

The entity of bagassosis and the role of these fungi in the etiology of hypersensitivity lung diseases were discussed. It is recommended not to employ atopic subjects in sugar industry and to use precipitin tests in labourers exposed as epidemiological parameter by analogy to tuberculin test.

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Hypersensitivity pulmonary diseases are now better understood. The most characteristic outcome of antigen/antibody reactions affecting the respiratory tract is that of bronchial asthma. Another form of insult occurs at the alveolar interstitial level. This form of reaction is represented by late "semi-retarded" hypersensitivity, the classical example of which is farmers lung. Thus one can describe two main types of alterations in hypersensitivity pulmonary diseases, one affecting the bronchi on one hand and the alveolar and interstitial tissues on the other hand (Molina 1976). This distinction could be more documented by pulmonary function tests which make it possible to characterize an obstructive syndrome or a restrictive alveolo-capillary block (ventilation-perfusion defect). Alveolitis in an urban and industrial environment was described as in detergent factories and from exposure to organic dust (Flindt 1969, Pepys et al. 1969, Radermecker 1971, Gothe et al. 1972). An example of hypersensitivity pneumonitis was provoked by inhalation of bagasse, that is the fibrous residue of sugar cane after the removal of sugar juice. Bagassosis was described in 1941 by Jamison and Hopkins and was reported from many countries (Castleden and Hamilton — Paterson 1942, Clearkin 1943, Manas 1945, Cangini 1951, Salfelder 1956).

Pepys in 1962 showed the presence of precipitins against extracts of mouldy hay in subjects affected by bagassosis. However Salvaggio et al. (1969) identified moulds in the bagasse and essentially *Micropolyspora faeni*.

The aim of this study is to investigate some workers in sugar refinery factory in Qouse near by Luxor in Upper Egypt. This is to search for mycoflora in their sputum and for precipitins in blood to fungi and to Thermophilic actinomycetes e.g. : *Micropolyspora faeni*, *Thermoactinomyces vulgaris* which are known to cause bagassosis or Farmers lung.

Description of steps adopted in manufacture of sugar in Qouse Factory :

Sugar cane is transferred from the field to the factory in special railway cars. The cars are first weighed on a scale. Sugar cane is then carried by cranes to be thrown in front of the

first press, where cutters cut the amount thrown into small pieces. These pieces are then squeezed in the first press and the resultant juice is transferred to the diffusion apparatus and the remnant bagasse is returned to the second press where it is mixed with saccharated water to be re-squeezed. The juice from the two presses is transferred through tubes to evaporation wards where it is boiled under high pressure and decreasing temperatures ranging from 120°C to 60°C. This process transforms the juice to syrup. The syrup passes into tubes to the cooking ward where the nucleus on which the sugar crystallises first appears. After formation of sugar crystals the whole mixture is transferred to centrifuges where sugar crystals are separated and then transferred to ventilators to dry up completely and to be then automatically poured into sacs of 100 kgs each. The Molasses which remains after separation of sugar crystals is stored in big tanks to be later used as mother substance for production of perfumes and alcohol in Hawamdyaa factory. The bagasse resulting from the whole process is transferred in bales to be used as fuel in special ovens and boilers for production of the power needed for all processes in the factory.

MATERIAL AND METHODS

33 labourers from different departments volunteered to be examined clinically and accepted to give about 7 ml. of blood samples for serological examination. Sputum was collected from those with productive cough. After a mouth wash and by a deep cough the sputum was raised and collected in a sterile container with a tight lid. Blood was collected (7ml) in sterile test tubes. Samples were sent within 24 hours in ice-box to Ain Shams Mycology Laboratory in chest medical unit. Samples of bagasse residue were also collected. These were cultured directly on the surface of sabouraud agar and incubated at room temperature. Moreover, plates of sabouraud agar were opened in the air of the factory for 15 minutes to trap the fungal spores present in the air. The sputum samples were cultured for fungi after being liquified and homogenized with pancreatine. For isolation of moulds and yeasts sputum samples were inoculated on plates and in tubes of sabouraud dextrose agar to which chloramphenicol was added to inhibit bacterial growth. Incubation was

carried out both at 25°C and 37°C for up to 3 weeks. The samples were also inoculated on nutrient agar, to which yeast extract was added, for the recovery of thermophilic actinomycetes. The latter media was incubated at 50°C. The culture were read after 7, 14 and 21 days. Pure colonies were picked and transferred on Czpeck's agar and Sabouraud's agar. Identification of the isolate was done macroscopically and microscopically.

Precipitins test were done by double immunodiffusion and counterimmuno-electrophoresis. The antigens used were ethanol and acetone fractions of fungal extracts isolated. Antigens of Actinomycetes were provided by Dr. D.W.R. Mackenzie, Mycology Reference Laboratory, London.

RESULTS

Table I shows different type of fungi isolated from air and residues collected from different stages of processing in the tactory. From all departments fungi isolated were *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Mucor*. *Penicillium* was found near diffusion apparatus, mechanical department and in offices. *Rhizopus* was cultured near diffusion apparatus. The culture of bagasse (sugar cane fibre residue) revealed isolation of *Aspergillus flavus*, *Mucor*, *Rhizopus*, *Penicillium* and Thermophilic actinomycetes. Table II shows comparison of occupational classification, respiratory manifestations and serological results in 38 workers who volunteered in this investigation. From them 5 were office workers (office attendants and accountants). 29 were symptomatic, most of them suffering from generalised obstructive airway disease (18 with GOAD). Precipitins were found to *Aspergillus flavus* in 22 workers, 19 of them were involved in processing departments while 3 were office workers. From them 21 were symptomatising and only one was asymptomatic. Positive precipitins to the Thermophilic actinomycetes i.e. *Microspora faeni* were found in 6 labourers, 5 of them came from processing departments and were symptomatic.

Table II also shows results of those with negative serology. It is interesting to note that 3 workers had at the same time precipitins for *Aspergillus flavus* and *Microspora faeni*, also

Table I
 Isolates Cultured from air and
 residues collected from different stages
 in Qouse sugar factory*

Isolate	Cranes	Cutters	Ist press	Diffusion Apparatus	Packing	Mechanical	Offices & medical Dept.
Asp. niger	+	+	+	+	-	+	+
Asp. fumig.	+	+	-	+	-	+	-
Asp. flavus	+	+	+	+	-	+	+
Mucor	+	+	+	+	+	+	+
Penicillium	-	-	-	+	-	-	+
Rhizopus	-	-	-	+	-	-	-

*: Bagasse culture revealed presence of *Aspergillus flavus*, *Mucor*, *Rhizopus*, *Penicillium* and Thermophilic actinomycetes.

Table II
Comparison of occupational classification, respiratory
manifestations and serological results in 33 labourers

	No. of Labourers	No. with precipitins to Asp. flavus	No. with precipitins to Microsporysora faeni	No. with —ve serology (No precipitins detected)
Total No.	33	22*	6	8
Workers from different departments	27	19	5	7
Office workers	5	3	1**	1
Asymptomatic	4	1	1	3
Asymptomatic :-	29	21	5	5
— Cough	—	21	5	5
— Expectoration	—	13	3	4
— Dyspnoea	—	21	5	5
— Wheeze	—	18	5	5
— Crepitations	—	3	2	—

* Some of these workers have also +ve precipitins to Therm. actinomycetes. case no. 17, 19, 21.

** Case no. 17 office worker suffering from bronchial asthma. having Asp. niger cultured from sputum and +ve serology for Asp. flavus and microsporysora faeni.

from one office workers who was suffering from bronchial asthma, *Aspergillus flavus* and *Micropolyspora faeni* were detected. It is evident from clinical examination that most of labourers examined had cough which in many was productive and wheezing of the chest was detected in 18 of them.

Table III shows the frequency of fungi isolated from sputum of different labourers who entered this study. *Aspergillus niger* was the commonest isolate followed in order of frequency by *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium*, *Paecilomyces* and lastly *Aspergillus ornatus* or *Mucor* isolated each from one labourer. The Thermophilic actinomycetes, *Sacharo-monospora Viridis* was isolated from the sputum of one labourer who was working in Press department and suffered from generalised obstructive airway disease. His blood showed presence of precipitins to *Aspergillus flavus* and *Micropolyspora faeni* as well. In press departments where sugar cane is squeezed and bagasse residue is left and scattered, the humidity and hot weather in Upper Egypt will favour growth of fungi and actinomycetes.

DISCUSSION

Castleden Hamilton-Paterson (1942) were the first to suspect a possible pathogenic role for moulds and other micro-organisms in the bagasse. There are 240 million fungal spores per gram of bagasse as well as numerous bacteria (Molina 1976). Since the product becomes very powdery, it is easy to predict how these spores can provoke alveolar or bronchial manifestations. Although bagassosis and Farmers Lung Characterized by exertional dyspnoea and disturbances mainly of alveolo-capillary permeability, many of our workers did have clinically evident generalized obstructive pulmonary diseases manifested by wheezing of the chest. This although rare could also be present with bagassosis (Molina 1978). The evolution of the disease tends towards spontaneous regression if the workers are excluded from exposure to contaminated atmosphere. Patients on continued exposure may progress to pulmonary fibrosis (Sodeman 1949, Ganguly and Pal 1955) and Corpulmonale (Buechner et al. 1958). In our study the main symptom of workers examined was cough with effort dyspnoea and many of them were diagnosed by resident physici-

Table III
Type of fungus cultured from sputum and number
of labourers having it in different departments

Isolates	Press Department	Evaporation & Cooking	Other(*) Departments
<i>Asp. flivus</i>	1		4
<i>Asp. niger</i>			10
<i>Asp. fumigatus</i>	2		2
<i>Asp. ornatus</i>			1
<i>Penicillium</i>	1		2
<i>Mucor</i>			1
<i>Cand. albicans</i>	1	1	5
<i>Paecilomyces</i> sp.			2
<i>Cladosporium</i> sp.	1		
<i>Therm. actinomycetes</i>	1) saccharo-monospora (**)		
	2) Viridis (case 19)		

(*) Others : porters, mechanics, carpenters, guards, office workers, welders, supervisors and electricians.

(**) Case no. 19; Labourer in Press department who was suffering from generalised obstructive air way disease and with precipitins to *Asp. flavus* and micropolyspora faeni detected. by serological tests .

ian as generalised obstructive pulmonary disease, other did have rales scattered on both lungs with productive cough. It is interesting that in these patients positive serology to *Aspergillus flavus* was detected and in one of them this was coupled with presence of the same fungus in his sputum as well. In only one labourer Thermophilic actinomycete (*Saccharomonospora viridis*) was isolated from sputum culture and the serology of this worker showed presence of precipitins to micropolyspora faeni. The presence of precipitins against Micopolyspora faeni provides us with evidence of very high value. The precipitins are evidence of subject contact with the antigen and this the clinician must correlate with other signs of disease. Hapke et al. 1968 found precipitins in 90 percent of cases of acute form of Farmers lung whilst they were only found in 50% of chronic cases. Thus their absence does not exclude the disease when it is chronic. The presence of different moulds in the residue of sugar cane and in the environment of the factory suggest their possible role in the mechanism of the positive results reported in this study. The spores of *Aspergillus* average about 2.5 microns in diameter, which is a convenient size for bronchial deposition and their inherent antigenicity is at least partly responsible for their pathogenic role in producing allergic bronchopulmonary aspergillosis (ABPA). It is important to note that finding the precipitins is not pathognomonic for ABPA but probably does indicate exposure to antigen (Novoy and Wells 1979). Presence of precipitins to *Aspergillus flavus* in serum of workers investigated confirms that these labourers have been exposed to this type of fungus. To confirm the pathogenic role of this fungus and its relation to their symptoms more criteria for diagnosis of ABPA are required i.e. peripheral blood eosinophilia, immediate skin reactivity to *Aspergillus* antigen, elevated serum immunoglobulin E concentrations and history of pulmonary infiltrates (transient or fixed) and in some cases with ABPA central bronchiectasis could be found. The previously described pattern of the disease is that of a full-term picture of ABPA, but undoubtedly on the other end of the scale some cases with early manifestation of the disease may present only with exertional dyspnoea and positive precipitins to *Aspergillus* before the condition proceeds to the full blown picture of ABPA. Efforts to diagnose these cases early and to apply treatment with

gressive bronchiectasis and pulmonary fibrosis (Molina 1976). Detection of the diseases at an earlier, treatable stage is a considerable challenge to clinical acumen. The application of immunodiffusion test for *Aspergillus* precipitins and perhaps immediate skin test reaction will serve well as screening tests for case detection helping to direct attention for this type of hypersensitivity lung syndromes. In skin tests (Salvaggio et al. 1966) 29.5 percent give immediate type positive reactions and 17.5 percent give semidelated reactions to extracts of bagasse. Hargreave et al. (1968) showed the value of inhalation tests with *Micromonospora vulgaris* or of mouldy bagasse for the confirmation of the diagnosis of farmers lung. This could have been done if it was easy to bring these labourers 600 kilometers to Cairo for bronchial challenge. Molnia (1976) reported positive precipitins to bagasse containing moulds only in three subjects out of five who were suffering from chronic cough and were working in posts of an exposed nature in sugar refinery and not handling bagasse. Our results shows 22 labourers with positive precipitins to *Aspergillus flavus* and in six labourers to Thermophilic actinomycetes i.e. *Micropolyspora faeni*. Lacey (1971) isolated from mouldy bagasse *Thermoactinomyces sacchari* which differs from *T. vulgaris* by the existence of spores carried by short sporophores. The *T. vulgaris* is of one micron in diameter surrounded by a covering consisting of at least six layers with an indented appearance of the superficial layer. This could be inhaled easily to the periphery of the bronchial tree causing diffuse interstitial granulomata with giant cell containing birifringent particles. This was reproduced in rabbit by experimental inhalation of dust of bagasse (Gerstl, Tager and Marinaro 1947). At a later stage of the disease pulmonary fibrosis, emphysema and bronchiectasis may occur.

From this study it can be concluded that the labourers in this sugar factory are subjected to continuous inhalation of moulds, especially *Aspergillus* species, and Thermophilic actinomycetes, especially, *Micropolyspora faeni*. The later organism was isolated for the first time in Egypt from sputum of these labourers. Both *Aspergillus* species and *S. viridis* were found to produce antibodies in the serum of patients which could easily be detected by precipitation test. Thus serological analysis for the presence of these precipitins could be applied as screening test. The use of corticosteroids are believed to be successful in preventing pro-

test for the inhalation of these fungi and Thermophilic actinomyces which may be the cause of hypersensitivity pulmonary reactions. The value of precipitins as an epidemiological test by analogy with tuberculin reaction in subjects exposed to Koch's bacillus is at present the object of several studies (Molina 1976) and their presence should be considered of clinical value. For prevention of bagassosis in workers involved in sugar industry it is recommended not to employ from the beginning atopic subjects and to handle all the process of sugar industry mechanically, thus decreasing the incidence of the disease. Opening the sacks of bagasse under water or drying it before handling will definitely minimize the growth of fungi and exposure of workers. From the results obtained in this work it seems necessary to pay more attention to the application of immunological tests in the routine diagnosis of fungal affection of respiratory system.

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