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Experimental Aspergillosis in Mice

Part I. Pathogenic potential of Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger

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RENON (1893) advocated that pulmonary aspergillosis was more common in those persons who by their occupation were exposed frequently to spores of Aspergillus. Recently, MONOD, PESLE and SEGRETAIN (1957) also stressed occurrence of the primary form of aspergillosis. However, SIDRANSKY and FRIEDMAN (1959) in their experimental studies found that *A. flavus* spores inhaled by the healthy mice did not produce a significant lesion in the lungs. The present study has been devised to compare experimentally in mice the pathogenic potential of *A. fumigatus, A. flavus* and *A. niger.*

Material and methods

Three groups of mice with 32 mice in each group were exposed individually to the spores of *A. fumigatus, A. flavus* and *A. niger* in a chamber similar to that described by PIGGOT and EMMONS (1960). A puff of air was injected every two minutes for ten minutes and in end the mice were left in the chamber for another two minutes to allow the spores to settle. One mouse from each group was sacrificed immediately after the exposure, its left lung removed and homogenized in 10 ml. of sterile normal saline and plate counts performed using suitable dilutions of the homogenate. The mice retained approximately 90,000 viable spores per left lung.

The animals were observed for four weeks or until death and were sacrificed at the suitable intervals. All the mice were necropsied, a portion of lung cultured and other fixed in 10 per cent formol-saline. The tissue sections were stained with Haematoxylin and Eosin and Silver methenamine. The findings were subjected to statistical analysis. The "Fisher's exact test for a $2' \times 2'$ table" was employed in calculating the probability values.

Forty eigth unexposed mice were studied simultaneously to exclude any possibility of a natural infection by Asperillus in these animals.

Results and observations

All the control mice survived and lungs from these animals were culture negative and showed normal histology (**Table I**). The mice exposed to spores of *A. fumigatus, A. flavus*

Table I: The mortality, culture and histopathological findings in the mice exposed to spores of A. fumigatus, A. flavus and A. niger

Group of mice	No. of mice	Mortality in ist week		Positive Culture		Histopathological findings Tissue reaction						Presence of hyphae	
						Extensive		Moderate		Mild/ Absent			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Exposed:													
A. fumigatus	32	5	15.6	11	34.4	10	31.2	8	25	14	43.7	0	0
A. flavus	32	2	6.2	8	25	6	18.7	12	37.6	14	43.7	0	0
A. niger	32	0	0	8	25	2	6.2	14	43.7	16	50	0	0
Unexposed:	48	0	0	0	0	0	0	0	0	48	100	0	0

and *A. niger* showed a low rate of mortality (**Table I**) which had no relation to culture or histology findings. The animals which showed positive culture from lungs were those which died or were killed within first three days of the exposure. A varying degree of tissue reaction could be demonstrated in lungs from the mice exposed to spores of *A. fumigatus*, *A. flavus* and *A. niger* but no fungal hyphae could be demonstrated. The mortality, culture and histology findings in the mice exposed to spores of *A. fumigatus*, *A. flavus* or *A. niger* compared to findings in the unexposed mice were statistically insignificant.

Lungs from the mice killed one day after the exposure to spores showed acute bronchitis and bronchopneumonic changes (**Fig. I**). A macrophage response around the bronchi appeared in the animals sacrificed after four days (**Fig. II**) and complete clearance of the

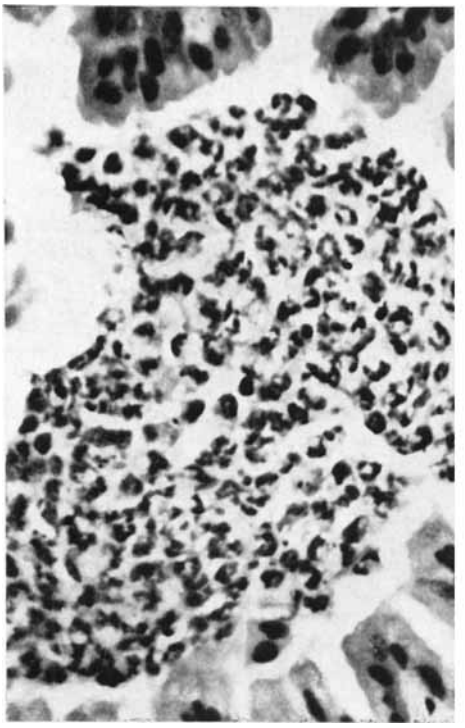

Fig. I: Section of lungs from the untreated mice exposed to spores of *A. fumigatus* and sacrificed one day after the exposure showing acute inflammatory exudate in lumen of the bronchus. H. & E. × 450

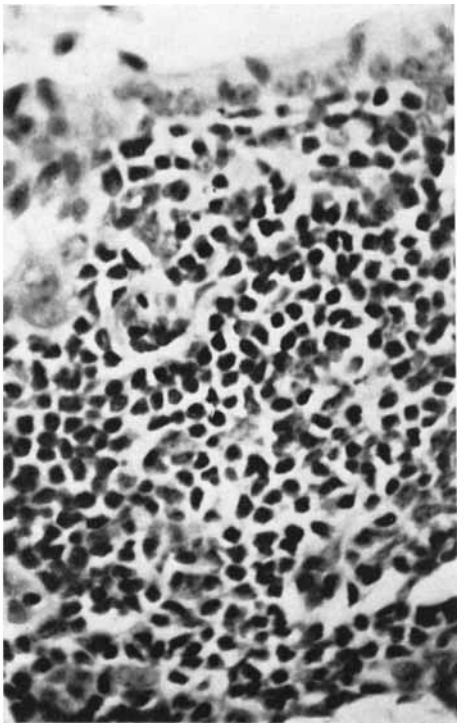

Fig. II: Section of lungs from the untreated mice exposed to *A. fumigatus* and sacrificed four days after the exposure showing macrophage response around the bronchus H. & E. × 450

lungs could be seen in the mice sacrificed six days after the exposure. The tissue reaction in lungs was most extensive in the mice exposed to *A. fumigatus* (**Fig. III**), comparatively less in those exposed to *A. flavus* (**Fig. IV**) and least marked in those exposed to *A. niger* (**Fig. V**).

Discussion

The mice exposed to high concentration of the viable spores of *A. fumigatus*, *A. flavus* or *A. niger* developed only a transient lesion in the lungs which cleared up within one week of the exposure. The few animals which died after exposure to the spores showed

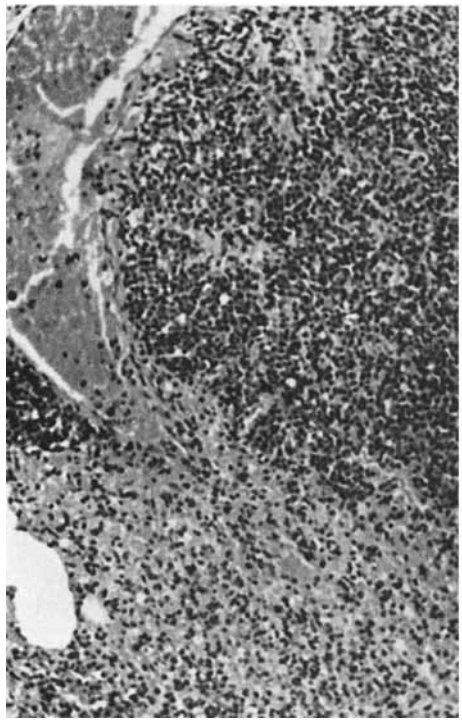

Fig. III: Section of lungs from the untreated mice exposed to *A. fumigatus* showing acute bronchitic and bronchopneumonic changes. H. & E. × 35

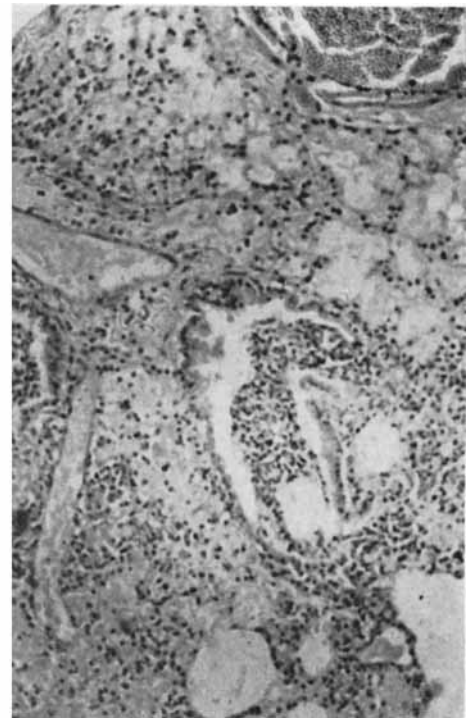

Fig. IV: Section of lungs from the untreated mice exposed to spores of *A. flavus*. Bronchopneumonic changes are less severe. H. & E. × 35

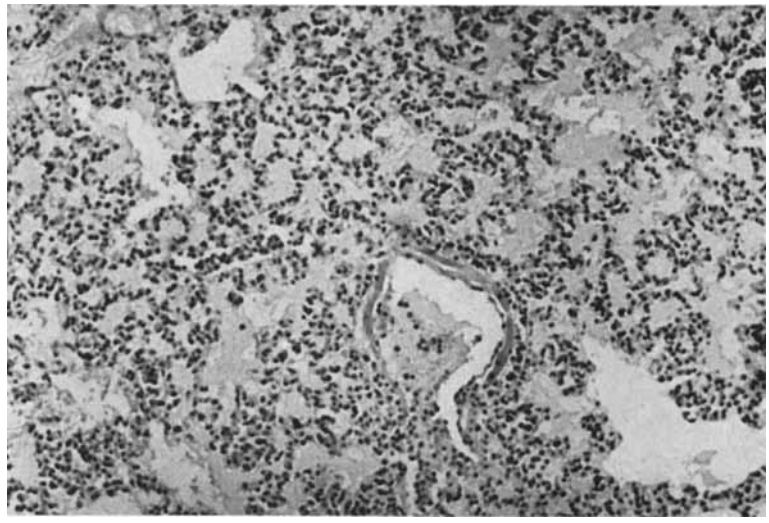

Fig. V: Section of lungs from the untreated mice exposed to spores of *A. niger*. The bronchopneumonic changes are minimal. H. & E. × 35

no fungal hyphae in the lungs and were culture negative. It appears, therefore, that death of these animals was simply a chance finding. The positive culture obtained from lungs of the mice dying within first three days of the exposure may be due to viable spores remaining in the lungs. Thus, except for a slight difference in severity of the tissue reaction, the low infectivity reported for *A. flavus* (SIDRANSKY and FRIEDMAN, loc. cit.) remains true also for *A. fumigatus* and *A. niger*. The evidence to date connecting pulmonary aspergillosis with certain occupations notably those concerned with birds, grains and their associated trades does not seem to be convincing (ORIE *et al.* 1960). The cases with respiratory diseases may harbour Aspergilli without suffering from the disease (PEPYS *et al.*, 1959) and workers exaposed to a heavy concentration of the spores do not necessarily show a rise in the incidence of the disease (STALLYBRASS, 1961). The low pathogenicity of Aspergillus is also suggested by the fact that disease often remains stationary for years and while Aspergillus is growing in one cyst, the other cysts may exist in the same lung which do not show any sign of the fungal growth (ORIE *et al. loc. cit.).*

The present study also suggested that *A. fumigatus, A. flavus* and *A. niger* differ in their pathogenic potential. This difference was noticed also in the experiments carried out in the cortisone treated animals (BHATIA and MOHAPATRA, 1969). This finding may be explained on the basis of the work done by CLAYTON (1957) who confirmed the presence of an endotoxin in *A. fumigatus* and also reported that extracts from other species of *Aspergillus* possessed toxigenicity to a lesser and varying extent.

Summary

Three groups of 32 mice each were exposed individually to a high concentration of the viable spores of *A. fumigatus, A. flavus* and *A. niger*. The mice developed a transient lesion in the lungs which cleared up within one week of the exposure. The tissue reaction was most extensive with *A. fumigatus*, less so with *A. flavus* and least marked with *A. niger*. No fungal hyphae could be demonstrated in the sections from lungs of these mice and positive culture was obtained only from lungs of those mice which died within first three days of the exposure. The findings also suggested that *A. fumigatus, A. flavus* and *A. niger* differ in their pathogenic potential.

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