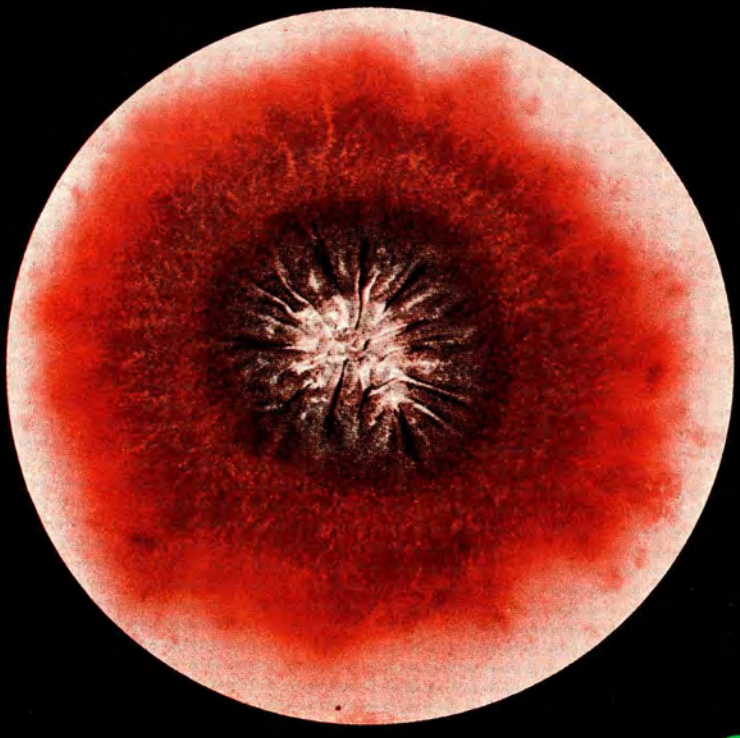
A 2547 E

mykosen

Herausgeber und Schriftleiter: Hans Götz, Essen, Heinz Grimmer, Wiesbaden
Detlev Hantschke, Essen, Wolf Meinhof, München, Hans Rieth, Hamburg



2/1970

1. Februa

Grosse Verlag GmbH. · 1 Berlin 31 (Halensee) · Kurfürstendamm 15

mykosen 13 (2) : 105—110 (1970) Eingegangen am 26. Juni 1969

Department of Microbiology, All India Institute of Medical Sciences, New Delhi - 16, India
(Chief: Prof. L. N. MOHAPATRA)

Experimental Aspergillosis in Mice

Part II. Enhanced susceptibility of the cortisone treated mice to infection with Aspergillus fumigatus, Aspergillus flavus und Aspergillus niger

V. N. BHATIA and L. N. MOHAPATRA

SIDRANSKY and FRIEDMAN (1959) reported a marked infectivity of inhaled spores of *A. flavus* in the cortisone treated mice. No such study has been carried out with other species of Aspergillus. The present paper deals with a comparative study of the lesion produced by *Aspergillus fumigatus, A. flavus* and *A. niger* in lungs of the cortisone and antibiotic treated mice.

Material and methods

Three batches of mice were exposed individually to the spores of *A. fumigatus, A. flavus* and *A. niger*. Each batch consisted of the four groups as follows:

I) the untreated group
II) the cortisone treated group
III) the antibiotic treated group
IV) the group treated with cortisone and antibiotics.

Each of these groups consisted of twelve mice out of which eight were exposed to the respective spores and four served as unexposed controls.

Table I: The mortality, culture and histopathological findings in the cortisone & antibiotic treated mice exposed to the viable spores of A. fumigatus

Group of mice	No. of mice	Mortality in Ist week		Positive Culture		Histopathological findings: Tissue reaction: Extensive		Tissue reaction: Moderate		Tissue reaction: Mild/ Absent		Presence of hyphae	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Untreated:													
Exposed	8	1	12.5	3	37.5	3	37.5	2	25	3	37.5	0	0
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic treated:													
Exposed	8	2	25	2	25	2	25	3	37.5	3	37.5	0	0
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Cortisone treated:													
Exposed	8	8	100	8	100	8	100	0	0	0	0	8	100
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Cortisone & antibiotic treated:													
Exposed	8	8	100	8	100	8	100	0	0	0	0	8	100
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0

Table II: The mortality, culture and histopathological findings in the cortisone & antibiotic treated mice exposed to the viable spores of A. flavus

Group of mice	No. of mice	Mortality in Ist week		Positive Culture		Histopathological findings: Tissue reaction: Extensive		Moderate		Mild/ Absent		Presence of hyphae	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Untreated:													
Exposed	8	0	0	2	25	1	12.5	3	37.5	4	50	0	0
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic treated:													
Exposed	8	0	0	3	37.5	2	25	2	25	4	50	0	0
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Cortisone treated:													
Exposed	8	8	100	8	100	8	100	0	0	0	0	8	100
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Cortisone & antibiotic treated:													
Exposed	8	8	100	8	100	8	100	0	0	0	0	8	100
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0

Table III: The mortality, culture and histopathological findings in the cortisone & antibiotic treated mice exposed to the viable spores of A. niger

Group of mice	No. of mice	Mortality in Ist week		Positive Culture		Histopathological findings: Tissue reaction: Extensive		Moderate		Mild/ Absent		Presence of hyphae	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Untreated:													
Exposed	8	0	0	2	25	1	12.5	2	25	5	62.5	0	0
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotic treated:													
Exposed	8	0	0	2	25	2	25	3	37.5	3	37.5	0	0
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Cortisone treated:													
Exposed	8	3	37.5	4	50	5	62.5	1	12.5	2	25	4	50
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0
Cortisone & antibiotic treated:													
Exposed	8	4	50	5	62.5	5	62.5	2	25	1	12.5	5	62.5
Unexposed	4	0	0	0	0	0	0	0	0	0	0	0	0

Cortisone (2.5 mg) was injected subcutaneously two days before the exposure, on the day thereof and then at two days intervals till the animal died or for fourteen days. Tetracycline hydrochloride was added to water (5 mg per 100 ml) which these mice drank throughout the course of the experiment. Exposure to the spores and further study of the animals was done according to methods described in our previous paper (Bhatia and Mohapatra 1969).

Results and observations

The unexposed controls in all the groups survived and lungs from these animals were culture negative and showed normal histology. The untreated and the antibiotic treated groups exposed to the spores of *A. fumigatus*, *A. flavus* and *A. niger* showed a low rate of mortality (**Table I, II and III**) which had no relation to culture or histopathology findings. The tissue sections from lungs of these mice showed a varying degree of tissue reaction but no fungal hyphae could be demonstrated. The macrophage response around the bronchi was seen in lungs of the mice died or killed on fourth day of the exposure and complete clearance of the lungs was noticed in the animals which died or were killed after seventh day of the exposure. The positive culture was obtained from lungs of those mice which died or were killed within first three days of the exposure.

The cortisone treated mice exposed to *A. fumigatus* and *A. flavus* showed 100 per cent mortality in first week of the exposure (**Table I and II**). Lungs from these animals were culture positive and showed extensive tissue damage and proliferating hyphae in the sections (**Figure I and II**). The findings were statistically significant with a probability

Fig. I: Section of lungs from the cortisone treated mice exposed to spores of *A. fumigatus* showing fungal hyphae penetrating through bronchus and invading the alveolar tissue. H. & E. × 100

value ranging from 0.0014 to 0.026. The mortality and culture findings in the cortisone treated groups exposed to spores of *A. niger* (**Table III**) showed only a statistically insignificant difference from those in the untreated groups. The histological findings in these groups were, however, statistically significant with a probability value of 0.025. The tissue reaction and fungal invasion was most marked in the mice exposed to

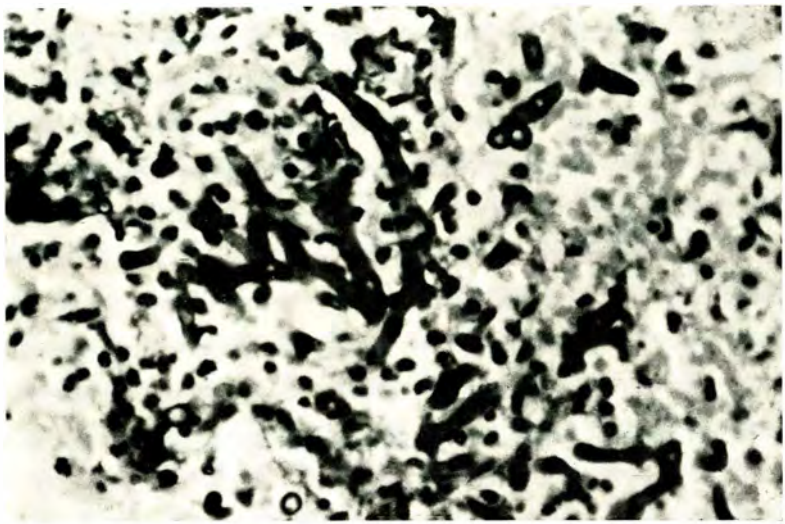


Fig. II: Section of lungs from the cortisone treated mice exposed to spores of *A. fumigatus* showing extensive haemorhagic bronchopneumonia with characteristic branched and septate hyphae in the alveolar tissue. H. & E. × 450

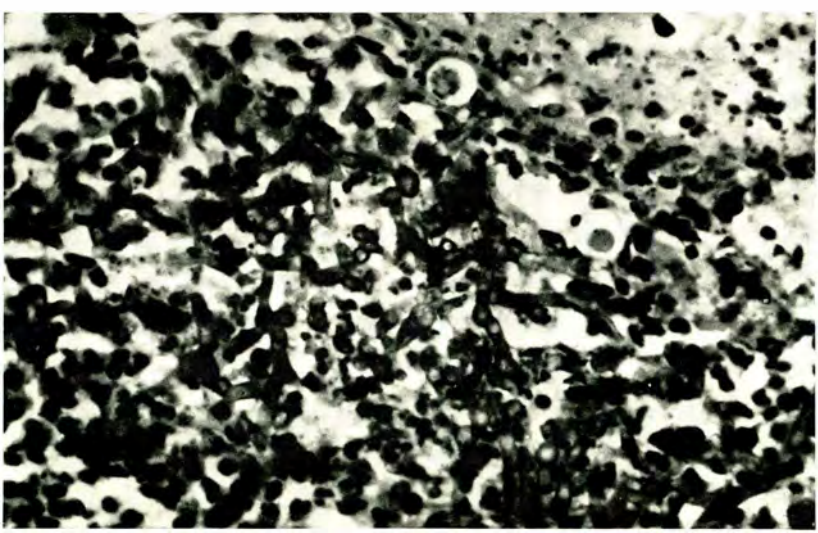


Fig. III: Section of lungs from the cortisone treated mice exposed to spores of *A. flavus* showing extensive haemorhagic bronchopneumonia and branched and septate hyphae. H. & E. × 450

A. fumigatus (Fig. II), comparatively less marked in those exposed to *A. flavus* (Fig. III) and least marked in those exposed to *A. niger* (Fig. IV and V). Findings in the cortisone treated group and those in the group treated with both cortisone and antibiotics showed no significant difference.

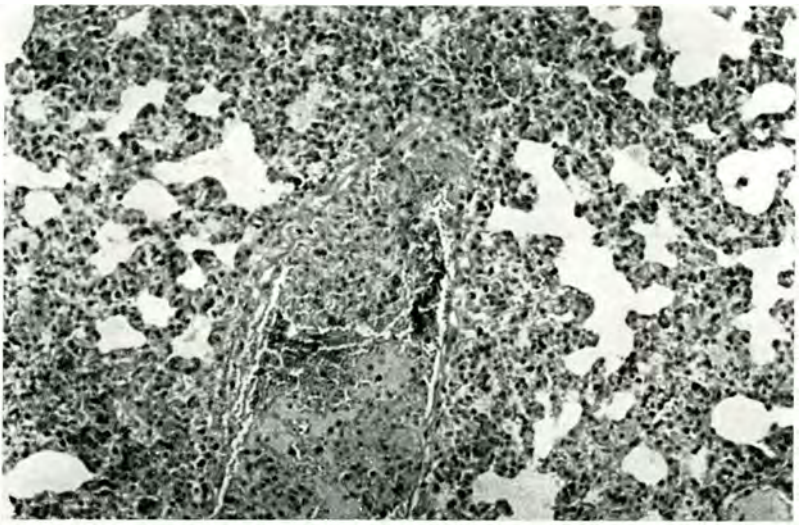


Fig. IV: Section of lungs from the cortisone treated mice exposed to spores of *A. niger* showing bronchopneumonic changes. H. & E. × 35

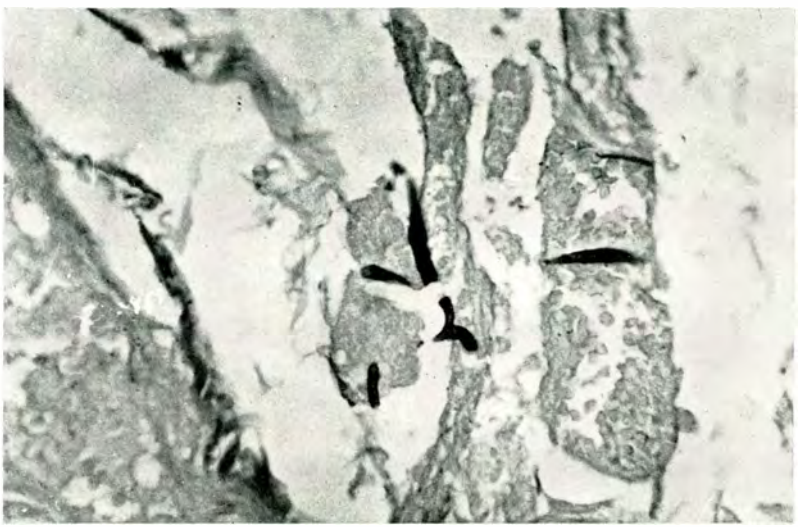


Fig. V: Section of lungs from the cortisone treated mice exposed to spores of *A. niger* showing bronchopneumonic changes and small fragments of hyphae. H. & E. × 400

Diskussion

Present study shows that cortisone rather than antibiotics is chiefly responsible for enhanced susceptibility of the host to the experimental infection with Aspergillus species. SIDRANSKY and FRIEDMAN *(loc. cit.)* also reported inconsistant results of antibiotic admi-

nistration on the experimental infection with *A. flavus* in the mice. Zimmerman (1950) cautiously implicated penicillin therapy as a possible etiologic agent in Aspergillus endocarditis in two patients, but one of his cases was suffering from Rheumatic fever and the other had an amputation. Kligman (1952) also reported Aspergillus fruiting bodies in the bowel mucosa of two patients who came to autopsy and had received succinyl sulphathiazole and streptomycin by mouth for long period but the cases had a primary disease of unknown nature causing severe bowel involvement. Obviously, aspergillosis could be superimposed on primary disease present in these cases.

Severity of the lesion produced in lungs in the present study was comparable to that in the study by Sidransky and Friedman *(loc. cit.)* although number of the viable spores retained by mice in the present series was only 90,000 per left lung as compared to 360,000 per left lung in the other series. Thus it appears that number of spores do not affect severity and progress of the lesion. Sidransky and Friedman *(loc. cit.)* suggested that number of spores affect severity of the lesion, but they have also reported an incidence when a leak in their exposure chamber infected several of the unexposed cortisone treated mice. They also cited an example from a study by Sagi and Lapis in 1956 on tumour transplants where many of their cortisone treated rats died of pulmonary aspergillosis. Obviously, the number of spores inhaled by animals in these incidents must have been very small.

The present study also suggested a difference in the pathogenic potential of three species of aspergillus. The lesions were more severe, progressive and fatal with *A. fumigatus* and *A. flavus* than with *A. niger*. This difference may be explained by the findings of Clayton (1957) that extracts of different species of Aspergillus differ in the degree of their toxigenicity. Thus, present study suggests that progress of the lesion in the experimental aspergillosis is governed not only by the adverse effect of cortisone but also by the pathogenic potential of the species of Aspergillus used in the experiments.

Summary

The untreated mice and the mice treated with cortisone, antibiotics and both cortisone and antibiotics, were exposed individually to the spores of *A. fumigatus, A. flavus* and *A. niger*. The cortisone but not the antibiotics seemed to enhance susceptibility of the mice to infection with Aspergillus species. The species of Aspergillus also affected the severity and progress of the lesion.

Acknowledgement

The authors are grateful to Dr. K. Ramachandran, Assistant Professor of Statistics in the Department of Social and Preventive Medicine, All India Institute of Medical Sciences, New Delhi, for statistical analysis of the data and to Mr. Sat Dev, Technical Assistant in the Department of Microbiology for the technical assistance.

References

Bhatia, V. N. & Mohapatra, L. N.: Experimental Aspergillosis in mice. Part I: Pathogenic potential of *Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger*. Mykosen **12** (11) : 651—654 (1969).

Clayton, Y. M. (1957) cited in Campbell, M. J. & Clayton, Y. M.: Bronchopulmonary aspergillosis. Amer. Rev. Resp. Dis. **89** : 186 (1964).

Kligman, A. M.: Are fungus infections increasing as result of antibiotic therapy? Jour. Am. Med. Assoc. **149** : 979 (1952).

Sidransky, H. & Friedman, L.: The effect of cortisone and antibiotic agents on experimental pulmonary aspergillosis. Amer. Jour. Path **35** : 169 (1959).

Zimmerman, L. E.: Candida and Aspergillus endocarditis with comments on role of antibiotics in dissemination of fungal diseases A. M. A. Arch. Path. **50** : 591 (1950).

Authors' address: Department of Microbiology, All India Institute of Medical Sciences, New Delhi - 16, India