STUDIES ON FUNGI ENCOUNTERED IN THE ATMOSPHERE

I. The Presence of Fungous Spores and of Pollens in KOH Preparations*

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In the diagnosis of superficial fungous infection direct microscopic examination of clinical material by means of the KOH preparation is the simplest and yet the most useful single laboratory procedure. From suspected mycotic skin lesions the materials for examination are obtained by scraping the more active borders with a scalpel or a currette, or by snipping off the tops of any available vesicles or vesiculo-pustules. These skin scrapings and/or tops of vesicles are placed on a microscopic slide, mounted with a drop or two of 10–20% potassium hydroxide solution under a coverslip, flamed and then examined under the microscope for the characteristic branching hyphal filaments of the superficial pathogenic fungi such as the dermatophytes. By reason of the ease of performance and well-defined results of the test, it has become a routine procedure in many dermatologic clinics.

This technic of obtaining the test material by scraping the skin for scales and cutting off the roofs of vesicles, does not necessarily exclude the different materials present on the skin surface. The skin, by nature of its being interposed between the body and its environment, is continually exposed to the outside surroundings. Acting as a protective barrier, its external surface catches and becomes a kind of reservoir for materials and substances coming from the atmosphere and other physical, chemical and biological environment it comes in contact with. In setting up the KOH mount these foreign materials are carried over with the skin scrapings, become an integral part of the slide preparation and are discerned under the microscope among the cutaneous epithelial cells. An examiner, intent in his search for the diagnostic hyphal filaments and other tissue forms of the superficial pathogenic fungi, more likely than not, misses the presence of these extraneous structures, or dismisses them as not pertinent to the problem, at hand, of finding the dermatophyte. During the last few years, however, closer attention was paid to their presence in the routine KOH mounts performed in the Dermatological Outpatient Clinic of the University of Michigan Hospital. There were commonly encountered fibers of all sorts, dirt and ointment particles, vegetable granules, pollens, spores, crystals and amorphous substances. The presence of these pollens and particularly, of fungous spores in these KOH mounts and their possible significance were considered intriguing.

The ubiquity of fungi is well-known. Approximately 80,000 species are said to exist. Some of these are parasitic on animal and plant life, but most are saprophytic, found living in the soil or dead vegetation. Many are extremely prolific and are capable of discharging enormous numbers of reproductive spores which, by virtue of their small size and high buoyancy,

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are anemophilous. Feinberg (1) dates the knowledge of the presence of these fungous spores in the atmosphere to Blackley. In 1873 he published his book on "Hay Fever: Its Causes, Treatment and Effective Prevention: Experimental Researches" wherein he described finding these spores in his slides exposed to the air for counting pollens. Extensive aerobiological studies, notably those of Durham, Feinberg and Prince, have definitely established the existence in the atmosphere of these fungous spores, their types of flora and their seasonal fluctuations. They have been found at altitudes of over 10,000 feet and over the Arctic regions. The Mold Aerosurvey Committee of the American Academy of Allergy have been conducting annual atmospheric surveys in different parts of the country. Mold sampling is made by the Petri dish and the glass slide technics. The plate method consists of exposing standard Petri dishes with a suitable agar medium for a certain period of time and identifying the fungous colonies upon sufficient maturation. The slide method consists of exposing slides and counting the fungous spores according to standards set for pollen counts.

About 60 genera of fungi have been recovered and identified from the atmosphere during these aerosurveys, but only about a dozen comprise the great majority of the atmospheric fungous flora. Schaffer, Seidmon and Bruskin (2) found that these 12 genera made up 95% of the total amount of fungi isolated from the air. A similar finding was reported by Targow and Plunkett (3). Christensen and Swaebly (4) reviewed the literature from 1935 through 1950 for the more commonly culturable atmospheric fungi and gave the following list:

1. Very Common
   Alternaria, hormodendrum (cladosporium), penicillium, aspergillus.
2. Common
   Mucor, helminthosporium, monilia (candida), fusarium, yeast (genus not given) phoma, pullularia, torula, rhizopus, trichoderma.

The differences in the fungous flora of the outdoor atmosphere, indoor air and house dust were also pointed out (2, 5, 6). Generally speaking, alternaria and hormodendrum predominate in the outdoor atmosphere while penicillium and aspergillus are more commonly found in the indoor air and in house dust.

Each of these four most common atmospheric fungi has its own characteristic type of spore and sporulation. Alternaria has short, unbranched conidiophores, usually single but may be branched. The spores are relatively large, on the average, 15 microns wide and 50 microns long. They are easily recognized by their clavate shape with cross striations in two planes, acquiring a muriform appearance. Their dark brown color adds to their easy identification. The conidiophores give rise to short and long simple chains of these large spores which are readily torn off singly or in short chains into the atmosphere. Hormodendrum is characterized by conidial "tree-like" branching chains supported by conidiophores. The conidia are much smaller, averaging 2–8 by 5–20 microns in size, oval in shape and may be one or two celled. They are brownish green in color. A useful identifying structure is the dark disjunctor at one end of each spore. Although these small hormodendrum spores may be broken off from the chains singly, they occur much more commonly in the atmosphere as clusters or branching chains. Aspergillus is characterized by an unbranched conidiophore which swells at its tip into a "vesicle" bearing small flask-shaped sterigmata. From the latter arise unbranched rows of tiny ovoid to spherical conidia measuring 2–4 microns in diameter. The spore formation is so abundant that these are discharged into the air as a fine powdery dust. Penicillium has a fruiting body that resembles a brush or broom, hence, its name derivation from "penicillus". Unlike aspergillus the conidiophore of penicillium divides into primary and secondary branches called metulae bearing the sterigmata which, in turn, give rise to chains of small conidia similar to those of aspergillus in size and shape. These are also easily discharged into the atmosphere.

Experience with the slide method of counting atmospheric fungous spores has demonstrated that of the four types of spores described previously, those of alternaria are the easiest to identify because of their size and characteristic appearance. It is not so difficult to recognize hormodendrum spores with their a

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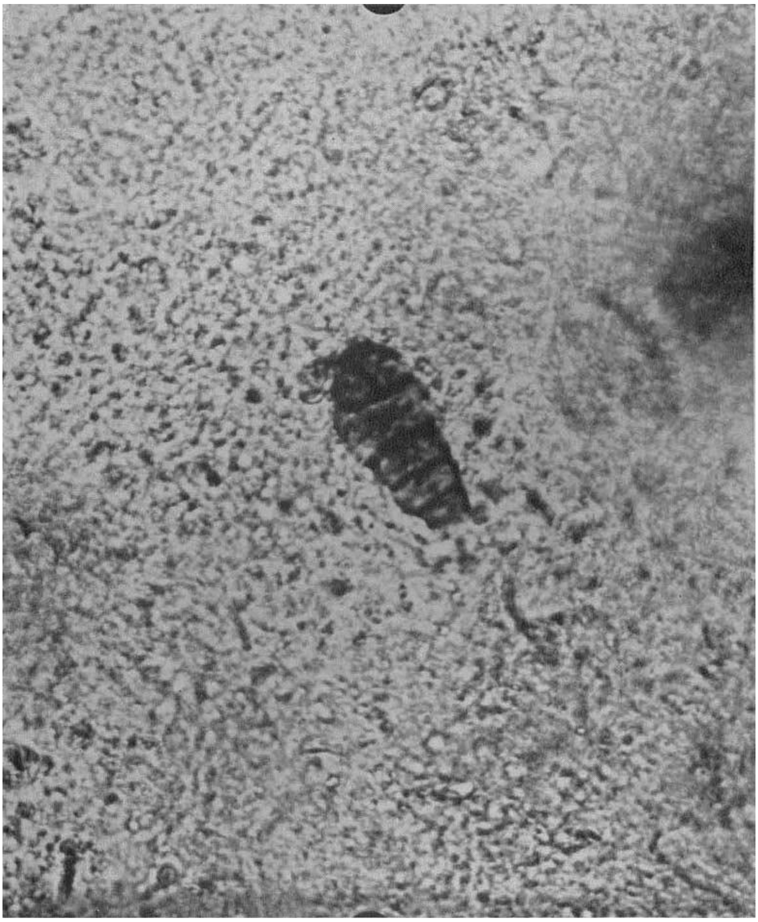

FIG. 1 (a) and (b). Alternaria spores in KOH mounts

FIG. 2. Helminthosporium spore in KOH mount

disjunctors, but they are more apt to be missed than alternaria spores, explaining the discrepancy between the results of the slide and of the plate methods of fungal aerosurvey. The conidia of aspergillus and penicillium, because of their transparency and small size, are well-nigh impossible to single out in the slide preparations exposed to the air. A similar situation was obtained in our examination of KOH mounts. Alternaria spores were almost exclusively the only type of fungous spores encountered. Fig. 1 depicts how they appear in KOH preparations lying among the epithelial cells of the skin scraping material. On a few occasions helminthosporium spores were also seen (Fig. 2). These can attain a size of 50–100 microns, even larger than alternaria spores. They are ovoid and multicellular with only cross septations, not very much unlike the fuseaux or macroconidia of Epidermophyton floccosum. Only once was the presence of hormodendrum spores held with reasonable certainty when a cluster of small, oval spores with disjunctors were seen. These smaller spores of hormodendrum and particularly, those of penicillium and aspergillus, if present, are obscured by the other elements of the KOH preparation such as the numerous epithelial cells, fat globules, air vacuoles and crystals, making it most difficult to pick them out even under high power magnification of the microscope. At no instance were the spores of penicillium and aspergillus recognized, but their possible association with skin scrapings may be suggested by another observation. It has also become customary to complement the KOH examination with cultural procedures. This is usually done by directly inoculating the skin material on Sabouraud's dextrose agar slant with the same scalpel used in obtaining the scrapings. A notoriously known shortcoming of this technic is the growth of contaminants, either bacterial or fungal, which has occasioned attempts to inhibit their growth by incorporating other compounds in the media such as penicillin and streptomycin against bacteria, and oxgall and actidione against fungal contaminants. The majority of these contaminating colonies are those of alternaria, hormodendrum, penicillium and aspergillus. Their initial growths are frequently seen to arise from the implanted skin materials or along the tracks made by the scalpel during inoculation. This may be construed as rather suggestive of contamination carried over with the inoculant and not of contamination directly from the air as a result of errors in aseptic technic in which case the contaminating colonies would appear in any part of the agar slant surface.

During the last year the KOH mounts prepared in the clinic everyday for diagnostic purposes, which averaged 4–6, were gathered and examined for the presence of these fungous spores, essentially those of alternaria. The whole area

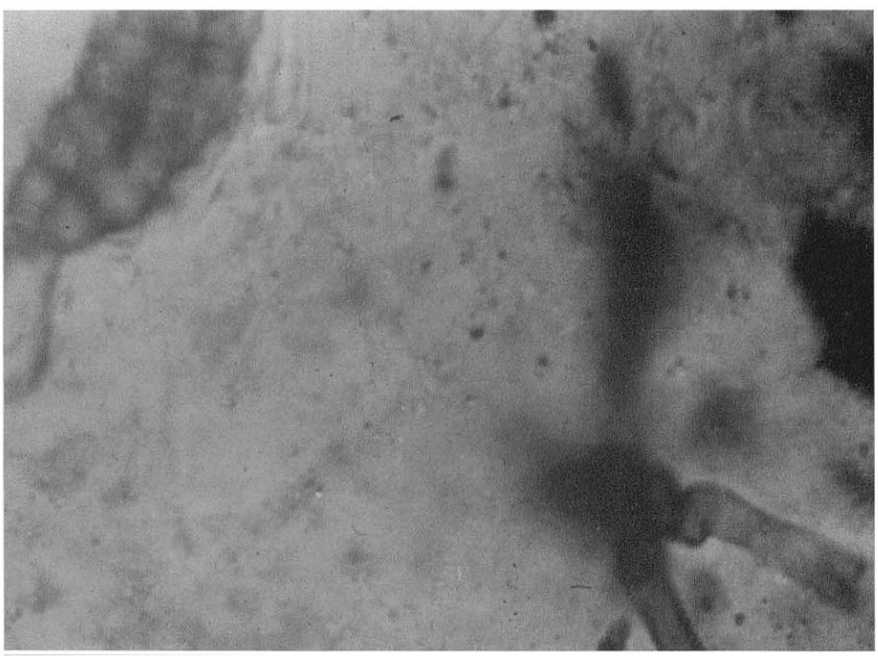

FIG. 3. At the upper left hand corner is an alternaria spore while at the lower right hand corner is a filamentous hypha-like structure.

under the coverslip was covered by making parallel sweeps across the entire width of the coverslip in much the same way followed in pollen counts. Even under the low power magnification the brownish alternaria spores stood out. They were usually found enmeshed among the epithelial cells of the scales.

There are a number of factors that may be considered as affecting the incidence of these spores in KOH mounts such as the type of the residential area of the patient, whether rural or urban, his occupation, his activity previous to the clinic visit, his hobbies, his cleansing habits, the season of the year when examined, and others. No attempts were made to evaluate the influence of these factors. However, the frequency of finding these fungous spores in KOH mounts follows approximately their curve of incidence in the atmosphere as determined by aerosurveys which revealed definite seasonal trends, particularly for the northern regions of the country. The alternaria season generally starts in May, reaching peak concentrations during the months of July, August and September, accounting for about 80% of the total annual spore count. The spores are at a

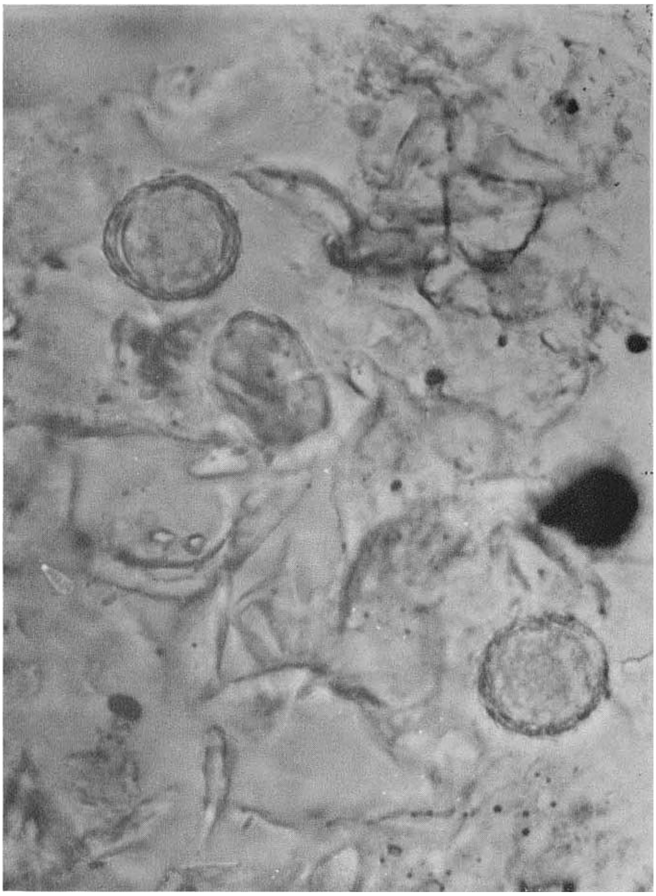

FIG. 4. Ragweed pollens in KOH mount

minimum during winter months. A similar seasonal tendency is obtained in the general vicinity of Ann Arbor, Michigan as seen in the annual reports of the Mold Survey Committee of the American Academy of Allergy, the latest of which was for 1952 (7). This incidence curve of alternaria spores in the atmosphere was roughly reflected in our experience with their presence in KOH mounts. They were most frequently encountered in these preparations during the peak summer months. Not a single spore was found during the winter months beginning December through March.

Most of the KOH mounts found positive for spores were taken from lesions on exposed portions of the body, particularly the hands and feet. This is to be expected inasmuch as the spores from the atmosphere logically contaminate the exposed areas of the skin. However, they were occasionally encountered in preparations taken from unexposed regions, even from the groins.

These KOH mounts prepared and examined for purposes of diagnosis were obtained from skin lesions. This does not mean, however, that fungous spores can be found only in scrapings from these lesions inasmuch as contamination of the skin with spores from the atmosphere obviously is not selective as to the specific areas of the skin they land on. A number of skin scrapings were taken from unaffected normal areas of the skin and KOH mounts prepared. Spores were also encountered in these preparations.

Blank runs of the KOH examination were also done by going through all the motions followed in performing the examination, except the actual scraping of the skin. These were carried out during the peak periods when the alternaria spores were abundantly present in the atmosphere and there were more chances of direct contamination from the atmosphere of the KOH mount during the performance of the test. There were no spores seen in these blank KOH preparations without the skin scrapings.

On a few occasions there were also encountered darkish, septate, branching, relatively thick-walled filaments that might conceivably have been fungal hyphae. It is possible that pieces of hyphae are broken off from the main mycelial masses by strong winds or by other mechanical means. Such a filamentous hypha-like structure is shown in Fig. 3, associated with an alternaria spore.

This search for fungous spores in KOH mounts also revealed the presence of air-borne pollens, even more frequently and in greater numbers. This is particularly true with ragweed pollen although pollens of trees, grasses and other weeds were encountered as well during their respective pollinating periods. Fig. 4 shows the typical regularly spiculed pollens of ragweed in KOH mounts. The ragweed season generally starting in late July is shorter than that of alternaria. Greatest pollution of the atmosphere with ragweed pollens occurs around the end of August and beginning of September. During these peak days, some skin scrapings were obtained which appeared studded with ragweed pollens under the microscope. It was also not unusual during these summer months to find both ragweed pollens and alternaria spores in the same KOH preparations inasmuch as the periods of pollination of ragweed and sporulation of alternaria overlap at these times. Fig. 5 illustrates the association of the two.

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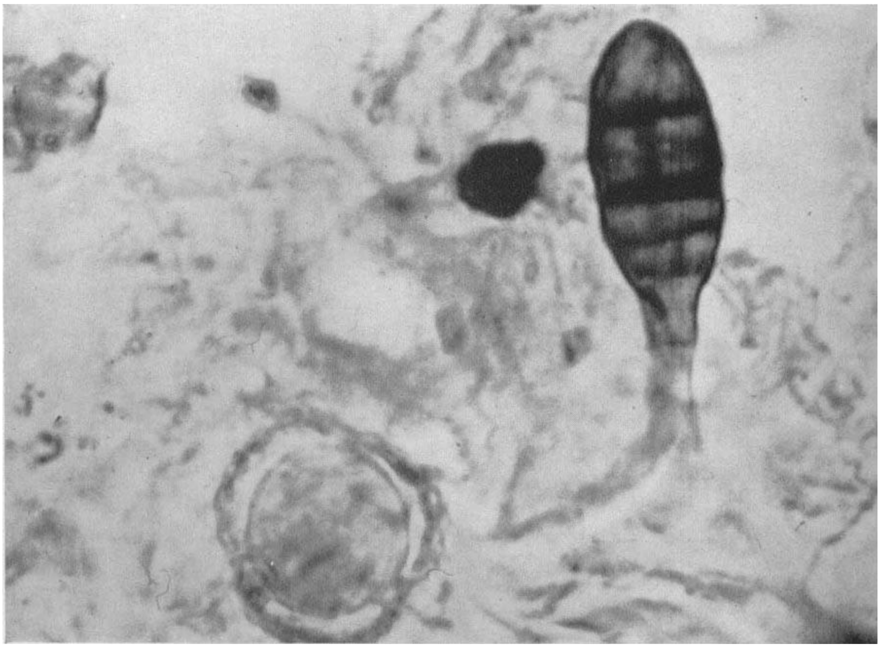

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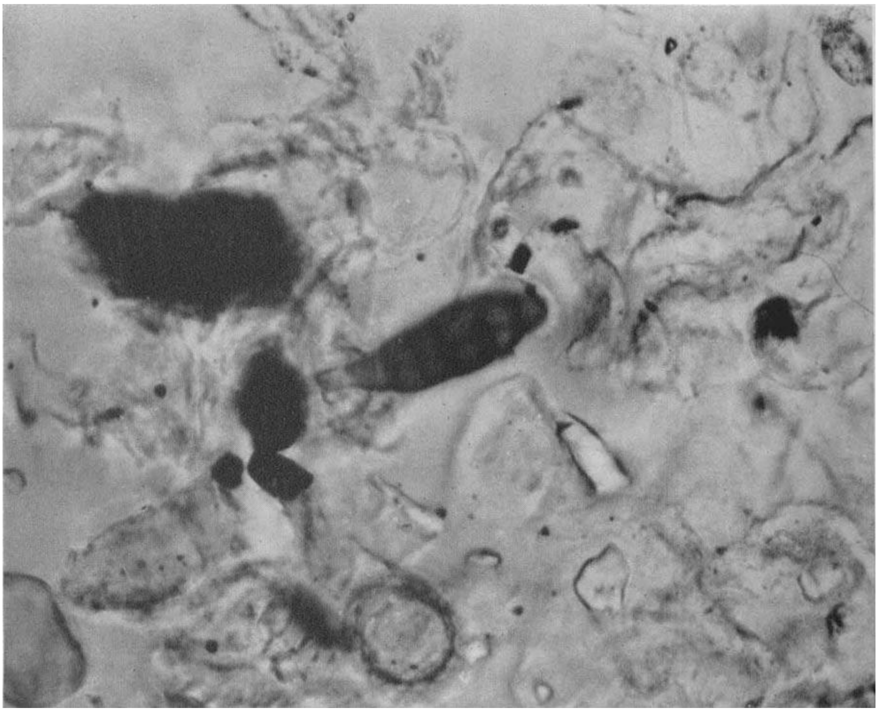

FIG. 5 (a) and (b). Alternaria spores and ragweed pollens encountered together in the same KOH mounts.

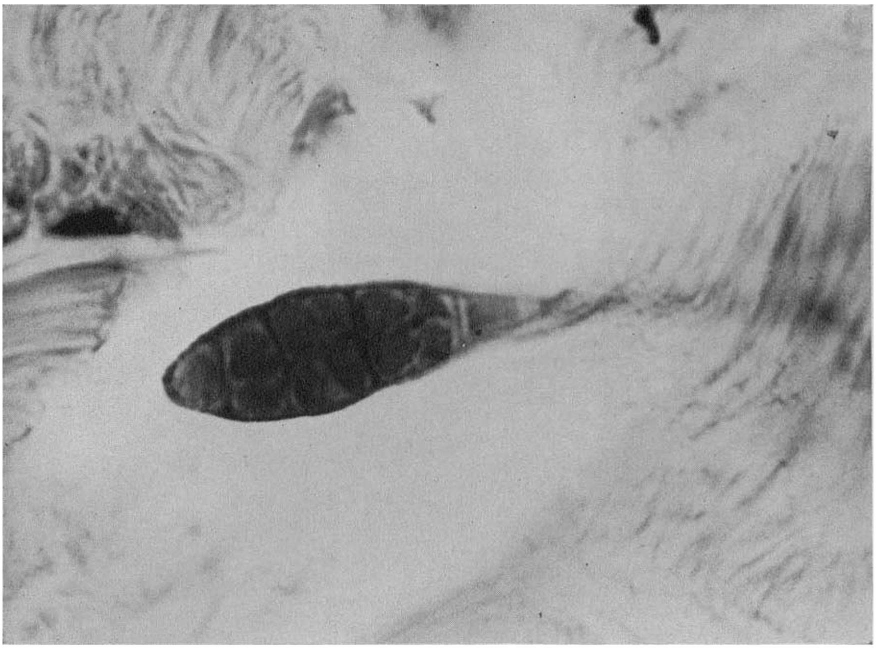

FIG. 6. Alternaria spore found in a histopathological section of a skin biopsy tissue at a level of the upper dermis.

In 1939 Ebertz and Lobitz (8) in Cincinnati reported finding ragweed pollens in histopathological sections of biopsy tissue taken from skin lesions of a patient with atypical scleredema. The pollens were located within or on top of the tissue sections. They considered this as merely a contamination during the preparation of the tissue sections in the pathology laboratory from an adjacent wind-swept field filled with ragweed plants. We had a similar experience, this time with alternaria. During the study of a routine skin biopsy section from a clinic patient, there was found an alternaria spore in the upper corium without any surrounding tissue reaction as shown in Fig. 6. It was speculated that instead of being a direct contamination of the slide from the air during the process of fixing and staining the tissue, this particular spore was present on the skin surface of the biopsy tissue and was carried down to the level of the dermis by the microtome knife during the sectioning.

SUMMARY

In examining the routine KOH mounts of skin scrapings from suspected superficial mycotic lesions the presence of fungous spores and of pollens was encountered.

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