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Determination of the Time of Death by Fungal Growth

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Summary. A woman was found in her bedroom, which had been kept at a constant temperature of 12° C, several weeks after she had been murdered. The establishment of the time of death was attempted by examination of fungal development on two parts of her body. Agar slopes were inoculated with the fungal growth present on an eyelid and on the inguinal skin. After 1 day at 20° C, the cultures were incubated at 11–12° C. The cultures were then observed daily until growth was comparable to that on the tissues that had been frozen. By these means it could be established that the victim died at least 18 days before her body was discovered. This, in fact, turned out to be the case. Hyphomycetes growth on a corpse may be helpful in determining the time of death when the event happened 10–20 days earlier, provided that the average surrounding temperature is known.

Key words: Time of death, post-mortem fungal growth – Fungi – Hyphomycetes

Zusammenfassung. In ihrem Schlafzimmer fand man die Leiche einer vor mehreren Wochen ermordeten Frau. Pilze hatten sich auf dem rechten Augenlid und auf der Haut der rechten Leistenfalte bis zum Stadium des arealen Myzeliums mit Sporenbildung entwickelt. Da die Temperatur des Schlafzimmers konstant bei 12° C geblieben war, wurde versucht, anhand der Pilzentwicklung auf Nährböden den Zeitpunkt des Todes zu bestimmen. Die Nährböden wurden täglich kontrolliert, bis die Pilzentwicklung ein Stadium erreicht hatte, das mit dem Myzelium auf den Geweben, die tiefgefroren konserviert worden waren, übereinstimmte. Dies ermöglichte die Feststellung, daß der Tod frühestens 18 Tage vor dem Auffinden der Leiche eingetreten war (30. Dezember 1979). Wie sich später herausstellte, war die Frau tatsächlich am 30. Dezember 1979 ermordet worden. Die Untersuchung der auf einem Körper vorgefundenen Hyphomycetes kann also bei der Bestimmung des Todeszeitpunkts sehr hilfreich sein, wenn dieser 10–20 Tage zurückliegt und man über die Temperaturumstände, denen die Leiche ausgesetzt war, informiert ist.

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Schlüsselwörter: Todeszeitbestimmung, Pilzbildung postmortem – Fungi – Hyphomycetes

Introduction

On January 16, 1980, the body of a 57-year-old baroness was found in her bedroom in an old mansion where she lived alone. She had died as a result of stab wounds to her chest, that had pierced her lungs and heart. She was lying almost naked and uncovered on her bed. The growth of intestinal flora had not yet commenced, and the interior body temperature was 12° C. Intense drying of the fingertips, lips and eyes and development of fungi on the right eyelid and on the skin of the right inguinal region indicated that death had occurred several weeks before. Since the whole mansion had been kept at a temperature of 12° C by central heating with thermostats in every room, the authors found it worthwhile to perform fungal cultures under controlled conditions to simulate what had happened on the victim's corpse after death. No information on this post-mortem phenomenon was found in the literature.

Material and Methods

Mycology

Three media were used for fungal growth: potato dextrose (PdT Difco B 13), Sabouraud (Sab. Difco B 109) and Trypticase Soy Agar (TSA Difco B 369). Chloramphenicol (20 µg/ml) was added to all media to inhibit bacterial overgrowth.

At autopsy the skin parts with fungal growth were removed and kept at 4° C until the next day when further examinations were carried out; from that day on, the skin parts were kept in a frozen state. The fungal growth media were then inoculated both with heavy inoculation (pieces of the skin) and with light inoculation (invisible on the loop). The tubes were incubated for 24 h at 20° C (the mean skin temperature of day 1 after death) and then placed at 11–12° C, the ambiant temperature of the bedroom. The fungal growth was recorded daily and compared with the state of the fungal development upon the pieces of skin which had been frozen.

The experiment was repeated twice, but the second time the inoculation was made at three points successively without a new inoculum on the loop. When the experiment was repeated, photographs were taken each day, beginning when visible growth appeared. For identification, the fungi were cultivated in petri dishes on 10 ml PdT-agar and also with the same medium on microscope slides. This technique allows the fine structures to be detected. Identification of the genus was made on the basis of the characteristics given by Gilman [3], Arx [1], Barron [2] and Raper [4].

Results

When a heavy inoculum (a piece of tissue) of the eyelid was used, growth was visible after 4 days on PdT-medium. Maximum development was obtained after 11 days. With a light inoculum the three media did not show any difference in visible growth: first growth was recorded at day 11, and heavy growth reached the edge of

Table 1. Time of visible fungal growth in days at 12° C

Material of autopsy	Medium					
	PdT		Sab		TSA	
	Visible	Heavy[a]	Visible	Heavy[a]	Visible	Heavy[a]
Infra orbital						
Heavy inoculum	4	11	—	18	4	18
Poor inoculum	11	18	11	18	11	18
Inguinal region						
Heavy inoculum	11		11	[b]	11	18
Poor inoculum	11		11	[b]	11	[b]

PdT, Potato dextrose medium; Sab, Sabouraud medium; TSA, Trypticase Soy Agar; —, Not carried out

[a] No heavy growth after 21 days

[b] Heavy: heavy growth means that the colony covers the slant until the glass edge

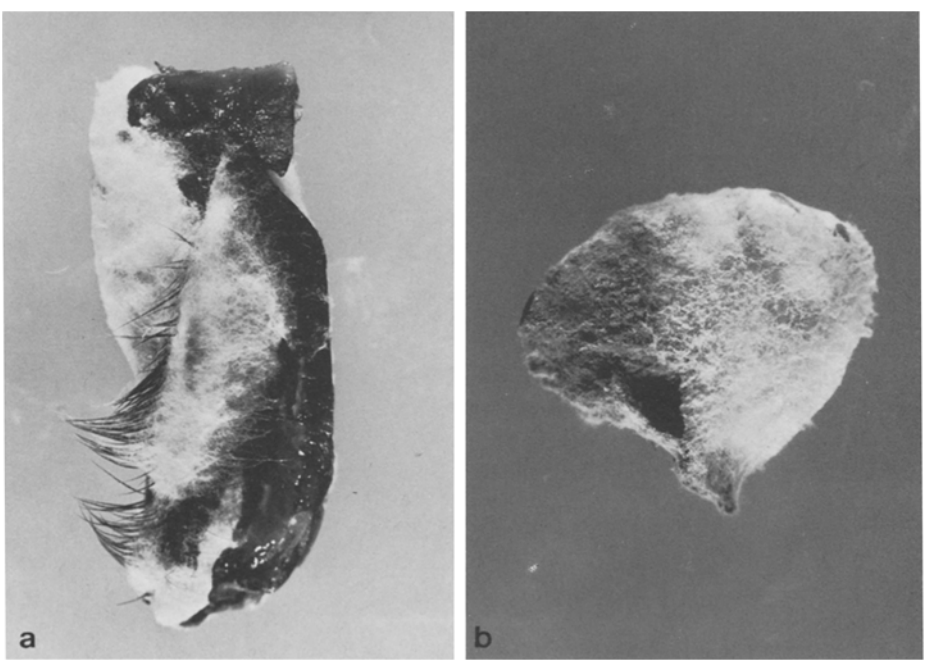

Fig. 1 a, b. Post-mortem fungal growth on the eyelid (**a**) and inguinal skin (**b**) removed at the autopsy and preserved at 4° C. (×2)

the testtube at day 18. Growth on Sabouraud-agar mostly resembled the fungal development on the eyelid, spreading with abundant aerial mycelium.

In the inguinal skin heavy growth was seen on TSA after 18 days and on the other media only after 21 days. The original growth on the inguinal skin was less

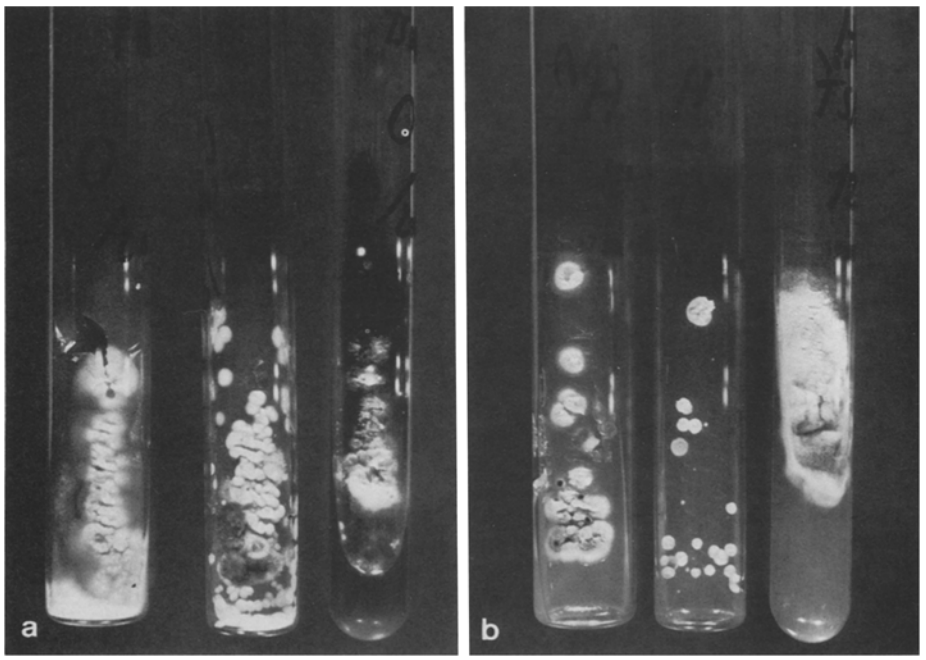

Fig. 2a, b. Cultures from tissues after autopsy, from the infra orbital region on three different media (a) and of inguinal tissue (b) (from left to right: Potato dextrose, Sabouraud, and Trypticase Soy Agar)

than that upon the eyelid, hence the slower development in vitro. These data are summarized in Table 1.

Figure 1 shows the pieces of tissue removed at autopsy after preservation for 21 days under refrigeration. Figure 2 illustrates some of the cultures from both tissues several days after the end of the growth recording but preserved at 12°C. These figures show mixed growth of different genera, but predominantly of one genus, which forms a white layer on the medium.

From these data it was deduced that the victim died at least 18 days before January 16, 1980 (day of the discovery of the body), the rationale being that one could expect two extreme conditions: heavy fungal contamination of the eyelid and groin skin prior to death and starting its growth immediately after death, or contamination by only some fungal spores starting to grow at any possible moment after death, the latter being the most likely.

The experiment was repeated twice, giving maximal and minimal time lapses of 23 and 11 days. Although this method is not very accurate, it was the only indirect method of estimating the post-mortem interval. The different genera were then

Fig. 3a–l. Microscopic mycology of the fungi. **a–c** The growth on tissue from the eyelid, *Geotrichum* (objective ×30) from the inguinal region, *Penicillium* (×8), and idem (×30). **d–l** Microscopic preparations of cultures: **d–i** from the eyelid; **d–f** *Geotrichum* (×8), idem (×30), and the sporangia of *Mortierella* (×30); **g–i** *Mortierella* spores in the sporangium (×8), *Cladosporium*, chain of conidia (×8), and a *Fusarium* (×30). **j–l** Cultures of the inguinal skin, Penicillium (×30), *Hormodendrum* (×8), and a *Cladosporium* (×30) (always left to right)

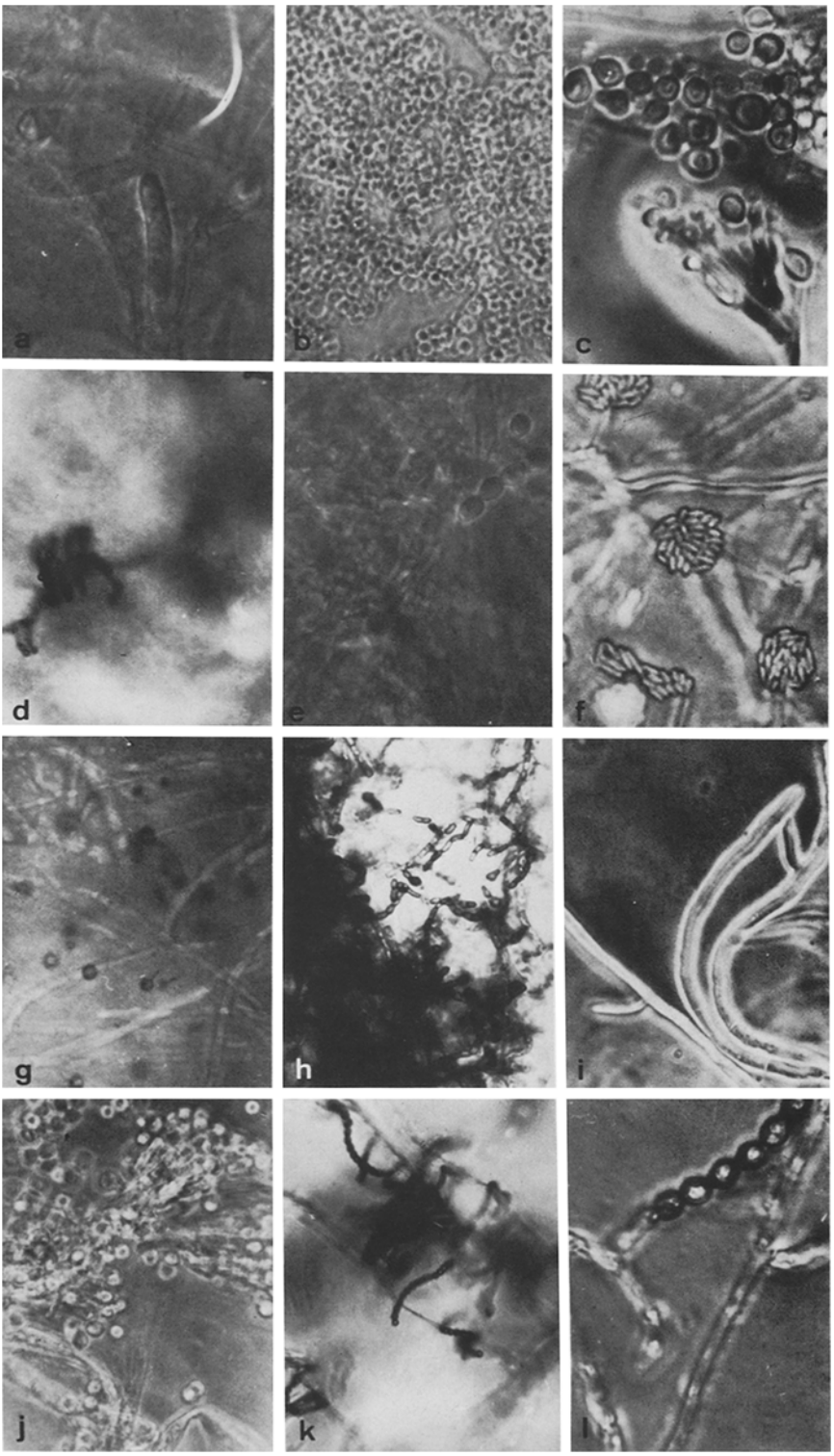

Fig. 3

identified following their morphology. From the palpebral region four different strains were isolated: *Geotrichum (candidum), Mortierella sp., Fusarium sp.* and *Cladosporium sp. Geotrichum sp.* growth was predominant on all media. From the inguinal region, three genera were obtained: *Geotrichum, Penicillium (notatum)* and *Hormodendrum sp.*

It is noteworthy that, after preservation of the tissue of the groin, the *Penicillium sp.* covered the entire surface. On the eyelid piece, however, there was only proliferation of *Geotrichum* (Fig. 1). The identification of the strains is illustrated in Fig. 3. The photographs show typical morphological characteristics of the genera. Identification of the species name was not attempted since it was not relevant to the purpose of this study, which was the determination of the time of death.

Discussion

The rate of fungal growth depends principally on inoculation and temperature and thus the moment of starting growth may be predicted by means of these factors. It may not always be possible to duplicate exactly the original conditions. As is shown with the inguinal skin piece, another fungus may overgrow heavily. For this reason the pieces must be frozen if confirmation is desired.

The determination of time of death is limited to the distinct phases of fungal growth: formation of substrate mycelium (first visible), aerial mycelium development (mostly white), sporulation (colour formation) and changes in colour after sporulation. The identities of the fungal genera probably are of no particular importance for this kind of work, since the growth of fungi strains after death depends on the environment in which the victim has lived. The described phenomenon is—to some extent—comparable to growth on stored grain [5].

The authors are fully aware that determining the time of death by this procedure is not an easy task. However, if all conditions are favourable it can lead to good results. In fact, several weeks after the laboratory work the offender was apprehended and admitted having committed the murder on December 30, 1979, i.e. 18 days before the corpse was found.

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