FUNGI (*Trichophyton interdigitale, Trichophyton violaceum, Epidermophyton inguinale, and Aspergillus oryzae*) AND FUNGICIDES.

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Introduction.

Reports on the isolations of fungi such as *Trichophyton interdigitale, Trichophyton violaceum Epidermophyton inguinale, Aspergillus*, and other fungi from infected nails, feet, skin lesions and even from the sweat of

normal person have been made by various investigators.[1] Studies concerned with their reactions to chemotherapy, and with *Aspergillus oryzae* in relation to food have been made. Certain substances such as mercurial compounds,[2] thallium acetate,[3] an ointment containing salicylic or benzoic acid[4] or both,[5] volatile oils,[6] various dyes[2][7][8], etc. have been found effective to prevent the growth or to kill the fungi at certain concentrations and under given conditions. However the concentrations required are beyond both the toleration of the human palate and the safety line for use either on the body or for preservation of food. Therefore it seemed important to study at first hand the influence both of the *p*H of the culture medium and of the concentration of the chemicals in relation to the worth of the various fungicides.

Experimental.

Description of Fungi. *Trichophyton interdigitale* (Kaufmann-Wolf) was found to be a fluffy white colony which grew rapidly in a rounded mass, and then spread over the medium. The colony later became cream-coloured, powdery and areolar.

Epidermophyton inguinale was greenish buff in colour, had a pyramidal form of Ascia and gradually covered the entire surface of the medium. No conidia were formed, but it was easily identified by fuseaux which were found on aerial hyphae.

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(1) E. M. Rockwood, *Arch. Dermat. and Syph.*, **22** (1930), 395; F. D. Weidman, ibid., **2** (1920), 703; **13** (1926), 374; S. O. Chambers and F. D. Weidman, ibid., **18** (1928), 568; A. Castellani, ibid., **17** (1928), 194, 354; J. F. Burgees, ibid., **14** (1926), 851; O. S. Ormsby and J. H. Mitchell, *J.A.M.A.*, **67** (1916), 711; F. W. Tanner and B. Feuer, *Arch. Dermat. and Syph.*, **1** (1920), 365; M. B. Hartzell, ibid., **1** (1920), 1; M. M. Keston, B. K. Ashford, R. W. Benham, C. W. Emmons, and M. C. Moss, ibid., **25** (1932), 1046; A. Strickler, E. A. Ozeller and R. P. Zaletel, ibid., **25** (1932), 1028; R. C. Jamieson and A. McCrea, ibid., **25** (1932), 321; C. W. Emmons, ibid., **25** (1932), 987; G. M. Mackee and G. M. Lewis, ibid., **23** (1931), 445; H. Fox and R. W. Fowlkes, ibid., **11** (1925), 446; H. Fox, ibid., **13** (1926). 398; M. F. Engman, ibid., **13** (1926), 352; M. B. Sulzberger, ibid., **18** (1928), 891; H. Odlan and R. E. Hoffstadt, ibid., **20** (1929), 335; A. G. Goud and E. K. Carter, ibid., **22** (1930), 225; S. M. Peck and G. Salomon, ibid., **24** (1931), 554; B. Blcck, *Brit. J. Dermat.*, **42** (1930), 549; O. L. Levin and S. H. Silvers, *Arch. Dermat. and Syph.*, **23** (1931), 1094.

(2) J. F. Schamberg and Kolmer, *Arch. Dermat. and Syph.*, **6** (1922), 746.

(3) B. E. Felden, ibid., **17** (1928), 182.

(4) J. H. Stokes, *J. Am. M. A.*, **98** (1932), 1127.

(5) J. H. Mitchell, *Arch. Dermat. and Syph.*, **5** (1922), 174.

(6) H. B. Myer and C. H. Thirnes, *J. Am. M. A.*, **84** (1925), 1985.

(7) D. L. Farley, *Arch. Dermat. and Syph.*, **2** (1920), 459.

(8) L. H. Leonian, ibid., **25** (1932), 1016.

Trichophyton violaceum developed (within four days) with a glistening surface. Within nine days a black violet colour developed from the center of the colony. No conidia were formed.

Aspergillus oryzae grew in a fluffy rounded white colony, rapidly spread over the medium, and gradually became dark yellowish green. Finally it became powdery and brownish green. It had characteristic conidiophores which made its identification easy.

Culture Medium. A specific group of fungi has a specific preference for a culture medium and produces a colony of definite shape and colour. For the present experiment Sabouroud's culture medium was prepared from the following ingredients:

Distilled water	Agar-agar	Peptone	Dextrose	Glycerine	Sodium chloride
1000 c.c.	15 gr.	15 gr.	20 gr.	5 c.c.	5 gr.

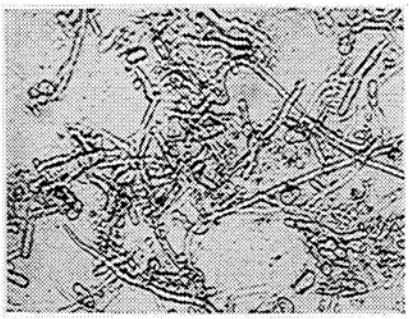
The mixture was boiled gently for one and a half hours until it became clear. The solution was then made up to the original weight with water and filtered through absorbent cotton. It was found, by potentiometric method using quinhydrone and calomel electrodes, to have a *p*H of about 5.4 which is similar to that of normal skin surface.[9] However the *p*H values of both sweat and blood of a normal person were found to be 7.33 which is also the optimum *p*H of pathogenic fungi. The *p*H of this medium was therefore adjusted approximately to 7.3 by means of about 5 c.c. of normal sodium hydroxide. Seven cubic centimeter portions of this solution, in test tubes of 20 c.c. capacity, plugged with absorbent cotton, were sterilized in an autoclave under 15 pounds pressure for 30 minutes. This treatment lowered the *p*H of the medium to 6.9 (±2). Nevertheless it gave rich growth. The tubes were stored in an ice-box to prevent evaporation of moisture.

The various fungicide solutions were made up in concentrations varying from 0.3 to 100 per cent. depending upon their effectiveness as fungicides.

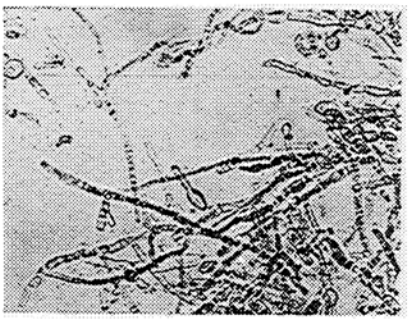
For each fungicide four series, each consisting of (usually) seven tubes of the medium, were taken. These were melted in a hot water bath, and then cooled to about 40°C. With a sterile 1 c.c. graduated pipette, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 c.c. of a fungicide solution were carefully added to the respective series. These were well mixed and the surface of each of the four series of media was then inoculated with a different fungus by means of a sterile platinum wire.

(9) O. L. Levin and S. H. Silvers, *Arch. Dermat. and Syph.*, **25** (1932), 823.

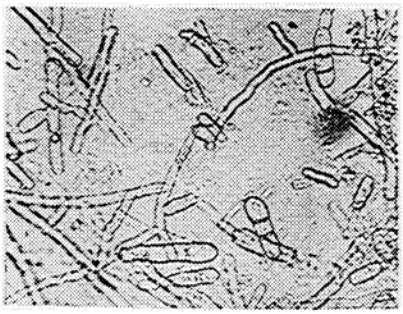
Microphotographs of Fungi



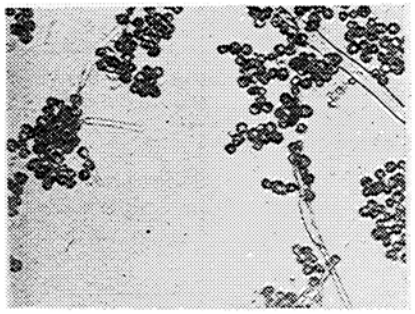
Trichophyton interdigitale ×450
Fig. 1.



Trichophyton violaceum ×450
Fig. 2.



Epidermophyton inguinable ×450
Fig. 3.



Aspergillus oryzae ×450
Fig. 4.

The tubes were kept at room temperature for more than three months of the period from April to September (of 1932 and 1933) which is the most favourable season for the growth of most of fungi. They were examined from time to time and the observations recorded. The results are tabulated giving the ratios of the chemicals to the amounts of medium. The figures at the left indicate the concentration at which the fungi grew while the figures at the right indicate the concentration too high for growth. However these values represent approximations only.

Discussion.

Solutions such as those of copper sulphate, phenol, boric acid, cobalt sulphate, hydrazine sulphate, chloramine T., Carrel-Dakin's solution,[10] etc.

(10) H. D. Dakin, *J. Am. M. A.*, **67** (1916), 1777.

TABLE. Relation between Fungi, Fungicides and the *p*H of media.

Fungicides	*Trichophyton interdigitale*	*Trichophyton violaceum*	*Epidemophyton inguinale*	*Aspergillus oryzae*
Boric acid	—	—	1:1,217–740 *p*H 6.37	1:486–370 *p*H 5.98
HgI_2	1:7,200–4,867 *p*H 6.44	—	1:14,200–7,200 *p*H 6.52	—
$HgCl_2$	—	—	1:12,000–8,110 *p*H 6.00	1:2,367–1,200 *p*H 5.31
C_6H_5OH	1:1,217–925 *p*H 6.45	—	1:1,217–925 *p*H 6.45	1:1,217–925 *p*H 6.45
Salicylic acid	1:1,217–925 *p*H 5.40–5.00	1:1,217–925 *p*H 5.40–5.00	1:1,217–925 *p*H 5.4–5.00	1:750–550 *p*H 4.17–4.20
Sodium salicylate	—	—	—	1:38–35
Sodium benzoate	—	—	—	1:12–9
$C_6H_5SO_2Cl$	1:660–536 *p*H 4.12	—	1:1,286–869 *p*H 4.62	1:537–452 *p*H 3.98
Pheylthiourea	1:1,100–800 *p*H 6.65	1:2,433–1,850	1;1,100–800 *p*H 6.68	1:293–227 *p*H 6.60
Thiourea	1:253–195 *p*H 6.78	—	1:720–487 *p*H 6.70	1:253–195 *p*H 6.78
$CuSO_4$	1:710–360 *p*H 4.02	—	—	1:710–360 *p*H 4.02
$Co_2(SO_4)_3$	1:1,286–652 *p*H 5.64	—	1:1,217–925 *p*H 5.64	—
Hydrazine sulphate	1:720–487 *p*H 6.22	—	1:720–487 *p*H 6.22	—
$NaHSO_3$	1:710–360 *p*H 6.41	—	—	1:710–360 *p*H 6.41
$Al_2(SO_4)_3$	1:243–185 *p*H 4.39	—	1:360–243 *p*H 4.73	—
$ZnSO_4$	1:710–360 *p*H 5.77	—	1:360–243 *p*H 5.64	—
Quinine sulphate	1:410–275 *p*H 6.20–6.00	—	1:701–334	—
Chloramine T.	1:710–360 *p*H 6.91	—	1:1,217–925 *p*H 6.74	1:710–360 *p*H 6.91
$NH_2OH \cdot HCl$	1:486–370 *p*H 4.97	—	—	1:710–360
Carrel-Dakin's solution (0.5% sodium hypochlorite)	1:18–12	—	1:18–12	1:9–5
Iodine	—	—	—	1:3,600–2,433
Acetic acid	—	—	—	1:93–75 *p*H 4.50–4.08
Lactic acid	—	—	—	1:19–15 *p*H 3.52–3.02
NaC	—	—	—	1:8–5

are of acidic or alkaline character. Therefore when they are added to the medium as described above, the *p*H is lowerd or elevated to a certain degree. For this reason the *p*H values of the media which prevent the growth or kill fungi are recorded underneath. However the *p*H values represent approximations only, since they are not determined at a constant temperature but over an interval between 35°C and 45°C.

Addition of fungicide to the medium influences the *p*H according to the nature of the compound. Certain chemicals as shown on the table do not act merely as fungicides, but their acidity is also responsible for the prevention of the growth of the fungi. This consideration is very important for practical application in chemotherapy.

As is seen in the table, HgI_2, iodine, $HgCl_2$ have the highest power as fungicides, and salicylic acid, phenyl-thiourea, $C_6H_5SO_2Cl$, chloramine T, etc. follow in order. However the effective power of $C_6H_5SO_2Cl$, $NH_2OH \cdot HCl$, sulphates, etc. may be due largely to their high acidity. Acetic acid is much more toxic than lactic acid. For example, *Aspergillus oryzae* can not grow in acetic medium at a *p*H of 4.08 while it grows in lactic medium even at a *p*H of 3.52. Sodium benzoate is a much weaker fungicide than lactic acid. If it is present in the medium in the ratio of 1:12 or about 8 per cent., *Aspergillus oryzae* will grow upon it. Sodium chloride has the weakest position among the chemicals. That is to say a ratio of about 1:5 or a concentration of 20 per cent. is necessary to prevent the growth of *Aspergillus oryzae*. This is of importance with regard to food preservation. It is interesting to note that thiourea exhibits a definite toxic value for the fungi combined with neutral and harmless properties for human tissue.

Summary.

The effectiveness of various fungicides on *Trichophyton interdigitale*, *Trichophyton violaceum*, *Epidermophyton inguinale*, and *Aspergillus oryzae* was studied, when added to Sabouroud's culture medium, previously adjusted to *p*H 6.9 (±2) and containing 0.5 per cent. of sodium chloride.

The results are tabulated giving the concentrations which permitted, and those which prevented, growth. The *p*H values at the concentration which prevented growth are recorded.

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