for agglutinins. Of the 8 carp that survived until the tenth week, at death, 2 had titers of 1:20, 2 had titers of 1:40, one had a titer of 1:80, and one of 1:640 (see table). The remaining fish died during the first 2 weeks of the experiment and had no agglutinins. In earlier experiments it has been shown that carp in nature do not have these agglutinins.

The other fish, adult rainbow and brown trout, held in 10°C running water, were subjected to weekly injections of 1 ml of *Bact. salmonicida* vaccine containing approximately 5 billion heat-killed virulent bacteria. Because of the conditions under which they were held, few trout survived longer than 5 weeks, and most of them died during the second and third weeks. Post mortem blood samples taken after the first week showed agglutinins, the results being recorded in the table. It will be noted that a number of trout had no agglutinins; but most of these died early in the study. Three of 74 control animals were shown to have titers as high as 1:80. This does not necessarily indicate the presence of natural agglutinins. The presence of agglutinins for *Bact. salmonicida* in these trout, which had received no vaccine injections, is more readily explained by the fact that these animals have been exposed to trout furunculosis throughout most of their several years of life. It is very possible that the antibody has been developed in response to this natural exposure of the trout to the infection.

These experiments indicate that agglutinin production takes place at 10°C. It is believed that earlier investigators failed to detect this phenomenon because their animals were not given the antigen over a long enough period of time.

Experiments are now in progress with gold fish, which tolerate extremes of temperature, to determine the effect of temperature on the rate of agglutinin production.

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Comparison of Growth of *Trichophyton interdigitale* on Wool Fabric With and Without Additional Nutritive Media.

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In connection with the Department of Agriculture's investiga-

tions on the sterilization of wool,[1-3] studies have been made of the growth of *Trichophyton interdigitale* on wool. This fungus is one of the micro-organisms responsible for the ringworm infection "athlete's foot."

Gould and Carter[4] inoculated wool with *T. interdigitale* and at the end of 2 months observed no evidence of growth. On the other hand, Bonnar and Dreyer[5] who studied the thermal death periods for *T. interdigitale, Microsporon lanosum, Epidermophyton cruris,* and *Trichophyton rosaceum,* grew these organisms on undyed flannel and found that they had permeated the fabric and, in some cases, apparently digested it. Berberian[6] obtained growth of *T. interdigitale* on a woolen stocking after sterilization and inoculation with the spores of this organism.

Materials and Methods. The material used was an undyed, laundered, wool-blanket fabric which weighed 23.9 ounces per square yard and was 0.159 inch thick. For this study the fabric was cut into ½-inch squares, and each square was sterilized in a separate Petri dish at 15 pounds steam pressure for 30 minutes.

The isolate of *Trichophyton interdigitale* used was a transfer of No. 4808 of the American Type Culture Collection, 1939. An inoculum was prepared by growing this culture at room temperature on slants of Sabouraud's agar for 10 days. The growth was carefully removed from the slant, transferred to sterile distilled water, and shaken with glass beads. The suspension was then centrifuged and the sediment washed with sterile distilled water. This process was repeated 2 additional times, and the final suspension of the spores made in sterile distilled water.

Four media were used in this work: Sabouraud's agar, peptone agar, mineral salts agar, and water agar. The composition of the Sabouraud's agar was as follows: agar, 18 g; peptone, 20 g; dextrose, 40 g; and distilled water 1 liter. The formula for the peptone agar was: agar, 18 g; peptone, 20 g; and distilled water, 1 liter. The mineral salts agar contained: agar, 18 g; $NaNO_3$, 3 g; K_2HPO_4, 1 g; $MgSO_4$, 0.25 g; KCl, 0.25 g; and distilled water,

1 Humfeld, H., Elmquist, R. E., and Kettering, J. H., U. S. Dept. Agr. Tech. Bull. No. 588, 1937, 26 pp.

2 Humfeld, H., Kettering, J. H., and Elmquist, R. E., U. S. Patent No. 2,204,360, 1940.

3 Elmquist, R. E., Humfeld, H., and Kettering, J. H., U. S. Patent No. 2,207,630, 1940.

4 Gould, G., and Carter, E. K., *Am. J. Hygiene,* 1931, **14**, 694.

5 Bonar, L., and Dreyer, A. D., *Am. J. Public Health,* 1932, **22**, 909.

6 Berberian, D. A., *Arch. Dermat. and Syphil.,* 1938, **38**, 367.

1 liter. The water agar consisted of: agar, 18 g; and distilled water, 1 liter.

All the media were adjusted to pH 5.8 by the colorimetric method using brom thymol blue and sterilized at 15 pounds steam pressure for 20 minutes. Each media was poured into sterile Petri dishes, 20 ml to each dish, and allowed to harden at room temperature. The plates were incubated 48 hours to test sterility.

Sabouraud's agar was considered a satisfactory nutrient media to support the growth of *T. interdigitale.* Peptone agar differed from Sabouraud's in that the carbohydrate carbon source, dextrose, was omitted. In the mineral salts agar, both dextrose and the nitrogen source, peptone, were omitted, but certain inorganic salts were added. The media which contained only agar and water was considered a non-nutritive base.

The sterile wool squares were transferred to the hardened agar plates by placing one piece of fabric in the center of each plate and pressing firmly against the media by means of sterile forceps. Ten squares were thus transferred to each of the 4 media, and 5 plates of each media were maintained as controls; that is, no fabric was added to the plate. Each plate containing the fabric was inoculated with a 0.1 ml suspension of the fungus by placing one-half of the suspension on the center of the wool squares and the remainder on the fabric edges. Each control plate also was inoculated in the center of the dish with the same amount of the organism. All plates were incubated for 14 days at room temperature.

Results and Discussion. All 5 controls of the peptone agar, as well as those of the Sabouraud's agar, furnished sufficient nutrient for the growth of *Trichophyton interdigitale,* but no growth occurred on any of the 5 replicates of the mineral salts media nor on those of the water agar media. Growth of the fungus was obtained on each of the 10 wool squares placed on Sabouraud's agar, on peptone agar, on mineral salts agar, and on water agar. These results are illustrated in Fig. 1, which shows the various controls and samples after incubation for 10 days at room temperature.

The results show that *T. interdigitale* was able to utilize wool fabric for its growth and multiplication in the absence of added dextrose or peptone, as evidence by its excellent growth on the fabric when either the mineral salts agar or the water agar were used. *T. interdigitale* apparently utilized peptone as a source for carbon and nitrogen, since peptone agar without wool supported a good growth.

The growth of *T. interdigitale* on wool was apparently stimu-

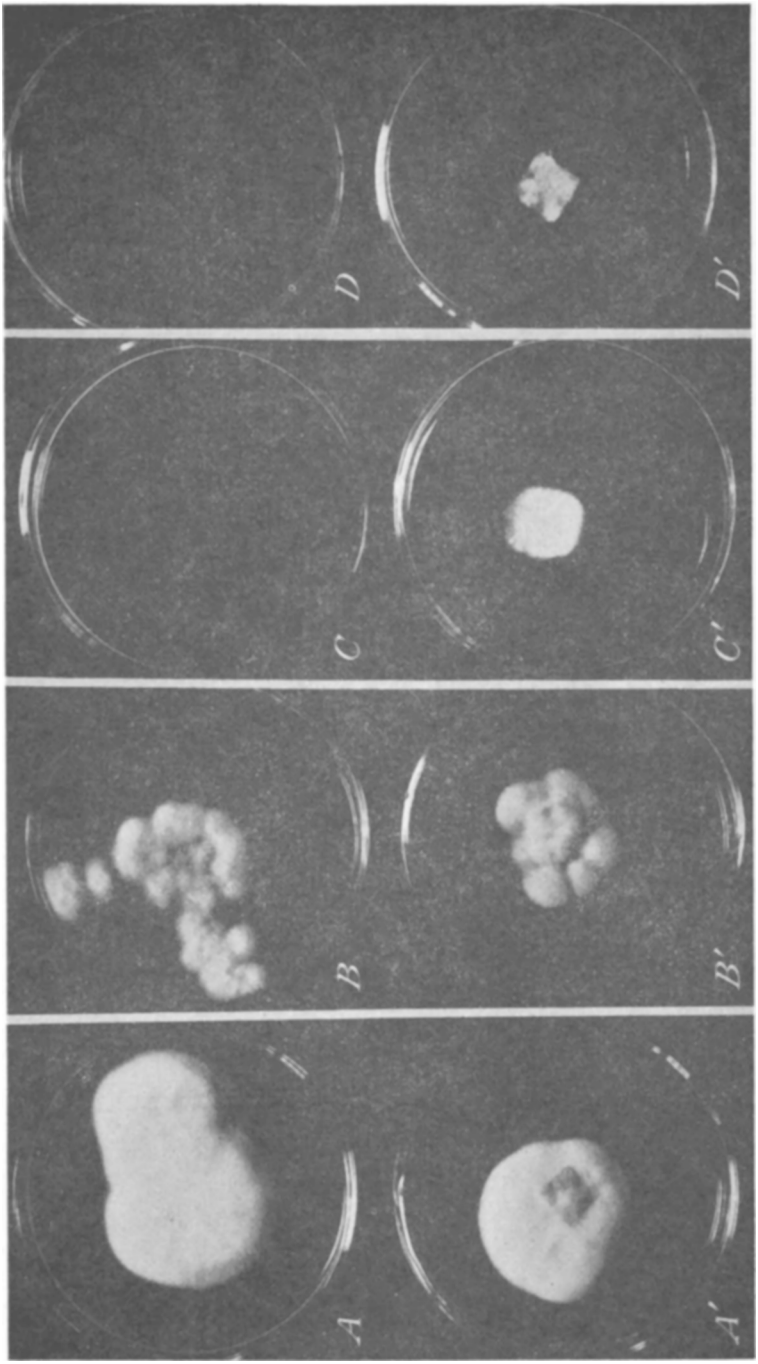

FIG. 1.

Growth of *Trichophyton interdigitale*: A, Sabouraud's agar without wool; A', Sabouraud's agar with wool; B, Peptone agar without wool; B', Peptone agar with wool; C, Mineral salts agar without wool; C', Mineral salts agar with wool; D, Water agar without wool; D', Water agar with wool.

lated by the addition of mineral salts. Examination of Fig. 1 shows increased growth of the fungus on the wool fabric where mineral salts agar was used in place of Sabouraud's agar, peptone agar, or water agar.

It was noted that the growth of *T. interdigitale* was slower on the wool when the mineral salts agar or water agar was supplied than when the Sabouraud's or peptone media were used. At the end of 4 days' incubation, growth was observed only on the two latter media.

Observation of Fig. 1 indicates that *T. interdigitale* utilized the media carbohydrate source of carbon in preference to the wool within a 10-day period. The growth on Sabouraud's agar without wool was profuse; whereas the growth on the wool placed on this agar was moderate. It is interesting to observe that this fungus showed the protein-sparing action of carbohydrates reported by Kendall[7] for bacteria.

When the dextrose is omitted, as in peptone agar, the wool was readily attacked and showed a good growth at the end of 10 days' incubation. At the end of 14 days, however, excellent growth also was observed on the fabric placed on the Sabouraud's media. This may indicate that after the media nutrient source is utilized, the wool offers a satisfactory substitute.

The findings reported here are of practical value, for wool hose are frequently worn by individuals suffering from "athlete's foot." These hose may be a constant source of infection since wool fabrics are not washed at sufficiently high temperatures, either in the home or in the laundry, to kill microorganisms. Legge[8] found that the most persistent cases of "athlete's foot" occurred when wool hose were worn. Also, because this fungus uses wool as a source of food, it causes deterioration of the fabric and hence decreases its serviceability. Possibly the mineral salt contained in perspiration and absorbed by wool hose aid in the growth of the fungus.

Summary and Conclusions. *Trichophyton interdigitale* was incubated on Sabouraud's, peptone, mineral salts, and water agar, both with and without the addition of wool. It was found that this fungus grew on the Sabouraud's and peptone media but not on the mineral salts or the water agar media. It grew on wool, however, when the wool was placed on all 4 media. Mineral salts appeared to stimulate the growth of the organism on the wool.

[7] Kendall, A. I., *Physiol. Rev.*, 1923, **3**, 438.

[8] Legge, R. T., *Am. J. Public Health and the Nation's Health*, 1931, **21**, 648.