riheme prosthetic nucleus and were it to occur in one of the components of a delicately poised enzyme system might modify its activity.

The only other suggestion as to the chemical nature of choline esterase has resulted from the investigations of Nachmansohn and Lederer.[18] They concluded that its activity was conditioned by a sulfhydryl group because of its inactivation by heavy metal ions; treatment with reduced glutathione was followed by reactivation. The same facts, however, can be cited in support of the ferriheme theory of its constitution. Ferrihemoglobin forms well defined compounds with each of the ions of the b family of the heavy metals of Groups I and II of the periodic system[19] (these incidentally also form insoluble sulfides) and the metalloferrihemoglobin combination is broken by its treatment with any reducing agent.

The hypothesis of the ferriheme nature of choline esterase and its corollary, that the enzyme may actually be a cytochrome, peroxidase, catalase, ferrihemoglobin and/or myomethemoglobin will have its scrutiny hampered by the universal distribution of iron compounds in living tissues.

[18] Nachmansohn, D., and Lederer, N., *Bull. Soc. Biol. Chim.*, 1939, **21**, 797.

[19] U. S. Patent No. 2,296,377 (assigned to Armour and Company).

14392

Antibacterial Filtrates from Cultures of *Aspergillus flavipes.*

EDWIN C. WHITE. (Introduced by J. Howard Brown.)

*From the Department of Bacteriology, Johns Hopkins Medical School.**

The production of a powerful, non-toxic antibacterial substance by *Penicillium notatum* has stimulated search in many quarters for other molds showing similar behavior. In addition to the work with *Aspergillus flavus* already reported,[1] the author has examined about 30 molds. Most of them were aspergilli, obtained from the collection of Dr. Charles Thom. A number have shown antibacterial activity of varying degree, but one, *Aspergillus flavipes,* has been of particular interest. As this work has been interrupted, it seems appropriate to record the results at this time.

The mold was designated in the Thom collection "175.4303.46-Texas soil." As received on an agar slant there was a heavy, tight mycelial growth which could be broken with difficulty, and spores for transfer were not easily obtained from this part of the growth. However, a light sandy area of sporulation was present at the tip of the slant where it was thinnest. This type of growth persisted in subcultures on slants, but by growing on Czapek-Dox agar in a Petri dish an even, sandy growth with ample sporulation was obtained. The growth at room temperature is colorless for about 3 days, then takes on a light brown sandy appearance as sporulation develops, and after 4 or 5 days a characteristic camphor-like odor is noticed. This odor has been developed only on solid media.

Little or no antibacterial activity was noted when the mold was grown on Czapek-Dox solution, but it appeared when a protein digest was used. With this medium (Tryptone-Difco) the best results were noted without any adjuvants at all, and sugar was definitely deleterious. The best medium of all, however, consisted solely of corn steep liquor, in the concentration of 5 or 10 ml to 100 ml of water. There was no definite advantage in the

* This work was done while the author was a guest in the Department of Bacteriology, with the aid of a grant from the Fluid Research Fund of the Rockefeller Foundation. The author gratefully acknowledges the helpful interest of Dr. J. Howard Brown, Dr. Ruth Wichelhausen, and Mrs. Lucille B. Robinson.

[1] White, E. C., and Hill, J. H., *J. Bacteriology*, 1943, **45**, 433.

higher concentration. This strongly acid solution was neutralized, and the precipitate formed on autoclaving was disregarded. Some variation was noted in the results with steep liquor of different origins, and results here recorded were obtained with corn steep liquor secured from the Corn Products Refining Co.

The 5- to 6-day filtrate, which was always alkaline, was neutralized, filtered through paper, clarified by centrifugalization if necessary and Seitz filtered. This filtrate was serially diluted with infusion broth, or, when *Strep. pyogenes* or a pneumococcus was the test organism, with broth containing 3% ascitic fluid. Further addition of one drop of defibrinated rabbit blood per ml did not alter the results, showing that presence of small amounts of blood does not interfere with the activity. One ml of each dilution (made in two-fold steps) was inoculated with a 4 mm loopful of the appropriate bacterial culture and incubated for 24 hours. In the case of *Cl. welchii* the incubation time was 48 hours in the anaerobic jar. The highest inhibitory dilutions noted are shown in the table. Although lower limits were found at times, the highest limits as recorded on the table were those generally observed. Indeed, the behavior of these filtrates has been more regular than those of any other mold worked with, including *P. notatum*. Within the period of observation, about 9 months, the constancy of appearance of the stock cultures and the constancy of antibacterial activity of the filtrates have suggested that the spontaneous formation of variants noted with other molds has been absent here.

TABLE I.

Inhibitory Dilutions of Filtrates from *A. flavipes* Grown on Diluted Corn Steep Liquor.

Organism	Inhibitory titer of filtrate
Strep. pyogenes C 203. (Old culture transferred daily for nearly a year, without mouse passage)	1280
Strep. pyogenes C 203. A virulent culture, from a recent mouse passage. (Obtained from Dr. Eleanor Bliss)	320
Pneumococcus, Type III, recently isolated from a case of meningitis	640
Pneumococcus, Type IV	1280
Cl. welchii, 24-hr growth in thioglycollate broth	640
Cl. welchii spores, 14-day growth anaerobically in sugar-free broth	640
Staph. aureus	5 to 10
Strep. fecalis	0 to 5
E. coli	No activity
A. aerogenes	" "

Without the isolation of a highly purified product one can only speculate about the nature of the antibiotic substance formed by *A. flavipes*. However, the pattern of behavior of these filtrates resembles that of filtrates from *P. notatum* tested during the course of this work, using the same organisms with both filtrates. The similarity is particularly noted in the case of *Staph. aureus*, where, with both molds, the activity was very low as compared to that against other Gram positive organisms. Filtrates from *A. flavipes* tested against *Staphylococcus aureus* by the Oxford cup and plate method showed zones of inhibition as wide as 25 mm in diameter. When the filtrate was diluted with one, 2 or 3 parts of broth, the zones were not narrowed proportionately, but only reduced to 22, 18 and 15 mm respectively. With this strain of staphylococcus the zones of inhibition were generally narrower with crude penicillin filtrates than with *A. flavipes* filtrates.

Preliminary experiments showed that the active material was largely, but not completely removed by charcoal at pH 4.0. No success was had in attempts to elute the material with buffers and various solvents. As much as 50% of the activity was recovered by concentrating the filtrates *in vacuo*, precipitating with 10 volumes of acetone and evaporating the solvent after filtering off the gummy precipitate.

The isolation of penicillin-like products from the author's strain of *A. flavus*[2] and from another strain of *A. flavus* by Bush and Goth,[3] together with the results here reported, suggest that further search may reveal that substances identical with penicillin or closely related products may be formed fairly commonly by aspergilli.

[2] McKee, C. M., and MacPhillamy, H. B., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 247.

[3] Bush, M. T., and Goth, A., *J. Pharm. and Exp. Therap.*, 1943, **78**, 164.