Kirby and Rantz[3] and 5 parts of Leventhal blood filtrate, prepared from equal parts of the above basal medium and horseblood by boiling and filtering through cheese cloth and a Seitz funnel. This medium was also found to be relatively free of sulfonamide inhibitors, using as a guide the action of low concentrations (10^{-5} and 10^{-6} M) of sulfathiazole on *E. coli*. The growth measurements were taken at times during which the increase in population was approximately linear (period of retarded growth) as determined by separate experiments. With the inocula used, this time was usually from 4 to 6 hours. The drugs chosen for study were again sulfathiazole, *p*-aminobenzoic acid and urea. Urea is of interest as a potentiator of the sulfonamides. In kind, the results paralleled those obtained on *E. coli*.

Fig. 1 shows most of the experimental results. Optical densities, as obtained from the drum of a Coleman Universal Spectrophotometer, are plotted against temperature. In the case of this organism, the density is directly proportional to population, and a density of 0.20 corresponds to a population of about 2.7 billion organisms per ml. The action of *p*-aminobenzoic acid alone has not been plotted, but at a concentration of 10^{-5} M it increased the growth rate up to the optimum growth temperature, and at higher temperatures exerted a marked inhibition. From the figure, it is clear that up to about 40°, *p*-aminobenzoic overcomes most of the bacteriostatic effects of sulfathiazole. At higher temperatures, the bacteriostatic action of this pair becomes increasingly pronounced. Again, the sulfathiazole shows greater bacteriostatic activity at the higher temperatures. Urea alone greatly diminishes growth at elevated temperatures, but, with this method of study, shows no synergistic action with sulfathiazole, except at the highest temperature.

Summary. The effect of sulfathiazole, *p*-aminobenzoic acid and urea, separately and in combinations, on the growth rate of *Streptococcus pyogenes* has been studied as a function of temperature. The bacteriostatic action of all these substances has been shown to increase with temperature. The sulfonamide-like action of *p*-aminobenzoic acid at higher temperatures is considered to be of particular significance. With the method used, urea shows no synergistic action with sulfathiazole on this organism. These results with *Streptococcus pyogenes* have been found to be analogous to those obtained with *E. coli*.

[3] Kirby, W. M., and Rantz, L. A., *J. Exp. Med.*, 1943, **77**, 29.

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An Antibiotic Substance Produced by Submerged Cultivation of *Aspergillus flavus*.

CLARA M. MCKEE AND HAROLD B. MACPHILLAMY. (Introduced by Geoffrey Rake.)
From the Divisions of Microbiology and Organic Chemistry, Squibb Institute for Medical Research, New Brunswick, N.J.

The strain of *Aspergillus flavus* used by White[1] and by Jones, Rake and Hamre[2] for the production of aspergillic acid has been grown in submerged culture with the production of an entirely different antibiotic substance. Erlenmeyer flasks containing a basic Czapek-Dox medium with the addition of adjuvant substances were inoculated with a heavy spore suspension of the mold. The flasks were subjected to constant, moderate agitation at a temperature of approximately 25°C. Within 48 hours there was heavy mycelial growth, and as this progressed, the

[1] White, E. C., *Science*, 1940, **92**, 127.

[2] Jones, H., Rake, G., and Hamre, D. M., *J. Bact.*, 1943, **45**, 461.

medium became increasingly alkaline and the antibiotic substance began to appear. Maximal activity, which coincided with a pH of about 8.4, appeared from 7 to 14 days after inoculation, depending upon the medium and method of cultivation.

The ability of this culture of *Aspergillus flavus* to produce an antibiotic substance different from aspergillic acid was not due to a change in the organism since cultures which produced this substance in submerged culture have been shown to produce aspergillic acid again in a typical manner under suitable conditions of surface growth.

The biological characteristics of this antibiotic substance, as so far demonstrated, suggest that it is either penicillin or a very similar substance. Both substances are highly active *in vitro* against gram positive cocci and are relatively inactive against gram negative bacilli. *In vivo* studies in mice reveal that the therapeutic efficacy of the two substances is the same when comparable doses, as determined by titration *in vitro*, are given. This is illustrated in Table I.

Other similarities between the two substances lie in the fact that both are excreted by the kidneys at about the same rate, and both are relatively non-toxic. A true estimate of comparative toxicity, however, can only be attained when purer preparations of the new substance than are now available are obtained. Further likenesses are shown by the fact that cultures which have been rendered resistant to one of the substances are resistant also to the other, although not to other antibiotic substances, and that a bacterial enzyme obtained from a culture of *Staphylococcus aureus* destroys the activity of both substances while it is inactive against aspergillic acid, gliotoxin and gramicidin.

Chemical studies have shown that, in regard to solubility and stability under various conditions, the active agent behaves much like penicillin. It is rapidly inactivated at room temperature below pH 4, and somewhat more slowly above pH 8. It can be extracted from ice-cold aqueous solutions at pH 2 with ether, amyl acetate or chloroform, recovered from the organic solvent with pH 6.5 phosphate buffer, and transformed into a relatively stable sodium salt in the same manner as penicillin. The active agent is also amenable to further purification by the chromatographic methods which have been applied successfully to the fractionation of crude penicillin.

A sodium salt, assaying 240 F.U./mg, which was obtained by chromatographing, showed the following analytical composition: C, 45.36%; H, 4.16%; N, 3.02%; Na, 13.36%. The specific rotation $[\alpha]_D$ was $+108^\circ$ (in water). These data, especially the comparatively high dextrorotation, are not incompatible with the assumption that penicillin or a very similar substance was a constituent of the mixture. Some α-furoic acid, identified by its melting point (129°) and by analysis, was later isolated from this fraction. The presence of this substance would account roughly for the deviation of the analytical figures from those found for penicillin sodium salts in the range of from 500 to 1000 Florey units.

Summary. An antibiotic substance entirely unlike aspergillic acid has been produced by submerged cultivation of a strain of *Aspergillus flavus*. This substance closely resembles penicillin biologically and chemically.

TABLE I.
Comparative Protection of Mice with Penicillin and the Antibiotic Substance from *Aspergillus flavus* Against a Type I Pneumococcus Infection.

Dose	Penicillin No. of mice L.	D.	Antibiotic substance from *Aspergillus flavus* No. of mice L.	D.	Control No. of mice L.	D.
100 Florey Units	9	1	10	0		
10 " "	8	2	7	3		
1 " "	4	6	8	2		
—					0	10

The mice were infected with 1 cc of a 10^{-7} culture.