LETTERS TO THE EDITORS

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Nitrogen Fixation by a Species of *Pullularia*

During the past few years an increasing number of organisms, other than *Azotobacter* and *Clostridium* species, have been found to fix small quantities of atmospheric nitrogen. *Chromatium* sp.[1], *Aerobacter aerogenes*[2], *Methanobacterium omelianskii*[3], *Pseudomonas*-like soil bacteria[4] and soil yeasts (*Saccharomyces* and *Rhodotorula* species[5]) have all been reported in the past three years as fixing atmospheric nitrogen. Nitrogen fixation by various lichens has also been described[6].

In the course of investigations by one of us (M.E.B.) concerning the stimulation of nitrogen-fixing organisms in the rhizosphere of clover plants grown in a clay–loam soil (*p*H 6·8) a black yeast-like organism regularly appeared on nitrogen-deficient agar plates. It has been identified as a species of *Pullularia*. On transfer to 10-ml. quantities of glucose–phosphate liquid medium containing no source of combined nitrogen the *Pullularia* grew well. After 14 days, nitrogen content assessments were made on replicate cultures by the micro-Kjeldahl method; compared with controls, gains of nitrogen of 0·20–0·26 mgm. per culture were found, corresponding to a fixation rate of 4–5 mgm. of atmospheric nitrogen per gm. of glucose supplied.

An estimation of the numbers of *Pullularia* in the clay–loam soil gave an approximate count of 8×10^3 cells per gm. of soil. The fungus was no more abundant in the rhizosphere of clover plants than in the surrounding soil.

Independently, Metcalfe has isolated what appears to be the same fungus from chalk soil, heath soil and a woodland mull soil and also from the thallus of the lichen *Cladonia uncialis*. These isolates grew readily in nitrogen-deficient washed agar media and in liquid media without added nitrogen compounds.

Grown on nitrogen-rich media, the fungus very rapidly loses its ability to fix atmospheric nitrogen. On nitrogen-deficient agar media, most of the growth is within the agar as rhizoid-like outgrowths from a small surface colony. Dark brown or black pigmentation occurs after a few days growth. On malt or yeast agar, pigmentation is usually slight.

We are indebted to Dr. Beryl L. Brady for identification of the organism as *Pullularia* sp.

Margaret E. Brown
Rothamsted Experimental Station,
Harpenden, Herts.
George Metcalfe
Department of Botany,
King's College,
University of London,
London, W.C.2.
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Origin of Antibiotic-Resistant Staphylococci

It is probable that antibiotic-resistant variants of bacteria arise by spontaneous mutation, and their further spread depends upon selective factors in the environment. Thus an antibiotic-resistant variant has a greater chance of survival when growing in the presence of the antibiotic, and this would explain the increase in the number of penicillin-resistant strains of *Staphylococcus pyogenes* isolated from hospital patients that has occurred since the introduction of the antibiotic[1].

However, a peculiar and significant finding is that the proportion of penicillin-resistant staphylococci in hospitals is increasing not only among those isolated from patients treated with penicillin, but also among those found in the noses of healthy carriers among the medical and nursing staff, most of whom have not been treated with the antibiotic. Thus, more than 90 per cent of the nasal carriers in a hospital may harbour penicillin-resistant *Staph. pyogenes*[2] and new personnel entering hospital with carrier strains which are penicillin-sensitive are, for the most part, rapidly colonized by the penicillin-resistant forms[3]. It has been suggested that these penicillin-resistant strains are acquired from the patients by contact and cross-infection, but it is unlikely that the penicillin-resistant strains are more suited to colonization of the healthy nose than the penicillin-sensitive strains, for, if this were so, one would expect their spread among the general non-hospital population. Our observations over the past few years show no evidence that there is a significant increase in the proportion of penicillin-resistant staphylococci among the general population.

A possible explanation is that the nurses and medical staff receive enough antibiotic on their hands and fingers and from the air to maintain a selective concentration in their noses which inhibits the penicillin-sensitive staphylococci. Confirmation of this possibility was sought by investigating the incidence of resistant strains in healthy carriers in an environment contaminated with antibiotic but which lacked treated patients as a source of resistant strains. Such an environment was a factory which handles and dispenses penicillin.

The staff of this factory, and a group of individuals from a general practice in the same geographical locality who had no contact whatsoever with the factory or its personnel, were first examined for nasal carriage of *Staph. pyogenes*. The carrier-rates for these groups and the antibiotic sensitivity and bacteriophage type of the carrier strains of *Staph. pyogenes* are shown in Table 1.

The carrier strains in the factory personnel resemble those commonly found in hospitals both in their resistance to penicillin (due to penicillinase production) and the limited number of types present. The majority of strains were of group III types and quite distinct from those isolated from the carriers among the general population group, which, in turn, are typical of the strains found elsewhere in the non-hospital population.

The penicillin content of the air was measured next, using an impinger-sampler collecting on to agar plates. The results are shown in the last column of Table 1. The concentration of penicillin present in the air of the penicillin filling-room and packing-room appears to be sufficient to depress the growth