and the necessary lengths of ½ in rod. A series of holes are drilled in the long rod to allow the ruling pens to be inserted; threaded holes at right angles to the pens contain the locking nuts.

The screens are inserted by resting the ruling pen on a sharp edge such as the base of the unit and picking up the grids from the edge of a glass microscope slide. The entire assembly is then placed in position in the rod. Suspensions are placed on the screen in any convenient manner.

FURTHER EVIDENCE FOR INDUCIBILITY OF STAPHYLOCOCCAL PENICILLINASE[1]

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Received for publication February 24, 1958

We have presented previously data supporting the conclusion that penicillinase is an inducible enzyme in a typical strain of penicillin resistant staphylococcus (Geronimus and Cohen, J. Bacteriol., **73**, 28, 1957). These observations were obtained with suspensions of intact staphylococci; they included experiments with organisms dried with acetone and ether in an attempt to determine whether the increased activity of penicillinase in staphylococci exposed to penicillin was the result of changes in rate of substrate transport to pre-existing enzyme or to an increase in concentration of enzyme (Barrett *et al.*, J. Bacteriol., **65**, 187, 1953). No evidence of changes in permeability of the staphylococci to penicillin was found in these experiments. However, in order to decide this point definitively, we have assayed the penicillinase activity of suspensions of untreated and penicillin treated staphylococci prior to and after disruption of the cells by sonic oscillation. The results give no evidence of a barrier between substrate and enzyme in the intact cells.

Staphylococcus aureus strain 55-C-1 was grown overnight at 37 C in static culture in 300 ml of a tryptic digest of beef heart broth. The resulting culture was diluted with twice its volume of 0.025 M $NaHCO_3$ and apportioned equally into two 1000 ml conical flasks, one of which received 0.9 g of Na penicillin G. The flasks were gassed for 5 min with 95 per cent air-5 per cent CO_2 and agitated on a rotary shaker for 90 min in the incubator at 37 C. During this time the concentration of staphylococci (calculated from optical density at 540 mμ in the Coleman Jr. spectrophotometer) in the penicillin treated suspension rose from 0.01 mg bacterial N per ml to 0.016 mg while that of the untreated suspension rose to 0.024 mg. The cells were collected by centrifugation at 2 C, washed once with 0.017 M $NaHCO_3$, and then resuspended in sufficient fresh broth to give untreated and penicillin treated suspensions containing 0.35 and 0.34 mg, respectively, of bacterial N per ml. A 12 ml aliquot of each suspension was treated for 40 min in a Raytheon 10 kc magnetostrictive sonic oscillator. An aliquot of the resulting sonically treated suspension was clarified by centrifugation at 2 C for 15 min at 20,000 × G. Penicillinase activity was assayed manometrically in each of the resultant prepara-

TABLE 1

Comparison of penicillinase activity of intact and sonically disrupted staphylococci

	Penicillinase Activity μL CO_2/hr/ml		
	Washed staphylococci	Disrupted cells	Disrupted cells, clarified
Untreated	69.5	55.5	40
Penicillin treated	2000	1032	1120
Ratio, penicillin treated to untreated	29	19	28

[1] This investigation was supported by research grants from the United States Public Health Service (E-883 (C-2)), the Massachusetts Heart Association and by a research contract DA-49-007-MD-892 between the Office of the Surgeon General, Department of the Army, and Harvard University.

tions (Henry and Housewright, J. Biol. Chem., **167,** 559, 1947). Table 1 summarizes results from one of two concordant experiments. The data demonstrate that the activity of staphylococcal penicillinase was increased 19- to 29-fold by exposure to penicillin, in confirmation of our previous reports. The activity of the intact staphylococci paralleled closely that of the corresponding disrupted cells. Therefore, these experiments indicate that the cell membranes of untreated staphylococci do not present any barrier between substrate and enzyme adequate to account for the 19- to 29-fold increase in penicillinase activity of the penicillin treated cells.

The findings support the interpretation that, in this strain of staphylococcus, the observed increase in penicillinase activity is the result of inducible biosynthesis of the enzyme.

ROUTINE USE OF SABOURAUD MALTOSE AGAR FOR THE RAPID DETECTION OF THE BLUISH-GREEN PIGMENT OF *PSEUDOMONAS AERUGINOSA*

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Received for publication March 10, 1958

In the clinical laboratory are often encountered difficulties in the identification of the members of the genus *Pseudomonas*, especially that of the type species *Pseudomonas aeruginosa*, because its characteristic pigment is not always formed in routine bacteriological media. In the absence of pigment, the distinction from the other members of the lactose nonfermenting genera (*Proteus, Salmonella, Shigella, Paracolobactrum*, and *Alcaligenes*) is not readily accomplished.

Gaby has reported the confusion in the literature concerning the characteristics of the genus *Pseudomonas* (Bacteriol. Proc., **1954**, p. 43).

On routine examination of clinical specimens for pathogenic bacteria and fungi, we noticed in some instances that Pseudomonas strains, which did not produce pigment on ordinary agar slants or on Sabouraud glucose agar, always gave a marked bluish-green pigment on Sabouraud maltose agar. A closely related phenomenon is observed with *Trichophyton rubrum* which produces its red pigment on Sabouraud glucose agar only.

Following up this first observation, we examined four Pseudomonas strains isolated from feces giving a light brown pigment only, six strains isolated from the ear canal which did not produce any pigment on ordinary agar slants, and one typical strain of *P. aeruginosa*.

Table 1 shows the advantage in using Sabouraud maltose agar for the rapid detection of the characteristic bluish-green pigment, even if the strains do not produce pigment at all on ordinary agar slants.

The addition of beef extract to Sabouraud maltose agar seemed to antagonize the production of pigment, especially with the strains which

TABLE 1

Pigment production by Pseudomonas strains on various media

No. of Strains of *P. aeruginosa*	Ordinary Agar Slant, pH 7.2 or 5.5	Glucose Agar, pH 7.2 or 5.5	Maltose Agar, pH 7.2 or 5.5	Sabouraud Maltose Agar	Sabouraud Glucose Agar
1 Typical pigmented strain	Bluish-green pigment	Light brown	+	++++	Light brown
4 Feces strains	Light brown pigment	Light brown	+	++++	Light brown
6 Ear strains	No visible pigment	No visible pigment	No visible pigment	++++	No visible pigment

Results read after 24 hr at 37 C; + to ++++ = intensity of the bluish-green pigment.