thresholds prior to treatment. One of these patients (J.B.) was also given deoxycorticosterone alone (10 mg/day), for 5 days. The detection thresholds after treatment with this drug were essentially the same as those prior to treatment, despite changes in serum sodium and potassium concentrations and a gain in body weight.

Thus we found that thresholds for the basic modalities of taste and for corresponding smells were lowered in cystic fibrosis. Sensitivity of taste in the patients was roughly 100 times (range 40 to 13,000 times) that of normal subjects; sensitivity of smell in the patients was roughly 10,000 times (range 10^3 to 10^8 times) that of normal subjects.

Whereas, after treatment with carbohydrate-active steroids, taste and smell thresholds for all test substances returned to normal in patients with adrenal insufficiency, no significant change was noted among patients with cystic fibrosis. Treatment with deoxycorticosterone alone did not alter the abnormal threshold in patients with adrenal insufficiency or in the one patient with cystic fibrosis who received it. These results call attention to another set of findings common to these two groups. Concentrations of sodium and chloride in the sweat of patients with untreated adrenal insufficiency and in patients with cystic fibrosis are abnormally high (*5–7*). After treatment with deoxycorticosterone or adrenal cortical extract the sweat-electrolyte concentration in patients with Addison's disease returns to normal levels (*5*). It has been reported that after restriction of sodium intake and treatment with deoxycorticosterone or 9α-fluorohydrocortisone the sweat-chloride concentration in patients with "fully manifest" cystic fibrosis does not return to normal levels (*7*). Changes in serum sodium and potassium concentrations do not alter taste or smell thresholds in patients with either adrenal insufficiency or cystic fibrosis.

No satisfactory explanation can be found for the normal taste and smell threshold exhibited by patient D.S. In view of the subjective nature of the test and the youth of the patient it is possible that there was some misunderstanding of the test instructions, which resulted in inaccuracy of threshold measurements. This patient was also the only female in the group. It is possible that not all patients with cystic fibrosis respond in like manner. That patient B.B. exhibited a normal smell threshold while exhibiting an abnormal taste threshold may be related to an acute exacerbation of this patient's severe chronic pansinusitis at the time of testing.

The mechanisms by which these phenomena occur are not clear. The underlying defect may reside in (i) the specialized end organ—that is, the taste bud or olfactory hair cell; in (ii) the cranial nerves that conduct the impulse —the 7th, 9th, or 1st; or in (iii) the brain itself. Preliminary data suggest no consistent gross abnormalities in the electroencephalographic responses or in the ulnar nerve conduction velocities of patients with cystic fibrosis (*8*), whereas these responses are abnormal in animals and patients with adrenal insufficiency (*8, 9*). Thus, the taste bud or olfactory hair cell—the end organs themselves—may be the site of the sensory abnormality in cystic fibrosis, whereas a general alteration in nervous system activity may be the basis of the sensory abnormality in adrenal insufficiency (*4*).

Taste and smell thresholds for NaCl and other modalities in a variety of acute and chronic debilitating diseases were comparable to those observed in healthy subjects (*1, 3, 8*). Cystic fibrosis is the second disease state (adrenal insufficiency was the first) in which a consistently abnormal threshold for taste and smell has been found.

These phenomena may lead to a better understanding of cystic fibrosis and of the physiology of taste and smell.

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Penicillinase Production in Some Bacilli

Abstract. Penicillinase production in strains of bacillus in which such production is constitutive, as well as in strains in which it is inducible, was found to be a function of the type of penicillin to which the organism was exposed. A repressive effect in two constitutive strains was noted. An increase in the level of penicillinase produced by the constitutive strains was noted for some of the penicillins.

It has long been known that a specific hydrolytic enzyme, penicillinase, is elaborated by certain micoorganisms in response to exposure to penicillin (*1*). While the same organisms produce penicillinase without having been exposed to this antibiotic, the basal level is much lower than the maximum observed when an organism is exposed to any of a large number of penicillins. In *Bacillus cereus* this has been observed to be a 300-fold (*2*) and 800-fold (*3*) increase.

Penicillinase is a clear-cut example of an inducible enzyme—that is, one that is produced in increased quantities in response to specific chemical stimuli. Penicillinase production is enhanced by several penicillins (*3, 4*). Mutants of *B. cereus* which produce penicillinase at a very high level without the stimulus of an added penicillin have been isolated (*5*). The level actually observed is higher than the maximum induced level so far observed in the closely related strains in which production of the enzyme is not constitutive. We report here the results of studies made to determine the effects of certain penicillins on penicillinase production in both inducible and constitutive strains of bacilli.

The penicillins used were benzyl penicillin (G), 6-aminopenicillanic acid (APA), 6-*N*-α-(phenoxy)pentanoylaminopenicillanic acid (PPA), 6-*N*-α-(*o*-benzy-*p*-chlorophenoxy) propionylaminopenicillanic acid (BCP, 6-*N*-α-(*p*-benzyloxyphenoxy)-propionylaminopenicillanic acid (BPP) (*6*), and cephalosporin C (CC). The microorganisms used were *B. cereus* [strains 5/B (NCTC 9946), 569/H (NCTC 9945), and 569 (NRRL 569)] and *B. subtilis* (strain ATCC 6633). Strains 5/B and 569/H are constitutive producers of penicillinase; the other two are strains in which production can be induced.

Suitable quantities of a casein hydrolyzate medium (CH/C) (*7*) were inoculated, usually by wire-loop transfer of the appropriate organism from an

agar slant. Growth at 37°C on a Burrell wrist-action shaker (size T) was allowed to proceed for no more than 16 hours—that is, within a few hours of the end of the log growth phase. The culture was then subdivided into several equal portions to allow comparison of the effect of the penicillins on different lots of the same batch.

Induction was carried out by three methods. The "continued growth" method differed only slightly from Pollock's "standard" induction procedure (*2, 3*). It consisted of taking log phase cells in their growth medium and adding enough of the penicillins to the respective vessels to produce the desired concentration. The vessels were incubated at 37°C with shaking for up to 5 hours; aliquots were removed at appropriate time intervals and assayed. The "cold pretreatment" technique was similar to Pollock's procedure (*2*). For this, the subdivided culture was centrifuged in a refrigerated centrifuge and the cells were washed with water. The cells were then resuspended in ice-cold water or medium containing the penicillins. This cell suspension was stored at 0°C for 1 hour. The cells were again centrifuged, washed, and suspended in fresh CH/C medium. The cells were then incubated at 37°C for up to 5 hours, aliquots for assay being taken at appropriate intervals. The third method involved exposure of the cells to the penicillins from the time the medium was inoculated. In every case, after growth, absorbance was measured in a Bausch and Lomb Spectronic 20 colorimeter. Penicillinase activity was assayed by a method essentially that of Perret (*8*).

All of the strains of bacilli investigated, with the exception of CC, showed growth inhibition when treated with the penicillins at concentrations of about $10^{-4}M$. The degree of growth inhibition varied for the various penicillins, but the relative effects on the four strains remained constant: APA < G < PPA < BCP < BPP. The maximum inhibition, under conditions of the continued growth method, was 30 percent. This relatively small inhibition is to be expected for the concentration of penicillins used and the large mass of cells; Wallmark and Finland have noted the effect of the size of the inoculum on penicillin sensitivity (*9*). With a small vegetative inoculum (0.1 ml of late log phase 5/B cell suspension added to 10 ml of CH/C; penicillins at about $10^{-6}M$), no inhibition of growth by CC was detected. Relative inhibition of growth was found to be as follows for the various penicillins: CC(~0) < PPA < BPP < APA ≪ G. Under these conditions G was almost completely bacteriostatic whereas the inhibitory effects of the others were relatively mild.

Table 1. Penicillinase production in some bacilli. The values represent the ratio of enzyme activity in the treated aliquots to the enzyme level in the control (basal level). The basal levels are shown in row 7 in terms of units of penicillinase per milliliter. A, values obtained by the continued growth method 2½ hours after addition of the penicillins; B, values obtained by the cold pretreatment method 2½ hours after resuspension in CH/C; C, values obtained from a small inoculum after 9 hours of growth.

Penicillin	*B. cereus* 569		*B. cereus* 5/B		
	A	B	A	B	C
APA	72	105	0.97	0.79	0.73
G	72	103	1.01	1.11	0
PPA	81	106	1.43	1.16	1.39
BCP	99	108	1.21	1.23	
BPP	103	111	1.33	1.25	1.24
CC					1.99
Basal	24	45	6800	7050	120

As may be seen in Table 1, the penicillins are inducers for the inducible strain 569 of *Bacillus cereus*. *Bacillus subtilis* responded entirely analogously. The two constitutive strains of *B. cereus*, 569/H and 5/B, exhibit an interesting phenomenon—an enhancement of the levels of penicillinase production above that of cells not exposed to the penicillins when the cells are treated with PPA, BPP, BCP, and CC. In contrast to this is a second phenomenon which may be seen from the tabulated data: APA lowers the level of penicillinase production in the constitutive strain 5/B (the effect in strain 569/H is similar). While the differences were not always large, they were seen consistently.

Many studies have been made of penicillinase induction by a variety of penicillins on several organisms. The results show considerable variation in response, depending on a number of variables. Among the more thorough studies are those of Pollock (*4*), Wallmark and Finland (*9*), and Steinman (*3*). Our purpose is not to compare our results with those of the many studies of growth inhibition but to discuss briefly the two phenomena mentioned above—enhancement of penicillinase production in constitutive penicillinase producers by some of the penicillins examined and lowering of penicillinase production in constitutive mutants by APA. Acting as an inducer or enhancer, CC had the most marked effect on the strains studied; BCP was very similar to BPP in its effect, whereas PPA was closer to G. Curiously, G seems to have the smallest effect on the constitutive strains, the degree of effect depending somewhat on the conditions of the study. Several possible explanations come immediately to mind, ranging from the possibility that strains 5/B and 569/H are in reality "semiconstitutive" to interpretations in the light of models of enzyme induction such as Szilard's (*10*).

The repressive effect of APA might be considered to be part of a general growth inhibition pattern. This material is in fact a growth inhibitor. Here, however, the inhibitory effect occurred only when cells were exposed to APA under the conditions of the continued growth method, or with the small inoculum, where the APA remained in contact with the cells for an indefinite period. With the cold pretreatment method of induction, in which excess APA is removed from the medium, it was found that there was no growth inhibition that could be detected by changes in optical density of the culture. Increasing the concentration of APA 100-fold produced no detectable growth inhibition under these conditions. It can be concluded that repression of the production of penicillinase in the constitutive strains is distinct from any gross effects on growth (*11*).

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