glionic depolarization induced by acetylcholine did not reappear when perfusion with lithium-Locke solution was extended beyond 10 minutes (Fig. 2). However, in each of eight experiments depolarization of the ganglia produced by potassium chloride (1 mg) was not materially altered during perfusion with lithium-Locke solution (Fig. 2). Both ganglionic transmission and acetylcholine-induced depolarization returned to control value upon reintroduction of sodium-Locke solution. The time-course of the several changes produced by lithium ions in ganglionic activity is illustrated graphically in Fig. 2.

In view of work by others (*2*), it can be assumed that conduction of impulses in the pre- and postganglionic neurons of the ganglia would be essentially normal during the period of perfusion with lithium-Locke solution. Inability of lithium to support transmission of impulses in sympathetic ganglia can be attributed, therefore, either to a decrease in release of acetylcholine from nerve terminals or to failure of lithium to substitute for sodium at the postjunctional membrane. Although it is not possible at present to decide between the alternatives, it is noteworthy that failure of transmission and the depolarization of ganglion cells by injected acetylcholine had a parallel time-course.

One of the most important differences in the cellular disposition of sodium and lithium is the relative difficulty with which the latter is extruded from intracellular sites (*2*). Therefore, it is possible that the failure of lithium to support ganglionic responses to acetylcholine is due to persistent ganglionic depolarization that results from accumulation of lithium ions within the ganglion cells. It has been shown that a low-amplitude, sustained depolarization of the superior cervical ganglion produced by tetanic preganglionic stimulation, pilocarpine, or anticholinesterase agents will suppress the depolarization evoked by injected acetylcholine (*3*). Because of the inherent property of the resistance coupled preamplifier to drift several hundred microvolts in 30 minutes, it was not possible to determine directly whether the ganglion was depolarized by lithium-Locke solution. It can be reasonably argued, however, that if the ganglion cells were depolarized as a result of an intracellular sequestering of lithium ions, then the ganglionic depolarization produced by potassium chloride, like that evoked by acetylcholine, should have been depressed. Since there was no change in ganglionic response to potassium, it is unlikely that ganglion cells were depolarized by lithium.

By exclusion, it appears that the inability of lithium to substitute for sodium in the superior cervical ganglion was due to the fact that mechanisms involved in the acetylcholine-induced ganglionic depolarization are able to discriminate between the two ions. This is in marked contrast to those mechanisms concerned with conduction of impulses in peripheral nerve and in skeletal muscle fibers, although the difference between junctional and conducting tissues with regard to the ability of lithium to substitute for sodium may be only quantitative in nature.

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References and Notes

1. C. J. Armett and J. M. Ritchie, *J. Physiol.* **165**, 141 (1963); D. H. Jenkinson and J. G. Nicholls, *ibid.* **159**, 111 (1961); A. J. Pappano and R. L. Volle, *J. Pharmacol.*, in press; A. Takeuchi and N. Takeuchi, *J. Physiol.* **154**, 52 (1960); N. Takeuchi, *ibid.* **167**, 141 (1963).
2. A. L. Hodgkin and B. Katz, *J. Physiol.* **108**, 37 1949); R. D. Keynes and R. C. Swan. *ibid.* **147**, 626 (1959); J. M. Ritchie and R. W. Straub, *ibid.* **136**, 80 (1957).
3. C. Takeshige and R. L. Volle, *J. Pharmacol.* **146**, 335 (1964).
4. Supported by NIH grant NB-06374-01.

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Ornithine Transcarbamylase Enzymes: Occurrence in Bacillus licheniformis

Abstract. *Two separate ornithine transcarbamylase enzymes have been found in extracts of late exponential-phase cells of* Bacillus licheniformis *grown on glucose and* L-*arginine. One enzyme presumably has a biosynthetic function and is repressed by arginine. The other is induced by arginine, is relatively heat-stable, and can be separated from the first by diethylaminoethyl chromatography.*

A recent report (*1*) demonstrated that ornithine transcarbamylase (OTC) (*2*) activity could be both repressed and induced by arginine in late exponential-phase cells of *Bacillus licheniformis*. Since it was clearly established that the induction of the enzyme activity could not be a derepression, a control system that would allow both events to occur in a single cell was difficult to envisage. Such a phenomenon has never been demonstrated; therefore it was important to determine whether the repressible and inducible OTC activities found in extracts of *B. licheniformis* have separate or identical physical identities.

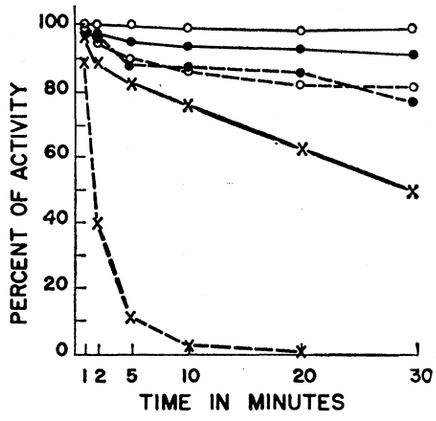

Fig. 1. Heat denaturation of induced and normal enzyme activities. Enzyme extracts were diluted to about 0.4 unit of OTC activity per milliliter in 5 m*M* tris-HCl buffer, *p*H 7.5, containing 1.0 m*M* mercaptoethanol (TM buffer) and incubated at 22°, 37°, and 55°C. At the times indicated, 0.1-ml samples were removed and immediately diluted to 0.9 ml with a solution (at 0°C) containing 100 μmole of tris-HCl buffer, *p*H 8.5, and 5 μmole of L-ornithine. After warming the solution to 37°C and adding 5 μmole of carbamyl phosphate, the OTC activity was then determined. Open circles, 22°C; closed circles, 37°C; *x*'s, 55°C; broken lines, induced activity; solid lines, normal activity.

Bacillus licheniformis, strain A-5, was grown in 1-liter lots to late-exponential phase in a minimal medium (*3*) with 20 m*M* glucose and 50 m*M* ammonium lactate as the carbon and nitrogen sources. Cells were harvested and washed twice at 0°C by centrifugation at 10,000*g* in a Sorvall RC-2 centrifuge. They were suspended in 2 m*M* tris-HCl buffer, *p*H 7.5, containing 1 m*M* mercaptoethanol and were disrupted for 2 minutes in a 20-kc MSE sonic oscillator. The crude extract was centrifuged at 0°C for 45 minutes at 37,000*g* and the supernatant solution was dialyzed against several changes of the same buffer and then frozen. The OTC activity in these extracts ranged from 0.2 to 0.5 unit per milligram of protein, one unit being defined as that amount of activity producing 1.0 μmole of citrulline per minute when measured by the method of Archibald (*4*). Protein concentration was determined by the

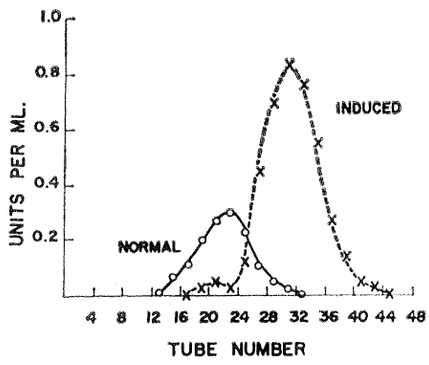

Fig. 2. Ornithine transcarbamylase activity after DEAE-column chromatography of extracts from normal and induced cells. The DEAE-cellulose was washed with TM buffer (see Fig. 1) and packed into columns (1 × 10 cm). The extracts (15.5 mg of the induced activity, 1.68 μ/mg; 33.0 mg of the normal activity, 0.24 μ/mg) were adsorbed onto separate columns and followed by 10 ml of TM buffer. Gradient elution was achieved by siphoning from 200 ml of TM buffer in a mixing flask attached to a flask containing the buffer plus 0.5*M* KCl. Fractions (5 ml each) were collected and OTC activity in the eluate was determined as in Fig. 1. Dotted line is the induced culture; solid line, normal culture.

method of Lowry *et al.* (*5*); the OTC assay was that described by Rogers and Novelli (*6*).

The OTC activity in crude extracts of late exponential-phase cells can be increased about tenfold by adding 10 m*M* L-arginine to the growth medium (*1*). The *p*H optimum of the "induced OTC" was determined and found to be the same as that of the "normal OTC"—about *p*H 8.5. The activity in extracts of these induced cultures was compared with the activity in normal cultures by two physical methods: (i) the heat stability of the activities, and (ii) the elution patterns from diethylaminoethyl (DEAE) cellulose chromatography columns. Both extracts, induced and normal, were prepared in an identical manner by the procedure outlined above.

Figure 1 shows the heat stability of the induced OTC and the normal OTC activities. Each of the points is an average of assays on three different preparations of each enzyme. At 55°C, the half-life of the induced enzyme activity is less than 2 minutes while that of the normal enzyme is 30 minutes. Thus, it appears likely that the activities reside in the different protein molecules.

Figure 2 demonstrates that the two activities elute in separate fractions from DEAE-cellulose columns. The normal enzyme is found in greatest quantity in tubes 20 to 25, whereas the induced activity elutes later, in tubes 28 to 34. It should be noted that a small amount of OTC activity in the extract of the induced culture eluted at the position of the normal enzyme. Both the amount and position of this activity are consistent with the proposal that the normal OTC activity in the genus *Bacillus* is repressible (*7*), and this small fraction would then be the residual, highly repressed, normal enzyme in the induced extract.

Several authors (*7*) have shown that OTC is under enzyme repression control in vegetative cells of *B. subtilis*. In addition, the induction of OTC activity in a nonrepressible strain of *Escherichia coli* has been demonstrated (*8*). Recently, Ramos *et al.* (*9*) suggested that cell extracts of a pseudomonad grown on citrulline as the only nitrogen source may contain two OTC enzymes. The enzyme activity in their extracts exhibited two *p*H optima and the amounts of activity at either *p*H varied with growth conditions.

The cellular control of the biosynthesis of OTC activity in *B. licheniformis* can now be explained with conventional mechanisms. During growth of this microorganism on glucose, L-arginine causes a repression of the normal biosynthetic enzyme. Although the induction of the second OTC is repressed by 10 m*M* glucose (*10*), this enzyme is synthesized rapidly near the end of the growth cycle. In the absence of added arginine, the repressible enzyme is formed and the inducible enzyme cannot be detected.

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References and Notes

1. R. F. Ramaley and R. W. Bernlohr, *J. Mol. Biol.* **11**, 842 (1965).
2. Ornithine carbamoyltransferase, No. 2.1.3.3 in *Enzyme Nomenclature* [Recommendations (1964) of the Int. Union of Biochem.] (Elsevier, New York, 1965), p. 94.
3. R. W. Bernlohr and G. D. Novelli, *Arch. Biochem. Biophys.* **103**, 94 (1963).
4. R. M. Archibald, *J. Biol. Chem.* **156**, 212 (1944).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *ibid.* **193**, 265 (1951).
6. P. Rogers and G. D. Novelli, *Biochim. Biophys. Acta* **33**, 423 (1959).
7. H. I. Lehrer and M. E. Jones, *ibid.* **65**, 360 (1962); R. H. Vogel and H. J. Vogel, *ibid.* **69**, 174 (1963).
8. L. Gorini and W. Gunderson, *Proc. Nat. Acad. Sci. U.S.* **47**, 961 (1961).
9. F. Ramos, V. Stalen, A. Pierard, J. M. Wiame, *Arch. Int. Physiol. Biochem.* **73**, 155 (1965).
10. E. Laishley and R. W. Bernlohr, *Bacteriol. Proc.*, in press.
11. I am indebted to Mrs. N. Minahan for technical assistance and to the U.S. Public Health Service for financial support (grants AI-05096 and GM-K3-7709).

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Terminology of Vertebrate Melanin-Containing Cells: 1965

At the Third Conference on the Biology of the Normal and Atypical Pigment Cell, held in New York in the fall of 1951, terminology for the pigment cell was suggested that has subsequently been accepted and utilized by scientists throughout the world (*1*). Since that time it has become evident that this terminology should be revised in the light of new findings in the biochemistry, ultrastructure, and cytophysiology of melanin-forming cells.

A questionnaire about the adequacy of terms in common use was sent to a large number of pigment-cell biologists during the winter of 1964–65 and later to all members of the Sixth International Pigment Cell Conference held in Sofia, Bulgaria, in May 1965. The results of this questionnaire will soon be published in the proceedings of the conference (*2*). The definitions that follow are consistent with the consensus of opinions expressed in answers to the questionnaire. It seems highly desirable to bring them to the attention of scientists in various fields. In summary (Tables 1 and 2), they constitute basically a restatement, with appropriate deletions and additions, of the terminology recommended in 1951 (Table 3).

The term "melanosome" (*3*) was introduced by Seiji and his co-workers (*4*) to describe a specialized organelle that develops within the melanocyte. This term was suggested because the term "melanin granule" had, until that time, been used indiscriminately to describe the pigmented particles of widely different size and structure that are present in melanocytes, macrophages and malpighian cells (keratinocytes). In 1963 Seiji *et al.* (*5*) proposed that three terms "premelanosome," "melanosome," and "melanin granule," be used to refer to these specialized organelles in different stages of development, melanization, and electron density. As originally proposed and used, these terms were defined as follows: *premelanosome*, a distinctive particulate protein matrix upon which melanin is usually deposited with consequent