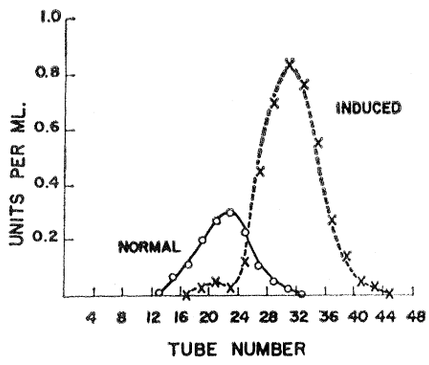

Fig. 2. Ornithine transcarbamylase activity after DEAE-column chromatography of extracts from normal and induced cells. The DEAE-cellulose was washed with TM buffer (see Fig. 1) and packed into columns (1 × 10 cm). The extracts (15.5 mg of the induced activity, 1.68 μ/mg; 33.0 mg of the normal activity, 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.

ROBERT W. BERNLOHR
Departments of Microbiology and *Biochemistry, University of Minnesota, Minneapolis*

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).

25 January 1966

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

formation of the melanosome; *melanosome*, a premelanosome after the onset but prior to the completion of melanin synthesis (melanosomes characteristically possess an active tyrosinase system); *melanin granule*, a melanin-containing organelle in which melanization is complete and no tyrosinase can be detected (may consist of a single melanosome or multiple melanosomes imbedded in a homogeneous matrix).

This nomenclature has been quite widely used, although certain difficulties arise in its application. For example, it has not yet been determined whether degree of melanization is the only factor that determines the electron density of the melanin-containing organelle. Furthermore, it is difficult to determine precisely the onset of melanin synthesis and therefore clearly to distinguish between premelanosomes and melanosomes. Accordingly, it is now proposed that the term *melanosome* be used to designate the fully pigmented melanin-containing organelle only. This suggestion that the term melanosome be applied to the distinctive terminal product of melanin synthesis is consistent with the nomenclature applied to other cell organelles (for example, ribosomes and lysosomes). The term *premelanosome* would then be applied to all stages in the genesis of melanosomes that precede the fully developed state. At the discretion of the investigator, and within the restrictions of his definition, the premelanosomal stage might be subdivided into early, intermediate, and late phases. The term melanin granule could be appropriately retained to include all melanin-containing particulates that can be observed with light microscopy. The additional terms (premelanosome and melanosome) for pigmented organelles would provide the amplification required by the electron microscopist and biochemist.

The term *melanocyte* is proposed to embrace all cells that produce melanin. Melanocytes are generally considered to be fully differentiated cells which have embarked on pigment synthesis. A melanoblast becomes a melanocyte with the formation of melanosomes. Strictly speaking, then, a melanoblast which can synthesize premelanosomes may become a melanocyte while undergoing embryonic migration toward its definitive location. Such a cell might properly be designated a migratory melanocyte. Although not a solution to all problems, this distinction between melanoblasts and melanocytes is based on a criterion that is not entirely arbitrary. In organisms of certain genotypes a failure of melanin synthesis leads to the formation of nonmelanized premelanosomes as terminal products; the presence of such bodies would confer melanocyte status on the cell that produced them.

The term *melanophore* has been commonly applied to the melanocytes of cold-blooded vertebrates that form an integral part of the mechanism for rapid change of color in the integument. This is consistent with tradition and with the new findings.

Regional differences in the location of melanocytes and melanophores may be indicated simply by use of the appropriate anatomical adjectives; for example, melanocytes in the retina, heretofore variously called "retinal pigmented cells" or "pigmented epithelial cells," could be described satisfactorily as *retinal melanocytes*; melanocytes of the epidermis as *epidermal melanocytes*; melanocytes of the dermis as *dermal melanocytes*, and so on.

The terms melanophage and macrophage are deleted from the present terminology. It is widely felt that the macrophage should be regarded only as an incidentally pigmented cell in which the incorporation of melanin granules is but one expression of its generalized phagocytic activities; accordingly, it does not clearly warrant a distinctive designation as a melanophage or inclusion in the melanocyte series.

The great majority of respondents favored the inclusion of the Langerhans cell in the proposed terminology. The status of the Langerhans cell as a precursor to melanocytes and, accordingly, its relationship to the melanoblast await clarification.

Table 1. Terminology proposed in 1965 for vertebrate melanin-containing cells, their precursors, and related cells.

*Melanocyte.** A cell which synthesizes a specialized melanin-containing organelle, the melanosome.
Melanophore. A type of melanocyte which participates with other chromatophores in the rapid color changes of animals by intracellular displacement (aggregation and dispersion) of melanosomes.
Melanoblast. A cell which serves at all stages of the life cycle as the precursor of the melanocyte (and melanophore).
Langerhans cell. A distinctive cell of the mammalian epidermis and dermis presumed to belong to the "melanocyte" series. It is revealed by gold impregnation and contains distinctive nonmelanized disc-like organelles.

* Included here are differentiated cells which synthesize nonmelanized or partly melanized premelanosomes as terminal products. It is suggested that in albinism the melanocytes containing nonmelanized premelanosomes be called *albino melanocytes*.

Table 2. Ontogenetic stages in the formation of melanin.

*Melanosome.** A discrete melanin-containing organelle in which melanization is complete; shown to be more or less uniformly "electron dense" by electron microscopy; tyrosinase activity not usually demonstrable.
Premelanosome. All distinctive particulate stages in the maturation of melanosomes. Electron density variable; possesses an active tyrosinase system after the onset of melanin synthesis.

* Multiple melanosomes imbedded in supporting matrices, for example, as in the macrophages and malpighian cells of mammals, may be designated *melanosome complexes*.

Table 3. Terminology recommended in 1951 (see Table 1) for pigment cells.

Melanocyte. Mature melanin-forming cell.
Melanoblast. Immature melanin-forming cell.
Macrophage (or melanophage). Cell with phagocytized melanin.
Melanophore. "Contractile" cell.

THOMAS B. FITZPATRICK
Harvard Medical School, Massachusetts General Hospital, Boston

WALTER C. QUEVEDO, JR.
Brown University, Providence, Rhode Island

ARNOLD L. LEVENE
Royal Marsden Hospital, London, England

VINCENT J. MCGOVERN
Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia

YUTAKA MISHIMA
Wayne State University School of Medicine, Detroit, Michigan

A. GEORGE OETTLE
South African Institute for Medical Research, Johannesburg, South Africa

References and Notes

1. T. B. Fitzpatrick and A. B. Lerner, *Science* **117**, 640 (1953).
2. *Structure and Control of the Melanocyte*, G. Della Porta, Ed. (Springer-Verlag, Berlin-Heidelberg-New York, 1966), in press.
3. H. Braunsteiner, F. Mlczoch, F. Pakeschi, *Klin. Wochschr.* **36**, 262 (1958), quite independently and prior to the introduction of the term by Seiji *et al.* (*4*), described a single case of human melanoma and compared melanin granules to ferritin and hemosiderin granules called "siderosomes." They used the term "melanosome" to describe melanin-containing "peculiar formations surrounded by a fine envelope which apparently had no connection to the well-known organelles."
4. M. Seiji, T. B. Fitzpatrick, M. S. C. Birbeck, *J. Invest. Dermatol.* **36**, 243 (1961).
5. M. Seiji, T. B. Fitzpatrick, R. T. Simpson, M. S. C. Birbeck, *Nature* **197**, 1082 (1963).

27 December 1965