Pleiotropy and the Detection of Point Mutations in the Mammal

by George L. Wolff*

Several point mutations in the mouse profoundly affect the metabolic characteristics of the animal. As examples may be cited the “muscular dystrophy” mutation (dy), the “obese” and “diabetes” mutations (ob,db), the “yellow” mutations (Ay,Awy, etc.) at the agouti locus, the two “pituitary dwarf” mutations (dw,df), and several mutations affecting body size, e.g., “pygmy” (pg)(1). In none of these cases has the polypeptide directly altered by the mutation yet been identified. In the case of the obese obob mutant the primary lesion remains unknown in spite of more than 20 years of effort by numerous investigators.

Most “visible” mutations, such as those affecting coat color, eye color, and other morphological and neurological characteristics, also fall into this category of mutations in which the primary polypeptide lesion is unknown. The affected characteristics, in general, result from the interactions of many developmental processes and metabolic pathways. The mutations presumably alter polypeptides which are involved at branchpoints or critical rate-limiting steps in one or more of the interacting metabolic pathways. Thus the influence of these mutations is exerted at some point prior to the final definition of the particular characteristic by which each mutant is recognized. These considerations suggest that the majority of these muta-

*Department of Health, Education, and Welfare, Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079.

December 1973 211
specific enzyme proteins as determined by electrophoretic techniques. Here, the primary lesion provides the basis for identification and localization of the mutation. However, in most cases the effects of the altered enzyme protein on the metabolic economy of the animal are unknown.

Methodology for detecting electrophoretically altered enzyme proteins and identification of the responsible mutations is at an advanced stage. However, no information regarding the metabolic importance of the detected variants can be obtained by sole reliance on these techniques.

At present, methodology for detecting mutations in which the phenotypes are expressed pleiotropically is limited to the specific locus test. This test is an easy, reliable, and accurate method for detecting mutations which have been induced at specifically identified loci. Its major limitation is the relatively small number of loci, at which "visible" mutations occur, which can be carried as recessive homozygotes in one or a few tester stocks because of deleterious effects on viability and fertility. Because different loci have varying degrees of sensitivity to different mutagens, this limitation is a serious one, especially from the point of view of assessing the relative mutagenicity of different substances and of different formulations of the same substance. In order to obtain valid data in this area, it is necessary to assay as large a proportion of the total genome as possible. This requirement is especially important if only one or, at best, a few assay systems are to be used for assessing the relative mutagenicity of large numbers of unrelated compounds.

Little, if anything, is known about the particular types of mutagenic changes represented by "pleiotropic" mutations. It is not known, for example, whether such mutations are induced generally by different classes of mutagens than mutations characterized solely by electrophoretic alterations. If the latter type of mutation has no apparent metabolic effects on the individual in its present environment, it may represent a mechanism of "preadaptation" for survival in an altered environment. Such mutations appear to be widespread in wild mouse populations and may represent metabolically and selectively "neutral" mutations.

Since mutations which alter only the electrophoretic properties of enzymes may differ from those which induce numerous pleiotropic effects in the relative threats they pose to the individual and to the population, it seems important to determine whether they, indeed, are induced by different classes of mutagens. In order to answer this question it is necessary first to develop assays to detect chemically induced pleiotropic mutations at a much larger number of loci than is possible with the present specific locus test.

From the viewpoint of a regulatory agency the information of particular interest is whether a substance is mutagenic and, if it is, its degree of mutagenicity in relation to other substances. To determine whether a substance is mutagenic, it is not necessary to identify the loci at which it has induced mutations. From a scientific viewpoint, however, the identification and localization of the individual chemically-induced mutations is of great importance. This is especially true of those mutations which may have potential utility in other areas of bio-medical research and also in those cases where it may be necessary to quantify risk.

On the basis of the foregoing rationale, Mammalian Genetics Branch, Division of Mutagenic Research, plans to develop methods to detect chemically induced pleiotropic mutations in genetically identified and controlled mice. The plans include use of assays of a series of enzyme activities to detect heritable alterations in certain characteristics of enzyme behavior such as thermolability, inducibility, degradation, allosteric regulation, and kinetic response to changes in the hormonal balance. In general, these enzymatic parameters are governed by conditions in the intracellular environment. Therefore, much of the data obtained in these studies will relate to mutations which,
directly or indirectly, affect the intracellular environment, either in specific tissues or in the animal as a whole. Breeding tests will be used to ascertain whether specific alterations are heritable or largely environmental in origin. It is anticipated that potentially useful mutations may be identified and preserved as a result of these tests.

Assays for detecting those mutations which appear to alter only the electrophoretic properties of specific enzyme proteins will also be employed in conjunction with the same experimental systems. Therefore, it should be possible to explore in some detail whether these supposedly “neutral” heritable changes actually do exert more or less subtle influences on the metabolic equilibrium or are truly without effect.

REFERENCES
1. Green, E. L., Ed., Biology of the Laboratory Mouse, 2nd ed. McGraw-Hill, New York, 1966.
2. Wolff, G. L., and Pitot, H. C. Response to hepatic enzyme activities to castration and sarcoma 37 growth in different mouse genotypes. J. Nat. Cancer Inst. 49: 405 (1972).
3. Wolff, G. L., and Pitot, H. C. Influence of background genome on enzymatic characteristics of yellow (A⁻⁻/⁻, A⁻⁻⁻⁻) mice. Genetics 73: 109 (1973).