sible trophic factors provided by peripheral inputs on the survival of central nervous system neurons during normal development and in certain types of injury or neurologic disorders.

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References and Notes

1. M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon, A. T. McPhail, *J. Am. Chem. Soc.* **93**, 2325 (1971).
2. J. Parness and S. B. Horwitz, *J. Cell Biol.* **91**, 479 (1981).
3. P. B. Schiff, J. Fant, S. B. Horwitz, *Nature (London)* **277**, 665 (1979).
4. P. B. Schiff and S. B. Horwitz, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1561 (1980).
5. M. DeBrabander, G. Geuens, R. Nuydens, R. Willebrords, J. DeMey, *ibid.* **78**, 5608 (1981); L. D. Simone, S. L. Brenner, L. J. Wible, D. S. Turner, B. R. Brinkley, *J. Cell Biol.* **91**, 337 (Abstr.) (1981).
6. E. B. Masurovsky, E. R. Peterson, S. M. Crain, S. B. Horwitz, *Brain Res.* **217**, 392 (1981); *IRCS Med. Sci.* **9**, 968 (1981).
7. R. Levi-Montalcini and P. U. Angeletti, *Pharmacol. Rev.* **18**, 619 (1966).
8. H. Thoenen and Y.-A. Barde, *Physiol. Rev.* **60**, 1284 (1980); G. P. Harper and H. Thoenen, *Annu. Rev. Pharmacol. Toxicol.* **21**, 205 (1981).
9. S. M. Crain and E. R. Peterson, *Brain Res.* **79**, 145 (1974).
10. ———, *Science* **188**, 275 (1975); *Dev. Brain Res.* **2**, 341 (1982).
11. S. M. Crain, *Neurophysiologic Studies in Tissue Culture* (Raven, New York, 1976).
12. E. R. Peterson and S. M. Crain, *Soc. Neurosci. Abstr.* **7**, 55 (1981).
13. ———, M. R. Murray, *Z. Zellforsch. Mikrosk. Anat.* **66**, 130 (1965); S. M. Crain and E. R. Peterson, *Science* **141**, 427 (1963).
14. A. F. W. Hughes, *Aspects of Neural Ontogeny* (Academic Press, New York, 1968).
15. J. I. Johnson, C. Hamilton and J.-C. Hsung, *Brain Res.* **38**, 421 (1972).
16. M. Jacobson, *Developmental Neurobiology* (Plenum, New York, ed. 2, 1978).
17. E. R. Peterson and S. M. Crain, *Dev. Brain Res.* **2**, 363 (1982).
18. S. M. Crain, E. R. Peterson, M. Leibman, H. Schulman, *Exp. Neurol.* **67**, 205 (1980).
19. P. D. Gorin and E. M. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5382 (1979); E. M. Johnson, P. D. Gorin, L. D. Brandeis, J. Pearson, *Science* **210**, 916 (1980); L. Aloe, C. Cozzari, P. Calissano, R. Levi-Montalcini, *Nature (London)* **291**, 413 (1981).
20. Taxol (1 m*M*) in dimethyl sulfoxide (DMSO) was diluted in culture medium to 1 to 2 μ*M*. Explants exposed to these levels of DMSO were not significantly affected.
21. E. R. Peterson, S. M. Crain, E. B. Masurovsky, S. B. Horwitz, P. B. Schiff, *J. Cell Biol.* **87**, 77 (Abstr.) (1980); in preparation.
22. Cell counts were made during microscopic observations of the living cultures with an oil-immersion objective lens (×40) and verified after fixation and staining.
23. Horseradish peroxidase was injected iontophoretically into the DRG's through glass micropipettes (tip diameter, 5 to 10 μm) filled with 20 percent HRP by applying two 100-msec pulses per second for 30 minutes. After 1 hour a modified Hanker-Yates histochemical procedure (*24*) was used prior to fixation (D. R. Friedlander and S. M. Crain, in preparation).
24. N. R. Smalheiser, E. R. Peterson, S. M. Crain, *Dev. Brain Res.* **2**, 383 (1982).
25. K. Stoeckel, M. E. Schwab, H. Thoenen, *Brain Res.* **89**, 1 (1975).
26. J. A. Kessler and I. B. Black, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 649 (1980); *Brain Res.* **208**, 135 (1981).
27. U. Otten, M. Goedert, N. Mayer, F. Lembeck, *Nature (London)* **287**, 158 (1980).
28. M. Goedert, K. Stoeckel, U. Otten, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5895 (1981).
29. G. M. Menesini-Chen, J. S. Chen, P. Calissano, R. Levi-Montalcini, *ibid.* **74**, 5559 (1977); E. M. Johnson, *Brain Res.* **141**, 105 (1978).
30. P. Dustin, *Microtubules* (Springer-Verlag, Berlin, 1978).
31. S. Varon, S. D. Skaper, M. Manthorpe, *Dev. Brain Res.* **1**, 73 (1981).
32. Supported by research grants to S.M.C. from the National Institute of Neurological and Communicative Disorders and Stroke (NS-14990) and from the Dysautonomia Foundation. Taxol was provided by S. B. Horwitz and NGF by Collaborative Research, Inc. Tissue culture facilities were kindly provided by M. Bornstein. Thanks are due to B. Crain for skillful technical assistance in the HRP labeling and electrophysiologic experiments.

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Histocompatibility and Isoenzyme Differences in Commercially Supplied "BALB/c" Mice

Abstract. *BALB/c mice obtained commercially were found to differ significantly from the standard phenotype of BALB/c strain mice. Isoenzyme tests and H-2 haplotype analyses indicated that the majority of mice from two of the three sources tested appeared mixed, frequently heterozygous, and did not consistently express either the expected H-2 or glucose phosphate isomerase type.*

In experiments conducted by us during the past year, several hundred commercially bred BALB/c mice were obtained from Charles River Breeding Laboratories to supplement BALB/cAu females produced at the University of Wisconsin. These animals were used in long-term experiments whose aim was to produce chimeric mice of BALB/c ↔ CBA/T6 constitution by injecting CBA/T6 cells into BALB/c embryos. In the course of analyzing internal chimerism among the 48 mice that were ultimately born to foster mothers during the period of December 1980 to July 1981, it became apparent that there was a serious discrepancy in the phenotype of the majority of these mice. Normally, the two parental strains are characterized by differences in the isozyme glucose phosphate isomerase (GPI) and H-2 haplotype (BALB/c: *Gpi-1*^{a}, *H-2*^{d}; CBA/T6: *Gpi-1*^{b}, *H-2*^{k}), in addition to external pigmentation and chromosome markers. Electrophoretic analysis of the isozyme samples from the injected animals, derived from embryos of mice obtained from Charles River Breeding Laboratories, showed that the GPI pattern exhibited by the majority of the animals

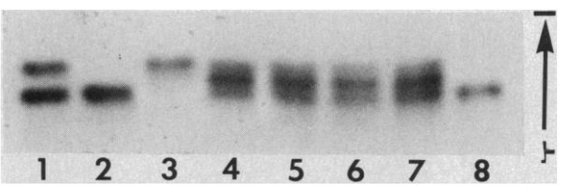

Fig. 1. GPI isozyme patterns of mice from inbred strain controls and of mice received from Charles River. Blood lysates were subjected to electrophoresis in tris-glycine buffer, *p*H 8.5, on cellulose acetate plates (Titan III, Helena Laboratories). (Slot 1) A 1:1 mixture of blood lysates from BALB/cAu and CBA/T6Au; (slot 2) BALB/cAu; (slot 3) CBA/T6Au; (slot 4) BDF1; and (slots 5 to 8) four different BALB/c animals received from Portage.

Table 1. MLC reactions among individual BALB/c mice from different facilities.

Stimulating cell*	Responding cell incorporation of tritiated thymidine†	
	BALB/c-IRC-M	B10.M
BALB/c-IRC-M	6,312 ± 2,053	63,952 ± 7,101
BALB/c-S_1	51,354 ± 5,983	63,091 ± 6,374
BALB/c-S_2	4,412 ± 1,938	35,870 ± 10,790
BALB/c-S_3	108,155 ± 10,792	55,353 ± 5,358
BALB/c-P_1	125,802 ± 25,739	47,909 ± 12,277
BALB/c-P_2	8,286 ± 2,018	70,973 ± 6,436
BALB/c-P_3	5,954 ± 1,046	57,644 ± 6,684
BALB/c-W_1	6,389 ± 1,552	56,479 ± 7,754
BALB/c-W_2	6,250 ± 1,830	59,068 ± 5,679
BALB/c-W_3	8,170 ± 1,136	37,238 ± 6,706
B10.M	123,045 ± 27,737	4,850 ± 1,717

*IRC-M, BALB/c^J colony, Immunobiology Research Center, University of Minnesota; S, C.R. Stoneridge; P, C.R. Portage; W, C.R. Wilmington; B10.M, H-2^f allogeneic control. †Results are given as number of counts per half-minute ± the standard deviation. Average of six replicate cultures of 5×10^5 stimulating spleen cells plus 5×10^5 responding spleen cells. Stimulating cells were exposed to x-rays (2500 roentgen) prior to mixing with responding cells. Tritiated thymidine was added on day 5 for 6 hours before assay.

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was not GPI-A or GPI-B, nor a chimeric mixture of these, but an F_1 GPI-AB pattern.

Neither the vasectomized mice used to produce pseudopregnant foster mothers nor any of the mice originating from our own colony were found to be anything other than the expected type. Inbred mice were examined by electrophoretic analysis of GPI isozymes and cytotoxicity and flow cytometric analysis for H-2 haplotype. By these same criteria, however, many of the animals received from Charles River that were not used for experiments and thus were available for testing, including the males used to generate all our BALB/c embryos, were not BALB/c by either GPI or haplotype analysis. The GPI-A, B, or F_1 AB band patterns were nearly equally prevalent, and many showed specificities other than H-2^d, as determined by testing with NIH alloantiserum D25 which reacts with specificities that include H-$2K^k$, K^q, and K^r but not with K^d or D^d.

Table 2. Summary of H-2 antibody-mediated cytotoxicity tests with individual BALB/c mice. Lymph node cells were placed in Terasaki plates for 15 minutes with antibody at twofold dilutions of 1/50 to 1/800 or with no antibody. The cells were rinsed and complement was added for 30 minutes at 37°C. The cells were stained with nigrosin dye, and the percentage of dead cells at each dilution was scored; NT, not tested.

Mouse*	H-2 antiserum†				MLC stimulation of BALB/c-IRC-M‡
	αD^d	$\alpha K^d I^d$	$\alpha H\text{-}2^v$	αK^q	
BALB/c-IRC-M	+	+	−	−	−
BALB/c-S_1	+	±	−	+	+
BALB/c-S_2	+	weak +	−	−	−
BALB/c-S_3	−	−	+	+	+
BALB/c-P_1	−	−	+	+	+
BALB/c-P_2	+	+	−	−	−
BALB/c-P_3	−	weak +	−	−	−
BALB/c-W_1	+	+	−	−	−
BALB/c-W_2	+	+	−	−	−
BALB/c-W_3	+	+	−	−	−
B10.G	−	−	+	+	NT

*Symbols as in Table 1; B10.G, H-2^q. †The antiserums are identified as follows: αD^d, MD-2 9 (B10.AKM × ASW)F_1 anti-ATH; $\alpha K^d I^d$, MK-3(31) (A × B10)F_1 anti-B10.D2; $\alpha H\text{-}2^v$, E-d.V (B10.D2 × DBA/2)F_1 anti-B10.sm; αK^q, E-17 (B10.A × 129)F_1 anti-AQR. ‡From Table 1.

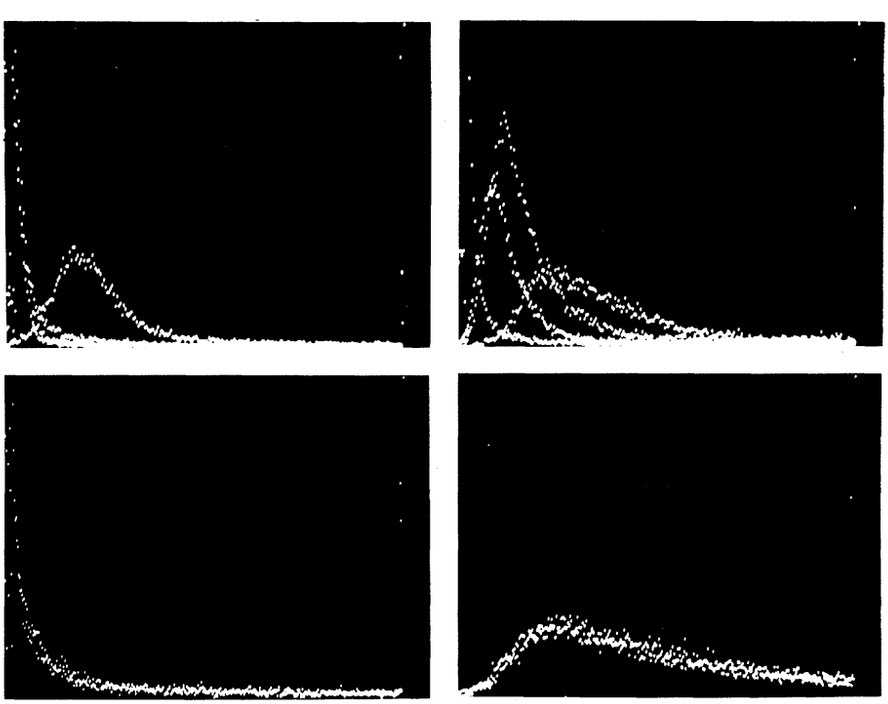

Fig. 2. (Left) Peripheral blood lymphocytes were treated with typing antiserum to H-2^q (D17, obtained from the National Institutes of Health), then with fluorescein isothiocyanate (FITC)–labeled rabbit antiserum to mouse immunoglobulin (IgG, H + L chain) and examined by means of a FACS-IV cell sorter to quantify fluorescence. (Top panel) Profiles of four "Balb/c" mice from the Stoneridge (Kingston) facility; one of these gave a positive reaction with antiserum to H-2^q; (bottom panel) typical BALB/cAu profiles (two mice shown). (Right) Peripheral blood lymphocytes were treated with typing H-2^d serum (NIH Y1-8-03-29-01), then with FITC-labeled rabbit antiserum to mouse IgG. (Top panel) The same four Stoneridge animals, one negative for H-2^d, two with weak reactivity, and one with typical BALB/c pattern; (bottom panel) BALB/cAu response to the same serum.

Although the Charles River mice included two separate shipments (October 1980 and January 1981), and although all BALB/c mice utilized in these experiments were maintained in a separate room containing only BALB/c animals and were not bred except for experimental material, we considered the possibility that we might have inadvertently mislabeled boxes at the University of Wisconsin or that a shipping clerk had accidentally shipped incorrect animals. We therefore purchased additional BALB/c mice from Charles River, requesting shipments from the several different animal breeding units including Wilmington and Stoneridge (Kingston), the sources of our initial animals, as well as Portage. On arrival, animals were housed in a separate building in a room with no other mice, and one of us (B.K.) personally uncrated and caged the animals.

Tests begun on these animals 2 days after their arrival confirmed our original findings that many of the animals (from two of the three Charles River facilities) were not typical BALB/c mice. Glucose phosphate isomerase analyses showed that of four Stoneridge BALB/c mice only one was the expected GPI-A, one was GPI-B, and two were F_1 GPI-AB. Portage mice included two GPI-A and three F_1 animals (Fig. 1); all six Wilmington mice tested were GPI-A. Mixed leukocyte culture (MLC) tests and alloantiserum binding analysis (flow cytometric) also indicated differences between our BALB/c and the Stoneridge BALB/c mice, and showed as well that the Stoneridge animals were themselves not uniform in their expression of H-2 antigens (Fig. 2).

Because of the serious implications of the above findings to other investigators, it was necessary to have the observations confirmed by another laboratory. Mice were shipped directly from the Wilmington and Portage facilities to the Immunobiology Research Center, University of Minnesota, where two of us (B.A. and F.B.) had agreed to carry out both MLC and microcytotoxicity assays. In addition mice from our latest Stoneridge shipment were transported by car to Minneapolis to be included in these tests since BALB/c mice from the Stoneridge facility would have been back-ordered for at least 2½ months.

In essence the tests at the University

of Minnesota confirmed those at the University of Wisconsin. MLC results showed that both Stoneridge and Portage BALB/c mice included animals that could stimulate strong MLC responses by BALB/c mice from the University of Minnesota colony, while no Wilmington BALB/c animals did so (Table 1). The ability to stimulate was paralleled by antibody-mediated cytotoxicity tests indicating disparate H-2 expression (Table 2).

The seriousness of our findings cannot be overemphasized. Since shipments received in January 1981 and September 1981 from the Stoneridge facility and in September 1981 and October 1981 from the Portage facility were incorrectly identified it may well be that shipments in general made from these facilities over many months may have led to erroneous conclusions in research experiments. For example, experiments indicating that hybridoma cells (usually of BALB/c origin) failed to develop as ascites tumors may have been due to the use of histoincompatible hosts rather than functionally limited tumor cells. Similarly, results of experiments on NK activity, tumor susceptibility, and immune responsiveness may need to be reassessed.

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References and Notes

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Histocompatibility and Isoenzyme Differences in Commercially Supplied BALB/c Mice: A Reply

Kahan, Auerbach, Alter, and Bach emphasize the need for genetic monitoring of inbred strains of rodents (*1*). Genetic characteristics have always been considered an important factor in the selection of animals for use in biomedical research. However, only in the last few years have genetic monitoring procedures become available for assessing the integrity of these inbred strains. Even now, availability of these procedures is largely restricted to academic or government institutions. In such institutions, procedures are primarily research oriented; thus, routine monitoring has a low priority, and it is almost impossible to maintain an adequate population survey based on such limited testing.

Until recently, assurances as to the genetic integrity of inbred strains of rodents in commercial breeding operations was primarily based on records provided by the supplier of the original breeding stock. Even if such breeding stock were truly inbred, potential for human error always exists. These relative weaknesses were a continuing—albeit minor—concern until a few years ago when the field of immunology became one of intense investigation resulting in rapid advances in knowledge. With the recent increased demand for both inbred and hybrid mice and rats, a common potential variable is the lack of genetic integrity in animal models used, regardless of whether they were acquired from commercial sources or from the investigators' own breeding colony.

Charles River Breeding Laboratories, Inc., through its close liaison with the scientific community, is cognizant of the need for genetic monitoring as part of its overall quality control program. In order to better assess the various methodologies available for genetic monitoring, a colloquium was convened by the company in Boston on 30 July 1981. Participants who attended the meeting came from the United States and Europe and had expertise in many different areas of genetic monitoring. After this colloquium, our professional and technical staff visited various laboratories to acquire skills for biochemical markers (*2, 3*), immunogenetic markers such as skin grafting (*3, 4*), serologic methodologies (*3, 5, 6*), and mandibular analysis (*3, 7*). A comprehensive, routine genetic monitoring program was established in our laboratories in October 1981 to supplement existing colony management practices developed to produce inbred strains of rodents. We believe that this program is reflective of the long-standing progressive attitude of Charles River since in the currently published guidelines (*8–10*) there is no mention of genetic monitoring.

Since the inception of this program, we have monitored more than 2500 animals, representing various strains of mice and rats, for their genetic integrity. If the test results are suspect, or even equivocal, the entire subline or production colony is eliminated. It should be noted that Charles River breeds BALB/c mice at nine different locations throughout the world, in 13 separate rooms, and suspicion of a problem in one room at one site represents a small percentage of the production animals available to investigators.

In addition, the company has retained, since the fall of 1981, a consultant mammalian geneticist who makes periodic scheduled visits to our laboratory. More recently, we have engaged the consulting services of two immunogeneticists who are assisting in the genetic monitoring of our inbred strains of rats.

We at Charles River Breeding Laboratories, Inc., would like to maintain an open policy of sharing information derived from its quality control diagnostic program with investigators using these animals. We urge investigators using inbred strains of mice and rats to monitor the genetic makeup of these animals in their own laboratories upon receipt, or request from the supplier current results of their genetic monitoring program.

It is our belief that a mutual responsibility must be exercised by both the supplier of laboratory animals and the user of animals to promptly report to each other any discrepancy in results which may provide an early warning that a potential problem might exist.

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References and Notes

1. B. Kahan, R. Auerbach, B. J. Alter, F. H. Bach, *Science* **217**, 379 (1982).
2. J. E. Womack, *Genetics* **92**, 55 (1979).
3. H. A. Hedrich, in *The Mouse in Biomedical Research*, H. L. Foster, D. J. Small, J. G. Fox, Eds. (Academic Press, New York, 1981), vol. 1, pp. 159–176.
4. R. E. Billingham and P. B. Medawar, *J. Exp. Biol.* **28**, 385 (1951).
5. H. A. Hoffman, K. T. Smith, J. S. Crowell, T. Nomura, T. Tomita, in *Animal Quality and Models in Biomedical Research*, A. Spiegel, S. Erichsen, H. A. Solleveld, Eds. (Fischer, Stuttgart, 1981), p. 307.
6. T. J. Gill, D. V. Cramer, H. W. Kunz, *Am. J. Pathol.* **90**, 735 (1970).
7. M. F. W. Festing, *Nature (London)* **238**, 351 (1972).
8. *Guide for the Care and Use of Laboratory Animals*, DHEW Publication No. (NIH) 78–23 (Institute of Laboratory Animal Resources, National Research Council, Washington, D.C., 1978).
9. Good Laboratory Practice Regulations, Nonclinical Laboratory Studies, DHEW, FDA, *Fed. Regist.* **43**, No. 247 (22 December 1978).
10. Toxic Substances Control Act, U.S. Environmental Protection Agency, 11 October 1976.

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0036-8075/82/0723-0381$01.00/0