iance within groups. We therefore see little possibility that pupillary response is appropriate for use as a physiological measure of intelligence.

Task-evoked pupillary responses thus appear to index the processing load imposed on the nervous system in the performance of mental activities (*4*). The magnitude of the physiological response systematically increased with task complexity or difficulty. Over all levels of problem difficulty for which correct performance was possible, more intelligent individuals showed smaller pupillary dilations than did their less intelligent counterparts. This result argues for more efficient and automatic information processing in individuals of higher psychometrically defined intelligence. These results provide evidence that physiological differences between individuals of differing psychometric intelligence emerge during mental activity (*9*).

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References and Notes

1. C. Spearman, *Am. J. Psychol.* **15**, 201 (1904).
2. D. Kahneman, *Attention and Effort* (Prentice-Hall, Englewood Cliffs, N.J., 1973).
3. D. Norman and D. Bobrow, *Cognit. Psychol.* **7**, 44 (1975).
4. J. Beatty, in *The Human Brain*, M. Wittrock *et al.*, Eds. (Prentice-Hall, Englewood Cliffs, N.J., 1977).
5. B. Goldwater, *Psychol. Bull.* **77**, 340 (1972).
6. W. Kintsch, *J. Math. Psychol.* **2**, 330 (1965).
7. E. H. Hess and J. M. Polt, *Science* **143**, 1190 (1964); D. T. Payne, M. E. Parry, S. J. Harasymiw, *Percept. Psychophys.* **4**, 139 (1968).
8. J. Bradshaw, *Psychonom. Sci.* **14**, 271 (1969).
9. One other report has been published examining intelligence and pupillary dilation during cognitive processing. F. Boersma, K. Wilton, R. Barham, and W. Muir [*J. Exp. Child Psychol.* **9**, 142 (1970)] measured pupillary responses during addition in a group of twenty 10- to 11-year-old children, half of whom were educable mentally retarded (EMR) (mean IQ = 72) and the other half normal (mean IQ = 112). They found smaller responses during addition in the EMR children, but these data are difficult to interpret: (i) EMR children may not be representative of the distribution of normal intelligence. (ii) In an unpublished investigation of task-evoked pupillary responses in learning disabled and normal children (ages 9 to 11), we found marked variations in the responses of the normal children with respect to the adult pattern of pupillary response. (iii) Boersma *et al.* included both correct and incorrect responses in their pupillary averages. For the normal group, 92 percent of all problems were correctly solved versus 25 percent for the EMR group. The smaller pupillary responses for the EMR group may simply indicate that most problems were not processed. Any generalization of the Boersma data to the adult population is tenuous.
M. P. Janisse [*Pupillometry* (Wiley, New York, 1977)] has summarized three previously unpublished experiments on pupillary response and intelligence. R. M. Daly [thesis, Loyola University, Chicago (1966)] compared groups differing in performance on a problem-solving test, with psychometrically measured intelligence held constant. The results, therefore, do not bear on the subject of this report. D. G. Crough [*Diss. Abstr.* **32**, 1870 (1971)] studied the pupillary response to items from the Raven Progressive Matrices Test and the Differential Aptitude Test Battery as a function of intelligence, which was estimated from performance on another subset of the Raven test and the SAT. His results were in accord with those presented here. Finally, S. Kuk and M. P. Janisse in two unpublished reports measured pupillary response in the digit span test for subjects differing in performance on the Digit Span Forward subscale of the Wechsler Adult Intelligence Scale. The amplitude of the task-evoked dilation for correctly solved problems was larger for the low-intelligence than the high-intelligence group, the pattern of results reported here, and contradictory to Kuk and Janisse's conclusions (figure 6-1 in Janisse).
10. Supported by the Office of Naval Research under contract N00014-77-C-0616.

21 May 1979

Developmental Fate of Skeletal Muscle Satellite Cells

Abstract. *Radioisotopically labeled satellite cells from clonal cultures were implanted into normal muscle of the original donor. Implanted cells invariably retained their myogenic potential by participating in the regeneration of damaged myofibers or in the development of existing fibers.*

Striated skeletal muscle is capable of rapid and widespread regeneration (*1*). In view of the established postmitotic nature of the nuclei in syncytial myofibers, the source of the regenerating cells in adult muscle has been considered to be the mononucleated satellite cells (*1*, *2*, *2a*) that lie beneath the external (basal) lamina of myofibers (*2a*, *3*). Investigations in vitro have demonstrated that satellite cells recapitulate the normal embryonic development of skeletal muscle through proliferation and fusion to give rise to cross-striated, contractile myofibers (*4*). Therefore, on a functional basis, satellite cells are developmentally indistinguishable from embryonic myoblasts in that both serve as myogenic precursors.

When grown under established culture conditions (*5*), satellite cells become myoblasts by faithfully repeating the sequence of muscle cytodifferentiation as it is observed in vivo. However, if grown in less than optimal conditions, or in an environment simulating that of diseased muscle, the developmental expression of these muscle precursors is reversibly altered (*6*). As a result of this modulation, the myogenic cells assume fibroblast or adipocyte morphology and behavior as long as they are grown in the altered environment.

Do satellite cell–derived myoblasts grown in vitro under optimal or less than optimal culture conditions truly reflect the developmental behavior of satellite cells in intact tissue, or are the cultured cells merely responding to an artificial growth environment? As a first step toward answering this question, the fate of satellite cells must be studied in vivo. To identify the developmental potential of satellite cells, experiments have been carried out by others (*2a*, *7*) to label intact muscle tissue with [^{3}H]thymidine and injure the labeled muscle in situ or transplant it into different hosts. Although these studies suggest that satellite cells participate in the normal regenerative response, the muscle used in these investigations is composed of a heterogeneous population of labeled cells that include satellite cells, fibro-

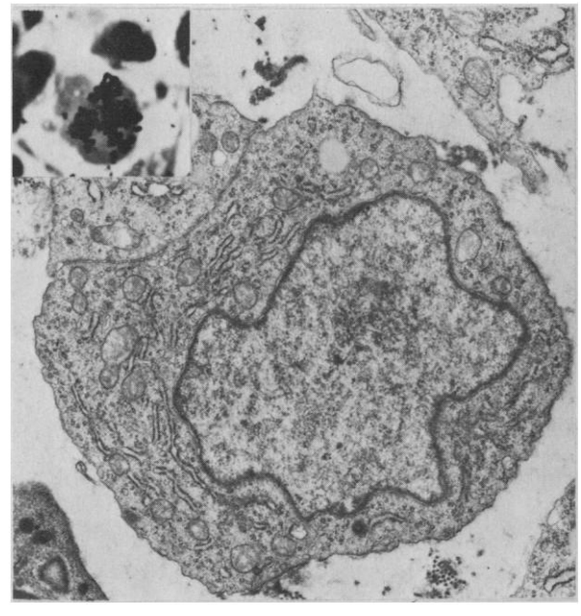

Fig. 1. Adjacent thick and thin sections of rat tibialis anterior muscle showing the same cultured myogenic cell 48 hours after implantation. The thick-section radioautograph (inset) shows silver grains over implanted cell (×1300). Electron micrograph (below inset) of the same cell illustrates typical cytology of a rat myoblast (×7400).

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blasts, endothelial cells, Schwann cells, and other migratory cells such as leukocytes and monocytes. Moreover, one cannot rule out the possibility that during muscle regeneration, cells recruited from distant sources migrate through the tissue or are delivered to the site of injury by the circulatory system (*2, 2a, 8*). To identify the potential of satellite cells to differentiate, one must accurately assess the developmental fate of a pure population of these myogenic cells. This report presents the results of a new procedure in which cultured satellite cells are implanted to permit accurate identification of their fate in normal muscle (*9*).

In the experiments described below, myogenic cells were obtained from juvenile rat and quail muscle tissue and from adult quail muscle tissue. In rapidly growing juvenile muscle, some embryonic myoblasts may still be present; however, identical results were obtained with adult tissue in which no myoblasts had been identified. Therefore, it is reasonable to assume that whether they are derived from juvenile or adult tissue, the myogenic cells in this culture-implant system accurately reflect the developmental potential of satellite cells.

In studies with rats, enzymatically dissociated cells from excised hamstring muscles (*10*) were inoculated at clonal densities and grown in complete culture medium (*5*) containing [^{3}H]thymidine (0.4 μCi/ml). Myoblast colonies, selected on the basis of cell morphology (*6*), were encircled with rings of Tygon tubing. Cells within the rings were released from the dish with trypsin (*11*) and the suspension was pelleted by mild centrifugation (600*g*). Immunological rejection was avoided by incubating the pellet in defined media (*12*) to ensure removal of any horse serum or chick embryo extract components that might have adhered to the plasmalemma of the cultured cells. The cell pellet was then implanted beneath the connective tissue fascia of the exposed tibialis anterior muscle of the original donor. The rats were killed after 2 to 7 days and the implanted areas were processed for electron microscopy. Developed autoradiographs prepared from thick sections were photographed and the prints used as guides in identifying labeled nuclei in adjacent thin sections (*13*).

Rat cells are capable of undergoing only 8 to 12 divisions in cell culture. To obtain greater yields of labeled satellite cells, we used Japanese quail (*Coturnix coturnix japonica*) muscle, since it has a greater proliferative capacity in vitro (*14*). Wing muscle tissue was enzymatically dissociated and filtered and the resulting single-cell suspension was plated at mass culture density (100,000 cells in a 100-mm gelatin-coated culture dish). After 24 hours, secondary cultures were plated with myoblasts selectively released from the primary culture by trypsinization (*5*). Secondary cultures containing 99.9 percent myoblasts (*6*) were given [^{3}H]thymidine-labeled medium on day 4, trypsinized on day 6, and the liberated cells subsequently pelleted. After incubation in defined medium, the pellet was implanted into the breast muscles of the original donor. After 2 to 12 days, the muscle implant was prepared for light and electron microscopic radioautography.

The fate of more than 120 labeled rat and quail cells were identified in muscle that was fixed 2 to 12 days after implantation of the cell pellets. No structural or functional differences were noted in the development of the implanted cultured cells in either rat or quail muscle. Within 48 hours after implantation, labeled cells were primarily found within the interstitial connective tissue and myofibers of the muscle. Many cells underwent extensive migration away from the injection site and were cytologically indistinguishable from normal embryonic myoblasts (Fig. 1) (*6*). By day 4, most of the labeled cells penetrated the basal lamina of intact myofibers. Some labeled nuclei were in satellite fibers, short myofilament-containing cells in the parent fiber's external lamina but not fused with the parent myofiber (Fig. 2, a, b, and c). By day 7,

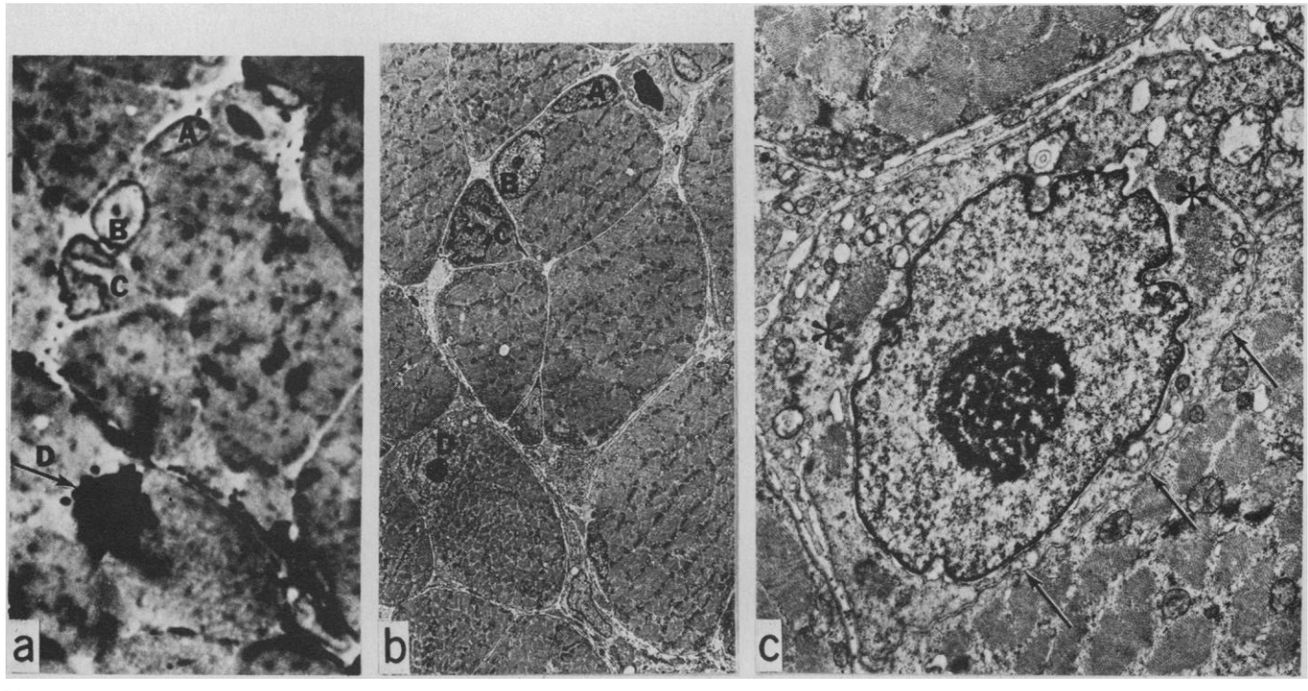

Fig. 2. Adjacent thick and thin sections showing the fate of culture-implant rat satellite cells. (a) Thick-section radioautograph shows heavy deposits of silver grains (arrow) over a [^{3}H]labeled myogenic cell (*D*) (×1200). (b) In an adjacent thin section, the exact position of the labeled nucleus (*D*) in (a) can be identified by using landmarks *A*, *B*, and *C* (×1200). (c) At higher magnification the labeled cell can be identified as a satellite fiber. The cell, which contains myofibrils (*), is situated between the external lamina and plasmalemma of the parent muscle fiber but remains separated by an interspace (arrows) that is devoid of external lamina material (×7400).

nearly all labeled nuclei were incorporated within the syncytial architecture of the original fibers (Fig. 3a). Labeled myofibers resulted from a fusion of labeled cells with preexisting myofibers. This is documented by the presence of both labeled and unlabeled nuclei within single well-differentiated syncytial fibers (Fig. 3b). Although more than 95 percent of the labeled nuclei were eventually found with existing parent fibers, there was a modest development of apparently new interstitial fibers that were primarily evident in areas of extensive structural damage (such as the incision and injection site). These cytologically immature fibers may have arisen in situ from the implanted cells or they may represent regeneration of damaged fibers in which the implanted cells took part. However, in areas away from the implantation site, labeled nuclei were present only within existing mature myofibers.

When the developmental potential of the cultured cells was challenged by implanting the pellet into a nonmuscular, subcutaneous site, the labeled cells exclusively gave rise to new myofibers. Although the resulting tissue was relatively disorganized, the new myofibers possessed the typical cytodifferentiative features of skeletal muscle. The implants had a connective tissue matrix derived from recruited (unlabeled) fibrogenic cells. Even under such unusual conditions, none of the cultured satellite cells resembled fibrogenic cells. However, this negative evidence does not rule out the possibility that under other circumstances, satellite cells may reversibly change into fibroblasts. Although the migratory nature of the labeled cells was revealed in these experiments, labeled cells from the subcutaneous implants did not penetrate the coarse epimysium. Still, satellite cell migration through major connective tissue septa is a possibility.

The results clearly demonstrate that labeled, satellite-derived myogenic cells reintroduced into normal muscle tissue exclusively retain their developmental potential and recapitulate the normal growth and development of embryonic muscle. Despite their association with existing fibers, some of the implanted cells participated in the formation of new interstitial fibers. That the fate of satellite cells was predetermined was supported by the fact that no labeled fibrogenic, endothelial, or other nonmuscle cells were observed.

After their reintroduction in pellet form into the muscle, the labeled cells rapidly dispersed and migrated away from the injection site until neither an intact pellet nor a labeled cell mass remained within the muscle. However, when the pellet of satellite cells was placed into a nonmuscular site, the cells essentially remained intact. In that case, host cells invaded the cell pellet and participated in the deposition of a connective tissue matrix.

It has been reported (*15*) that the basal lamina is an impenetrable connective tissue sheath that restricts the migratory behavior of the enclosed satellite cells. This supposition, however, is contradicted by the "homing" behavior of the transplanted cells. Clearly, the endomysial sheath did not restrict the migration and penetration of labeled cells; nor did it interfere with their subsequent fusion with intact myofibers. The ability of satellite cells to pass through the external lamina and to undergo extensive migration may be significant in normal regenerative behavior. It has been suggested (*16*) that the small population of satellite cells could not account for the rapid regeneration of injured muscle. However, our observations suggest that after an injury, satellite cells associated with undamaged myofibers may leave their ensheathing external lamina, migrate to the site of injury, and participate in the regeneration of new myofibers.

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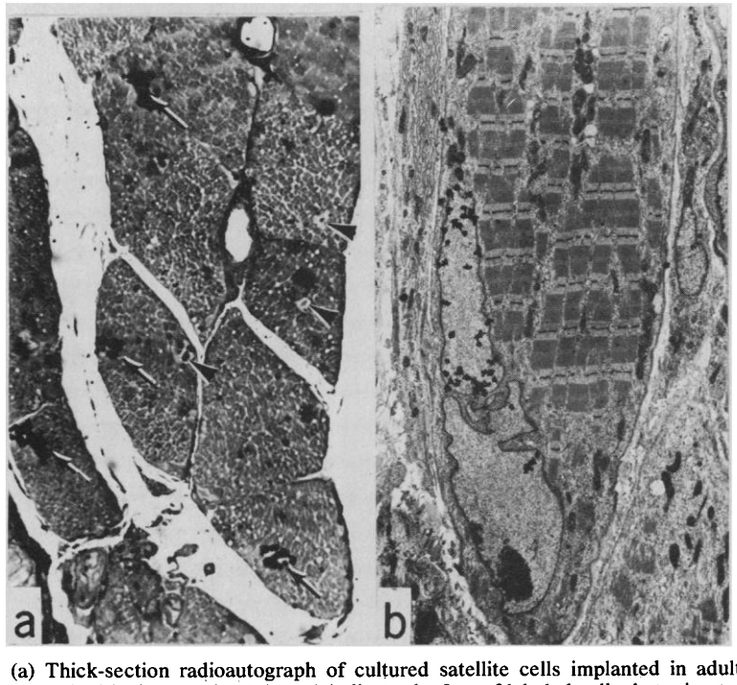

Fig. 3. (a) Thick-section radioautograph of cultured satellite cells implanted in adult quail muscle. Nuclei with silver grains (arrows) indicate the fate of labeled cells that migrated from the implantation site to fuse with mature fibers. Nuclei of the original fibers are unlabeled (arrowheads) (×520). (b) Adjacent labeled and unlabeled nuclei within the syncytium showing mosaic nature of a mature myofiber. Grains over one of the nuclei reveal the fate of a satellite-derived myogenic cell (×2500).

References and Notes

1. B. M. Carlson, *Am. J. Anat.* **137**, 119 (1973).
2. J. C. T. Church, in *Regeneration of Striated Muscle and Myogenesis*, A. Mauro, S. A. Shafiq, A. T. Milhorat, Eds. (Exerpta Medica, Amsterdam, 1970), p. 101; H. Schmalbruch, *Tissue Cell* **8**, 673 (1976).
2a. M. H. Snow, *Cell Tissue Res.* **186**, 535 (1978).
3. A. Mauro, *J. Biophys. Biochem. Cytol.* **9**, 493 (1961); A. R. Muir, in *Regeneration of Striated Muscle and Myogenesis*, A. Mauro, S. A. Shafiq, A. T. Milhorat, Eds. (Exerpta Medica, Amsterdam, 1970), p. 91; E. Schultz, *Am. J. Anat.* **147**, 49 (1976).
4. U. R. Konigsberg, B. H. Lipton, I. R. Konigsberg, *Dev. Biol.* **45**, 260 (1975); R. Bischoff, *Anat. Rec.* **182**, 215 (1975).
5. I. R. Konigsberg, *Dev. Biol.* **26**, 133 (1971).
6. B. H. Lipton, *ibid.* **60**, 55 (1977).
7. M. H. Snow, *Anat. Rec.* **188**, 201 (1977); J. S. Neerunjun and V. Dubowitz, *J. Neurol. Sci.* **24**, 33 (1975).
8. R. G. Bateson, D. F. Woodrow, J. C. Sloper, *Nature (London)* **213**, 1025 (1967); J. C. Sloper, R. G. Bateson, D. Hindle, J. Warren, in *Regeneration of Striated Muscle and Myogenesis*, A. Mauro, S. A. Shafiq, A. T. Milhorat, Eds. (Exerpta Medica, Amsterdam, 1970), p. 157.
9. B. H. Lipton, in *Muscle Regeneration*, A. Mauro, Ed. (Raven, New York, 1978), p. 31.
10. Minced muscle is dissociated in 0.2 percent trypsin and 0.04 percent collagenase for 20 minutes; cold medium is added to stop enzyme action.
11. I. R. Konigsberg, *Science* **140**, 1273 (1963).
12. C. P. Emerson, in *Pathogenesis of Human Muscular Dystrophies*, L. P. Rowland, Ed. (Exerpta Medica, Amsterdam, 1977), p. 799.
13. This technique was used by F. P. Moss and C. P. Leblond [*Anat. Rec.* **170**, 421 (1971)].
14. L. Hayflick, *Am. J. Med. Sci.* **265**, 432 (1973).
15. R. Bischoff, *Anat. Rec.* **180**, 645 (1974). See also R. Bischoff in (*4*).
16. M. Reznik, *Differentiation* **7**, 65 (1976).
17. Supported by NIH grant NS12171, NSF grant PCM 80787, and by the Muscular Dystrophy Association.

26 June 1979