extracellular electrode with current strengths sufficient to activate the adjacent muscle fibers. Such "failures" might reflect a nonspecific tissue antagonism, immaturity, or inexcitability of the neurons. A more interesting alternative is that the synapses between neurons and those between neurons and muscle exhibit the same type of specificity demonstrated in the central nervous system (*13*).

In sum, isolated nerve and muscle cells, plated in cell cultures at relatively low densities, differentiate sufficiently to exhibt action potentials and to form chemically mediated synapses. The occurrence of synaptic potentials is consistent with the electron microscopic description of nerve-muscle contacts in a nearly identical culture system by Shimada *et al.* (*2*). The direct visualization of both pre- and postsynaptic elements in this culture system should permit a detailed study of the ontogeny and specificity of synaptic relations between cells.

GERALD D. FISCHBACH
Behavioral Biology Branch,
National Institute of Child
Health and Human Development,
Bethesda, Maryland 20014

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6. Small fragments of pectoral muscles of 11-day chick embryos were exposed for 30 minutes to 0.25 percent trypsin in a balanced salt solution free of Ca^{2+} and Mg^{2+}, mechanically agitated in complete culture medium (see below), and filtered through a double layer of lens paper. The resulting mononucleated cells were plated in collagen-coated [M. Bornstein, *Lab. Invest.* **7**, 134 (1958)], 60-mm plastic petri plates at densities ranging from 100,000 to 500,000 cells per plate. Spinal cord cells prepared in an identical manner from minced fragments of whole cords of 7-day embryos were either plated in separate dishes or added to the muscle cultures 2 to 4 days after the muscle was plated, a time when young myotubes predominated. The cells were maintained at 37°C in a medium consisting of Eagle's minimum essential medium, horse serum, embryo extract, and a mixture of penicillin and streptomycin (in a proportion of 8 : 1 : 1 : 0.1) which was changed every 2 to 3 days. The medium was equilibrated with a mixture of 95 percent air and 5 percent CO_2.
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8. The cultures were mounted on the stage of an inverted phase-contrast microscope, and one or two KCl-filled microelectrodes which measured 20 to 50 megohm were introduced from above. Each microelectrode was connected in a bridge circuit such that it could be used simultaneously for transmembrane recording and stimulation. The stimulus was applied through a 10^9-ohm resistor in series with the microelectrode. During the recording periods, the medium was equilibrated with 5 percent CO_2 and maintained at 35° to 37°C.
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Turnover of the Brain Specific Protein, S-100

Abstract. *Rats were killed after intraventricular administration of [^{3}H]leucine, and the turnover rates of total soluble proteins and of the brain specific S-100 protein were determined. The half-life of S-100 was estimated to be 16 days. The S-100 protein, thus, has a turnover not unlike that of the average water-soluble protein of brain.*

The S-100 protein has been found in an immunologically cross-reacting form in the brains of all vertebrates and invertebrates examined (*1*). The fact that S-100 is present only in the nervous system but in no other organ (*2*), taken in conjunction with its apparent evolutionary stability, suggests that the protein may have an important, as yet undetermined, role in the function of the nervous system. Evidence obtained by several independent methods has also established that S-100 is localized predominantly, if not exclusively, in glial cells within the central nervous system (*3*). In an attempt to further characterize this glial protein, we have determined the turnover rate in vivo of S-100. Although it has been suggested that the S-100 protein turns over extremely rapidly in the brain (*4*), our work indicates a much slower turnover rate.

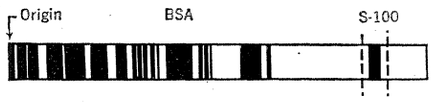

Fig. 1. Polyacrylamide-gel (7.5 percent) electrophoretic pattern of the DEAE-cellulose fraction containing the S-100 protein. The dotted line indicates the portion of the gel which was used to determine the specific activity of the S-100 protein.

Each of 14 male Holtzman rats, approximately 35 days of age, was stereotaxically implanted, under Nembutal anesthesia, with a lightweight cannula in the lateral ventricle according to standard stereotaxic procedures (*5*). Animals of this age were selected because the biosynthesis of the S-100 protein increases rapidly from birth to approximately 50 days in the developing rat brain (*1*), and we were thus assured of a relatively high labeling of the protein. At the end of a 5-day postoperative recovery period, the unanesthetized animals were infused with 200 μc of [4,5-^{3}H]leucine (New England Nuclear) in 100 μl of 0.15*M* NaCl over a 30-minute period by means of a chronic infusion system (*5*). Rats were killed by decapitation either at 5 hours, 24 hours, 48 hours, 7 days, 14 days, or 28 days after infusion. In all cases the brains were removed within 1 to 1½ minutes, quickly frozen on a block of dry ice, and stored at −80°C. Immediately before chemical analysis the brains were thawed, and the placement of each cannula in the lateral ventricle was grossly determined by sectioning the brain through the cannula track. The data reported here are based only on those animals in which the cannula appeared to rest in the lateral ventricle.

The brains were then weighed and homogenized in glass hand-operated homogenizers in ten volumes of the cold barbital-NaCl buffer which was used in subsequent complement-fixation assays (*6*); the homogenate was centrifuged at 25,000 rev/min for 90 minutes. The supernatants were saved, and total soluble proteins were determined by the method of Lowry *et al.* (*7*). Radioactive label in total soluble proteins was determined by precipitating the proteins in 0.3 ml of the supernatants with trichloroacetic acid (TCA) and then centrifuging at 2000 rev/min. The supernatants were discarded, and the precipitate was resuspended in 5 percent TCA and centrifuged again. Only one TCA washing was necessary since total radioactivity in the supernatant was negligible at this point. The resulting precipitate was then completely dissolved in 0.5 ml NCS (Nuclear-Chicago Solubilizer) reagent, 10 ml of toluene-base scintillation liquid was added, and the samples were counted in a Packard Tri-Carb scintillation counter.

The remainder of the original brain supernatants were chromatographed on a diethylaminoethyl (DEAE)-cellulose

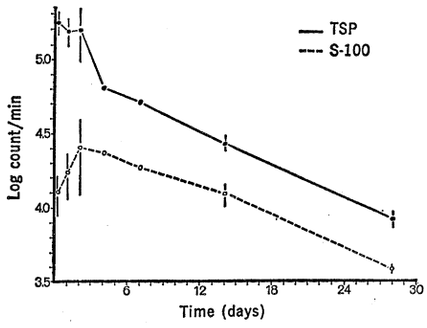

Fig. 2. The log of the specific activity (count/min per milligram of protein) of total soluble brain proteins (TSP) and the S-100 protein for each of the survival periods.

column (*1*). The 15 fractions resulting from this separation were concentrated by pressure dialysis and subjected to electrophoresis on 7.5 percent polyacrylamide gels (*8*) which were then stained with amido black. Figure 1 shows the electrophoretic pattern of the DEAE-cellulose fraction containing the S-100 protein. The S-100 moved faster than any other protein and was, thus, easily identified and isolated. The S-100 band was cut out (Fig. 1), dried in a vacuum overnight, and dissolved in hydrogen peroxide (30 percent) in a 75°C water bath (*9*). To each of these samples was added 0.5 ml of NCS reagent and 10 ml of toluene-base scintillation liquid.

That S-100 was, in fact, the only protein in this fast-moving band (Fig. 1) was established in the following way. First, the amount of S-100 in the DEAE-cellulose fraction applied to the gels was measured by complement fixation (*6*), a method which is both highly sensitive and specific. Second, the S-100 band was cut from the gels (Fig. 1), and the stain was eluted by incubation with 1*N* NaOH for 24 hours. The S-100 content was estimated by comparing the absorbance of the extracted dye solution with that from standards of pure beef S-100 subjected to the same procedure. The amount of S-100 applied to the acrylamide gels was 7.0 μg as determined by complement fixation (for one representative sample), and the dye elution method yielded a value of 6.7 μg of S-100 in the S-100 band cut from the gel. These data, together with the fact that the band presumed to contain S-100 in these experiments moved identically with purified beef S-100, indicate that the S-100 protein, and no other protein, comprised the fast-moving band isolated in our experiments.

As a further control for the validity of our procedure, a known amount of pure beef S-100 was incubated with previously labeled rat liver homogenate, in which no S-100 is found (*1*), and then this mixture was subjected to electrophoresis as described. Total counts in the resulting S-100 band did not exceed background levels. Thus, our results indicate specific incorporation of [^{3}H]leucine into the S-100 protein itself.

The specific activity of total soluble proteins in the brain and the S-100 protein are plotted in Fig. 2 for each of the survival periods. In close agreement with earlier studies of brain protein turnover (*10*) our results confirm that there are at least two populations of soluble proteins in the brain, a small group turning over very rapidly (2 to 4 days) and a larger group with a considerably slower turnover rate of 15 to 16 days. The S-100 protein has a half-life similar to that of the soluble brain proteins that turn over more slowly, that is, approximately 16 days. Of particular interest, is the delay in the incorporation of the radioactive label into the S-100 protein whose specific activity did not reach a peak until nearly 48 hours after the infusion of [^{3}H]leucine. The reason for the delay in the peak labeling of the S-100 protein in these experiments is still unknown.

The results of our experiment are not in agreement with those described in a study by McEwen and Hyden (*4*) in which they reported that the half-life in vivo of the S-100 protein was extremely short. Although the reason for this apparent discrepancy is not immediately clear, it seems likely that the chemical or isotopic purity of the S-100 protein isolated and counted to determine specific activity may be a factor. Detergent extracts were used in the earlier experiments, and these were applied directly to acrylamide gels without prior purification. Under the conditions in our experiment, which may be the first to unequivocally demonstrate the turnover of a single brain specific protein, we conclude that the S-100 protein turns over at approximately the same rate as the average water-soluble brain protein.

THEODORE J. CICERO
BLAKE W. MOORE
Department of Psychiatry,
Washington University School of Medicine,
St. Louis, Missouri 63110

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Pagophagia in the Albino Rat

Abstract. *Pagophagia, or ice-eating, is a common symptom of iron-deficiency anemia in humans. Rats made anemic by withdrawal of blood consume a significantly greater proportion of their daily water in the form of ice than nonanemic controls. Recovery from the anemia eliminates the pagophagia.*

Pica, the consumption of nonnutritive substances, has been correlated with iron-deficiency anemia in humans (*1*). One of the commonest forms of pica associated with iron deficiency is ice-eating, or pagophagia (*2*). People who are severely deficient in iron may consume up to several large glasses of ice a day in preference to cold water (*2*). Ice-eating ceases after recovery from the iron deficiency. However, the evidence suggesting a relation between