Leucine Pools in Normal and Dystrophic Chicken Skeletal Muscle Cells in Culture*

Peter A. Schneible‡ and Ronald B. Young§

From the Department of Biological Sciences, University of Alabama, Huntsville, Alabama 35899

The specific radioactivity of \([^{3}H]\)Leu in the extracellular, intracellular, and Leu-tRNA pools of normal (white leghorn) and dystrophic (line 307) embryonic chick breast muscle cultures was analyzed as a function of equilibration time and extracellular Leu concentration (0.05–5 mM). The primary results were the following 1) \([^{3}H]\)Leu equilibrated to a constant specific radioactivity in the intracellular and Leu-tRNA pools within 2 min after addition to both normal and dystrophic cultures. 2) After equilibration, the extracellular \([^{3}H]\)Leu specific radioactivity in dystrophic cell culture medium was lower than that of medium exposed to normal cells (especially at low Leu concentrations), probably because of increased release of unlabeled Leu from the dystrophic cells as a result of faster protein breakdown. Accordingly, the specific radioactivities in the intracellular and the Leu-tRNA pools were also lower in dystrophic cells. 3) At 5 mM extracellular Leu, the specific radioactivity in the Leu-tRNA pool was approximately 40% lower than the specific radioactivity in the intracellular pool in both normal and dystrophic cells. Thus, high concentrations of extracellular Leu cannot be used to "flood out" reutilization of unlabeled Leu (released by protein degradation) during protein synthesis. 4) At 5 mM extracellular Leu, the specific radioactivity of \([^{3}H]\)Leu in the intracellular pool was comparable to that in the extracellular pool in normal and dystrophic cells; however, the specific radioactivity of Leu-tRNA (i.e. the immediate precursor to protein synthesis) was only 55–65% of the extracellular specific radioactivity in normal and dystrophic cells. In conclusion, reutilization of Leu from protein degradation is higher in dystrophic muscle cell cultures than in normal muscle cell cultures, and accurate rates of protein synthesis in cell cultures can only be obtained if specific radioactivity of amino acid in tRNA is measured.

One of the early defects in muscular dystrophy may be an alteration in plasma membrane structure and function. Dystrophic muscle shows increased permeability to enzymes and ions (Herman and Fernandez, 1977; Weinstock and Jones, 1977) and exhibits a number of morphological alterations associated with the plasma membrane (Mokri and Engel, 1975; Schotland et al., 1977). Other membrane functions, such as amino acid transport, may also be affected in dystrophic cells, and these changes could lead to alterations in cellular amino acid pools and ultimately muscle protein metabolism. Most studies of protein metabolism employ radiolabeled amino acids. Unfortunately, many invalid assumptions about the specific radioactivity of the precursor pools and about the reutilization of amino acids have been made. Recently, several investigators have measured protein synthesis by analyzing the specific radioactivity of amino acids bound to tRNA, and hence the immediate precursor to protein synthesis (Airhart et al., 1979; Hildebran et al., 1981; Martin et al., 1977; McKee et al., 1978; Schneible et al., 1981; Schneible and Young, 1981). These studies convincingly illustrate the need for accurate measurements to ensure the validity of using amino acid data to calculate true rates of synthesis.

Airhart et al. (1979) and McKee et al. (1978) have proposed models for compartmentation of amino acids for protein synthesis within the transport system of the cell membrane and therefore link membrane function intimately to the protein metabolism mechanisms within the cell. This possibility is especially intriguing in relationship to genetic muscular dystrophy where changes in both the cell membrane and protein breakdown may occur. Thus, the primary goal of this study was to determine the relationships among the extracellular, intracellular, and Leu-tRNA pools in skeletal muscle cells cultured from normal and dystrophic muscle. These relationships were studied both to examine possible alterations caused by muscular dystrophy and to enable more accurate measurements of the rates of protein synthesis and breakdown in normal and dystrophic muscle cells. A preliminary report of some of these results has appeared previously (Schneible and Young, 1981).

EXPERIMENTAL PROCEDURES

Experimental Design—The relationships among cellular amino acid pools were examined by studying the specific radioactivity of Leu in the pools at various times after administration of \([^{3}H]\)Leu and by examining the effect of Leu concentrations on the steady state therefore link membrane function intimately to the protein metabolism mechanisms within the cell. This possibility is especially intriguing in relationship to genetic muscular dystrophy where changes in both the cell membrane and protein breakdown may occur. Thus, the primary goal of this study was to determine the relationships among the extracellular, intracellular, and Leu-tRNA pools in skeletal muscle cells cultured from normal and dystrophic muscle. These relationships were studied both to examine possible alterations caused by muscular dystrophy and to enable more accurate measurements of the rates of protein synthesis and breakdown in normal and dystrophic muscle cells. A preliminary report of some of these results has appeared previously (Schneible and Young, 1981).
ment, cells were incubated in unlabeled experimental medium (85% Eagle's minimum essential medium without Leu, 10% horse serum, 5% embryo extract, 10^{-5} \text{ M} \text{fluoro-deoxyuridine, and various concentrations of Leu}) for 30 min to allow cellular amino acid pools to equilibrate with that particular medium. (4,5-^{3}H)Leu was then added in a small volume of buffered saline, and the kinetics of equilibration of this newly added (4,5-^{3}H)Leu into cellular pools was followed.

### Cell Culture—Preparation of skeletal muscle

Embryonic breast muscle was dissected from 13-day-old embryos. The tissue was disaggregated into individual cells by vortexing the suspension on a Vortex mixer for 30 s, and cells were recovered by centrifugation. The cells were counted in a hemocytometer and plated at approximately 4 × 10^{4} cells/60-mm collagen-coated tissue culture plate. Culture medium was changed every 24 h, and the cells were incubated at 37 °C in a 5% CO\textsubscript{2} atmosphere. When muscle cell proliferation began to decline (usually day 3 of culture), 10^{-4} \text{ M} \text{fluoro-deoxyuridine was added to the culture medium to inhibit fibroblast proliferation. Cultures were examined after 6-8 days, at which time the cells had reached a steady state with respect to protein synthesis and degradation (Young et al., 1981).

### Specific Radioactivity of Cellular Leu Pools—Incorporation into Protein

After incubation with (4,5-^{3}H)Leu for various times, the medium was removed from the plate and saved for measurement of extracellular specific radioactivity as described later. Cells were washed by successive immersion in five beakers, each containing 200 ml of ice-cold buffered saline.

Intracellular free Leu and Leu bound to tRNA were isolated and analyzed for specific radioactivity as detailed by Airhart et al. (1979). Briefly, the cells were lysed in 0.5 ml of a buffered detergent solution, and a 0.2-ml aliquot was removed to determine intracellular amino acid specific radioactivity. The remaining solution was extracted with phenol, and nucleic acids were precipitated by addition of ethanol. Amino acids were dissociated from tRNA by base hydrolysis, and the amino acid solution was evaporated to dryness under vacuum (Airhart et al., 1979).

The portion of the cell lysate set aside for intracellular amino acids was treated immediately with 5 volumes of 10% (w/v) trichloroacetic acid. The solution was cooled to 4 °C and the precipitated protein was pelleted by centrifugation. This pellet was used for measurement of (4,5-^{3}H)leucine incorporation into protein (see below). The supernatant, containing free amino acids, was extracted eight times with ether to remove trichloroacetic acid. A sample was evaporated to dryness for determination of Leu specific radioactivity in the intracellular pool. Similarly, the culture medium was deproteinized by addition of 100% (v/v) trichloroacetic acid to a final concentration of 10%. The precipitated protein was pelleted by centrifugation, and the trichloroacetic acid was removed from the supernatant by five extractions with ether. The extracellular samples were not evaporated, however, because the amino acid concentration was high enough for direct measurement of specific radioactivity.

The dried samples of aminoaeryl-tRNA and intracellular amino acids were taken up in bicarbonate buffer as described by Airhart et al. (1979) to a final pH of 8.5-10. A small aliquot of the deproteinized labeling medium was also brought to pH 8.5-10 with this buffer. All samples were then reacted with [^{14}C]dansyl chloride, and two-dimensional chromatography was performed on Cheng Chin micropolyamide thin layer plates (Airhart et al., 1979). The Leu spot was cut out, and the [^{14}C]dansyl chloride, the ratio of the two isotopes in the resulting dansyl-leucine could be used to measure the specific radioactivity of the Leu by the following formula: $\text{SA}_{\text{tRNA}} = (\text{CPM} / \text{CPM}) \cdot (\text{SA}_{\text{tRNA}} / \text{K})$, where $\text{SA} = \text{specific radioactivity in disintegrations/min/mole}$, DNS CI = dansyl chloride, and K = moles of dansyl chloride bound per mole of amino acid and equals 1 for Leu.

### Radiolabeled Leu Incorporation into Protein

The precipitated protein from the extracellular fraction was filtered through glass fiber filters. The filters were washed with 19 ml of 5% trichloroacetic acid and 5 ml of 95% ethanol, dried, and placed in scintillation vials. NCS tissue solubilizer (0.5 ml) was added, and the vials were heated at 50 °C for at least 1 h to solubilize protein. After the vials were cooled to room temperature, radioactivity was measured by liquid scintillation spectrometry.

### Cell Counts

Replicate muscle cell cultures in 60-mm tissue culture plates were stained with Giemsa stain as described by Young et al. (1975). At least 600 nuclei were counted in a minimum of five randomly chosen fields. The number of nuclei within multinucleated myotubes, the number of mononucleated cells, and the percentage of nuclei within multinucleated myotubes were calculated and used as indices of growth and differentiation of muscle cell cultures and as a normalization factor for incorporation of [^{3}H]Leu into protein.

### Measurement of protein synthesis

Since the [^{3}H]Leu was reacted with [^{14}C]dansyl chloride, the ratio of the two isotopes in the resulting dansyl-leucine could be used to measure the specific radioactivity of the Leu by the following formula: $\text{SA}_{\text{tRNA}} = (\text{CPM} / \text{CPM}) \cdot (\text{SA}_{\text{tRNA}} / \text{K})$, where $\text{SA} = \text{specific radioactivity in disintegrations/min/mole}$, DNS CI = dansyl chloride, and K = moles of dansyl chloride bound per mole of amino acid and equals 1 for Leu.

### Results

Figure 1 shows the equilibration kinetics of [^{3}H]Leu in the extracellular, intracellular, and cellular pools in dystrophic muscle cell cultures.
in intracellular, and Leu-tRNA pools of normal and dystrophic muscle cultures in medium with Leu concentration of 0.05 mM, the lowest concentration used in this investigation. In both cases, the [\textsuperscript{3}H]Leu was added at zero time to the culture medium; thus, the extracellular [\textsuperscript{3}H]Leu specific radioactivity attained its highest value immediately upon initiation of the experiment. Because the quantity of Leu in the extracellular pool was large compared to the quantity in the other two pools, its specific radioactivity diminished only 10–15% during the 30-min incubation period. This decrease in extracellular specific radioactivity presumably was caused by the release of nonradioactive amino acids from cellular protein by protein degradation. In both normal and dystrophic cultures, the intracellular and Leu-tRNA specific radioactivities reached a steady state within 2 min and remained essentially constant thereafter. Thus, [\textsuperscript{3}H]Leu was rapidly distributed to various pools within the muscle cell, and the dystrophic condition had no apparent effect on the time course of this distribution. Additional measurements at 60 and 120 min after addition of [\textsuperscript{3}H]Leu showed that the specific radioactivity in all three pools was not significantly different from the values observed in Fig. 1 after 30 min (data not shown). In both normal and dystrophic cultures, the equilibrated specific radioactivities of the intracellular and Leu-tRNA pools were lower than the extracellular specific radioactivity (Fig. 1, A and B). The intracellular and Leu-tRNA pools receive Leu from both the external medium and degradation of endogenous protein. Because the Leu given off by protein degradation is not radioactively labeled and because some of this nonradioactive Leu is reutilized for synthesis of new proteins, the specific radioactivities of these two pools are lower than that of the extracellular pool. This condition should persist until an equilibrium is reached between the rate of [\textsuperscript{3}H]Leu incorporation into protein (i.e. when all proteins are uniformly labeled) and the rate of release of [\textsuperscript{3}H]Leu by protein degradation. Although the time required for uniform protein labeling was not measured, results of experiments carried out for up to 2 h were identical with the distributions shown in Fig. 1 after 30 min.

If A and B of Fig. 1 are compared, one primary difference between normal and dystrophic cultures is observed. The values for extracellular Leu specific radioactivity in dystrophic cultures were lower by approximately 45% compared to those in normal cultures, even though the same quantity of [\textsuperscript{3}H]Leu was added to the medium bathing both cell types. For example, the specific radioactivity of [\textsuperscript{3}H]Leu in the extracellular pool of normal cells after 30 min was 998 dpm/pmol (Fig. 1A), but only 531 dpm/pmol in the extracellular pool of dystrophic cells after 30 min (Fig. 1B). The values for steady state intracellular and Leu-tRNA specific radioactivity in dystrophic cells were also proportionally decreased compared to normal cultures. The best explanation for the lower extracellular specific radioactivity in dystrophic cells even at zero time after addition of [\textsuperscript{3}H]Leu is a consequence of the experimental design (see “Experimental Procedures”). Prior to addition of [\textsuperscript{3}H]Leu directly to the cultures, the cells were preincubated for 30 min in culture medium containing the appropriate concentration of nonradioactive Leu. This action was taken to allow the cells to adjust to the altered extracellular Leu concentration before the [\textsuperscript{3}H]Leu was added. In dystrophic cells, where protein degradation was apparently faster, a larger quantity of nonradioactive Leu was released during the 30-min preincubation period, and this faster release was then manifested as a lower extracellular specific radioactivity immediately upon addition of [\textsuperscript{3}H]Leu.

The effect of extracellular Leu concentration on the equilibrated specific radioactivity of cellular [\textsuperscript{3}H]Leu pools is shown in Table I. Note that the values for specific radioactivity decreased in all cases as the Leu concentration was increased. This occurred because the quantity of [\textsuperscript{3}H]Leu was not increased as a constant proportion of the total quantity of Leu in the cell culture medium. Three practical considerations dictated that a constant extracellular specific radioactivity could not be employed over the range of 0.05–5.0 mM Leu. First, the cost of conducting adequate numbers of experiments with 5 mCi/ml of [\textsuperscript{3}H]Leu would have been prohibitive. Second, 5 mCi/ml of [\textsuperscript{3}H]Leu is a sufficiently high level of radioactivity to risk radiation toxicity to the cells and thereby induce aberrant results. Third, the embryo extract and horse serum components of cell culture medium both contain small quantities of nonradioactive Leu. This exogenous Leu would have prevented rigorous definition of [\textsuperscript{3}H]Leu specific radioactivity, especially at 0.05 mM leucine. For these reasons, direct comparisons of data in Table I can be made only among samples analyzed at the same medium Leu concentration. Comparison among the different Leu concentrations is described later.

As expected from data in Fig. 1, the extracellular specific radioactivity in dystrophic cultures was considerably lower than that in normal cultures at 0.05 mM Leu (Table I). At 0.2 mM Leu, the dystrophic extracellular [\textsuperscript{3}H]Leu specific radioactivity was only slightly lower than the normal value; but at 5.0 mM Leu, the difference was unexplainably large again. Additionally, the intracellular and Leu-tRNA specific radioactivities in dystrophic cultures are lower than the corresponding values in normal cells at all three Leu concentrations (Table I).

Table II also shows the effect of Leu concentration on cellular Leu pools; however, the specific radioactivities of the equilibrated intracellular and Leu-tRNA pools from Table I have been recalculated as percentages of the corresponding extracellular [\textsuperscript{3}H]Leu specific radioactivities. Recalculation in this manner more clearly illustrates a crucial point. As the extracellular Leu concentration was increased, the intracellular [\textsuperscript{3}H]Leu specific radioactivity values approached the values for the extracellular Leu pool. Stated differently, extracellular Leu contributed more and more Leu to the intracellular pool as the external Leu concentration was raised, and the proportion of intracellular free Leu coming from protein breakdown was concomitantly decreased. At the highest Leu concentration studied (5.0 mM), the intracellular specific radioactivity equaled the extracellular specific radioactivity in normal cells and was only slightly lower in dystrophic cells.

In contrast to the intracellular pool, the specific radioactivity of the Leu-tRNA pool was only minimally responsive to changes in Leu concentration (Table II), implying that the proportions of amino acids from the extracellular pool and from protein degradation were not significantly affected. Therefore, high extracellular Leu does not appear to eliminate or even significantly alter reutilization of amino acids from breakdown of cellular proteins as is frequently assumed. Indeed, the fact that Leu-tRNA specific radioactivity in both normal and dystrophic cells was only 55–60% of extracellular specific radioactivity implies that 40–45% of the Leu incorporated into muscle protein originates from reutilization.

**Total Protein Synthesis Rate in Dystrophic Muscle Cultures**

As expected from the protein synthesis in normal and dystrophic cultures, muscle cells were pulse-labeled for 2 h with 0.1 mCi/ml of [\textsuperscript{3}H]Leu in complete culture medium containing a physiological concentration of Leu (0.2 mM).
Leucine Pools in Dystrophic Muscle Cultures

Cultured cells from normal and dystrophic muscle were exposed to medium containing [3H]Leu at the indicated concentrations. The specific radioactivities of cellular Leu pools were measured 30, 60, or 120 min after addition of [3H]Leu (0.05 mM, 50 μCi/ml; 0.2 mM, 100 μCi/ml; 5.0 mM, 500 μCi/ml). As indicated in the text, specific radioactivities measured at either 30, 60, or 120 min were not significantly different, and the data from all three times were therefore pooled for this table. Data are expressed as the mean ± S.E. from two to 12 experiments with multiple samples.

| Leucine conc.* (mM) | Extracellular† | Intracellular‡ | Leucyl-tRNA§ |
|---------------------|---------------|----------------|--------------|
|                     | Normal | Dystrophic | Normal | Dystrophic | Normal | Dystrophic |
| 0.05                | 885 ± 94\(^{a}\) | 499 ± 78**\(^{b}\) | 272 ± 36\(^{a}\) | 239 ± 36\(^{a}\) | 435 ± 49\(^{a}\) | 310 ± 46**\(^{a}\) |
| (n = 21)            | (n = 23)   | (n = 16)      | (n = 14)  | (n = 14)    | (n = 11) | (n = 13)   |
| 0.20                | 635 ± 21\(^{a}\) | 576 ± 40**\(^{b}\) | 401 ± 43\(^{a}\) | 290 ± 69**\(^{b}\) | 355 ± 29\(^{a}\) | 318 ± 28\(^{a}\) |
| (n = 38)            | (n = 43)   | (n = 15)      | (n = 14)  | (n = 14)    | (n = 20) | (n = 22)   |
| 5.0                 | 175 ± 10\(^{a}\) | 114 ± 22**\(^{b}\) | 175 ± 12\(^{a}\) | 102 ± 20**\(^{c}\) | 98 ± 11\(^{a}\) | 17 ± 9**\(^{f}\) |
| (n = 12)            | (n = 9)    | (n = 12)      | (n = 9)   | (n = 9)     | (n = 6)  | (n = 9)    |

- The concentration of Leu listed reflects only that added as nonradioactive Leu. The actual Leu concentration was higher because of the Leu in horse serum and embryo extract. Assuming the concentration of Leu in serum and embryo extract is approximately 0.245, 0.05, and 5.0 mM, respectively.
- Data from dystrophic cells were compared to their normal counterparts in each cellular compartment using a t test. * indicates p < 0.05; ** indicates p < 0.01.
- Means for normal cells in the same row not bearing the same superscript are different from each other at the 0.05 level using a t test. Note that this comparison is only made within each row of data for each concentration of Leu.
- Data from dystrophic cells in the same row not bearing the same superscript are different from each other at the 0.05 level using a t test.
- Refers to means that are compared according to the conditions in Footnotes c and d.

### TABLE II

**Leucine specific radioactivity**

Cultured cells from normal and dystrophic muscle were exposed to medium containing [3H]leucine at the indicated concentration. The specific radioactivity of cellular leucine pools was measured (see Table I). Data are expressed as the mean ± S.E. from two to seven experiments with multiple samples in each experiment. Data are per cent of extracellular leucine specific radioactivity.

| Leu conc. (mM) | Intracellular | Leucyl-tRNA |
|----------------|--------------|-------------|
|                | Normal | Dystrophic | Normal | Dystrophic |
| 0.05           | 30.7   | 47.9       | 49.1   | 62.1       |
| 0.20           | 93.1   | 51.9       | 55.9   | 55.2       |
| 5.0            | 100    | 89.5       | 56.0   | 62.3       |

### TABLE III

**Effect of muscular dystrophy on muscle differentiation and protein synthesis at 0.2 mM leucine**

Muscle cells at 7 days of incubation were pulse-labeled for 2 h with 100 μCi/ml of [3H]Leu. Specific radioactivity of [3H]Leu in the Leu-tRNA pool was employed to calculate the rates of synthesis from incorporation data. Data are expressed as the mean ± S.E. from six experiments with at least duplicate measurements for each parameter.

| Parameter                  | Normal | Dystrophic |
|----------------------------|--------|------------|
| Percentage fusion          | 58.8 ± 1.0 | 59.5 ± 3.4 |
| Myotube nuclei/culture (×10⁶) | 1.98 ± 0.1 | 2.37 ± 0.17 |
| [3H]Leucine dpm/culture (×10⁶) | 23.8 ± 2.4 | 24.3 ± 1.9 |
| Synthesis rate (pmol Leu/h×10⁶ nuclei) | 15.4 ± 2.6 | 21.8 ± 3.2 |

Results from these experiments (Table III) illustrated three important points. First, normal and dystrophic cultures were morphologically similar after 7 days in culture as evidenced by comparable percentages of nuclei within multinucleated myotubes and total number of nuclei within myotubes. Second, when the rates of incorporation of [3H]Leu into total protein was compared during the pulse labeling period, no differences in the apparent synthesis rate were observed (Table III, line 3). Third, conversion of the incorporation data (or apparent synthesis rates) into actual protein synthesis rates using Leu-tRNA specific radioactivity revealed that total protein synthesis was approximately 42% higher in dystrophic cells than in normal cells. Because muscle cell cultures are at a steady state with respect to protein synthesis and degradation after 7-8 days and because the synthesis rate must equal the degradation rate at steady state, the data in Table III also indicate that protein degradation is approximately 42% higher in cultured dystrophic cells.

### DISCUSSION

The amino acids that are polymerized into peptide chains can originate from either the extracellular environment as a result of active amino acid transport or from utilization of amino acids that were liberated by proteolysis of pre-existing proteins. When the relative contribution of amino acids from these two sources is unknown, it is difficult to accurately measure rates of protein synthesis or breakdown. Our previous research (Riebow and Young, 1980; Young et al., 1981), which suggested that myofibrillar protein synthesis and breakdown were elevated in dystrophic muscle cells, employed pulse label and pulse-chase techniques. These data were therefore subject to the following assumptions. 1) The contribution to protein synthesis of amino acids from utilization was negligible compared to the contribution from active transport, and 2) normal and dystrophic muscle cells derive the same fraction of amino acids from utilization and transport. From Tables I and II, it is clear that neither of these assumptions was completely valid. For example, in normal and dystrophic cells, the tRNA specific radioactivity was 55% of the extracellular specific radioactivity, implying that approximately 45% of the amino acids for protein synthesis originated from utilization. Moreover, the specific radioactivity of tRNA in dys-
trophic cells is less than the specific radioactivity of tRNA in normal cells (Table 1).

In contrast to the differences reported here for line 307 dystrophic chick cultures, Wolitsky et al. (1982) have found no differences in protein synthesis and degradation in cultured muscle cells from line 412 normal and 413 dystrophic muscle cultures. Line 412 is a better genetic control for line 413 than the white leghorn chick is for line 307 dystrophic chickens, and some of the differences reported here undoubtedly reflect genetic divergence unrelated to the dystrophic lesion. In any case, discovery of the mechanism by which protein degradation is accelerated so drastically in line 307 dystrophic chick cultures, Wolitsky et al., 1981). The model of muscle atrophy is characteristic of muscular dystrophy, and changes in protein metabolism must occur to explain this loss of muscle tissue. Many studies have described such changes but differ in whether the change in metabolism is due to a change in synthesis, degradation, or both (Battelle and Florini, 1973; Riebow and Young, 1980; Weinstock et al., 1981). The underlying key assumption of studying muscular dystrophy in cell culture is that the genetic lesion is expressed independently of physical or biochemical contact with other dystrophic tissues or organs (i.e. via nerve or blood). Several major lines of evidence support this assumption for genetic muscular dystrophy of the line 307 dystrophic chick (discussed by Young et al., 1981). Even though muscle cell cultures artificially represent the in vivo situation, evidence for abnormalities in the proteolytic systems that metabolize myofibrillar proteins is reasonably convincing (Askanas et al., 1971; Battelle and Florini, 1973; McConnell et al., 1981a and b; Goldberg et al., 1977; Riebow and Young, 1980; Rourke, 1975; Weinstock and Jones, 1977; Young et al., 1978 and 1981).

Acknowledgments—We appreciate the assistance of Sara Bradley with muscle cell cultures and Casey Vaughn for measurement of specific radioactivities.

REFERENCES

Airhart, J., Kelley, J., Brayden, J. E., Low, R. B., and Stirewalt, W. E. (1979) Anal. Biochem. 96, 45-55
Askanas, V., Shafiq, S. A., and Milhorat, A. T. (1971) Arch. Neurol. 25, 259-265
Battelle, B. A., and Florini, J. R. (1973) Biochem. J. 12, 635-643
Goldberg, A. L., Griffin, G. E., and Dice, J. F. (1977) in Pathogenesis of Human Muscular Dystrophies (Rowland, L. P., ed) pp. 376-385, Excerpta Medica, Amsterdam.
Hammer, J. A., Jr., and Rannels, D. E. (1981) Biochem. J. 198, 53-65
Herman, B. A., and Fernandez, S. M. (1977) Fed. Proc. 36, 585
Hildebran et al., 1981; Schneible et al., 1981). Negligence in measuring the tRNA specific radioactivity could therefore lead to errors of up to 35-40% in quantitating protein synthesis or degradation rates in cell cultures.

The possibility that calculated rates of protein synthesis using Leu-tRNA specific radioactivity contain additional errors other than those discussed above cannot be eliminated. An inherent assumption is that all isoaccepting species of Leu-tRNA are randomly utilized from the extracellular pool and that these isoaccepting species are then randomly utilized for protein synthesis. If different isoaccepting species of Leu-tRNA were to receive their amino acid from unique pools within the cell, measurement of the averaged specific radioactivity of these isoaccepting species would accurately reflect the specific radioactivity of the individual species. Direct proof that this does not occur can only be obtained by comparing the specific radioactivity of tRNA with the specific radioactivity within nascent chains of specific proteins. In at least one reported instance, indirect evidence indicates that selective amino acid pool utilization does not take place (Hildebran et al., 1981). The model of interaction of proline pools in human lung cells illustrated by Hildebran et al. (1981, Fig. 4) may be generally applicable to other amino acids and is useful in visualizing the complexity of intracellular amino acid pools.

Muscle atrophy is characteristic of muscular dystrophy, and changes in protein metabolism must occur to explain this loss of muscle tissue. Many studies have described such changes but differ in whether the change in metabolism is due to a change in synthesis, degradation, or both (Battelle and Florini, 1973; Riebow and Young, 1980; Weinstock et al., 1981). The model of muscle atrophy is characteristic of muscular dystrophy, and changes in protein metabolism must occur to explain this loss of muscle tissue. Many studies have described such changes but differ in whether the change in metabolism is due to a change in synthesis, degradation, or both (Battelle and Florini, 1973; Riebow and Young, 1980; Weinstock et al., 1981). The underlying key assumption of studying muscular dystrophy in cell culture is that the genetic lesion is expressed independently of physical or biochemical contact with other dystrophic tissues or organs (i.e. via nerve or blood). Several major lines of evidence support this assumption for genetic muscular dystrophy of the line 307 dystrophic chick (discussed by Young et al., 1981). Even though muscle cell cultures artificially represent the in vivo situation, evidence for abnormalities in the proteolytic systems that metabolize myofibrillar proteins is reasonably convincing (Askanas et al., 1971; Battelle and Florini, 1973; McConnell et al., 1981a and b; Goldberg et al., 1977; Riebow and Young, 1980; Rourke, 1975; Weinstock and Jones, 1977; Young et al., 1978 and 1981).

Acknowledgments—We appreciate the assistance of Sara Bradley with muscle cell cultures and Casey Vaughn for measurement of specific radioactivities.
Leucine pools in normal and dystrophic chicken skeletal muscle cells in culture.
P A Schneible and R B Young

*J. Biol. Chem. 1984, 259:1436-1440.*

Access the most updated version of this article at [http://www.jbc.org/content/259/3/1436](http://www.jbc.org/content/259/3/1436)

**Alerts:**
- When this article is cited
- When a correction for this article is posted

**Click here** to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/259/3/1436.full.html#ref-list-1](http://www.jbc.org/content/259/3/1436.full.html#ref-list-1)