Protein Synthesis in Single Cell Suspensions from Rat Liver

I. GENERAL PROPERTIES OF THE SYSTEM AND PERMEABILITY OF THE CELLS FOR LEUCINE AND METHIONINE*

(Received for publication, May 4, 1972)

GERHARD SCHREIBER AND MARGOT SCHREIBER
From the Biochemisches Institut, Universität Freiburg, 7800 Freiburg im Breisgau, Germany

SUMMARY

Suspensions of single cells were prepared from normal adult rat liver by perfusion of liver with solutions of hyaluronidase and collagenase (5, 6). The suspension was obtained after incubation of the cells at pH 7.17. The incorporation of L-[1-14C]leucine into protein was observed at a puromycin concentration of 1.4 μg per ml, or 5.1 × 10⁻⁵ M L-methionine. An inhibition of 50% of the incorporation of amino acids into protein was observed if the cells were incubated under nitrogen. Optimal incorporation was obtained in 3 mM to 12 mM phosphate buffer at pH 7.17. The incorporation was inhibited at higher phosphate concentrations, and it was also less efficient in Tris-HCl buffer, Tris-maleate buffer, and Tris-phosphoric acid buffer.

The incorporation of L-[1-14C]leucine into protein was directly proportional to the concentration of the cells in the suspension. High doses of L-leucine or L-methionine (~2 mM) did not inhibit the incorporation of amino acids into protein. The cells were freely permeable for L-leucine and L-methionine. The system incorporating amino acids into protein was half-saturated with 7.2 × 10⁻⁵ M L-leucine and 5.1 × 10⁻⁵ M L-methionine. An inhibition of 50% of the incorporation of L-leucine or L-methionine into protein was observed at a puromycin concentration of 1.4 μg per ml, or 0.8 μg per ml respectively. Changing the incubation temperature from 22° to 32° caused a 4-fold increase of the initial rate of incorporation of L-leucine or L-methionine.

whereas no albumin synthesis could be observed in cell-free protein-synthesizing systems from normal or regenerating rat liver, provided that albumin had been purified to constant specific radioactivity (13).

In this paper, protein synthesis in suspended single rat liver cells is characterized in detail. The cells were found to be freely permeable for leucine and methionine. The treatment with collagenase and hyaluronidase probably destroys the active transport system of the cell membrane. The high efficiency of protein synthesis (amino acid incorporation is linear with respect to time for 1 to 2 hours, and several per cent of offered radioactively labeled amino acid is incorporated into protein (8)) together with the possibility of controlling part of the intracellular environment of the protein-synthesizing apparatus, may render the system most suitable for studies on the mechanisms of liver protein synthesis and its regulation.

MATERIALS AND METHODS

Animals and Operations—All cell suspensions were prepared from male Buffalo rats weighing between 200 g and 400 g and kept under conditions as previously described (12, 14). Operations were performed between 10 a.m. and 2 p.m. under ether anesthesia, which does not influence protein synthesis in the liver (15, 16).

Chemicals and Isotopes—All chemicals were of scintillation or analytical grade. L-[1-14C]leucine (50 to 62 mCi per mmole), 98% to 99% pure, L-[4, 5-3H]leucine (290 mCi per mmole), 97% to 98% pure, L-[1-14C]methionine (55 to 58 mCi per mmole), 97% to 98% pure, L-[1-14C]leucine (1000 mCi per mmole), 98% pure, and L-[U-3H]methionine (290 mCi per mmole), 95% to 98% pure, were purchased from the Radiochemical Centre (Amersham, United Kingdom).

Dissociation and Incubation Media—The composition of the dissociation medium used for perfusion and shaking was published elsewhere (8). The incubation medium consisted of 139 mM NaCl, 5.37 mM KCl, 0.81 mM MgSO₄, 1.26 mM CaCl₂, 6 mM NaH₂PO₄-Na₂HPO₄ buffer of pH 7.17, 0.02% heparin (to inactivate hyaluronidase activity), 0.00006% sodium penicillin G, 0.00004% potassium penicillin G, 0.002% streptomycin, amino acids as described by Waymouth (17), 0.56 mM l-alanine, and 0.21 mM l-serine.

Preparation of Cell Suspensions—The procedure for preparing single cell suspensions was a modification (8) of that of Berry and Friend (6). An artificial lung, similar to that described for rat liver perfusion systems (18-22), with a cooling jacket (22), was used for saturating the dissociating perfusion medium with...
oxygen and carbon dioxide. On the average, 2.1 g ± 0.2 g of
cells (wet weight of packed cells) were obtained per 100 g
of rat.

Acidity of Proteins—Acid hydrolysis of protein was
performed as described by Keutmann and Potts (23).

Paper Chromatography—Amino acids obtained from protein
hydrolysates were separated either in the system of Lewin and
Wei (24) or on Schleicher and Schuell chromatography paper in
pyridine-ethyl acetate-water, 5:12:4 (v/v/v). Radioactivity
on the paper was determined by liquid scintillation counting.

Determination of Radioactivity—Radioactivity was deter-
mined as described previously (12). In all double labeling ex-
periments, [3H]toluene and [14C]toluene were used as a standard
for the determination of the absolute counting efficiency.

RESULTS AND DISCUSSION

Isolation of Radioactive Proteins from Cells—Cell suspensions
have the advantage that many identical samples may be ob-
tained from one incubation mixture. A fast and convenient
procedure is therefore necessary for the isolation of radioactive
proteins from those samples. The method of Mans and Novelli
(25) could be successfully applied to liver cell suspensions without
homogenization of the samples prior to the application to the
paper disks. The standard deviation was in the range of the
pipetting error and increased only slightly toward the end of the
incubation.

Effect on Amino Acid Incorporation of Varying Atmosphere
of Incubation, Concentrations of Hydrogen Ions, Albumin,
Glucose, Buffers, and Cells—Similar results on the incorporation
of L-[1-14C]leucine into protein were obtained for cells incubated
in air and for cells incubated under an atmosphere of 95% O2 +
5% CO2. However, no incorporation at all was observed if the
cells were incubated under nitrogen.

A pronounced dependence of the incorporation of L-[1-14C]-
leucine into protein on the concentration of H+ ions was found
with a sharp maximum of the incorporation rate at a pH of 7.17.
The same value had been measured previously for a cell-free
amino acid-incorporating system composed of microsomes and
of a pH fraction from rat liver.1 The width of the pH depend-
cy curve at half-maximal incorporation rate corresponded to
about one pH unit. This was narrower than found for many
enzymatic reactions, e.g., those catalyzed by α-amylase (26),
or aspartate aminotransferase (27), or fumarate hydratase (28).

Addition or omission of glucose or bovine serum albumin did
not affect the rate and the amount of incorporation. Among
the buffers tested for the incorporation of L-[1-14C]leucine into
protein, 3 mM to 12 mM Na2HPO4-NaH2PO4 buffer of pH 7.17
seemed to be the most suitable buffer system. Since this buffer
is relatively weak, the pH of the incubation mixtures was read-
justed immediately before start of the incorporation reaction.
The change of pH during an incubation of 120 min did not exceed
0.2 pH unit. Lower incorporation rates were observed in bi-
carbonate-phosphate buffer, Tris-HCl, Tris-maleate, and Tris-
phosphate buffer in the range of 10 to 20 mM. Incorporation was
strongly inhibited at buffer concentrations above 20 mM.

The incorporation of L-[1-14C]leucine into protein was directly
proportional to cell concentration in the range of 2.6 × 10^6
to 4.7 × 10^7 cells per ml. Increasing the cell concentration in the
incubation mixture did not lead to a decrease of the incorpora-
tion of amino acid into protein.

Effect of Prior Incubation, Termination of Incorporation


Reaction by Isotope Dilution—A sigmoid shape of the incorpora-
tion curve was usually obtained if the suspended cells were
brought to 37° and if the reaction was started immediately there-
after by addition of L-[1-14C]leucine. This sigmoid shape was
not seen if the cells were previously incubated for 25 min at 37°
before L-[1-14C]leucine was added.

Addition of a 45-fold or a 450-fold excess of L-[14C]leucine
carried almost instantaneous cessation of measurable L-[1-14C]-
leucine incorporation into protein, suggesting very fast equilibra-
tion of extracellular and intracellular leucine pools.

Effect of Increasing Concentration of [14C]Amino Acids
Added as Precursor on Their Incorporation into Protein—
The concentration of added L-[1-14C]leucine in the incubation
mixture was varied between 0.0018 mM and 1.41 mM, that of
L-[1-14C]methionine was varied from 0.0036 mM to 1.52 mM.
For L-leucine incorporation, a typical saturation curve was ob-
tained with half-saturation of the system at a concentration of
about 0.07 mM (Fig. 1). For L-methionine, no saturation was
obtained even at a concentration of 1.52 mM. However, at high
concentrations of added methionine, the saturation curve seemed
to approach asymptotically a straight line with a positive slope.
If a parallel to this straight line was drawn through the origin
and if this parallel was subtracted from the original curve, a
typical saturation curve was obtained also for methionine.
From this curve, the concentration necessary for half-saturation
of the system with L-methionine was found to be 0.05 mM.

The saturation curves observed for the dependence of amino
acid incorporation on the concentration of amino acid added to
the incubation mixture may be explained in one of the following
ways.

1. High concentrations of amino acids may possibly inhibit
protein synthesis. The toxic effect of large doses of methionine
on guinea pig liver has been interpreted to be mediated by a de-

Fig. 1. Incorporation of L-[1-14C]leucine into protein as a function of the concentration in the incubation mixture. Start by
rapid warming up to 37° (warming up time, <30 s), termination by
rapid cooling to 0°. Incubation time, 55 min.
creased ATP (29-31). A decrease of ATP concentration would also lead to a decrease of protein synthesis. Therefore, we studied the incorporation of L-[1-14C]leucine, varying L-methionine concentration in the incubation mixture from 0.004 mM to 1.8 mM, and that of L-[1-14C]methionine varying L-leucine concentration from 0.003 mM to 1.6 mM. No change in the rate of the incorporation of L-[1-14C]methionine or L-[1-14C]leucine into protein was observed. Only if 10 mM mercaptoethanol, a component of many amino acid-incorporating systems, was added, incorporation of L-[1-14C]leucine was inhibited to 58.7% when the concentration of L-methionine in the incubation mixture was increased to 1.4 mM.

2. Isotope dilution might be another explanation for curves similar to those of Fig. 1. Added [14C]-labeled amino acid (z) is taken up by the cells from the incubation medium. It is then diluted by the intracellular pool of [14C]-amino acid (a). The specific radioactivity of protein isolated from the cells after incubation will be proportional to the specific radioactivity of the precursor amino acid. Thus, if the incorporated amount of [14C]-labeled amino acid is designated by y, and if k is a proportionality constant, the following equation holds:

\[ y = k \frac{x}{z + a} \]  

or

\[ y = -a \frac{y}{z} + k \]  

The second equation is linear in y and y/z. Indeed, if we plotted y against y/x, a straight line was obtained for the incorporation of L-leucine. At least two straight lines were found for the incorporation of L-methionine. The size of the amino acid pools, a, was calculated from the slopes, -a, of those lines. For L-leucine, 0.072 µmole of L-leucine per ml of incubation mixture or 68 µmole of L-leucine per 100 g of cells were obtained. This value was in the same range as the concentration of L-leucine in rat liver reported by other authors (32, 33).

3. Equation 2 is analogous to that proposed by Hofstee (34) for a reaction with Michaelis-Menten kinetics, y corresponding to r, and y/x corresponding to r/s. In Fig. 2, v was plotted against r/s for L-leucine incorporation. It cannot be decided from mathematical analysis if the data presented in Figs. 1 and 2 have to be interpreted by isotope dilution or by the existence of an enzymatic process with Michaelis-Menten kinetics. The concentration of leucine in rat liver (32, 33) would be in the appropriate range to explain our data by isotope dilution. Enzymatic processes with Michaelis-Menten kinetics explaining the data could be the active transport of amino acids through the cell membrane and the activation of the amino acids by their corresponding aminoacyl-tRNA synthetases. The capacity for concentrative uptake would also be necessary for the explanation of our incorporation data by dilution of the added precursor in the intracellular amino acid pool. The following experiments describe the kinetics of amino acid uptake by isolated liver cells in suspension.

Permeability of Single Rat Liver Cells in Suspension for L-Leucine and L-Methionine—The almost instantaneous cessation of the incorporation of L-[1-14C]leucine into protein after addition of excess L-[14C]leucine suggested fast penetration of leucine into the cells or fast equilibration between intracellular and extracellular L-leucine pools. Cells with an active transport system for amino acids show the phenomenon of concentrative uptake. If such cells are washed in suspension medium or saline, intracellular amino acid is released only slowly into the washing medium. Duck erythrocytes, for example, were losing slowly 60% of intracellular glycine in six 1-hour washes, 20 volumes each, with NaCl-CaCl₂-NaHCO₃ medium (35).

The system for the concentrative uptake of methionine and leucine in suspended rat liver cells was tested as follows. A cell suspension of 2.25 × 10⁶ cells per ml was divided into four equal portions of 5 ml each. To the first portion, 0.018 mM L-methionine containing 1 µCi per ml of L-[1-14C]methionine was added (Fig. 3, black columns). To the second portion, 1.8 mM L-methio-
nine, which also contained 1 μCi per ml of L-[1-14C]methionine, was added (Fig. 3, white columns). To the third and fourth portion, 0.016 mM (Fig. 3, hatched columns) and 1.6 mM L-leucine (Fig. 3, dotted columns), both containing 1 μCi per ml of L-[1-14C]leucine, were added. All portions were incubated for 35 min at 37°C under shaking in a Warburg apparatus. The incubation was terminated by cooling to 0°C and 5 aliquots of 0.9 ml each were removed from each portion. From the first aliquot of each portion, cells were separated by centrifugation for 10 min at 50 X g, lysed in 0.5 ml of 1 M HClamino hydroxide, and radioactivity was determined as described under "Materials and Methods." The cells from the second aliquots were washed once in 10 ml of cold 0.9% NaCl before lysing and counting, cells from the third aliquots were washed twice, cells from the fourth aliquots were washed three times, etc., before lysing and counting. About the same proportion of the amino acid was found in the cells, both for the low methionine and for the high methionine concentration (Fig. 3, black and white columns of the first tetrad). Almost all of the radioactivity could be removed from the cells during the first wash. Similar results were obtained for the two L-leucine concentrations differing also by a factor of 100. The results suggest that the cells have lost the capacity for concentrative uptake of L-leucine and of L-methionine.

Kinetics and temperature dependence of the penetration of L-leucine and L-methionine into isolated rat liver cells in suspension were studied in further experiments. From a cell suspension, four 12-ml portions were prepared containing about 12 μCi of L-[1-14C]leucine in 1.6 mM L-leucine (Fig. 4, LEU H) or in 0.016 mM L-leucine (Fig. 4, LEU L), or about 12 μCi of L-[1-14C]methionine in 1.8 mM L-methionine (Fig. 4, MET H) or in 0.018 mM L-methionine (Fig. 4, MET L). All suspensions were kept at 0°C. An aliquot of 1 ml was removed from each suspension. Ten μCi of L-[4,5-3H]leucine (1000 mCi per mmole) were added to the L-leucine-containing aliquots, and 10 μCi of L-[U-3H]methionine (290 mCi per mmole) were added to the L-methionine-containing aliquots. The cells were recovered from the aliquots by centrifugation for 10 min at 50 X g, lysed with 0.5 ml of 1 M HClamino hydroxide in methanol, and the ratio of 14C-labeled amino acid to 3H-labeled amino acid was determined. The remaining portions of the cell suspensions of 1 ml each, which contained only 14C-labeled L-leucine or L-methionine were then incubated for 35 min at 37°C under shaking in a Warburg apparatus. One ml was removed from each portion after 35 min of incubation, cooled to 0°C, L-[3H]methionine or L-[3H]leucine was added in the cold as described for the first aliquot, and the ratio of 14C-labeled amino acid to 3H-labeled amino acid was determined. To the residual portions of 10 ml each, 0.1 μCi of L-[4,5-3H]leucine (1000 mCi per mmole) was added to the L-[4C]leucine-containing portions, and 0.1 μCi of L-[U-3H]methionine (290 mCi per mmole) was added to the L-[4C]methionine-containing portions (Fig. 4, arrows). Incubation at 37°C was continued, and 1-ml aliquots were removed every 3 min. The aliquots were rapidly cooled to 0°C, and cells were collected by centrifugation. The ratios of 14C-labeled amino acid to 3H-labeled amino acid were determined and plotted against incubation time (Fig. 4). A constant ratio of 14C-labeled amino acid to 3H-labeled amino acid was established instantaneously independent of temperature and of amino acid concentration, even if amino acid concentrations varied over 100-fold. This is incompatible with the existence of an intact active transport system for L-leucine and L-methionine. It suggests free exchange of L-leucine and L-methionine between the extracellular and the intracellular compartment.

The estimation of L-methionine and L-leucine uptake by cells incubated with various concentrations of L-methionine or L-leucine gave further evidence for the lack of an active transport system for these amino acids (Fig. 5). A cell suspension was

Fig. 4. Kinetics of equilibration of L-leucine and L-methionine between the intracellular and the extracellular compartments. Cells were incubated with two different concentrations of 14C-labeled L-amino acid for 35 min at 37°C. 3H-labeled amino acids were then added (arrows) and the ratio of 14C dpm:3H dpm was determined at intervals of 3 min. The zero point sample was prepared and measured at 0°C. For further experimental details see text.

Fig. 5. Uptake of L-leucine and L-methionine as a function of their concentration in the incubation medium. The concentration of the cells was 2.25 X 10⁶ cells per ml. Incubation time 5 min. Start by rapid warming up to 37°C, stop by rapid cooling to 0°C.
divided into 22 fractions of 1 ml each. L-[1-14C]Leucine (0.08 mCi per mmole, closed circles) or L-[1-14C]methionine (0.08 mCi per mmole, open circles, dashed line) was added in the concentrations indicated on the abscissa of Fig. 5. All fractions were then incubated at 37° for 5 min. After incubation, cells and incubation medium were separated by centrifugation, and radioactivity in both was determined. The intracellular amount of L-[1-14C]-amino acid was plotted against its extracellular concentration (Fig. 5). No saturation of the uptake of L-leucine or L-methionine was observed, even at a concentration of 10 mM.

The results of the experiments described in Figs. 3 to 5 suggest that the saturation curve of Figs. 1 and 2 cannot be explained by isotope dilution or by the saturation of an intact amino acid transport system of the cell membrane. The saturation curve of Figs. 1 and 2 probably represents the saturation of the leucine-activating enzyme of the liver cell. For L-methionine, a single species of an activating enzyme (36-38) as well as the resolution of the methionine-activating activity into two different bands by chromatography on DEAE-cellulose (39) or by electrophoresis in polyacrylamide gel (40) have been described. From the observation of two components of the saturation curve one may suspect the existence of two different methionine-activating enzymes. Therefore, amino acid incorporation into protein of the cells was further characterized. The inhibition by puromycin, the temperature dependence of the reaction, and the composition of the reaction product were studied.

Inhibition by Puromycin—Incorporation into protein of L-[1-14C]leucine from an incubation medium with 1.613 mM L-leucine and incorporation into protein of L-[1-14C]methionine from incubation media containing either 0.036 mM or 3.636 mM L-methionine was measured at 18 different concentrations of puromycin, ranging from 0 to 0.4 mg per ml (Fig. 6). An inhibition of 50% was observed for the incorporation from 1.613 mM L-leucine at a puromycin concentration of 1.4 μg per ml, for the incorporation from 0.036 mM L-methionine at 0.8 μg per ml of puromycin, and for the incorporation from 3.636 mM L-methionine at 70 μg per ml of puromycin. Thus, the concentration of puromycin producing 50% inhibition was almost 50-fold larger for incorporation from the high methionine concentration than for that from the low methionine content of the incubation mixture. An inhibition of 100% of L-leucine incorporation, and an inhibition of 96.4% of L-methionine incorporation from 0.036 mM L-methionine was caused by 0.4 mg per ml of puromycin, but incorporation from 3.636 mM methionine was only inhibited to 40% of the value observed without puromycin added.

Temperature Dependence of Incorporation of L-Leucine and L-Methionine into Protein—Incorporation into protein was studied at various temperatures for 1.613 mM L-[1-14C]leucine (Fig. 7, closed circles), 0.036 mM (Fig. 7, open circles, dashed line) and 3.636 mM L-[1-14C]methionine (Fig. 7, open squares, dotted line). The initial rate of the incorporation was plotted against incubation temperature in percentage of the value observed at 37°. Between 20° and 30°, all three curves showed a similar increase of the reaction rate with the increase of temperature. Below 20°, only the incorporation of L-leucine and of L-methionine from the low methionine concentration were further diminishing with decreasing incubation temperature.

Analysis of Reaction Product—Protein was isolated after incubation of the cells with L-[1-14C]methionine by a procedure similar to that described by Mans and Novelli for paper disks.

![Fig. 6](http://www.jbc.org/...)

![Fig. 7](http://www.jbc.org/...)

---

**Fig. 6.** Inhibition by various puromycin concentrations of L-[1-14C]leucine or L-[1-14C]methionine incorporation into protein for 1.613 mM L-leucine containing 5 μCi per ml of L-[1-14C]leucine ( ), or for 0.036 mM (○, ---) and 3.636 mM L-methionine (□, ---) containing 2 μCi per ml and 20 μCi per ml of L-[1-14C]-methionine, respectively. Incubation at 37° for 50 min, cell concentration 1.4 X 10⁸ cells per ml, start by rapid warming to 37°, termination by cooling to 0°.

**Fig. 7.** Temperature dependence of the initial rate of incorporation into protein of L-[1-14C]-labeled L-amino acids from 1.613 mM L-leucine (Leu), 0.036 mM (Met₁), or 3.636 mM L-methionine (Met₂). Leu contained 5 μCi per ml, Met₁ 2 μCi per ml, and Met₂ 20 μCi per ml of L-[1-14C]-labeled L-amino acid.
(25), but carried out in liquid and including additional washes in 5% trichloroacetic acid. Amino acids were obtained from the isolated protein by acid hydrolysis and were separated by paper chromatography. Recovery of the radioactivity was between 70% and 100%. Paper chromatograms were analyzed for radioactivity as described under "Materials and Methods." Two radioactive peaks were obtained for the L-[1-14C]methionine preparation used as precursor, one corresponding to methionine, the other to methionine sulfoxide. The proportion of methionine sulfoxide to methionine increased with the time the chromato-
grams were kept in air for drying before starting the chromatography. No trailing of the methionine peak was observed, suggesting that no conversion of methionine to methionine sulfoxide took place during the chromatography itself. The protein from an incubation mixture containing 0.036 mM L-[1-14C]methionine (57 mCi per mmole) was analyzed, a similar pattern was obtained, except that a small amount of radioactivity was found before the methionine sulfoxide peak and after the methionine peak. The result of the analysis of protein from an incubation with 3.636 mM L-[1-14C]methionine (57 mCi per mmole) without (closed circles), and with 0.4 mg per ml of puromycin added (open circles, dashed line), is shown in Fig. 8. Five peaks could be distinguished. Puromycin clearly inhibited only the incor-
poration of material of Peak II, which corresponded to methionine sulfoxide, and Peak III, which corresponded to methionine. Peak I was not inhibited at all by puromycin, whereas some inhibition may have taken place in the region of Peak IV and the left flank of Peak V. Thus, only the low \( K_m \) value which may be calculated for saturation of the system by methionine corresponded to methionine incorporation by protein biosynthesis, whereas the high \( K_m \) value was caused by radio-
active contaminants. These were enriched by binding to pro-
tein during incubation. The amino acid-activating system had been saturated already by a very small concentration of L-
methionine, and a further increase of the concentration of L-
[1-14C]methionine could not lead to an increase of incorporation of radioactivity.

**Concluding Remarks** Uptake of amino acids by the active transport system through the cell membrane depends on tempera-
ture (35, 41). Its rate is relatively slow (cf. Refs. 41–56). Equil-
ibration times of 1 hour and more have been reported (55, 56). In our experiments, equilibration between the extracellular and the intracellular space occurred almost instantaneously, even at 0°C. This argues against the participation of an intact active transport system of the cells in the equilibration. It is more likely that the curve of Fig. 1 may be explained by saturation of the L-leucine-activat-
ing enzyme. The pool of tRNA\(_{\text{Leu}}\) is too small to account for isotope dilution at the tRNA level.

The results on the incorporation from large doses of L-[1-14C]-
methionine demonstrate the danger brought about by using large doses of radioactively labeled precursor amino acids, even if those amino acids are of very high purity. The amino acid-activating enzymes have a great affinity for their substrate amino acid and, therefore, they are saturated at low precursor doses. Further increasing of the precursor dose will lead to en-
richment of contaminants binding to protein. In contrast to a widely spread opinion, more specific results are obtained on incor-
poration of amino acids into protein if radioactively labeled amino acids of low purity, but with high specific radioactivity, are used, instead of highly pure amino acids of low specific radio-
activity. The high specificity of the activating enzyme comp-
pensates for the lack in purity of the precursor amino acid. Fur-
thermore, the proportion of precursor amino acid which was incor-
porated rises sharply with a decrease of the offered dose (Fig. 9), thus rendering incorporation experiments more eco-
nomical.

---

**FIG. 8.** Chromatography of the protein hydrolysate from cells incubated with 3.636 mM L-[1-14C]methionine (57 mCi per mmole) without (●) and with 0.4 mg per ml of puromycin (○) added. Incubation at 37°C for 50 min, start by rapid warming to 37°C, termination by rapid cooling to 0°C. Chromatography in pyridine-
ethyl acetate-H\(_2\)O = 5:12:4 (v/v/v) on Schleicher and Schüll 2043 b MgL chromatography paper.

**FIG. 9.** Proportion of L-[1-14C]leucine or L-[1-14C]methionine incorporated into protein as a function of their concentrations in the incubation mixture.
Whereas incorporation of L-[1-\(^{14}\)C]leucine was completely inhibited by puromycin (Fig. 6), incorporation of L-[1-\(^{14}\)C]methionine could not be entirely suppressed by puromycin (Figs. 6 and 8). Chain initiation was reported to take place in the presence of puromycin (57). A small incorporation of L-methionine in the presence of puromycin could also be due to unspecific binding or to the action of methionine aminopeptidases such as described for the removal of methionine after chain initiation (5%-63%). Experiments to discriminate between those possibilities are currently in progress.

REFERENCES
Protein Synthesis in Single Cell Suspensions from Rat Liver: I. GENERAL PROPERTIES OF THE SYSTEM AND PERMEABILITY OF THE CELLS FOR LEUCINE AND METHIONINE
Gerhard Schreiber and Margot Schreiber


Access the most updated version of this article at http://www.jbc.org/content/247/19/6340

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/247/19/6340.full.html#ref-list-1