Müller-Eberhard, H. J., Dalmasso, A. P., & Calcott, M. A. (1966) *J. Exp. Med. 123*, 33–54.
Pangburn, M. K. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 1755.
Pangburn, M. K., & Müller-Eberhard, H. J. (1980) *J. Exp. Med. 152*, 1102–1114.
Pangburn, M. K., Schreiber, R. D., & Müller-Eberhard, H. J. (1977) *J. Exp. Med. 146*, 257–270.
Rapp, H. J., & Borsos, T. (1963) *J. Immunol. 91*, 826–832.
Tack, B. F., & Prahl, J. W. (1976) *Biochemistry 15*, 4513–4521.
Tack, B. F., Harrison, R. A., Janata, J., & Prahl, J. W. (1980a) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 700.
Tack, B. F., Harrison, R. A., Janatova, J., Thomas, M. L., & Prahl, J. W. (1980b) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5764–5768.
Vogt, W., Schmidt, G., von Buttlar, B., & Dieminger, L. (1978) *Immunology 34*, 29–40.
Von Zabern, I., Nolte, R., & Vogt, W. (1980) *J. Immunol. 124*, 1543.
Vuk-Pavlović, S., Blatt, Y., Glaudemans, C. P. J., Lancet, D., & Pecht, I. (1978) *Biophys. J. 24*, 161–174.

Isolation of Immunoglobulin Messenger Ribonucleic Acid from Human Lymphoblastoid Cell Lines†

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ABSTRACT: Eight human lymphoblastoid cell lines were screened for levels of synthesis and secretion of immunoglobulins G and M (IgG and IgM). These cell lines produced between 2 and 20% of immunoglobulin, as a proportion of total protein synthesis, and secreted the nascent immunoglobulins with characteristic half-times varying between 4 and 40 h. The secreted immunoglobulins exhibited equimolar ratios of heavy (H) and light (L) chains, consistent with the formula for complete immunoglobulins (H_2L_2); IgG was secreted in the monomer (7S) form, whereas IgM was secreted in varying proportions of the monomeric (8S) and pentameric (19S) forms. One cell line (RPMI 1788) produced a 2-fold molar excess of light over heavy chains, but did not secrete the excess light chains. The conditions of tissue culture were varied to optimize the production of immunoglobulins. Using RPMI 1788, which produced IgM(λ), it was found that substituting swine for calf serum and harvesting cells in the late-exponential phase of growth stimulated total protein synthesis, preferential immunoglobulin synthesis, the rate of immunoglobulin secretion, and the assembly of pentameric IgM. Messenger ribonucleic acid (mRNA) was isolated from two of the cell lines, one (RPMI 1788) producing μ and λ chains and the other (Bristol 7) producing γ and κ chains. The messenger RNA was fractionated by sucrose-gradient centrifugation and translated in a rabbit reticulocyte lysate, and the translation product was characterized by polyacrylamide gel electrophoresis before and after immunoprecipitation with specific antisera. RPMI 1788 contains a minor μ-chain-encoding mRNA, which sediments at 19 S and codes for a 67 400-dalton polypeptide, in addition to the major μ-chain-encoding mRNA, which sediments at 18 S and codes for a 65 000-dalton polypeptide in vitro. The RPMI 1788 λ-chain mRNA sediments at 13 S and codes for a polypeptide 31 000 daltons, longer than the mature λ chain synthesized in vivo. Bristol 7 yielded just two messenger activities, one at 16 S coding for a γ chain of 51 000 daltons, and the other at 12–13 S coding for a κ chain of 28 650 daltons. Sucrose-gradient fractionation resulted in a 10-fold purification of the immunoglobulin messenger RNA in the isolated fractions relative to that of the total. Immunoglobulin heavy or light chains comprised about 1% of the translation product of the gradient fractions. The proportion of immunoglobulin synthesized in the cells in culture (ca. 10% of total protein synthesis) was 2 orders of magnitude greater than that observed on translation of the isolated messenger RNA in vitro (ca. 0.1% for the unfractionated RNA). Further work is required to establish the basis for this large discrepancy.

The cloned genes for specific human immunoglobulins would be useful tools in molecular immunogenetics and might also provide, through bacterial expression, a useful source of proteins for immunotherapy. The technology for the stimulation, selection, and immortalization of human lymphocytes producing immunoglobulins of defined specificity, and for cloning the cells producing homogeneous idiotypes, is now available [see, e.g., Steinitz et al. (1979a,b)]. We show here that the isolation of messenger ribonucleic acid (mRNA) coding for immunoglobulin chains in vitro, suitable for enzymatic synthesis of the genes and cloning in bacteria, is possible, despite the fact that the levels of messenger RNA seem to be considerably lower than in mouse myelomas, the usual source of immunoglobulin mRNA, or in hybridomas made by fusing human lymphocytes with mouse myelomas (Dolby et al., 1980).

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Materials and Methods

Cell Lines. RPMI 1788, BRI 7, BRI 8, BEC 11, and MICH were supplied by Searle Research Laboratories. SMI 4, TAY 3, and DAUDI were provided by Dr. C. M. Steel. The

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Table I: Human Lymphoblastoid Cell Lines

cell line	source	chromosome analysis	virology	doubling time (h)
RPMI 1788	peripheral lymphocytes from normal donor (ATCC CCL 156)	diploid, male	no EB virus-like particles	20–25
MICH	spleen patient with idiopathic thrombocytopenia	subtetraploid, female	no EB virus-like particles, contains EB antigens	20–25
BEC 11	lymphoid cells of patient with tonsillitis	diploid, female	no EB virus-like particles, contains EB antigens	20–25
BRI 7	peripheral lymphocytes from normal donor	diploid, male	no EB virus-like particles, contains EB antigens	20–25
BRI 8	peripheral lymphocytes from normal donor	diploid, female	no EB virus-like particles, contains EB antigens	20–25
SMI 4	cord blood of healthy infant	not examined in this study		
DAUDI	Burkitt lymphoma			
TAY 3	lymphoid cells of patient with acute myeloblastic leukemia			

origins and some of the characteristics of the cell lines are summarized in Table I.

Cell Culture. Cells were grown in culture medium RPMI 1640, pH 7.4, modified as described by Birch et al. (1979) and supplemented with either 10% v/v fetal calf serum (Gibco) or 5% v/v swine serum (Medical and Veterinary Supplies, Ltd.). Cultures with swine serum contained 1 g/L Pluronic F68 (Wyandotte Chemical Corp.) to prevent precipitation of serum protein.

Cells were inoculated at 10^5 cells/mL and grown in 50-mL cultures in 250-mL conical flasks, either static ("nonagitated") or rotated at 200 rpm on an orbital shaker or "stirred" in 100-mL Wheaton Celstir vessels. The temperature of cultures was 37 °C and the atmosphere 5% CO_2 in air. Growth kinetics were similar in static and agitated cultures, the cells growing exponentially to a maximum cell density of (9–12) × 10^5 cells/mL.

Incorporation of [^3H]Leucine. The methods used were similar to those originally described by Melchers (1970) and Parkhouse (1971). Samples of 10^7 cells were harvested, washed twice with serum-free RPMI 1640 medium minus leucine (GIBCO), resuspended in 2 mL of the same medium with added [^{3}H]leucine (56 Ci/mmol from Amersham) at 125 μCi/mL (2 μM), and incubated for 4 h at 37 °C. The suspension was centrifuged at 600*g* for 10 min, and the supernatant fraction or culture medium was further clarified by centrifugation at 4500*g* for 10 min. This supernatant was made 100 mM in iodoacetamide (Sigma), 1 mM in phenylmethanesulfonyl fluoride (Sigma), and 0.5% in Nonidet P-40 (BDH) and was then centrifuged at 27000*g* for 30 min. An aliquot of 20 μL was taken for trichloracetic acid (Cl_3CCOOH) precipitation to determine total radioactivity in the medium fraction; 1-mL aliquots were taken for immunoprecipitation and gel electrophoresis. The pelleted cells were lysed by the addition of 1 mL of 0.5% Nonidet P-40 in 0.15 M NaCl, 0.05 M phosphate buffer, pH 7.6, 100 mM iodoacetamide, and 1 mM phenylmethanesulfonyl fluoride. After freezing and thawing this suspension twice, it was centrifuged at 50000*g* for 30 min. An aliquot of 10 μL was taken for Cl_3CCOOH precipitation; 0.5-mL aliquots were taken for immunoprecipitation and gel electrophoresis.

Immunoprecipitation. The synthesis of immunoglobulin protein was estimated by the double-antibody precipitation or the "sandwich" technique using combinations of either rabbit anti-human immunoglobulin serum plus goat anti-rabbit immunoglobulin serum or goat anti-human immunoglobulin serum plus donkey anti-goat immunoglobulin serum (Miles). Anti-human immunoglobulin serum was specific for IgM, IgG, or purified μ or λ chains. The mixtures of anti-IgM plus anti-IgG were used as the first antibody in the initial screening experiments (Figures 1 and 2). In a typical experiment, 6 μL of anti-human serum at 1 mg/mL was added to 0.5–1.0 mL of the cell lysates or medium fractions and incubated for 15–30 min on ice with occasional shaking. The appropriate second antiserum, about 50 μL at 10 mg/mL, was then added and incubated for a further 30–45 min on ice. The suspensions were layered onto a discontinuous gradient of 50 μL of 0.5 M sucrose and 100 μL of 1 M sucrose, 0.5% Nonidet P-40, in 0.14 M NaCl, and 0.05 M phosphate buffer, pH 7.6, in polypropylene microfuge tubes (Beckman) and centrifuged at 10000*g* for 5 min. The tubes were then frozen in liquid N_2, and the tips were cut off with a razor blade. After the pellets thawed, they were inverted into 1.5-mL Eppendorf tubes and centrifuged at 4000*g* for 5 min. The pellets were dissolved in electrophoresis buffer, and aliquots were taken for Cl_3CCOOH precipitation.

Isolation of Microsomes. The frozen cell pellets were broken up and pulverized with a pestle and mortar under liquid nitrogen. The resulting powder was allowed to warm up to 0 °C and was suspended at 10 mL/g of cells in homogenization buffer [cf. Marcu et al. (1978)] containing 0.25 M sucrose in TKM (10 mM Tris-HCl, pH 7.5, 25 mM KCl, and 10 mM $MgCl_2$) plus 7 mM 2-mercaptoethanol, 0.2 mM cycloheximide, spermidine tetrachloride, and heparin as described below.

Two methods of cell disruption were found to be satisfactory: (1) The cells were hand homogenized in a tight-fitting glass homogenizer (40 strokes) in buffer containing 1 mg/mL heparin and 0.25 mg/mL spermidine tetrachloride. (2) The cells were disrupted by nitrogen cavitation essentially as described by Wall et al. (1977) in a homogenization buffer containing 0.25 mg/mL heparin and 0.05 mg/mL spermidine tetrachloride, using a 30-mL stainless-steel high-pressure cell (Yeda Press, Yeda).

Nuclei and cell debris were removed from the homogenates by centrifugation 2 times for 5 min at 1100*g* (3000 rpm in a Sorvall SS34 rotor). The supernatants from homogenates produced by cavitation were supplemented with heparin to a final concentration of 1 mg/mL. Microsomes were recovered from the postnuclear supernatant by centrifugation through a 5-mL 0.75 M sucrose cushion in TKM for 40 min or without a cushion for 30 min at 50000*g* (20000 rpm, SS34 rotor).

The main difference from previously published procedures is the inclusion of at least 250 μg/mL heparin during cell disruption. We found that significant degradation of polysomes or added labeled RNA occurred without the nuclease inhibitor. The nuclear lysis which would usually occur at this heparin concentration was prevented by the presence of

spermidine at the concentrations indicated above.

Isolation of RNA from Microsomes. Pellets were suspended in the RNA isolation buffer [0.1 M NaCl, 0.01 M Tris-Hcl, pH 7.5, 0.001 M EDTA, 0.2% sodium dodecyl sulfate (NaDodSO$_4$), and 500 μg/mL proteinase K (Boehringer)] and allowed to stand at room temperature for 20 min before shaking the suspension with a 1:1 phenol–chloroform mixture, equilibrated with 0.1 M NaCl, 0.01 M CH_3COONa, pH 6.0, and 0.001 M EDTA, as described by Perry et al. (1972). The final aqueous phase was precipitated with 2.5 volumes of ethanol, washed 2 times with 2 mL of 3 M sodium acetate, pH 6.0, and reprecipitated with ethanol, as described by Palmiter (1974), before dissolving in water. The final yield of RNA was 0.5 mg/g of cells.

Isolation of RNA from Cells. RNA was isolated directly from cells by using guanidinium thiocyanate following a procedure recommended by Dr. P. Butterworth (unpublished experiments), modified from that of Chirgwin et al. (1979). A 4 M guanidinium thiocyanate (Fluka) solution, containing 0.1 M 2-mercaptoethanol and 0.1 M potassium acetate, was adjusted to pH 5.0 with glacial acetic acid and cleared by 5 min of centrifugation at 50000g (20000 rpm, SS34 rotor). Cells pulverized as described above were added to the guanidinium thiocyanate solution (10 mL/g of cells) and homogenized for 45 s in a Virtis homogenizer at setting 40. The homogenate was layered over a 1-mL cushion of 5.7 M cesium chloride containing 0.1 M EDTA, pH 7.5, and centrifuged at 185000g (33000 rpm, SW41 rotor) at 15 °C for 18 h. After the supernatant and cushion were removed and the sides of the tubes were washed and drained, the RNA pellets were suspended in 0.5 M NaCl, 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, and 0.2% NaDodSO$_4$.

Isolation of Poly(A+) RNA by Oligo(dT)–Cellulose Chromatography. Poly(A+) RNA was isolated by oligo(dT)–cellulose chromatography [using oligo(dT)–cellulose type T3 (Collaborative Research, Waltham, MA) or T-1 (Boehringer)] essentially as described by Faust et al. (1979). Typically, 1–2% of the total RNA was recovered in the final oligo(dT)-bound fraction.

Fractionation of RNA by Sucrose-Gradient Centrifugation. Poly(A+) RNA and *Escherichia coli* ribosomal RNA (rRNA) and transfer RNA (tRNA) as size markers were analyzed in 5–30% linear sucrose gradients in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.5, 0.001 M EDTA, and 0.2% NaDodSO$_4$ and centrifuged at 20 °C for 16 h at 115000g (26000 rpm, Beckman SW41 rotor). The RNA samples in 1 mM EDTA and 0.2% NaDodSO$_4$ were heated for several minutes at 65 °C and quickly cooled before adjusting to the gradient salt and buffer concentrations. The gradients were analyzed in ISCO gradient monitor. Fractions of 0.4 mL were collected, and 2.5 μg of calf liver tRNA, 15 μL of 3 M sodium acetate, pH 6, and 1 mL of ethanol were added, and RNA was allowed to precipitate at −20 °C overnight. The RNA precipitates were collected by centriguation and washed 3 times with 95% ethanol before being dried and dissolved in 20 μL of water. A 1-μL aliquot of each fraction was used in the translation assay.

Messenger RNA Translation in Vitro. The RNA was translated with the mRNA-dependent rabbit reticulocyte lysate essentially as described by Pelham & Jackson (1976). Samples were translated in a final volume of 12.5 μL or multiples of this volume, containing 5 μL of the lysate, up to 2.0 μg of the total or polysomal RNA, and either 3 μCi or L-[^{3}H]leucine (135 Ci/mmol, Amersham) or 11.5 μCi of [^{35}S]methionine (1400 Ci/mmol, Amersham) plus a mixture of the other 19 amino acids, each at 100 μM concentration. The translation mixture also contained 10 mM *N*-2-(hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (Hepes), pH 7.5, 10 mM creatine phosphate, 80 mM KCl or 100 mM potassium acetate, 0.3 mM spermidine tetrachloride, and 0.4 mM ATP. Creatine phosphokinase (50 μg/mL) and hemin (100 μM) were added to the lysate before micrococcal nuclease treatment as described by Pelham & Jackson (1976). Translations were carried out for 60 min at 37 °C. Incorporation into hot trichloroacetic acid precipitable material was assayed by spotting 1–2-μL aliquots onto 3 MM filter papers (Whatman) as described by Mans & Novelli (1961).

Immunoprecipitation of Translation Products. Specific antigen–antibody complexes were precipitated with *Staphylococcus aureus* Cowan I (Pansorbin, Calbiochem) as described by Kessler (1975). Indirect immunoprecipitation with a second antibody was also used in some experiments. Immunoglobulin G fractions of rabbit sera raised against human IgG, IgM, and κ- and λ-light chains and swine anti-rabbit IgG sera were supplied by DAKO (Copenhagen, Denmark).

Aliquots (10–20 μL) of lysate were diluted into about 100 μL of NET buffer (0.15 M NaCl, 0.05 M Tris-HCl, 0.005 M EDTA, pH 7.4, and 0.02% sodium azide) containing, in addition, 0.05% Nonidet P-40, either 1 mM methionine or 1 mM leucine (the same as the radiolabeled amino acid), 1 mM phenylmethanesulfonyl fluoride (PMSF), and 50 mM iodoacetamide and centrifuged for 10 min at 27000g (15000 rpm, SS34 rotor) to remove aggregated material.

The samples were pretreated with 10 μL of the 10% *Staphylococcus aureus* suspension by incubation for 15 min at 0 °C, followed by centrifugation for 30 s in an Eppendorf centrifuge to remove the bacteria. Immunoprecipitation was then carried out by incubation with 0.2–1.0 μL of the appropriate antibody for 30–60 min at 0 °C, followed by the addition of 10 μL of the *Staphylococcus aureus* suspension and, after a 15-min incubation, collection of the immune complexes bound to the bacteria by centrifugation as described above. The supernatants were retained for further immunoprecipitation, and polypeptide chains were liberated by boiling the bacteria in 20 μL of protein electrophoresis loading buffer (Laemmli, 1970).

Radioactivity in immunoprecipitates was determined by Cl_3CCOOH precipitation onto GF/C filters as described by Pelham & Jackson (1976) except that the products were eluted from the filters using Soluene 350 (Packard) before liquid scintillation counting. Total Cl_3CCOOH-precipitable protein in the lysate was similarly determined in order to estimate the percentage of immunoprecipitable protein synthesized in vitro.

Total translation products and immunoprecipitates were analyzed by electrophoresis on 10% acrylamide gels as described by Laemmli (1970). Electrophoresis was for 2–3 h at 25–35 mA in approximately 1-mm-thick gels. After electrophoresis, the gels were fixed and prepared for fluorography as described by Bonner & Laskey (1974) or by using ENHANCE (New England Nuclear) according to the manufacturer's instructions and exposed at −70 °C on preexposed Kodak X-Omat X-ray film (Laskey & Mills, 1975).

Results

Cellular Synthesis and Secretion of Immunoglobulin Chains. Immunoglobulin chains synthesized in the eight human lymphoblastoid cell lines examined in this study were identified and quantified by specific immunoprecipitation and gel electrophoresis, as described under Materials and Methods and in the figure and table legends. The electrophoretic profiles of the labeled immunoglobulin chains in cell lysates

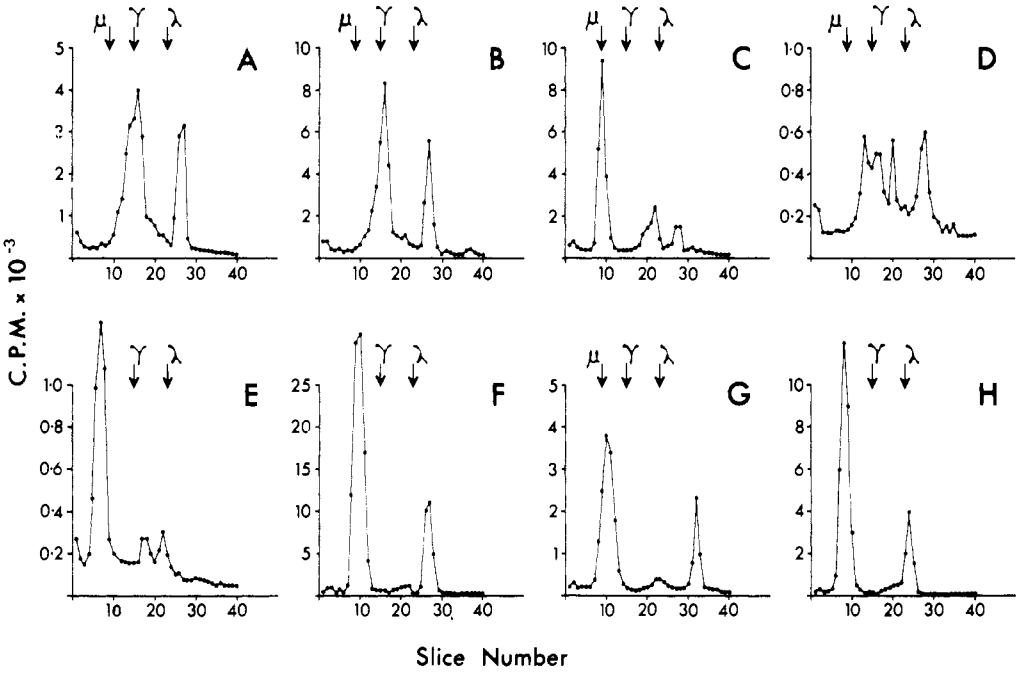

FIGURE 1: Identification of immunoglobulin chains produced by eight human lymphoblastoid cell lines in tissue culture. Cells were grown to the mid-log phase in nonagitated suspension culture in fetal calf serum, and 10^7 cells were then incubated with [^{3}H]leucine, proteins were isolated and analyzed by immunoprecipitation with equal parts of rabbit anti-IgG and anti-IgM plus goat andi-rabbit immunoglobulin, as described under Materials and Methods. Radioactivity profiles are shown for (A) BEC 11, (B) BRI 7, (C) BRI 8, (D) MICH, (E) DAUDI, (F) SMI, (G) TAY, and (H) RPMI 1788. The positions of radioiodinated immunoglobulin chain markers are indicated by arrows. Electrophoresis was from left to right.

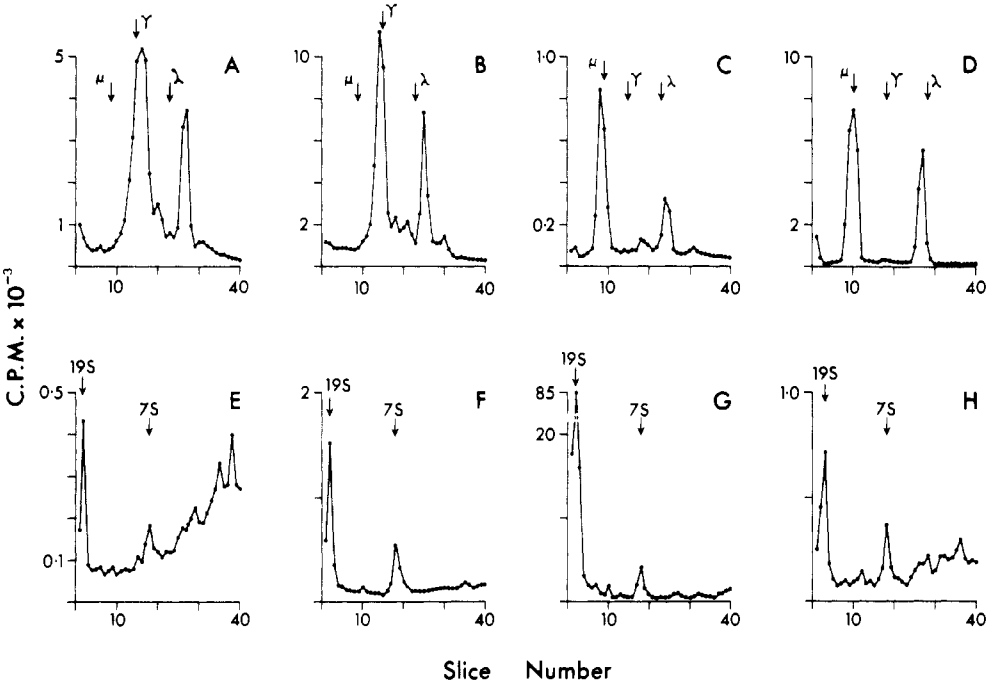

FIGURE 2: Identification of immunoglobulin proteins secreted by human lymphocytes in tissue culture. Proteins from the media of cells incubated as described in the legent to Figure 1 were analyzed by gel electrophoresis. In addition to the usual electrophoresis in10% gels of reduced and alkylated samples (see Materials and Methods), untreated samples were run on 4% polyacrylamide gels. Reduced samples of (A) BEC 11, (B) BRI 7, (C) RPMI 1788 and (D) SMI and unreduced samples of (E) BRI 8, (F) RPMI 1788, (G) SMI, and (H) TAY are shown, together with the positions of marker immunoglobulin chains (A–D) and 19S and 7S IgM.

and the culture media are shown in Figures 1 and 2, respectively. These results reveal that SMI 4 (Figures 1F and 2D), RPMI 1788 (Figures 1H and 2C), and BRI 8 (Figure 1C) produce μ-heavy and λ-light chains, BRI 7 (Figures 1B and 2B) and BEC 11 (Figures 1A and 2A) and probably MICH (Figure 1D) produce γ-heavy and κ-light chains; and TAY 3 (Figure 1G) produces μ-heavy and κ-light chains. The electrophoretic profiles of the unreduced immunoglobulins from BRI 8, RPMI 1788 SMI 4, and TAY 3 (Figure 2) reveal that the secreted immunoglobulins are assembled into complete (7–8S) protein and that the IgM is predominantly in the pentameric form. A breakdown of the chain synthesis and secretion as a percentage of total protein synthesis and secretion is presented in Table II. Here, it is seen that immunoglobulin synthesis comprised proportions of the total protein synthesis varying from less than 2% (MICH) up to

Table II: Levels of Immunoglobulin Synthesis by Human Lymphoblastoid Cell Lines

cell line	Ig chains[a]	total protein synthesis[b] [cpm × 10^{-5} (4 h)$^{-1}$ (5×10^6 cells)$^{-1}$]			Ig protein synthesis[c] [cpm × 10^{-5} (4 h)$^{-1}$ (5×10^6 cells)$^{-1}$]			% secreted/4 h[d]		% Ig synthesis[e]	% Ig in medium[f]
		cells	medium	total	cells	medium	total	Ig	non-Ig		
SMI 4	$\mu + \lambda$	471	80	551	58	51	109	47	7	20	63
RMPI 1788	$\mu + \lambda$	256	14	270	25	3.2	28	11	4	11	23
BEC 11	$\gamma + \kappa$	231	20	252	10	5.5	15	36	7	6	28
DAUDI	$\mu + \lambda$	100	5	105	6.1	0.4	6.5	6	5	6	8
BRI 7	$\gamma + \kappa$	347	40	387	10	20	20	50	8	5	25
BRI 8	$\mu + \lambda$	338	28	366	15	3.5	19	19	4	5	13
TAY	$\mu + \kappa$	373	15	388	14	1.9	16	12	3	4	13
MICH	$\gamma + \kappa$	223	13	236	3	1	4	25	5	2	8

[a] Identified from electrophoretic profiles (see Figures 1 and 2). [b] Estimated from Cl_3CCOOH-precipitable radioactivity in fraction. [c] Estimated from Cl_3CCOOH-precipitable radioactivity in anti-Ig precipitates of fractions. [d] 100 × (Ig or non-Ig in medium total Ig or non-Ig). [e] 100 × (Ig protein synthesis/total protein synthesis). [f] 100 × (Ig in medium/total protein in medium).

Table III: Levels of Immunoglobulin Synthesized by RPMI 1788 in Different Tissue Culture Conditions

culture[a]	growth phase[b]	total protein synthesis[c] [cpm × 10^{-6} (4 h)$^{-1}$ (5×10^6 cells)$^{-1}$]			anti-Ig[d]	Ig protein synthesis[e] [cpm × 10^{-6} (4 h)$^{-1}$ (5×10^6 cells)$^{-1}$]			% Ig secreted[f]	% Ig synthesis[g]
		cells	medium	total		cells	medium	total		
NASC	early log	45.6	8.1	51.7	μ	6.2	3.3	9.5	35	18
					λ	6.8	2.6	9.4	28	17
NASC	late log	55.8	12.1	67.9	μ	6.3	6.1	12.4	49	18
					λ	6.1	3.2	9.3	34	14
SC	early log	20.6	2.2	22.8	μ	1.5	0.8	2.3	34	10
					λ	2.3	0.7	3.0	34	10
SC	late log	57.8	14.1	71.9	μ	4	8	12	67	17
					λ	5	4.5	9.5	47	13

[a] NASC = nonagitated suspension culture; SC = spinner culture. [b] Early exponential = 3.4×10^5 (NASC) and 3.5×10^5 (SC) cells/mL; late exponential = 7.1×10^5 (NASC) and 6.5×10^5 (SC) cells/mL. [c] Estimated from Cl_3CCOOH-precipitated radioactivity in fraction. [d] μ = anti-IgM; λ = anti-λ-antiserum. [e] Estimated from Cl_3CCOOH-precipitable radioactivity in anti-Ig precipitates of fractions. [f] 100 × (Ig in medium/total Ig). [g] 100 × (Ig protein synthesis/total protein synthesis).

20% (SMI 4). About 3–8% of the nonimmunoglobulin protein synthesized in the 4-h labeling period was secreted into the medium. In contrast, proportions of immunoglobulin varying between this base level (around 5% for DAUDI) and 50% of the total (SMI 4 and BRI 7) were secreted from the cells. This represented between 8 and 63% of the total secreted protein.

In further experiments with RPMI 1788 and BRI 7, the cells were cultured in swine serum instead of fetal calf serum. Swine serum was initially chosen because it was found to be equally effective in supporting cell growth in the case of RPMI 1788 (doubling time 24 h) at one-fifth of the usual serum concentration (2%, as against 10% v/v) and one-tenth of the cost of fetal calf serum (Birch et al., 1979). This effect was not seen with all the cell lines examined; e.g., SMI 4, which requires 20% fetal calf serum for optimal growth, does not grow well in swine serum. In the case of RPMI 1788, the substitution of swine serum for fetal calf serum stimulated total protein synthesis by a factor of four, immunoglobulin synthesis by a factor of five, the representation of IgM on the membrane surface at least 10-fold, and the rate of immunoglobulin secretion by a factor of eight (results not shown).

The tissue culture conditions and the time of harvesting the cells may also affect the specificity of cellular protein synthesis in human lymphoblastoid cells, as shown in Table III. The proportion of immunoglobulin synthesis is higher in cells harvested in the late-exponential, as compared with the early exponential, phase of growth, particularly in the case of cells grown in the highly aerated spinner cultures, as compared with static cultures. More detailed studies have revealed that RPMI 1788 cells synthesize an excess of λ-light chains but secrete only complete immunoglobulin protein (Cramer, 1977). The high proprotion, quantitatively estimated to be around 18%, of immunoglobulin synthesis, relative to total protein synthesis, is reflected qualitatively in the prominence of peaks in the profile of total protein synthesis, which correspond to the heavy and light immunoglobulin chains (Figure 3). We return to this point.

Isolation of Messenger RNA and Translation in Vitro. (*a*) *Translation of BRI 7 Messenger RNA.* Poly(A+) RNA was isolated from either total RNA or microsomal RNA, prepared as described under Materials and Methods, and translated in vitro. Comparison of the translation products by gel electrophoresis (Figure 4A, tracks 2 and 12) reveals a qualitatively similar pattern (more evident after a shorter exposure of track 12 than shown in Figure 4A). In each case, immunoprecipitation with anti-IgG leads to the appearance of a product (tracks 4 and 9) that migrates slightly faster than the γ chains synthesized in the cell (track 7). The κ and γ bands are much weaker or absent in the control immunoprecipitations with *Staphylococcus aureus* alone (tracks 3 and 11) or following precipitation with a heterologous (anti-IgM) antibody (tracks 6 and 8). The small amount of κ chain precipitated with anti-IgM probably reflects the recognition of light-chain determinants by this antiserum (cf. the precipitation of λ chains from RPMI 1788 by anti-IgM shown in Figure 5C). The minor bands accompanying γ chains, observed in tracks 8–11, are presumably contaminants because they are of equal intensity in samples precipitated with anti-IgG and the heterologous antisera.

(*b*) *Translation of RPMI 1788 Messenger RNA.* The corresponding results for RPMI 1788 are shown in Figure 4B. Immunoprecipitation of the total translation products (Figure 4B, lane 1) with anti-IgM (lane 3) reveals an intense band at a mobility corresponding to 65 000 daltons, which is not present

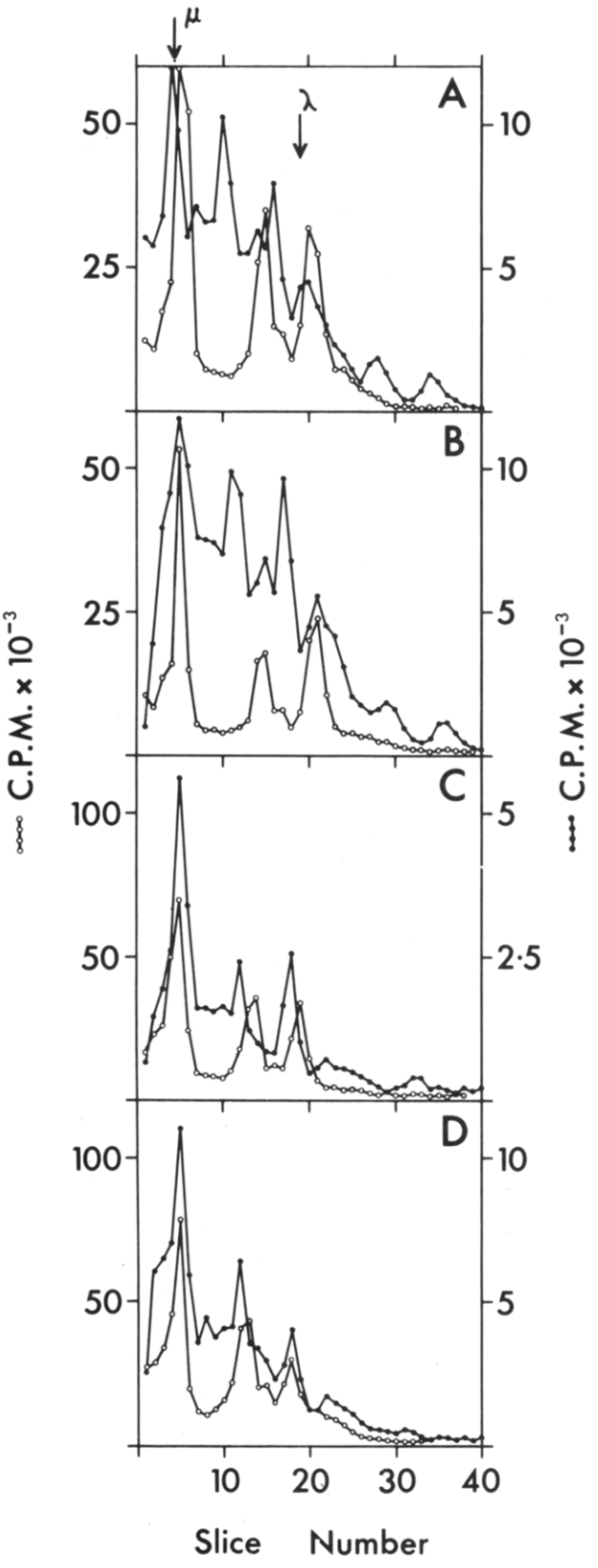

FIGURE 3: Qualitative analysis of polypeptide chains precipitated with antiimmunoglobulin antiserum. RPMI 1788 was grown in nonagitated suspension culture (NASC) or in spinner culture (SC) to the late phase of growth; then 2.5×10^5 cells were harvested and incubated under standard conditions with [^{3}H]leucine. Gel profiles of Cl_3CCOOH precipitates of total protein (●) and protein immunoprecipitated with rabbit anti-human IgM plus goat anti-rabbit IgG from the cell lysate fraction (○) of (A) SC-grown and (B) SC-grown RPMI 1788 and the medium fractions of (C) NASC-grown and (D) SC-grown RPMI 1788 are shown.

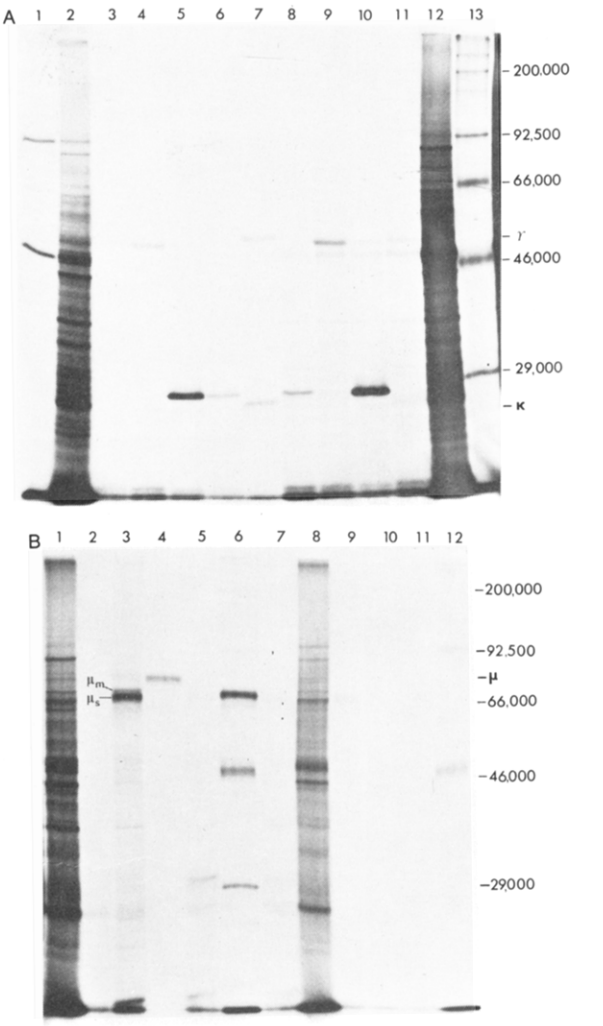

FIGURE 4: Translation of Bristol 7 and RPMI 1788 mRNAs. mRNA was translated in the reticulocyte lysate in the presence of [^{35}S]-methionine, and the product was analyzed by polyacrylamide gel electrophoresis as described under Materials and Methods. Aliquots of 1 μL of the lysate were loaded in the tracks showing total translation products, 10 μL was immunoprecipitated, and the entire immunoprecipitate was loaded on gel. (A) Bristol 7 RNA. (Tracks 2–6) Translation of poly(A+) RNA isolated from the membrane-bound polysomes, giving 30 800 cpm/μL above background. (Tracks 8–12) Translation of poly(A+) RNA from total RNA isolated by the guanidinium thiocyanate procedure, giving 73 000 cpm/μL above background. (1) Translation without added RNA; (2) total translation products; (3–11) immunoprecipitates with (3) *Staphylococcus aureus* alone, (4) anti-IgG, (5) anti-κ, (6) anti-IgM, (7) [^{3}H]leucine-labeled product of cells, secreted into the medium and precipitated with anti-IgG, (8) anti-IgM, (9) anti-IgG, (10) anti-κ, and (11) *Staphylococcus aureus* alone; (12) total translation product; (13) ^{14}C-labeled molecular weight standards, listed in Table IV, ca. 4×10^{-4} μCi/protein. The mobilities of the γ and κ chains labeled in the intact cell are indicated. (B) RPMI 1788 RNA. (Tracks 1–3, 5, and 7) Translation of poly(A+) RNA isolated from microsomes, giving 35 000 cpm/μL above background; (Tracks 8–11) poly(A−) RNA from the same microsomes, giving 11 400 cpm/μL above background. (1) Total translation products; immunoprecipitates with (2) *Staphylococcus aureus* alone, (3) anti-IgM, (5) anti-λ, and (7) anti-IgG; (4) [^{3}H]leucine-labeled product of cells, isolated from the cell lysate and precipitated with anti-IgM; (6) ^{14}C-labeled molecular weight standards (Table I); (8) total translation product; immunoprecipitates with (9) anti-IgM, (10) anti-λ, and (11) anti-IgG; (12) translation product with no mRNA added. The mobility of the μ chain labeled in the intact cell is indicated.

in the control immunoprecipitates with *Staphylococcus aureus* alone (lane 2) or with anti-IgG (lane 7). We also observed a minor component of 67 400 daltons. This minor component was routinely observed, and evidence is given below to suggest

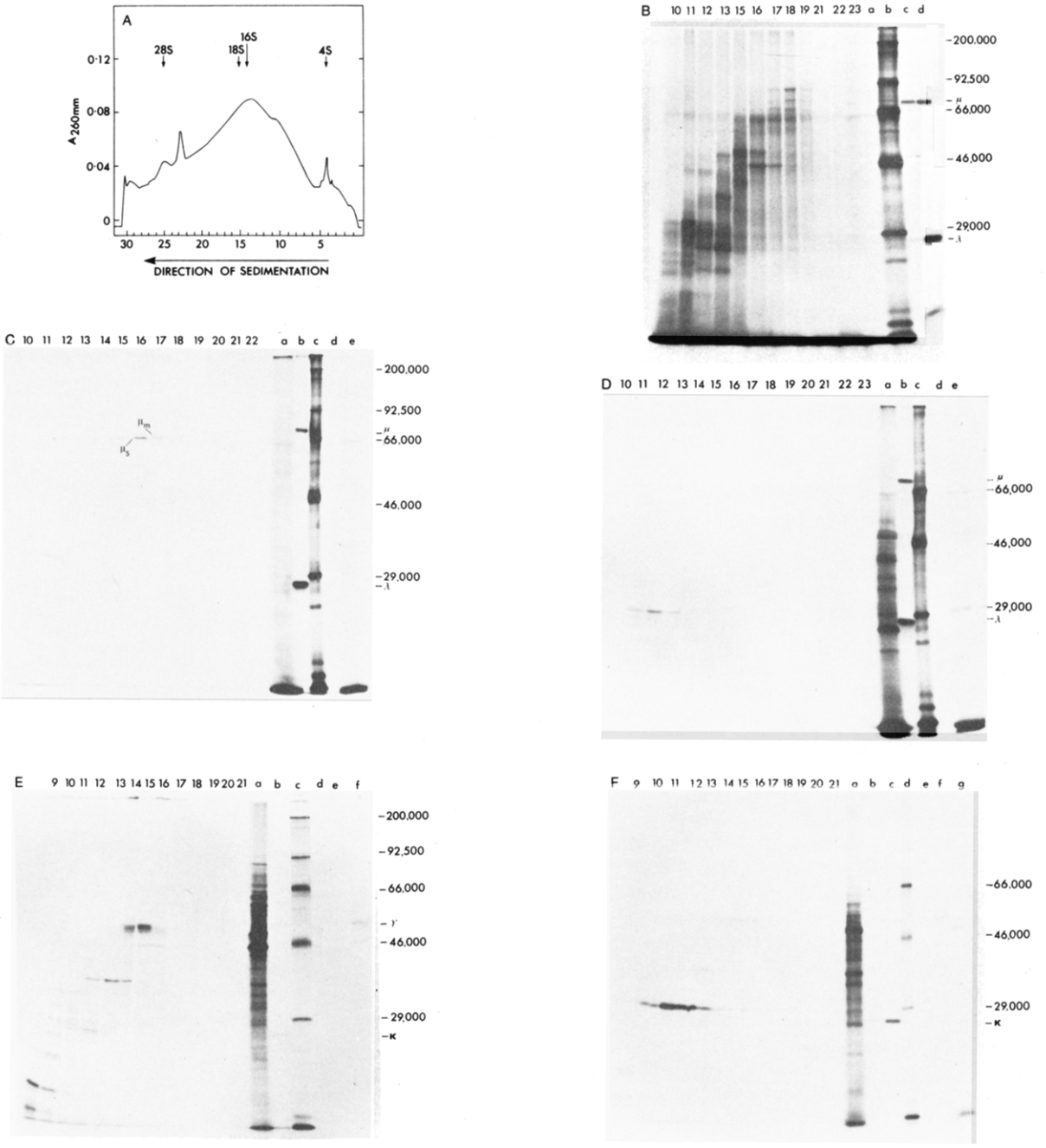

FIGURE 5: Sucrose-gradient analysis of RPMI 1788 and Bristol 7 mRNA. (A) Absorbance profile (A_{260}) of 23 μg of RPMI 1788 poly(A+) RNA fractionated as described under Materials and Methods, with positions of rRNA and tRNA standards indicated. (B) Total translation products of gradient fractions in (A) using [^{3}H]leucine as label, with 2000 cpm/μL incorporation above background and 2 μL loaded in each track. Gel tracks are labeled to correspond with gradient fractions 10–23, excluding fractions 14 and 20, presented instead in parts C and D of Figure 3, respectivel]: (a) lysate with no RNA added; (b) ^{14}C-labeled molecular weight standards (Table I); (c) anti-IgM precipitate of cell lysate after incubation of cells with [^{3}H]leucine; (d) anti-λ precipitate of same cell lysate as in (c). (C) Immunoprecipitate with anti-IgM of translation products, 9 μL of each lysate, of RPMI 1788 sucrose gradient shown in (s), tracks 10–23 numbered to correspond to the gradient and to patterns of the total product shown in (B): (a) 2 μL of total translation product of gradient fraction 20; (b) anti-λ precipitate of cell lysate of [^{3}H]leucine-labeled cells; (c) ^{14}C-labeled molecular weight standards (Table I); (d) anti-IgG precipitate of translation products of gradient fraction 20; (e) total translation product of lysate with no RNA added. (D) Immunoprecipitate with anti-λ of translation products, 9 μL of each lysate, of RPMI 1788 sucrose-gradient fractions (A), tracks 10–23 numbered to correspond to the gradient and to patterns of the total product shown in (B): (a) total translation products of gradient fraction 14; (b) immunoprecipitate of cell lysate of [^{3}H]leucine-labeled cells; (c) ^{14}C-labeled molecular weight standards (Table I); (d) anti-IgG precipitate of translation products of fraction 14; (e) total translation products of lysate with no RNA added. (E) Immunoprecipitate with anti-IgG of translation products, 5 μL of each lysate, of sucrose-gradient fractions of 28 μg of poly(A+) total cellular RNA from Bristol 7, tracks 9–21 numbered to correspond to gradient. RNA was fractionated in parallel with RPMI 1788 RNA shown in (A), and labeled amino acid incorporation was ~30000 cpm/μL above background in peak fractions: (a) total translation products of fraction 16; (b) anti-IgG precipitate of cell lysate of [^{3}H]leucine-labeled Bristol 7 cells; (c) ^{14}C-labeled molecular weight standards (Table I); (d) anti-IgM precipitate of fraction 16; (e) *S. aureus* precipitate of fraction 16; (f) total translation product of lysate with no mRNA added. (F) Immunoprecipitate with anti-κ of translation products, 5 μL of each lysate, of sucrose-gradient fractions described in (E), tracks 9–21 numbered to correspond with gradient fractions: (a) total translation products of sample 14; (b) anti-IgG and (c) antiprecipitate of cell lysate of [^{3}H]leucine-labeled Bristol 7 cells; (d) ^{14}C-labeled protein molecular weight standards (Table I); (e) anti-IgM precipitate of fraction 14; (f) immunoprecipitate with *S. aureus* alone; (g) total translation products of lysate with no mRNA added.

Table IV: Apparent Molecular Weights of Immunoglobulin Chains Produced by Translation of mRNA in RPMI 1788 and Bristol 7 Cells and in a Cell-Free System

	mol wt[a]				
	γ	κ	μ(1)	μ(2)	λ
cell product	52 700 ± 200 (4)	27 260 ± 600 (7)	74 100 ± 800 (7)		28 100 ± 300 (6)
cell-free system product	51 000 ± 300 (2)	28 650 ± 250 (7)	65 500 ± 850 (6)	67 400 ± 650 (3)[b]	31 100 ± 650 (4)
difference in M_r	1 700 ± 500	1 400 ± 900	8 500 ± 1200		3 000 ± 950
% difference in M_r	3		11		
difference in amino acids		13			27

[a] Molecular weights were determined from the electrophoretic mobility in polyacrylamide gels containing $NaDodSO_4$ (see Materials and Methods) with reference to a calibration curve obtained with ^{14}C-methylated markers (Amersham), assuming the following molecular weights: myosin, 200 000; phosphorylase *b*, 92 500; bovine serum albumin, 66 210; ovalbumin, 46 000; carbonic anhydrase, 29 000. Results are presented as mean ± SD (number of measurements). [b] Minor component (see text).

that is is the translation product of a minor μ-chain mRNA, of a slightly larger size (19 S) than the main (18S) species (Figure 5C).

We identify the most intense band in track 5 as the λ chain, as it is not present in the control immunoprecipitations (lanes 2 and 7) and its apparent molecular weight (31 000) is consistent with that of the λ chain synthesized in vivo. More definitive results were obtained in experiments using [^{3}H]-leucine to label the λ chain (Figure 5D). Methionine is often absent from the mature λ chain (Dayhoff, 1972), and therefore [^{35}S]methionine, as used for the experiment shown in Figure 4, may label only the N-terminal precursor peptide.

Molecular Weights of the Translation Products. The molecular weights of the four immunoglobulin chains, synthesized in vivo and in vitro, were measured from the electrophoretic mobility, with reference to a calibration curve made from the proteins of known molecular weight, and the results are summarized in Table IV. The greater size of the in vitro products of light-chain messenger RNA is consistent with the presence of the 20 amino acid N-terminal peptide that is normally cleaved off in vivo, judging from the earlier results on the mouse system (Burstein & Schechter, 1977a,b). The smaller size of the in vitro products of heavy-chain messenger RNA is consistent with the degree of carbohydrate modification in vivo, 3% of the mass for γ chains and 11% for μ chains (Nisonoff et al., 1975), which retards the polypeptide chain more than the 20 amino acid precursor peptide (Jilka & Pestka, 1977; Early et al., 1980a) retained on the in vitro product.

Fractionation of Heavy- and Light-Chain Messenger RNAs. The size distribution of RPMI 1788 poly(A+) mRNA, analyzed by sucrose-gradient centrifugation, is shown in Figure 5A. Immunoprecipitation of the translation products of the individual gradient fractions (Figure 5B) with anti-IgM (Figure 5C) and anti-λ chain (Figure 5D) antisera reveals the activities of heavy- and light-chain messenger RNAs. The μ-chain mRNA can be resolved into two components, a major one at 18 S and a minor one at 19 S in the gradient, and the λ-chain mRNA sediments at about 12 S.

A sample of BRI 7 poly(A+) mRNA was analyzed in an identical parallel gradient in the same experiment, and the result of the immunoprecipitation of the translation products of the gradient fractions is shown in Figure 5E,F. The strongest γ-chain precipitate (track 15, Figure 5E) and the strongest κ-chain precipitate (tracks 11 and 12, Figure 5F) occur at approximately 16 S and 12–13 S, respectively. Traces of κ chain are detected over a wide region of the gradient due to the intensity of labeling. The numerous products smaller than γ chains in tracks 9–13 of Figure 5E are due to nonspecific precipitation. This sometimes occurred because of the relatively poor yields of the γ chains, as compared, for example, to those of the κ chains, making background contamination more significant.

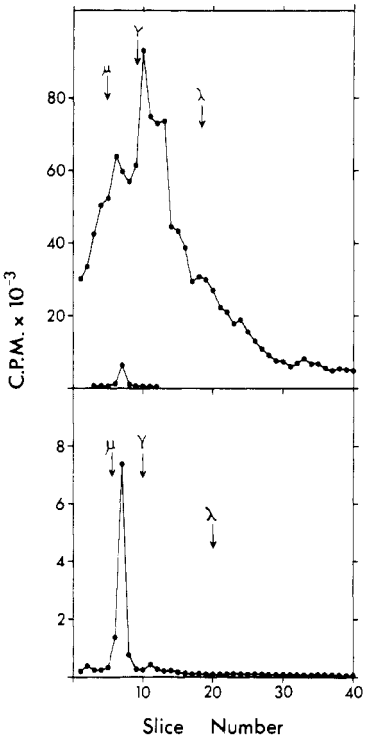

FIGURE 6: Messenger activity of RNA isolated from RPMI 1788 cells. RPMI 1788 cells were grown in spinner cultures in swine serum supplemented medium up to the late-exponential phase, and messenger RNA was extracted from microsomes, purified by oligo(dT)–cellulose chromatography, and translated in a reticulocyte lysate with a labeled amino acid precursor ([^{3}H]leucine). The translation products were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis before and after immunoprecipitation with antiserum against IgM. The total translation product is shown in the upper panel, and the specifically precipitated IgM is shown on an expanded scale in the lower panel, and replotted on the original scale in the upper panel (lower curve). Summation of the counts in the two curves shows that μ-chain messenger RNA represents about 0.1% of the total messenger activity in the isolated RNA.

Purification of Immunoglobulin Messenger RNA from Human Lymphoblastoid Cells. In the unfractionated mRNA, immunoglobulin mRNA represents about 0.1% of the total activity in the cell lines we have studied. This can be seen by comparing the radioactivity profiles of total protein synthesis and immunoglobulin synthesis, as for RPMI 1788 in Figure 6. About 1% of the total labeled protein coded by the peak fractions of the Bristol 7 mRNA gradient can be precipitated with specific antisera against IgG and κ chains. This corresponds to about 0.1–0.2% for the sum of γ plus κ in the total translation product, estimated from the sum of the counts for total protein synthesis across the gradient. Sucrose-gradient

centrifugation thus results in a 10-fold purification of the immunoglobulin messenger RNAs.

Discussion

Immunoglobulin Synthesis in Vivo. The synthesis of one class of heavy chain (μ or γ) and one type of light chain (λ or κ) by each of the eight human lymphoblastoid cell lines surveyed in this study (Figures 1 and 2) is consistent with the presumed monoclonal origins (Steel et al., 1971; Fialkow et al., 1973; Bêchet et al., 1974). Our results are also consistent with previous qualitative studies of immunoglobulin chain synthesis by some of the cell lines (Steel et al., 1971) and with previous studies demonstrating that the cell line DAUDI is a "nonsecretor" (Klein et al., 1968; Sherr et al., 1971; Evans et al., 1974).

In this study, we have shown that the different human lymphoblastoid cell lines examined, grown in standard conditions, exhibit widely varying capacities for immunoglobulin synthesis and secretion (Table II). The rate of synthesis extends from 2 to 20% of the total protein synthesis, and the rate of secretion also showed a 10-fold variation. For the purpose of messenger RNA purification, the choice of cell lines relatively active in immunoglobulin synthesis would be expected to be advantageous. However, the expected agreement between cellular immunoglobulin synthesis and immunoglobulin messenger RNA activity in vitro was not upheld by our results.

It may be calculated from the results presented here that one of the more active lines, RPMI 1788, grown in swine serum and harvested in the late-exponential phase to optimize immunoglobulin production, synthesizes 70 000 molecules of IgM cell^{-1} h^{-1}. We have calculated from the earlier results of Watanabe and co-workers that the same cell line produced 130 000 molecules of IgM cell^{-1} h^{-1} in their hands (Watanabe et al., 1973). The production of about 10^5 molecules IgM cell^{-1} h^{-1} thus seems to have been a fairly stable characteristic of this cell line between 1973 and 1979. This large capacity for immunoglobulin production is not unusual for human lymphoblastoid cell lines, as shown by previous authors (Hutteroth et al., 1973; Nilsson, 1978).

Previous quantitative surveys of immunoglobulin synthesis in lymphocyte culture have mainly been limited to mouse systems, either normal B lymphocytes grown in short-term culture or mouse myelomas. Melchers and co-workers have estimated the rate of immunoglobulin synthesis by normal mouse B lymphocytes in short-term tissue culture. This cell population is heterogeneous, in respect to both clonal origin and stage of cell differentiation. The bulk of relatively immature, small B lymphocytes may in fact be physically separated from the minority of mature, large B lymphocytes. The latter are about 10 times more active in immunoglobulin synthesis than the former, producing 10^5 molecules of IgM cell^{-1} h^{-1} (Andersson et al., 1974a). However, the small B lymphocytes can evidently be stimulated to increase their immunoglobulin production in culture to the same level by either concanavalin A (Andersson & Melchers, 1973) or bacterial lipopolysaccharide (Melchers & Andersson, 1974a,b). The rates of immunoglobulin synthesis and secretion by the more active human cell lines examined in this study are similar to the levels in mitogen-stimulated mouse B lymphocytes in short-term culture (Melchers & Andersson, 1974b).

For the mouse myeloma lines, those synthesizing IgG are generally more active than those synthesizing IgM. The IgM lines, like our human lymphoblastoid cell lines, show a 10-fold variation in the output of IgM, reaching a maximum level of about 10^5 molecules cell^{-1} h^{-1} (Andersson et al., 1974b). The IgG lines reach levels an order of magnitude higher, around 10^6 molecules cell^{-1} h^{-1} (Baumal & Scharff, 1973). None of the IgG-secreting human lymphoblastoid cell lines were as active as this. The activity in protein synthesis can be rationalized in terms of cell morphology, which is in turn correlated with the state of cell differentiation. The electron micrograph of a typical RPMI 1788 cell shown in Figure 7 reveals the "immature" characteristics of this cell line, notably its large nucleocytoplasmic ratio and dispersed chromatin, its poorly developed endoplasmic reticulum, its irregular cell shape and its pseudopodia (Nilsson, 1971, 1978). Consistent with the activity in protein synthesis is the low yield of microsomal RNA from RPMI 1788, 0.5 mg/g of cells, about one-third of that obtained from mouse myeloma cells [see, e.g., Marcu et al. (1978)].

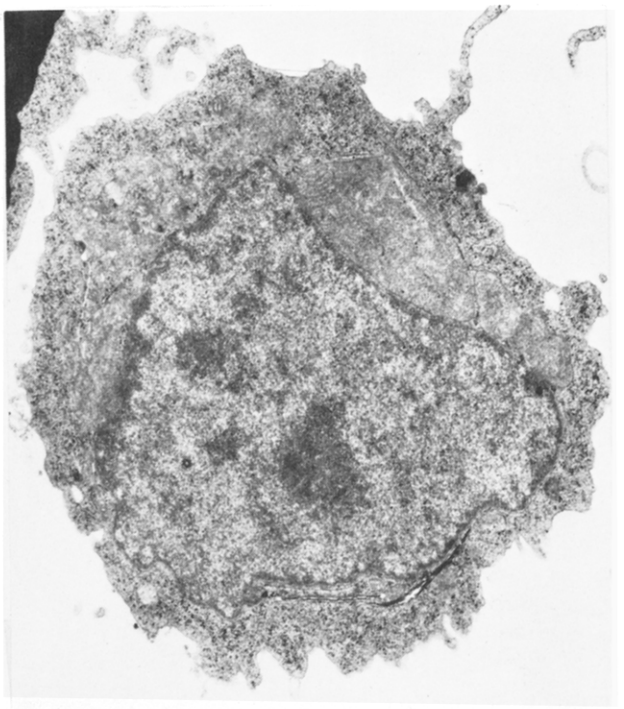

FIGURE 7: Electron micrograph of RPMI 1788 cells. Electron micrograph of RPMI 1788 cells at magnification 11.780×. Cells were harvested at late-exponential phase from a medium containing fetal calf serum. Cells grown in swine serum were similar in appearance.

Immunoglobulin Synthesis in Vitro. No systematic study of immunoglobulin mRNA from any source but mouse myeloma has previously been made. Our results on the human lymphoblastoid cell lines must perforce be compared with those on mouse myelomas.

Neither the size of the human immunoglobulin mRNAs nor the size of their translation products in vitro, found in this study, differed in any significant way from the counterparts in the mouse. Our resolution of two μ-chain mRNAs from RPMI 1788 may likewise correspond to the membrane and secreted forms of this immunoglobulin chain found in mouse lymphocytes, the larger, membrane from arising in less differentiated cells and differing from the smaller, secreted form in mature lymphocytes at the C-terminal end (Alt et al., 1980; Early et al., 1980b). Previous authors (Singer et al., 1980) have observed that DAUDI, a nonsecretor, synthesizes mainly a 67 000-dalton μchain, whereas RPMI 1788, an active IgM secretor, synthesizes mainly a 65 000-dalton μ chain. The presumptive membrane form of the μ chain is thus more prominent also in human lymphoblastoid cells of a less mature lineage. In fact, the earlier authors failed to detect it in RPMI 1788, consistent with our evidence that it is a relatively minor

product. From the approximate sedimentation coefficients of 18 and 19 S, using the equation $s^0_{20,w} = 0.02M^{0.5}$ (McPhie et al., 1966), we have calculated a difference of about 300 nucleotides in the size of the two human μ-chain mRNAs. This is larger than necessary to account for the difference of about 25 amino acids in the lengths of the encoded μ chains (Table IV). The two mouse mRNAs also differ in length by about 300 nucleotides, while the encoded polypeptide chains differ by 20 amino acids. This is explained by the differential splicing of a common mRNA precursor, which results in mRNAs with different lengths of untranslated sequence at the 3′ termini (Alt et al., 1980; Early et al., 1980b; Rogers et al., 1980).

BRI 7 and RPMI 1788 were chosen for the isolation of human γ- and κ- and μ- and λ-chain mRNAs, respectively, because immunoglobulin synthesis in these cell lines approached the levels found in mouse myelomas, from which immunoglobulin mRNAs had previously been isolated. The apparent concentrations of the immunoglobulin mRNAs in the extracted RNA, estimated from their translational activity, were, however, 2 orders of magnitude lower than those expected from our studies of cellular protein synthesis, since the immunoglobulin chains comprised only about 0.1% of the total product in vitro, as compared with about 10% in vivo. This large discrepancy between immunoglobulin synthesis in vivo and in vitro is strikingly illustrated by Figures 3 and 6, where specific antibodies have been used to precipitate the immunoglobulins, and the resulting electrophoretic patterns have been compared with the patterns of total protein synthesis. The situation is clearly different from mouse myelomas, where the immunoglobulins comprise a major part of the translation product of the mRNA in vitro [see, e.g., Marcu et al. (1978)]. Further work is required to explain the anomalous results. After sucrose-gradient fractionation of the human messenger RNA, one obtains a 10-fold purification of the immunoglobulin mRNA, and the peak fractions, in which the heavy- and light-chain mRNAs comprise about 1% of the total activity, have been used for the synthesis of complementary DNA. Attempts to clone the DNA complementary to immunoglobulin mRNAs of a number of different human lymphoblastoid cell lines are currently in progress.

Mouse myelomas provided a convenient starting point for the isolation of the first immunoglobulin mRNA, since these cells are very active in immunoglobulin synthesis and indeed prove to contain high concentrations of the message. In contrast, methods do not yet exist for the establishment of human myelomas in culture on a routine basis, and the less mature lymphoblastoid cell lines appear to yield relatively low concentrations of immunoglobulin mRNA, judging from our results. Despite the relatively low concentrations of the mRNA, our results show that the inclusion of nuclease inhibitors in the preparation makes it possible to isolate intact heavy- and light-chain immunoglobulin mRNAs from these cells in sufficient amounts for the purpose of molecular cloning.

Recently, human immunoglobulin-secreting cell lines have been established by fusion of human lymphocytes with mouse myelomas, and the human immunoglobulin mRNAs have been isolated and used to synthesize and clone the complementary DNA (Dolby et al., 1980). As with hybridomas, lymphoblastoid cell lines can be selected for the production of specific antibodies (Steinitz et al., 1979a,b). Either hybridomas or lymphoblastoid cell lines then can provide a source of cells for the isolation of immunoglobulin mRNA and the synthesis of potentially useful genetic products.

Acknowledgments

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References

Alt, F. W., Bothwell, A. L. M., Knapp, M., Siden, E., Mather, E., Koshland, M., & Baltimore, D. (1980) *Cell (Cambridge, Mass.) 20*, 293–301.

Andersson, J., & Melchers, F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 416–420.

Andersson, J., Lafleur, L., & Melchers, F. (1974a) *Eur. J. Immunol. 4*, 181–188.

Andersson, J., Buxbaum, J., Citronbaum, R., Douglas, S., Forni, L., Melchers, F., Pervis, B., & Stott, D. (1974b) *J. Exp. Med. 140*, 742–763.

Baumal, R., & Scharff, M. D. (1973) *J. Immunol. 111*, 448–456.

Bêchet, J. M., Fialkow, P. J., Nilsson, K., Klein, G., & Singh, S. (1974) *Exp. Cell Res. 89*, 275–282.

Birch, J. R., Cramer, F., Edwards, D. J., Cartwright, T., & Gould, H. J. (1979) *Dev. Biol. Stand. 42*, 165–169.

Bonner, W. M., & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83–88.

Burstein, Y., & Schechter, I. (1977a) *Proc. Natl. Acad. Sci. U.S.A. 74*, 716–720.

Burstein, Y., & Schechter, I. (1977b) *Biochem. J. 165*, 347–354.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1969) *Biochemistry 18*, 5294–5299.

Cramer, F. (1977) Ph.D. Thesis, University of London.

Dayhoff, M. I., Ed. (1972) *Atlas of Protein Sequence and Structure*, Vol. 5, pp D254–259.

Dolby, T. W., Devuono, J., & Croce, C. M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6027–6031.

Early, P., Huang, H., Davis, M., Calame, K., & Hood, L. (1980a) *Cell (Cambridge, Mass.) 19*, 981–992.

Early, P., Rogers, J., Davis, M., Calame, K., Bond, M., Wall, R., & Hood, L. (1980b) *Cell (Cambridge, Mass.) 20*, 313–319.

Evans, J., Steel, M., & Arthur, E. (1974) *Cell (Cambridge, Mass.) 3*, 153–158.

Faust, C. H., Heim, I., & Moore, J. (1979) *Biochemistry 18*, 1106–1119.

Fialkow, P. J., Klein, E., Klein, G., Clifford, P., & Singh, S. (1973) *J. Exp. Med. 138*, 89–102.

Hutteroth, T. H., Cleve, H., Litwin, S. D., & Poulik, M. D. (1973) *J. Exp. Med. 137*, 838–843.

Jilka, R. L., & Pestka, S. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5892–5996.

Kessler, S. W. (1975) *J. Immunol. 115*, 1617–1624.

Klein, E., Klein, G., Nadkarni, J. S., Nadkarni, J. J., Wigzell, H., & Clifford, P. (1968) *Cancer Res. 28*, 1300–1310.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Laskey, R. A., & Mills, A. D. (1975) *Eur. J. Biochem. 56*, 335–341.

Mans, R. J., & Novelli, G. D. (1961) *Arch. Biochem. Biophys. 94*, 48–53.

Marcu, K. B., Valbuena, O., & Perry, R. P. (1978) *Biochemistry 17*, 1723–1733.

McPhie, P., Hounsell, J., & Gratzer, W. B. (1966) *Biochemistry 5*, 988–993.

Melchers, F. (1970) *Biochem. J. 119*, 765–772.

Melchers, F., & Andersson, J. (1974a) *Eur. J. Immunol. 4*, 181–188.

Melchers, F., & Andersson J. (1974b) *Adv. Cytopharmacol. 2*, 225–234.

Nilsson, K. (1971) *Clin. Exp. Immunol. 9*, 785–793.

Nilsson, K. (1978) *INSERM Symp. No. 8*, 307–317.

Nisonoff, A., Hopper, J. E., & Spring, S. B. (1975) *The Antibody Molecule*, Academic Press, New York.
Palmiter, R. D. (1974) *Biochemistry 13*, 3606–3615.
Parkhouse, R. M. R. (1971) *Biochem. J. 123*, 635–661.
Pelham, H. R. B., & Jackson, R. J. (1976) *Eur. J. Biochem. 67*, 247–256.
Perry, R. P., La Torre, J., Kelley, D. E., & Greenberg, R. (1972) *Biochim. Biophys. Acta 262*, 220–226.
Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L., & Wall, R. (1980) *Cell (Cambridge, Mass.) 20*, 303–312.
Sherr, C. J., Schenkein, I., & Uhr, J. W. (1971) *Ann. N.Y. Acad. Sci. 190*, 250–267.
Singer, P. A., Singer, H. H., & Williamson, A. R. (1980) *Nature (London) 285*, 294–299.
Steel, C. M., McBeath, S., & O'Riordan, M. L. (1971) *J. Natl. Cancer Inst. (U.S.) 47*, 1203–1214.
Steinitz, M., Koskimies, S., Klein, G., & Makela, O. (1979a) *Curr. Top. Microbiol. Immunol. 81*, 156–162.
Steinitz, M., Seppala, I., Eichmann, K., & Klein, G. (1979b) *Z. Immunitactsforsch. 156*, 41–47.
Wall, R., Lippman, S., Toth, K., & Federoff, N. (1977) *Anal. Biochem. 82*, 115–129
Watanabe, S., Yagi, Y., & Pressman, D. J. (1973) *J. Immunol. 111*, 797–804.

Choline and Acetylcholine Metabolism in PC12 Secretory Cells†

William P. Melega and Bruce D. Howard*

ABSTRACT: PC12, a clonal line of rat pheochromocytoma, synthesizes, stores, and secretes dopamine and acetylcholine. The cells take up choline by a saturable process and rapidly convert the accumulated choline to acetylcholine. This choline transport has a K_m of 12 μM, is Na^+ and energy independent, and is relatively insensitive to hemicholinium-3 ($IC_{50} \sim 50$ μM). Different ionic conditions can modulate the choline transport. Uptake was increased by pretreatment with 55 mM K^+ whereas it was decreased in the presence of 55 mM K^+. Choline uptake had similar characteristics in PC12 cells that had been induced to extend neurites by treatment with nerve growth factor. In undifferentiated PC12 cells, storage of newly synthesized acetylcholine was found in bound and free compartments as evidenced from subcellular fractionation. The free pool had a faster turnover rate. Most of the newly synthesized acetylcholine was rapidly degraded in the absence of a cholinesterase inhibitor while continuous incubation with labeled choline resulted in a slow incorporation of newly labeled acetylcholine into a bound pool. The accumulation of acetylcholine in the bound pool, but not acetylcholine synthesis, was inhibited by each of several agents that are known to interfere with the generation or maintenance of proton electrochemical gradients. The newly synthesized acetylcholine could be released from PC12 cells by incubation of the cells with 55 mM K^+. These properties indicate that PC12 cells are a good system for studying acetylcholine metabolism by secretory cells.

Studies on a variety of nervous tissues have revealed several important characteristics of the processes by which acetylcholine is stored and secreted by neurons. However, little has been learned about the molecular mechanisms involved in these processes. Progress has been impeded by technical problems inherent to the experimental systems (e.g., vertebrate neuromuscular junction, mammalian brain, mammalian autonomic ganglion, electric organ of rays) commonly used for biochemical studies of acetylcholine metabolism.

To overcome these technical problems, we have begun to study acetylcholine storage and secretion with another system that allows us to exploit the experimental advantages of cell culture. This system is PC12, a clonal line of rat pheochromocytoma (Greene & Tischler, 1976). PC12 cells synthesize acetylcholine and catecholamines (primarily dopamine), store each in separate granules, and secrete each by a Ca^{2+}-dependent process (Greene & Tischler, 1976; Greene & Rein, 1977a,b; Schubert & Klier, 1977; Rebois et al., 1980).

In nerve terminals, acetylcholine is synthesized to a great extent from choline that is transported into the terminals by a carrier-mediated process (Kuhar & Murrin, 1978; Jope, 1979), and a sizeable fraction of the newly synthesized acetylcholine is loaded into synaptic vesicles (Zimmermann & Denston, 1977). Upon depolarization of the nerve terminals, the acetylcholine is released from the terminals by a Ca^{2+}-dependent process; in several systems, there is preferential release of newly synthesized acetylcholine (Collier, 1969; Potter, 1970; Richter & Marchbanks, 1971; Dunant et al., 1972; Molenaar et al., 1973).

Here, we show that choline and acetylcholine metabolism in PC12 is similar to but not identical with that previously characterized in cholinergic neurons. Our results indicate that PC12 cells will have much utility for studying certain aspects of acetylcholine metabolism by secretory cells.

Materials and Methods

Chemicals. Bromopyruvate, eserine sulfate, iodoacetic acid, neostigmine bromide, oligomycin, ouabain, sodium fluoride, and valinomycin were obtained from Sigma Chemical Co. Sodium azide was from Matheson Coleman and Bell; hemicholinium-3 and *N,N'*-dicyclohexylcarbodiimide (DCCD)[1]

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[1] Abbreviations used: BETA, (2-benzoylethyl)trimethylammonium; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; S-13, 2',5-dichloro-*N-tert*-butyl-4-nitrosalicylanilide.

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