

SYNTHESIS, ASSEMBLY, AND SECRETION OF GAMMA GLOBULIN BY MOUSE MYELOMA CELLS

I. ADAPTATION OF THE MERWIN PLASMA CELL TUMOR-11 TO CULTURE, CLONING, AND CHARACTERIZATION OF GAMMA GLOBULIN SUBUNITS* ‡

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The fully assembled gamma G globulin molecule consists of two heavy and two light polypeptide chains linked by disulfide bonds. The synthesis and assembly of this multichained molecule can be effectively studied in transplantable mouse plasma cell tumors which synthesize large amounts of homogeneous gamma globulin (1). In some of these tumors blocks at different stages in the assembly process (2, 3) make it possible to identify precursors of the assembled gamma globulin molecule.

In previous studies Merwin plasma cell tumor-11 (MPC-11)¹ myeloma cells freshly dissociated from tumors were found to secrete fully assembled molecules (H_2L_2), half molecules (HL), light chain dimers (L_2), and free light chains (L) (4). The production of relatively large amounts of partially assembled molecules could have been due to significant numbers of nonviable cells and to the suboptimal metabolic conditions in the incubation mixture. Since some of the subunits appeared to be end products while others were precursors of the fully assembled molecule (4), it was important to determine whether all the precursors could be produced by an individual cell.

These possibilities have now been explored in MPC-11 myeloma cells adapted to growth in continuous culture. Such cultures provided a uniform population of viable cells which could be studied during logarithmic growth to ensure

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¹ Abbreviations used in this paper: H, heavy chain; H_2 , heavy chain dimers; H_2L_2 , fully assembled molecules; HL, half molecules; L, light chain; L_2 , light chain dimers; ME, 2-mercaptoethanol; MPC-11, Merwin plasma cell tumor-11; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid.

optimal metabolic conditions and could be cloned to ensure cellular homogeneity. Adaptation to culture resulted in the selection of a minor cell type which synthesized and secreted fewer HL molecules and free L chains than did the parent tumor. Cloning of the tumor cells in vivo and of the cultured cells in vitro indicated that individual cells were capable of synthesizing all the partially and fully assembled gamma globulin molecules.

Materials and Methods

Tumor and Cultures.—The MPC-11 plasma cell tumor, originally induced in BALB/c mice, secretes gamma 2b globulin, was provided by Dr. John Fahey 5 yr ago, and has been maintained in this laboratory by serial subcutaneous passage in the same strain of mice. Its pattern of synthesis of fully and partially assembled gamma globulin molecules has not changed in that period. The tumor cells were adapted to culture using the technique described by Buonassisi et al (5). A tumor was removed from the animal, and cut into 2–3 mm pieces which were suspended in Eagle's basal medium supplemented with nonessential amino acids (6), 20% horse serum, and 4 mM glutamine. The tissue fragments were shaken vigorously in 10 ml of medium in a rotatory shaker, the tumor fragments were allowed to settle, and the supernatant cell suspension was diluted to $1-2 \times 10^6$ cells in the same medium and 5 ml transferred to each Petri dish. At 2-day intervals $5-10 \times 10^6$ cells were resuspended in 0.5 ml of medium, and reinjected into BALB/c mice. The tumors that developed from cells which had survived in culture for the longest period of time were processed as described above and again placed in Petri dishes. After the first three culture-animal passages, Dulbecco's modified Eagle's medium supplemented with nonessential amino acids, 4 mM glutamine, and 20% horse serum was used instead of Eagle's medium. Subsequent experiments demonstrated that adaptation to culture was not facilitated if Dulbecco's medium was used from the outset. After a number of culture-animal passages, each of which resulted in longer survival in vitro, the cells began to grow continuously. Two main MPC-11 lines were established in continuous culture. One, designated MPC-11 (cult), was established in the sixth adaptation cycle; the second designated clone 1 (cult), was established directly from a spleen clone (see below) derived from MPC-11 tumor cells which had undergone three adaptation cycles.

Once adapted to culture, the cells could be injected back into animals and the tumors which developed could be used to start a new continuous culture without additional adaptation cycles. The established cultures did not attach to either plastic or glass, and were maintained at 37°C in a 5% CO₂ atmosphere in plastic Petri or tissue culture dishes (Falcon Plastics, Los Angeles, Calif.) at a cell concentration of between 3×10^5 and 2×10^6 cells/ml. The cultures had a doubling time of about 20 hr and were fed three times a week by the addition of an equal volume of fresh medium. After several months, the cells were also transferred to a suspension culture in spinner bottles (7). Both lines have been maintained in dishes and in spinner bottles for over a yr. The cells have been checked repeatedly for the presence of mycoplasma by Dr. Elliot Levine of this department and found to be negative. The cells were still highly tumorigenic after 11 months of growth in continuous culture. When inoculated intraperitoneally, about 100 cells produced a tumor in 50% of the animals. Much higher numbers of cells were needed in order to produce a tumor when inoculated subcutaneously. Electron microscope preparations prepared by Dr. E. Robbins and Miss G. Jentsch showed the characteristic pattern of myeloma cells with a relatively large amount of rough endoplasmic reticulum. The cultured myeloma cells also contained the type A particles in the cisternae of the rough endoplasmic reticulum seen in other myeloma tumors (8, 1).

In addition to the MPC-11, we have examined two other myeloma tumors: MOPC-173, which secretes gamma 2a globulin, and X5563, which produces gamma G globulin. Both

tumors were kindly supplied by Dr. Michael Potter of the National Institutes of Health, Bethesda, Md.

Cloning Procedures.—Cells which had not been adapted to culture were cloned *in vivo* in the spleen of BALB/c mice by the technique of Till and McCulloch (9). A cell suspension prepared from a tumor was filtered through a 100-mesh stainless steel wire screen, and 10^4 – 10^5 single cells injected intravenously into the tail vein of BALB/c mice. Depending on the number of cells injected, between one and five colonies could be detected as discrete nodules 2–3 wk after injection. Individual tumor nodules enucleated from spleens which contained no more than three well separated nodules were immediately reimplanted into animals.

Cells which had been adapted to culture were cloned in soft agar as described by Pluznik and Sachs (10) and Bradley and Metcalf (11). 3T3 mouse fibroblasts or primary embryonic mouse cells were grown in small plastic Petri dishes until a confluent monolayer had formed, when they were overlaid with 5 ml of 0.5% Bacto-agar (Difco Laboratories, Inc., Detroit, Mich.) in Dulbecco's medium supplemented as described above. Cells were then resuspended in 0.34% agar in the same medium at 45°C and 1 ml of this mixture containing 10^2 – 10^5 cells was poured on top of the 0.5% agar layer. Macroscopic colonies which developed after 10–14 days were removed from the soft agar with a sterile Pasteur pipette, and were either injected directly into animals or grown in dishes which contained fresh confluent monolayers of 3T3 mouse fibroblasts. Cells obtained from the parent MPC-11 tumor and cloned directly on agar as described above formed small colonies of up to 18 cells in size, but only if the plates were seeded with a high cell concentration (10^5 cells per dish). The average cell-doubling time in these colonies was much longer than that of the cells adapted to culture, and they failed to grow when transferred onto fresh feeder layers or to produce tumors when injected back into animals.

Incubation of Cells with Radioactive Precursors.—Tumor fragments were suspended in a 25 ml Erlenmeyer flask in 10 ml of Ca-free Eagle's medium for spinner culture supplemented with 0.0018 M CaCl_2 and containing $\frac{1}{20}$ of the normal amounts of threonine, valine, and leucine (6). After 5 min of vigorous shaking in a rotatory shaker at 37°C, the tissue fragments were allowed to settle and the supernatant containing dissociated cells filtered through a 100-mesh stainless steel screen.

Cells obtained from the tumor or culture were washed twice in round bottomed tubes with the amino acid-depleted medium described above, and resuspended in the same medium at a concentration of 1 – 5×10^6 cells/ml. 3–15 μC each of ^{14}C -L-threonine, valine, and leucine (U.L., specific activity > 0.15 Ci/mmmole, New England Nuclear Corp., Boston, Mass.), or 15 μC each of ^3H -labeled threonine, valine and leucine (specific activities: 0.132, 0.600, and 40 Ci/mmmole, respectively, Schwarz Bio Research Inc., Orangeburg, N. Y.) were added to each of the incubation mixtures. In the earliest experiments, ^{14}C -L-threonine, valine, arginine, and lysine were used for labeling. No difference in the pattern of gamma globulin synthesis was observed when the two labeling mixtures were compared. In some of the experiments the cultured cells were labeled in the presence of 10 % horse serum. The cells suspension was gently agitated in a rotatory shaker at 37°C. To prepare cytoplasmic proteins, portions of the incubation mixture were removed after 30 min, chilled, and the cells pelleted and resuspended in an isotonic buffer containing 0.145 M NaCl, 0.0015 M MgCl_2 , and 0.01 M Tris-HCl, pH 7.2, at 4°C. The detergent Nonidet P-40 (NP-40) (Shell Chemical Co., London SE1, Waterloo 1212, U.K.) was then added to a final concentration of 0.5% and the nuclei and ribosomes removed by sedimentation at 105,000 g for 30 min at 4°C (12).

Secreted material was prepared from the supernatant medium of cells incubated for 3 hr at 37°C.

Immunological Precipitation of Gamma Globulin Fraction.—Completed gamma globulin molecules and unassembled subunits were precipitated from either the cytoplasm or culture fluid by "direct" or "indirect" precipitation methods (13). All precipitations were done in

antibody excess. The anti-MPC-11 gamma globulin serum was kindly supplied by Dr. Susan Zolla (New York University College of Medicine). This antiserum was obtained from rabbits immunized with MPC-11 myeloma globulin purified from the serum of mice bearing MPC-11 tumors by starch gel electrophoresis and ammonium sulfate precipitation and contained approximately 40 mg/ml of gamma globulin. In the direct precipitation procedure, 20 μ l of antiserum was added to cytoplasm obtained from 2.5×10^6 cultured cells, or 10 μ l of antiserum was added to 5×10^6 tumor cells, in a volume of 0.5 ml. The mixture was incubated for either 3 hr or overnight at 4°C and the precipitate washed twice with 0.5 ml of cold phosphate-buffered saline (PBS) (0.02 M phosphate buffer, pH 7.0, 0.85% NaCl). The precipitate was then resuspended in 0.01 M sodium phosphate, pH 7.0 containing 2% sodium dodecyl sulfate (SDS) and dissolved by heating at 100°C for 1 min (14). Iodoacetamide was added to a final concentration of 0.03 M and incubation continued for 30 min at 37°C. The sample was then dialyzed against the 0.01 M sodium phosphate, pH 7.0, 0.1% SDS, 0.001 M sodium azide, and electrophoresed on acrylamide gels as described below.

In the indirect method of immunologic precipitation, 0.75 ml of cytoplasm prepared from 0.5×10^6 cells was incubated with 5 μ l of antiserum for 30 min at 37°C. 0.25 ml of sheep antiserum directed against rabbit gamma globulin was then added, and the incubation continued for 2 hr. The resulting precipitate was washed three times with 0.5 ml of cold PBS and treated in the same way as the precipitate obtained by the direct method. An anti- λ phage rabbit antiserum and sheep anti-rabbit gamma globulin were used to determine nonspecific precipitation (13).

Acrylamide Gel Analysis of Labeled Proteins.—Separation of proteins by size on SDS-containing acrylamide gels has been described by Maizel (15, 16) and in a previous publication (4). Soluble cytoplasmic proteins, secreted material, or immune precipitates were treated with 2% SDS either for 30 min at 37°C or for 1 min at 100°C. To prevent disulfide bond interchange the soluble proteins were alkylated by adding iodoacetamide to a final concentration of 0.03 M. While the covalent bonds of gamma globulin are not disrupted by heating at 100°C for 1 min, this treatment completely dissociates immune precipitates.² The samples were then dialyzed for 16–24 hr at room temperature against SDS-phosphate buffer (0.1% SDS, 0.01 M sodium phosphate, pH 7.0, and 0.001 M sodium azide).

In those experiments in which disulfide bonds were reduced in order to dissociate the gamma globulin molecules into heavy and light chains, 2-mercaptoethanol (ME) at a final concentration of 0.075 M was added to the SDS-treated proteins for 1 hr at 37°C. The samples were then either alkylated immediately with 0.1 M iodoacetamide for 30 min at 37°C, or first dialyzed against SDS-phosphate buffer containing 0.015 M ME and 16 hr later alkylated with 0.03 M iodoacetamide. In either case the proteins were then dialyzed against the same buffer without ME. The protein solution was concentrated to a volume of 0.2–0.3 ml by dialysis against 60% sucrose and then applied on a 20 cm 5% acrylamide gel containing 0.1% SDS and 0.1 M sodium phosphate buffer (pH 7.0). The reservoir buffer was the same as that present in the gels, and the electrophoresis was carried out at room temperature for 16 hr at 60–70 volts (6–7 ma per gel). For the determination of the relative numbers of heavy and light chains, electrophoresis was carried out on 10 cm 7.5% SDS containing gels for 3–4 hr at 90–100 volts. The gels were fractionated mechanically as described by Maizel (15) and the samples collected on planchets or in scintillation vials. The planchets were counted in a Nuclear-Chicago low background counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Samples labeled with both ¹⁴C and ³H were counted in the Beckman 1200 scintillation counter after 10 ml of modified Bray's solution (containing naphthalene, dioxane, and PPO) had been added to each vial. In some of the experiments the appropriate corrections for channel overflow

² Scharff, M. D. Unpublished data.

were programmed into a computer, which calculated and plotted the results in terms of percentage of the total counts in the gel.

Column Chromatography of Tryptic Peptides of Heavy and Light Chains.—MPC-11 tumor cells were incubated separately with either ^{14}C - or ^3H -labeled threonine, valine, and leucine, while the cultured cells were incubated with only ^{14}C -labeled threonine, valine, and leucine. The incubation conditions were the same as those described above. After 3 hr, the cells were separated from the medium and the supernatant fluid containing the secreted protein was centrifuged at 105,000 g for 30 min to remove cell debris. The ^3H -labeled protein secreted by the tumor was mixed with the ^{14}C -labeled secretion from the cultured cells. In the control the same ^3H -labeled material was mixed with ^{14}C -labeled secretion from the tumor. Both samples were dissociated and reduced by boiling for 1 min in 2% SDS and 0.15 M ME and then incubated for 30 min at 37°C. The samples were dialyzed overnight at room temperature against SDS-phosphate buffer containing 0.015 M ME. Recrystallized iodoacetamide was added to a final concentration of 0.03 M for 30 min at 37°C, and the samples were again dialyzed overnight against SDS-phosphate buffer. The heavy (H) and light (L) chains were separated on 10 cm 7.5% SDS-containing acrylamide gels as described above. The gels were fractionated mechanically (15) and approximately 35 fractions were collected into test tubes. After 16 hr at 4°C to allow the protein to elute from the gels, a small sample from each tube was counted for both ^3H and ^{14}C radioactivity. The tubes corresponding to peaks of radioactivity of the H and L chains were pooled separately, the gel removed by centrifugation, and 500 μC of human gamma globulin (Pentex Inc., Kankakee, Ill.) added as carrier. After precipitation with 10% trichloroacetic acid (TCA), and successive washing with 5% TCA, 1:1 ethanol-ether, and ether (17), the dried pellet was resuspended in 1.0 ml of water, the pH was adjusted to neutrality with 0.05 M NH_4HCO_3 , and each of the samples was digested with 1 mg of trypsin (Worthington Biochemical Corp., Freehold, N. J.) twice recrystallized) for 5–8 hr at 37°C. The pH of the digestion mixture was monitored with a small amount of phenol red, and readjusted to pH 7.0 when necessary. The small amount of insoluble material which still remained after treatment with trypsin was removed by centrifugation at 2000 rpm for 10 min and 3 ml of 0.05 M pyridine acetate buffer, pH 4.0 was added to stop the digestion. The samples were then chromatographed (18) on a 1 by 25 cm. Dowex 50 sulfonated polystyrene resin (SPHERIX, type XX907, Phoenix Precision Instrument Co., Philadelphia, Pa.) column at 60°C. The elution buffer was generated in a Varigrad (Phoenix Precision Instrument Co.) which contained the following pyridine acetate buffers in successive chambers: (a) 100 ml of 0.05 M, pH 4.0; (b) 100 ml of 0.5 M, pH 5.0; (c) 100 ml of 0.5 M, pH 5.0; and (d) 100 ml of 2.0 M, pH 6.0. One hundred 3 ml fractions were collected directly into scintillation vials, the pyridine acetate was removed by evaporation, and 1 ml of water and 10 ml of Bray's solution were added to each vial. The samples were counted as described above.

Although few, if any, differences were detected in the peptides examined in this paper, the technique as described has revealed differences in the tryptic peptides of the major adenovirus proteins (19). In addition, comparison of γ_{2a} (MOPC-173) and γ_{2b} (MPC-11) heavy chains revealed many peptides which were not common to both chains.²

RESULTS

Pattern of Gamma Globulin Synthesis and Secretion by MPC-11 Tumor Cells and Splenic Clones.—The intracellular and secreted proteins produced by MPC-11 tumor have been analyzed on sodium dodecyl sulfate (SDS)-containing acrylamide gels. Dissociation with SDS solubilized all of the protein, and migration in the SDS-gel system is proportional to the molecular weight of the protein rather than to its charge (20, 21). The electrophoretic patterns

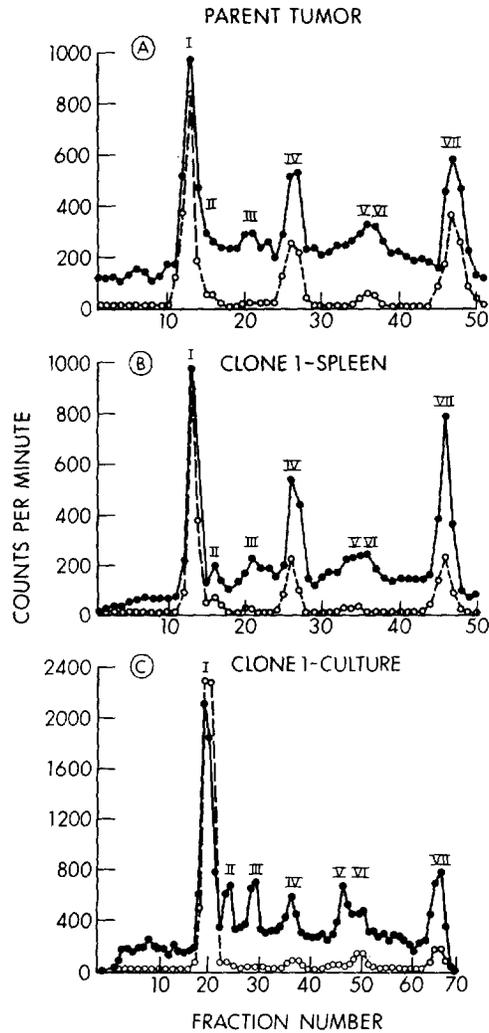


Fig. 1. Gel electrophoresis patterns of intracellular and secreted proteins of the parent MPC-11 tumor-(A), of a spleen clone derived from the parent tumor-(B) and of the cultured cells derived from the spleen clone-(C). Labeled cytoplasm (●—●) and secretion (○---○) were prepared as described in the Materials and Methods and electrophoresed on 5% 20 cm SDS-acrylamide gels for 16 hr at 70 v. The gels were fractionated into planchetes and the radioactivity in each of the planchetes determined. Each of the figures presents the results of separate analysis of cytoplasm and secretion done on two gels. The various protein peaks are marked from I to VII.

of (a) the cytoplasm of cells incubated with radioactive amino acids for 30 min, and (b) material secreted into the medium after 3 hr of incubation are shown in Fig. 1A. Several distinct peaks of radioactivity seen in the cell extract were also found in the medium (Fig. 1A). Seven of these peaks (I-VII) were shown to be gamma globulin by treating the cytoplasmic extract (Fig. 2A) or secretion (Fig. 2C) with antiserum specific for mouse gamma globulin. The immunological precipitates were analyzed on SDS-containing acrylamide gels (Figs.

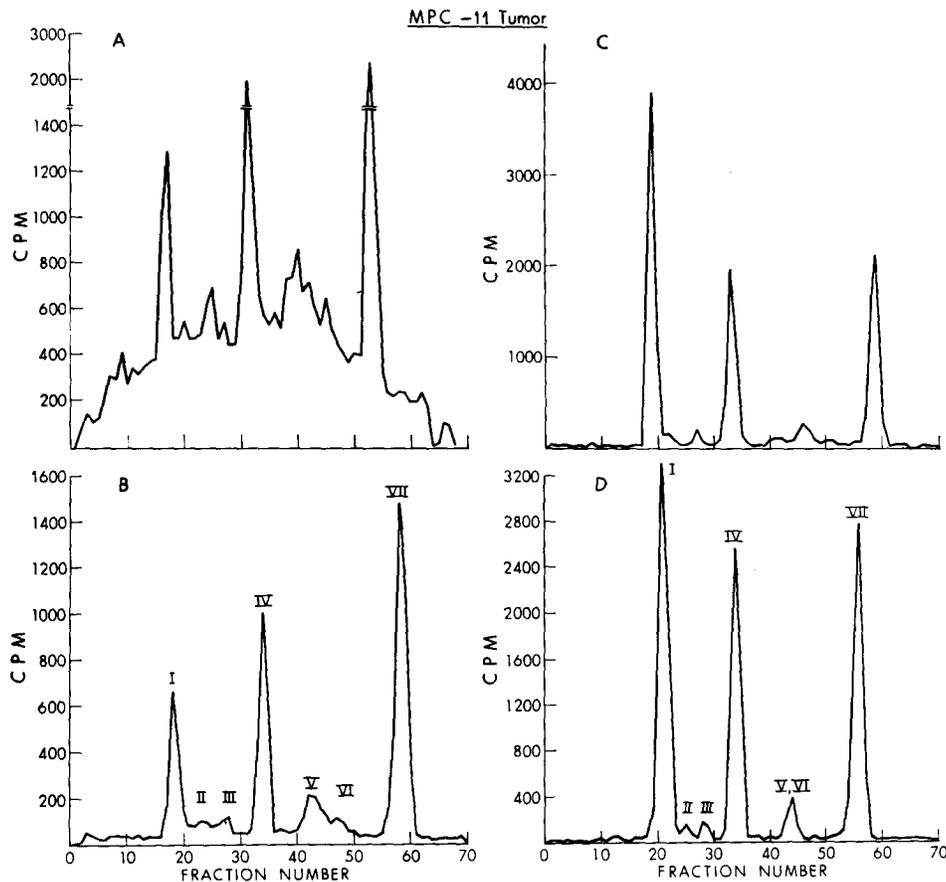


FIG. 2. Gel electrophoresis patterns of immune precipitates of cytoplasm and secretion of the parent MPC-11 tumor. Labeled cytoplasm and secretion were prepared as described in Materials and Methods and treated with antiserum against MPC-11 myeloma protein. The immune precipitates were washed, dissolved in 2% SDS at 100°C for 1 min, and then electrophoresed on 5% 20 cm SDS acrylamide gels using the conditions described in Materials and Methods. Radioactive profiles of the gels are shown: A, cytoplasm; B, immune precipitate of cytoplasm; C, secretion; and D, immune precipitate of secretion.

2B and 2D). Peak I of the secreted material had been previously shown to be assembled 7S gamma globulin molecules (H_2L_2), peak IV to contain half molecules (H L disulfide linked), and peak VII to be free light chains (4). As will be shown below, peaks II, III, V, and VI, also represented either free chains or partially assembled gamma globulin molecules.

To determine whether all of these gamma globulin molecules could be synthesized by an individual cell, MPC-11 tumor cells were cloned in the spleens of BALB/c mice. The clones were passed into animals, and the cytoplasm and secretion of each of the resulting tumors were examined by acrylamide gel electrophoresis. Fig. 1B shows the results with one clone, and the relative

TABLE I
Characterization of Spleen Clones from MPC-11 Tumor Cells

Clone No.	Total cpm in secreted gamma globulin peaks*				
	I, II	III	IV	V, VI	VII
	%	%	%	%	%
1	58.1	1.6	16.8	2.4	21.2
2	48.2	3.2	23.0	3.6	22.1
3	53.1	1.7	20.6	3.9	20.6
4	45.6	2.2	18.4	4.4	29.5
5	48.6	2.1	18.9	3.7	26.7
6	45.0	2.2	17.4	5.8	29.6
Average	49.8	2.2	19.2	4.0	25.0
Parent tumor	46.8	1.8	18.9	4.9	27.5

* Cells from the parent MPC-11 tumor and from tumors derived from spleen clones were labeled with ^{14}C -threonine, valine, lysine, and arginine, and the secretion analyzed. The sum of radioactivity in peaks I-VII was determined and the per cent of each peak calculated. Since peak II was relatively small and poorly resolved from peak I, they were considered together. Peaks V and VI were also not well enough resolved to be calculated separately.

amounts of radioactivity in the peaks obtained from six clones are summarized in Table I. Although there were small differences in the amount of L chains produced, the fact that all the clones synthesized and secreted approximately the same relative amounts of each of the gamma globulin peaks indicated that a single cell was capable of synthesizing H_2L_2 , HL, and L, as well as the other partially assembled molecules. This experiment did not, however, prove that the parent tumor was homogeneous, since cells which were trapped by the spleen and formed detectable tumors might not have been a random sample of the injected cells.

Adaptation of MPC-11 Tumor Cells to Culture.—The pattern of gamma globulin synthesis by cells which had been maintained in culture (Fig. 1C) was significantly different from that of the parent tumor (Fig. 1 A, B). Although all

the gamma globulin molecules found in the parent tumor were synthesized by the cultured cells, the latter contained less half molecules and free light chains. The ratio of light to heavy chains in the original MPC-11 tumor and the parent clone 1 tumor was approximately 3.5:1 but only 1.7:1 in the cultured

TABLE II

Pattern of Gamma Globulin Secretion by MPC-11 Cells after the Onset of Continuous Culture

Exp. No.	Origin of cells	Cells grown as:		Time in culture <i>days</i>	Total cpm in secreted gamma globulin peaks*			
		Tumor	Culture		I, II %	IV %	V, VI %	VII %
1	Parent MPC-11	+	-	-	48.5	19.0	4.3	28.2
2	MPC-11	-	+	5	61.4	7.0	7.4	24.2
4	MPC-11	-	+	7	62.5	5.9	11.4	20.0
12	MPC-11	-	+	10	71.0	3.6	12.9	12.5
	MPC-11‡	+	-	-	78.5	4.1	6.7	10.7
13	MPC-11	-	+	42	65.4	4.1	11.1	19.4
30	MPC-11	-	+	90	81.5	3.9	7.0	7.7
1	Clone 1§	+	-	-	59.1	17.1	2.3	21.6
4	Clone 1 (12/7/67)	-	+	22	60.0	5.8	12.5	21.5
11	Clone 1 (1/30/68)¶	-	+	2	73.9	4.8	6.5	14.9
	Clone 1 (1/30/68)	-	+	2	72.5	5.1	7.7	14.7
13	Clone 1	-	+	35	73.1	5.2	8.9	12.8
30	Clone 1	-	+	90	76.5	3.7	10.5	9.3
50	Clone 1	-	+	180	73.1	5.2	8.9	12.8

* Cells were labeled with ¹⁴C-threonine, valine, and leucine and the relative amount of the various gamma globulin peaks in the secretions calculated as described in Table I. Peak III was omitted from the calculations since in the cultured cells it amounts to $\leq 0.5\%$ of the total cpm of the gamma globulin.

‡ The same MPC-11 tumor had been used to start a culture and to transfer into animals. 10 days later, cells from the culture and the tumor were examined.

§ Cells from a tumor derived from spleen clone 1.

|| Indicates date of initiation of continuous culture from spleen clone 1 cells.

¶ This line was started on 1/30/68 from a tumor that had grown in animals injected subcutaneously with clone 1 (12/7/67) cultured cells. These cells were labeled in the absence (upper row) and in the presence (lower row) of 10% horse serum.

cells. About 20% of the total radioactivity of the cytoplasm and almost 100% of the radioactivity of the secretion was precipitated with antiserum directed against MPC-11 myeloma globulin. As with the parent tumor, the seven peaks common to both the cytoplasm and secretion of cultured cells were all precipitable with this antiserum. Cells examined a few days after establishment in continuous culture seemed to secrete relatively more free light chains than did later cultures (Table II). At this time the culture contained both cells

which resembled the original tumor and cells which were synthesizing relatively fewer L chains (cf. Table III).

The pattern of gamma globulin secreted by the MPC-11 cultured cells stabilized after 90 days and that of clone 1 after 35 days in continuous culture with no significant differences between them (Table II). The MPC-11 cultured line has remained stable in both Petri dishes and spinner cultures for $1\frac{1}{2}$ yr. On the other hand, the clone 1 culture in Petri dishes stopped producing com-

TABLE III
Characterization of Agar Clones Derived from Clone 1 Cultured Cells

Exp. No.	Origin of cells		Total cpm in secreted gamma globulin peaks			
			I, II	IV	V, VI	VII
			%	%	%	%
13*	MPC-11,	parent tumor	50.0	11.1	8.4	30.5
	Clone 1,	tumor grown from parent culture	76.4	3.3	8.1	12.2
	Clone 1-(1),	tumor from agar clone	82.7	3.5	7.1	7.3
	Clone 1-(2),	tumor from agar clone	79.9	3.5	8.2	8.6
	Clone 1-(3),	tumor from agar clone	48.8	20.6	9.5	21.0
20‡	MPC-11,	parent tumor	48.1	11.7	7.7	32.4
	Clone 1,	parent culture	79.0	1.7	9.0	10.3
	Clone 1-(4),	tumor from agar clone	83.8	2.9	6.4	7.0
	Clone 1-(5),	tumor from agar clone	84.7	2.4	6.2	6.6
	Clone 1-(6),	tumor from agar clone	83.0	2.8	6.9	7.1
	Clone 1-(7),	tumor from agar clone	87.0	2.3	5.1	5.8
	Clone 1-(8),	tumor from agar clone	81.6	2.9	6.3	9.2
	Clone 1-(9),	tumor from agar clone	86.3	3.2	4.4	6.2
	Clone 1-(10),	tumor from agar clone	85.8	3.7	4.1	6.3

* These agar clones were derived from clone 1 cultured cells 2 wk after they were established in continuous culture.

‡ These agar clones were derived from clone 1 cultured cells which had been maintained in continuous culture for 8 wk.

plete gamma globulin molecules after 8 months in culture, and subsequently secreted only light chains.

The relative amounts of the various gamma globulin molecules were not significantly affected when the cells were transferred from culture into animals and the tumor cells then analyzed in short term culture, (Table II, Experiment 12). Addition of serum to the labeling medium did not affect the relative number of counts in each of the peaks (Table II, Experiment 11).

Cloning of Cultured Cells in Soft Agar.—The plating efficiency of the cultured myeloma cells when cloned in soft agar 2 wk after their establishment in culture was 0.1–1%, and increased to 10% 9 months later. It was later pos-

sible to increase the cloning efficiency to 40% by substituting agarose for the Bacto-agar (22). When the agar-clones were injected into animals and the pattern of gamma globulin secretion of the resulting tumors determined, 9 of 10 clones examined proved similar to the parent culture (Table III) and

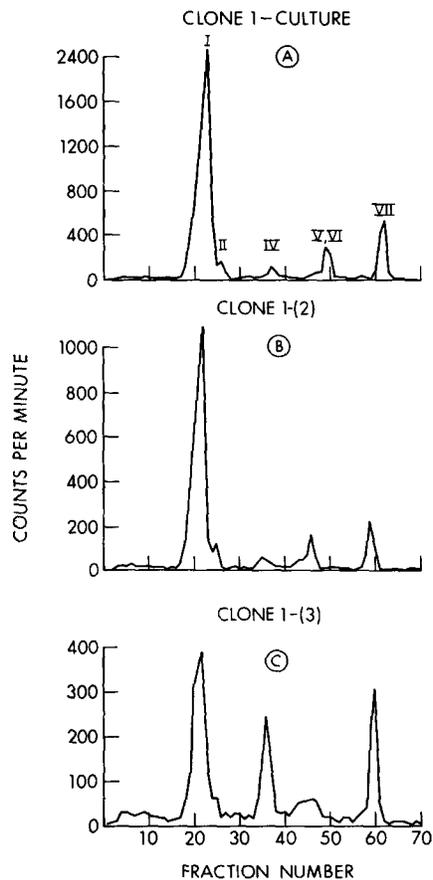


FIG. 3. Gel electrophoresis secretion patterns of agar clones derived from clone 1 cultured cells. The cells were cloned on soft agar as described in Materials and Methods. Individual clones were picked and injected intraperitoneally into mice. Cells from the tumors that developed were labeled with ^{14}C amino acids and the secretion analyzed. A, Clone 1 cultured cells; B, agar clone 1-(2); and C, agar clone 1-(3).

were capable of producing all of the intermediate gamma globulin molecules observed in the parent culture. Clone 1-(3), however, resembled the parent tumor in the large number of half molecules and free light chains present in its cytoplasm and secretion (Fig. 3). Although this difference persisted after

repeated animal passage, on two different occasions, when clone 1-(3) was reintroduced into culture its pattern of secretion became similar to that of the rest of the agar-clones (Table IV). During readaptation of clone 1-(3) to culture many of the cells died and it took 4-8 wk before the remaining cells began to grow well. Since the cloning efficiency in these experiments was relatively low and since only 10 clones were examined, more extensive studies will be required to determine the degree of heterogeneity in the parent culture. However, the results do suggest that the majority of the cells had a similar pattern of gamma globulin synthesis and secretion.

Characterization of Partially Assembled Molecules in the Cytoplasm and in the Secretion of Cultured and MPC-11 Tumor Cells.—Each of the various gamma globulin peaks in the cytoplasm and secretion was further characterized by

TABLE IV
Stability of Clone 1-(3) in Animals and Its Adaptation to Culture

Origin of cells	Tumor-transfer* No.	Date of initiation of culture	Date of examination of culture	Total cpm in secreted gamma globulin peaks			
				I, II	IV	V, VI	VII
				%	%	%	%
Clone 1‡				76.4	3.3	8.1	12.2
Clone 1-(3)	1	—	—	48.8	20.6	9.5	21.0
Clone 1-(3)	6	—	—	52.5	20.6	4.7	22.2
Clone 1-(3)	9	6/25/68	8/13/68	81.2	4.1	4.4	10.3
Clone 1-(3)	11	7/2/68	8/13/68	82.6	3.9	4.2	9.2

* Clone 1-(3) was maintained by animal transfer.

‡ A tumor grown from the parent clone 1 culture was analyzed.

determining its molecular weight on SDS-containing acrylamide gels (Fig. 4). When ^3H -labeled secreted material from the MPC-11 parent tumor was mixed with ^{14}C -labeled cytoplasm from the cultured cells, the corresponding gamma globulin molecules migrated identically on acrylamide gel. It was concluded that peak I is the complete H_2L_2 molecule because it had the same mobility as other mouse IgG myeloma proteins (MOPC-173 and X5563)², and as the 6.5S myeloma protein found in the serum of animals bearing the MPC-11 tumor (23). Peak I also coelectrophoresed with five different human IgG myeloma proteins. Upon reduction, the H and L chains of MPC-11 secreted protein had the same mobility as the respective chains from human myelomas.³ On the basis of recently determined molecular weight values for a human myeloma protein and its polypeptide chains (24) it was concluded that the MPC-11 H_2L_2 molecule and the L chain have molecular weights of 150,000 and 22,000,

³ Zolla S., and J. Buxbaum. In vitro immunoglobulin synthesis by human myeloma cells. In preparation.

respectively. The experimentally determined molecular weights as derived from Fig. 4 and the suggested structure of each of the intermediary molecules are shown in Table V.

Since the molecular weight determination was only an approximation, and in some cases more than one structure was possible, the relative amount of H and

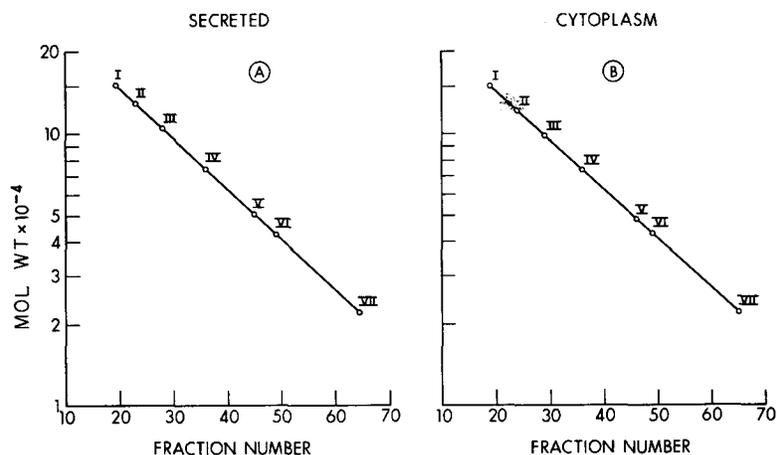


FIG. 4. Determination of the molecular weights of gamma globulin subunits produced by clone 1 cultured cells. The molecular weights of the complete gamma globulin molecule (peak I) and the free light chain (peak VII) were assumed to be 150,000 and 22,000, respectively. The electrophoretic mobilities of peaks I and VII were plotted as a linear function of the logarithm of their molecular weight values. The molecular weights of the rest of the gamma globulin peaks were determined by inserting their electrophoretic mobilities onto the straight line which connects peaks I and VII (see Table V).

TABLE V

Estimation on Acrylamide Gel of the Molecular Weights of the Gamma Globulin Peaks in the Cytoplasm and Secretion of Clone 1 Cultured Cells

Peak No.	Suggested structure	Mol wt $\times 10^{-3}$ calculated for suggested structure*	Mol wt $\times 10^{-3}$ determined from the gel†	
			Cytoplasm	Secretion
I	H ₂ L ₂	(150)	(150)	(150)
II	H ₂ L	128	123	129
III	H ₂ , HL ₂	106, 97	98.5	104
IV	HL	75	73.5	74
V	H	53	48.5	50.5
VI	L ₂	44	43.0	42.5
VII	L	(22)	(22)	(22)

* The mol wt of H₂L₂ was assumed to be 150,000 and that of L, 22,000. These numbers are in parentheses.

† Determined from Figs. 4a, b.

L chains in each of the partially assembled molecules was determined by elution of the individual peaks from the gel and reelectrophoresis after reduction and alkylation. Peaks I-VII from the cytoplasm of the cultured cells were recovered both from gels containing whole cytoplasmic extracts, and from gels containing immunological precipitates. ^3H -labeled secreted material from the MPC-11 parent tumor was added to each of the peaks in order to identify the

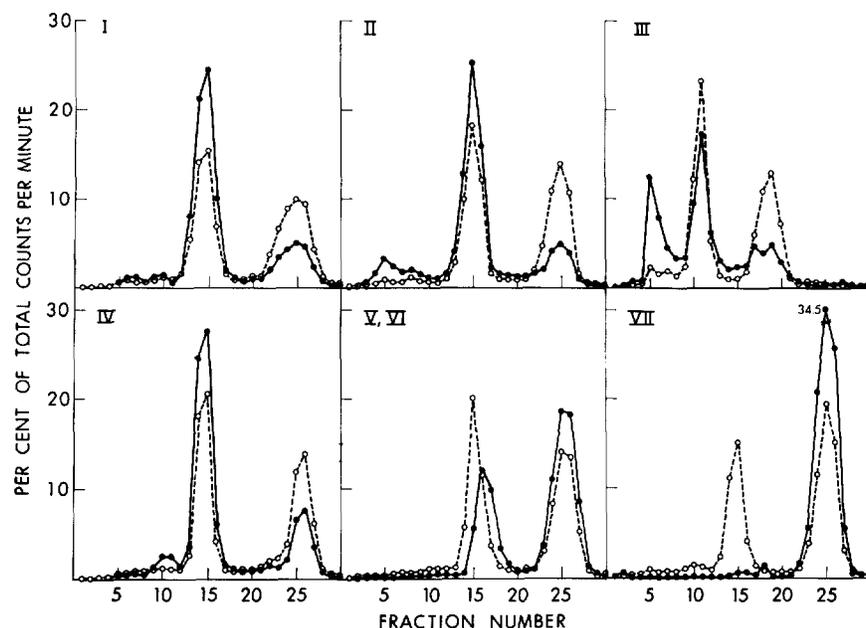


FIG. 5. Determination of the relative amounts of heavy and light chains in immunologically precipitated cytoplasmic gamma globulin peaks of clone 1 cultured cells. 5×10^6 cells were labeled in 1 ml with $15 \mu\text{c}$ each of threonine, valine, and leucine for 30 min at 37°C . The labeled cytoplasmic gamma globulin was indirectly precipitated with antiserum against MPC-11 myeloma protein. The immune precipitate was washed and dissolved in 2% SDS and electrophoresed on 5% 20 cm SDS acrylamide gels. The gels were fractionated and collected into test tubes, which were left overnight at 4°C to allow elution of the protein from the gel. The electrophoresis pattern was evaluated by counting the radioactivity of 50 λ samples from each of the test tubes. The amount of heavy and light chains in peaks I-VII was determined by treating each of the peak tubes with 0.075 M 2-mercaptoethanol in the presence of 2% SDS and alkylating with 0.1 M iodoacetamide (Materials and Methods). The dissociated chains were then separated on 7.5% 10 cm acrylamide gels. ^3H -labeled secreted material from the MPC-11 parent tumor served as a marker (○--○) and was mixed with the ^{14}C -labeled experimental samples (●—●) before dissociation into heavy and light chains. The amount of radioactivity under the heavy and light chain peaks were calculated and the ratio of H:L chains determine (see Table VI, first row). The total number of ^{14}C cpm found in each of the gels was: I, 18,552; II, 7623; III, 2138; IV, 9837; V, VI, 11,634; and VII, 11,837.

electrophoretic migration of the H and L chains. The relative amount of H and L chains in the immunologically precipitated cytoplasmic peaks I-VII is shown in Fig. 5, and the ratio of H:L chain radioactivity in the various peaks is compared to that of the parent tumor in Table VI. The presence of H and/or L chains in each of the seven peaks confirmed that all contained gamma globulin molecules. In three experiments using either immunologically precipitated or nonimmunologically precipitated cytoplasm from the cultured cells, the average ratio of H:L chain radioactivity in peak I was 3.0 (range 2.5-3.5). When peak I of the secreted material, which contained little cell protein, was similarly examined (Table VII and Fig. 6), in four different experiments, the average H:L chain ratio was 3.2 (range 2.5-4.0).

TABLE VI
Characterization of Cytoplasmic Gamma Globulin Peaks

Origin of cells	Ratio of radioactivity in heavy and light chains (H:L)						
	I	II	III	IV	V	VI	VII
Clone 1 culture*	2.90	3.30	1.90‡	2.70	0.54		0.03
MPC-11 parent‡ tumor	2.47	3.09	0.61	2.30	1.87	1.09	0.05
Suggested structure based on molecular size§	H ₂ L ₂	H ₂ L	H ₂ , HL ₂	HL	H	L ₂	L

* Cytoplasm was precipitated by the "indirect" method and the immune precipitate analyzed (see Materials and Methods and Fig. 5).

‡ Cytoplasm was precipitated by the "direct" method and the immune precipitate analyzed (see Materials and Methods).

§ See Table V.

Assuming molecular weights of 53,000 for the H chain and 22,000 for the L chain, the ratio of H:L chain radioactivity in peak I should be 2.4. The difference between the theoretical and experimental values may be due to several reasons. Although the variation between duplicate determinations was less than 5%, when the same material was reduced, alkylated, and analyzed at different times, the observed variation was as great as that described above for different samples. There might also be (a) unequal labeling of the H and L chains due to different proportions of valine, threonine, and leucine residues in the two chains, (b) preferential loss of L chains, or (c) assembly of significant numbers of unlabeled L chains present in the cellular pool prior to the addition of radioactive precursors. Since duplicate determinations showed little variability, the ratio of H:L chain radioactivity in each of the peaks could be reliably compared to that found in peak I of the same experiment.

In any one experiment, the ratio of H:L chain radioactivity in peak IV was

comparable to that in peak I (Table VI, VII). Since the molecular weight of peak IV was half that of peak I (Table V), it seemed clear that peak IV contained half molecules (HL).

The molecular weight of peak II was compatible with a structural formula of H_2L . Its H:L chain ratio was higher but not significantly different from peak I. The individual chains of peak II were the same size as the marker H and L chains (Fig. 5). The ratio of H:L chains in peak II may reflect spill-over from the adjoining peak I which was present in much larger amounts. However, it was clear that peak II contained both H and L chains, since comparison of the individual fractions between peaks I and II and throughout peak II revealed an absolute increase in both H and L chains in that peak.

TABLE VII
Characterization of Secreted Gamma Globulin Peaks

Origin of cells	Ratio of Radioactivity in heavy and light chains (H:L)						
	I	II	III	IV	V	VI	VII
Clone 1 culture*	2.90	3.50	4.50	2.90	1.22	0.10	0.02
Clone 1-(15)*· ‡	2.47	—	1.62	2.70	2.36		0.09
Clone 1 culture§	4.00	3.70	—	3.40	0.35		0.02
MPC-11 parent tumor*	2.67	—	—	2.84	0.55		0.01
Suggested structure based on molecular size	H_2L_2	H_2L	H_2, HL_2	HL	H	L_2	L

* The secretion was analyzed without immunological precipitation.

‡ Agar clone derived from the Clone 1 cultured cells.

§ Secretion was precipitated by the "indirect" method and the immune precipitate analyzed (see Materials and Methods and Fig. 6).

|| See Table V.

Experiments with a variant tumor which accumulates peak II may permit a definitive determination of its structure.

The molecular weight of peak III suggested that it might contain H chain dimers (H_2). When isolated from nonimmunologically precipitated cytoplasm of the cultured cells, peak III (Fig. 7) was found to contain cellular material (fractions 1-11) and heavy chains, (fractions 13-17) but few, if any, light chains. The recovery of peak III in immunological precipitates has been highly variable, and the ratio of H:L chains in these precipitates also varied greatly (Table VI). Although it is possible that the antiserum reacts poorly with H_2 , experiments now in progress suggest that when cytoplasmic extracts are incubated in the presence of antiserum, H_2 may assemble into larger subunits. The ratio of H:L chains in the immunologic precipitated peak III indicated that in addition to H_2 there were also some L chains containing molecules in this peak. Askonas and Williamson (25) have also demonstrated the presence of H_2 in the cytoplasm of X5563.

Peak V had a molecular weight comparable to that of free H chains (Table V). As in the case of peak III, it was more prominent in total cytoplasmic lysates than after immunological precipitation, and might have been either incompletely precipitated or undergone self-assembly. When dissociated with

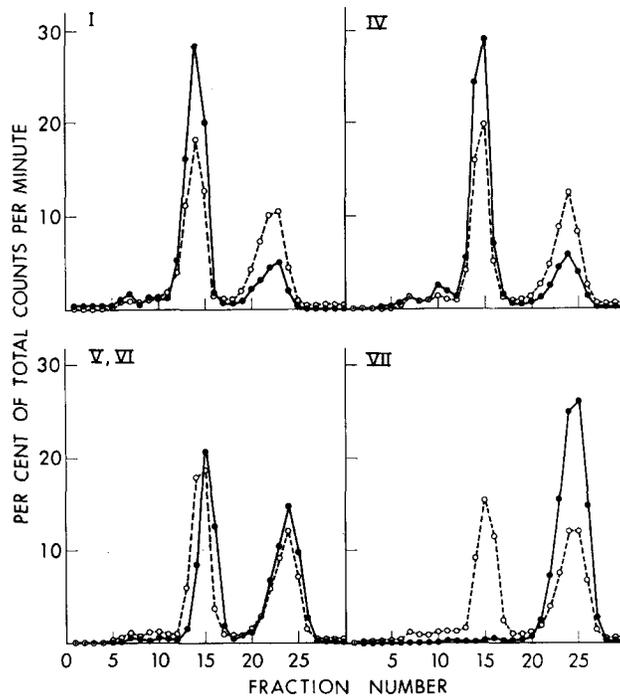


FIG. 6. Determination of the relative amounts of heavy and light chains in immunologically precipitated secreted gamma globulin peaks of clone 1 cultured cells. 5×10^6 cells were labeled with $15 \mu\text{c}$ each of threonine, valine, and leucine for 3 hr at 37°C . The secreted material was separated from the cells and treated with antiserum against MPC-11 myeloma protein. The immune precipitate was analyzed as described in the legend of Fig. 5. The results of the analysis of the four major peaks in the secreted material are shown. ³H-labeled secreted material from the MPC-11 parent tumor, (O---O), ¹⁴C-labeled secreted material from clone 1 cultured cells (●—●). The amount of radioactivity in heavy and light chains was calculated and the ratio of H:L chains determined (see Table VII, third row). The total number of ¹⁴C found in each of the gels was: I, 21,838; IV, 6080; V, VI, 6586; VII, 5898.

mercaptoethanol, material 5–10% smaller than the marker H chains was found in both the unprecipitated cytoplasm (not shown here) and in the immunological precipitates of both cytoplasm and secretion (Figs. 5, 6). We have concluded that peak V contained H chains, perhaps lacking some or all of their carbohydrate moiety. The presence of a defective H chain was also demonstrated in a freshly isolated agar clone derived from clone 1 cultured cells—clone 1-(15). (Results not shown.) In contrast to the cultured cells, peak

V from the tumor had the same electrophoretic mobility as the marker H chain.

Peak VI contained much less H than did peak V, had the molecular weight of L chain dimers (L_2), was immunologically precipitable, and when reduced, some of it was converted to single light chains.

In sum, the molecular weights and polypeptide composition of the various gamma globulin molecules produced by cultured myeloma cells have been

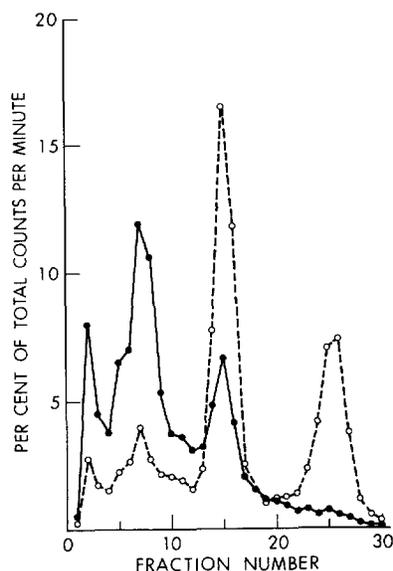


FIG. 7. Relative amount of heavy and light chains in peak III of nonimmunologically precipitated cytoplasm of clone 1 cultured cells. Conditions of labeling, separation of the various cytoplasmic peaks, and the dissociation into heavy and light chains of the individual peaks were the same as described in the legend of Fig. 5. ^3H -labeled secreted material of the parent MPC-11 tumor served as a marker for heavy and light chains ($\text{O}---\text{O}$) and was mixed with the ^{14}C -labeled cytoplasmic peak III ($\bullet---\bullet$) before reduction, alkylation, and separation of the two polypeptide chains.

examined and compared to those produced by the parent tumor. All the peaks contained gamma globulin polypeptide chains. Peak I contained H_2L_2 , peak IV contained HL, and peak VII contained L. Peaks II, III, and VI were present in relatively small amounts in both the cytoplasm and secretion of cultured cells, and in the parent tumor. We cannot yet assign a structure for peak II. Peak III probably contained H_2 , as well as a small amount of some L chain-containing molecules. Peaks V and VI contained free H chains and L_2 . Since the amounts of peaks II, III, V, and VI present in the secretion were very small, they may have been released into the medium as a result of cell lysis

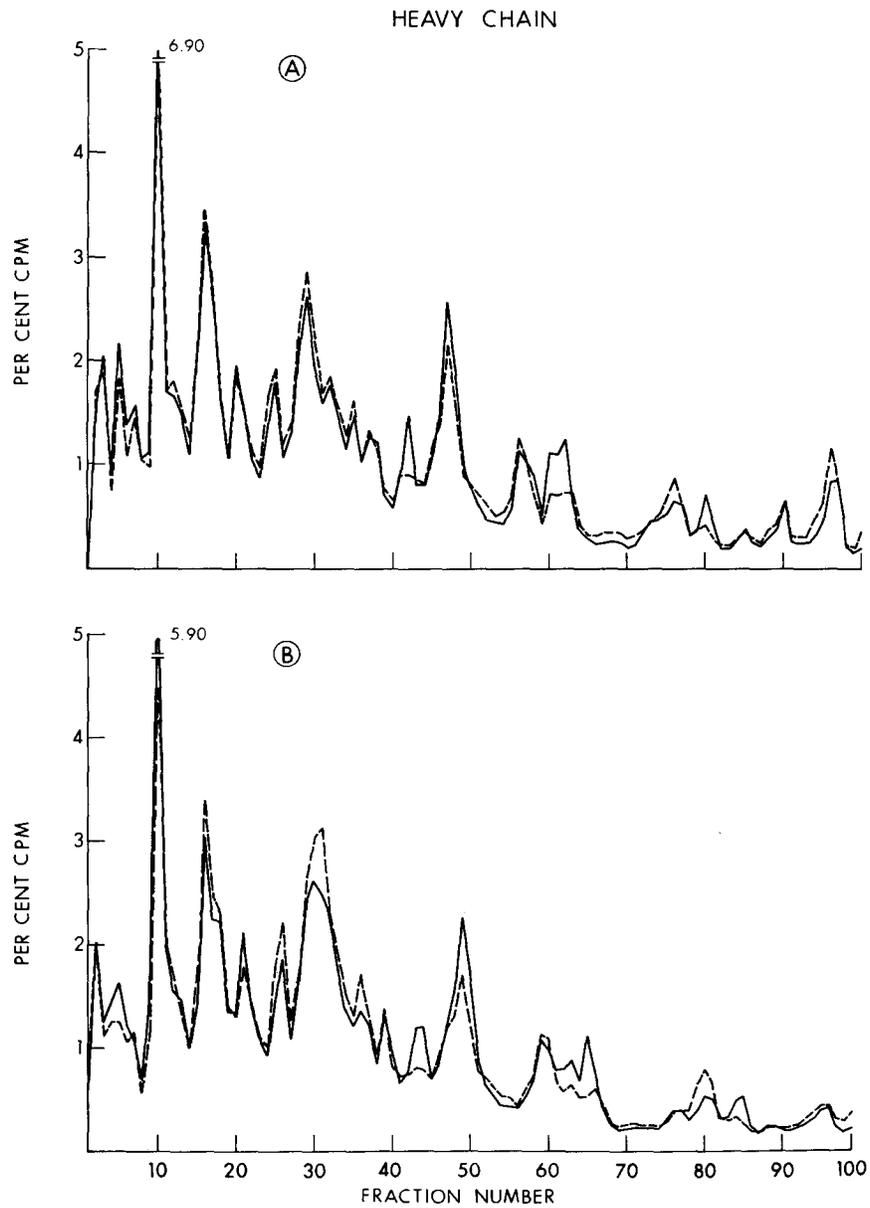


FIG. 8. Peptide column analysis of the heavy chains of MPC-11 parent tumor and clone 1-(15) cultured cells. Secreted material from myeloma tumor cells labeled with ^3H amino acids were mixed with ^{14}C -labeled secreted material from the cultured cells and dissociated into heavy and light chains (Materials and Methods). The heavy and light chains were separated on 7.5% 10 cm SDS-acrylamide gels. The chains were collected, treated with trypsin, and the peptides chromatographed on a Dowex 50 column as described in Materials and Methods. A, ^3H -labeled heavy chain of MPC-11 parent tumor (---) mixed with ^{14}C -labeled heavy chain of the same tumor (—). B, ^3H -labeled heavy chain of the MPC-11 parent tumor (---) mixed with ^{14}C -labeled clone 1-(15) heavy chains (—).

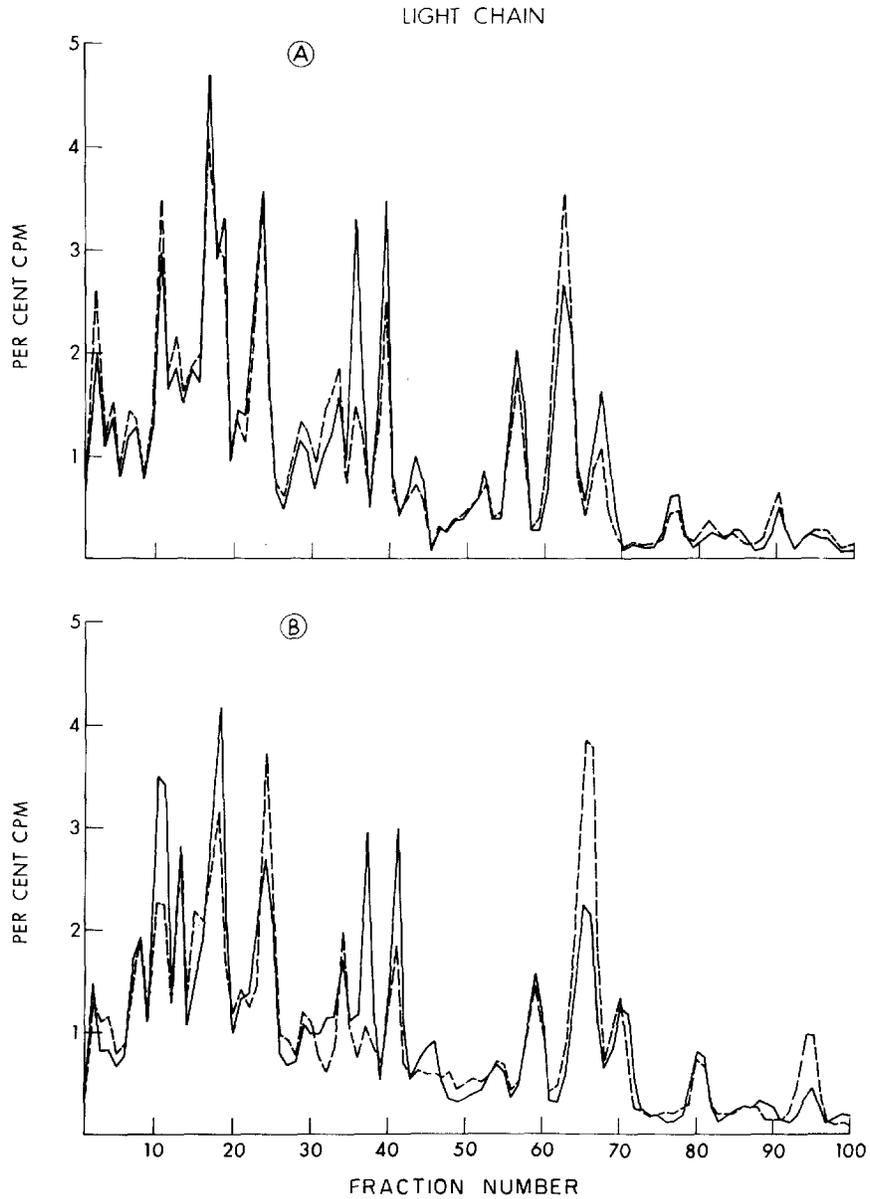


FIG. 9. Peptide column analysis of the light chains of MPC-11 parent tumor and clone 1-(15) cultured cells. Peptides of light chains from the secreted material of MPC-11 parent tumor and clone 1-(15) cultured cells were prepared and analyzed as described in the Material and Methods. A, ^3H -labeled light chains from MPC-11 parent tumor (---) mixed with ^{14}C -labeled light chains from the same tumor (—). B, ^3H -labeled light chains from MPC-11 parent tumor (---) mixed with ^{14}C -labeled light chains from clone 1-15 (—).

and may not be true secretory products. Finally, all these molecules were found, albeit in varying amounts, in the parent tumor, the cultured cells, and in individual clones derived from the cultured cells.

Comparison of the Tryptic Peptides of the H and L Chains Secreted by the Culture and Parent MPC-11 Tumor.—Since the pattern of gamma globulin synthesized by the cultured cells differed from that of the parent tumor, it was important to determine whether there were also changes in the primary structure of their H and L chains. ^3H -labeled secreted material from the tumor was mixed with ^{14}C -labeled secretion from an agar-clone (clone 1-(15)), reduced and alkylated, and the H and L chains isolated and subjected to tryptic digestion and subsequent analysis by column chromatography (Figs. 8, 9). In all cases simultaneously prepared ^3H - and ^{14}C -labeled material from the tumor were also compared as a control, since small differences had been noted in such control comparisons of differentially labeled, but otherwise identical, material (Figs. 8A, 9A). Those differences are probably attributable to the different relative specific activities of the individual amino acids within each mixture. The small differences between the H chains of the tumor and the culture (Fig. 8B) were also present in the control (Fig. 8A), and it was concluded that within the sensitivity of this method the cultured and tumor H chains were identical. The small difference between the light chains of the tumor and the culture in fractions 32 and 33 (Fig. 9B) was not observed in the control (Fig. 9A), and was also present when the label was reversed, i.e., when ^3H -labeled light chains from the culture were compared with ^{14}C -labeled light chains from the tumor. Because this difference is not present in a major peptide, further experiments will be necessary before it can be concluded that there is a real, though small, difference in the primary structure of the light chains of the culture and the tumor.

DISCUSSION

Cells obtained from the MPC-11 mouse plasma cell tumor were adapted to continuous culture and the gamma globulin molecules produced by the parent tumor and the cultured cells were compared. Molecules which were specifically precipitated with antiserum against mouse gamma globulin were further characterized with respect to their molecular weight on SDS-containing acrylamide gels and to the relative numbers of H and L chains which they contained.

Both parent MPC-11 tumor and cells cultured from that tumor produced fully assembled gamma globulin monomers (H_2L_2) and six other unassembled and partially assembled gamma globulin molecules. The H chains produced by the tumor and cultured cells contained identical tryptic peptides. The light chains were also very similar, although they may differ in one tryptic peptide. The culture and tumor differed in that the ratio of light:heavy chains was 3.5:1 in the tumor, but only 1.7:1 in the culture. In addition, the

tumor secreted relatively more free light chains and half molecules. Clones derived from single cells were found to synthesize all seven types of gamma globulin molecules, indicating that individual cells produced a spectrum of partially assembled and fully assembled gamma globulin molecules. The production of both fully assembled gamma globulin and its subunits by single cells is also supported by the finding that most of these subunits were precursors of the completely assembled molecule.⁴ All gamma globulin molecules found in the cytoplasm of the myeloma cells were present also in the secreted material. However, some of these gamma globulin subunits, including the presumptive free H chains, were found in such small amounts in the medium that their presence could have been due to a low level of cell lysis.

The cultured cells produced the same relative amounts of all seven types of gamma globulin molecules when incubated in the presence and absence of serum, and maintained the same pattern of gamma globulin synthesis after passage through animals. The pattern of gamma globulin synthesis by the tumor was also the same whether the cells were labeled *in vivo*⁴ or *in vitro* either as a cell suspension or as tissue fragments (4). These findings indicated that the patterns of gamma globulin synthesis which are characteristic of the parent tumor and of the cultured cells were quite stable, were not due to an artifact produced by the *in vitro* labeling conditions, and reflected the events as they were occurring in individual cells.

It is to be emphasized that the use of SDS, which disrupts noncovalent bonds, precluded the determination of the natural state of any of the gamma globulin molecules. All that could be concluded was that there were seven different species of covalently linked heavy and light chains. As a matter of fact, in a following paper⁴ it will be shown that most of the half molecules of the tumor exist as whole 7S monomers which lack a disulfide bond between the heavy chains.

A number of investigators have described the adaptation of mouse myeloma cells to continuous culture (26-28). The cultured cells described here probably represent a minor cell type which was either present in the parent tumor or which developed during "adaptation" to culture, and had a selective advantage. That such selection had occurred was supported by the fact that many of the tumor cells died during the initial stages of adaptation, and that the characteristic pattern of gamma globulin synthesis by the cultured cells remained stable and did not revert to that of the parent tumor when they were subsequently maintained by serial animal passage. Based on karyotypic analysis, Schubert and Horibata (29), have also concluded that a minor population of myeloma cells had been selected for during adaptation to culture.

⁴ Scharff, M. D., R. Laskov, and R. Lanzerotti. Synthesis, assembly, and secretion of gamma globulin by mouse myeloma cells. II. Assembly of the gamma G globulin molecule. In preparation.

After about 8 months in continuous culture all the clone 1 subcultures maintained in Petri dishes began to produce less H_2L_2 molecules and finally secreted L chains only. Another subculture of clone 1 which had been maintained in spinner bottles has continued to produce 7S gamma globulin for over $1\frac{1}{2}$ yr, but 12% of its cells were also found to secrete only light chains (22). The experiments reported here were performed before any major change was observed in the pattern of gamma globulin synthesis. However, since even these early cultures could have contained significant numbers of L chain-producing variants, most of the experiments were also performed on a freshly isolated 7S gamma globulin-producing agar-clone. Such clones contained less than 0.1% of cells secreting only light chains (22).

The structure of the various gamma globulin molecules was established by determining their size and polypeptide composition. H_2L_2 , H_2 , HL, H, L_2 , and L were identified in the cultured cells. Another molecule which may be H_2L was also present but its structure could not be definitively determined. The presumptive free H chain found in the cytoplasm of the cultured cells (mol wt 49,000) was smaller than the H chain in the assembled molecule (mol wt 53,000). This smaller H chain was not due to a minor cell type, since it was also present in a freshly isolated clone. We are currently examining the possibility that the difference in size between the two H chains is due to a lack of carbohydrate. Other myeloma tumors have also been found to contain a number of gamma globulin subunits. Askonas and Williamson (30) reported a pool of L chains in the X5563 myeloma tumor, and later presented evidence for the presence of small amounts of H_2 and H_2L in the same tumor (25). Schubert (31) reported the presence of HL in MOPC-21, and Namba and Hanaoka (28) recently found L, L_2 , HL, and free H chains in MOPC-31B. Free H chains have also been demonstrated intracellularly (32) and in the culture fluid of Burkitt lymphoma cells (33).

It is not known why many of the myeloma tumors secrete excess L chains while tumors which secrete excess H chains or only H chains (34, 35) are very rare. Excess production of L chains is not unique for the tumor cells. Lymph nodes derived from immunized rabbits have also been shown to secrete about two-fold molar excess of L chains (36). The overproduction of L chains could be due to the presence of a mixture of two myeloma cell types: one with balanced synthesis and the other which synthesizes L chain only (37). However, synthesis of excess L chains does occur in clones derived from both MPC-11 tumor and cultures. Although both tumor and cultured cells produced an excess of L chains, this excess was greater in the tumor cells. Overproduction of light chains could be due to (a) different numbers of genes actively synthesizing light and heavy chain messenger; (b) different rates of transcription of light and heavy chain messenger; (c) intermittent transcription during the cell cycle (38), with a longer period of transcription for light chain messenger;

(*d*) messengers with different stability or efficiency of attachment to ribosomes; (*e*) different rates of translation of heavy and light chain messenger; and (*f*) selective degradation of the heavy polypeptide chain.

Although degradation of both heavy (3) and light chains (31) has been suggested by others, pulse-chase experiments with both MPC-11 tumor (39) and cultured cells⁴ did not indicate any significant degradation of either heavy or light chains. Previous experiments have shown that the translation time of the heavy and light chains of the tumor cells were approximately proportional to their molecular weights (39). In the presence of 1 μ g/ml actinomycin-D the synthesis of the heavy and the light chains by the tumor and the cultured cells declined at a similar rate, with a half life of 3-5 hr,⁵ implying that at least by this method, the half lives of the messengers for the two chains were similar. There is as yet no experimental data available as to whether excess production of light chains is due to gene dosage, or to a control mechanism at the transcriptional level.

There were fewer half molecules in the cultured cells. It will be shown in a following paper that the half molecules are precursors for the whole 7S gamma globulin in the cultured cells, but are end products in the tumor.⁴

SUMMARY

MPC-11 myeloma tumor cells were adapted to growth in continuous culture. The cultured cells resembled the parent tumor in that they produced the fully assembled gamma globulin molecules as well as six unassembled molecules. Although cultured and tumor cells synthesized excess light chains, the molar ratio of light (L) to heavy (H) chains was approximately 1.7:1 in the culture, and 3.5:1 in the tumor. The cultured cells also produced fewer half molecules and free light chains than the parent tumor. Peptide column analysis did not reveal differences in the primary structure of the H chains derived from the parent tumor and the culture. The L chains may have differed by a minor peptide.

As much as 20% of the newly labeled cytoplasmic proteins and almost 100% of the proteins secreted by the cultured myeloma cells could be precipitated by specific antiserum. The immune precipitates contained seven different gamma globulin molecules, six of which were characterized according to their molecular size and H and L chain content as fully assembled molecules (H_2L_2), heavy chain dimers (H_2), half molecules (HL), H, light chain dimers (L_2), and L chains.

All gamma globulin subunits as well as the complete H_2L_2 molecule were produced and secreted by splenic clones of the parent MPC-11 tumor, and agar clones of the cultured cells. This indicates that the various gamma globulin

⁵ Laskov, R., and M. D. Scharff. Unpublished results.

subunits were produced by the same cell and did not reflect cellular heterogeneity with respect to gamma globulin synthesis.

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