embryo cells *in vitro*. Moreover, the cells of tumors induced in hamsters by MSV (Moloney) carry a defective viral genome that can be 'rescued' by co-cultivation of the hamster tumor cells with mouse embryo cells in the presence of a murine leukemia virus (4). The antigenicity of the "rescued" sarcoma virus is determined by the particular leukemia virus used as a "helper" virus in the system.

Our results suggest that infection of hamster cells with high concentrations of MSV may result in the selection of a non-defective sarcoma virus capable of replicating in hamster cells. However, the complex dose-response curve of hamster grown MSV in mouse embryo cells would not support this hypothesis. Alternatively, a "helper-virus" capable of replicating in hamster embryo cells may have been selected. These preliminary results indicate the need for a detailed comparison of mouse and hamster grown MSV.

Summary. Preliminary studies on the transformation of hamster embryo cells by a murine sarcoma virus are reported. Although hamster embryo cells are readily transformed by high concentrations of this virus, they are much less sensitive than are mouse embryo cells. The transformed hamster cells are spindle-shaped and stain very densely with May Grünwald-Giemsa. They grow vigorously and are easily maintained *in vitro* with continuous release of virus into the culture medium. The hamster grown virus shows a complex dose-response curve when assayed on mouse embryo cells. Virus-producing tumors are induced by inoculation of the hamster grown virus into newborn hamsters.

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1. Harvey, J. J., Nature, 1964, v204, 1104.
2. Chesterman, F. C., Harvey, J. J., Dourmashkin, R. R., Salaman, M. H., Cancer Res., 1966, v26, 1759.
3. Moloney, J. B., Nat. Cancer Inst. Monog., 1966, v22, 139.
4. Huebner, R. J., Hartley, J. W., Rowe, W. P., Lane, W. T., Capps, W. I., Proc. Nat. Acad. Sci., 1966, v56, 1164.
5. Hartley, J. W., Rowe, W. P., ibid., 1966, v55, 780.
6. Ting, R. C., Virology, 1966, v28, 783.
7. Simons, P. J., Dourmashkin, R. R., Turano, A., Phillips, D. E. H., Chesterman, F. C., Nature, 1967, in press.

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Production of Free Light Chains of Immunoglobulin by a Hematopoietic Cell Line Derived from a Patient with Multiple Myeloma.* (32327)

Y. MATSUOKA, G. E. MOORE, Y. YAGI, AND D. PRESSMAN
Departments of Biochemistry Research and Surgery, Roswell Park Memorial Institute, Buffalo, N. Y.

A number of hematopoietic cell lines have been derived from biopsy tissue of patients with Burkitt's lymphoma(1,2,3,4) and from the buffy coat of patients with various leukemias and lymphomas(5,6,7,8), other malignancies(9), and of normal individuals(10). Many of these cell lines produce various classes of immunoglobulins or their component polypeptide chains(11,12,13). Cultured cells and culture media generally showed reactions with antibodies specific to both heavy chains and light chains of human immunoglobulin, indicating that whole globulin molecules are synthesized in these cultured cells. However, some indication that the cells do not always synthesize equivalent amounts of heavy and light chains was obtained from the results of quantitative radiolabeled antibody technique(12). Indeed, light chains in free form were observed together with whole globulins in products of some of the cell lines(13, and unpublished results).

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We are reporting a cell line derived from a patient with multiple myeloma which produced and secreted only free, light chains of λ-type in culture. No indication for the presence of heavy chains was obtained, either in the cells or in the media. The cells resemble immature plasma cells by electron microscopy. No herpes virus-like particles which are frequently found in these cell lines were observed in this cell line.

Materials and methods. The materials used to establish and maintain these human hematopoietic cell lines have been described (14). The γ-, α-, and μ-heavy chains of immunoglobulins were examined by staining the cells with fluorescein-conjugated globulin of antisera specific to each heavy chain. These heavy chains and κ- and λ-light chains were also examined by immunodiffusion and immunoelectrophoresis of the spent culture media using antisera specific to each heavy or light chain. Specific antisera used have been described(12). Rabbit antiserum against Type II Bence-Jones protein was purchased from Antibodies, Inc., Davis, Calif., characterized by immunodiffusion and immunoelectrophoresis, and used as anti-λ chain reagent.

Spent culture media were obtained from suspension cultures containing about 1×10^6 cells per ml. Media were concentrated by pervaporation approximately 5- to 25-fold for immunodiffusion and 25-fold for immunoelectrophoresis. Immunodiffusion and immunoelectrophoresis were done in a 1.2% agarose layer on microscope slides. Light chains of λ-type used as a standard for estimating the amount of free λ-chain were obtained from a purified myeloma protein of λ-type IgA by the method of Fleischman *et al*(15).

Urine of the patient from whom this cell line was derived was used for comparison. It contained approximately 1-2 mg/ml of Bence-Jones protein of λ-type.

Case history. A 61-year-old man was hospitalized because of severe back pain. A diagnosis of multiple myeloma was established and the patient was transferred to RPMI on 5/31/66. A definite diagnosis could not be made from several bone marrow studies and there were no plasma cells in smear preparations of the peripheral blood. The serum protein level was 9.4 g% with 3.6 g% albumin and an electrophoretic analysis revealed an increase in γ-2 globulin and α-2 lipoproteins. The myeloma protein was found to be IgG of λ-type. Bence-Jones protein of λ-type was found in the urine.

The diagnosis of myeloma was confirmed by biopsy of a tumor mass on the chest wall. Cell cultures were set up from this biopsy tissue and from bone marrow but none survived more than several weeks except for fibroblasts. On 6/16/66 leukophoresis was done with 2 units of blood and 15 cultures were started. Several original cultures were combined and 66 days later an increase in growth rate and cell clumps was noted in one culture. The cell culture became self-sustaining and was designated as RPMI #8226.

Morphological characteristics. The cultured cells resembled the lymphoblastoid cells of other cell lines developed from peripheral leukocytes. No typical mature plasma cells were seen, however, there were immature cells with deep staining cytoplasm. Electron microscopy of almost all of the cells revealed a circum cellular arrangement of swollen endoplasmic reticulum which has not been noted in any of the other cell lines (Fig. 1). These structural features resemble the developing plasma cell. The cells are diploid (major) and polyploid with some abnormal chromosomes (H. Kitamura and G. E. Moore, unpublished results).

No herpes virus-like particles (leukovirus) which are commonly found in other cultured human hematopoietic cells(10,14) were found in this cell line.

Immunoglobulin studies. Smeared cells were stained with fluorescein-conjugated globulin of antisera monospecific to γ-, α-, or μ-heavy chains of human immunoglobulins. None of the cells were stained with any of the anti-heavy chain reagents indicating that the cells do not contain any of these 3 heavy chains.

Concentrated media from suspension culture were examined by immunodiffusion and immunoelectrophoresis against the above 3 anti-heavy chain sera and against antisera monospecific to either κ- or λ-type light chain.

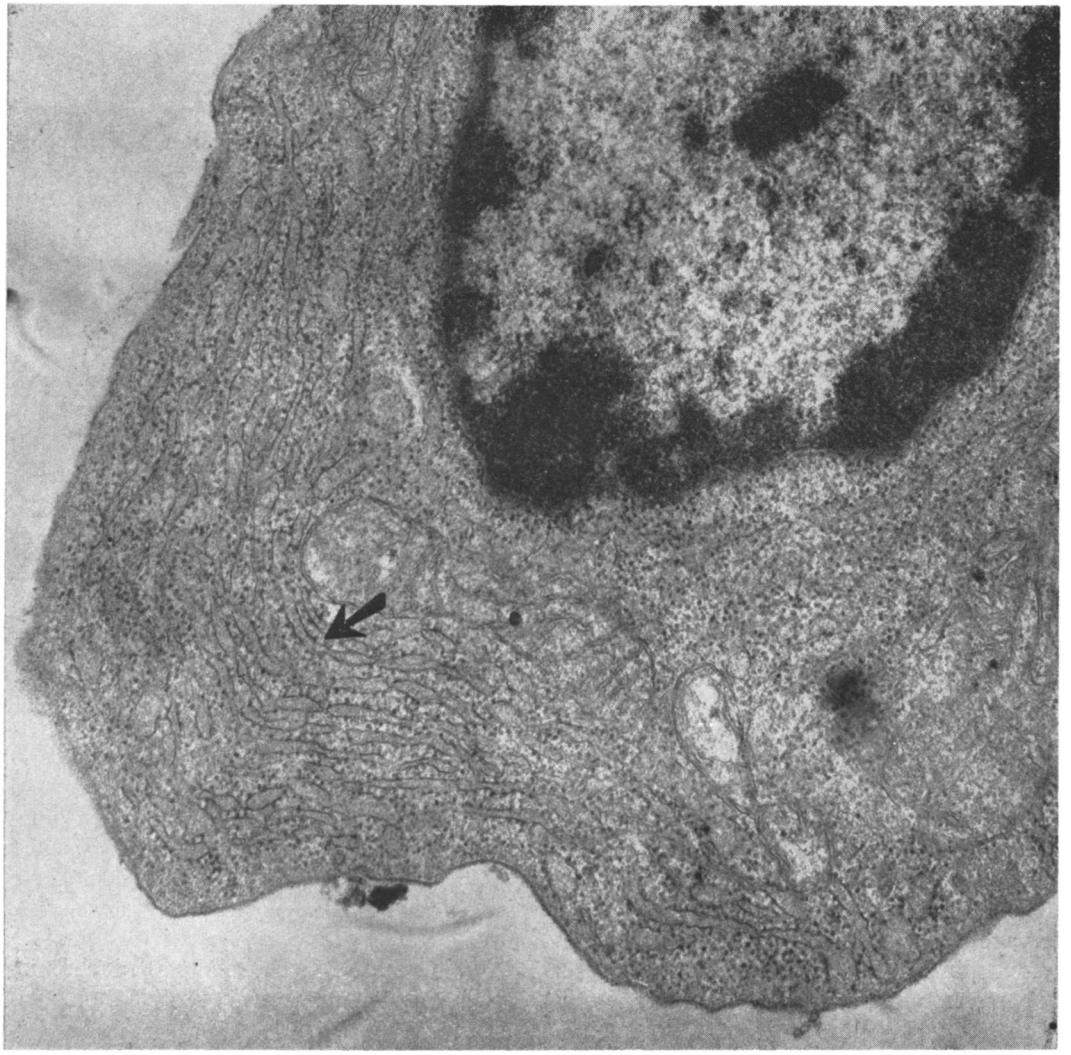

FIG. 1. Electronmicrograph of cultured cell (RPMI #8226). Note the circum cellular arrangement of endoplasmic reticulum shown by the arrow. This degree of development of these structures is not seen in lymphoblastoidal cells derived from patients with other diseases. × 32,000; uranyl acetate and lead citrate stain.

No reaction was observed with anti-heavy chain reagents and with anti-κ light chain reagent, but anti-λ light chain reagent showed a sharp precipitate arc with the culture media.

The protein reactive to anti-λ reagent was partially purified by electrophoresis and gel filtration from the culture media and the urine of the patient from whom the cell line was derived. For the recovery from culture medium, concentrated medium was first submitted to zone electrophoresis in 1.2% agarose at pH 8.6 (veronal buffer) to remove most of the proteins mainly derived from the fetal calf serum used for culture. The major part of the component reactive to anti-λ reagent was found in a narrow segment including the starting zone. Proteins in this segment were eluted by freezing and thawing, concentrated by pervaporation, and then the concentrated solution was applied on a column of Sephadex G-100. Three peaks of proteins were obtained (Fig. 2A). After concentration, the first peak was shown to contain mostly 7S γ-globulin of bovine origin. The second peak contained mostly albumin of bovine origin. The third peak contained

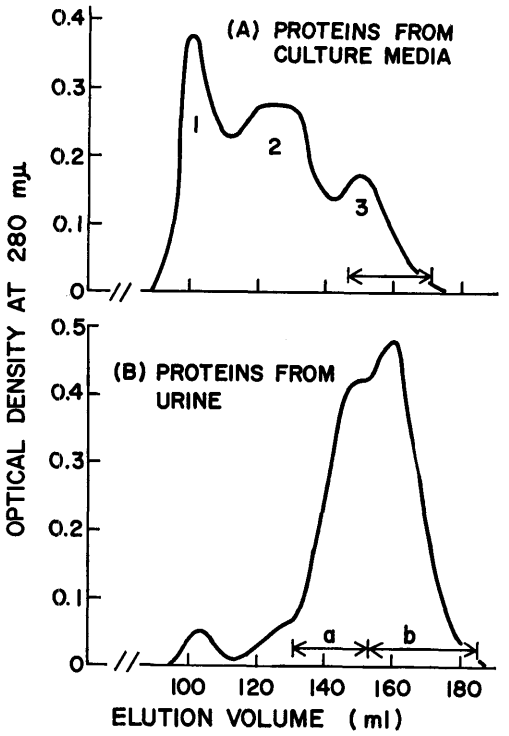

FIG. 2. Gel filtration pattern on Sephadex G-100 of partially purified fractions from culture media (A) and patient's urine (B). Fractions containing proteins reactive to anti-λ chain reagent were pooled as indicated and used for immunodiffusion and immunoelectrophoresis. 2.8 × 50 cm column with borate buffer pH 8.0. Sample volume: 1.4 ml for (A), 1.2 ml for (B).

large amounts of the component reactive to anti-human λ chain reagent along with some bovine serum albumin.

The Bence-Jones protein in the patient's urine was purified in an identical manner. The Bence-Jones protein eluted from the starting zone after agarose electrophoresis was concentrated and applied on the same Sephadex G-100 column used above. The Bence-Jones protein was eluted as a major peak which appeared to be a composite of two peaks, presumably due to dimer and monomer forms of the protein (Fig. 2B). The elution volume of this major peak corresponded to that of the third peak with proteins from the culture medium. Thus, it is clear that the component reactive to anti-λ chain reagent in the culture medium has a molecular size very similar to that of Bence-Jones protein in the urine. Edelman and Gally have shown that Bence-Jones proteins are light polypeptide chains of immunoglobulins in free form(16).

Immunodiffusion with the anti-λ chain reagent indicated the reaction of identity among the concentrated medium, the original urine, the purified proteins from the medium and the urine, and a λ-chain from a purified myeloma protein of IgA class. An example is shown in Fig. 3A. Upon immunoelectrophoresis (Fig. 3B), the reactive component from the culture medium migrated at the same rate as the Bence-Jones protein from the urine. However, in both cases, the reactive components in the starting materials migrated more slowly than those in the purified materials. The reason for this difference is not clear.

The above results indicate that this cell line from a myeloma patient produced λ-type light chains of human immunoglobulins in free form which are antigenically indistinguishable from the Bence-Jones protein in the patient's urine. No indication was observed for the presence of heavy chains, γ-, α-, or μ-chain.

The amount of free λ-chains produced by this cell line was estimated by the method of Fahey *et al*(17) by diffusing concentrated culture medium into an agarose layer containing antiserum. Using free λ-chain isolated from a λ-type IgA myeloma protein as standard, it was estimated that this cell line produced about 15 μg λ-chain per day per 10^6 cells (45×10^7 molecules per cell per day). This is about 20 times higher than the amount of immunoglobulin produced by some other cell lines on weight basis (unpublished results) and indicates that the cells are very actively producing free λ-chains.

A number of mouse plasma cell tumor lines producing only light chains of immunoglobulin *in vivo* have been reported(18).

Summary. A cell line derived from a myeloma patient was found to produce only λ-type light chains of immunoglobulin which were secreted into the culture medium. There appears to be no formation of γ-, α-, or μ-heavy chains or of intact immunoglobulins. The light chains produced gave a reaction of identity with the Bence-Jones protein of the

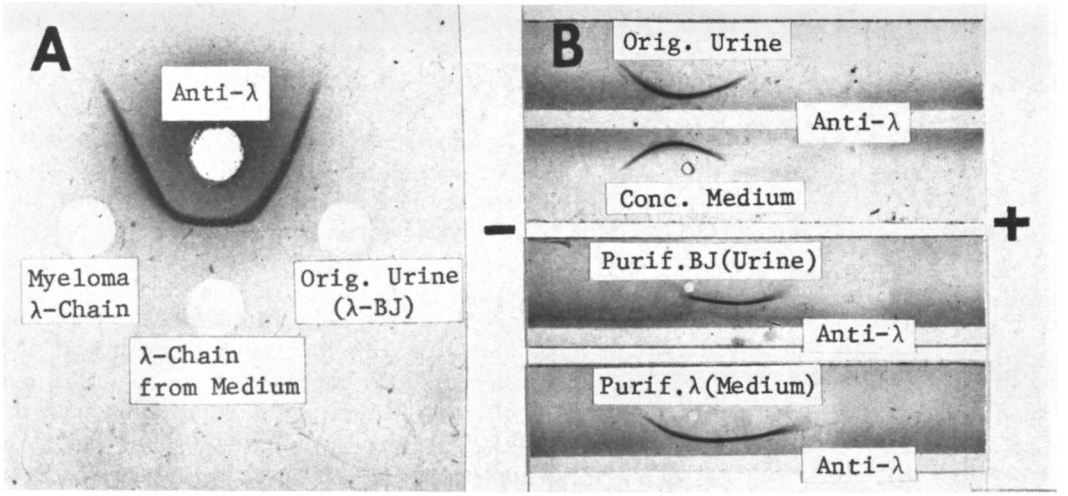

FIG. 3. Immunodiffusion (A) and immunoelectrophoresis (B). Sephadex fraction *a* from the urine was used as purified BJ in (B). Fraction *b* gave the same pattern. Electrophoresis was done in an agarose layer with the LKB 6800A-1 apparatus at 225 V for 60 min.

original patient's urine upon immunodiffusion and showed a similar molecular size upon gel filtration. The cells produced about 15 μg λ-chain per day per 10^6 cells (45×10^7 chain molecules per day per cell) under the culture conditions used.

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1. Epstein, M. A., Barr, Y. M., J. Nat. Cancer Inst., 1965, v34, 231.

2. Pulvertaft, R. J. V., Lancet, 1964, v1, 238.

3. Stewart, S. E., Lovelace, E., Whang, J. J., Ngu, V. A., J. Nat. Cancer Inst., 1965, v34, 319.

4. Minowada, J., Moore, G. E., Gerner, R. E., Toshima, S., Takagi, N., Sandberg, A. A., Special issue of East African Med. J., in press.

5. Iwakata, S., Grace, J. T., Jr., N. Y. State J. Med., 1964, v64, 2279.

6. Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., Boone, B. A., McCarthy, R. E., Cancer, 1965, v18, 522.

7. Armstrong, D., Proc. Soc. Exp. Biol. and Med., 1966, v122, 475.

8. Lucas, L. S., Whang, J. J., Tjio, J. H., Manaker, R. A., Zeve, V. H., J. Nat. Cancer Inst., 1966, v37, 753.

9. Moore, G. E., Gerner, R. E., Minowada, J., in The Proliferation and Spread of Neoplastic Cells, 21st Ann. Symp. on Fundamental Cancer Research at Univ. of Texas, M.D. Anderson Hosp. and Tumor Inst., in press.

10. Moore, G. E., Gerner, R. E., Franklin, H. A., J.A.M.A., 1967, v199, 519.

11. Fahey, J. L., Finegold, I., Rabson, A. S., Manaker, R. A., Science, 1966, v152, 1259.

12. Tanigaki, N., Yagi, Y., Moore, G. E., Pressman, D., J. Immunol., 1966, v97, 634.

13. Wakefield, J. D., Thorbecke, G. J., Old, L. J., Boyse, E. A., Fed. Proc., 1966, v25, No. 2, Part I, Abstr. No. 2664.

14. Moore, G. E., Grace, J. T., Jr., Citron, P., Gerner, R. E., Burns, A., N. Y. State J. Med., 1966, v66, 2757.

15. Fleischman, J. B., Pain, R. H., Porter, R. R., Arch. Biochem. Biophys., 1962, Suppl. 1, 174.

16. Edelman, M. D., Gally, J. A., J. Exp. Med., 1962, v116, 207.

17. Fahey, J. L., Lawrence, M. E., J. Immunol., 1963, v91, 597.

18. McIntire, K. R., Potter, M., J. Nat. Cancer Inst., 1964, v33, 631.

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