Synthesis of M Protein by Mouse Myeloma Tumor: Correlation of In Vitro and In Vivo Methods

By Wayne E. Truax and Juanita P. Bray

AN IN VITRO method demonstrating protein synthesis by neoplastic cells or tissue, particularly multiple myeloma, would offer several useful approaches to the chemotherapy of this cancer: (1) It could be used as a means of exploring basic mechanisms of protein synthesis and turnover; (2) it could serve as a means of studying paraprotein formation; and (3) it might serve as a screening system for therapeutic agents in which the tumor tissue, rather than the patient, would be used to assess the effects of new compounds. A system which has permitted the demonstration of the probable synthesis of myeloma protein by plasma cells in multiple myeloma has been reported.[1] This was accomplished by incubating bone marrow with tritiated d,l-leucine and thereafter determining radioactivity in protein fractions separated by filter paper electrophoresis. Correlation and confirmation of these findings with in vivo studies in the human is not economically practical because of the large quantity of tritium required. An animal system for such a comparative study utilizing the transmissible mouse myeloma tumors of Pilgrim and Potter[2] has been adopted for extension of these studies and in vitro methods comparable to the human myeloma system have been correlated with in vivo studies. The comparability of these results lend validity to the human myeloma in vitro system.

Methods

C3H mice of the Heston strain obtained from Bar Harbor, Maine, were used. Tumors of each type, 5563 and 5647 were obtained from M. Potter at the National Institutes of Health, Bethesda, Md. After samples of blood were taken for base line serum electrophoretic studies, tumors were planted subcutaneously in the right flank of the animals by trochar. Tail blood samples were taken weekly until tumor growth was established and serum protein abnormalities were demonstrated. Half of the animals in each group were sacrificed, bled, and the tumor removed aseptically. The tumor was minced with a scalpel, suspended in 2 cc. of mouse serum and 50 μc. H_3-d,l-leucine per cc. was added, Incubation was carried out at 37 C. for 20 hours. The serum was removed after centrifugation and dialyzed against saline at 4 C. for 48 hours. The samples were then placed on Schleicher and Schuell 2043A paper strips and electrophoresis carried out at 3 ma. for 17 hours in veronal buffer (pH 8.6, ionic strength 0.075) in a Spinco Durrum-type cell. After drying and fixation the strips were dyed in brom phenol blue, rinsed with 5 per cent acetic acid, developed in NH_4OH atmosphere, and scanned with a Spinco analytrol. Radioactivity assay was carried out in a Nuclear gas-flow chromatographic scanner using a direct writing recorder. The electrophoretic strips were compared with the radioactivity scan for localization and identification of protein components.

The second group of animals were injected intraperitoneally with 500 μc. H_3-d,l-leucine

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and sacrificed in 24 hours by exsanguination under ether anesthesia. The serum was dialyzed as above, subjected to electrophoresis and the strips were scanned to determine localization of radioactivity. Autoradiographs of tumor tissue sections were prepared from both groups of animals using Eastman NTB-2 liquid photographic emulsion and developed after 14–17 days dark room exposure at 4 C.

Results

The 5563 Pilgrim-Potter tumor characteristically produced a gamma type hyperglobulinemia. This pattern was identical in all animals bearing the tumor. The 5647 tumor produced a beta-2 type protein with similar characteristics. Figure 1, A–D present the electrophoretic patterns of sera from the respective mice before and after tumor "take" of the two protein types. Figure 1, E, F show the radioactivity scan of the sera after incubation of the tumors with the tritiated amino acid. Specific incorporation into the respective abnormal protein peaks is observed. This is similar to the results obtained with incubation of human myeloma marrow treated similarly.[1] Figure 1, G, H depict the radioactivity scan of the serum electrophoretic strips following injection of H_3-d,l-leucine into the animals. As in the tumor incubation, the major concentration of radioactivity is seen in the respective abnormal protein peaks. In addition, however, incorporation of radioactivity into albumin and alpha globulin is observed. Autoradiographs were prepared from both the incubated tumors and the tumors of injected animals. These are seen in figure 2 and show radioactivity in tumor cells.

Discussion

Any in vitro biological system is open to the criticism that in vivo physiologic conditions are not being duplicated. Therefore, whenever possible, parallel experimentation with both systems should be conducted so that similarities and differences may be detected. Since a human in vivo system is not available, an animal system which bears remarkable similarities to its human counterpart has been studied. The advantages of the mouse myeloma tumors in a study of myeloma protein synthesis include the constancy of the protein produced. Whereas, in the human, each myeloma patient produces a protein which is different chemically, physically and immunologically from all others,[3] mouse myeloma No. 5563 always produces the identical gamma type protein in all animals inoculated, and 5647 always produces a beta-2 globulin.

The in vitro incubation of the myeloma tumors with tritiated d,l-leucine resulted in the appearance of the two typical myeloma proteins tagged with radioactivity. Cell-free mouse serum incubated similarly with tritiated leucine failed to do this. That this in vitro incubation probably reflects physiologic processes is supported by similar protein incorporation of H3-d,l-leucine when the amino acid was injected into the living animal. In addition to incorporation of the amino acid into the myeloma globulin, radioactivity was also observed in the albumin and alpha globulin fraction and was presumably produced by the liver or other tissues. It would appear therefore, that in vitro incubation offers certain advantages over in vivo studies, in that tissues isolated from the whole animal can be studied individually, and can be used to demonstrate

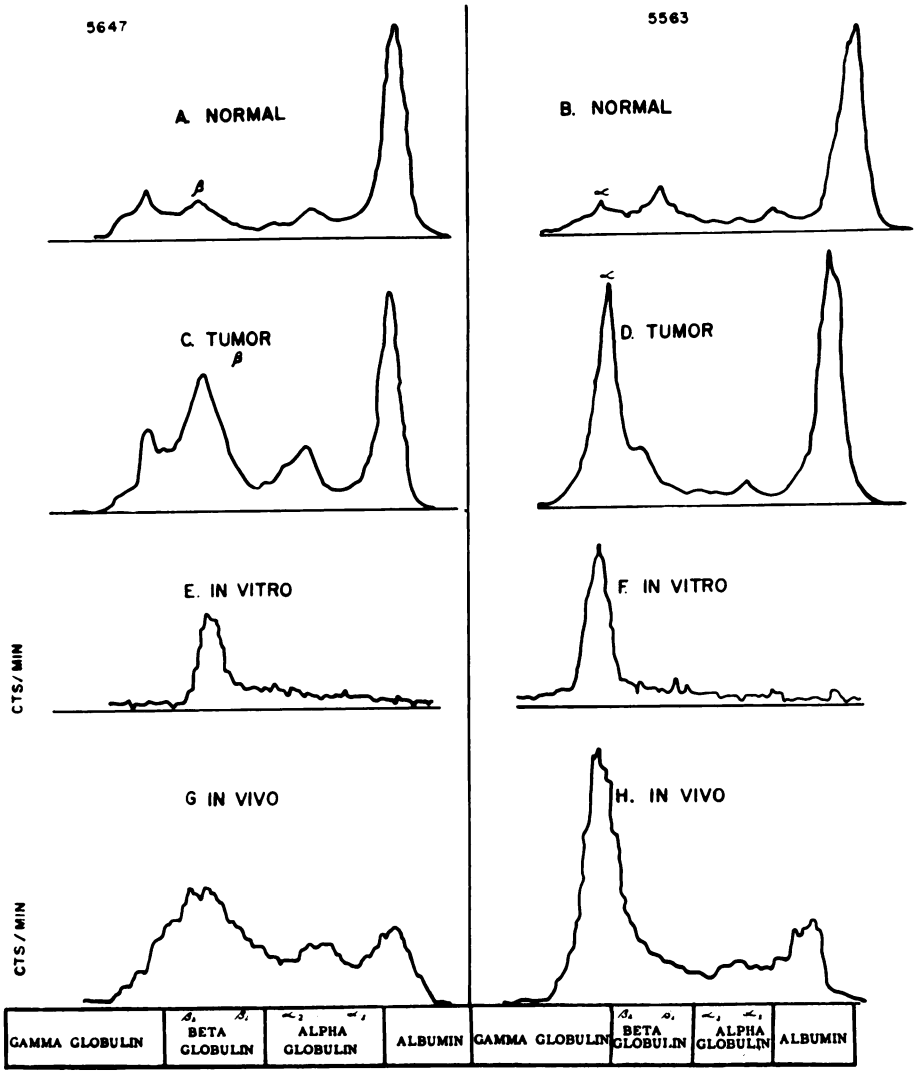

Fig. 1.—(A, B) Serum electrophoresis of normal mice prior to tumor implantation. **(C)** Ibid. 28 days following implantation of beta myeloma tumor No. 5647 **(D)** Ibid. 28 days following implantation of gamma myeloma tumor No. 5563. **(E, F)** Radioactivity scan of C and D following 20 hour in vitro incubation with H_3-d,l-leucine. **(G, H)** Radioactivity scan of serum electrophoretic strips following injection of H_3-d,l-leucine.

gamma and beta globulin synthesis only. Autoradiographs of in vitro incubated tissues and in vivo injected animals both revealed radioactivity in the tumor cells and indicated their involvement in the synthetic process (figs. 2a and 2b). Autolysis, though present in the incubated tumors, did not prevent the identification of active cells.

These data support the validity of previously reported in vitro human myeloma studies in which the bone marrow was shown to incorporate tritiated

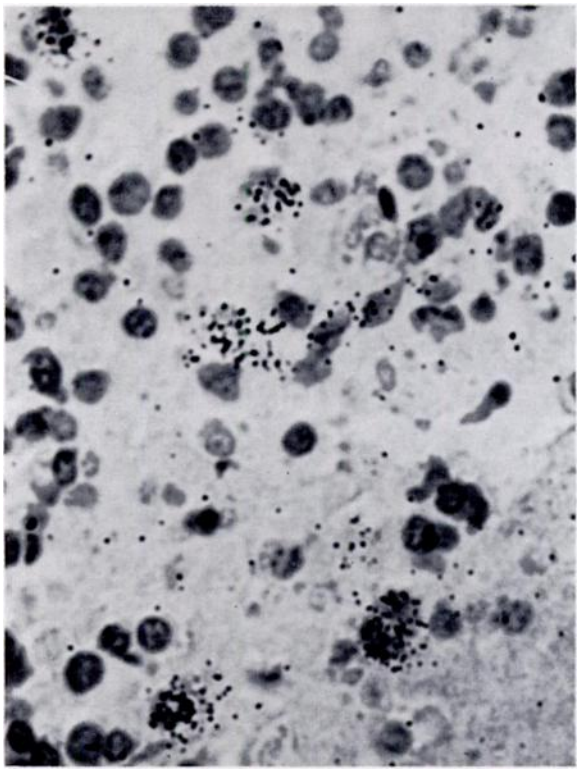

Fig. 2a.—Autoradiograph of myeloma tumor following intraperitoneal injection of H_3-d,l-leucine; 17 days exposure; 570 X.

leucine into protein and implicated the myeloma cell as the site of such incorporation.

This confirmation with animal tumor studies supports the premise that the in vitro human system may be a useful tool for the study of protein synthesis and turnover, of paraprotein production, and that it may serve as a possible chemotherapeutic assay system in the search for agents effective in the management of human neoplasia.

Summary

1. In vitro incubation of Potter-Pilgrim No. 5563 and No. 5647 mouse myeloma tumors with tritiated-d,l-leucine produced demonstrable radioactivity in the respective myeloma proteins.

2. In vivo inoculation of H_3-d,l-leucine in tumor bearing animals showed similar incorporation and in addition showed incorporation of radioactivity into albumin and alpha globulin.

3. It is suggested that the in vitro studies better serve to identify the isolated tissue function in protein synthesis.

4. Mouse myeloma studies confirm previous results in human multiple myeloma and indicate that in vitro incubation of protein producing tissues with H_3-d,l-leucine can demonstrate protein synthesis.

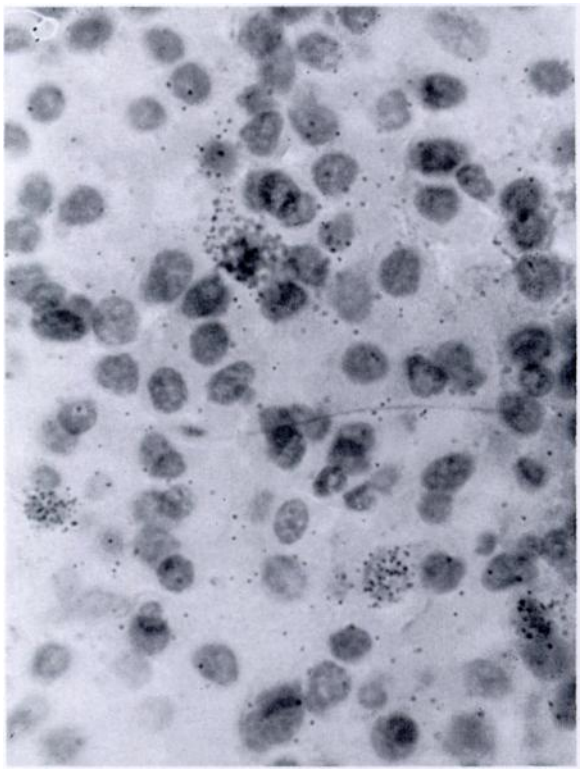

Fig. 2b.—Autoradiograph of myeloma tumor following in vitro incubation of tumor with H_3-d,l-leucine; 14 days exposure; 1080 X. (Note: Despite autolysis during incubation, the radioactivity can easily be localized over the tumor cells.)

Summario in Interlingua

1. Le incubation in vitro de tumor myelomatose murin Potter-Pilgrim No. 5563 e No. 5647 con tritiate d,l-leucina produceva demonstrabile grados de radioactivitate in le respective proteinas de myeloma.

2. Le inoculation in vivo de d,l-leucina a H_3 in animales tumorifere monstrava un simile incorporation e in plus le incorporation de radioactivitate in albumina e globulina alpha.

3. Es presentate le conclusion que studios in vitro servi melio a identificar le function de tissu isolate in le synthese de proteina.

4. Studios con myeloma murin confirma previe resultatos de studios con multiple myeloma human e indica que le incubation in vitro de tissu que produce proteina con le presentia de d,l-leucina a H_3 pote demonstrar le synthese de proteina.

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