however, prevent the loss of infectivity of SFV and WEE at 37°C in Earle's solution (Table I). Non-specific polymer interaction and specific protein-protein interactions are other mechanisms through which albumin could exert a stabilizing effect. Even highly purified preparations of albumin may contain significant amounts of other materials such as fatty acid(12), and such contaminants may be the stabilizing agent.

The mechanisms by which amino acids provide some measure of stability to SFV and WEE are also obscure. Cysteine has been reported to stabilize both EEE and WEE by acting as a reducing agent(13,14). Which amino acids are responsible for the increased resistance to inactivation indicated in Table I is not yet known; preliminary studies indicate that more than one may be effective. It is of interest that insects, a frequent vector of arboviruses, characteristically have high levels of free amino acid in their blood(15).

Summary. SFV retained a high level of infectivity from pH 6 to above pH 10 when serially diluted in 0.1 M phosphate buffer. After incubation of such serial dilutions at 37°C, infectivity persisted at 10^{-3} dilutions only above pH 6. The presence of 0.1% bovine serum albumin resulted in a marked increase in thermal stability at 37°C above pH 6 but not in more acid solutions. On dilution in several tissue culture media both SFV and WEE retained a higher level of infectivity in media containing a mixture of amino acids or glutamine than in media containing glucose and inorganic salts only.

We wish to acknowledge the able technical assistance of Mr. Richard Charlat.

1. Bauer, J. H., Mahaffy, A. F., *Am. J. Hyg.,* 1930, v12, 175.
2. Howitt, B. F., *J. Inf. Dis.,* 1934, v55, 138.
3. Speir, R. W., *Virology,* 1962, v17, 588.
4. Reed, L. J., Muench, H. A., *Am. J. Hyg.,* 1938, v27, 493.
5. Duffy, C. E., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 333.
6. McLimans, W. F., *J. Immunol.,* 1947, v56, 385.
7. Duffy, C. E., Stanley, W. M., *J. Exp. Med.,* 1945, v82, 385.
8. Finkelstein, H., Marx, W., Beard, D., Beard, J. W., *J. Inf. Dis.,* 1940, v66, 117.
9. Dick, G. W. A., Taylor, R. M., *J. Immunol.,* 1949, v62, 311.
10. Theiler, M., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 380.
11. Charney, J., Machlowitz, R. A., Spicer, D. S., *Virology,* 1962, v18, 495.
12. Foster, J. F., in *The Plasma Proteins,* Academic Press, 1960, v1, 180.
13. Bang, F. B., Herriott, R. M., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 177.
14. Labzoffsky, N. A., *Canad. J. Res., Sect. E., Med. Sci.,* 1946, v24, 119.
15. Buck, J. B., in *Insect Physiology,* John F. Wiley & Sons, Inc., 1953, 174.

Received June 5, 1963. P.S.E.B.M., 1963, v114.

Immunofluorescent Studies of Human Plasma Cells in γ and β_2A Myelomas. (28617)

PIERRE BURTIN AND DENISE BUFFE (Introduced by B. Waksman)
Laboratoire d'Immunochimie, Institut de Recherches Scientifiques sur le Cancer, Villejuif (Seine).

In human myeloma one can find a marked increase of γ globulins or β_2A globulin in the serum, and, in both cases, proliferation of plasma cells. We have tried to establish a correlation between myeloma protein production and cytologic abnormalities, using fluorescent specific antibodies.

Material and methods. Myeloma plasma cells were obtained by bone marrow aspiration. The aspirate (except for a part which was smeared and stained with May-Grünwald-Giemsa) was mixed with Hanks' solution containing heparin, centrifuged at 500 r.p.m. for 5 min., washed twice with Hanks' solution, smeared on coverslips, air dried and fixed by alcohol for 5 minutes at room temperature. In some experiments osmotic lysis of red cells was obtained, at least for most

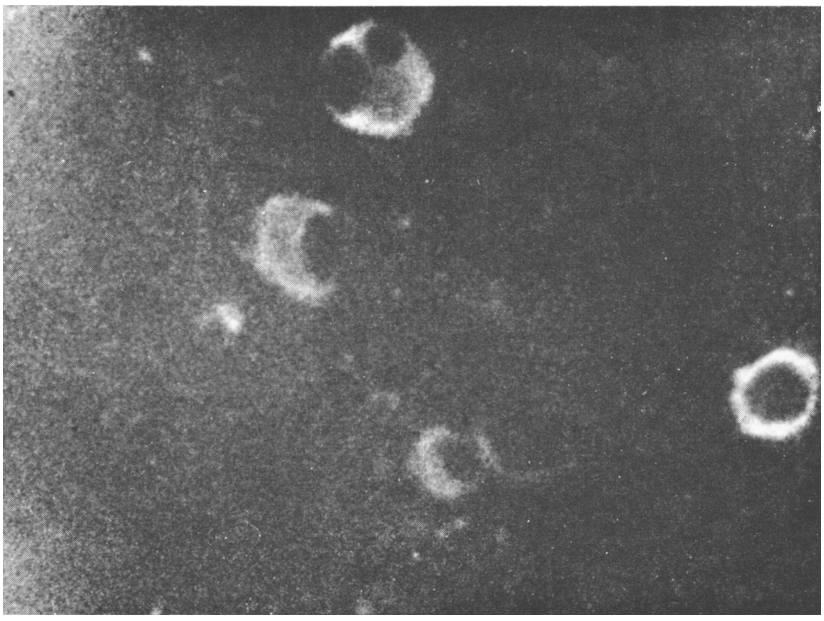


FIG. 1. γ myeloma cells stained by ITCF conjugated anti γ globulin antiserum: 2 plasma cells, 1 binucleated plasma cell, 1 round cell (plasmablast?) ($\times$ 500).

of them, by washing the aspirate once with Hanks' fluid diluted with an equal volume of distilled water.

Rabbit antisera against γ globulin absorbed by human milk globulins (containing a large amount of β_2A globulin and no γ globulins), and against β_2A globulins absorbed by cord serum (free of β_2A globulin), were conjugated to fluorescein isothiocyanate or sulforhodamine chloride, then purified by filtration on Sephadex G 25 and chromatography on DEAE cellulose, according to Goldstein *et al.* (2).

Cover slips were placed in humidified Petri dishes and flooded with fluorescent antiserum for 20 minutes, then washed twice with buffered saline, once with distilled water, and air dried. In some cases they were flooded again with another antiserum having different specificity and fluorescence, using the same procedure.

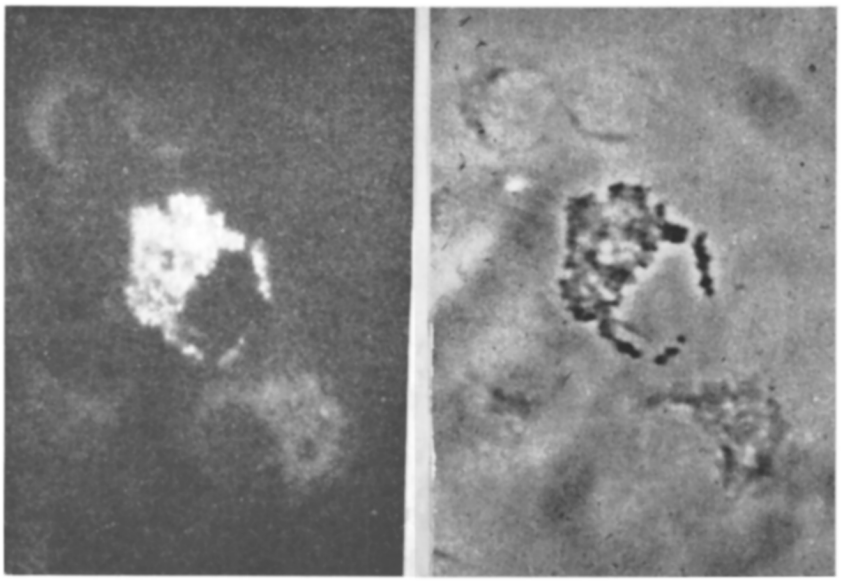
Fluorescence microscopy was carried out using a Wild microscope, an ultraviolet light source Osram HBO 200, and as filters a UG1 excitating filter, a BG 23 transmitting filter and a GG4 absorbing filter. Microscopic fields were systematically studied using phase contrast. Photographs were taken with Super Anscochrome Daylight film with an exposure time from 2 to five minutes.

Sera of patients were always studied by immunoelectrophoresis, using horse antisera against normal human serum (from Pasteur Institute), and rabbit antisera against γ and β_2A globulin.

Results. Bone marrow of 32 patients was examined, among them 13 γ myelomas, 16 β_2A myelomas, one γ and β_2A myeloma, one case showing Bence Jones Protein in the serum, and one case with a marked decrease of γ and β_2A globulin. As some patients had bone morrow studies 2 or more times, 37 bone marrow aspirates were studied. Nearly all of them were examined with anti γ and anti β_2A antibodies; some were examined only with antiserum corresponding to the immunoelectrophoretic type of myeloma.

In 20 experiments, no fixation of fluorescent antibodies occurred, although many plasma cells were present in aspirates. In 17, cells were observed fixing fluorescent antibodies and could be analyzed.

Fluorescent cells were always plasma cells, of different types. Their size was variable, even in the same patient. Nucleus size and location were different from one cell to another, indicating antigen presence in mature plasma cells with an eccentric nucleus and an



A B

FIG. 2. A. Large plasma cell from a γ myeloma, stained by ITCF conjugated anti γ globulin antiserum: note the fluorescent granulations ($\times$ 500). B. Same field seen in phase contrast. Note other plasma cells, which fix fluorescent antibodies very little or not at all ($\times$ 500).

abundant cytoplasm, as well as in immature cells with a big central nucleus (Fig. 1). We observed some bi- or multinucleated plasma cells showing fluorescence. Some cells having a buddy irregular nucleus were found in one case.

The proportion of fluorescent cells was sometimes high, sometimes low. In all cases, some of the plasma cells were not labelled, and in some cases, most of them were unlabelled. There was no correlation between the type of plasma cells and the fixation of fluorescent antibodies, except in one way; the fluorescent cells often had a granular structure, generally better seen in phase contrast than in fluorescence, sometimes very impressive: the big fluorescent granulations seemed very close to Russel Bodies (Fig. 2).

Fluorescence was always cytoplasmic, evenly distributed throughout the cytoplasm or most marked in the perinuclear region. Archoplasm often seemed to be less fluorescent. Fluorescent intranuclear granulations were sometimes seen, but they may be due, at least in some case, to the projection of cytoplasmic granulation onto the nuclear area.

The relationship of immunofluorescence and myeloma type was carefully studied. In 13 γ myelomas, a positive reaction with fluorescent anti γ antibodies was obtained 8 times. Some plasma cells were sometimes seen giving a positive reaction with anti β_2A antibodies, even if β_2A globulin level in serum was very low. Fluorescent plasma cells of different color and specificity could thus be seen in the same field. In one case, fixation of anti γ antibodies was negative, but a few granular plasma cells gave a strong positive reaction with anti β_2A globulin.

Among 16 β_2A myelomas, immunofluorescence reaction was negative in nine. When positive, they were obtained with anti β_2A antibodies (Fig. 3) and, to a lesser extent, with anti γ antibodies. Plasma cells of different color were also seen in the same field. We could never demonstrate double labelling in the same cell. The three other cases were negative.

Discussion. One of the most important findings is the high number of plasma cells which do not fix fluorescent antibodies. Is our technical procedure responsible for this? Protein loss during washing cells may have occurred, but studies of unwashed aspirates demonstrate no increase in number of fluorescent cells detectable. Non-penetration of fluo-

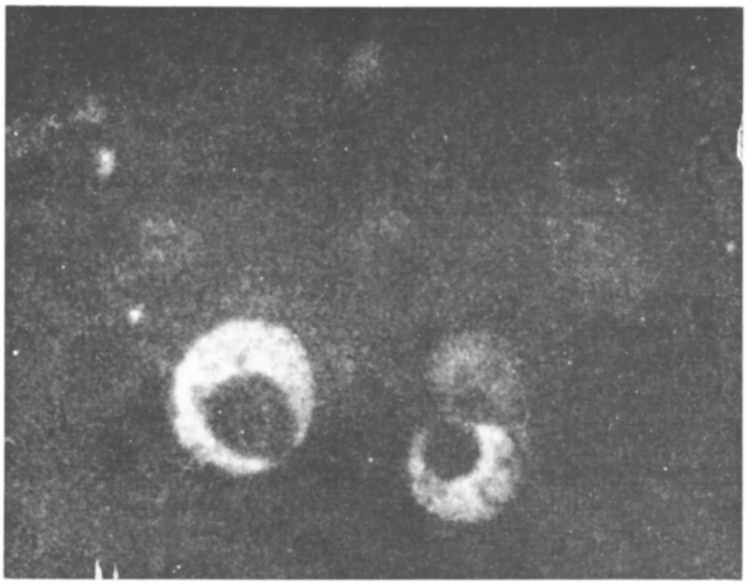


FIG. 3. β_2A myeloma plasma cells stained with SR conjugated anti-β_2A globulin serum ($\times$ 500).

rescent antibodies into cells is unlikely, as alcohol fixation and air drying alter the permeability of cellular membranes. Lack of sensitivity of the direct immunofluorescent method in comparison with the indirect one has to be taken into account, as it may explain the discrepancy between our results and those of Solomon *et al.*(3). Solomon, using the indirect immunofluorescent technique, found labelling of all the plasma cells.

Even if this explanation is correct, apparent non-fixation of fluorescent antibodies by cells means that those cells contain at most a small amount of γ globulins, or β_2A globulin, at the time of examination. Did they already excrete the protein? Are they not producing these proteins at time of study? The latter explanation is the most likely, as judged by the positive correlation between the granular structure of cytoplasm, visible in phase contrast microscopy, and the fixation of fluorescent antibodies: cytoplasmic granulations may correspond to ergastoplasmic vesicles seen on electron micrographs, and reveal cell secretory activity. If bone marrow aspirate does not contain secretory plasma cells, or very few, one has to admit that myeloma proteins are synthetized in other places, and therefore that, in myeloma patients secretory and non-secretory plasma cells exist together. A sternal marrow puncture explores only a very small part of the lymphoplasmocytic system, and foci of secretory plasma cells could probably be easily found in different parts of the skeletal system.

Positive findings establish a correlation between plasma cell proliferation and myeloma protein secretion. They are in good agreement with Vazquez's results in γ myelomas (4), Solomon's in γ and β_2A myelomas(3). However there is no strict correlation between myeloma type and immunofluorescence findings, in contrast to Solomon's conclusions. In γ myelomas, some plasma cells do fix anti β_2A antibodies, and anti γ antibodies are fixed in β_2A myelomas. In both cases, there often is a double population of plasma cells, having a specific capacity of synthetizing either γ globulins or β_2A globulin. This is in agreement with our findings in Waldenström's macroglobulinemia lymph nodes(1), where cells were found to contain γ globulins or β_2M globulin, but never both. Preliminary studies in human normal spleen confirm the specific synthesis of one immunoglobulin by plasma cells. It seems therefore that human plasma cells can elaborate only one immune globulin, at least at a specific moment of their life.

Summary. Thirty-seven bone marrow as-

pirates of human γ and β_2A myelomas were smeared and labelled by fluorescent antibodies against γ and β_2A globulin. Never were all the observed plasma cells reactive with fluorescent antibodies, and in half of the cases, no fixation by any plasma cell was noted. Plasma cells of different types were fluorescent, most of them having a granular structure. They were found to fix fluorescent antibodies against γ globulin or β_2A globulin, but never both.

1. Burtin, P., *Rev. Fse. Et. Clin. Biol.,* 1961, v6, 1044.

2. Golstein, G., Slyzys, I. S., Chase, M. W., *J. Exp. Med.,* 1961, v114, 89.

3. Solomon, A., Fahey, J. L., Malmgren, R. A., *Blood,* 1963, v21, 403.

4. Vazquez, J. S., *J. Lab. Clin. Med.,* 1958, v51, 271.

Received June 17, 1963. P.S.E.B.M., 1963, v113.

"Cyclopin": A Trypsin Sensitive Constituent of *Penicillium cyclopium* With Antiviral Properties.* (28618)

KIARASH NAFICY AND DAVID H. CARVER (Introduced by J. F. Enders)

Research Division of Infectious Diseases, Children's Hospital Medical Center, Department of Pediatrics, Harvard Medical School, and Children's Hospital Medical Center, Boston, Mass.

During an experiment with Sindbis virus in human amnion cell cultures, it was noted that in one culture the expected cytopathic changes did not occur. It was then observed that a fungus was growing in this tube. The fungus was cultured in Sabouraud's medium and identified as a penicillium by Dr. George Foley and Professor William H. Weston of Harvard University and subsequently as *Penicillium cyclopium* by Dr. Kenneth Raper of University of Wisconsin.

Preliminary tests revealed that cytopathic effects of Sindbis virus were regularly suppressed by extracts prepared from the penicillium. Experiments described below were then performed in attempts to elucidate the mechanism involved in inhibition of Sindbis virus by the extracts and to define some of the physical and chemical properties of the inhibitor. Information was also sought regarding the spectrum of viruses inhibited by this factor (or factors) in the fungal extract which for convenience of reference is here provisionally designated "cyclopin."

Materials and methods. Cell cultures. Primary cultures of human amnion (HA) and chick embryo (CE) cells were prepared as previously described(1,2). Human amnion cells were kept for at least 3 weeks prior to inoculation of arboviruses(3). Grivet monkey kidney *(Cercopithecus aethiops)* (MK) cell cultures were obtained commercially.†

Maintenance medium. Bovine amniotic fluid (BAF) was used in all cultures(4).

Viruses. The following viruses were employed:

Sindbis: Strain AR339 Taylor, A. R. CE 10, HA 4.

Western Equine Encephalitis (WEE) Johnson, H. Bird 628, Tc 00218, P15, cl 1 HA8.

West Nile: Sarafend Strain, Appendix cell line 6, CE 8.

Chickungunya: Suckling Mouse Brain 3, HA 2.

ECHO 11: Gregory Strain, MK 6, HA 4.

Poliovirus Type I: Brunhilde. Stock strain.

Herpes simplex: Zacchardi Strain, rabbit cornea 1, chick amniotic sac 5, CE3, HA 6.

Adenovirus Type II: Laboratory Strain Norian, WS 2 (stable line of HA) HA 4.

Coxsackie A9: Laboratory Strain Berkman, MK 4.

Coxsackie B2: Laboratory Strain Kransler, MK 6.

Coxsackie B5: Laboratory Strain Keating, MK 5.

Antisera. Sindbis rabbit antisera were pre-

* Supported by Research Grant from Nat. Inst. of Allergy and Infect. Dis., U. S. P. H. S.

† Microbiological Associates, Bethesda, Md.