Intranuclear Inclusions in Multiple Myeloma and Macroglobulinemia

By GEOFFREY M. BRITTIN, YASUKAZU TANAKA AND GEORGE BRECHER

ANY DISTINCTIVE inclusion bodies have been described in the plasma cells of inflammatory and neoplastic diseases and in plasma cell tumors. Most of these structures have been in the cytoplasm of the plasma cell, and hematologists are familiar with Russell bodies,1 Mott cells,1 "grape cells,"2 "morula cells,"3 and crystalline precipitates.4,5 In addition, Sorensen has recently described inclusions resembling viral particles in the cytoplasm of plasma cells of a case of multiple myeloma.6 There are only a few reports of inclusions in plasma cell nuclei,7-13 and some of these structures actually represent cytoplasmic invaginations.14 Various authors have attributed diagnostic significance to intranuclear inclusions in plasma cells.12,13

It is the purpose of this report to present evidence that inclusions in plasma cell nuclei may be of nuclear origin, that they may represent nuclear elaboration of protein, and that they may be found in several immunoelectrophoretic varieties of multiple myeloma as well as in macroglobulinemia.

Materials and Methods

Intranuclear inclusion bodies were found in the plasma cells of nine patients with plasma cell malignancies and in a single plasma cell of a patient with an apparently reactive plasmacytosis. They were found repeatedly in all patients except one (DR), in whom they were present in only a single bone marrow specimen. Six patients had multiple myeloma, two of the gamma type, three of the beta-2A type, and one with Bence Jones protein only. Three patients had macroglobulinemia, and two of them elaborated Bence Jones protein in addition to macroglobulins. An additional three cases of macroglobulinemia with intranuclear inclusion bodies in lymphoid plasma cells have been reported from this institution.12 The total of six cases represents approximately 30 per cent of the 20 cases seen at this institution. The three cases of beta-2A myeloma with inclusion bodies also happen to represent about 30 per cent of the nine cases seen here. No attempt was made to review all of our cases of gamma myeloma. We have examined both imprints and paraffin sections of transplantable plasma cell tumors of mice that elaborate abnormal serum proteins analogous to beta-2A myeloma protein of man. The following tumors were examined histologically: 5847, MPC-2, MPC-15, MPC-23, MPC-25, and MPC-26.

Plasma cells were obtained by bone marrow aspiration and by surgical biopsies of a plasmacytoma; in addition, autopsy specimens of bone marrow or plasma cell tumors were obtained in two of the cases. Serum proteins were characterized with the ultracentrifuge and by immunoelectrophoresis. Bone marrow smears were fixed in absolute methanol and particles for sectioning in Zenker formol or sodium acetate mercuric chloride formaline.15 For lipid staining with Sudan black B, smears were fixed in formalin vapor. Standard histochemical methods were employed.1,5 Staining of the bone marrow smears with acridine orange was done by the method of Schiffer.16 Ultraviolet television microscopy was done by the method of Williams.17 The plasma cells of a single case (LM) were
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examined with an immunofluorescent technic using specific rabbit antiserum to human 6.6S gamma myeloma protein. For electron microscopy, bone marrow obtained by needle aspiration was immediately spread on a wax plate, and a portion of the aspirate rich in particles was transferred into a Wintrobe pipette to a large amount of 2 per cent osmic acid. No anticoagulant was used. Dehydration and polymerization were carried out according to Luft, except that the following mixing ratios were used: Epon 812 10 ml., DDSA 25 ml., DMP 0.6 ml. Sections were stained with either uranyl acetate or lead acetate.

LIGHT MICROSCOPY

In Giemsa stained smears, the intranuclear inclusions had several appearances. In the plasma cells of the two patients with gamma myeloma protein, one with beta-2A myeloma protein, two with macroglobulinemia, and of the one with a reactive plasmacytosis, the inclusions clearly consisted of two portions, a central bluish hyaline spherule (or spherules) and a thin peripheral rim of dark staining material (fig. 2 & 3). The smallest inclusions were colorless or only faintly blue (fig. 4). They were sometimes single and at other times numbered as many as seven in a single nucleus. Mutiple inclusions

Fig. 1.—Patient L. M. with gamma myeloma. Acridine orange smear. Nuclear DNA yellow-green, cytoplasmic RNA orange-red, intranuclear inclusions not stained.

Figs. 2–10.—Giemsa stained smears.

Fig. 2.—Patient F. C. with macroglobulinemia. Three faintly blue hyaline spherules surrounded by a rim of dark material.

Fig. 3.—Patient D. R. with multiple myeloma and Bence Jones protein. Single spherule surrounded by rim of dark material.

Fig. 4.—Patient T. P. with beta-2A myeloma. Two faintly blue spherules. One of the spherules in cytoplasm; the other apparently in transit from nucleus to cytoplasm. Note variation in size and nuclear-cytoplasmic ratio of plasma cells.

Fig. 5.—Patient H. B. with beta-2A myeloma. Three entirely separate small spherules.

Fig. 6.—Patient L. E. with gamma myeloma. Spherical inclusions in both nucleus and cytoplasm. Note that cytoplasmic spherules are not surrounded by dark material.

Fig. 7.—Patient L. E. with gamma myeloma. Inclusions with peripheral dark material enclosing blue spherules of variable size. Larger spherules appear to form from smaller ones.

Fig. 8.—Patient L. M. with gamma myeloma. Large blue inclusions in naked nucleus.

Fig. 9.—Patient H. B. with beta-2A myeloma. Large, homogeneous colorless inclusion without spherules.

Fig. 10.—Patient F. C. with macroglobulinemia. Large inclusion containing many small colorless spherules.

Fig. 11.—Patient L. M. with gamma myeloma. PAS smear. Inclusion is PAS negative.

Fig. 12.—Patient N. S. with beta-2A myeloma. PAS paraffin section. Inclusion consists of PAS positive central spherule and thin rim of dark material. The colorless area separating these two elements is probably artefact.

Fig. 13.—Patient M. T. with macroglobulinemia and Bence Jones protein. PAS smear. Inclusion with many small spherules and little amorphous material. Note that only the amorphous substance between the spherules is red, making the inclusion appear faintly PAS positive.

Fig. 14.—Patient M. T. PAS smear. Large inclusion with much amorphous material stains red, making the inclusion appear strongly PAS positive.

Fig. 15.—Patient M. T. PAS paraffin section of plasma cell tumor in liver, autopsy. Inclusion contains several strongly PAS positive spherules.
Figs. 1–15.—See legend, facing page. All magnifications at 1500x.
might be entirely separate (fig. 5) or might all be surrounded by the same
dark staining material (fig. 2). Inclusions were observed to involve one or
both nuclei of binucleate plasma cells. They were present in nuclei contain-
ing nucleoli, and were easily distinguished from them. Only very rarely were
the inclusions observed in the cytoplasm (fig. 6), and they were never found
exclusively in the cytoplasm. Cytoplasmic inclusions consisted only of bluish
spherules without peripheral dark staining material. Rarely a small inclusion
appeared to be in transit from nucleus to cytoplasm (fig. 4). Larger inclusion
bodies appeared to form by conglomeration of smaller ones; these larger
bodies always stained blue with Giemsa (fig. 7). Plasma cells containing small
inclusions were indistinguishable from their non-involved neighbors; on the
other hand, plasma cells with large inclusions often showed fraying of the
peripheral cytoplasm, or fragmentation, or even complete loss of cytoplasm.
Their nuclei sometimes appeared pyknotic. Inclusions were not uncommonly
found in naked nuclei (fig. 8).

Plasma cells of several of the patients with beta-2A myeloma and with
macroglobulinemia contained inclusions not observed in patients with gamma
myeloma protein or Bence Jones protein. These inclusions consisted of homo-
genous colorless material without spherules (fig. 9) or inclusions composed
of many small colorless spherules (fig. 10). Even when they were very large
and involved almost the entire nucleus, these inclusions were not hyaline or
blue. However, multiple spherules were usually surrounded by a thin rim of
dark staining material (fig. 10), and then resembled the multiple inclusions
with a band of dark staining material already described (fig. 7).

Intranuclear inclusion bodies were not observed in any of the transplantable
mouse plasmacytomaks which elaborate abnormal proteins analogous to beta-
2A myeloma protein of man. In none of the mouse tumors and in none of
the specimens from the nine patients with multiple myeloma or macroglobu-
linemia were Russell bodies, "grape cells," "morula cells," "flaming plasma
cells,"20 or "thesaurocytes"20 found.

**ELECTRON MICROSCOPY**

Plasma cells of three patients were examined, one with gamma myeloma
(LM) and two with macroglobulinemia (FC and MT). Electron microscopic
findings in cases LM and FC were identical and are considered separately from
those of case MT.

**Patients LM and FC:** Inclusions were round or oval, of variable size, and
always located centrally in the nucleus. Even the largest inclusions occupying
almost the entire nucleus were not related anatomically to the nuclear en-
velope. Inclusions were surrounded by a distinct single membrane approxi-
mately 70 A thick, or about the same thickness as the inner layer of the
nuclear envelope. This membrane had tiny protrusions and invaginations

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*These cytoplasmic inclusions were readily distinguished by their larger size and bluish
color from cytoplasmic vacuoles which are not uncommon in cells of the bone marrow
and peripheral blood1 and which were accidentally included in plasma cells of figures 2
and 10.
In a single instance an inclusion was observed without a limiting membrane (patient FC), and this inclusion did not have spherules.

Inclusions consisted of two parts, corresponding to the two parts discernible in Giemsa stained smears: 1) an amorphous portion of low electron density; 2) dense osmophilic spherules with perfectly smooth margins (fig. 19). These two portions were not separated by a membrane, and neither had a crystalline or periodic structure. Several spherules could all be surrounded by the same amorphous material or each could be entirely separate. The proportion of osmophilic to amorphous substance was variable: some inclusions consisted mostly of dense spherules, whereas others of similar size contained large amounts of amorphous material. None of the inclusions contained characteristically cytoplasmic structures such as mitochondria or endoplasmic reticulum, and cytoplasmic invaginations of the nucleus were never observed. Examination of a large number of intranuclear inclusions suggested that the different appearances may represent different stages of maturation. A possible sequence of development is shown in figures 16 through 19. In what is believed to be the initial stage, the osmophilic bodies were small and irregular. Subsequently they enlarged, conglomerated, and formed larger osmophilic bodies that were spherical. The final stage of development consisted of a single large dense spherule, round or lobulated, occupying almost the entire inclusion.

Ergastoplasmic sacs of the plasma cells containing inclusions were flat, and there was no evidence of accumulation of substances in either the endoplasmic reticulum or between the layers of the nuclear envelope. Mitochondria were morphologically normal: they were not swollen and the cristae were intact. In the electron microscopic studies, no inclusions were found in transit from nucleus to cytoplasm or from cytoplasm to an extracellular location. Inclusions were found in cells with nucleoli, but were never related to them (figs. 18 and 19). Inclusions were never observed in cells undergoing mitosis.

**Patient MT:** Several different types of intranuclear inclusions were identified. Some of the inclusions consisted of closely arranged amorphous material containing a few osmophilic spherules; these inclusions were similar to those already described for patients LM and FC. In addition, there were large inclusions surrounded by a single limiting membrane and composed of flocculent, loosely arranged material without osmophilic spherules (fig. 2). These inclusions were not associated with cytoplasmic invaginations nor did they contain endoplasmic reticulum or mitochondria; however, the loose arrangement of the contents of the inclusion resembled that of the cytoplasm. Some nuclei contained pseudoinclusions (cytoplasmic invaginations) filled with endoplasmic reticulum and mitochondria.

Rarely were inclusions observed within nucleoli or closely associated with them. These inclusions were not surrounded by a limiting membrane and did not contain osmophilic spherules. They therefore resembled the "nucleolus-associated" bodies described by others, but had the distinguishing feature of a central area of increased opacity (fig. 22). A striking composite intranuclear inclusion was discovered (fig. 23). It clearly contained a large cytoplasmic...
Figs. 16–19. (above and on facing page)—Patient F. C. Nuclear (N) inclusions consisting of amorphous material (A) and opaque spherules (S). No relationship exists between nucleolus (n) and nuclear inclusions. Arrows indicate limiting mem-
brane. The sequence of figs. 16-19 is believed to constitute developmental stages of maturing inclusions. (Magnification 29,000x.)
Table 1.—Serum Proteins and Frequency of Inclusions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Serum Proteins</th>
<th>Bone Lesions</th>
<th>Per Cent Plasma Cells in Bone Marrow</th>
<th>Per Cent Plasma Cells with Intranuclear Inclusions</th>
<th>PAS Staining of Inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. M.</td>
<td>MM</td>
<td>63</td>
<td>γ</td>
<td>+</td>
<td>25</td>
<td>25</td>
<td>negative</td>
</tr>
<tr>
<td>L. E.</td>
<td>MM</td>
<td>63</td>
<td>γ, BJ</td>
<td>+</td>
<td>95</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>D. R.</td>
<td>MM</td>
<td>54</td>
<td>BJ</td>
<td>+</td>
<td>&gt;95</td>
<td>&lt;1</td>
<td>negative</td>
</tr>
<tr>
<td>N. S.</td>
<td>MM</td>
<td>63</td>
<td>β_{2A}</td>
<td>+</td>
<td>34</td>
<td>2</td>
<td>positive</td>
</tr>
<tr>
<td>H. B.</td>
<td>MM</td>
<td>63</td>
<td>β_{2A}</td>
<td>+</td>
<td>85</td>
<td>2</td>
<td>positive</td>
</tr>
<tr>
<td>T. P.</td>
<td>MM</td>
<td>72</td>
<td>β_{2A}</td>
<td>+</td>
<td>55</td>
<td>&lt;1</td>
<td>positive</td>
</tr>
<tr>
<td>F. C.</td>
<td>MG</td>
<td>61</td>
<td>MG</td>
<td>-</td>
<td>45</td>
<td>6</td>
<td>positive</td>
</tr>
<tr>
<td>F. R.</td>
<td>MG</td>
<td>65</td>
<td>MG, BJ</td>
<td>-</td>
<td>90</td>
<td>&lt;1</td>
<td>not done</td>
</tr>
<tr>
<td>M. T.</td>
<td>MG</td>
<td>65</td>
<td>MG, BJ</td>
<td>+</td>
<td>75</td>
<td>1</td>
<td>positive</td>
</tr>
<tr>
<td>E. M.</td>
<td>Leukemia</td>
<td>42</td>
<td>None</td>
<td>-</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>not done</td>
</tr>
</tbody>
</table>

BJ = Bence Jones.
MG = Macroglobulinemia.

invagination with ergastoplasmic sacs distended with loosely arranged floccular material. Thin parallel fibrils extended from the invaginated contents through the site of invagination into the cytoplasm. A double membrane surrounded the cytoplasmic contents. Abutting the cytoplasmic portion of the inclusion was a typical intranuclear inclusion composed of amorphous material and osmophilic spherules. It was surrounded and separated from the invaginated cytoplasmic contents by a single membrane. The clearly cytoplasmic portion of the inclusion contained a few osmophilic spherules surrounded by endoplasmic reticulum and within ergastoplasmic sacs, but otherwise identical to the spherules of the nuclear portion. These cytoplasmic spherules were indistinguishable from Russell bodies.\(^{22,23}\)

**Histochemistry**

Identification of the chemical nature of the inclusion bodies was attempted in both smears and paraffin sections. The spherules stained green with Sudan Black B; the peripheral portion stained blue-black even after extraction with chloroform or hot pyridine. The spherules were strongly osmophilic and the peripheral portion faintly so. The inclusions were Feulgen negative, were not stained either orange-red or yellow-green with acridine orange (fig. 1), and did not absorb ultraviolet light at 265 m\(\mu\) when examined with the ultraviolet television microscope. These findings indicated that the inclusions do not contain DNA or RNA. Both the spherical and peripheral portions of the inclusions absorbed light at 280 m\(\mu\), indicating that they contained proteins. The inclusions were colorless or faintly eosinophilic with hematoxylin and eosin; they did not take Perl's stain for iron; and they were not metachromatic with toluidin blue or crystal violet. When observed by phase contrast microscopy the inclusions were sometimes refractile and sometimes opaque. They were not doubly refractile. They stained a brilliant red with Masson's trichrome, black with Weigert's fibrin stain, yellow with Van Gieson's picric-fuchsin, and red-brown to pink with acid fuchsin. With Mallory's PTAH they stained a mahogany color without blue bodies.

As indicated in table 1, the results of PAS staining were variable. PAS posi-
activity appeared to be related to the reported hexose content of the patient's abnormal serum protein. The inclusions of the patients with gamma myeloma protein and Bence Jones protein, both of which have low hexose contents, were uniformly PAS negative (fig. 11). On the other hand, inclusions of patients with beta-2A myeloma protein and macroglobulins, both of which have high hexose contents, were often brilliantly PAS positive and were apparently identical (fig. 12). PAS positive inclusions were found more readily in paraffin sections than in smears.

Examination of PAS stained smears and paraffin sections of the bone marrows of patients with beta-2A myeloma and macroglobulinemia revealed that not all of the intranuclear inclusions were strongly PAS positive. Frequently, individual spherules were PAS negative and only the amorphous material about and between them stained red (fig. 13). Therefore, inclusions composed mostly of amorphous material, with few or no spherules, were strongly and diffusely PAS positive (fig. 14); those composed of multiple spherules with relatively little amorphous material were only faintly PAS positive. However, spherules rarely stained well with PAS (fig. 15).

Smears of the bone marrow of one patient (LM) with gamma myeloma protein were examined by an immunofluorescent procedure using specific rabbit antiserum to human 6.6S gamma myeloma protein. The cytoplasm of the plasma cells fluoresced strongly, and in the nuclei of several of these cells there were thin rings of fluorescence corresponding to the peripheral portions of the inclusion bodies. The central spherical portion of the inclusions did not fluoresce. Unfortunately, this immunofluorescent examination was carried out in only one case; nevertheless, the finding of intranuclear fluorescence with specific antiserum indicates that the peripheral amorphous portion of the intranuclear inclusions contains the same abnormal protein present in the cytoplasm of the plasma cells.

**DISCUSSION**

The electron microscopic studies presented show that the inclusions of the present report are true nuclear inclusions. Their central location, single...
Fig. 21.—A different type of inclusion in patient M. T., with coarsely flocculent material, but without dense spherules. Note double contour of nuclear membrane (Nm) and single membrane of nuclear inclusion (arrow). (Magnification 29,000x.)

Fig. 22.—"Nucleolus-associated" inclusion in patient M. T. nu = nucleolus, I = inclusion, Nm = nuclear membrane. P = pseudo-inclusion, representing invagination into nucleus containing cytoplasmic vesicles surrounded by a double membrane (Nm). (Magnification 36,000x.)
Fig. 23.—Compound inclusion in patient M. T. composed of true intranuclear inclusion body and a cytoplasmic invagination into nucleus (N). Arrow indicates double membrane characteristic of cytoplasmic invaginations. The intranuclear inclusion body is delineated by a single membrane and consists of amorphous substance and dense spherules (S). Similar spherules (S1) in the cytoplasmic invagination are indistinguishable from Russell bodies. f = fibrillar material. (Magnification 15,000x.)
limiting membrane, lack of anatomical relation to the nucleolus or nuclear envelope, and lack of cytoplasmic attachments or structures all support this view. Structurally the inclusions consist of two parts, an amorphous portion of low electron density and densely osmophilic spherules; the proportion of these two substances is variable. Although both portions are proteins, they are different both histochemically and structurally. The immunofluorescent data suggest that the amorphous portion contains the same abnormal protein present in the cytoplasm of the plasma cells, and for the most part it is this amorphous portion that contains a high carbohydrate content, as judged by PAS staining. The significance of the osmophilic spherules is not known, but the proposed sequence of development of the inclusions suggests that they form in or from the amorphous material, perhaps by a process of coacervation.

In a case of Waldenström's macroglobulinemia, Dutcher and Fahey\textsuperscript{12} have described PAS positive intranuclear inclusions that fluoresced with specific antimacroglobulin serum. In their published photomicrographs, the inclusions stain diffusely; fluorescence is not localized. We do not believe that our results are at variance with those of Dutcher and Fahey, for we have shown that intranuclear inclusions in cases of macroglobulinemia may consist of amorphous material, and our findings suggest that the amorphous material contains the abnormal protein present in the cytoplasm of the plasma cells.

The inclusions described by Thiery\textsuperscript{11} in the plasma cells of the mouse spleen and the mesenteric nodes of the rat are intranuclear like those described here; however, they resemble vacuoles. In only one electron micrograph do they appear to be composed of multiple spherules, but even in this case their lack of osmophilia makes it unlikely that they are related to the inclusions of this report.

The intranuclear inclusion bodies of the present report superficially resemble Russell bodies, which arise as osmophilic structures (often spherules) in distended ergastoplasmic sacs.\textsuperscript{22,23} Russell bodies contain glycoprotein,\textsuperscript{27} and their behavior with PAS and Romanowski stains is variable.\textsuperscript{23,24} They contain gamma globulin\textsuperscript{29} and, when stained with fluorescein-tagged antisera to gamma globulin, there is membrane-like fluorescence sharply localized to their peripheries, while the central portions remain unstained.\textsuperscript{29,30} Fluorescent studies indicate that only the peripheries of the bodies stain, not the entire ergastoplasmic contents;\textsuperscript{30} and since there is no morphologic evidence for a membrane-like structure surrounding Russell bodies, White has suggested that perhaps the large tagged antibody molecules cannot penetrate the dense layers of the Russell bodies.\textsuperscript{29} Such an explanation need not be invoked to account for the peripheral localization of fluorescence in the present study, since the inclusions clearly consist of two portions. None of our histochemical studies of intranuclear inclusions, except the absence of strong fuchsinophilia, has produced findings different from those reported for Russell bodies.\textsuperscript{27} We feel, therefore, that the intranuclear inclusions in plasma cells are analogous to Russell bodies. However, we wish to emphasize a fundamental difference between the two structures: Russell bodies are clearly of cytoplasmic origin; intranuclear inclusions are nuclear products not derived from cytoplasm.
It is unlikely that the intranuclear inclusions represent a degenerative change, for many plasma cells containing inclusions are otherwise morphologically indistinguishable from their non-involved neighbors. However, plasma cells containing large inclusions bodies may show evidence of damage, and it is possible that the accumulation of large amounts of inclusion material in the nucleus interferes with cell metabolism, ultimately leading to cell death, as proposed by Paraskevas, Heremans, and Waldenström. After the death of the plasma cell, the inclusion material may then appear to be extracellular. We have not observed actual transit of inclusions from the cytoplasm to an extracellular location, as did Thiery in plasma cells of the mouse.

We believe that our electron microscopic and histochemical studies of the intranuclear inclusion bodies provide morphologic evidence of nuclear elaboration of protein other than nucleoprotein. Although there is considerable biochemical evidence for such a process, morphologic evidence is scant. O'Brien has shown that hemoglobin appears in the nucleus of the embryonic chick erythroblasts before it is detectable in the cytoplasm, and Schmid, Schwartz, and Sundberg have described inclusions containing hemoglobin within the nuclei of normoblasts in congenital erythropoietic porphyria. Richter has provided electron microscopic evidence that apoferritin may be elaborated by the nucleus in response to iron loading. It is noteworthy that White, Coons, and Connolly have described spots, rings, and strands of fluorescence in plasma cell nuclei in addition to diffuse cytoplasmic fluorescence in their studies of antibody production in experimental animals. These patches of antibody were noted frequently in the nuclei of immature cells and less often in more mature ones. Although no structures corresponding to the intranuclear fluorescence were seen in the Giemsa stained preparations, the studies not only provide evidence for nuclear elaboration of gamma globulin, but the more frequent occurrence of fluorescence in immature rather than mature cells suggests that antibody production may actually begin in the nucleus.

There is also recent evidence for nuclear elaboration of substances other than protein that are ordinarily considered to be exclusively cytoplasmic. Hines and Pollister have described intranuclear production of glycogen by the parenchymal cells of the tadpole liver. They interpret their findings as evidence for a distortion of what is probably a normal process, the formation of nuclear products that subsequently enter a cytoplasmic organelle to become involved in cytoplasmic metabolism. It is likely that accumulation of protein inclusions in the nuclei of plasma cells in multiple myeloma and macroglobulinemia represents a comparable distortion of a normal course of events.

Various authors have attributed diagnostic significance to intranuclear inclusion bodies in plasma cells. Dutcher and Fahey have suggested that PAS positive inclusions may be peculiar to Waldenström's macroglobulinemia, although they felt that one might rarely find similar inclusions in other diseases. Recently, Paraskevas et al. have described intranuclear inclusions in two cases of beta-2A multiple myeloma and they have suggested that these inclusions, along with “flaming plasma cells” and “thesaurocytes,” are characteristic morphologic features of this particular variety of myeloma. Our findings should serve to dispel any notions of the specificity of intranuclear
inclusions in plasma cells, either for a certain kind of plasma cell as defined morphologically, or for a certain protein defined immunoelectrophoretically. We have found PAS positive inclusions in cases of beta-2A myeloma identical to those of macroglobulinemia, as has Christiensen, and we have found hyaline, bluish inclusions in patients elaborating gamma, beta-2A, Bence-Jones, and macroglobulin proteins. We have also found a typical bluish intranuclear inclusion in a case of reactive plasmacytosis without an abnormal serum protein. We may also note that we could not distinguish the plasma cells in beta-2A myeloma from those of other plasmacytomas, although in general the plasma cells of beta-2A myeloma were large and their cytoplasm was PAS positive as described by Waldenström. We have not encountered "flaming plasma cells" and "thesaurocytes," claimed to be characteristic of beta-2A myeloma, in any of our cases, but we have seen them in two patients with reactive plasmacytosis.

Rask-Nielsen et al. have reported "flaming plasma cells" in a transplantable mouse leukemia associated with an abnormal serum protein analogous to beta-2A myeloma protein of man. There is no mention of intranuclear inclusions in the report. We have examined both imprints and paraffin sections of seven transplantable mouse plasmacytomas which produce abnormal serum proteins analogous to beta-2A myeloma protein of man. In none of the tumors did we find intranuclear inclusion bodies, "flaming plasma cells," or "thesaurocytes."

Our findings may be of interest from the point of view of deranged nuclear metabolism and of nuclear elaboration of protein; however, it is likely that intranuclear inclusion bodies in plasma cells, like Russell bodies, have no more diagnostic significance than does the morphology of the plasma cell.

**SUMMARY**

Inclusion bodies in plasma cell nuclei of six patients with multiple myeloma and of three patients with macroglobulinemia have been studied histochemically. In three cases they have been studied with the electron microscope. The inclusions are readily identified in Giemsa stained smears of bone marrow and in paraffin sections. Their PAS positivity may be related to the hexose content of the patient’s abnormal serum protein. Evidence is presented that the inclusions are true nuclear inclusion bodies, that they represent nuclear elaboration of glycoprotein, and that they may be found in all three immunoelectrophoretic varieties of multiple myeloma, as well as in macroglobulinemia.

**SUMMARIO IN INTERLINGUA**

Corpores de inclusion in nucleos de plasmocytos ab sex patientes con myeloma multiple e ab tres patientes con macroglobulinemia eseva studiate con le methodos del histochimia. In tres del casos ille corpores eseva studiate per medio del microscopio electronic. Le inclusiones es facilemente identificabile in frottis de medulla ossee a tincturation Giemsa e in sectiones in paraffin. Lor positivitate in tincturation PAS es possiblemente relationate con le hexosa
INTRANUCLEAR INCLUSIONS

continite in le seroproteina anormal del patiente. Es presentate evidentia que le inclusiones es ver corpores de inclusion nucleari, que illos representa un elaboration nucleari de glycoproteina, e que illos pote esser incontrate in omne tres varietates immunoelectrophoretic de myeloma multiple, como etiam in macroglobulinemia.

ACKNOWLEDGMENTS

We thank Dr. John L. Fahey of the National Cancer Institute for characterizing the serum proteins of these patients, Dr. Richard A. Malmgren of the National Cancer Institute for performing the immunofluorescent study, and Dr. George Z. Williams of the Clinical Pathology Department for performing the analyses with the ultraviolet television microscope.

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