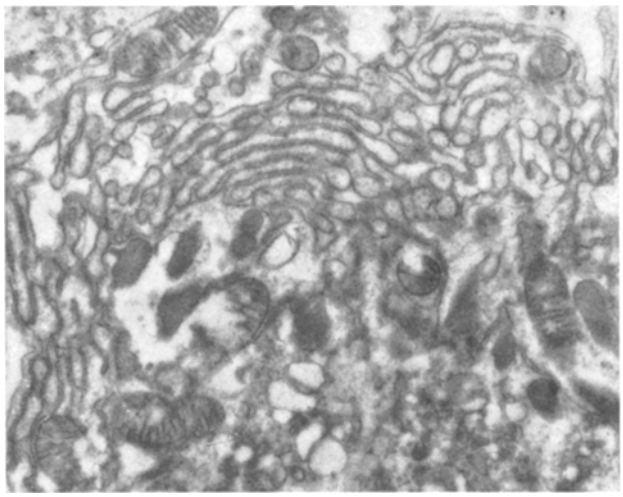

Fig. 4. Electron micrograph of myeloma cell demonstrating a variety of cell organelles including ergastoplasm, mitochondria, Golgi complex and crystalline structures. Note electron lucidness of these organelles. No electron staining. × 10,000.

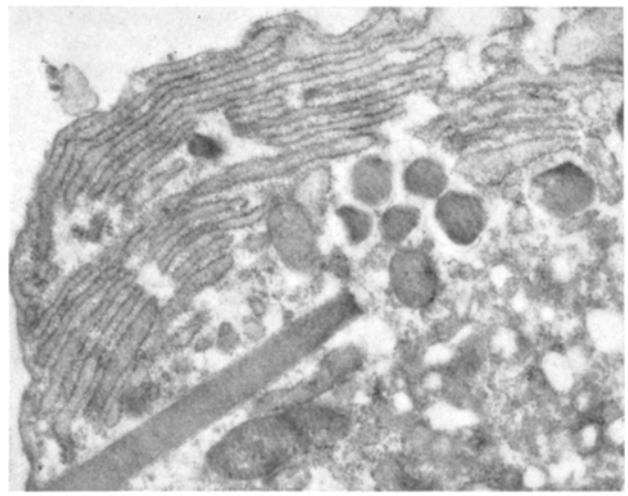

Fig. 5. Myeloma cells reacted with peroxidase-conjugated anti-$\varkappa$ antibody, showing the existence of electron-dense reaction precipitate on ergastoplasm, ergastoplasmic ribosomes and intracisternal cavity. In comparison with Figure 4, it is noted that electron density of other cell organelles remains unchanged by peroxidase reaction. No electron staining. × 15,000.

cytoplasmic crystalline structures which apparently originated from Golgi complex in the reaction with peroxidase label seems of importance. Whether this unresponsiveness reflects the functional abnormality of Golgi complex following malignant transformation in neoplastic plasma cells, or it merely represents a degeneration of proteins resulting in the loss of antigenicity of immunoglobulin molecules during the storage of produced immunoglobulin inside Golgi complex awaits further investigation[19].

*Zusammenfassung.* Menschliche Myelomazellen wurden immunchemischelektronenoptisch untersucht. Durch die Behandlung mit peroxidasekonjugiertem Antikörper gegen die Immunoglobuline H- oder L-Kette wurde keine H-Kette beobachtet.

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*Tokyo (Japan), 28 July 1969.*

[19] We thank Dr. S. Ito of the Anjo Hospital of Aichi for providing the myeloma specimen.

## Ultrastructural Localization of Heavy- and Light-Polypeptide Chains in Human Long-Term Culture Cells Detected by Peroxidase-Conjugated Antibodies

A long-term culture line, R.P.M.I. No. 4666 was originally established from peripheral blood of a patient with chronic myelogeneous leukemia and since has been propagated in suspension culture[1]. Extensive studies revealed that this culture consisted of very uniform cell types and was producing monoclonal immunoglobulin A (IgA) of $\varkappa$-type along with free $\varkappa$-type light chain simultaneously in the same cells[2].

In an attempt to reveal electron microscopic localization of component polypeptide chains of human immunoglobulin in the cytoplasm of the culture cells of human hematopoietic origin, we employed in the present study peroxidase-conjugated antibodies directed to human immunoglobulin component chain. By reacting fixed cells with peroxidase-conjugated antibody and revealing peroxidase activity cytochemically, ultrastructural localization of immunoglobulin was readily demonstrated as distinct electron-dense reaction product.

Antibodies monospecific either for $\alpha$-heavy chain or for $\varkappa$-light chain were purified specifically by use of immunoadsorbents as described previously[3,4], and purified antibody was coupled to horseradish peroxidase using *p,p'*-difluoro-*m,m'*-dinitrodiphenyl sulfone as a coupling reagent as described by Nakane and Pierce[5]. The anti-

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body reactivity and enzyme activity in peroxidase-antibody complex was tested by immunoelectrophoresis. Both peroxidase-conjugated anti-α and anti-ϰ produced a single precipitin line against normal human sera or purified IgA or Bence Jones protein of ϰ-type. Enzyme activity was revealed cytochemically exactly on the precipitin line.

Procedures for reacting cell specimens with peroxidase-conjugated antibody reagents and for subsequent electron microscopic observation closely followed the method of those authors[5].

When cell sediment fixed with 10% formalin in phosphate buffer (pH 7.2) was treated either with peroxidase-conjugated anti-α or anti-ϰ antibody, intracellular immunoglobulin was revealed as conspicuous electron-dense regions resulting from cytochemical reaction of peroxidase[6,7]. Since fixed cell sediment can be well-dispersed and therefore contact between individual cells and peroxidase-conjugated antibody was assured, there seemed to be no difficulty in penetration of antibody into cytoplasm. Such difficulty in diffusion was reported when cell block was stained with similar antibody-enzyme complex.

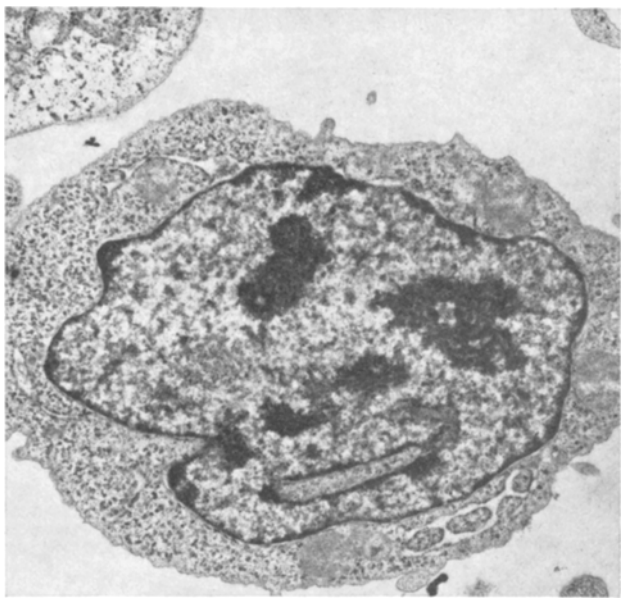

Fig. 1. Electron micrograph of a R.P.M.I. No. 4666 cell treated with peroxidase-conjugated anti-ϰ. The free polysomes increased in electron density. Lead citrate and uranyl acetate. × 8000.

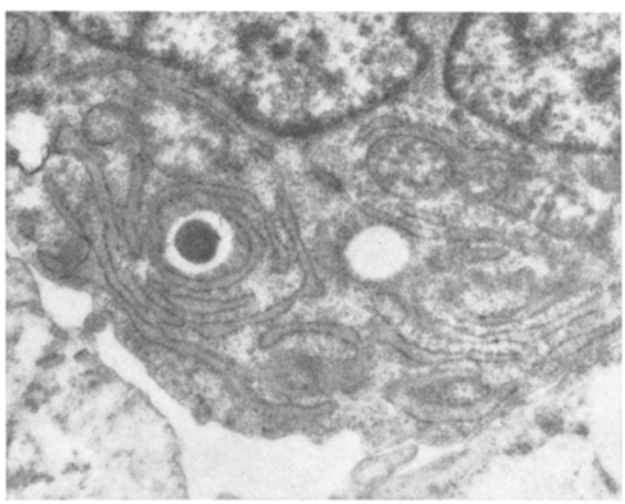

Fig. 2. Positive reaction in ergastoplasm, ergastoplasmic ribosomes and nuclear membrane is demonstrated in R.P.M.I. No. 4666 cell treated with peroxidase-conjugated anti-ϰ antibody. No electron staining. × 10,000.

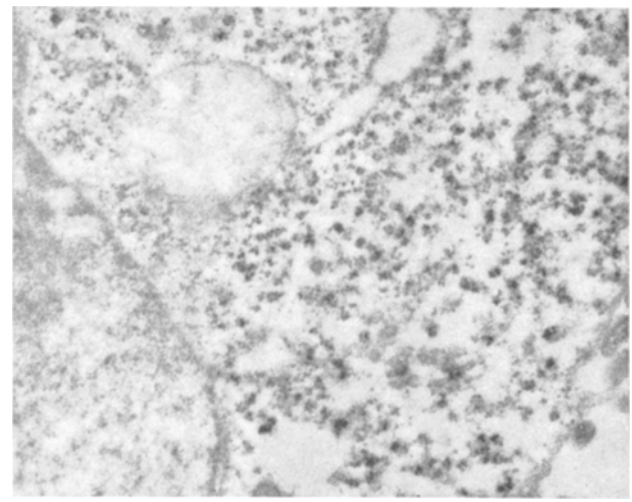

Fig. 3. A part of R.P.M.I. No. 4666 cell treated with anti-α antibody, showing positive reaction of many but not all cytoplasmic ribosomes in small cluster forms. Note that electron-dense reaction precipitates are observed exactly on ribosome element comprising polyribosomal aggregate. No electron staining. × 20,000.

Approximately 30% of cell was positive with the enzyme label of either anti-α or anti-ϰ antibody. In contrast to the report of LEDUC et al.[8], immunoglobulin or its subunit was localized to certain limited regions of cytoplasm even in lymphoblastoid cell in the present experiment (Figure 1).

All the controls, either cells treated with peroxidase label or other antibody specificity, or cells treated with peroxidase label but not placed in the enzyme substrate, or cells treated with substrate alone, or cells reacted with peroxidase-conjugated normal globulin were negative.

Of particular interest was the substantial difference in subcellular distribution pattern of α-heavy chain and ϰ-light chain as revealed by this method. When cells were treated with peroxidase-conjugated anti-α antibody, free polysomes in the cytoplasm were selectively stained indicating that they are the dominant site of α-chain accumulation. Ribosomes lining the ergastoplasmic membrane were weakly stained but intracisternal cavity of ergastoplasm was in large part negative for the reaction of α-chain (Figure 3).

On the other hand, ϰ-type light chain was not detected on free polysomes in the small cluster forms. Instead, the ϰ-chain was localized mainly to ergastoplasm, its membrane and ribosomes lining the ergastoplasmic membrane. In contrast to α-heavy chain, ϰ-light chain was detected also in intracisternal cavity of positive ergastoplasmic lamellae. The external layer of nuclear membrane was

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another dominant site of $\varkappa$-chain accumulation except for nuclear pores (Figure 2).

Ribosomes in direct relation with nuclear membrane were often stained with the same enzyme label. Sparse and poorly developed Golgi complex was always unresponsive either to peroxidase-conjugated anti-$\alpha$ or anti-$\varkappa$ antibody.

SCHAPIRO et al.[9] reported that polysomes are the active unit of gamma globulin synthesis in 2 transplantable murine plasma cell tumors, and heavy and light polypeptide chain molecules are formed separately on polysomes of different size, i.e., on 270s and 190s polysomes, respectively. WILLIAMSON and ASCONAS[10] obtained similar observations in their $^{14}$C-amino acid incorporation experiment using ascitic murine plasma cell tumors.

We could not determine so far whether observed differential localization of heavy and light polypeptide chain, in our culture line revealed by electron microscopy, is the morphological counterpart of biochemical results by those authors, or whether this phenomenon is an inherent property of culture cells.

*Zusammenfassung.* In langfristiger Kultur wurde mit peroxidasekonjugiertem Antikörper (monospezifisch gegen H- und L-Kette) die submikroskopische Lokalisation der Immunoglobulin H- und L-Kette in den menschlichen Lymphoblastoidzellen an das endoplasmatische Retikulum gebundene beziehungsweise freie Polysoma gefunden.

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[11] We thank Dr. G. E. MOORE, of the Roswell Park Memorial Institute, Buffalo (New York, USA), for supplying culture cells.

## Separation of Germ Layers in Presomite Rat Embryos

The method of excision of presumptive ectodermal, endodermal and mesodermal areas from early amphibian embryos and the subsequent cultivation of isolated pieces under various experimental conditions has contributed a great deal to the understanding of some fundamental phenomena occurring in early development[1,2]. In avian and mammalian embryos any attempt to isolate particular prospective organ-forming areas must include the separation of germ layers from one another. By reason of the intricate mechanism of the formation of germ layers, some relevant experiments on avian[3-5] and mammalian[6,7] embryonic shields cannot be considered as true separation of definite germ layers. It was the purpose of the present investigation to apply the common method of enzymatic 'splitting off' of epithelia to the separation of germ layers in presomite rat embryos.

Pregnant females of randomly bred albino rats were killed by ether 9 days after mating and the embryos were isolated in sterile Tyrode's solution. The majority of them belonged to stages 14 and 15 in the normal series of NICHOLAS[8]. The mesodermal wings approached each other underneath the neural groove, opposite to the primitive streak. The extraembryonic part was cut off and the cup-shaped egg cylinder was transformed into a flat bi-lobated shield by a longitudinal cut (Figure). Prepared in this manner the shields were submitted to the action of enzymes. A solution containing 0.5% trypsin (cryst., lyophyl., Worthington) and 2.5% pancreatin (Difco) in the calcium- and magnesium-free Tyrode's saline was used. The embryonic shields were kept in it for 30 min at 4 °C (in the refrigerator[9]). The action of enzymes was blocked by a mixture of Tyrode's saline and horse serum which was substituted by the pure saline after 15–20 min. After this treatment the ectoderm detached spontaneously from the underlying mesoderm except for the region of the primitive streak where a complete separation had to be achieved by tungsten needles. The very thin embryonic endoderm did not detach spontaneously, but it could be easily rolled up from the mesoderm. In this way the embryonic shield was divided into 4 parts: the ectodermal and endodermal layer, and 2 mesodermal wings. The histological control showed that a complete separation of germ layers had taken place.

In order to test the viability and developmental capacities of embryonic cells after the treatment with enzymes, single germ layers or 2 of them in different combinations were implanted by a braking pipette under the kidney capsule of adult male rats. After 15 days the hosts were killed, the grafts were removed and submitted to the routine histological procedure. In all, 96 grafts were made

Cross-section of a stretched 9-day-old rat embryonic shield. × 100.

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