ANNUAL REPORT OF THE INSTITUTE
FOR VIRUS RESEARCH KYOTO UNIVERSITY

VOLUME I

SERIES A: PATHOLOGICAL ARTICLES
ELABORATED BY THE DEPARTMENT OF PATHOLOGY
DIRECTOR: PROF. S. AMANO

IMMUNOCYTOPATHOLOGY VIRUS INFECTION AND LEUKEMIA

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SERIES A: PATHOLOGICAL ARTICLES
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Preface to the First Issue of Series A.

When I began my career in the Pathological Institute of Kyoto University, it was under the direction of Professors Fujinami and Kiyono alternately. One year before retiring with the title of Emeritus Professor, Doctor Fujinami was concentrating his final research in chicken sarcoma, which he introduced in 1909 for the purpose of experimental cancer research. I highly admired and respected him from the stage of university student. My first contribution to Pathology and to Professor Fujinami was entitled : " Influence of blocked r.e.s. upon the heterotransplantation of chicken sarcoma in rats."

At that time, Professor Kiyono was feverishly engaged in the anthropological investigation of prehistoric Japanese, excavating in all parts of Japan for skeletons. He had just completed his " Histiocytic Cell Theory " followed by his elaborated and prodigious monograph : " Studies on Vital Staining ", which brought exciting advances in biology, anatomy and physiology.

This environment so impressed me that I tried to visualize a bridge connecting cancer and the defensive organs. With the kind support of Professor Katsunuma, the founder of the Japan Hematological Society, the result of my first research work, applied with cytochemical techniques, was reported at its first annual meeting, as the chief report : " On the Pathoanatomy of blood diseases, especially on the tissue hyperplasia in hematopoietic organs ",

Thereafter, my keen interest in the study of monocytic leukemia brought forth new theory on the independence of monocytic series from r.e.s. and other blood cell series. And this was extended to general hematology and to inflammation pathology.

Here, my desire to obtain facilities with which I could observe inflammatory cell in tissues in the same degree as peripheral blood cells was realized. This was made possible by using a device for extending tissues by which supravital observation as well as Giemsa staining could be observed.

Thus the peculiar generating modus of plasma cells from the adventitial cells of small blood vessels came into our observation in the experiments utilizing this method, and these findings were ascertained agains and again till to a birth of a new plasma cell theory, which includes the antibody production of these cells. This view-point promised from the beginning a genetical independency of plasma cells from lymphocytes. Here, reexamination of lymphocyte generation was considered with an introduction of lymphogonia, the youngest and transitional cells from the lymphatic reticulum cells.

Since Metschnikoff, the macrophage system was presumed to be the source of antibody, however, now the time has come when our plasma cell claims its due responsibility to produce antibody. Our further studies testing the co-work of plasma cells and macrophages were extended all over the problems of classical inflammations as well as new viral infections, which were published at the Annual Meeting of the Japanese Pathological Society in 1949.

In 1952, just after Japan had regained her independence, the International Congress of Geographical Pathology was held in Liége. This gave me an opportunity to report on our study of infectious hepatitis in Japan. From there I went to Argentina to attend the IV International Congress of Hematology held in Mar del Plata. I gave a report of the Atomic Bomb Casualties in Hiroshima ; this was the first time for such a report to be given by a Japanese abroad. This trip included a visit to the Karolinska Instituet where I talked with Professor Caspersson about our ultraviolet microscopic studies performed in Japan just before the II World War, to prove the existence of nucleic acid in cytoplasm (1938–1940). I also had the pleasure of visiting Professor Büchner in Aschoff-Haus, at the Pathological Institute of Freiburg University, where my old teachers, Prof. Fujinami and Prof. Kiyono, under the direction of Prof. Ziegler and Prof. Aschoff respectively, began their brilliant careers.

The Institute for Virus Research has been established in Kyoto since 1956, and the Department of Pathology has planned electron microscopical investigation of leukemia and cancer regarding their causative viruses with the full cooperation of all members of the new Institute. This brings me back to my starting point of cancer study in pathology. Because of my new responsibility as Director of this Institute, our research is now meeting some inhibition due to my lack of time. However, in 1958, we were able to publish an annual report of the Institute, and the Department of Pathology was the first contributor to present paper concerning pathological articles (Series A). Other contributors, such as the Departments of Biophysics, Biochemistry, Sero-Immunology and Prevention-Therapy will in turn present the reports of their investigations for publication.

Here I would like to introduce the present contributors, most of whom have been my long year collaborators. By their cooperation they helped to promote our pathological activities since the period of post-World War II.

Dr. Hanaoka opened the cytological pathways to the immunocytology of plasma cells, following in the foot steps of his predecessors, Dr. Hirata and Dr. Fujii of the Osaka Womens Medical School (This school provided me with female researchers during the II World War period to compensate for our lack of male researchers at that time). Now, Dr. Fuji, a young promising co-worker, is extending this theme immunochemically.

Dr. Tanaka brilliantly developed the preliminary work of the late

Dr. Unno in the study of lymphatic cell series with special reference to lymphogonia. In connection with the study of lymphatic reticulum cells, Dr. Tanaka performed an epoch-making systematic study of reticuloendothelial system utilizing electron microscope and ultra-thin sectioning. Meanwhile, he has made much progress in the new ultracytology which is not contained in the present publication.

Dr. Matsumoto (formerly called Dr. Dohi) began his career in pathology with extensive inoculation experiments of varied viruses, and is now especially concentrating in the observation of ectromelia mice with his skill in electron microscopy hoping to open a pathway to the problem of viral proliferation in vivo.

Dr. Yamamoto tackles the problem of posthepatitic pathology of infectious hepatitis. His monograph concerning liver lesions in this disease will be published shortly.

With the establishment of the Virus Institute, Dr. Ichikawa and Dr. Iwakata simultaneously joined our group. Dr. Ichikawa keeps abundant mice strains in his room, investigating the role of the virus in the spontaneously produced leukemia and tumor. Dr. Iwakata detected a new viral reservoir in chicken erythroblastosis, the role of r.e.s. in viral leukemia as being a new theme.

Dr. Hamamoto is persuing the distributing modus of Shope papilloma virus according to the development of tumor in benign and malignant conditions.

There is no pathology confined merely to virus research. Only the fundamental knowledge in pathology in a wider range will enable us to approach the essential feature of the viral manifestations. To meet this requirement it is essential for pathologists to possess the knowledge of various new techniques to deal with the virus and host cell, especially in phase contrast and electron micrography, physicochemistry aud immunocytology. Therefore, we have entitled this report : "Immunocytopathology, Virus Infection and Leukemia". Only the development of my pathological researches can explain the internal relationship between these factors in the title. However, I believe that some external relationship between the data will also support this understanding.

Professor SHIGEYASU AMANO

Institute for Virus Research, Kyoto University

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CONTENTS

Ann. Report. Inst. Virus Research. Kyoto Univ.
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1. Studies on Plasma Cells —Cytogenesis, Defensive Function and Ultracytophysiology. A Review of our Original Studies since 1944*

SHIGEYASU AMANO**

CONTENTS

I. GENESIS OF PLASMA CELLS

The study on the problem of antibody production were promoted by three centers during the second world war, as we may retrospect it from the present state. Namely, (1) the antibody production of plasma cells of Scandinavian school,[1,2] (2) the antibody production of lymphocytes worked by Ehrich[3] and Dougherty[4] in United States and (3) the antibody production

* These studies have been done at the Pathological Institute of Medical Faculty and the Institute for Virus Research, Kyoto University, Japan.

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of plasma cells advocated by our Kyoto School[7-16] based on a new datum in histogenesis—Advential theory.

Japanese theory was started from the findings (Amano, Hirata &. Fujii, 1944)[9,10] that the plasma cell generation follows quite different modus from that of the lymphocytes and histiocytes, which necessitated an unique functional qualification of the plasma cell. Thus the ability producing antibody-globulin was attributed to plasma cells. And this stand point allowed directly the birth of the theory of antibody production of the plasma cells (Amano, Hirata &. Fujii, 1944, 1945).

The Ehrich's lymphocyte theory of antibody production started from an experiment with the sensitized lymph nodes (1945)[3] was converted, afterwords, to the plasma cell theory (1949).[5] Therefore, the world wide opinion seemed to be unified to the Scandinavian theory of antibody production, so to speak, the plasma cell theory.

However, without strict discrimination of plasma cells from other cell series, regarding morphological characteristics it might be practically meaningless, that one talk only about the antibody production of plasma cells. For instances, the transitional cells of Fagraeus[2] which, according to her understanding, intermadiate the transformation of reticulum cells to plasma cells do not belong to plasma cell series but really to a youngest type of lymphatic cells—to avoid misunderstanding, we call these cells as lymphogonia (Amano)[54,66] since 1951—though most of the European schools accord to Fagraeus unjustifiablly.

Therefore, we would like to point out here that the concept of plasma cells, too emphasized among the European School, involves practically undissolved problems referring to plasma cell morphology and morphogenesis. We may seek a forerunner of the Fagraeus' theory in Rohr (1936),[17] whose observation of plasma cell generation (or reticulum cell theory) was only confined to the observation of the free cells of the bone marrow obtained by marrow puncture and, therefore, lacks in the histological basis from the start—.

Our studies in Japan were carried out independently from the European and American researches during the second world war, with a device of a technique to observe supravitally connective tissues of the skin by way of extending method,[8] describing fibrocytes, histiocytes, monocytes, lipoblasts of the vascular walls in the inflammatory tissues precisely (1943).[7]

In these observations the plasma cell metamorphosis from the vascular

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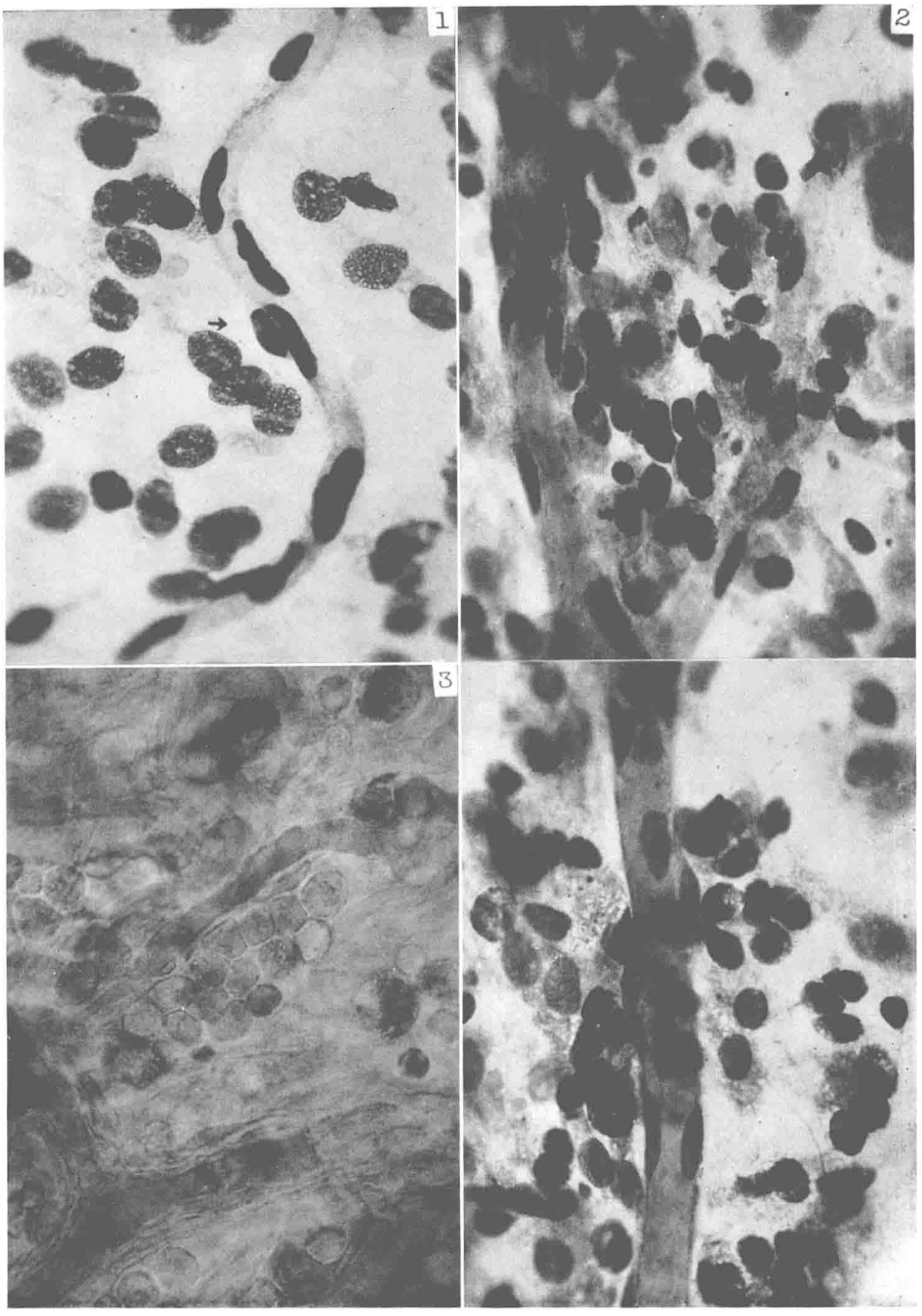
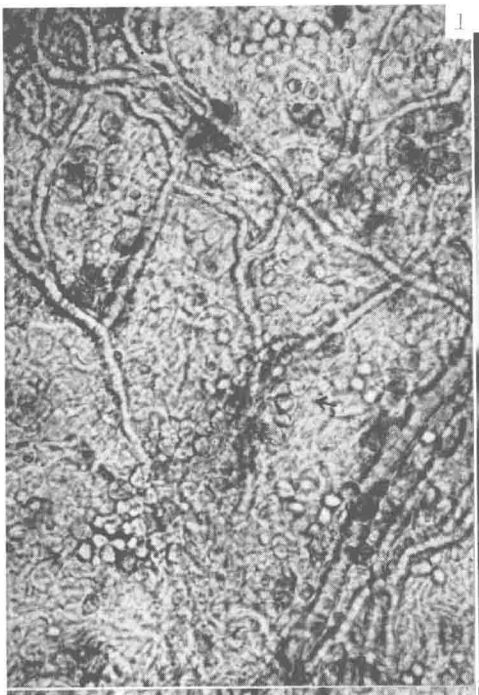
Plate. I.

1. An adventitial cell of a omental capillary which will take part in plasma cell metamorphosis. (Extending method, Giemsa-stained)
2. The Arthus phenomenon-provoked cutaneous tissue with a plasma cell cluster alongside of a newly formed fine capillary. (Extending method, supravital state)
3. The same locus after fixation and Giemsa-staining. The formation of plasma cells is remarkable. (Amano, Hirata & Fujii, 1944)





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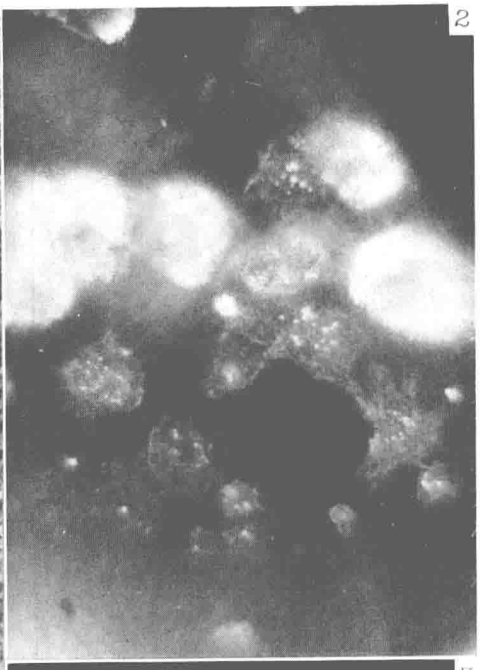

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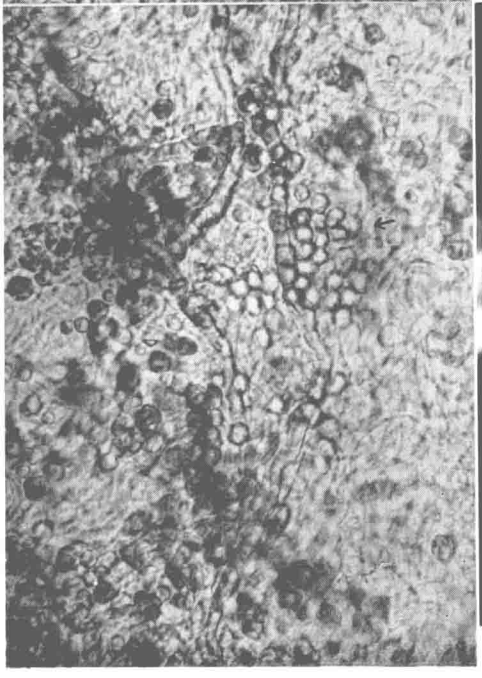

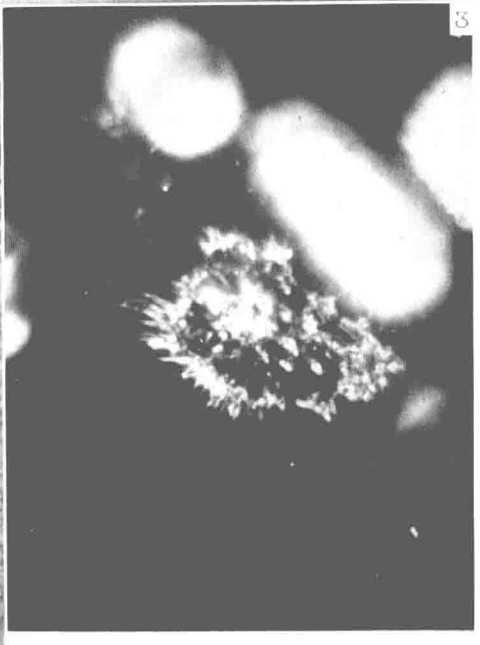

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adventitial cells in sensitized and the Arthus phenomenon provaked cutaneous tissue was described for the first time correctly (1944).[9] This plasma cell metamorphosis was ascertained by the materials without the contamination of lymphocytes. Therefore, the Marchand's theory[18] which assumed lymphatic origin of plasma cells was abandoned and a new theory of plasma cell genesis from the adventitial cells was established by us.

According to our observations of supravitally extended connective tissues, the adventitial cells we deal here demonstrate no phagocytic ability reverse to the Kiyono's understanding[20,21] which identified adventitial cells with the Zimmermann's pericytes[23] morphologically. So we should define here the "adventitial cells" to be a resting cells but determinated to give rise plasma cells through metamorphosis. Therefore, we proposed a new name of " Adventitia-plasma cell system " (1944)[9].

Though these model experiments were carried out with cutaneous connective tissues where neither myeloid nor lymphoid cells coexist normally, this new rule of the plasma cell generation from adventitial cells was applicable to myeloid and lymphatic organs,[14] as it was revealed immediately after that.

Namely, the distribution of plasma cells in the bone marrow is confined mostly alongside of small blood vessels, especially arterioles, which is demonstrated prominently in the case of aplastic marrow. The latter presents a most eclatant example to reject the Rohr's propose which insists the plasma cell genesis from reticulum cells.

Besides, as referred to lymph nodes, the plasma cell foci are confined to the medullary pulp where vascular elements are concentrated, but not to cortical region. Though the spleen has a complex structure and inadequate to investigate this problem, but the plasma cell foci are found principally alongside of arterioles and distant from lymph follicles.

Therefore, the theory of plasma cell generation from vascular adventitial cells provide a wide scope to rule the normal and pathological distributions of the plasma cells in tissues and organs (1944). Later Björneboe (1947)[1] reported the plasma cell reaction in the fatty tissue of the kindney which accords to the grade of sensitization. Our observations initiated from the findings of the skin with the Arthus phenomenon were extended immadiately to that of that of the hematopoietic organs as well as the con-

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Plate. II.

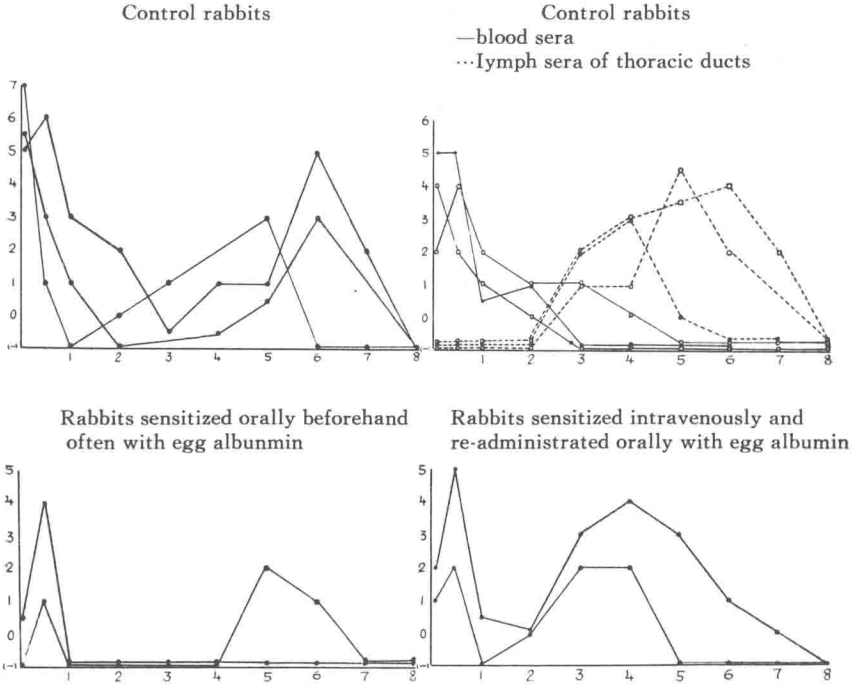
1. The Arthus phenomenon-provoked cutaneous tissue in supravital state. Plasma cell clusters are scattered along the newly formed vascular net-work. (Amano, 1944)
2, 3. Living plasma cells obtained from subcutaneum with the coverglas which were inserted beforehand and picked out 5 days after the provocation of the Arthus phenomenon. By the dark field illumination, plasma cells strech out some rigid processes, being cytoplasm almost transparent except mitochondria and neutral red vacuoles (rosette formation), while histiocyte turbid white burdened with heterogenous substances. This type represent the real form of plasma cells in tissues which are so slow in changing the cellular shape. (Yamamoto, 1950)[147]

nective tissues of the whole body. As to the problems whether reticulum cells have any genetical relation with the plasma cell system will be discussed later.

II. Function of Plasma Cells.

The generation of plasma cells which follows quite different mode from that of lymphatic cells, necessitated our new explanation concering the unique distribution of plasma cells in the whole body in normal and pathological conditions.[39–44]

Parallel with the studies on the distribution of plasma cells in hematopoietic organs, the mode and grade of the distribution of the plasma cells in the mucous membrane of the intestine were tested in autoptic materials, which called our interest very much.[24] The findings in severe malnutrition in the second world war period, with consideration of other varied diseases and in varied ages, were observed systematically by us (Hanaoka 1947)[25]. Following this, an experimental observation of the peroral sensitized animals with the consideration of antigen distribution tested by serological method,



Text-fig. 1. Egg albumin titers referring to serum or thorocicus lymph (dotted line) tested by way of precipitation of egg albumin (after the administration of 10% egg albumin 10 ml prokilo peroral). Ordinate : antigen titers, abscissa : hours (Hanaoka, 1951)

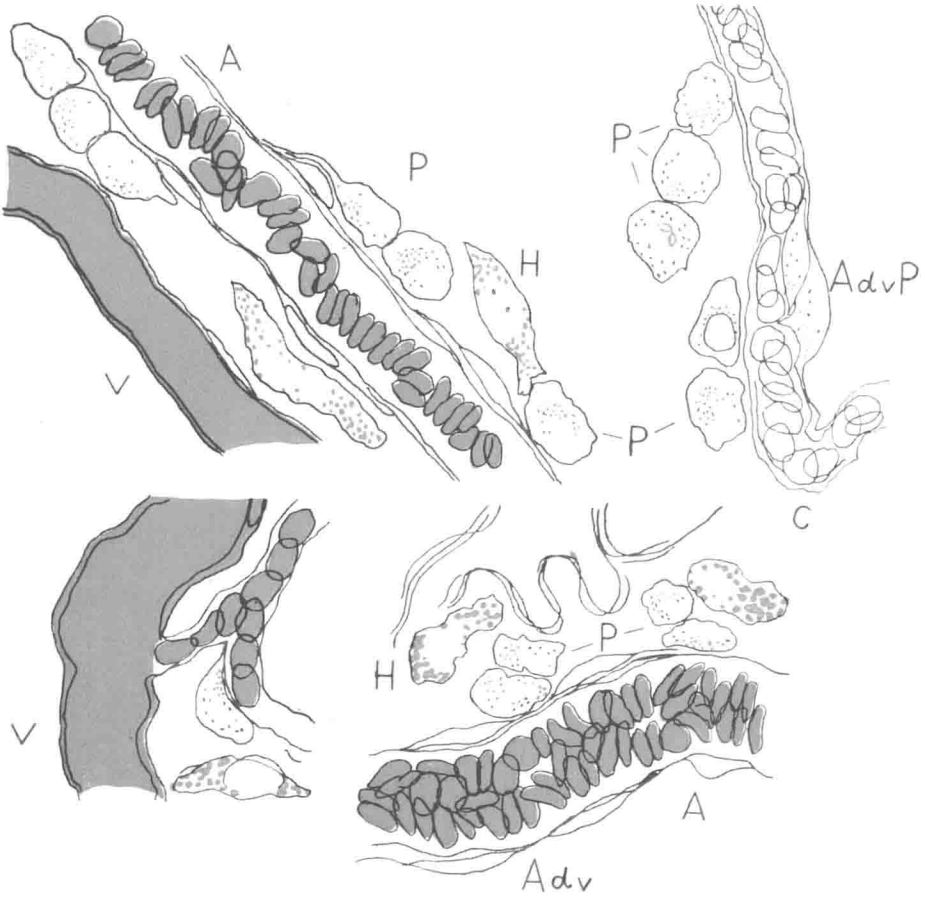
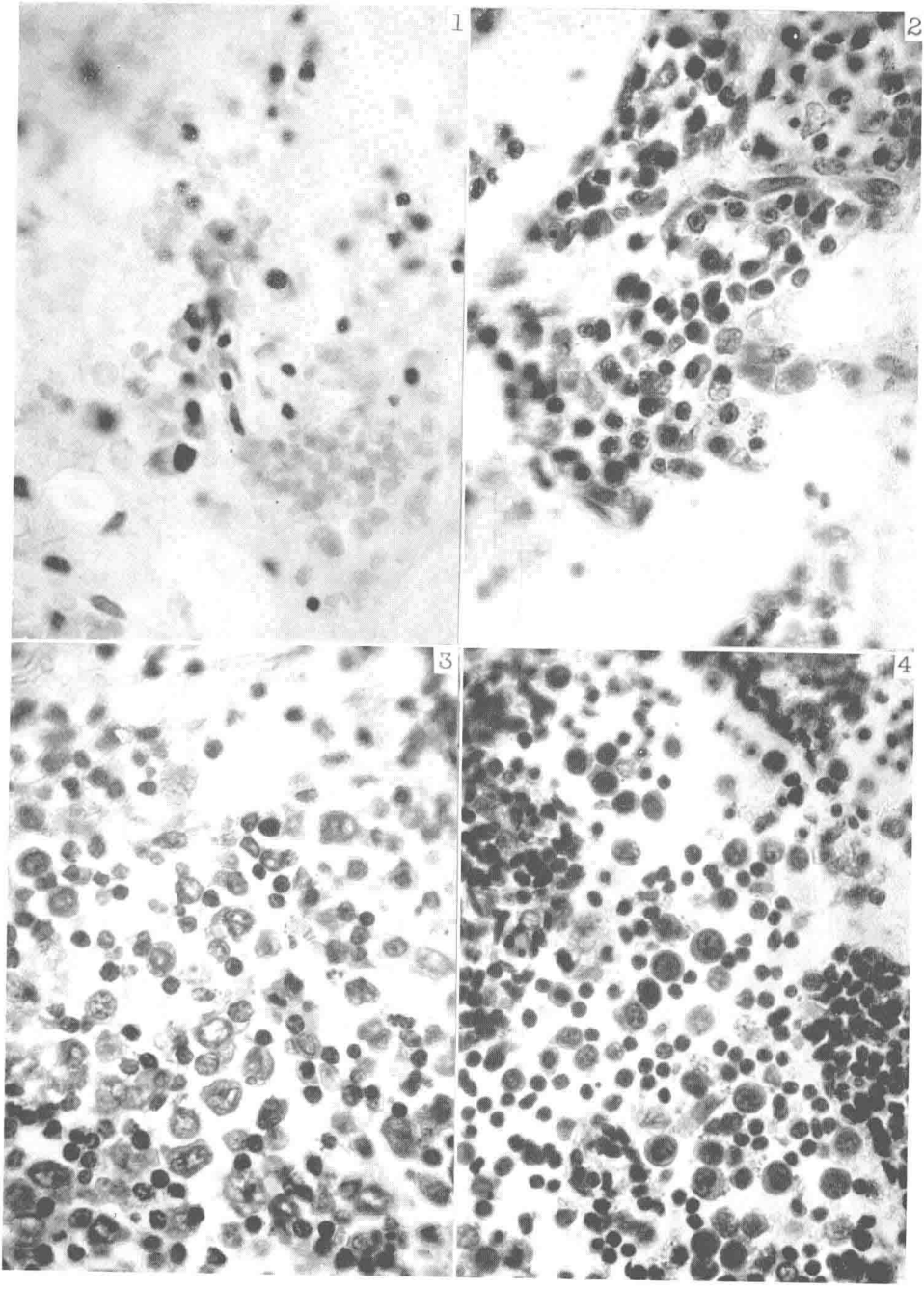


Plate III.

Supravital finding cutaneous tissue which demonstrates the genetical relationship between adventitial cells (Adv) and plasma cells(P). (Neutral red-Janus green stain of extending tissue, 5 days after the Arthus phenomenon). Young plasma cells in adventitial position exhibit (AdvP) the same supravital staining modus as free plasma cells. See the neutral red rosette formation and scattered mitochondrial distribution. Histiocytes (H) show diffusely distributed neutral red vacuoles. A : arterioles ; C : newly formed small blood vessels in granulation ; V : venules. (Compare with the electronmicrographs in Plate VIII)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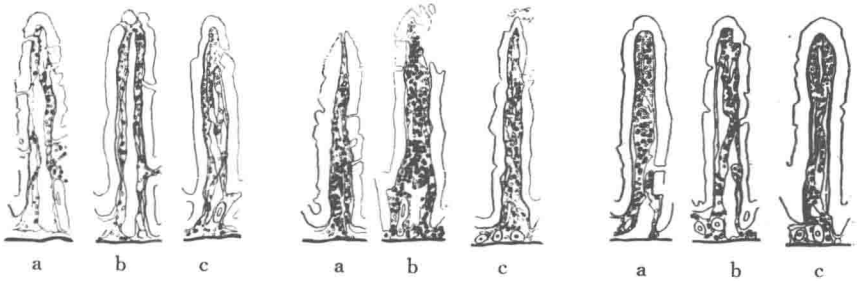
revealed that the plasma cells in the intestinal mucosa serve as the barricade against the invasion of foreign protein entering orally (Hanaoka 1951)[26].

Egg albumin was administered into the intestine of rabbits availing canules and its appearance in the peripheral blood was measured availing precipitation titers. In the course of this observation, two peaks were remarked. The first sharp peak locates after 30 minutes which disappear one hour after, while the second peak forms a plateau between 4 and 6–7 hours (Text–fig. 1). The throwing-away of thoracicus lymph by way of the opening of ductus thoracicus nullify the formation of the second peak, which means that the second peak owes to egg albumin absorbed from the lymph channel of the intestinal wall. On the other hand, the injection of egg albumin into the peritonal cavity brings forth only the second peak. The fact is observed referring to rabbits which do not accustomed to egg albumin absorption. On the other hand, frequent oral administra-tions of egg albumin into the intestinal lumen of rabbits provoke the antigen antibody reaction like the Arthus phenomenon at the intestinal wall which may be verified from the attitude of minimal egg albumin appear in the peripheral blood and the predominant reaction of plasma cells of the intestinal mucosa of those animals. (Text–fig. 2)

That the intestinal mucosa has a plasma cell barricade is an interesting



Text-fig. 2. Distribution of plasma cells in the mucous membrane of the small intestine. left : control, middle : intravenous sensitization and 3 successive peroral sensitizations; right : frequent peroral administrations. a. duodenum, b. jejunum, c. ileum. Black cells in the figure express the plasma cellular distribution (Hanaoka, 1951)

Plate IV.

Distribution of plasma cells in hematopoietic organs.

1. Spinal bone marrow in aplastic anemia (from an autopsy case). Note the distribution of plasma cells alongside of an arteriole. As this tissue contains no parenchymatous cells, the adventitial localization of plasma cells is seen prominently.
2, 3, 4. A mesenterial lymphnode of a coli-bacilli administered rabbit. Usual localisation of plasma cells (1) is mostly confined to the medullary pulp near the sinus where vascular tissues are concentrated, and therefore, adventitial generation of plasma cells attributable to the adventitial cells. On the other hand, large lymphogonia are distributed diffusely to cortical pulp but not to medullary pulp. (2, 3) These large lymphogonia in mobilization are seen in the medullary sinus (4).