REVOLUTION
OF
BIOLOGY & MEDICINE

— A NEW THEORY ON THE LIFE SCIENCE
AND
ITS PRACTICAL APPLICATION TO
HEALTH & DISEASE —

By
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"Error runs down an inclined plane, while Truth has to laborioriously climb its way up hill."

-- Oriental old Proverb --

"There is no religion higher than truth."

-- Satyât Nâsti paro dharmah --

"Was ist das Schwerste von Allem ?
Was dir das Leichteste dünkt
Mit den Augen zu sehen
Was vor den Augen dir liegt."

-- J. W. von Goethe --

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PREFACE

"My aim is to establish entirely renewed science against the extremely old object"

—Galileo Galilei —

I wrote this book as the ninth volume of the ten serial volumes which include my complete works concerned with eight new fundamental and revolutional principles on the biological and medical sciences carried out by me for the past fifty years.

And also the practical application of these principles to health, prolongation of life and daily living are included. Furthermore, this book still more contains the scores of my own original papers published in English.

But I hope that the persons who wish to know the whole details of my work, will refer to my following serial works: "CHISHIMA'S COMPLETE WORKS REGARDING BIOLOGICAL AND MEDICAL SIENCES" (See pp.5-16)

Aims of this book

◎ Modern society which lays great emphasis on technological progress and making money, brought us startling material prosperity but lack of wisdom of the ultimate effect abusing them is bringing about many kinds of dangerous chemical pollution on our earth, and mankind is faced with a critical moment.

Moreover, it has changed people into economic animals and kept them at a distance from one another.

If we leave the matter as it is, this material trend will be promoted more and more, and, as a result, our mental decay and physical decline will be promoted more and more, too.

It is essential that we should change our way of thinking on the view of value.

Now modern material civilization is confronted with a turning-point in history.

We must change our thinking method (scientific methodology or logic) from the materialism, mechanism and formal logic to "bio-dialectic",

designated by the present author, which is a dialectic unifying the body and mind, in other word, unifying (Aufheben in German) material dialectic and spiritual dialectic.

◎ It is generally believed that modern sciences, especially biological and medical sciences have attained to the marvelously advanced state.

But we must not overlook the contradiction between the modern medical sciences and diseases. That is to say, the more medical sciences advances, the more the patients suffering from incurable chronic diseases such as cancer, heart diseses, apoplexy and so on increase in number.

This suggests that modern Occidental medicine probably has some defects in its principles. According to my opinion, those defects may be attributed to the following two reasons; one is the inadequate thinking of life, that is formal logic, mechanism and materialism are taken.

And another is the misunderstanding about blood, especially the origin and function of the red blood corpuscles.

In this book I will present my findings about these problems.

Ranging from my first principle to the seventh principle, I will describe the fact that the haematopoietic organ is not the bone marrow but is the intestinal villus, and that the red blood corpuscles hold the polypotency of their differentiating into all kind of somatic cells and germ cells, even into the cancer cells or several other pathologic cells under the pathological conditions.

Even if such a heterodox theory may be considered an incredible one, I believe confidently that it is true, because it is based on positive facts and it coincides with a correct thinking (scientific methodology, bio-dialectic).

◎ Recently there appears a new tendency in the field of medical science, not only in Japan but also in Europe and America.

This is the revaluation of the Oriental medicine which differs from the Occidental medicine on the following points: ①Emphasizing the harmony between mind and the body, ②Laying stress on the wholeness of an organism instead of the analytical thinking, ③Making more of prevention than treatment, ④Considering herbs than new chemically-synthesized remedies, ⑤Putting a more value on the natural cure than the arfificial remedies and surgical operation for certain chronic diseases.

◎ In this book I intend to unify Oriental and Occidental medicines by picking up the merits, and throwing away the demerits included in them respectively.

And I have attempted to show a vision of the third medicine or the medicine-tomorrow.

Acknowledgment

I am deeply indebted to my colleaugues Prof. F. Tsukiji and Mr. M. Saito for their helping to English translation and proof reading.

I am also very grateful to the many persons whose names are described in each paper and monograph published in the past fifty years.

I must express my gratitude to Y. Horita and Y. Ito for their encouragement, stimulation and advice to me, by which I have decided to publish this serial book (Chishima's complete works).

Finally, I must express my appreciation to the members of "the Neo-hematological society" (superintended by me), and many other persons helping me with my publication.

KIKUO CHISHIMA

October 1972

INTRODUCTION

In the sixteenth century, the discovery of Copernicus that the earth revolves around the sun evoked a storm of indignation because it displaced the earth that had been thought to be the center of the universe to the less important position.

Namely, the earth was proved to be only one of the several planets depending on the sun. In these day such a revolutional idea was, inevitably, thought an insult at the earth's chief inhabitants, mankind.

Nowadays sciences iucluding physics and biology, also are confronted with a turning point (Copernican conversion), as has been pointed out by some of the eminent scientists.

If we read the history of science, we shall find that there are many revolutional discoverings the discoverers of which were exposed to ridicule at first.

But if those revolutional finding are correct in both fact and theory, peo-

ple in the next generation (especially scientists) will gradually come to accept the newly presented heterodox theory. And then the heterodoxy will became the next orthodoxy instead of the present-day one and this history repeats itself.

My findings and opinions are so widely and basically opposed to, the orthodox biological and medical sciences that their practical application to human life has a great important meaning.

Therefore my new theory may be at first, considered to be a curious and doubtful heterodoxy by people.

But I believe that if one reexamines it practically or theoretically one will find that it is true.

Now I will mention here the outline of "my eight fundamental and revolutional principles concerned with biological and medical sciences.

◉ Eight fundamental new principles

Most of my works and opinions are opposed to the orthodox principles of the biological and medical sciences and to the orthdox scientific methodology. They are as follows:

① The first principle :

Red blood corpuscles with polipotency differentiate into all kinds of somatic cells and germ cells, in accordance with their cellular environmental conditions (milieu).

② The second principle :

Reversible differentiation between the red blood corpuseles and the fixed cellular elements under the different nutritional conditions or the developmental stages.

③ The third principle :

Bacteria and viruses arise spontaneously from organic matter by means of the AFD process (Aggregation, Fusion and Differentiation).

④ The fourth principle :

Cells increase in number, mainly, by the new-formation of them from organic matter but not by the so-called mitotic cell division.

⑤ The fifth principle :

Haematopoietic organ of the red blood corpuscle is not the bone marrow but the intestinal villus in the adult and the placental villus in the embryonal stage.

⑥ The sixth principle :

Orthodox genetics contains some basic mistakes. For instance, according to my finding, the germ cells such as spermatozoa and ova arise newly from the somatic element, the red blood corpuscles.

⑦ The seventh principle :

Darwinism involves some important contradictions of the origin of life, the mutation theory, existence of microorganisms (amoebae, bacteria) which remained as they were without evolution, and then the negligence of symbiosis (mutual aid) as an important evolutional factor, etc.

⑧ The eighth principle :

I have presented a new scientific methodology, bio-dialectic instead of formal logic or material dialectic.

○ I will describe in the latter chapter of this book the practical application of the above-mentioned eight principles to people's health and prolongation of life, etc.

○ For convenience sake to foreigners who may not read all my "complete works-serial books" in Japanese language, I will mention here the contents of my serial books in ten volumes (covering more than 5500 pages published in Japan beween 1971 and 1972)

Contents of "Chishima's Revolutional Complete Works on the Biological and Medical sciences.

(1) VOLUME I : LIFE, CELLS, THE ORIGIN OF BLOOD CORPUSCLES, HAEMATOPOIESIS IN THE INTESTINE (pp. 1~488)

In this volume I have criticized the following fundamental orthodox principles,

① Negation of spontaneous generation of microorganism (Pasteur and Oparin's theory)

② Orthodox cell theory (cell increases in number by means of mitotic cell

division only)

③ Intramedullary hemopoitic theory.

Against the above-mentioned three orthodox princples, I have presented quite new theories, based on the facts and theoretical point of view.

According to my new principles the orthodox views regarding with epidemiology, bacteriology, life, cell and blood must be changed revolutionary.

Contents

〔I〕 Cell and biology
〔II〕 New-formation of cell without mitotic cell division.
〔III〕 Disputations about spontaneous generation of life (bacteria, virus)
〔IV〕 Origin of bacteria and virus.
〔V〕 Origin of cell and microorganism in relation with symbiosis.
〔VI〕 Relation between digestion and intestinal symbionts in higher vertebrates.
〔VII〕 Phagocytosis theory and amoebism (Phagocytosis, Amoebism, Pinocytosis and Potocytosis)
〔VIII〕 Phylogenetic studies on haemopoesis (invertebrates)
〔IX〕 Phylogenetic studies on haemopoiesis (vertebrates)
〔X〕 Haemopoiesis in intestinal villi:
〔XI〕 Summary. Appendices: Literature and Index.

〔2〕 VOLUME II : REVOLUTION OF HEREDITY, EMBRYOLOGY, AND EVOLUTION IN RELATION TO THE ORIGN OF GERM CELLS

(pp. 1—476)

According to my view "Germ cells arise from blood corpuscles, and not from their mitotic cell division but from new cell formation of blood cerpuscles", the following problems are discussed. ①Antagonism between Mendelism-Morganism and Lysenkoism, ②Blind spots and contradictions included in evolutionism and embryology, especially on the non-evolutional half side of evolutionism, and on the evolutional thought… "The stronger prey upon the weaker" should not be applicable to the man-kind in future. On the contrary the symbiosis (mutual aid) is the most impotant evolutional factor of man-kind henceforward. This principle has very important meaning against the war-provokers.

. And my finding on "the germ cells are derivatives of blood corpuscles" is decicive factor on the judgment on the disputation between Mendelism-Morganism and Lysenkoism.

Contents

〔I〕 Origin of embryonal germ cells and the differentiation of blood corpuscles.
〔II〕 Origin of the functional ovum and the differentiation of blood corpuscles.
〔III〕 Origin of the functional spermatozoa in relation to the differentiation of blood corpuscle (Spermatogensis in epididymis)
〔IV〕 Summary and conclusion as to the origin of germ cells.
〔V〕 Criticism on the disputation between Mendelism-Morganism and Lysenkoism.
〔VI〕 Criticism about Weismannism and Morganism from point of my new theory regarding the origin of germ cells
〔VII〕 On the contradiction between the orthodox genetics (negation of the inheritance of acquired character) and evolutionalism based on mutation theory.
〔VIII〕 Criticism on the orthodox embryology and genetics based on mitotic cell division theory.
〔IX〕 Gene theory contradicts to the true evolutional thought
〔X〕 Relation among genetics, embryology and "the repeating of past life" (designated by me)

Appendix

(1) My impression of "the Tenth International Congress of Genetics" (in TOKYO)
(2) Suggestion to the Mendelists-Morganists
(3) Literature and Index.

〔3〕 VOLUME III : ON THE FATE OF RED BLOOD CORPUSCLE : REVOLUTION OF MEDICAL AND FROM A BIOLOGICAL SCIENCES (ESPECIAIIY CYTOLOGICAL AND HISTOLOGICAL POINT OF VIEW) (pp. 1~646)

According to my opinion, the very important mistakes of modern biology and medicine are due to the misunderstanding about the function and fate of the red blood corpuscles which occupy majority part of formed elements of blood.

I have found that the red blood corpuscles have poly-potential or totipotential ability to differentiate into all kinds of somatic cells and germcells and tissues.

And under the pathological conditions they differentiate into cancer cells, the cellular elements of inflammatory region.

Furthermore, they play important role in the case of wound healing or regeneration. Therefore, the orthodox histology, haematology, pathology, cytology, blood transfusion, hygiene, prolongation of life, medical treatments and the other fields of modern medicine, must be fundamentally reexamined.

Especially, cancer is the most important problems of the world, but there is no one who knows that cancer cells are increasing in number by differentiation from red blood corpuscles, but not by the mitotic cell division.

According to my opinion, the most important factor of the prevention and therapy of cancer is to promote the production of healthy, normal blood, especially the red blood corpuscles which are newly formed in intestinal villi by means of AFD process of digested food substances.

I should say that, if scientists in the world do not accept my finding the cancer problems will never be solved.

Contents

〔4〕 VOLUME IV : REVOLUTION IN THE EIGHT FUNDAMENTAL PRINCIPLES ON THE MODERN BIOLOGY AND MEDICINE AND REEXAMINATION ON THE INTRAMEDULLARY HAEMATOPOIETIC THEORY. (pp. 1~506)

〔1〕 Revolution in the eight fundamental principles on modern biology and medicine-and its practical application to health and prolongation of life

(1) The first principle. Red blood corpuscles differentiate into all kinds of somatic cells and germ cells. And its practical use to the daily life.

(2) The second principle. Reversible differentiation between red blood corpuscles and fixed fissue elements according to the nutritional conditions or embryonal stage. And practical application of this principle to the daily life.

(3) The third principle. Spontaneous generation theory of bacteria and villus. And application of this principle to the health and daily ljfe.

(4) The fourth principle. New formation of cells from organic substances. And application of this principle to the health and daily life.

(5) The fifth principle intestinal hemopoietic theory. And its practical use for daily life.

(6) The sixth principle. Irrationality included in the orthodox genetics-principle. Especially, my finding on the origin of germ cells which are derived from blood corpuscles must bring profound influence on the orthodox genetic theory. And application of this principle to eugenics and the daily life.

(7) The seventh principle. Illogicality included in evolutionism. Especially "the negligence of the symbiosis which is the most important factor of evolution", "biological meaning of war and peace in man-kind", "the stronger prey upon the weaker" now must not be applied to human society any longer.

(8) The eighth principle. True scientific methodology of the biological sciences must be the "Bio-dialectic".

Almost all bio-scientists in the world are the followers of formal logic or some of scientists are materialistic dialectic's followers. But I have presented "biodialectic" that is an unifying dialectic both of spiritual dialectic and materialistic dialectic following the progressing law of dialectic itself.

I think that the most important cause of the theoretical stand still of the modern biology and medicine may be attributed to their inadequate scientific methodology (philosophy). We must find a way out of the deadlock in modern biology and medicine.

I believe firmly that there is no other way with the exception of scientists changing their thinking and adopting "bio-dialectic.

〔II〕 Reexamination of the intramedullary haematopoiesis theory

(1) Relation between the origin of red (or yellow) bone marrow and the differentiation of red blood corpuscle under the well-fed condition, (with English résumé).

(2) Intramedullary hemopoiesis under the starved condition, especially the reverse differentiation from fat globule into red blood corpuscles.

(3) Reversible differentiation between the bone (or cartilage) and the blood corpuscles.

(4) Criticism on the so-called extramedullary hemopoietic theory.

(5) Answers to the questions contributed to me from forty-six scientists in the world.

〔III〕 Revolution of medical science and the medical treatment system in Japan

(1) Reexamination and reflection of superstitious belief of persons to the modern medical theory and practice.

(2) Ethics of physicians.

(3) Improvement of the medical education system.

(4) Improvement of the medical treatment system.

Appendix: Literature. Intedex.

〔5〕 VOLUME V: STUDIES ON THE HEN'S EGG

(pp. 1~506)

This volume contains (i) Reprint of the monograph, KEIRAN ZENKO (Studies on the hen's egg) published by the present auther in 1933, and (ii) my twelve original papers regarding bird eggs.

Part 1. Contents

① Structure, formation and physical constitution of hen's eggs.

② Chemical constitution, nutrient value and preservation of hen's eggs.

③ Studies on the egg-laying capacity of hen.

④ Studies on the artificial incubation.

⑤ Inspection, judgment of hen's egg.

⑥ Preservation and manufacturing of hen's egg.

⑦ Trading and selling price of hen's egg.

Appendix: Literature, Index.

Part 2. Eleven original papers published by the present author (published in 1930~1936)

① On the revision of erroneous explanatory diagrams of hen's egg which prevailed, hitherto, world wide (J. appl. zool. 3. 3. 1931).

② Correlation among the shape-index, egg-weight and the direction of egg-laying. (lay down from the blunt end or from sharp end of the egg at first) (I) (Ibid. 3. 3. 1931)

③ Ibid.…(II) (Ibid. 3. 4. 1931)

④ Spirality of bird's egg and of hen's oviduct. (Ibid. 3. 3. 1931)

⑤ Comparison between hen's egg and a Japanese large turtle (*Chelonia Japonica* (THUMB). (1930) (Jap. J. zoology. 42. 502. 1930)

⑥ An malformed turkey's egg and a malformed hen's oviduct. (I bid. 43. 507. 1931)

⑦ On the ultraviolet fluorescence of hen's egg and oviduct as an indicator of the detection of old or new egg. (Botany and Zoology (Shyokubutsu to Dobutsu) 2. 7. 1934)

⑧ Ibid (II). (1934) (Ibid. 2. 8. 1934)

⑨ Studies on the birds' egg (Report I)
On the formation-mechanism of "chalazae" and spirality of hen's oviduct (with Dr. N. Yagi) (1934) J. Natural History (Hakubutsugaku zassi Bunrika Daigaku. 30. 47. 1934)

⑩ Ibid. (Report II) Studies on the microscopic structure, texture and translucent spots of egg-shell. (J. of Japan Zootechnical Science 7. 4. 1935)

⑪ Ibid. (Report III) On the relation between the texture of egg shell and the weight loss of hen's egg. (Ibid. 8. 2. 1936)

〔6〕VOLUME VI : CIVILIZATION AND LIFE(pp.1-493)

—— Decline of life owing to the unbalanced material civilization ——

Contents.

(1) Civilization and the pollution of food stuffs (harmful chemical treatment, artificial colouring of foods),

(2) Civilization and toxic effect from the agricultural spray of insecticides, pepticides, herbicides and other chemical substances.

(3) Civilization and the dangerous secondary effect of synthesizd new remedies.

(4) Civilization and the terrible effect from radioactive isotopes.

(5) Antihumanistic weapons (Biological, Chemical weapons)

(6) Public nuisance accompanied with the expansion of industry.

(7) Air pollution and the decline of life.

(8) Water pollution

(9) Unwonted sound (noise), vibration, tremor, sink of lands.

(10) Industrial hygiene

(11) Occupational diseases

(12) Harmonious control between mind and body.

(13) Religion and Medicine.

(14) Psycho-somatic diseases in relation with civilization.

(15) The cause, prevention and therapy of cancer and the harmony of three S principle (Spirit, Sang, Sport)

(16) Prevention of fatigue and recovery from it.

(17) Relation between sleeping and health.

(18) Food in relation to the human thought, health and longevity.

(19) Principle of harmony and "musubi" (peace, unity)

(20) Theory and practice regarding life, health and longevity of mankind.

(21) An opinion of USSR Scientist "man can live untile one hundred fifty years "(Dr. A. Bogomolet's book translated into Japanese and criticized on it by the present author)

Appedix. Literature. Index.

〔7〕VOLUME VII : WAVE AND SPIRAL TENDENCY OF LIFE PHENOMENA(pp.1~600)

——BIO-DIALECTIC——

This volume is my first publication of my life works secured through fifty years on the wave and spiral tendency of life phenomena. In this volume

I have tried to unify my theoretical biology and experimental biology.

Contents

Chap I. Wave and spiral tendency of structure, movement and life phenomena in organisms (Mainly from an ontogenetical point of view)

①Morphological waveness and rhythm. ②Morphological spirality. ③Asymmetry and spirality of morphology and functions. ④Relation between asymmetry and spiral. ⑤Rhythmic and spiral movement. ⑥Antagonism and spirality in the parasitism, sexual phenomena and other physiological phenomena. ⑦Wave and spirality in growth. ⑧Correlation between the rhythm in non-living world and in living world (rhythmic ecology). ⑨Wavy and spiral tendency of organisms and that of natural environment (the sun, season, month, day, weather, etc.)

Chap II. Wave, spiral tendency in organisms (mainly from an phylogenetic or an evolutional point of view)

⑩Wave and spirality of heredity and evolution. ⑪Wave and antagonism in instinct and psychological phenomena. ⑫Wave and spirality of the development of civilization. ⑬Wave and spiral tendency of the mechanism and the vitalism through the time, and the relation between living and non-living substances.

Chap. III. The ten principles on wave and spirality in the life phenomena

①Wavy and spiral outlook on the world (weltbild oder weltanschaung... G).···wave-spiral tendency in atom and universe. ②Principle of repeating the past record. ③Principle of the unity of inner (milieu) and outer environment of organism. Unity of mind and body. ④Antagonism and its unification. ⑤Probabilismic point of view ⑥Wholeness and correlativity of structure and function in organism. ⑦Non-living one side of organism. ⑧The eighth principle "there is no decided border line in every-thing and matter". ⑨Asymmetry is fundamental nature endowed by heaven. ⑩Strengthening rule of wave and spirality in organisms.

Chap. IV. Scientific methodology and Bio-dialectic

①Something binding up science, philosophy, and religion. ②Material and energy (new conception of energy, spiritual energy). ③Four scientific methodology (formal logic, spiritual dialectic, materialistic dialectic and bio-dialectic that is to say, mind and body are inseparably united one). ④Scientific theory and mental constitution of scientists. ⑤Scientific thought in relation to social form, the time (era).

〔8〕 VOLUME VIII : COMPLETE COLLECTION OF THE ORIGINAL PAPERS IN JAPANESE

(pp.1～538)

This volume contains one hundred and scores of Chishima's papers published on the scienfific periodics (Japan Zoological magazine.) Jap. Anatomical Ass. Jap. Physiological Ass. Bulletin of Gifu Univ. Jap. Zootechnical science. Jap. applied Zool. J. Insect. Biol. Med. Science (Tokyo Univ), Biol. Sci. (Iwanami) etc.

〔9〕 VOLUME IX : CHISHIMA'S NEW THEORY ON THE BIOLOGY AND MEDICAL SCIENCE TRANSLATED INTO ENGLISH

This volume is written in English and, on the part I. contains the revolutional eight principles on the biology and medicine presented by the present author, and on the part II. scares of the present authors English papers are also collected. (this volume) (pp. 1～460)

〔10〕 VOLUME X : SCIENTIFIC ESSAYS, CRITICISMS ON MEDICAL SCIENCE, AND THE AUTOBIOGRAPHY OF THIS AUTHOR

(pp. 1～550)

In this volume the writer's opinions regarding biological and medical sciences are collected. For instance, "campaign against the fluoridation of drinking water".

"On the danger of blood transfusion" "Material civilization and spiritual civilization". "Unifying of the oriental medicine and the occidental medicine". "Criticism on the opinion against rice eating". "Secondary reaction of chemical drugs". "Diseases caused by the modern medical treat-

ment ".

"Problems on sex". World peace and Bio-dialectic". "On the faults of the modern analytical dietetics". "Criticism against some Japanese opinion that "milk is not only useless but also harmful to human being". "Harmony between mankind and natural environment ", etc.

(11) VOLUME XI : APPENDIX

Index throughout the whole ten serial volumes of "Chishima's Complete works ".

Explanation of the Colour Photomicrographs(Figs. 1~30)

(Figs. 1~27 were photomicrographed from preparations; paraffin sections, fixed in Bouin's fluid and stained by haematoxylin and eosin ; Fig. 28~30. photomicrographs from stamp—smear preparations stained by Giemsa's solution. All of these photomicrographs are original with K.Chishima)

Fig. 1. Photomicrograph from a section of Frog's liver injected carbon colloid, showing the transition from erythrocyte into liver cell.

Black mass (a) located in the interstices of liver cells are derivatives from clumps of erythrocytes with carbon colloid. This mass shows transition into Kupffer's cells, from which it differentiates into liver cells (b ›). There is no sign of typical mitotic figure in the liver.

Fig. 2. Normal liver of frog.

Showing the transitional phases from erythrocyte, with an elongated nucleus indicated by an arrow into the liver cells with clear round nucleus or nuclei). At the lower part of the figure there are two black masses. These are the clumps derived from melanizated liver cells.

Fig. 3. Showing open system of the capillaries of spleen.

There can be seen a mingled state of the erythrocytes with dark stained nucleus, the spleen-cell (a kind of lymphocyte) with somewhat clear nucleus and the elements of several grades of transitional phases between erythrocyte and spleen-cell. Notice// there can be seen many of extravasated erythrocytes.

Fig. 4. Pancreas of mouse and Langerhan's islets.

There can be seen the continuation and transitional phase from erythrocytes (a) stained with eosin (red) localizing at the upper-right of the fig. into the pale cell-mass (Islet of Langerhans). Islet of Langerhans often contains erythrocytes in it, and it is well-known that the islet cell secrete a hormons (insulin) which play a role to prevent diabetes.

At the circumferance of the Islet there are many of dark stained cell-mass (panercaticacinus), according to my opinion Islet cells differente into acinus cells under well-nourished condition but under starved condition the acinus cells differentiate reversely into the Islet cells and at last return to erythrocytes.

Fig. 5. Differentiation from erythrocytes into primordial germ cell (tadpole's gonad).

At the left side (kidney) there are erythrocytes and its derivatives... lymphocyte like cells. From the middle portion of fig. toward the right side there can be seen the transitional phases from lymphcytoid cell-mass into the primordial cells with large clear nucleus by means of A.F.D. process (Aggregation, Fusion and Differentiation).

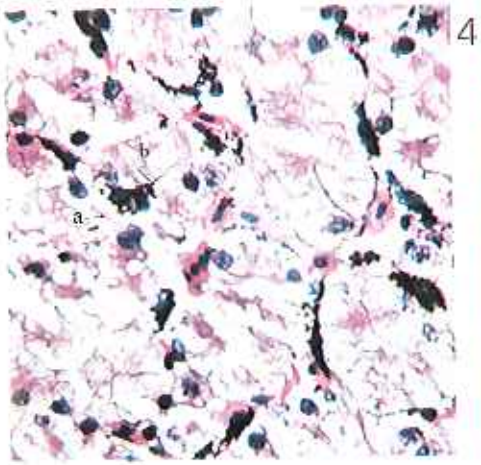
On the periphery of these germ cells these are many erythrocytes or the elements belonging to its differentiating phases with dark stained nucleus.

Fig. 6. New-formation of ovum from erythrocytes in the blood vessels. (Surface portion of frog's ovary).

two blood vessels are branching off toward the left-upper and the right-upper portion of fig. and in the blanted ends of these two blood vessels there can clearly be seen ovums growing through the A.F.D. process of erythrocytes.

There are figures resembling somewhat mitotic figure, but it is not mitosis, on the contrary it is the transitional phase of cell-fusion.

At lower-left there is a large ovum and at the periphery of it are several numbers of erythrocyt is nuclei and their transitional phases into yolk material.



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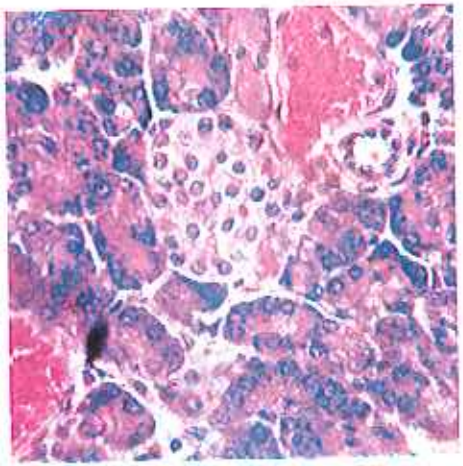

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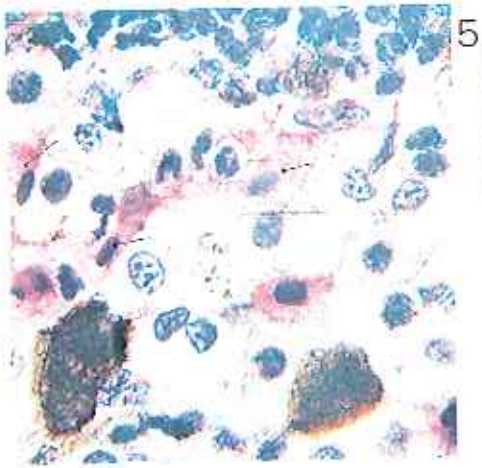

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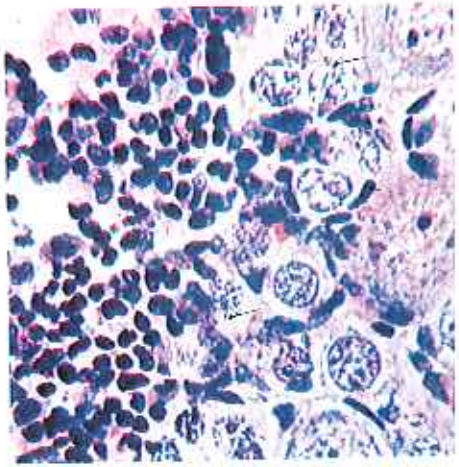

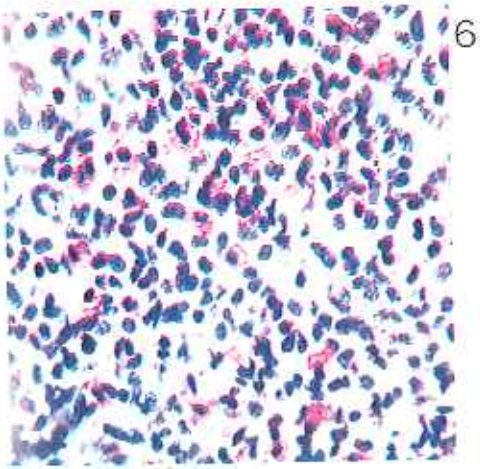

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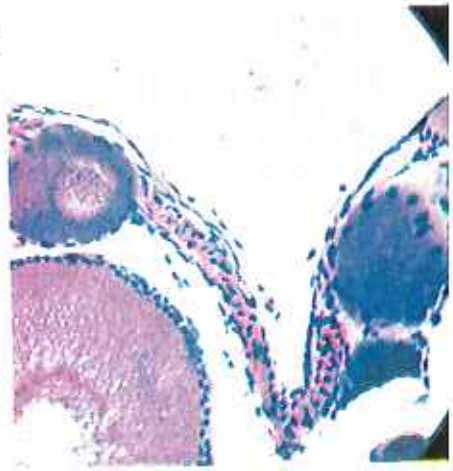


Fig. 7. Differention from erythrocyte into spermatozoa. (testis of mouse)

At the upper middle portion erythrocytes (stained red) are located in the interstices of seminiferous tubules and are two seminiferous tubules at lower left and right side.

Outside of seminiferous tubules are covered with erythrocytes (red) and show transition from erythrocytes into→cells of basement membrane →Sertoli cell →Spermatogonium →primary spermatocyte →Secondary spermatocyte→Spermatid→the so-called Spermatozoa.

But the so-called spermatozoas in the seminiferous tubule are not true functional sperm. While functional sperms with fertilizing capacity are generated only in the epididymis (cf. fig. 9).

Fig. 8. Functional sperms are generated from erythrocytes in the epididymis. (testis of mouse).

Red staining area transversing the middle portion of the fig. is the blood stream and there are transitional phases from erythrocyte into the so-called early spermatozoa through the same transitional stages described in fig. 7.

Fig. 9. True spermatogenesis take place in epididymis (tesis of mouse)

A cross section of a ductus epididymis. Erythrocytes (stained with red and indicated by arrow) are adhered on the surface of the ductus. There are transitions from erythrocytes into wall-cells of the ductus and→light stained wall cell→degeneration of nucleus→pale monera-like substance (a)→with approaching to the centre of the ductus there appear spontaneously spermatozoas (b). These spermatozoas show typical shape of sperm and functional as has been believed. There is no evidence that these sperm are not generated in the testis by mitosis but are spontaneously generated *in situ*.

Fig.10. Cancer cells are a derivative from erythrocytes (human uterine cancer)

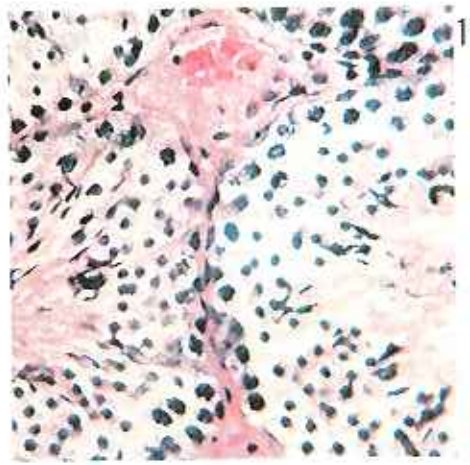
Two blood vessels are descending downward, and their ends are opening to the tissue. Many of erythrocytes are scattering about and they show transition into small-lymphocytoid elements,→immature small cancer cells.

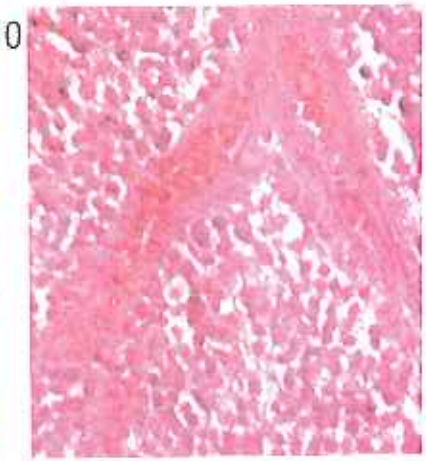
Fig.11. Human uterine carcinoma.

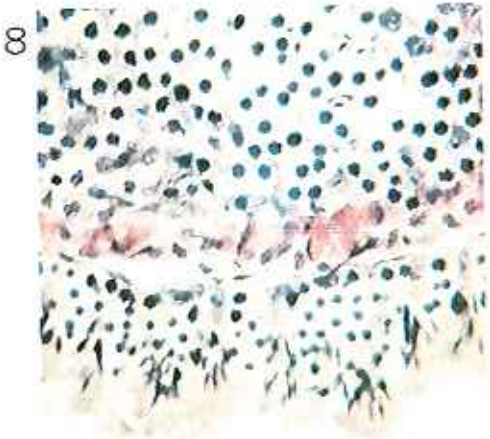
There can be seen many of extravasated erythrocytes (stained with red) are scattered here and there, and mingled with small primordial cancer cells. And there is transition between these two elements.

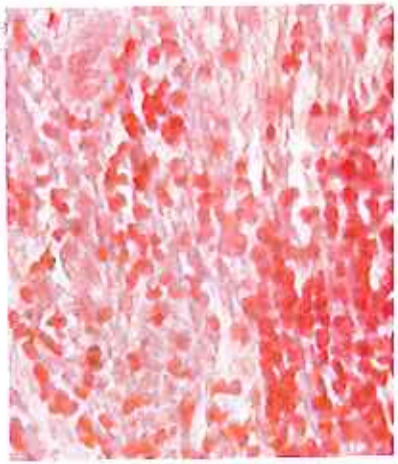
Fig.12. Cancer nest of human uterine sarcinoma.

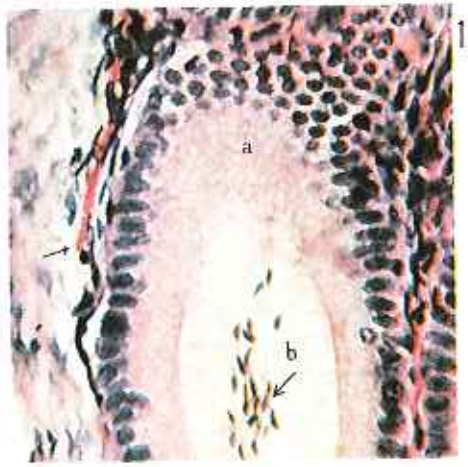
Tow clusters of cancer cells, the cancer nests, are shown at upper left and right side of the fig. And at the lower left side is a cancer nest at early stage of it's differentiating from a blood vessel which still contains some of erythrocytes.

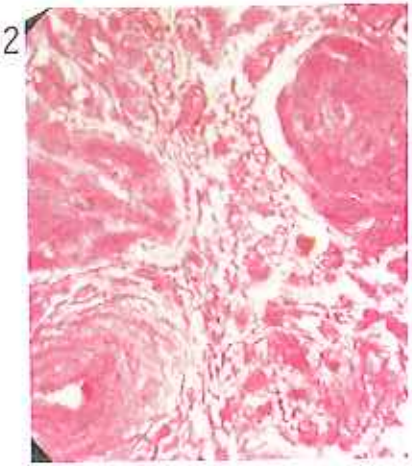












g.13. Reverse differentiation from yolk-spheres into erythrocytes under the starved condion (frog's ovary starved for five weeks)

The figure is a magnified upper left portion of an ovum. Yolk-spheres (a) (located from middle into right in the fig. , and stained with red). From which toward the left side there arising melanin pigment→vaguely stained nuclei (b) arise →clear nuclei (c)→typical erythrocytes (d) and then they are carried out by blood vessel. There is shown clearly the evidence of the reverse differentiation from yolk-spheres into erythrocytes.

g.14. Reverse differentiation from yolk-spheres into erythrocytes (frog's ovary, starved for five weeks)

There are an ovum differentiating reversely into erythrocytes at the middle portion of the fig. Yolk-spheres (a) stained with red. From the periphery of them arise dark brown colored melanin pigment (b)→transitional phases →typical erythrocytes (c) with dark stained nuclei and red colored cytoplasm→are carried out by blood vessel (d). Thus the ovary shrinks gradually. Around this ovum there are six ovums. These ovums also reverse into erythrocytes by and by. Lastly formed ovum begins reverse differentiation at first.

g.15. Yellow bone marrow (fatty tissue) reversely differentiate into blood corpuscles (bone marrow of cat's femur starved 12 day)

There arise many erythroblasts in a large fat globule (a), and then they transform into normoblasts (b). A normoblast gives rise to many of none-nucleated erythrocytes and they are carried out by blood vessel.

ig.16. Reverse differentiation from a ganglion cell into many of erythrocytes (frog starved for five weeks)

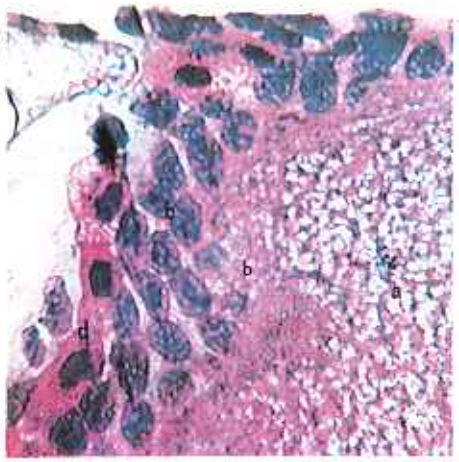
large ganglion cell (a) with clear cytoplasm (which indicates that it has contained lipoid substances) and with dark stained nucleus. Within that cell newly arise many of normal erythrocytes through transitional stages (b,c.)

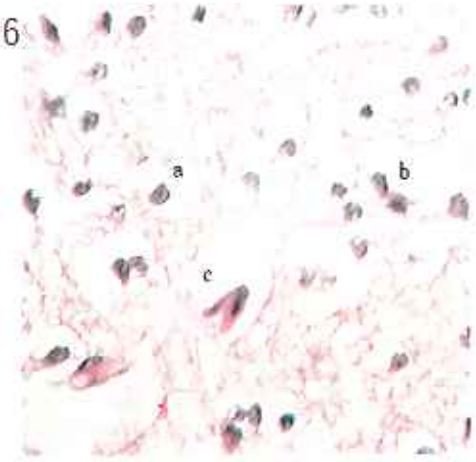
ig.17. Reverse differentiation from spinal cord into erythrocytes (spinal cord of frog starved for five weeks).

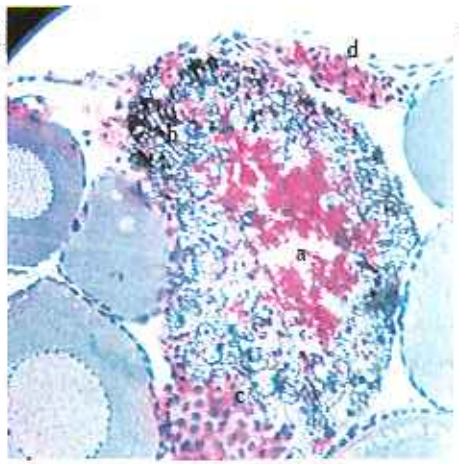
There is a capillary (a) stained with red at somewhat left side of centre line. In which contain two erythrocytes. Left and right side of the capillary there are many of reverse transitional phases (b) from nerve fibers into capillaries and erythrocytes.

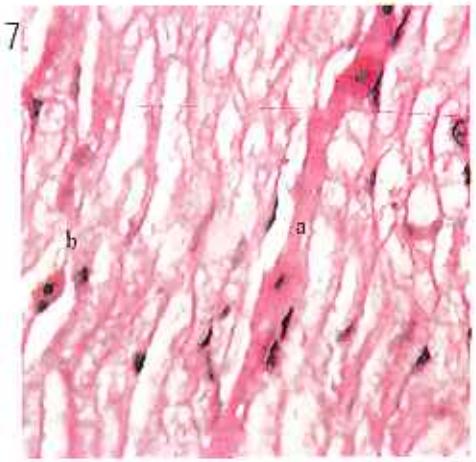
ig.18. Reverse differentiation from bone-tissue into erythrocytes (femur of a chick starved 9 days)

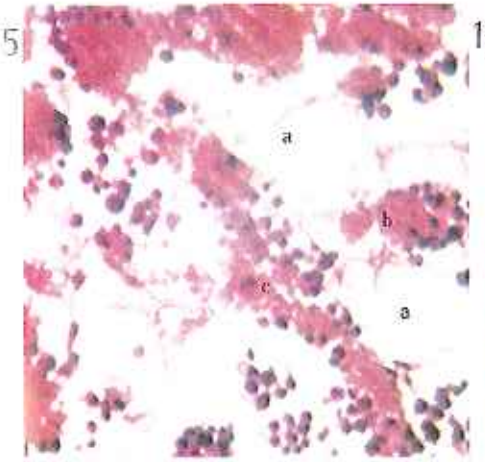
Under the well fed condition erytrocytes differentiate into cartilage tissue→bone-tissue, while under the starved condition they differentiate reversly into blood corpuscles. In this case Haverstan canal (a) become wide and bone-tissue become cartilage (b) and then bone marrow (c) and further into blood corpuscles. In the bone marrow there remains thin bone-edge (d) and texture of bone undergo porous and fragile.











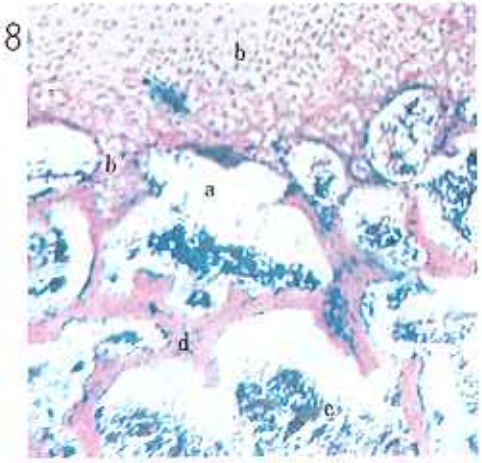


Fig.19. New formation of erythrocyte from yolk-sphere in chick embryo. (hematopoiesis in yolk sac)
Surface of yolk sac in chick. With going to upper portion the yolk-sphere (a) become to be fragmented →arise nuclei →erythroblasts→erythrocytes →prototype of blood vessel containing erythroblasts (b) and then they are carried out. Notice, the prototype of blood vessel has no well-defined boundary, (c) on the contrary, it continuous to yolk-spheres. Hematopoiesis in yolk-sac in human being is only rudimentary.

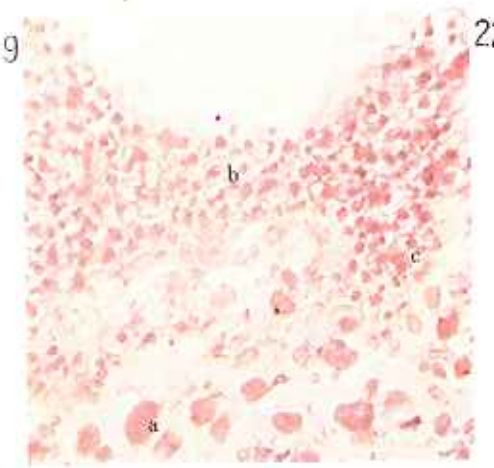
Fig.20. Hematopoiesis on the placenta in rabbit (Hematopoies on placental villus)
Second stage of hematopoiesis in developmental embryo in mammals including human being proceed throughout all embryonal stage. Upper right portion of the fig. shows maternal uterine wall (a). From where blood is poured out into the uterus →the maternal blood corpuscles are gathered together→primoridial placental villus (b)→fuse with embryonal placental villus →epithelial cells of villus → growth of them →hemocytoblasts→erythrocytes (c) stained with red→carried out by blood vessels formed newly.

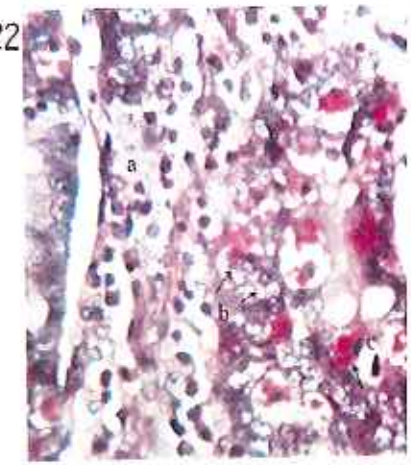
Fig.21. Magnified photomicrograph of the fig.20
Maternal blood corpuscles (a) adhere on the placental villus (b)→differentiate into the epithelial cells of villus by means of new cell formation *insitu*→from inner portion of the epithel transform into erythroblast →erythrocytes (c). In this case also there is no sign of mitotic cell division.

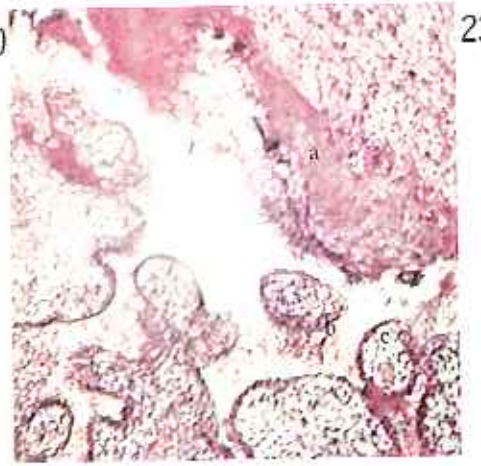
Fig.22. Hematopoiesis on the intestinal villus in goat's small intestine.
Epithelial cells (a) of intestinal villus grow gradually with approaching to inner portion of villus. and become a large cell (b) with large nucleus and stained with red (eosin). And these normoblast like cell transform into several numbers of non-nucleated mammalian erythrocytes by means of sporulation-like process.
In this case also these is no sign of mitotic cell proliferation.
Thus the embryonal erythrocytopoiesis takes place ① at first on the villus of yolk sac from yolk-spheres and second stage ② on the placental villus from maternal blood, and ③ after the birth, on the intestinal villus from digested food material.

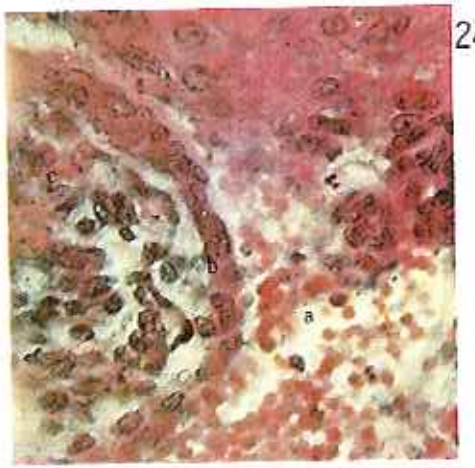
Fig.23. New-formation of erythrocytes from yolk-spheres (heart of tadpole just after hatching)
There are about ten primordial erythrocytes within the heart. Each primordial erythrocytes, (the so-called yolk-shere cell) includes about 20 -30 of yolk-spheres. Some of these show sign of synthesis of DNA in that mass (stained with hematoxylin). After a few days these primordial erythrocytes differentiate into typical erythrocytes with nucleus.

Fig.24. New formation of muscular tissue from the yolk-spheres (a portion of tadpole's tail)
Yolk-spheres (a) showing the appearance of red granules stand lengthways. Then they show transition into muscule fibers (b) staind with light red colour. Here and there can be seen striations.
After the yolk-spheres are consumed completly in the embryo, the erythrocytes take the place of yolk-spheres.









(B) ORIGINAL PAPERS PUBLISHED IN ENGLISH

I Relation Between the Histogenesis of the Wolffian Body and the Differentiation of Blood Cells in Chick Embryos*

By

Kikuo Chishima

(Dept. of Vet. Med. The Gifu Univ. Gifu-Ken, Japan.)

Six Figures

Introduction

Ever since wolffian body was first described by Wolff (1890) it has been generally believed by many workers (Brambell, '28, Lillie, '19 and others) that the glomeruli and Wolffian tubules begin their development as a result of the condensation and mitotic proliferation of mesenchymal cells. However it seems probable that this conception may demand further comprehensive evidence, because the most important problem with regard to the source or origin of these elements has not yet been settled so far as the writer is aware. It is widely accepted opinion that the erythrocytes are the most highly differentiated cells and so I can find no paper dealing with the behavior and differential potencies of erythrocytes, even though there have been published tremendous amount of the literatures regarding to erythrocytes.

The writer (Chishima (2–14, 29); have reported that the erythrocytes in several species of vertebrates show wonderful behavior and they may have very wide differentiating capacities. This paper is a description of the relation between the histogenesis of Wolffian body and the differentiation of the blood cells in chick embryos.

Acknowledgements

This study was carried out during the years 1940–1944 at the Kyushu University. I wish to express my gratitude to Professor Masaharu Tange for his helpful advice ahd criticism. My thanks are also due professor Hajime Mimura for his suggestion and counsel.

* Redaktion der Okajimas folia anatomica joponica
Anatomisches institut der Keio universität
Shinjuku, Tokyo
Japan

Band 23, Heft 6 pp. 337-351
(Okutober 1951)

(I) Materials and Methods

257 F_1 chick embryos obtained by crossing female Barred Plymouth Rock with male Rhole Island Red which were kept at the Laboratory of Zootechny, Dept. of Agriculture, Kyushu University were used as materials. Age of embryos ranged from 4 days to 21 days of incubation. As a fixative Bouin's fluid was used, and serial sections were cut 4 to 10 micra thick, stained with Delafield's hematoxylin and eosin, in some instances Flemming, Champy, Meves, Regaud and Allen Bouin's fluid were used as fixatives. Hydrogen Peroxyde, which was diluted with equal amount of Ringer's solution, were injected into the air chamber of some of incubating eggs, and then at certain days after the operation the Wolffiian bodies were examined.

The Wolffian bodies removed from embryos at 9–10 days of incubation, transplanted into the collio-allantoic cavities of the other incubating eggs at the same age through the small whole which had been made on the egg shell. The operated eggs returned to incubator, and after certain interval the transplanted tissues were studied microscopically.

Results

(a) *Direction and Phases of the Differentiation of the Blood Cells in Wolffian body.*

The embryonic erythrocytes (IA) migrated into wolffian body show the transitional phases into following four main directions, namely, IIA. IIB. IIC. and IID under the influence of induction of field at which the erythrocytes are located and then these four types of cells further show, transitional phases into III A. III B. III. C. and III D. respactively, according to lapse of time.

Thus all of elements of wolffian body in chick embryo belong to any one of these four phases. These classification of the cell types, however, are only for the convenience sake of studies, so that, in strict sense, it is unable to draw a strict line among these cell-types because they show continual or transitional phases in each other.

The characteristics of the cell-types described above are as follows :—

PHASE I. The embryonic erythrocytes belong to this type, and this type may further be subdivided into two types I A and I B.

puscles. (pituitary gland, pineal body, adrenal, thyroid gland, parathyroid, thymus especially Hassall's corpuscles, carotid body, aortic body and Coccygeal body, in relation to the differentiation of red blood corpuscles)

X. **Revolution of Medicine and Biology (Part 1) Problems on the Starvation and Fasting**

(History of famine, Histological changes due to starvation. Weight change of the organs under starved condition, Histological change of muscle, heart, blood, gonad, digestive organ, liver, pancreas, spleen, lung, kidney, mammary gland, thyroid, brain, nervous system, emotion, bone, bone-marrow, and skin under starved condition. And its relation with the reverse differentiation from the fixed tissues into blood corpuscles. Rejuvenation and prolongation of life span by means of fasting or semi-starvation. The starvation as a social issue.)

XI. **Revolution of Medicine and Biology (Part 2) Regeneration, and Wound Healing in Relation to the Differentiation of Red Blood Corpuscles** (Reexamination on the histogenesis of regeneration, wound healing). Regeneration bud (or blastema) and scar (connective tissue) are derived from differentiated red blood corpuscles.

Blood coagulation is induced by the destruction of red blood corpuscles. Metaplasie. Pluripotency of red blood corpuscle).

XII. **Revolution of Medicine and Biology (Part 3) Inflammation and Red Blood Corpuscle.** (Historical change of the meaning of inflammation, Cause of inflammation, Symptom in relation with blood vessel and blood cells. Leucocyte as a derivative from red blood corpuscle. All cellular elements of the inflammation area (site) are resultants from differentiated red blood corpuscles. Reaction to the injury in protozoa and invertebrates.

Lymphocytes which appeared in inflammatory tissue are mainly derivative of red blood corpuscles. Principle of therapy of inflammation).

XIII. **Revolution of Medicine and Biology (Part 4) Cancer cells proliferate by "AFD Process" of the Red Blood Corpuscles**

(Mechanism of the multiplication of cancer cell is basical problem and the first step of cancer studies. Distinctive feature of cancer cell. Cancer cell does not multiplicate by means of mitotic cell division, but by AFD process (Aggregation, Fusion and Differentiation) of red blood corpuscles. Intimate relation between tumor and blood vessel. Criticism on the orthodox theory on the outbreak of cancer. Carcinogenic substances and factors.

Nutritional condition and cancer, especially in relation to fasting (semistarvation) and cancer. Emotion as an important causal factor of cancer. Criticism on the result of the International Cancer Conference.

XIV. **Revolution of Medicine and Biology (Part 5) Relation Between the Nervous System and the Blood Vessel** (including blood corpuscles), **and the Correlation Between Mind and Body**

(Parallelism of the distribution-pattern between nervous system and blood vessels. Histogenesis of nerve cell from yolkspheres and blood cells at early developmental stage in Amphibia.

Intimate relationship among nerve, lymphatic canal and vein.

Reverse differentiation between nervous tissue and blood corpuscle under the different nutritional conditions. Psycho-Somatic Medicine. Theories of Pavlove, Cannon, Selye and Reilly and Oriental medicine etc.)

XV. **Problems of Death**

(Partial death, and Individual death. Cause of death, Devolution and extermination of species.

XVI. **A Retrospective Bird's-eye View on My Biological Research** Works for the last forty years. Literature published by the present author).

materials from the mother blood.

Therefore the propriety of mother's diet, mental life, mode of living, affects to more susceptible embryo than mother.

It is well known fact that in Germany, England and Japan the "phoconelia" with short legs and without hands were born of mothers who drank thalidmid sleeping drug.

It is only one instance among many. We can not deny that the above-mentioned several diseases are mainly due to the blood stained with unnatural chemical or physical factors by civilized and unnatural mode of life.

Chap.III. My Works Published, Previously, on the Differentiating Capacity of Erythrocytes

My works have been published by the following three monographs and many of scientific papers, from 1948 up to present.

For the sake of scientists and laymen who wish to know more detailed facts about this problem I will state briefly on the problem, (some of my papers have already been mentioned in the previous chapters)

(A) Monographs

◎ "REVOLUTION OF MEDICAL AND BIOLOGICAL SCIENCES" (Subtitle..... THE FATE OF RED BLOOD CORPUSCLE)., BY K. CHISHIMA. pp. 1-638 Pub. Neo-Haematological Assoc. Gifu. Japan 1961.)

This monograph has published by me as a companion book or an end (completing) volume of previously published three other monographs; "Basis of Neo-Biology Vol. I. .... Genetics, Embryology, and Evolution in relation to Origin of Germ Cell, 1957; "Basis of Neo-Biology. vol. II.

The Origin of Life, Cell and Blood Corpuscle, 1958:

I. Periodicity, Asymmetry and Spirality of Organisms and AFD process (Aggregation, Fusion and Differentiation) in Organisms as a moment of the Developmemt and Evolution

Notes: AFD process takes place on normal developmental process of life.

Reverse AFD process takes place on the death or decomposition of cell, so that virus is not an element on the way of evolution, but is only a resultants from the pathological and reverse AFD process of cell.

II. Cell Differentiation and Organization-grade (Organizer, Field, Polarity, Ontogenesis, Philogenesis). Theory of the repetition of past record of protoplasm. etc.

III. Growth and Degrowth

Mitotic cell division is not leading growth factor, and Reverse differentiation from fixed tissue cells into blood cells give rise to degrowth. etc.

IV. Orthodox View on the Histology

V. Revolutional Histology (Part I) Differentiating Potencies and Fate of Blood Cells. (Ancients' view on the blood. Fate of erythrocyte and erythrophage (or hemophage). Giant cell as a result of Syncytium (AFD process). The so-called extramedullary hemopoiesis is only a phase of reverse differentiation from tissues into blood cells. Life span of erythrocyte. Fate of lymphocyte, meaning of lymphoid area etc.

VI. Revolutional Histology (Part 2) Vascular System as a milieu of cells

A-V bridge. (A-V anastomoses). Open type of capillary. Passing out of erythrocyte through capillary wall. Blood vessel and nervous control. Intimate relationship among blood vessel, nerve and blood corpuscle, development of heart and capillary. etc.

VII. Revolutional Histology (Part 3) Differentiation of Blood Corpuscle and the Formation of Various Kinds of Tissues.

(Ectodernal involution organ and outgrowth organ with blood vessels. Epidermis, melanin pigments, connective tissue, adipose tissue, muscular tissue, notocord, cartilage and bone tissue, as a derivative from red blood corpuscles).

VIII. Revolutional Histology (Part 4) Structure and Histogenesis of Various Kinds of Organs

(Histogenesis of salivary gland and Liver. Langerhan's Island of pancreas, spleen, excrete organs, milk gland and respiratory organ in relation with the differentiation of red blood corpuscles)

IX. Revolutional Histology (Part 5)

Histogenesis of Endocrine organs and differentiation of red blood cor-

is necessary to avoid the danger from old degenerated or deteriolated blood.

Because the fore-said substitutes can be preserved without degeneration, and they are convenient and cheap in point of production and transportation.

It has been generally believed that if one liter of blood is lost, one liter of blood transfusion must be done.

Up to this time it was thought that blood must be supplied for the supply of oxygen and nutrients to tissues.

According to my opinion even if red blood corpuscles which carry oxygen decrease in number by blood loss, all tissues of body differentiate reversely into blood corpuscles, and erythrocytes in blood recover their normal number with comparative rapidity.

Therefore after the loss of a large amount of blood physicians have to inject into blood vessel blood substitutes enough volume to make the normal blood circulation smooth.

Moreover the side effects do not produce by this method.

About one million of Europe-American Christians started a movement against blood transfusion and denied blood transfusion.

Thus they have had good results to keep health of man.

(VI) Why does Hypertrophy in the Liver and the Spleen Take Place in the Case of Cancer, Anaemia, Atomic Bomb-disease, and Leukemia?

In the orthodox hematology and medical science we can not find any reliable explanation of this problem.

In these diseases hypertropy of the liver and spleen always occur respectively more than several times of normal size, in spite of emaciation of the body.

Most probably, in this case, we can not recognize almost any typical mitotic figure in these organs.

No one has not demonstrated the reliable evidence that the enlargement of these organs is done by mitotic cell division.

Why so? This is because pathologists have been believed that the orthodox cell theory of the principle of Virchow.... "Omnis cellula e cellula" --- is truth and is an iron law of biology.

According to my opinion, as damaged intestine of these diseases, hemopoietic

function is not fully done, the patient falls into anaemia and the erythrocytes reversely differentiated from various tissues of body are carried to liver and spleen and fixed there where the blood circulation is stagnated. And these stagnated erythrocytes differentiate, according to each milieu, into the cells of liver or spleen respectively, synthesizing DNA in the protoplasm of nonnucleated red blood corpuscles.

Therefore the first curing method for these diseases is to devise a countermove for the recovery of physiological function of whole body, especially of digestive canal.

But orthodox medical sciences overlook utterly this principle.

For the planning of recovering of digestive function, especially the villie-function of intestinal mucous membrane, we must devise dietary cure and improvement of living for promoting health.

For these diseases the application of radio-active isotopes and chemotherapy make the condition of the patient worse and no effect can be expected.

Even if temporarily improved, after all, it causes the vitality of the whole body to decrease. From olden times, in oriental medicine, several kinds of herbs (Sanzukon, Seed of Hishi, Hamachisha, and Hatomugi (Peare barley) have been used as special remedy of cancer.

It can not be said that these herbs are special remedies for cancer and anaemia alone, but these have the action of activating chiefly digestive organ generally.

After all, in the cases of anemia and cancer the principal object in view is to make the function of digestive organs normal and to activate normal erythrocyte poiesis in the intestine.

(VII) It Must be Regarded that Deformity, Weakmindedness, Infantile Spastic Displegia, Infantile Paralysis, Polymyelitis, and Yellow Baby etc. in Infants and Children Come Chiefly from Unnaturalness of Womb Environment or from the Irrational Life of the Pregnant Mother rather than Heredity.

Results of my studies on germ cells and all the cells in embryo give rise to their appearance from differentiation their blood corpuscles which take their

ceived the highest treatment. In spite of the utmost treatment they died unexpectedly earlier by the implication of other diseases owing to the fore-said three treatments of orthodox medicine.

In short, the "alpha and omega" of prevention and medical treatment of cancer is my opinion that "cancer cells are the derivatives from erythrocytes under pathological conditions".

It is of great importance that medical scientists of the world should accept my opinion and that general laymen should also understand this, and endeavour to prevent cancer.

(III) Inflammation and its Relation with Erythrocytes.---the basic Problem of Internal Medicine and Pathology---

As already stated, all inflammation, such as pneumonia, pleuritis, meningits, nephritis, dermatitis and so on, give rise to blood congesting in accordance with local stimulation. Inflammated parts show the five characteristics symptoms of inflammation, namely, red, swelling, heat (fever), pain and disturbance of the function.

In general, physicians practice allopathic therapy for such symptoms.

In order to exclude such symptoms physicians usually practice narcotics to pain, antipyretics to high fever, astringents (or binding remedies) to diarrhea, purgatives to constipation, sympathomimetic drugs to high blood pressure (hypertension) respectively.

These are only allopathic treatment and not fundamental therapeutics.

Therefore these are followed by harm side effects.

The fundamental principle to prevent and cure inflammation is to live natural living in harmony with mind, body, and exercise and above all to reduce quantity of blood. Therefore it is necessary to reduce hemopoiesis of intestinal villic by fasting, semifasting or reduced diet according to the kind and degree of diseases.

Modern medicine knows only plus-dietics and does not know the importance of minus dietics, and thus is liable to give diet excessively to various kinds of inflammation and to make it worse.

(IV) Most of the surgeons in the World don't Know the True Mechanism of Wound Healing, in whic is the Most Fundamental Problem in Modern Medical Science.

Erythrocytes in the blood which was shed to wounding portion, differentiate into the connective tissue and crusta which repair the wound part. Though they don't know my new theory, if physicians sew up the wound, it will naturally heal. But in order to prevent the wound from becoming septic, and to recover it early, it is necessary to apply the fundamental treatment, which was mentioned in the previous chapter, to external wound.

Especially reduction of diet, minus dietics prevents suppurative and quickens healing. It is necessary for physicians and laymen to know this principle.

During the Great World War, O.B. Lepeshinskaya Advocated the therapy of wound that "blood bandage" quickens wound healing and took good effect. She was unaware of the fact that the blood corpuscles differentiate into connective tissue (cicatrice) of wounding sites.

If bacterial dirtiness does not exist in the wounding part, the wound gets better by mere banding of the wounding part.

Therefore in the field of surgery it is extremely important to understand the intimate relationship between wound-healing and erythrocytes.

(V) On the Dangerous Side Effect of Blood Transfusion Before or After the Surgical Operation.

The physicians who belive the orthodox dietics in which plus dietics, is over-estimated use habitually blood transfusion after the operation and sometimes before the operation. Such treatment tend to accompany serum hepatitis as side effects of blood transfusion and makes the condition of the patient still worse beyond expectation by "refuse reaction" (antigen antibody reaction). By blood transfusion the patient sometimes is infected with malaria, syphilis, and other pathogenic micrroorganisms.

Therefore, as already stated in the previous chapter concerning cancer, it is safe to avoid blood transfusion to the utmost, to develop blood substitutes and to use them. To use blood substitute is very useful not only for prevention of occurence of serum hepatitis but infections of pathogenic microorganisms. It

titis virus or cancer virus are generated spontaneously.

Thus the condition of the patient is in danger of getting worse.

Because the protein in the serum and blood corpuscles is different among individuals respectively in the strict sense of the word, even if blood group type is the same.

When different kind of protein is poured directly into blood vessel, antigen-anti-body reaction takes place in living body, though there may be differences of degrees.

When the patient of cancer receives a large quantity of blood transfusion, he suffers with higher rate from a complication of serum hepatitis than generally be considered.

Therefore there are many instances in foreign countries that sustitute solution free from protein, for instance, Ringer's solution, physiological salt solution, gelatin solution, PVA, Dextlan, Riger-Lock solution and others were used instead of blood, and in these cases the serum hepatitis and death rate decreased remarkably.

Especially in hospitals relating to religion where blood transfusion is denied from the stand point of faith, good results have been obtained by the use of the above-mentioned substitute blood.

Hereafter the enlightenment of beneficial and harmless substitute blood is desired earnestly.

The principle that the red blood corpuscles in transfused blood are fixed within liver of slow blood flow or in cancer tumor and where they differentiate into respective cells.

Orthodox medical scientists, however, does not understand that virus is generated spontaneously.

Radioactive isotopes, X-ray and chemical remedies used generally today are originally carcinogenous substances.

Radioactive isotope; namely cobalt,^{60} radium, etc, and chemical remedies, namely nitrogen mustard, etc, which are widely used in modern medicine, affect harm action on living body.

It is the treatment of the western medicine of today that tries to kill cancer cells alone using the above-mentioned medical treatments.

But these treatments are not right in principle and also have not succeeded in practice. The idea that "poison quells poison" is liable to involve even healthy cells by poison. Furthermore it is not fundamental treatment to forget that cancer is a chronic general disease and cut off local diseased portion alone. To improve whole living conditions is first consideration.

It is not rational to try to seek a certain special remedy of cancer.

Because cancer is generated owing to the traditional accumlation of unnatural condition of living.

Therefore it is the most important to get rid of the cause.

It is due to this point that orthodox medicine, which mainly depends on radio isotopes, surgical knife and special chemotherapy, is powerless in treatment for cancer.

As cancer cells are the derivatives from blood corpuscles, which became abnormal, especially from red corpuscles, the fundamental countermeasure must be to make blood normal.

The foundation of making blood normal is to live a life depending on mind, diet and natural living.

(3) The Counter plan against Cancer must be Prevention First.

"To find disease early and to cure it early"…this is the motto of the countermeasure for cancer in almost every civilized country.

But this is not right treatment.

The principle of oriental medicine of olden times was mainly to prevent body from illness, that is, preventive medicine.

There is truth in the so-called proverb that "An ounce of prevention is worth a pound of cure". The meaning of the proverb is very applicable to cancer.

The reason why the Ministry of Welfare and the medical circles of Japan do not adopt this treatment, lies probably in the system of medical practitioners, commercialism of physicians, and misunderstanding of the basic principles of life.

In order to prefer preventive medicine to therapeutic medicine and to keep the health of the nation more rational, it is desirable to adopt the system of national expenditure of medical treatment.

In cancer problem, prevention is more important than medical treatment. In the cases of the late Premier Ikeda and Dr.Tasaki, Director of the National laboratory of cancer in Japan, their cancer were early discovered and they re-

or semi-starvation and thus reduce blood volume.

Synthesized chemical remedies which are widely used to hypertension should be avoided. Because it is not the fundamental treatment, but is mere an allopathic treatment.

It restraints temporarily the tension of sympathetic nerves and may decrease temporarily. But when the effect of such chemical substances is lost the patient may be returned to the same condition as was before or more.

For the prevention and cure of hypertension and cerebral apoplexy, it is the most efficient treatment to reduce blood volume by means of reduced diet, fasting cure and low feeding. But modern medicine forgets this point. It goes without saying that "KI,, (mind), "KETSU,, (blood and body), and "DO,, (exercise) should be in harmony" (according to oriental medicine, basic principle to keep health is the harmony between "KI" and "KETSU,, but I think there is necessity to add "DO,,).

(II) Prevention and Therapy of Cancer in Relation with Blood

(1) The Key to Solve the Problem of Cancer is to Clarify or Solve the Origin of Cancer Cells.

While almost all of modern medical scientists in the world believe that the cancer cells arise from normal epithelial cells by cell-mutation, and rapidly increase in number of them by means of mitotic cell division. So they are wrong in the starting point.

According to my studies, as already stated, erythrocytes differentiate into cancer cells under pathological condition of the body.

I have firstly found this fact in the world, and I have published in English my finding in Okajima's Folia Anat. Bd. 37, Helft 4–5, 1961.

So long as this finding of mine is not frankly adopted by the medical scientists, the solution of cancer problems will not be attained.

It is right that Warburg's opinion that under the oxygen deficient condition, cancer cells grow. There are many scientists who maintain lately that cancer cells arise by cancer virus.

But according to my opinion bacteria and viruses do not increase in number by means of dividing in two, but they are generated spontaneously in the degenerating protoplasm.

Most probably there is no virologists who have positively proved that virus, which invaded into the inside of living cells from the outside, and multiplied by means of dividing into two.

In the case of cancer cells, though virus does not invade into the cell through cell membrane (protoplasmic membrane), Cytoplasm itself changes into the so-called "cancer virus" when the cell is disorganized losing its normal activity and approach the condition of cellular death.

Therefore cancer virus is not the cause of cancer but the result of degenerated cell.

In short, cancer is a chronic general disorder which occurs when the vitality of body decreases remarkably and blood changes abnormally owing to long, unnatural irrational living.

The treatment of cancer, therefore, must be re-examined.

While orthodox medical typical cancer treatments are the following three kinds of methods.

(i) Surgical cutting off of carcinoma (tumor).

(ii) Radio isotope therapy.

(iii) Chemotherapy.

But these are, so to speak, allopathic treatments, and not fundamental treatments. Therefore the therapy of cancer of today does not produce any succesful result. Moreover, it often makes the condition of the patient worse.

(2) Blood Transfusion is liable to be Attacked With Serum Hepatitis. and so blood substitute solution should be used.

After surgical cutting off the cancer tumor, blood transfusion is always practiced.

But blood transfusion is threatened with the complication of serum hepatitis of very high rate, even though the adapted blood group and fresh blood is used. It is difficult to detect the existence of hepatitis virus in the blood before blood transfusion. Even if blood which does not contain hepatitis virus in transfused, the blood corpuscles in the transfused blood are fixed in the liver and in the cancer tumor of the patient and they become cancer cells because the vitality of the patient's body is low. So that the liver and cancer tumor swell and enlarge without exception.

And within such pathological liver cells and cancer cells, the socalled hepa-

CHAP. II. PRACTICAL APPLICATION OF THE FIRST PRINCIPLE - (THE THEORY OF DIFFERENTIATING POTENCY OF RED BLOOD CORPUSCLES) TO HEALTH AND DISEASES

(I) The Quality and Quantity of Blood and Blood Corpuscles are the Fundamental Factors Controlling Health, Longevity, Prevention of Diseases and Medical Treatment.

(1) Quality of Blood and Health

As has been mentioned above, the erythrocytes are the basic material of all kinds of cells in human being and other animals.

Therefore, if its quality and quantity are not normal it is matter-of-course that we can not maintain or promote the health.

To maintain physiologically clear and unpolluted state of blood we must be faithful to eat always unpolluted natural food in quality, small feeding in quantity, and masticate the diet well. I have designated this as a "three S dietary rule for health", that is to say, in Japanese "three S,, means the capitals of the following three words; "Shizen-Shoku,, (natural food), "Sho-shoku,, (Small feeding) and "Soshyaku,, (chewing well). Furthermore we must also make every effort to keep harmony of stable mind, fresh blood and muscular exercise. If our mode of living would be contrary to the above-mentioned necessary conditions for health we may not escape from various diseases, on some occasions, there is a possibility of newly deformed, weakminded, or sickly infants newly born, through the polluted blood of their pregnant mothers. Our health also depends upon the environmental conditions. So that, spraying of dangerous insecticides, pollution of air, water and soil by several, chemical poisons and radio-isotopes, from waste products of factories, polluted foods by artificial staining, treated with harmful chemicals, or with irradiation treatments, several new remedies with harmful side effects, all of them are absorbed into blood through the digestive canal or other parts of body and give dangerous effect every man and even to foetal life.

We shall be confronted more and more with issues vitally affecting environmental pollutions.

By physical exercise, the lung and heart increase in their activity, accordingly circulation of blood is promoted and the oxygen content and freshness of blood increase more and more, and it prevents the differentiation from erythrocytes into cancer cells, because the cancer cells develop in under the oxygen deficient conditions as has been pointed out by Warburg. As the river's water is purified by its flowing, blood also is refreshed by its active circulation.

For the sake of healthy life every one, of course, must preserve his blood normally by taking natural food, and by well-balanced, mental life, because by these factors' influence the constitution of blood changes remarkably. It is a well-known fact that the mental stresses, such as anger, fear, grief, discontent, and the other mental stresses always give rise to almost all kinds of chronic diseases.

Therefore, I have adovocated, "the harmony of "Ki-Ketsu-Do (気・血・動) is a fundamental principle of health and longevity,,

In Japanese, "Ki" (気) means mind (emotion or spirit), "Ketsu" (血) blood, and "Do" (動),,..... physical and mental exercise.

Moreover, to maintain normal constitution of the blood is not only necessity for every man's health but it is the most important problem for foetal life and the children of next generation.

(2) Quantity of Blood

It goes without saying that the quantative decrease of blood and blood corpuscles set cause anaemia, but on the other hand the excess of blood volume is apt to be attacked with hypertension, cerebral apoplexy, heart diseases and other disturbances.

Hitherto arterial sclerosis and mental stress were considered to be the main cause of hypertension. It may be true partially. But according to my opinion, it seems to be neglected or ignored that over quantity of the blood is the cause of these diseases. As will be mentioned in the later chapter, the site of erythrocyte-poiesis is in intestinal villie, so that any person whose digestive organ is healthy and who eats fancy food much, increases excessively in absolute volume of blood and is liable to the danger of cerebral apoplexy due to explosion of blood vessel.

Therefore any person who is liable to suffer hypertension and cerebral apoplexy, should live on vegetables, reduced diet and sometimes practice fasting

(5) Differentiation Process of Erythrocytes in Wound Healing

In this case, the erythrocytes differentiate, at first, into mesenchymes or so-called embryonal cells, and then, from the mesenchymes into epithelial cells. The so-called mesenchymes-B are very young elements just as differentiated from red blood corpuscles as described above. Thus the wound closure is completed within about seven to ten days by means of the differentiation of red blood corpuscles bled on the wound site. The present author confirmed the above-mentioned facts by experimental study on the wound healing of skin, gonad, and liver etc. in birds and mammals.

There is great variation in the ability of different kinds of cells and tissues to regenerate. Connective tissue, liver and blood vessels are very rapidly regenerated. Because those are the first step of differentiation from red blood corpuscles even though they pass through the intermediate stage such as mesenchyme, lymphocyte or other leucocytic stages. In these cases regenerated new cell does not arise by mitotic proliferation of preexisting cellular elements.

However the more highly specialized cells such as nerve cells or muscle in higher animals or man, lose regenerating ability.

In general, the reparing ability of the injured tissue lowered according to old age and to the more highly evolved animals.

(VII) Blood Coagulation and the Role of Erythrocytes

(1) Generally Accepted Opinion on the Mechanism of Blood Clotting

The coagulation of blood is essential for the protection against over blood loss from the circulation system when a blood vessel ruptures.

It is believed that in the most of invertebrates blood contains no fibrinogen, coagulation is effected by an agglutination of blood corpuscles only.

In the lower vertebrates clotting by agglutination of corpuscles has practically no fibrinogen and prothrombin within the vessels, and the thromboplastic substance on the outside constitutes a mechanism which provides perfectly for the fluidity of the circulating blood and its prompt clotting when shed.

In mammalian blood a new element appears, namely, the blood platelets when blood is shed, they promptly gather and agglutinate and disintegrate, so that they furnish thromboplastic substance which starts the process of blood coagulation.

The blood platelets which play the most important role in blood clotting have been believed that they are fragments of cytoplasm thrown off from megacaryocytes of the lung and bone marrow.

And the most generally accepted opinion as to the chemical and physical mechanism of blood coagulation is as follows;

(a) Platelets adhere to the broken edges of a vessel, then they disintegrate and produce thromboplastin.

(b) Thromboplation + Calcium + Prothrombin + factor V + Other questionable factors→thrombin.

(c) Thrombin+fibrinogen→fibrin.

(d) Fibrin thread entraps erythrocytes to form clot.

(2) My Opinion On Blood Coagulation

According to my studies the important role of platelets playing in the blood clotting can not be denied, but the above-described orthodox opinion as to the origin of platelets is very doubtful.

Because I can not find such an appearance or behaviour of megakaryocytes in marrow or lung, as they projects, pseudopodia-like excrescence into capillary wall, and pinched off the surface of the megakaryocytes and make them enter into the blood stream as platelets.

Therefore, the opinion of the megakaryocytic origin of platelets seems to me as an imaginactive and fancy theory.

Results of my observations on the blood clotting the platelets have no locomotive ability, inspite of the fact that they are agglutinated in coagulated blood. This fact shows that they were not gathered quickly there. On the contrary, there are well-grounded facts, that the erythrocytes shed out of blood vessels, some of erythrocytes soon rupture, and extruded cytoplasm of them rapidly change their staining capacity and become the same of the platelets.

Therefore, blood platelets are resultants of destructed erythrocytes and not the products of megakaryocytes.

Furthermore, according to my studies on the blood clotting of mammals, birds and amphibia it was found that the destructed erythrocytes play an important role in blood clotting as I have published in the following papers (Chishima '51)

item, "Differentiating capacity (ability) of red blood corpuscle,,

(2) Orthodox Conception About the Mechanism of the Wound Healing

It is generally accepted opinion about the mechanism of wound healing (regeneration) that the destroyed cells or tissues are repaired or replaced by similar or dissimilar cells by means of mitotic cell division and in parts by cell-migration.

So that according to the orthodox opinion the wound healing process begins at first hemarrhage, and the crust formation, removal of dead tissue or debris by phagocytosis or liquefaction of dead cells, leucocytes of all kinds gathered selectively into the injured part, replacement of lost cells and tissues is accomplished by cell migration, proliferation (mitotic division) and cell-migration, in wounds of skin, the fibrablasts play role chiefly by its migration into clot, these fibroblasts arise from the cells already present in connective tissue.

Fibroblasts put out processes and fuse with those of the near-by fibroblosts and at last they become shrunken cells→the mature connective tissue cells.

(3) Erythrocytes Play the Most Important Role on Wound Healing.

But the above-mentioned orthodox view does not agree to the facts.

Because there can be found no true mitotic division of fibroblasts in the wounding part.

Most probably, the above-mentioned orthodox view on the mechanism of wound healing is due to the misunderstanding of the differentiating process from erythrocytes into the cellular elements of the wound healing, as a mitotic proliferation.

Because the extravasated erythrocytes in the wounding region show certain phase resembling some stage of mitotic division, synthesizing DNA in their cytoplasm. In this process, at first, the erythrocytes are aggregated and graduary lose their red staining property with eosin (losing oxy-hemoglobin). And then DNA synthesis take place in the fused mass (blood monera). In this case chromatin substance (DNA) appears here and there in the blood monera. So that this figure is apt to be misunderstood as a mitotic figure.

Migration of fibroblasts in wound can not be denied as a slight function of wound healing, but, it can not be said as a chief factor of wound healing. Because there is no sign of such abundant supply of fibroblasts through mitotic

proliferation.

Hitherto the formation of new capillary and endothelium at the region of wound, has been considered to be the migration and mitotic proliferation of endothelial cells. But the chief source of their supply does not be proved satisfactorily. Granulation tissue of wound site composed of (a) newly formed capillaries, (b) fibroblasts, (c) leucocytes, and (d) red blood corpuscles.

According to my view the capillary and its endothelial cells are resultants from mechanically depressed red blood corpuscles and its differentiation. And the leucocytes in the site also arise from differentiation of red blood corpuscles transferred there out of the open end of blood vessels.

The leucocytes and fibrolasts too are the derivatives of the erythrocytes.

Thus almost all of the cellular elements of grannulation tissue which repair the wound are originated from erythrocytes bled there immediately after the injury.

(4) The phagocytosis Theory in Wound lesion is a Misunderstanding.

Crust formation on the surface of wound also takes place from the drying and hardened of coagulated blood bled there.

It has been believed that the dead cell fragments in the wound are removed by phagocytes.

However, it is most probably due to misunderstanding of the AFD process of red blood corpuscles.

That is to say, the process of new cell formation in the fused mass of several number of red blood corpuscles resembles the phagocytic behaviour of leucocyte or phagocytes.

But there is no firm evidence of phagocytosis.

On the contrary, there can be seen all transitional phases from the fused blood corpuscles into the so-called phagocytes or leucocytes.

The epithelium covering the healed wound of skin is generally believed as the results of migration of neighboring epithelial cells because there is no evidence of mitotic, proliferation of epithelial cells. It may not be denied that the very small portion of the source of epithelial cell is repaired by migration of the cells, but according to my opinion the migration from neighboring epithelial cells is not a chief or entire source of the wound healing, because almost all source of epithelial cells is the derivatives from red blood corpuscles.

of cancer cells and created a sensation in France.

At this time Dr.Stephanopoli of Pasteur Laboratory had already known my finding (Chishima '61) on the origin of cancer cell. So that he declared that "the first finder of the origin of cancer cell is not Dr.Halpern but Prof.Chishima of Japan".

(V) Inflammation and Erythrocytes

Whenever certain tissue is injured or irritated by physical, chemical, microorganismic agents or by emotional stress there follows a series of tissue reactions at the site irritated, that is the so-called inflammation.

Most of diseases originate or accompany with inflammation including either of inner medicine or surgical sphere.

In spite of the fact that the inflammation is the most basical problem in the medical sciences, it seems to me that the true mechanism of inflammation has been misunderstood by present day medical scientists.

That is to say, the most important point which differ from my view, is the negligence of an important role played by erythrocytes at the region of inflammation, and the overestimation of the function of leucocytes.

It is generally accepted opinion that in the site of inflammation there take place the widening of blood vessels, increased blood pressure, accelerated rate of blood flow at first, then increase in permiability of vessels, the escape of plasma from vessels into an area of inflammation.

So that there appears the following inflammatory five symptoms, namely; redness, heat, swelling, pain and functional disturbance. In this case, the increased permiabity due, chiefly, to increased size (diameter) of capillary pores (the so-called stigmata or stomata of capillary's wall). It is said, that, under the normal condition, the diameter of stigmata is about 38 ängstrom, and under the inflammation they increase in size to 50 to 200 ängstrom, so then blood stasis, plasm extrusion and edema take place, and then occurs emigration of leucocytes through capillary wall, some investigator describes that the erythrocytes also go through the wall's pores, and that the leucocytes also emigrate out fo blood vessels by means of amoeboid motion and by chemotaxis.

However, according to my studies on the capillaries of living body or stained section preparates, the capillary's ends are open here and there, so that the erythrocytes flow out, freely into the interstitial spaces of tissues.

The so-called a state "erythrocytes are ingested by phagocytic macrophages in the region of inflanmatory region" is, in my view, only a product of the AFD process of erythrocytes, because there is no evidence of the proliferation or emigration of macrophages. On the contoray there can be seen clear transitional phases from erythrocytic mass (monera) into macrophage.

According to my opinion, polynuclear, polymorphonuclear leucocytes and monocytes are derivatives of red blood corpuscles through their AFD process. Small lymphocyte only originates from differentiation of single red blood corpuscle.

Various kinds of leucocytes differing in morphologically and staining property may most probably be due to the difference of differential stage of them and the influences of cellular environments (milieu) where the extravascular erythrocytes are localized.

It is generally believed by pathologists that the pus in purulent inflammatory lesion is a corpse of leucocytes which are defeated in battle with pyogenic bacillus.

But such an opinion may not be true, because the results of my observations on the putrefaction of leucocytes or other cells the protoplasmic substance itself become into putrefactive bacteria without bacterial invasion into the cell. (See p. "spontaneous generation of bacteria")

(VI) Relation Between Wound Healing, Regeneration and Red Blood Corpuscles

Regeneration and wound healing have been already noted as one of the primitive reactions to injury, occurring in lower animal as well as men, though the capacity in higher animals and men is not better-developed than in the most of lower ones.

Replacement of the lost part (cells or tissue) by new ones occurs not only after injury but, in tissues, such as epidermis and blood, continuously, as old cells wear out. This case is known as physiological regeneration.

(1) Wound Healing and Erythrocytes

In this chapter this author will describe the former case, the wound healing. For as to the latter case, the physiological regeneration of such as muscle or several kinds of healthy tissues I have already mentioned before under the

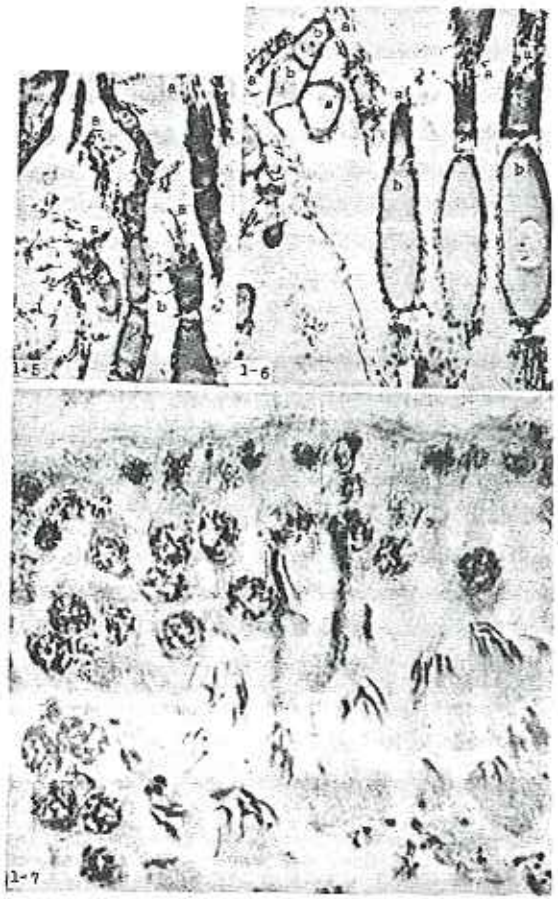


Fig.1—5&6. New formation of egg in the ovariole (ovarian tube) of grasshopper.

a···the so-called ovariole. The ovariole is a derivative from transformed trachea, because there can be seen the taenidium on the end portion of the ovariole.

b···egg (ovum) (K.C.)

Fig.1—7. Spermatogenesis of mouse.

Showing many spermatozoas arising from one spermatocyte without cell division.

Spermatogonias located upper most site of the figure are derivative of erythrocytes.(K.C.)

(IV) Origin of the Cancer Cell

···Cancer Cells Develop from Erythrocytes···

(1) Outline of My Studies on the Relation Between the Cancer Cells and Erythrocytes

Many of the Scientists believe that cancer may arise by way of the chromosomal mutation or by abnormality, so that the cancer cell multiply in the wild and unregulated manner. And the new cells resulting from these divisions have the same ability to escape control.

This opinion is based upon erroneous orthodox cell theory, chromosome theory and orthodox genetics.

These principles are unconfirmed and merely imaginative as already stated, cancer cell does not show typical mitotic figures.

The so-called abnormal mitotic figures of cancer cell are not true mitotic process, but it is only a phase of new formation of nucleus (DNA) in the aggregated mass of non-nucleated erythrocytes.

I have been designated such a fused mass of blood corpuscles as "blood monera" So that the abnormality of chromosomes (shape and number) is not the cause of cancer, but the result of cancer. (Fig. 1—2 and color plate)

Admitting one step, the abnormaly chromosomes of cancer cells in number and shape are only parallel change.

Virus

In virus diseases, virus is not the cause of the disease but a result of degenerating cytoplasm. In other word, viruses give rise spontaneously synthesized DNA (virus particles) in the pathologically degenerating protoplasmic substance.

It is so with the so-called cancer virus.

If one reflects frankly that cancer and virus diseases are neither hereditary nor infectious, one will understand it.

It was in 1961 (see Chishima '61) that I found and published that "cancer cells are derived from the erythrocyte's differentiation." After that V.G. Kryukov and O.P.Lepeshinskaya of Lab. Acad. Med. Sciences of the U.S.S.R. wrote a letter to me, supporting my paper (Chishima '61) and published their opinion resembling to my opinion, in 1963.

In 1965 Bernard Halpern published a new theory concerning to the origin

There is no such a case in Japan, that the paper applying for doctor was let alone for ten years long without reporting to faculty meating on one's finding.

So that I have published this paper (in English) on the Okajimas Folia Anatomica (Bd.24, Heft 3, 1948). Before this paper, I have published a resumé of this finding of mine in Japanese (Chishima '48 a.b). collectively,

And I have published, further, collectively, as a monograph (Chishima '57). on my studies on tke origin of germ cells in many kinds of animals.

Orthodox theory regarding germ cell-origin-Weismannism has been believed until now, that the germ cells are independent genetically from somatic cells, and the germ cells are continuative themselves through their cell division. But such orthodox theory does not agree with fact.

This finding of mine has great important meaning in relation with genetics, embryology, evolutionism and other biological principles.

Since my first publication on this new theory already 24 years glied away. But most of the biologists and medical scientists excepting few scientists, have been maintain noncomment for my theory.

(2) Suggestions to the Mendelists-Morganists

The "International Congress of Genetics" was held in Tokyo and Kyoto for a week begining on the 6th September 1956.

In this congress almost all investigators of the world excepting Glushchenko and Kushner, believed in Mendel-Morganism.

Allmost all of publications in this congress were orthodox genetics.

To my regret there was no scientists who published his studies about the origin of germ cells which are the foundation and starting point of genetics.

After that congress I expressed my impressions at the end of the monoglaph which I published in 1957.

"Suggestions to the Medelist-Morganists"

It is well to admire J.G.Mendel and T.H.Morgan for the sake of their precursory works on genetics. But nowadays unprogressive Mendelist-Morganists are still clinging to the ghost or the remains of the old Mendelian doctrine which has already-finished its function.

The orthodox genetics, the chromosome theory of heredity asserts that,

i) the gene or hereditary substance localized in chromosome is independent of environmental changes, and is never generated *de novo*,

ii) the germ cell is immortal and is independent genetically of somatic cell, and

iii) the hereditary alteration is produced only by sexual breeding or indefinite mutation which is independent of natural environmental change.

However, it must be clear to any progressive biologists that such an orthodox Morganism is a static concept dwelling, still, on the preformation theory, the Pre-Darwinian thought.

Because, recently, the following three fundamental discoveries contradicting with the leading idea of Morganism have been presented by the biologists, that is to say, the first is the proof of the inheritance of acquired character (by I.W.Michurin. T.D.Lysenko and their corroborators).

The second is the verification of new-formation of cells from living substances, i.e. yolk sphere, egg albumen etc.(by O.B.Lepeshinskaya, her cooperators and K. Chishima).

The third is the finding that,

a) the germ cells are produced as a result of differentiation and de-differentiation of the somatic cells, the erythrocytes,

b) reversible differentiaton between the blood cells and almost all kinds of fixed cells including germ cells, yolksphere, fat tissue, and bone tissue etc.(by K.Chishima). Even if Morganists may reject or take no notice of those new facts, or be regardless of the mental attitude of them, there is no longer room for doubt that the orthodox genetics must be reconstructed fundamentally, just as a certain insect larvae should be attained to an adult stage through the metamorphosis (histolysis and reconstructive histogenesis).

Thus the situation of nowaday Mendelism-Morganism is just like that of insect lave just before metamorphosis, or that of Ptolemaic system of astronomy immediately before the replacement by Copernican system.

The immortal saying of J.W. von Goethe,, "Stirb und Werde!" may deserve, in this case, the deepest contemplation for the present conservative Morganists.

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(See figs.1—3, 4, 5, 6, 7)

Difference of structure and nature of various sorts of reticulo endothelial system cells conform to the "field" where they are located.

(III) Origin of the Germ Cell

(1) Outline of the History of My Studies on the Origin of Germ Cells

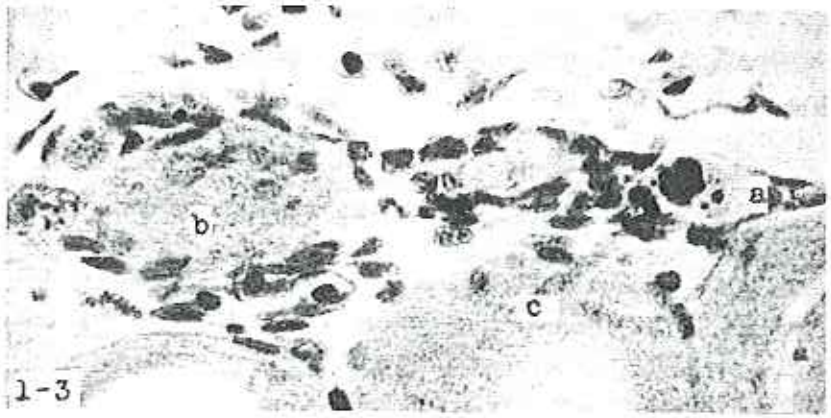
The origin of the primordial germ cells has been studied by Swift ('15), Brode ('28), Dantschakoff ('31), Matsumoto ('31), Goldsmith ('28) and other many scientists. But as all of these investigators are believers of orthodox cell theory, ever since Virchow, their opinions regarding the origin of germ cells differ with fact in certain important points.

I have studied about this problem on ckick embryos, at the laboratory of Zootechnical Science, Kyushu Imperial University. And I have found that the primordial germ cells of chick embryo arise, at first, on the blood island (outer side of embryo), where the primordial germ cells give rise to by means of new-formation of cell from yolk spheres, but not by mitotic cell division. And in later embryonic stage the germ cells and other all kinds of embryonic gonad cells are derived from erythrocytes.

In other word, the germ cells and other cellular elements of gonad are the derivatives from differentiated erythrocytes. And these embryonal germ cells increase in number by differentiation of erythrocytes, but not by mitotic cell division. I have, furthermore, studied on this problem using as materials, amphibia, reptiles, mammals, fishes and insects etc. and I have confirmed that my finding on the chick embryos is true and is applicable to the other animals mentioned above. (Chishima '48, '51, '52, '54, '55, '57).

I have presented in 1947 a thesis (title : Studies on the relationship between the histogenesis of gonads and differentiation of blood cells in chick embryos) to Kyushu Imperial University, to apply for a degree. That paper was received regularly by the university. But my opinion is so revolutional and so contradictory to the orthodox theory that my paper was let alone for ten years long, without examination of my thesis for doctorate.

The member of jury of my thesis did not lodge a protest against my thesis. Because there can be no gainsaying for my thesis as they have no foundation based on fact. As they were orthodox believers, that they were afraid of opposed opinion from orthodox scientists.



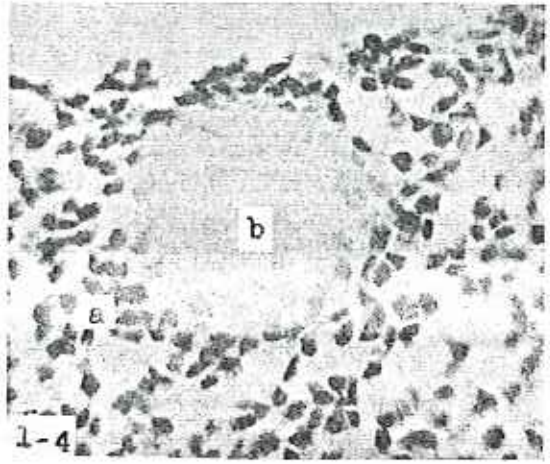


Fig.1—3. New formation of ovum in frog's ovary.
a···primitive ovum in the blood vessel.
b···early stage of the new formation of ovum through the AFD process of erythrocytes.
c···young ovum. Notice the transitions from peripheral cells (derivatives of erythrocytes) into yolk material. (K.C.)

Fig.1—4. New formation of ovarian follicle of rabbit.
a···mesenchymatous cells derived from erythrocytes.
b···new formation of follicle through the AFD process of mesenchymatous cells. (K.C.)

According to the general opinion, loose connective tissue cells develops from the mesonchymes which have embryonal nature and they give rise to the formed elements of blood, blood vessel (vascular endothelium) and various kinds of connective tissues.

It is said that the mesenchymal cells remain continuously in the interstices of tissue spaces until adult stage since embryonic stage.

But such an orthodox opinion as to the origin of mesenchymes is obviously incorrect. Because (i) there is no evidence of continuation by means of mitotic division since embryonic stage. (ii) according to my studies on the mammals, birds or amphibian's embryos the mesenchyme cells are, at first, derived from yolk spherss by means of AFD process (Aggregation, Fusion and Differentiation from yolk spheres in the developing ovum).

I have defined such mesenchymes arised from yolk sphere's AFD process as the mesenchyme-A. And I have found that these messenchyme-A with multipotential ability, differentiate into almost all kinds of embryonal cells including ecto-dermal, entodermal and mesodermal cells.

New cell formation (not mitotic division) of mesenchyme-A from yolk spheres through AFD process continue until the yolk spheres are completely consumed.

While another type of mesenchyme which I have defined as "the mesenchyme-B", a derivative of non-nucleated erythrocytes (in mammals) or of nucleated erythrocytes (in bird and lower animals).

The mesenchyme-B therefore can be found in prenatal or adult body only. Their shape, character and locality resemble mesenchyme-A.

So that generally both mesenchyme cells have been denominated indiscriminately as "mesenchyme"

Because most of histologists adhere to orthodox cell theory, there may be nothing for it but to be referred to "mesenchyme-A" as the ancestral cell of "mesenchyme-B".

(14) Reticuloendothelial Cells

The reticuloendothelial cells have been referred to be a cell type scattered all over the body.

And the best known function of them is phagocytosis and formation of antibodies in inflammation and immunity.

The reticuloendothelial system sometimes is defined as macrophage system.

It has been said that this system includes the following various kinds of cells; macrophage in connective tissue, adventitial cells of blood vessel, reticular cells in the lymph node, or in spleen, Kupffer cells in liver sinuses, lining cells of simuses in the adrenal and hypophysis, the dust cells of the lung, endothelial cells lining the blood vessel etc.

It is said that in embryonal early stage, the endothelium of blood vessels is identical in its potencies with the common mesenchymal cells, so that it has hemopoietic ability.

As the results of my studies it was found that the endothelial cells lining blood vessels arised from yolk spheres through the AFD process in amphibian embryo at the eariest stage, and in its later from erythrocytes.

Thus the endothelial cells are not the mother cells of erythrocytes but are progeny or derivatives of erythrocytes. This opinion can be applied to other animals (mammals, birds)

And all the reticuloendothelial system described above are the differentiated resultants from erythrocytes.

It is generally believed that the reticuloendothelial cells play a role to destruct the erythrocytes, in evidence of the presence of erythrocytes, hemosiderin and cell fragements within the reticuloendothelial cells.

There is apparently contradicted statements with orthodox scientists.

That is to say, they say on the one hand, the reticuloendothelial cells have erythrocytopoietic ability, while, on the otherhand, they say that the erythrocytes are destructed by endothelial cells.

Such a contradicted orthodox opinion can be clearly solved by my theory and agree with facts. According to my opinion, the erythrocytic fragements, and hemosiderin involved within the endothelial cells are not vesults of phayocytosis, but a phase of the AFD process of erythrocytes. That is to say, in certain case the erythrocytes may aggregate, fuse and differentiate into Kupffer cell or macrophage, and other sites of body erythrocytes adhered to vessel's or sinuse's walls differentiate into each of reticuloendothelial cell according to its localization. In the latter case, the differentiation of each erythrocyte can take place without AFD process.

So that all kinds of the reticuloendothelial cells belong to the very young differential stage just arised from erythrocytes.

must be impossible to explain it by mitotic cell division of their mother cells.

On the contrary, there can be seen every transitional phase from erythrocyte or erythrocytic monera (mass) into lymphocytes or other various kind of leucocytes according to their cellular environment (milieu).

Orthodox hematologists have believed that the lymphocytes are proliferated by mitotic cell division in the so-called the lymphocytopoietic nodule of lymph node. But it is next to impossible for hematologists to prove such a fact because there can hardly be shown the evidence of mitotic cell proliferation.

According to my investigations lymphocyte is the youngest stage of cell differentiated from erythrocyte, And it shows transitional phases into various kind of fixed cells in every site of interstitial spaces of tissues, showing the transitional figures from erythrocytes.

It has been believed generally that most parts of various kinds of leucocytes are formed in the bone marrow by mitotic cell division.

But there is no reliable sign of their mitotic proliferation.

On the other hand there can be seen the all transitional phases from erythrocytes into the leucocytes including lymphocytes. This tendency is more more remarkable in inflammatory region or in the bone marrow.

On the new formation of leucytes from erythrocytes by means of cytoplasmic extrusion I have already published by three papers (Chishima '49-'50) (see p.).

(11) The leucocyte is Only a Transitional Phase from Erythrocytes to the Fixed Tissue Cell.

The leucocytes, especially neutrophils, have been referred to be the most important element for protection against the bacterial invasion through their phagocytic action.

But this orthodox conception is very doubtful, because under the putrefaction process of leucocytes or other cells they degenerate into a bacterial mass without invading of bacteria from outside into these cells. So that the phenomenon, "the leucocytes engored bacteria", in reality, are the resultants of the decayed leucocytes or cells in which the bacteria spontaneously arised.

Therefore, it can be said that the orthodox conception, "the phagocytic function of leucocyte", is based on uncertain observation.

On the contrary, the most important function of leucocytes is rather its differentiating ability to become various kinds of fixed cells than their phagocytosis.

Prominent hematologists A.A. Maximow and W.Bloom ('50) have pointed out that "the small lymphocytes, and monocytes in inflammatory region, indirectly develop into macrophages and fibroblasts, a transformation not strictly reversible." This opinion is correct, but in my opinion, lymphocytes and other type of leucocytes have wide capacity of differentiation into various fixed cells under physiological conditions too. In the bone marrow and other fat forming positions, the leucocytes undoubtedly differentiate into adipose tissue through their fatty degeneration. The stem cells of various kinds of leucocytes, therefore, are not the derivatives from every different sort of their stem cells but an unitary stem elements, (in mammalian animals; non-nucleated erythrocytes) or nucleated erythrocytes (in bird and other lower animals).

Why the same elements, the erythrocytes can differentiate into so many kinds of other cells?

The answer to this question is "the influence of induction of the "field" where the erythrocytes and leucocytes are carried by circulation.

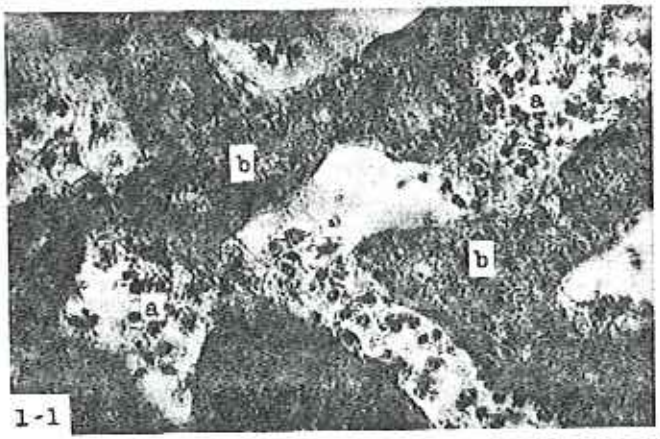
(12) Connective Tissue Cells, Fibroblasts and Vascular Endothelium

The majority opinion holds that fibroblasts are the highly differentiated cells which do not give rise to other types of cells, though it is generally believed that they can develop into osteocytes.

And it is also a majority opinion that the vascular endothelium of the enbryonic liver, bone marrow, and spleen may take part for a short time in the production of hemocytoblasts, and many histologists believes that the endothelium of vessels, even in adult stage, have erythrocyte poietic function.

However, according to my opinion, orthodox opinions described above are lacking in firm evidence. And the results of my studies, though, those of fibroblasts, small lymphocytes and vascular endothelium differ each other in shape; fibroblasts are slender spindles, small lymphocytes are spherical, endotheliums are thin flattened in shape, they all belong to the same differentiation stage from non-nucleated erythrocytes; the youngest stage just developed from erythrocytes. Because there is no conclusive evidence of the mitotic proliferation of these cells. And their difference in shape due, most probably, to their physico-chemical environmental conditions. About this problem I have published my papers since 1948 years (Chishima '48-'63).

(13) Mesenchyme-A and Mesenchyme-B



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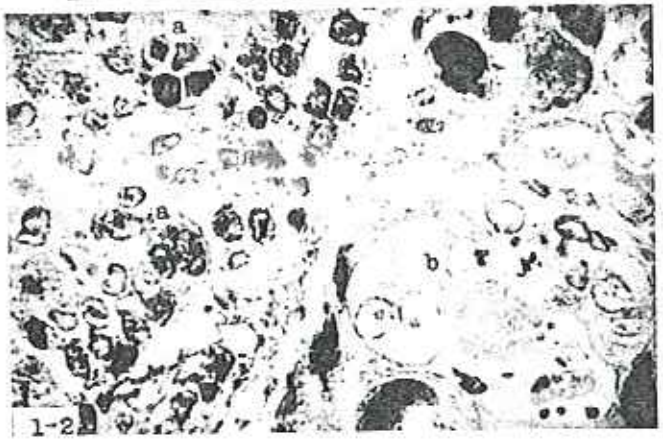

1-2

Fig.1—1. Photomicrograph showing the differentiation from erythrocytes (a) into liver cells of the liver cell-cord of 8 days chick embryo. (K.C.)

Fig.1—2. Showing the differentiation from erythrocytes into cancer cell of human uterine cancer through the AFD process of erythrocytes.

a···aggregated mass of mesenchyme-like cells derived from erythrocytes.

b···cancer cell derived from the fusion and differentiation of the (a).

There is no typical mitotic figures in the carcinoma tissue. (K.C.)

functions.

Their number is far smaller than that of the erythrocytes, averaging in the normal human blood 5,000 to 9,000 in a cu. mm.

It has been said that the number of leucocytes in the circulating blood varies at different times of the day, during digestion, in the various parts of the circulatory system and, in addition, may change rapidly under the influence of numerous conditions which are hard to control.

Consequently, many of the leucocyte counts that are made so frequently in the clinic have only a relative value. In my observations, they vary in number even during on the blood smearing process according to the destruction of erythrocytes.

In fresh human blood several types can be distinquished:

Small lymphocyte, about one half the diameter of an erythrocyte, spherical with a scanty, clear cytoplasm, relatively large spherical nucleus. There are also other leucocytes, middle or larger lymphocytes; monocyte with somewhat greater amounts of cytoplasm than lymphocytes, granular leucocytes, acidopil. eosinophil basophil, neutolophil leucocytes.

It has been said that leucocytes, are capable of amoeboid motion, phagocytic action (phagocytosis of bacteria by leucocyte hitherto referred to be of great biological or medical importance. But about this theory I have some doubts) about these problems of leucocytic functions I will mention later on.

(10) Orthodox Theory on the Leucocyte-Forming Tissue is not Reliable.

According to the orthodox leucocyte-genesis, the most lymphocytes arise by mitosis of preexisting lymphocytes within the lymphatic tissue, such as the lymph nodes, the spleen and bone marrow. However the results of my studies, there is no nodes, spleen and the bone marrow. According to my studies, there is no firm evidence of mitotic lymphocyte proliferation even in the postnatal or embryonal stage of mammalian animals.

The so-called mitotic fugures of lymphocytes are most probably a misunderstanding of the process of new formation of nucleus (DNA) in the non-nucleated erythrocyte or erythrocytic monera, by means of AFD process of erythrocytes.

It must be impossible to show that there is sufficient mitotic index value corresponding to increasing in great number of daughter lymphocytes.

On the same reason, increasing in number of other leucocytic elements also

is the most youngest stage of cell, and it must rather be referred to an element belonging to precellular stage than a cell.

If we expressed metaphorically, the non-nucleated human erythrocytes resemble infants. As their prospective occupation may be determined by their circumstances and education, the erythrocytes also differentiate into various sorts of cells according to their cellular environmental conditions such as different physical, chemical, physiological and pathological conditions. Because the erythrocytes with polypotentiality change their form and function adapting to their positions where they are brought by blood stream.

According to embryological term, this phenomena can be said as follows, "erythrocytes" with totipotential ability differentiate into all kinds of fixed or free cells according to respective influence or "induction,, from each "field" where erythrocyte is localized and comes in contact with another kind of cell.

Differentiation from erythrocytes into all kinds of other cells can be recognized by existence of transitional phase (intermediate elements) between erythrocyte and other kind of cell, if any one frankly observes a histological sectioned preparate or living culture specimen one would surely agree with my opinion.

(7) The so-called "Amitosis, and "Abnormal Mitosis, are misunderstanding

The so-called "amitosis" and "abnormal mitotic figure", are generally believed as the former correspond to the polynucleated liver cell, and the latter to the cancer cell with polynuclear or polymorphnucleus. (Figs.1~1, 1~2)

But this orthodox conception is most probably the misunderstanding of the AFD process, which gives rise to new large cell from an erythrocytic mass (blood monera). In the intermediate stages of this process, in fact, there arise one or more of nucleus in the protoplasmic substance and this intermediate figures are apt to be misapprehened as an amitosis or the abnormal mitotic figure. However, these orthodox view is not be substantiated by reliable fact, therefore such a conception is nothing but an imaginary opinion.

Thus almost all of the so-called plasmodium such as the giant polyspindle cells of malignant neoplasm, Langhan's giant cell in the tubercle in pathogenic conditions, and in normal healthy body, the giant polynucleated cell in the bone marrow, multinucleated liver cell, and of others has been referred to be the plasmodium cells which have arised by amitotic nuclear division.

But this orthodox view also lacks in positive evidence. On the contrary, in accordance with my view all of these plasmodium cells are the resultants of AFD process of the erythrocytes.

(8) Metaplasia and Cell Differentiation

The technical term "Metaplasia" has been used by histologists and pathologists, in the following meaning that is some tissue cells which have once differentiated, change further into another kinds of cells.

In other words metaplasia is a form of abnormal regeneration by which produce a type of cell different from that normally found in a given location, e.g. from fibrous tissue to bone, or squamous epithelium may replace columnar epitherlium.

It is a common sense of biology and embryology that the cells of developing individual gradually lose their power to form cells of different kinds. Thus the fertilized ovum is totipotential; it has the ability to differentiate into cells of all types. Cells of embryonal layers are multipotential; they retain, yet, the power of forming cells of several types. However, in adult stage, cells become unipotential, are able to form cells of a single type only, such as germ cell from germ cell, liver cell from liver cell only, and so on. According to the orthodox cell theory, certain cells under certain conditions regain embryonal property of being able to form cells of different other kinds, and the result is metaplasia.

And metaplasia referred to occurs under three conditions: Chronic inflammation, vitamin A deficiency, and neoplasia (neoplasm).

However, results of my studies on several kinds of animals including man, the conceptions of metaplasia must be changed fundamentally.

Because the term metaplasia has been based on orthodox cell division theory; cell proliferate only by its cell division, but, now, such an opinion can not be referred to be a true principle of biological sciences. Because I have found that erythrocytes have totipotential or multipotential power to differentiate into almost all kinds of cells, as described above and, further, may be mentioned in the following chapters.

(9) Erythrocytes Differentiate into all Kinds of Leucocytes

The blood of all animals contains a number of colorless corpuscles (leucocytes). Although the histogenesis and morphology of these leucocytes have been studied intensively, little is known of their origin and fundamental physiologic

TYPE I A. Round or oval normal embryonic erythrocytes, about 6 by 13 micra in size, belong to this type. But the erythrocytes at more later embryonic stages in incubation become more ellipsoidal and smaller in shape.

Type I A-cells have relatively small, basophilic, oval or round nuclei including a few chromatin granules and have eosinophilic cytoplasm of somewhat mottled appearance.

TYPE I B. The erythrocytes belonging to this type are more eliptical in form than I A and often show irregular outline of cells. The cytoplasm is characterized with two distinct zones, the inner zone surrounding the nucleus stained more intensely than the outer zone. So that it appears, as if, it represents a result of plasmolysis.

Type I B. perhaps is older stage than type I A, but this conception may need further investigation.

PHASE II. This phase includes the type II A. II B. II C. and II D. and each of them shows transittonal phase from phase I.

These four main directions of differnntiation in embryonic erythrocytes may be attributed to their localization in wolffian body and to the inductions of each fields.

Type II A. The cells belonging to this type exist most commonly in interstitial lumen of Wolffian body, and show transitional phases from type I A cells. Scanty, clear cytoplasm, relatively large round nucleus occupying nearly the whole cell body, are characteristic to the cells of this type. The inner structure of the nuclei of this type is characterized with the presence of dense, large, irregular and dark staining clumps of basochromatins. Within these clumps there are narrow clear spaces. The arrangement of the chromatin particle resembling to a form of spokes of wheel, as often being mentioned as a characteristic feature of lymphocyte, is by no means common to this type. Above-described feature is of the younger stage of type II A-cell and is not essentially different from the small lymphocytes. However, according to advancement of differentiation, they become somewhat resembling to middle-size-lymphocytes bearing larger, lighter nuclei and slightly increased clear protoplasm. (Fig. 3, 4)

Type II B. (Fat-laden cell-like) (Fig. 3.)

Type II B. cells, show also all of transitional phases from the type I A; however, contains no oxy-hemoglobin, therefore cytoplasm appears as clear as that of "fat-laden cell" which is found in embryonic gonad.

The outline of the cell is somewhat polyedral, owing to mutual pressure. The surface of nucleus, situated eccentrically, is somewhat

obscure and protrudes many of fine nuclear strands toward the cytoplasm. This type of cell may be seen in the inner part of glomeruli.

Type II C. (fibroblast or connective tissue cell-like) (Fig. 4)

This type also shows every transitional form from type I A and is essentially identical in structure of nucleus and cytoplasm to type II A, but differs in form, that is to say, both of nucleus and cytoplasm are elongated and spindle-like in shape. The spatial site, where this direction of differentiation occurs, is a very narrow space of the surface of tissue or organ, such as the outer surface of Wolffian body, wolffian tubules or of glomeruli.

TYPE II D (Eosinophilic granulocytes)

This group of cells takes its appearance, at first, in the intertubular lumen of Wolffian body in chick ambryo at about 14 days of incubation, but still there can, at that time, be seen no cosinophilic granulocytes in circulating blood. These cells are, most probably, derived from type I A and I B. The cytoplasm of these cells stains deeper red than normal erythrocytes, and includes intensely staining cosinophilic granules in it. There can be seen complete series of transitions from the eosinophilic granulocytes with abundant granules to that with very few of granules.

PHASE III (Mesenchymatous Cells)

This phase includes type III A, III B, III C and III D, and each of them shows transitional phases, from II A, II B, II C and II D, respectively, but these four types resemble to one another. (Fig. 3)

These four cell-types have usually, round or oval but semetimes polymorphous, so that they are variable in size and form. Slightly basophilic cytoplasm is often accumulated on the one side of nucleus The nucleus is larger, vesicular and stains lighter than that of type II A, so that this cell-type is identical in essential points to so-called mesenchyme cell. However, some of them situating at periphery of glomeruli, are larger in size and are multinucleated.

These multimecleated cells does not seems to be a resultant of amitosis, but they may be referred to a syncytium, and they often show transition to degenerating form. The existence of above mentioned diffrentiating direction and transitional phases in embryonic erythroytes may further be substantiated by the following facts:—

(b) *The Histogenesis of Glomeruli in Connection with the Differentiation of Erythrocytes in Chick Embryo.*

Development of Wolffian body begins by condensation of mesonephric elements, mesenchymatous elements, at first, but these mesenchy-

matous elements show every transitional phases from erythrocytes which have migrated into the lumen of Wolffian body through the mesonephric artery. Most of the newly formed glomerulis, in the early embryonic stage at 4 to 7 days of incubation, consist chiefly of erythrocytes (Type I A), however, the erythrocytes gradually decrease in number, thence-forth, on the other hand, phases II or III cells, increase in number, and some of glomeruli become at last consisted almost entirely of the phase II cells. Thus there can be seen every transitional phases from erythrocytes into mesenchymatous components (Phase III cells) within Wolffian body. The erythrocytes included in a glomerulus are usually more deeply stained with eosin than those in intertubular lumen. At the early embryonic stage (4–5 days) most of glomeruli lie on the upper and inner boundary of Wolffian body, the nearest place to the dorsal aorta. And there can often be seen the newly formed anlage of glomerulus which is composed of the glomeration of about thirty to fifty erythrocytes connecting with dorsal aorta through small capillaries. (Fig. 1–4)

New formation of glomeruli continues until the later stage at about 14–15 days of incubation. At that tage, some of the large Primordial glomeruli are often composed of 200–300 erythrocytes but then they become separated into five or more clumps. These erythrocytic clumps also show transitional phases into glomeruli. The sign of degeneration of glomeruli begines at later stage by taking appearance of older type of phase III cells on the surface of glomeruli. The older type of phase III cells with large vesicular nuclei or sometimes with polynuclei, isolate themselves from the surface of glomeruli and then they degenerate.

The so-called mensonephros tissue cells which are hither-to believed to be a source of the histogenesis of Wolffian body, may most probably, correspond to type II A cells or younger stage of phase III cells at the very early embryonic stage. This opinion may be supported by the fact that there can be seen extravascular erythrocytes scattered everywhere in the Wolffian body and they are almost always mingled with mesenchymatous cells which show the transition from erythrocytes.

Furthermore, in spite of vast increase in number of the components of glomeruli there can be recognizable very scarce mitotic figures. (Table 1) (Fig. 3, 4)

(c) *Histogenesis of Wolffian Tubules.*

The Wolffian tubules begin also their formation by the condensation of so-called mesonephrous elements of embryos at 3 to 4 days of incubation. But since that embryonic age there arise the clumped masses of

erythrocytes which then show transitional phases into the mass of mesonephrous elements mentioned above. (Fig. 5) In the center of these spheroid cell masses, appear then clear lumen, and the tubular structure may be completed by anastomosis or fusing together with these cell masses lying closely side by side. The source of the elements of wolffian tubules also may be referred to erythrocytes, because there are transitional phases between these two kinds of elements, and the mitotic indices of the mesonephrous tissue cells are very low value.

The basophilic properties of the elements of Wolffian tubules at early stage of the tubular formation, have become cosinophilic according to the advance of the tubular age, and at last degenerate reducing their staining capacity. Thus we can discriminate the cosinophilic tubules from basophilic tubules of a Woffian body in chick embryo at 7 days of incubation or at still older one. It is not uncommon that the type II C bells adhering on the outer surface of W. t. show transition, on the one hand, to the erythrocytes, and on the other to the cells composing W. tubules. The formation and development of W. t. in chick embryo do not cease on the 10th to 11th day of incubation as has been believed, but continue at 14–15 days of incubation in male embryos or at 15–17 days of incubation in femals embryos. The epithelial cells covering the surface of Wolffian body degenerate periodically; the first appears during 7–10 days of incubation and the second takes place during 14–21 days of incubation. As a result of that phenomenon, the intertubular blood cells flow out on the surface of Wolffian body, and they form a blood layer on the surfnce so that the blood layer attains sometimes about 20–40 micra in thick. (Fig. 6) The erythrocytes localized in the outer most of this layer show transition into type II C cells and at last into phase III cells.

(d) *Some of Experimental Results as to the Differentiation of Erythrocytes in Wolffian Body.*

Diluted hydrogen peroxyed solution has been brought by injection into the air chamber of egg at 13–15 days of incubation. After 2–3 days of the injection the mesonephros was dissected from embryo and examined. The Wolffian body of chick embryo, which has died suffering from mesonephritis caused by the injection, showed following symptom of servere inflammation viz., the increase in size of mesonephros about four to five times larger than normal one, new formation of large blood vessels on its surface and turning to brilliant pink or chocolate color in fresh condition. The serial sections of above described mesonephros were inspected under microscope.

Table 1. Mitotic index* in the mesonephros in the chick embryos.

Age of emb. in days & sex	No. of emb.	Glomeruli			Wolffian tubules			Intertubular cell		
		* a	b	c	a	b	c	a	b	c
3	2	637	3	0.470	253	4	1.581	220	2	0.909
3.5	2	358	2	0.559	204	2	0.980	211	5	2.370
4	2	390	6	1.539	232	3	1.293	242	7	2.892
5	6	1512	7	0.463	1103	23	2.085	1171	12	1.024
6	5	992	5	0.504	875	8	0.913	458	7	1.528
7	2	273	3	1.099	294	4	1.361	505	3	0.594
	2	894	4	0.498	510	8	1.568	502	8	1.594
8	3	1012	5	0.494	1003	7	0.698	1210	11	0.909
	1	450	1	0.222	453	2	0.442	450	2	0.444
9	1	187	1	0.535	221	2	0.904	163	1	0.380
	1	200	1	0.500	213	2	0.939	325	2	0.615
10	2	612	3	0.490	675	3	0.444	568	3	0.528
	2	953	3	0.315	818	3	0.367	732	5	0.683
11	2	950	2	0.211	1053	3	0.285	330	2	0.606
	1	506	2	0.395	601	2	0.333	315	1	0.317
12	1	411	1	0.243	502	1	0.199	297	1	0.337
	—	—	—	—	—	—	—	—	—	—
13	2	1105	2	0.181	970	2	0.206	622	2	0.322
	4	2207	5	0.227	2853	5	0.175	941	3	0.319
14	1	582	1	0.172	593	3	0.506	208	1	0.481
	2	1085	2	0.184	1200	3	0.250	486	2	0.412
15	1	611	0	0	633	1	0.158	203	1	0.493
	1	600	1	0.168	702	3	0.427	273	1	0.366
16	—	—	—	—	—	—	—	—	—	—
	1	851	0	0	809	0	0	535	1	0.187
17	—	—	—	—	—	—	—	—	—	—
	1	753	1	0.133	717	2	0.279	231	1	0.433
18	1	500	0	0	511	0	0	247	0	0
	4	2753	1	0.036	2620	2	0.079	853	1	0.012
21	3	1418	4	0.282	1724	1	0.058	895	1	0.116
	5	2788	3	0.108	2812	1	0.036	1217	1	0.082
	61									

* a..... No. of cells in resting in certain field.
b..... No. of cells in mitosis in the same field.
c..... Mitotic index which were culculated as follows:

$$\frac{M}{C} \times 100$$

M, represents the numbers of dividing cells countered, and *C*, the numbers of resting cells found in the same areas.

The intertubular sinusoid were filled with abundant erythrocytes, thus the walls of Wolffian tubules have been pressed by the erythrocytes and sometimes they atrophied so severely that the whole mesonephros appears as if a sac filled with blood. Sections were made of the mesonephros of chick embryo which were allowed to set on incubator for 2 days after they had died with artificial mesodephritis. In these sections the intertubular lumens or sinusoid were filled with small lymphoid cells, on the contrary, there could not be seen any of nomal erythrocytes or their degenerative forms, and moreover there were transitional phases from erythrocytes to the small lymphoid cells. So, the writer was compelled to have a conception that these lymphoid elements originated from the differentiation of the erythrocytes in situ.

Sometimes I have examined the mesonephric sections which have made from dead embryos which were allowed to set on the incubator for 2 to 3 days after they had died suffering from artifical asphixia by means of applying vaselin on the whole surface of incubating eggs. In such a section I found an interesting fact that all of mesonephroic elements showed a tendency to become uniform cell type with decreased amount of cytoplasm and reducing in staining capacities, rounding up of the outline of cell and nucleus, pycnosis of nucleus of younger cell, thus all of mesonephoric elements became resembling to the small lymphocytes. It is of interest that there is certain relationship between the age of cells and staining properties of their nuclei, that is to say, the nuclei of young, undifferentiated cells (Phase II cell) stained more intensely with basophilic dye than that of the more advanced stage (Phase III cell).

(c) *The mitotic indices in the Mesonephros of the chick embryos show very low values.* (Tab. 1)

Discussion

Some investigators in the present time hold an opinion that every kind of cells begets its own kind of cell by mitotic division, that is to say, the muscle cells are derived only from muscle cells, epithelial cells arise from epithelial cells only, and so on, while other investigators stand against the opinion. It seems to the writer that there is a general trend of the opinions passing gradually away from the former opinion toward the latter one, because the evidences substantiating the possibility of differentiation from certain undifferentiated cells into other kinds of cells, have steadily been increasing. The writer intend to discuss this

problem from the standpoint of view that the erythrocytes in chick embryo have differential potencies.

It is generally admitted view that the erythrocytes, in embryo or postnatal chick, are highly differentiated or senile cells when they begin their life work, and have no further differential capacities. But as far as chick embryos are concerned the writers can not agree with such opinion on account of the following facts:—

(1) *Existence of transitional form.*

Existence of transitional phases between erythrocytes and the elements of mesonephros as has been described in detail.

(2) *Mitotic index and cell migration.*

In spite of vast increase in number of the elements in developing Wolffian body the mitotic index shows so low values that the mitosis can hardly be referred to a chief means of supplying the increasing cells, if any, it may play an unimportant role. It has been believed by many authors that the mitotic index offers a usefull criterion for determining the part played in development by such process as cell migration or cell division. Schultz (28) studied the problem in relation with chick embryos of 18, 33, 48 and 72 hours of incubations, according to her data the mitotic index of entire embryo of 18–20, 33, 48 and 72 hours of incubation is 4.139, 2.516, 3.121 and 1.763, respectively. The duration of the period from prophase to telophase in mitosis of mesonephric elements, and the resting period have not been determined but it is said that the mitotic division of mesenchymal cells requires 67–267 minutes, on the average about 180 minutes. The results of present observations show that the mitotic index of the cells of the glomeruli, Wolffian tubules, intertubular lumen and average of these three parts in chick embyos at 4–7 days of incubation was 0.820, 1.444, 1.526 and 1.263, respectively. If we referred to the time of duration of mitotic division to be 3 hours, value of mitotic index, 1.263, means that there are 10.1 cells may arise from 100 cells for 24 hours. By this proliferating rate, it may require, logically, about 8–9 days for 100 cells increase twice by mitotic division. While, according to my observations (unpublished data) the Wolffian body in the chick embryo at 7 days of incubation reaches about twice as large as that of embryos at 4 days of incubation. This fact may support my opinion that the migration of erythrocytes into Wolffian body and differentiation of them, in loco, play the most important role in histogenesis of Wolffian body. Moreover, there are considerable large numbers of the elements of mesonephros are degenerating in the mesonephros, so that the cells proliferated by mitotic division may be insufficient even to supply the degenerating cells in mesonephros. Some of the workers assert that there is a diurnal mitotic rhythm in the bird's embryos, the writers could however, not confirmed such a fact in the Wolffian body of chick embryos sacrificed at every 1–2 hours interval in a day.

(3) *Migration of erythrocytes and Mesenchymal Cells.*

(i) The glomeruli of mesonephros receive the supply of blood through mesonephric artery. And there can always be seen many of extravasated erythrocytes which clumped in mass, within Bowman's capsul and they show transitional phases into the elements of glomerulis. Thus they may transform, most probably, into fixed elements, mesenchymal cells of glomeruli. (Fig. 1–2)

(ii) Wolffian Tubules. It is a view generally admitted that the new formation of Wolffian tubules ceases on the fifth day of incubation, because all of the mesonephrogenous tissue being then used up (Lillie (22) Lewis and Bremer, (21)). The writer observed, however, that the formation of Wolffian tubules at the most early stage, takes place by the condensation of so called mesonephric elements, mesenchymal elements, but these elements, also, belong to the type II A cells derived from erythrocytes. If the opinion of the previous workers is true, subsequent growth of Wolffian tubules since fifth day of incubation should be accomplished by multiplication of the tubular elements, While, the new formation and growth of Wolffian tubules continue to 14–15 days of incubation notwithstanding there is very low value of mitotic index, so that the mitosis can not be considered to be a leading factor of increasing number of the tubular elements. On the other hands, there can be seen all of transitional forms between the cluster of erythrocytes migrated into intertubular lumen through vena cava interior, and the elements of the primordium of Wolffian tubules.

(iii) Intertubular lumen of the Wolffian Body (Venous sinusoid). The venous sinusoid which is connected with vena cava inferior contains several kinds of cells, i.e. all the transitional forms from type II A cell into type III A and III D cells. And in this sinusoid the ratio of II A cells to I A cells (erythrocytes) is higher than that of vena cava inferior. This means, perhaps, that the erythrocytes migrated there have differentiated into these elements in loco. (Fig. 5)

Some of the authors have described that the interlubular stroma of the mesonephros is the hematopoietic foci of cosinophilic granulocytes (Muckmullc and Michels '32 in teleost; Jordan (16), in proteus anguinus). Present writers also recognized the existence of eosinophilic

granulocytes in that sinusoid in chick embryo (at 14 days of incubation) previous to the appearance of these cells in the blood stream. But there is evidence neither these cells proliferate by mitotic division nor emigrate from other site, on the contrary, there can be seen the transitional phases from erythrocytes to these elements. Jordan (16) claims that in teleost, there can be found the transitional forms between eosinophilic granouaocytes and lymphoid cell, and the latter differentiate into former. The present writer (Chishima (9)) has already published that eosinophilic granulocytes arised from differentiation of, or from cytoplasmic extrusion of erythrocytes in tissue culture of bone marrow of young chickens. The most suitable site of eosinophilic granulocyte-formation, perhaps is referred to be a place at which the blood current has stopped or stagnated.

(4) *The Fate or Differentiatial Potencies of Blood Cells.*

This subject has been throughly discussed or described by many authors, [(Kingsbury (19); Jordan (16–18) Frieländer (15); Rievel (26); Techow, (30), Nonidez (24); Maximow (23); Andrew and Andrew (1); Neuda (31) and others)] and the present writer (Chishima (2–12)) also has published an opinion as to this problem, consequently it is omitted to discuss again this problem in this paper.

Summary and Conclusion

Studies on the histogenesis of Wolffian body and the differentation of erythrocytes in chick embryos have been carried out. The results obtained in these studies may be summarized as follows:—

(1) Erythrocytes in the Wolffian body in chick embryo show transitional phases into the several sorts of elements of Wolffian body, such as small lymphoid cell, the cell resembling with fat-laden cell, fibroblast or connective tissue cell, eosinophilic granulocyte and mesenchymal cell.

(2) The primordium of glomeruli begins its development by condensation of erythrocytes. but there-after the erythrocytes included in that anlage gradually decreasing in number, on the contrary, the small lymphoid cells increase in number and there can be seen transitional forms between these two elements.

(3) The development of Wolffian tubules begins, at first, by condensation of so-called nephrogenous tissue cells, but then they are formed from condensed mass of erythrocytes by means of the differentiation of erythrocytes, formation of lumen in the center of mass and

fusing together with these masses, thus cord like tubule may be formed.

(4) Formation of Wolffian tubules of chick embryos does not cease on the 10 to 11 days of incubation, but continue to the later embryonic stage.

(5) The mitotic indices in glomeruli, Wolffian tubules and intertubular region are so small in value that mitosis can hardly be seen to be the chief factor of vast increase of cells in these tissues, on the contrary, there are many evidences that the leading factor of increasing in number of cells in developing mesonephros rests on the basis of the migration, and differentiation of erythrocytes into the fixed elements of Wolffian body.

(6) The experimental results show that the erythrocytes actually differentiate into small lymphoid cells in Wolffian bodies which were transplanted or received certain treatment.

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Explanation of Plate I

The histogenesis of Wolffian body and the differentiation of blood cells in the chick embryo.

Abrrebiations

AO, Aorta; BC, Blood cells; Bl, Blood layer; fWT, fusing Wolffian tubules; Gl, Glomerulus; Fl-I, Glomerulus at early developmental stage of its formation; Gl-2; Glomerulus at the advanced stage of its formation; Gon, Gonad; MA, Mesonephric Artery; Mst, Mesentery; Nch, Notochord; PWt. Primary Wolffian tubule; Vci. Vena Cave inferior; Vs, Venous sinusoid; I-A, Erythrocyte; LI-A, small lymphocyte; III-A, Mesenchymatous cell; II-B, fat-laden cell like; II-C, Fibroblast.

Fig. 1. Transverse section of 4-days chick embryo, showing the beginning of the formation of Glomerulus by the condensation of erythrocytes flowed into the lumen of Wolffian body from Aorta through the small mesonephric artery. ×150.

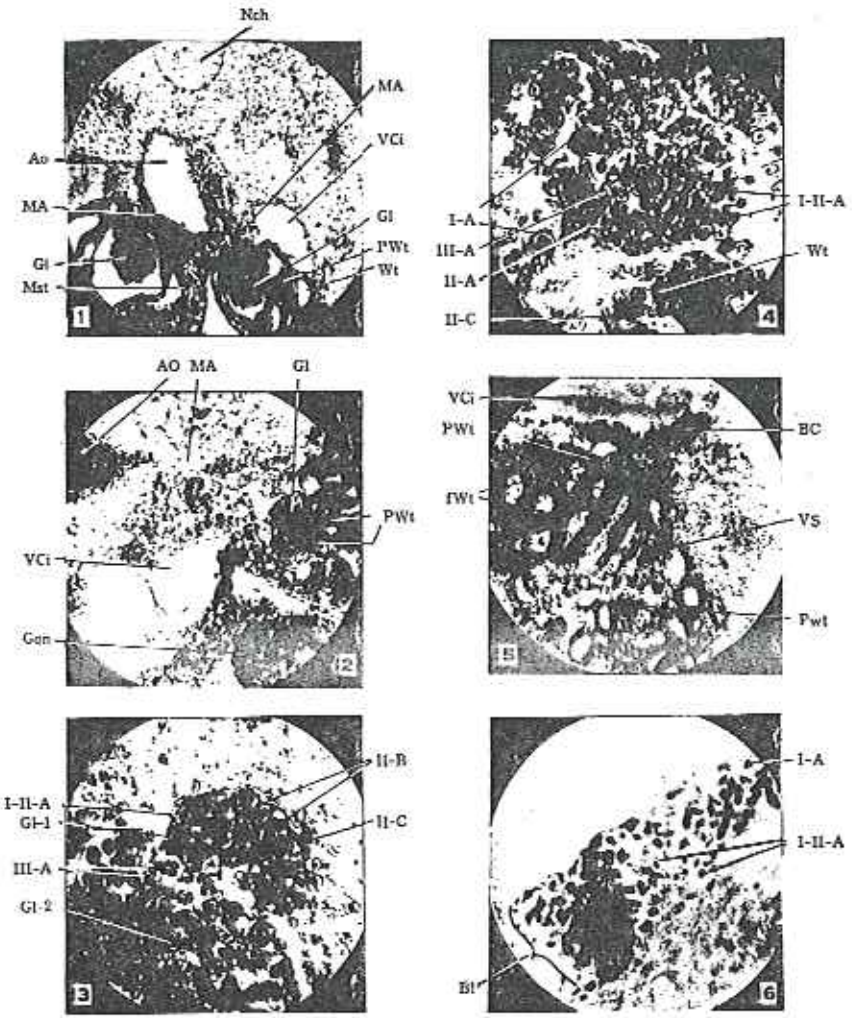
Fig. 2. Section of 6-days-chick embryo. The relation between mesonephric artery and early histogenesis of Wolffian body are shown. ×135.

Fig 3. Section of 6-days-chick embryo. It is able to be clearly seen that the newly formed glomerulus consists of almost all of blood cells at the stages of type I-II. ×800.

Fig. 4. Section of 6-days-chick embryo, indicating several differential phases of blood cells in a glomerulus and Wolffian tubules. ×800.

Fig. 5. Section of 8-days-chick embryo, the fuse of Wolffian tubules and continuity of blood in the venous sinusoid with that of vena cava inferior. ×150.

Fig. 6. A part of the Wolffian body in a 14-days-chick embryo, the blood layer on the surface of Wolffian body is presented. The migrated erythrocytes have fixed there and thence-forth they differentiate into the elements of Wolffian body. ×800.



II Studies on the Relationship Between the Histogenesis of the Gonad and the Differentiation of the Blood Cells in the Chick Embryos*

By

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Sixty-three Figures

CONTENTS

I. Introduction

Much has been reported regarding the development of the gonads in birds but the following remain still disputed, namely (1) the mode of formation of the sex cord and rete cord, (2) the possibility of germ cell differentiation from the elements of germinal epithelium, and (3) the relationship between the development of the gonad and the Wolffian body.

* Kedakfion der Okajimas Folia Anatomic Japonica
(Anat. Inst. der Keio Univ. Shinzuku Tokyo Japan)
Bd. 24. Heft 3. pp. 149-186. (1952)

So far as I am aware, no work seems to have done regarding the histogenesis of the gonad with special referrence to the differentiation of blood cells in chick embryos or other animals.

Since then I had published many works as to the behaviour and differential potencies of erythrocytes, I have been maintained that the erythrocytes in vertebrates are very young, undifferentiated cells with wide potencies, and are differentiated into several types of cells according to the cell environment.

In this paper the writer intends to describe the histogenesis of the gonad with special reference to the differentiation of blood cells especially of erythrocytes.

II. Materials and Methods

The materials used in this study were the 267 F_1 chick embryos obtained by crossing female Barred Plymouth Rock and Male Rhode Island Red and 30 chickens of newly hatched male White Leghorns. The embryonic age ranged from 3 to 21 days of incubation. Embryos at early stages of incubation were cut in toto but at later stage the gonads were removed from the embryo together with Wolffian body, and these blocks were fixed in Bouins fluid. Sections in paraffin of 5-8 μ were made, and stained mainly with Delafield's haematoxylin and eosin, and sometimes Flemming's, Champy's, Meve's and Regaud's fluids were used as fixatives and stained with iron haematoxylin.

For sufficient observation on the relationship between the gonad and the mesonephros, it is important that the serial sections should be made by cutting the gonad with the mesonephros, so as parallel to the long axis of the gonads. In order to ascertain whether there is or is not a certain diurnal rhythm of the cell division in germinal epithelium of embyryos, ranging from 5.5 days to 6.5 days of incubation, the materials were fixed at intervals of one or two hours throughout the day and the night.

The graft experiments of the gonads were carried out in the following manner, so that the small cut pieces of the gonad of chick embryos ranging from eight to nine days of incubation transplanted into the chorio-allantoic cavity through a small hole previously made in the egg shell of developing eggs at the same embryonic age as the donor embryos. After the operation the holes were sealed with small pieces of egg shell and paraffin, and then returned to the incubator. On certain days after the operation the transplanted tissues were

removed and sections were inspected. To examine the behavior of the blood cells, the embryonic blood was injected with a syringe through small holes made in egg shells, into the fertilized or unfertilized eggs which had been kept in the incubator. In some case the blood vessels on the chorion of the developing embryo were injured by small, sharp hooks through the small hole made in the egg shell to induce bleeding, then the hole was sealed with paraffin and replaced in the incubator. The blood cultured by the method mentioned above was examined at definite intervals after the operation by stained sections. In order to confirm the differentiation of the erythrocytes in the gonads in their most natural condition, the testis of newly hatched, living chickens were cut partially with small scissors in order to produce bleeding wounds. The chickens were killed after various intervals after the operations and stained sections were made and inspected.

III. Results

(A) Tendency and phases of the differentiation of blood cells in the gonads in chick embryos.

The erythrocytes migrated into the embryonic gonad show transitions into the following four directions, namely IIA, IIB, IIC, and IID cells according to the area in which the erythrocytes are located, and these four types of cells show further transitions, respectively, into IIIA, IIIB, IIIC and IIID, according to the lapse of time. Consequently, all of the elements of the gonads belong to one of these phases. (Fig. 1)

This classification of cell types, however, is only for convenience of study, so that, in a strict sense, it is impossible to draw a definite line between these three phases because they show unbroken transition. The characteristics of these cell-types are as follows:—

(a) Phase I. The embryonic erythrocytes belong to this type, and may further be divided into two types, IA and IB.

(1) Type IA. Round or oval normal embryonic erythrocytes, about 6 by 13 micra in size, belong to this type. But at later embryonal stages the erythrocytes become more elliptical and smaller in size. (Tab. 1) The type IA-cells, with relatively small, basophilic oval or round nuclei including a few chromatin granules, contain the eosinophilic cytoplasm showing a somewhat mottled appearance.

Table 1. Variation in size and shape of erythrocytes and their nuclei according to the age of chick embryos.

Sex & Age of embryo in day	No. of embryos	Size and shape of erythrocyte											
		Large				Intermediate				Small			
		L.	B.	$\frac{L.+B.}{2}$	$\frac{B.}{L.}$	L.	B.	$\frac{L.+B.}{2}$	$\frac{B.}{L.}$	L.	B.	$\frac{L.+B.}{2}$	$\frac{B.}{L.}$
2- 3	15	11.28	9.60	10.44	0.85	8.43	7.20	7.82	0.85	5.72	5.23	5.48	0.91
4- 6	15	10.88	8.80	9.84	0.81	8.48	6.56	7.52	0.77	5.28	4.48	4.88	0.85
7- 9	10	12.96	9.28	11.12	0.72	8.88	7.60	8.24	0.86	5.44	4.08	4.76	0.75
10–13 ♂	7	10.28	6.85	8.57	0.67	8.34	6.17	7.26	0.60	5.03	4.34	4.69	0.86
10–13 ♀	9	11.44	8.35	9.99	0.73	9.10	7.20	8.15	0.79	4.89	4.26	4.58	0.87
14–18 ♂	7	9.03	6.74	7.89	0.75	7.66	5.26	6.46	0.69	4.91	3.31	4.11	0.69
14–18 ♀	7	9.74	7.74	8.74	0.79	7.73	5.87	6.80	0.76	4.66	4.00	4.33	0.86
19–21 ♂	6	10.14	4.80	7.47	0.47	8.26	4.80	6.53	0.58	4.00	3.48	3.74	0.67
19–21 ♀	8	10.22	8.09	9.16	0.79	8.00	6.47	7.24	0.81	4.67	3.40	4.04	0.73

Sex & Age of embryo	No. of embryos	Size and shape of nucleus in erythrocyte											
		Large				Intermediate				Small			
		L.	B.	$\frac{L.+B.}{2}$	$\frac{B.}{L.}$	L.	B.	$\frac{L.+B.}{2}$	$\frac{B.}{L.}$	L.	B.	$\frac{L.+B.}{2}$	$\frac{B.}{L.}$
2- 3	15	6.09	5.48	5.79	0.90	4.56	4.18	4.37	0.92	3.38	3.32	3.35	0.98
4- 6	15	4.54	3.90	4.22	0.86	4.16	3.68	3.92	0.88	4.00	3.44	3.72	0.86
7- 9	10	4.70	3.84	4.27	0.82	4.00	3.04	3.52	0.76	3.60	2.96	3.28	0.82
10–13 ♂	7	3.89	3.40	3.15	0.62	3.43	2.40	2.92	0.70	3.20	2.69	2.95	0.84
10–13 ♀	9	4.26	3.46	3.86	0.81	3.60	3.11	3.36	0.86	3.38	3.02	3.20	0.89
14–18 ♂	7	3.88	3.86	3.37	0.74	3.77	1.93	2.85	0.51	2.97	2.51	2.74	0.85
14–18 ♀	7	3.86	2.80	3.33	0.73	3.46	2.18	2.82	0.63	3.06	2.54	2.80	0.83
19–21 ♂	6	4.00	2.66	3.33	0.67	3.74	1.80	2.77	0.48	2.94	2.40	2.67	0.82
19–21 ♀	8	4.40	2.67	3.54	0.61	3.30	2.00	2.65	0.61	3.22	2.83	3.03	0.88

L., Length; B., Breadth.

(2) Type IB. The erythrocytes belonging to this type are more eliptical in form than IA and often show an irregular cell outline. The cytoplasm is characterized by two distinct zones, the inner zone surrounding the nucleus stained more intensely than the outer zone, so that it appears, as if, it represents a result of plasmolysis. Type IB-cell seems to be an older stage of type IA, but it requires further research.

(b) Phase II. This phase includes types IIA, IIB, IIC and IID cells and each of them show transitions from Phase I. These four main directions of differentiation of embryonic erythrocytes no doubt, may be attributed to their localization in the gonad.

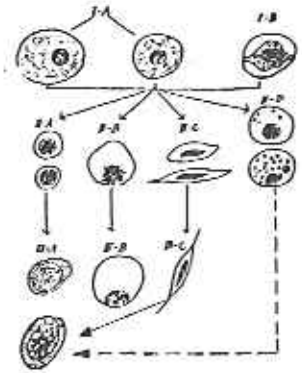

Fig. 1. Directions of differentiation of the embryonic erythrocytes in the gonad of chick embryo.

ABBREVIATIONS

I-A, I-B, and type I (normal erythrocytes); II-A, small lymphocyte; II-B, fat-laden cell; II-C, fibroblast; II-D, eosinophilic granulocyte; III-A, mesenchymatous cell (B); Dg., degenerating form. ×1000

(1) Type IIA. The cells belonging to this type exist most commonly in the interstitial lumen of the gonad and in germinal epithelium, and they show transitional phases from type IA-cells. Scanty, clear cytoplasm, a relatively large round nucleus occupying nearly the whole cell body, are the characteristics of the cell of this type. The inner structures of the nucleus are characterized by the presence of dense, large, irregular and darkely staining clumps of basochromatins. Within the clumps there are narrow clear spaces. The arrangement of the chromatin particles in the form of the spokes of a wheel, as is often mentioned as a characteristic feature of lymphocyte is not common with this type. These features are characteristic of the younger stages of type IIA-cells and do not differ essentially from the small lymphocytes. However, with the progress of differentiation, they come to resemble the medium size lymphocyte bearing the larger, lighter nucleus and slightly augmented clear protoplasm.

(2) Type IIB (Fat-laden cell)

Type IIB-cell shows also a transition from type IA-cell. Its cytoplasm almost equals in amount to that of type IA; however, it contains no oxy-hemoglobin, consequently the cytoplasm appears clear. The

outline of the cell is somewhat polyhedral owing to mulual pressure. The nucleus situated eccentrically is somewhat obscure and from it often protrude many fine nuclear strands from its surface towards the cytoplasm.

Typical cells of this type can be seen in the "cluster of fat-laden cells" in the ovary.

(3) Type IIC (Connective tissue cell and Fibroblast)

This type also shows transitions from type IA, and is essentially identical, in structure, to type IIA, but differ in form, namely, both nucleus and cytoplasm are elongated and spindle-like in shape. The sites, at which this direction of differentiation occurs, are the tunica albuginea and the outer surface of the sex-cord, cortical cord and the surface of the gonad, especially in the testis. (Figs. 3, 4, 21)

(4) Type IID (Eosinophilic granulocyte)

This type of cell is found in the interstices of the sexcords or medullary cords of the embryos at about 17 days of incubation and upwards. They are seen much more in the testis (at the later embryonic stage) than in the ovary. The cytoplasm of the these cells stain deeper red than normal erythrocytes, and contain intensely staining eosinophilic granules. (Fig. 5)

There is also seen the transition between type IID-cells and erythrocytes (type IA or IB) Some of the type IID-cells show disintergration scattering about the granules. But probably they transform into mesenchymatous cell B.

(b) Phase III (Mesenchymatous cell B)

This phase includes type IIIA, IIIB (fat cell) and IIIC-cell (connective tissue cell) and each of show transitions, respectively, from IIA, IIB and IIC. And the type IIIC cell often show transitions into type IIIA cell through its rounding up process.

Type IIIA cell are, usually, round or oval but sometimes polymorphous in form, so that it is variable in size and form. Slightly basophilic cytoplasm often accumlate on one side of nucleus, which is larger, vesicular and stain lighter than that of type IIA. Thus it is identical in essential points to so-called mesenchymal cell in chick embryo. (Figs. 1, 3, 4, 6)

Primordial germ cell, oögonia and spermatogonia may be considered as an old or riped form of this type which is developed by fusion and differentiation of young types of the mesenchymatous cell (type IIIA). The details of this will be described in later chapters. (Figs. 2, 3, 4, 5 and 6)

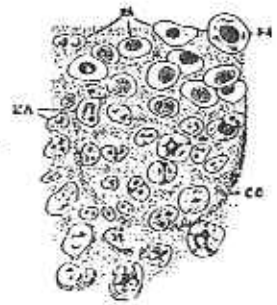

Fig. 2. A part of the surface of the left ovary in a 13-day chick embryo, showing the formation of a cortical cord and transformation phases of the erythrocytes into the elements of the cortical cord.
I-A, II-A, III-A, See Fig. 1;
CC, cortical cord. ×750

Fig. 3. The surface of testis in 21-days chick embryo, showing the blood layer and erythrocytes being transformed into the elements of germinal epithlium in situ.
I-A, II-A, III-A, II-C, III-C, see Fig. 1.
×650

(B) Experimental results on the differentiation from **erythrocytes into the other kinds of cells.**

(a) It was found that the erythrocytes, cultured in the fertilized or unfertilized eggs for 4 days, are transformed into small lymphoid cells. (Figs. 19, 20)

(b) Small pieces of the gonad of embryos at 8-9 days old were grafted into chorio-allantoic cavity of embryo of the same age as the donor, and 2-3 days after the operation the stained sections were made from the grafted tissues and inspected. In these sections, the gonadial elements showed a tendency towards transformation into a uniform type, by rounding up of the cells, into the small lymphoid or mesenchymal cell.

In this case, the staining properties of the nuclei are lowered with cellular age.

(c) The testes of newly hatched chickens, which had been given a small wound with scissors, were examined with stained sections at certain intervals after the operation. At 1 hour after the operation the wound area was filled with a wedge-shaped thrombus composed, almost entirely, of normal erythrocytes. At 6 hours after the operation the wound area was occupied by a thrombus composed of large amounts of small lymphoid cells and of a small number of erythrocytes.

1-2 days after the operation, the small lymphoid cells further increased in number, moreover, there appeared fibroblast or connective tissue elements (type IIC, IIIC) in that area, while the erythrocytes disappeared. (Figs. 21-24)

In this case there was no evidence that the large number of small lymphoid cells are originated by rapid assembling from other areas or by their mitotic proliferation. On the contrary, there were clear transitions from erythrocytes into these elements in loco.

(d) Some other evidences of the differentiation from erythrocytes into other fixed cells are as follows:

(1) *Existence of extravascular erythrocytes*

It is widely accepted opinion that the erythrocytes do not pass out into the lumens of tissues through the capillary wall. However, if we observe thoroughly the stained sections of the embryonic gonad we may find a noteworthy fact that there are many extravascular erythrocytes in the interstitial lumen of the gonad or on the surface of the gonad in normal chick embryo. Some workers may consider it as a venous sinusoid, but these sinusoids have no epithelial wall. Erythrocytes often are mingled with various elements of the interstice as has been described in a previous chapter. Moreover, they show transitions into these elements, according to their localization. (Figs. 6, 13, 14. 26-32, 33, 44-49, 51) Thus capillary system of the gonad is an open type.

The "blood cell cord" mentioned above may be referred to as a capillary, without definitive endothelial cells, in which the blood current has stopped physiologically. The extravascular erythrocytes can be found in every part of the embryonic body, so that route of blood in the embryonic body, most probably, changes from time to time and the blood cells in the lumens may be transformed into the fixed elements of several types of tissues.

(2) *The relationship between the situation of the gonad and the blood vessels.*

At an early embryonic stage, the primordial gonads of chick embryos lie closely under the subcardinal vein and at the inner side of the revehent vein, these veins as yet having no endothelial cells, so that the blood cells contained in these vessels are in direct contact with the primordial gonad. Some erythrocytes contained in these vessels adhere to the gonad and show transitions into the elements of gonad. (Fig. 25)

Though this relationship is more obvious in the early embryonic stages, there is still a close connection between the goned and the large blood vessels. Consequently the histogenesis of the embryonic gonad may not be solved without taking into consideration the important role of the blood cells and blood vessels.

Fig. 4. Part of the surface of testis in 21-day chick embryo, showing transitions from erythrocytes into connective tissue cells.
Abbreviations are same as in Fig. 1. ×700

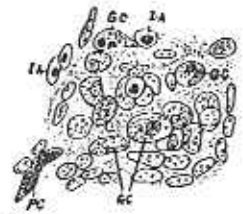

Fig. 5. Part of the interstice of a testis in 21-day chick embryo, showing transitional phases from type IA cells into type II-D cells.
I-A, see Fig. 1.
GC, eosinophilic granulocyte (type II-D)
PC, pigment cell. ×700

(C) The origin and the fate of the so-called primordial germ cell.

In chick embryos the mature, typical primordial germ cells (hereafter, the abbreviation, "P. G. C." will be used) are much larger than tha germinal epithelical cells, and are spherical in shape with large vesicular nuclei. At the nuclear pole there is a wide area of cytoplasm and it is there that the attraction sphere can be seen.

The attraction sphere, which is seen in the P. G. C., appears as a large condensed, flattened or cap-shaped substance lying close to the nuclear surface and shows a intermediate feature between cytoplasm and nucleus. (Figs. 7, 8, 9, 18) The cytoplasm of the most primitive or young P. G. C. included in germinal epithelium is basophilic in staining and consequently, shows no clear demarcation between nucleus and cytoplasm.

The primitive P. G. C. appears as a resultant of the fusion of two or more epithelial elements, because there can be seen transitional phases between these two elements. The primitive P. G. C. further fuse with adjacent or crescent-shaped mesenchymal cells which closely surround the surface of the young P. G. C. (Figs. 8, 9, 18) The basophilic cyto-

plasm of primitive P. G. C. gradually become lighter and finally become the typical P. G. C.

So-called P. G. C. in the capillary can be recognized in the neighbourhood of the coelomic angle. But these clear and large cells differ from the "Entodermallen Wander Zellen of Dantschakoff" in the circulating blood, because they show transitions from fused erythrocytes in the staganated capillaries. There is no evidence that the P. G. C, or "Entodermal Wander Zellen" move toward the germinal epithelium through the gonadial stroma by their own amoeboid movement.

The mitotic indices of the P. G. C. or germ cells in germinal epithelium show very low value. (Tab. 2)

Table 2. Mitotic index of primordial germ cells in the germinal epithelium of chick embryos

Sex & Age of embryos	No. of embryos	Mitotic index	
		Right gonad	Left gonad
5	7	0.28	0.51
6	5	1.00	1.20
7	4	0.74	0.56
8 ♂	2	0.02	0.03
8 ♀	2	0.02	0.04
9 ♀	2	0	0
10 ♂	2	0	0
10 ♀	4	0	0
11 ♂	2	0.02	0
11 ♀	1	0	0.03
12 ♂	2	0	0
12 ♀	2	0	0.03
13 ♂	2	0	0
13 ♀	4	0	0
14 ♂	1	0	0
14 ♀	2	0	0.01

Table 3. Number and distribution of primordial germ cells in embryonal gonad

Age in days	No. & Sex	Germinal epithelium		Under layer of ger. epi.		Medulla	
		Right g.	Left g.	Right g.	Left g.	Right g.	Left g.
5	6	2.67	7.08	1.00	3.50	0.50	1.33
6	5	1.00	5.30	1.50	6.00	0.30	0.30
7	4	0.88	3.62	0.75	2.25	0.38	0.40
8	♂ 3	0.50	3.00	0.33	1.83	0.83	0.83
	♀ 1	0.50	2.50	0.50	1.50	0.50	0.50
9	♀ 1	0	2.50	0	2.50	0	0.50
10	♂ 2	0	0	0	0	1.50	2.50
	♀ 3	0.23	1.67	0	0.50	0	0
11	♂ 2	0	0	0	0	1.50	1.73
	♀ 1	1.00	1.50	0	0	0.50	2.50
12	♂ 1	0	1.50	0	0	0	0
13	♂ 2	0	0.50	0	0	8.00	9.00
	♀ 4	0	0.86	0	0	0	0.70
14	♂ 1	0	0	0	0	5.00	5.50
	♀ 2	0	0.75	0	0	0	1.25

No. of P. G. C. in a field (ob. 4 X ok. 6)

The degenerating features of P. G. C. or germ cells can be seen in the deeper part of the embryonic gonad after the middle stage of incubation. On the other hand, primitive P. G. C. continuously arise from the germinal epithelial cell, the derivative of the blood layer, by fusion and differentiation of epithelial cells.

Thus so far as my observations on the origin and fate of P. G. C. in chick embryo are concerned, they lend no support to its origin from special, selfdifferentiating cell, "Ent. W. Z." of Dantschakoff ('08) or "large cells" of Swift ('14), and to the opinion that the P. G. C. continue to the definitive germ cell through mitotic division. Thus the majority of definitive germ cells are most probably, derived secondarily from the epithelial cells of the sex-gland. (Fig. 6, 7, 8, 9)

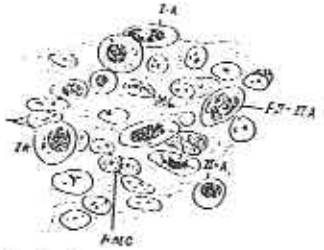

Fig. 6. Some part of the germinal epithelium in a 5-day chick embryo, showing several phases of differentiation and fusion of blood cells.

I-A, erythrocytes; II-A, lymphoid cell; MC, mesenchymatous cell (B) or (III-A); FII-III-A, a transitional phase from type II-A, to III-A, contains two nuclei; FMC, fusing mesenchymatous cells. ×750

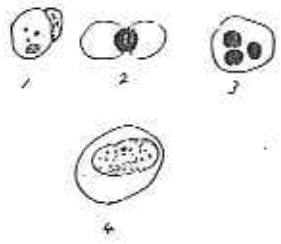

Fig. 7. Four curious blood cells from the blood vessel of early chick embryo, 1 3 are the blood cells from Aorta of 3.5-day chick embryo and 4 is that of 6-day embryo.

1, a blood cell, to which a mesenchymatous cell is adhered; 2, connection with two erythrocytes; 3, an erythrocyte containing three nuclei; 4, a large erythrocyte in which three nuclei are about to fuse. ×750

(D) The origin and formation of the sex-cord, medullary cord and cortical cords with special reference to the differentiation of erythrocytes.

Results of my observations indicate that sex-cord formation in chick embryos is not accomplished by a single mode as has been believed, but there can be seen three different modes, the Ist, IInd and IIIrd modes. (Figs. 10, 11)

(a) The Ist mode, *The sex-cord formation from the "blood layer"*

On the surface of the gonad, especially, on the polar regions of the gonad of chick embryos from 7 to 21 days of incubation, there can be found a layer of blood which has flowed out of the interstice onto the surface of the gonad through the spaces of the degenerated epithelium of the gonad. (Figs. 32, 34, 39, 41, 44, 47) The "blood layer" is seen markedly on the gonads of embryos of 13 to 15 days of incubation, especially on the left ovary. However, if we observe only a restricted region, the middle region of the gonadial surface, we are apt to overlook the blood layer. In the testis of embryos at 10-13 days of incubation and at later stages the extravascular erythrocytes in the interstice or under the connective tissue membrane of the

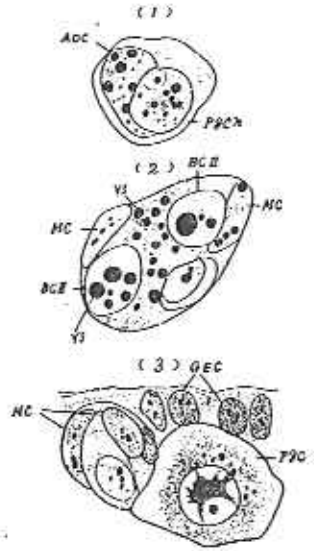

Fig. 8. So-called "Primordial Germ Cells" from the gonad or the mesoderm in 6-day chick embryo.

(1) A P. G. C. from the mesoderm near the coelomic angle. ADC, centrosphere containing yolkspheres in it. P. G. C. N. nucleus of primordial germ cell.

(2) Fusing blood cells from the same region as that of (1), but they are clumped together within a capillary and then they transform into P. G. C. by fusing completely with each other. BCII, Primitive blood cells which contain yolk spheres; MC, mesenchymatous cell (B); YS, yolk sphere.

(3) A P. G. C. in the germinal epithelium and a young primordial germ cell localized to the left of it, which is composed of five cells. Later it may be transformed into a P. G. C. by fusion of these five cells. GEC, cells of germinal epithelium. ×1200

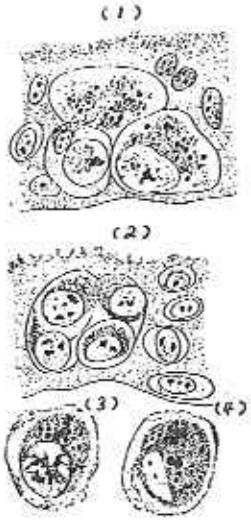

Fig. 9. Several phases of development of so-called P. G. C. by fusion of germinal epithelial cells.

(1) Three so-called P. G. C., at their younger stage, in the germinal epithelium. The germinal epithelial cell contains many of spheres blackened by osmic acid. This cell is attached to the surface of the nucleus of another adjacent cell, and later fuses with it. One undifferentiated P. G. C., situated at the left and lower part of figure, contains a mesenchymatous cell (B) in its cyotplasm. (2), Four large epithelial cells aggregated in a clear lumen, and they are about to fuse with each other. (3), A so-called P. G. C. with a centrosphere-like cap containing yolk sphere, which may have been derived from another cell fused with it. (4), Another view of the same cell (figs. 9~3) from a different focus. Showing that the centrosphere-like body covers almost all the surface of that nucleus.

×1000

surface of testis, condense into a small mass. (Figs. 32, 33, 35) The blood layer usually projects bud-like processes toward the medulla at regular intervals. (Figs. 41, 45, 47) Ten or more erythrocytes in the blood layer glomerate and show transition into the finger or bud-like primordium of the sex-cord which then separates from the germinal epithelium or blood layer. (Figs. 32, 34, 35, 39, 41, 42, 45, 47) These masses fill at first the spaces among the preexisting sex-cords, but after separation from the germinal epithelium they arrange themselves in a manner as if they were piled up into a stone wall. Later these masses are fused with adjacent ones to form the seminferous tubules pointing to the hilum of testis. (Figs. 11, 32, 33, 35) In general, in the blood layer, the differentiation from erythrocytes, into the germinal epithelial cells advance from the middle region towards the polar ends of the gonad. (Figs. 47, 62)

On the germinal epithelium of embryos at 4-6 days of incubation the blood layer has not yet made its appearance, but the lumen underlying the germinal epithelium, includes erythrocytes and transitional forms from erythrocytes to mesenchymal cells. (Figs. 26, 31)

Up to 4 to 5 days of incucation the germinal epithelium consists mainly of round, oval or cylindrical mesenchym cells, but the outline of these cells is often very obscure so that they appear as if they form a syncytium, having within it a few of the so-called primordial germ cells. (Figs. 31, 53)

Germinal epithelium is thicker in the left gonad than in the right. (Tab. 4)

Table 4. Thickness and number of cells of the germinal epithelium

Age of embryos	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21
No. of embryos	6	5	4	4	1	5	4	1	6	3	4	1	1	6	2	13
No. of cells Right g.	3.0	2.4	2.0	1.83	1.50	2.50	2.50	—	1.23	2.50	2.50	—	2.50	1.50	—	2.20
				2.50	-	2.17	1.50	2.0	1.50	1.50	2.50	2.50	—	2.10	1.50	1.67
No. of cells Left g.	4.0	4.0	3.0	2.83	—	3.75	2.50	-	1.25	2.50	3.33	—	2.50	1.50	-	2.20
				3.50	3.50	3.50	4.50	4.50	4.50	4.50	5.50	4.0	—	5.30	4.00	4.70

"No. of cells of R. or L. gonad" means number of cells composing a layer of germinal epithelium.

It is a noteworthy fact that despite of the great increase in number of the germinal epithelial cells there can be seen very few mitotic figures. (Figs. 26, 35)

Furthermore, there is no evidence of "localized intensive mitotic proliferation of germinal epithelium", as has been claimed by Swift ('15). (Figs. 26, 28) And there could not be found any evidence that the formation of the sex-cords cease abruptly at 6.1 days of incubation in the male as was described by Swift ('15). But the process continues to the time of hatching. On the contrary, there can be seen transitional phases from the erythrocytes which have migrated to the surface of the gonad into the elements of the germinal epithelium. (Figs. 2, 6, 32-35, 39, 41, 45-47, 62)

(b) The IInd mode of sex-cord formation.

Another mode of the sex-cord formation takes place by the condensation of the extravascular erythrocytes at the interstitial lumen of the stroma of the gonad. (Figs. 11, 14, 49)

A special veriety of cord was found in the stroma of the gonads in chick embryos of 5-8 days incubation and was designated as "the blood cell cord" by the present writer. (Figs. 14, 26-28)

That cord is composed of cells of type IIA and IIB (the detail characteristics of these cell-types will be described in a later chapter) which show transitions, from the erythrocytes into the elements of the sex-cord or medullary cord.

These transitional phases can be observed by serial stained sections. Consequently these cords may be referred to as derivatives, of erythrocytes, which were contained in the capillary, or which filled in the spaces of stroma, where the blood current had become stagnant or stopped. But the typical manner of sex-cord formation by the IInd mode can be seen in male embryos at about 10 day of incubation.

In these interstitial lumen we can find the transitional phases from condensed masses of erythrocytes into the primordium of sex-cord composed of so-called indifferent interstitial cells, i. e. mesenchymatous cells (type IIIA).

Eosinophilic granulocytes make their appearance in the interstices of gonad from the embryo at about 17 days of incubation and they are found much more in the testis than in the ovary. (Fig. 2)

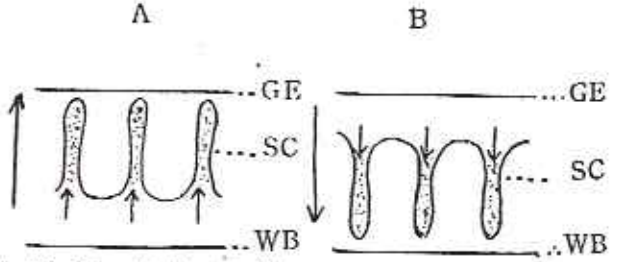

Fig. 10. Schematic diagrams illustrating two different antagonistic theories, published by previous workers, as to the mode of sex cord formation.
(A) The view holds that the sex cords are formed by evaginations of the Bowman's capsules and Wolffian tubules.
(B) Another view, that the sex cords are derived from the ingrowth of the germinal epithelium.
GE, germinal epithelium; SC, sex cord; WB, Wolffian body.
Notice, the arrows indicate the directions in the growth of sex cords.

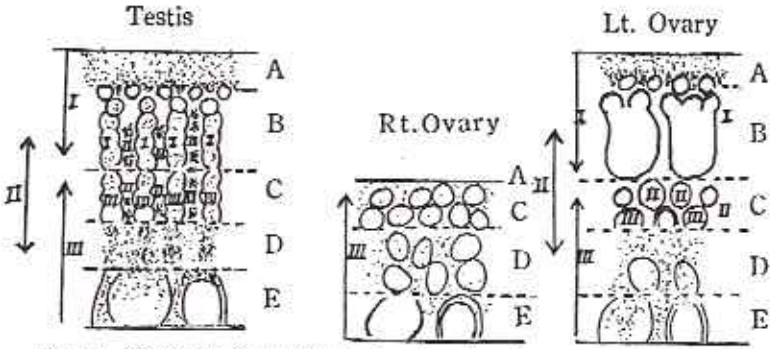

Fig. 11. Schematic diagram illustrating the view of present writer, regarding the formation of sex cords in chick embryo.
I. The first mode of sex cord formation from the germinal epithelial elements derived from the embryonic blood cells.
II. The second mode of sex cord formation, from the condensation and differentiation of blood cells migrated into the interstitial lumen of sex dords or medullary cords which have already been present.
III. The third mode of sex cord formation by means of transition of the elements of Wolffian body.
A. Germinal epithelium; B. the sex cord (I) or cortical cords (I) which have formed from the first mode of sex cords formation;
C. The sex cord II-III or medullary cords II-III formed respectively from the second or third mode; D, transitional portion from rete region (tissue of mesonephros) into gonadal area; E, mesonephric tissue about to be transformed into the embryonic gonad.
Note, the arrows at the side of each figure indicate the direcrions in growth of cords.

(c) The IIIrd mode of sex-cord formation

This mode of the sex-cord formation takes place by the rearrangement and differentiation of the elements of the Wolffian body into the gonadial tissues. (Fig. 11) This mode is more evident at the early stage of development or in the ovary than the later developmental stage or in the testis. And in the medulla of gonads we can often recognize the existence of well preserved glomeruli or Wolffian tubules or their derivatives. (Figs. 30, 53–56, 63)

This tendency is most cleary seen in the right ovary. (Fig. 63) The Wolffian body decreases in size as the gonad grows, owing to the transformation of mesonephric elements into gonadial elements.

Of course, the sex-cord does not start its formation till 4–5 days of incubation, but at that stage there is already seen the primordium of sex-cord or medullary cords as has been noticed by Witschi ('35). These masses of mesenchymal cells show transitions from glomeruli, Wolffian tubules or the elements of venous sinusoids of mesonephros. (Figs. 29. 30)

As the writer (Chishima '48, '51) has shown the glomerules and Wolffian tubules are also the derivatives from erythrocytes. The transition of mesonephric elements into gonadial tissue continues from the earliest stage of gonadial development till the time of hatching. But at a later embryonic stage the transformed elements of mesonephros give rise to the rete-cords, and the urinogenital connection. (Figs. 57–63)

(d) The mitotic index of the gonadial elements

The mitotic indices of the gonadial elements in chick embryos at 4 to 21 days of incubation are measured. (Tab, 2, 5)

It has been considered that the mitotic index offers an usefull criterion for determin whether the development of a certain part depends on cell division or cell migration.

At 11 days of incubation, the sex-cords arrange themselves in a line vertically from the periphery to the hilum of the testis and a single line of sex-cord is composed of about 100 to 120 cells. If these cells arise as a result of the mitotic division of the germinal epithelial cells during the five days from 6 to 11 days of incubation, it should be expected that the mitotic index of the sex-cord indicates 3.14. However, the actual values are very low ranging from 0 to 0.04. (Tab. 5)

Moreover, in the germinal epithelium and medulla, we can find

larger number of degenerating cells and fusing cells than the cells showing mitotic figure.

From this fact the mitotic proliferation of the elements of the germinal epithelium or sex-cord can not be considered as a chief factor in the development of the sex-cord. It is noteworthy that the mitotic index is very low at all parts of the gonad in chick embryo. From these facts the writer considers that the chief factor involved in the cellular increase of embryonic gonad is not a mitotic division but a migration and differentiation of blood cells.

The fate of erythrocytes in chick embryo.

In chick embryo, a vast number of erythrocytes arises from the blood island on the surface of the yolk sac, while there can not be found any sign of true degeneration of erythrocytes in any part of the embryonic body.

Consequently, if the erythrocytes have no potencies to differentiate into other kinks of fixed cells, the embryonic body should inevitably become a sac filled with blood, but this is not the case.

Table 5. The mitotic index of the epithelial cells in the germinal epithelium in chick embryos

Age of embryos	4	5	6	7	8	9	10	11	12	13	14	15	16	18	19	21
No. of embryos	4	7	5	4	4	2	6	3	4	6	3	4	2	5	1	15
Right gonad	0.01	0.02	0.01	0	0.02	—	0	0.02	0	0	0	0	0	0	—	0
					0.02	0	0	0	0	0	0	0	0	0	0	0
Left gonad	0.01	0.03	0.01	0.04	0.03	—	0	0	0	0	0	0	0	0	—	0
					0.04	0	0	0.03	0	0	0.01	0	0	0.01	0	0

$$\text{Mitotic index} = \frac{\text{No. of cells in active mitotic process}}{\text{total no. of cells countered}} \times 100$$

(c) The structure of the sex-cord

Up to 7–8 days of incubation the primitive sex-cords are small (30 by 40 micra in diameter) and composed, in a horizontal section, of about 8–10 cells, but with the advance in the embryonal age the cords increase in size owing to the growth of individual elements and their increaseg in numbers. The adherence of erythrocytes on the surface of the sex-cord and their differentiation into the components of the sex-cord through a stage of the cells of the basement membrane of the sex-cord causes their increase in numbers. (Fig. 37)

The sex-cords, that are derived from the three modes mentioned above, fuse with adjacent ones and form the seminiferous tubules to the hilum, and later become wavy by anastomosing with other tubules. The full-grown sex-cord consists of five kinds of cells viz. (i) elongated erythrocytes adhering to the surface of the cord. (ii) basement membrane cells (type IIc cell), (iii) meshenchymal cells (type IIIA cells), (iv) so-called primordial germ cells or spermatogonia, (v) degenerating cells lying at the innermost part of the cord. Transitions can be seen among all these five elements.

It is a noteworthy fact that the germ cells and the large clear cells in the sex-cord are most probably not a special, selfdifferentiating system, because they show the signs that they are derived from indifferent cells in the sex-cord.

There can be seen only very few mitotic figures in these clear, large cells. On the contrary, these large cells degenerate gradually from the inner part of the sex-cord. Consequently the five kinds of cells in the sex-cord may be referred to as a series of differentiating phases from erythrocytes into so called germ cells.

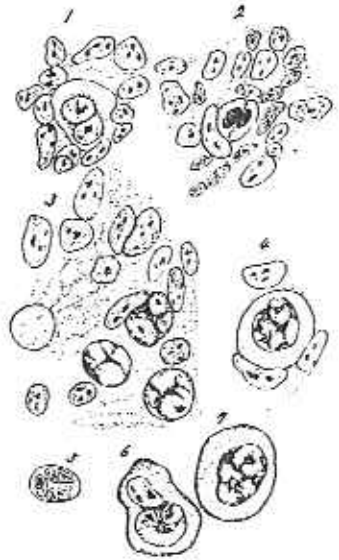

Fig. 12. Illustrating the enlargement or growth of germ cells as a result of fusion of two or more blood cells or their derivatives in the gonad of chick embryos.

1. A germ cell with two nuclei, at a phase of fusion of two nuclei into one, is surrounded by many mesenchymatous cells.
2. Two blood cells, from the germinal epithelium, about to fuse into one.
3. Germ cells from a sex-cord at various stage of cell-fusion.
4. A young germ cell from a sex-cord, surrounded by two mesenchimatous cells. And its nucleus with irregular outline and line of fusion.
5. A syncytial cell from a sex-cord. showing three nuclei which may have arisen as a result of cell-fusion but most probably not due to amitosis.
6-7. Two germ cells from a sex-cord which may be a product of the result of cell-fusion. ×1000

Table 6. The Diameter and number of cells composing the sex cord, medullary cord and cortical cord in chick embryo

Sex & age of embryo in days	No. embryos	Sex cord			Medullary cord			Cortical cord		
		Diameter (μ)		No. of cells*	Diameter (μ)		No. of cells*	Diameter (μ)		No. of cells*
		Length	Breadth		Length	Breadth		Length	Breadth	
5	7	42.75	34.35	10.83						
6	5	48.00	35.25	14.20						
7	4	57.00	42.38	16.00						
8-10 ♂	6	58.84	44.00	13.50						
8-10 ♀	6				56.00	44.00	13.20	44.00	36.00	12.50
11-15 ♂	10	70.40	56.00	18.30						
11-15 ♀	10				54.00	44.00	20.50	64.00	48.00	21.73
16-18 ♂	3	96.70	72.00	43.50						
16-18 ♀	4				80.00	48.00	18.50	65.00	61.00	50.00
19-21 ♂	8	128.00	112.00	45.30						
19-21 ♀	8				85.00	48.00	17.50	68.00	61.00	68.00

* The number of cells composing a transverse sectional plane of a cord.

Table 7. The variation in cell size in relation to their position in a sex cord

Position of cell	Size of cell (μ)		Size of nucleus (μ)	
	Length	Breadth	Length	Breadth
Basement membrane (outer most layer)	9	1.6	5	1.2
1st inner layer	6	3.2	4	1.6
2nd inner layer	7	5.0	5	3.2
3rd inner layer	7	6.0	5	4
4th inner layer	10	8	6	5
(Inner most layer)	17	14	8	8

(f) Formation of primitive sex-cord, cortical cord and medullary cord in female embryos.

In the female embryonic gonad the primitive sex-cord, cortical cord and medullary cord are also formed by the three different modes mentioned above, thus there is no radical difference between the testis and ovary.

These three modes appear rather more evident in the left ovary than in the testis. (Fig. 11) After 7-8 days of incubation, there can be seen, also, the blood layer, and between 8 to 12 days of incubation, the formation of the blood layer is relatively prominent, so that the transformation of that blood layer into the cortical cord, undoubtedly, corresponds to the so-called "second proliferation of germinal epithelium" which was defined by Swift ('15) and Brode ('28). (Figs. 39, 41-43) In spite of the vast increase of epithelial cells, mitotic figures of these elements are seldom. On the contrary, there can clearly be seen the transition from erythrocytes contained in the blood layer into the elements of the germinal epithelium of ovary. (Fig. 2)

The manner of arrangement of the condensed masses of the germinal epithelial elements, which correspond to the sex-cord in the male differs from the female. So that about 6-7 or more of the condensed masses of epithelial elements are grouped in an oblong or long oval shaped cortical cord surrounded by connective tissue elements, instead of the tubular formation as in the male. (Fig. 46) Thus the cortical layes or ovigerous layer with irregular projection into the stroma is formed.

The cortical layers are composed of thick, usually discrete cords of epithelial cells at first, which are then transformed into thick clear layers containing oögonia which show transitions from erythrocytes through a stage of the germinal epithelial elements. (Fig. 62)

The oögonia in the deeper parts of the cortex, which were formed early, are in more advanced stages of development than those in more peripheral regions. Most of the oögonia in deeper parts then degenerate. So-called follicular cells included in the cortical cord may be considered as indifferent, younger stages of oögonia.

Some parts of the medullary cord may have arisen from early formed sex-cords, but most of them are formed by IInd and IIIrd modes of the "formation of the sex-cord". (Figs. 46, 48)

(E) The cause of retrogression of the right ovary.

Asymmetrical development of the ovary in birds have been studied by many workers, but the subject has not been clarified.

The growth rate of the right ovary gradually decreases after the appearance of the distended medullary cord, and it become more loose and porous in texture and finally degenerate almost completely by time of hatching. Though there are thick cortical and blood layers on the left ovary of the embryo at 8–9 days of incubation, (Figs. 38–40), the right ovary of the same age has no such layer. Furthermore, in the right ovary of that age, the distended medullary cords lie near or immediately under the epithelium, and the medulla consists of the elements from the mesonephros, and they do not show the IInd mode of the sex-cord formation.

These facts undoubtedly are due to the lack of fixation of blood cells on the surface or in the medulla of the right ovary.

Consequently the growth of the right ovary depends merely on the transition of the Wolffian body. The mitotic indices in the right ovary are of so low value that it may be impossible even to supply the degenerating elements in that organ.

Hence the right ovary unavoidably follows retrogression with the retrogression of the Wolffian body.

(F) Significance of the nuclear characteristic of the oögonia in the ovigerous layer.

The oögonias composing the ovigerous layer of 18–19 day old embryos show a characteristic feature in the nucleus resembling much the true metaphase of mitosis, but this condition of the nucleus does not show any further advanced phase for the next 3 days, from 18 to 21 days of incubation, consequently these oögonias are not followed by true mitotic division, but, on the contrary, the oögonia in the deepest layer show degeneration. (Fig. 46)

Thus the specific nuclear structure resembling the mitotic figure of oögonia can not be considered as true mitotosis.

(G) Formation of the distended medullary cord and cluster of fatl-aden cells.

The distended medullary cord makes its appearance in the ovary at 8 days of incubation, and this is the most reliable critertion for

the discrimination of sex in the early embryonic gonad.

The distended medullary cords are derived from the medullary cord whose components have degenerated, and from the degeneration of the cluster of fat-laden cells (type II B cell), or from the mesonephrous tissue at later embryonic stages. (Figs. 40, 62)

At first, the lumens of the distended medullary cords are very small but they increase in diameter with the degeneration of their components and as the result of fusion with adjacent ones so as to form a net-work in that strands the blood circulates.

On the other hand, the cluster of fat-laden cells and the medullary cord show transitional phases from the condensed erythrocytes migrated into these places. (Fig. 40)

The so-called "cluster of fat-laden cells" makes its appearance in the medulla of the left ovary of the embryo after 12–14 days of incubation. The clusters of fat-laden cells, which are blackened with osmic acid are homologous in their formation-process with the medullary cords.

(H) Formation of the Rete cords.

The rete cords are connected with the Wolffian tubules and the glomeruli. In later stages they assume the shape of canals and often contain circulating blood. Even though they communicate with the sex-cords or medullary cords, there can be found no reliable evidence that most of them originate from the ingrowth of the germinal epithelium. On the contrary, there can be seen clear evidence of a transition from the glomeruli or Wolffian tubules. (Figs. 54–62)

Hence they may be referred to as modified Wolffian tubules or glomeruli rather than modified sex-cords. It seems that the rate cord may play, at certain developmental stages, a role in carrying the blood from the mesonephros into the gonad.

IV. Discussion

(A) The prospective potencies of erythrocytes.

(a) General consideration.

It is a widely accepted opinion that the function of the erythrocytes is restricted to the conveyance of Co_2 and O_2, and the most haematologists look upon the erythrocyte as a most highly differentiated cell

with no prospective potencies. But, it appears to the writer that this interpretation does not show sufficient evidence.

Ever since my previous report (Chishima, '48, '49, '50) on the differentiation and behaviour of erythrocytes in chick and other vertebrates, I have maintained that the erythrocytes are vary young, undifferentiated cells with wide prospective potencies, differentiating into other kinds of cells viz, several kinds of elements of the Wolffian body in chick embryos, small lymphocytes, neutrophilic granulocytes, eosinophilic granulocyte, several elements of bone marrow in chick; muscle tissue, pigment cell, osteoblast, epithelial cell etc. in tadpole; and other kinds of cell or tissue. Thus the erythrocytes play a most important role in the histogenesis or increase in cell numbers of growing organs in chick embryo and other animals.

The writer, therefore applied the term "differntiation" instead of "metaplasia or metaplasie" to express the transformation of erythrocytes into other kinds of cells.

It is generally accepted that all types of cells increase in number by mitotic division, but there is a group of investigators who hold the opinion that a certain type of cell can be transformed into another according to environmental factors. Fisher and Mayer ('31) Maximow ('28), Dürken ('28) Dantschakoff ('17) et al belong to this group. As to the differentiation of blood cells in the gonads, Bouin and Ancell ('08) say that the testicular cells in horse arise from the small lymphocytes which are located in the intertubular lumen, Goodale ('19) stated that the interstitial cells in the gonad of domestic fowl arise from primitive blood cells. Champy ('13) in frog, Courier ('22) in fish, also recognized the differentiation from leucooytes into interstitial cells, and Nonidez ('20) attributed the origin of interstitial cells to wandering blood cells in domestic fowl. Recently, Andrew and Andrew ('49) published a noteworthy opinion, the differentiation from lymphocytes into epithelial cells in man, the writer quite agrees with their opinions.

(b) Relationship between erythrocyte and mesenchyme cell.

It is the most common opinion that the mesenchymal cells give rise to the erythrocytes and other blood cells.

But so far as the chick embryo is concerned there is clear distinction between the mesenchyme cell founded in blood islands and that in the embryonic body. The former, the mesenchyme cell-A

designated by the present writer, is larger and stains deeper with basophilic dyes and is often more flat or crescent in shape than the latter which is designated by me mensenchyme cell-B.

It is true that the mesenchyme cell-A gives rise to erythrocytes, but the mesenchyme cell-B shows transitions from erythrocytes not only in the gonad but also in several areas of the embryonic body.

At these areas, mesenchyme cell-B and erythrocytes mingle together and the clear transitions between these two elements are seen. It is important to determine whether the erythrocytes arise from mesenchyme cell-B or mesenchyme cell-B arise from erythrocytes.

From results of my observation and experiment it may be concluded that the erythrocytes are differentiated into mesenchyme cell-B following the stagnation or stop in blood current in the embryonic tissues.

(B) The origin and fate of the Primordial germ cell.

Since Weismann (1834-1914) introduced the now familiar idea of the "continuity of germ plasm," the origin and the history of P. G. C. have become subjects of active investigation, however, the early history of the P. G. C. remains disputed. The point at issue is the possibility of differential capacity of P. G. C. from the somatic mesenchyme cells of the germinal epithelium.

Regarding this problem in chick embryos, there have also been contradictory opinions.

The first group of investigators believed in the strict independence of germ cells from somatic cells and they believe in the principle of Weismann's germ plasm theory which asserts that the sole source of definitive germ cells is the P. G. C. Adherents of this theory are: Nussbaum ('80, '01), Hoffmann ('92), Rubaschkin ('07) Dantschakoff ('08) Swift ('15, '16), Reagan ('16), Richards, Halpin and Goldsmith ('26, '28, '35), Brode ('28), Heys ('31).

The second group of investigators believe that the P. G. C. eventually degenerate and the majority of definitive germ cells are derived secondarily from the germinal epithelial cells. Adherents of this theory are; Waldeyer ('70), Semon ('87), dHollander ('40), Firket ('14, '20), Gatenby ('24). It is noteworthy that there are many adherents of the second group who have studied this problem with mammalian materials. (Simkins, '23, '28; Hargitt '26) In the work on rats, Hargitt ('26) was led to the conclusion that there is no segregation of germ cells

and no migration through a "germ track" into the gonad. As a result of his studies on germ cell origin and history, Hargitt is willing to discard entirely the Weismannian concept of the continuity, and said:

"Personally, I believe biology would be greatly the gainer by dropping the germplasm idea entirely and permanently". "Entodermallen wander Zellen" defined by Dantschkoff ('08, '31) and "large cells in blood vessels" observed by Swift ('14) may correspond to "the fused erythrocytes in stagnated capillaries" observed by mc. Dantschakoff ('0, '31), Swift ('14), Firket ('14) Goldsmith ('28). Stanley and Witschi ('40) believed that the P. G. C. with pseudopodia can migrate by active amoeboid movement.

While, Humphrey ('25), Matsumoto ('31) Asayama ('40 and the writer denied the presence of pseudopodia and active movement of P. G. C. Reagan ('16), Dantschakoff ('32) and Goldsmith ('35) tried experiments on cauterization or removal of germinal crescents of chick embryo to ascertain the origin of the P. G. C., but they failed. Dantschakoff ('31) and Witschi ('35) pointed out that the development of P. G. C. has a close connection with the celluar environment.

There is a group of workers who believe that the growth of certain types of cells may be attained by fusion with others. Allen ('04), Stockard ('15), Willson ('28), (In germ cells), Morita ('43) (in somatic cells) and Chishima ('48, '44, '51, '52), (in germ cells and somatic cells) belong to this group.

(C) Formation of the sex-cord etc.

Results of the various workers on the formation of the sex-cords in vertebrates including chick embryos, show many diversified opinions. This difference in view may not be due to difference in materials but may be due to discrepancies of interpretation. And, there have been published the following four different opinions regarding the modes of sex-cord formation in birds, namely:

(a) Sex-cords arise from Wolffian tubules. (Waldeyer, '70).

(b) Sex-cords arise as a results of the evagination of Bowman's capsule. (Hoffman, '89, '93; Semon, '87)

(c) Sex-cords arise from the condensation of migrated cells from the germinal epithelium into the underlying stroma. (Schmigelow, '82; Mihalkovics, '85; Laulanie, '86)

(d) Sex-cords are formed by the direct ingrowth of germinal

epithelial cells which are proliferated by marked local mitotic division. (Janosik, 85; Dantschakoff, '31; Brode, '28; Swift '15, '16; Goldsmith, '28, '32; Brambell, '32)

The opinion (d) has been up-held by Swift ('15, '16) and is the most widely accepted one today.

However, sex-cord formation can not be explained by any of these four opinions, even though, each of them agree with fact in some points but differ in others. That is to say, opinions (a) and (b) agree with the "IIIrd mode of sex-cord formation" described in this paper and the opinion (c) corresponds to my "Ist mode of sex-cord formation", but opinion (d) resembling the opinion (c) differs from my "Ist mode" in many important points.

The opinion (d), especially of Swift ('15) claims, that the germinal epithelium of chick embryos at 5.5–6.5 days of incubation sends finger or bud-like processes into the subjacent tissue as a result of localized intensive mitotic proliferation of the germinal epithelium, but I could not find such a localized intensive mitotic division of the germinal epithelium. Prenant ('89, quoted by Swift, '15) has also made the same observations as that of the present writer. Furthermore, Schmidt and Hoffmann ('41), Papanicoaou ('24), Evans and Swezy ('31) also denied the existence of mitotic proliferation of the germinal epithelial cells in mammals.

On the other hand, there can be seen migration of vast numbers of erythrocytes and their transitional phases into the elements of the germinal epithelium.

Asayama ('40) describes transition of mesenchmal elements into the sex-cord cells in the newt. Muckmull and Michels ('32) reported a very interesting work in teleost's testis, that is the formation of the "wedge-like clumps of macrophages, which have engored the injected carbon particles, on the surface of the testis. These clumps, most probably, correspond to the primordium of the sex-cord consisting of blood cells derived from my "Ist mode of sex-cord formation".

It is interesting that the classic views such as opinions (a), (b) and (c), even though they have been discarded, seem nearer to the truth than the most widely accepted opinion (d).

(D) Reexamination on the role (quantitative value) of mitotic proliferation of gonadal elements.

It is the most widely accepted opinion that several organs grow

chiefly by means of the mitotic division of their own components. But this conception, seems to me, has not yet been substantiated by reliable quantitative data, so far as I am aware.

The primary sex-cords at 6½ day chick embryos are composed of about 12 cells. If these cells are derived from local intensive mitotic division of an epithelial cell during 24 hours (5½—6½ day of incubation) it should be multiplied 3.5 times because of $12=2^{3.5}$.

One cycle of mitotic division, then, should de calculated as 7 hours ($24^h \div 3.5$).

The duration of mitotic division of mesenchymal elements in chick embryos has been recorded by Shultz ('22, quoted by Richards, '35), Belar ('29) and others as 37 to 87 minutes (60 min. in average). Therefore, in this case, resting period may be estimated as 6 hours. So that the expected mitotic index may be culculated as follows; $100 \div 7 = 14.3$.

While, mitotic index of the elements of germinal epithelium in 5½—6½ day chick embryos (16 individuals) was ranged from 0.01 to 0.03. From this, it seem most probable that the cells increased by means of mitotic division can do only a negligible parts $\left(\frac{1}{1430}-\frac{1}{477}\right)$ in the growing gonads. On the other hand, there can be seen large number of extravasated erythrocytes in the surface and interstice of the gonads, from which every transitions into the elements of primary sex-cord or of interstice can be seen.

On the contrary, the degenerating or fusing cells in the gonads increase in rate according to the advancing age. And the decreasing number of cells by these phenomenon exceeds by far number of cells showing mitotic figure. Furtheremore, there are large number of pseud mitosis of oögonias as described above.

Inspite of the exisistence of these antagonistic facts to the mitotic proliferation, the embryonic gonads grow steadily, and increase in numbers of gonadal elements.

From these facts we can not escape from the conclusion that the main factor of the growth of the embryonal gonad is the migration of erythrocytes into gonads, and their differentiation into the fixed elements of the gonad through mesenchymal or lymphoid cell stage.

So that I will present the following theoretical formula regarding with the growth of the gonad in chick embryo. It probably true in animal generally that this is applicable to the growth of several other organs;

$$Tn = M-(Pem+Cf+Cd)+Mig$$

and further,

$$\text{Growth} = [M-(Pem+Cf+Cd)+Mig]\,Gr+P.$$

Notice:

Tn ..... Total number of cells increased in certain growing period.
M ..... Increased cells by means of mitotic division.
Pem ..... Cells showing pseud or endo mitotic figure.
Cf ..... Cells at a stage of fusion.
Cd ..... Cells at a degenerating phase.
Mig ..... Cells migrated and differentiated into gonadal elements. (may be computed by, $Mig = Tn-M-(Pem+Cf+Cd)$)
Gr ..... Growth rate of cell.
P ..... Products of degenerated or de-differentiated cells, such as fat, ground substance of bone, yolk spheres, and other non-cellular, but living substances.

(E) The cause of retrogression of the right ovary.

The asymmetrical development of the reproductive organs in female birds have been of interest to biologists for many years. Witschi ('35) considered that the asymmetry in the gonad of birds is due to a primary, hereditary deficiency of the right cortical inductor. This deficiency was supposed by him to express itself in decreased attraction of the P. G. C. especially during the phase of their redistribution early on the third day. So that Witschi ('35) believed actual migration of P. G. C. from the right to left gonad. Even though this conception based on statistical finding it has not been substantiated by the actual evidence of migration of P. G C. from right gonad to left.

As has been described, the chief factor in the retrogression of the the right ovary is the deficiency of fixation and differentiation of erythrocytes in the right ovary, consequently the growth of the right ovary depends only upon the "IIIrd mode of sex-cord formation". So that the right ovary retrogresses in accordance with the retrogression of the right Wolffian body.

(F) Destiny or fate of the sex-cord and medullary cord.

Swift ('15) and Brode ('28) held the opinion that the primary sex-cord in chick embryos grew into the seminiferous tubules in male and into the medullary cords in female. Results of my observation agree with this opinion. Witschi ('35) described the existence of a primitive medulla, which was present in all gonads of 4 to 5 day old chick em-

bryos, and he considered it as a derivative from the residual remnant of the mesonephric blastema. Brode ('28) described that the medullary cords differentiate further into (1) distended medullary-cord, (2) cluster of "fat-laden" cells of Nonidez ('22), (3) isolated medullary cord cells. (5) cord of P.G.C. (6) cords of P.G.C. bounded together by a basement membrane and (7) mixed cords consisting of germ cells and clear "fat-laden" cells. It seems to me that these several types of cells or cell masses are only transitional forms from the condensed mass of erythrocytes to their degenerating form.

The origin and significance of the cluster of fat-laden cells have been studied by Waldeyer ('70), Descillens ('12), Firket ('14) Boring and Pearl ('17), Nonidez ('22), Fell ('22, '24) Poll and Fell ('23), and Masui and Hashimoto ('22, '24).

It is noteworthy fact that the "fat-laden" cells are found mainly in the left ovary. It suggests that the blood cells especially erythrocytes fall more rapidly and easily into fat degeneration in the left ovary than in the testis.

(G) The origin of the rete cord.

Regarding this there have also been many diverse opinions, namely, rete cords are (a) Derivatives of the Wolffian tubules. (Waldeyer '70)

(b) Evagination of Bowmann's capsule, (Schmigelow, '82; Mihalcovics, '85; Laulanie, '86; Semon, '87)

(c) Ingrowth of the germinal epithelium. (Janosic, '85, '90)

(d) Condensation of mesonephric mesenchymal cells. (Firket, '14; Witschi ('14, '35)

However, it is probable, that most of the rete cords are transitional forms from the Wolffian body, including the Wolffian tubules, glomeruli, mesenchymal cells and blood cells, into the sex-gland.

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V. Summary and conclusion

(1) In this paper the origin of the so-called primordial germ cell and the histogenesis of the gonad in chick embryo are described with special reference to the differentiation of erythrocytes.

(2) Erythrocytes in the gonad of chick embryos show transitional phases into several kinds of formed elements of the gonad such as small lymphoid cell, "fat-laden cell", fibroblast or connective tissue cell, eosinophilic granulocyte and mesenchymal cell-B, according to, (a) the cellular environment, where the erythrocytes are localized or remain stagnant, and (b) with the lapse of time.

(3) The differential potencies of erythrocyte were recognized by means of the following experiments, viz. (i) blood cell culture, (ii) gonad implantation and (iii) wound inducing experiment with testis.

(4) Gonad primordium in early embryonal stage is in direct contact with erythrocytes contained in the subcardinal vein and revehent vein, and some of these erythrocytes adhere to the gonad and show transitional phases into gonadal elements.

(5) So-called primordial germ cells with very low value of mitotic index show no reliable evidence of genetic continuity with oögonia or spermatogonia; on the contrary, they arise, most probably, from the incorporation (fusion) with many of the mesenchymal elements of the germinal epithelium in situ, and further the germinal epithelial cells show transitions from the erythrocytes.

(6) There are three different modes, from which the sex-cord may be formed. The first is from the germinal epithelium which is a derivative from the blood layer (since 8-9 day of incubation). In this case there can be found no evidence of local intensive proliferation of the epithelial elements, on the contrary, migration and differentiation of erythrocytes can be clearly demonstrable. The second mode is formation from the condensation and differentiation of erythrocytes migrated into the interstices of the gonad. The third mode is the transformation and rearrangement of mesonephric elements.

(7) Asymmetrical development of the embryonic gonad in the female chick is due to the lack of first and second modes of sex-cord formation of the right ovary. The right ovary, consequently retregresses by the hatching time according to the degeneration of the right mesonephros.

(8) There is no evidence that the oögonia composed of ovigerous

layer of the left ovary are produced by mitotic proliferation of their own kind. Their characteristic nuclear features, resembling the prophase of mitosis, are not true mitotic figures.

(9) So-called "cluster of fat-laden cells" show transitional phases from cluster of erythrocytes that have migrated into the interstices of the medullar region.

(10) Distended medullary cords are derived from the degeneration of the medullary cords and of the "cluster of fat-laden cells".

(11) Rete cords are transitional portions of the mesonephric tissues into the gonad.

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Since this article was writen in Sept. 1947 I have learned of following important papers on the blood or on the cell-differentiation;

(1) Boström, L., 1949: Blood sp. issue. 1; (2) Dawson, A.B., 1931: Anat. Rec. 110; (3) ——., 1951: J. Morph. 88; (4) Duran-Jorda, F. 19 8: Lancet. Sept. 18; (5) ——., 1949: Ibid. Apr. 16; (6) ——., 1950: Nature 165, Feb. 18; (7) ——., 1950: Acta. Med. Scandinav. 136; (8) ——., 1951: Ibid. CXL; (9) Farr, R.S., 1951: Anat. Rec. 109; (10) George, W.C., 1939: Quart. J. Micr. Sci. 81; (11) ——. & N., John, 1948: J. Morph. 83; (12) Hett, J., 1937: Zeitschr. f. Zellforsch. u. micr. Anat. 26; (13) Lepeshinskaya, O.B., 1951: Biol. Sci. 3, quoted by N., Kusano & H., Sato; (14) Lewis, W.H., 1931: Carnegie Inst. W.Y.B. 30; (15) Neuda, P.M. 1950: Proc. Soc. Exp. Biol. & Med. 74; (16) Torrey, T.W., 1950: J. Exp., Zool. 115; (17) Plum, C., 1947: Blood sp. issue 1; (18) Weiss, P., 1950: Quart. Rev. Biol. 25; (19) Wigglesworth, V.B., 1948: Symposia of Soc. Exper. Biol. No. II. Growth; (20) Zollinger, H.U., 1948: Am. I. Path.

These excellent works are not in conflict with my observations and conclusions, and supports in parts. Beside my papers cited above I have published the following works; (1) Nine papers cited in my previous work (Chishima, K., 1951: Okajima's Folia Anat. Jap. 23); (2) Res. Bull. Coll. Agr. Gifu. Univ. 1, 1951; (3) Biol. Sci. (Seibutsu Kagaku) 4, 1, 1952; (4) 57th General Meet. Soc. Anat. Nipponica, 1952; (5) 4th Symposium of Biol. Sci. (Seibutsu Kagaku) "Growth" 1952.

Explanation of Plates

Abbreviations

ACC, primordium of cortical cord.
AO, aorta.
AG, primordium of gonad.
ASC, primordium of sex cord.
BC, blood cell.
BL, blood layer.
BLC, cord of blood cells
BMC, cell of basement membrane.
BV, blood vessel.
CC, cortical cord.
CL, cortical layer.
CTC, connective tissue cell.
DFLC, degenerating fat-laden cell.
DGC, degenerated cell.
DMC, distended medullary cord.
EBL, erythroblast.
EWZ, "Entodermallen Wander Zellen" (termed by Dantschakoff)
FBC, blood cell at a stage of fusion
FCC, cell mass resulted from fusion with several of cortical cords.
FGC, germ cell at a stage in the fusion.
FLC, fat-laden cell.
FPGC, younger stage of so-called a "primordial germ cell" now going into fusion with others.
GBC, group of blood cells.
GE, germinal epithelium.
GL, glomerulus.
GO, gonad.
LGO, left gonad.
MC-A, mesenchymarous cell (A).
ME, mesentery.
MEC, medullary cord.
MED, medullary.
OG, oögonia.
OV, ovary.
PGC, so-called primordial germ cell.
RGO, right gonad.
REV, revchent vein.
ROV, right ovary.
SC, sex cord.
SCV, subcardinal vein.
SHM, shell membrane.
TA, tunica albuginea.
TGL, glomerulus transforming into the elements of gonad.
TT, testis.
TWB, transforming Wolffian body.
TWT, transforming Wolffian tubule.
WB, Wolffian body.
WT, mesonephric tubule.
I, II, cell of type I (erythrocyte) and type II (small lymphoid cell).
II-A, small lymphocyte.
II-B, fat-laden cell.
II-C, fibroblast.
III-A, mesenchymatous cell-(A).

PLATE I.

(13) Transformation phases of the erythrocytes in an interstice of the gonad in 6-day chick embryo. (4×6, This means ocular 4× objective 6, of a Zeiss microscope, the same denotation applies to those that follow)
(14) Blood cord in testis from 7-day chick embryo. (4×6)
(15) A portion of the blood island in 48-hour chick embryo, showing the erythroblasts arising from mesenchyme cell-A. In this case, mesenchymatous cell (A) arise spontaneously from the yolk material by means of coacervation but not by mitosis or amitosis. (4×6)
(16) The region between the subcardinal vein and mesonephros in 3-day chick embryo, showing the transitional phases from erythorcytes into mesenchymatous cell-(B). Notice, that there is no definite boundary line between the two elements. (4×6)

(17) The so-called "Entodermallen Wander Zellen" termed by Dentschakoff, and normal erythrocyres from Vena cava inferior in 3-day chick embryo. (4×18)
(18) A portion of germinal epithelium of the gonad in a 6-day chick embryo, showing the so-called primordial germ cell and its growing phase by fusion with germinal epithelial cells. (4×16)

PLATE II.

(19) Blood cells and their derivatives which were extravasated by rupture of the blood vessels distributed on the surface of the chorion. They were cultured there during two days after the operation. It can be seen that most of extravasated erythrocytes are transformed into lymphoid cells. (4×6)
(20) The cells treated in the same way as that in plate II-(19) with a fertilized egg of 10-days incubation. (4×6)
(21) Section of part of testis surface in newly hatched chick, on which a cut was made to bleed and the part fixed one hour after the operation. (4×18)
(22) The wound portion of the testis treated in the same manner as that of fig. 21. But it was fixed 4 days after the operation. Mejority of erythrocytes have been transformed into lymphoid cells (type II A, cell). (4×6)
(23) Transplanted blood cells from 21-day chick embryo into the hypoderm of newly hatched chick, fixed 48 hours after operation. Show the fusion of blood cells. (4×6)
(24) Section of part of the testis, fixed when 6-days had passed after the operation. The region of the wound shows wedgelike connective tissue cell mas and there can be seen transitions from fibroblast (type II-C) into connective tissue cells (type III-C cell). (4×6)

PLATE III.

(25) Transverse section of chick embryo, at 7-days of incubation, showing the close relationship of gonad to blood vessels. (4×2)
(26) A part of the gonad in a 6-days chick embryo, fixed at 20:00. A.M. We see no evidence of the so-called "first proliferation of germinal epithelial cell", that is to say, there is almost entirely no mitotic figure observable in the germinal epithelium, on the other hand, there appears a young primordial germ cell at a stage of fusion. Many of extravasated blood cells (type I, II & III cells) filled the interstice of tissue. (4×6) Fixed with Flemming sol. and stained with iron hematoxylin.
(27) A part of the gonad at 6 days of incubation, showing there is no evidence of mitotic figure but a cord with no endothelial cells. It contains no normal blood cells but contains the cells of type II-B. (4×6) Fixed with Flemming sol. and stained with iron hematoxylin.
(28) A part of gonad at 7 days of incubation, showing no evidence of mitotic figure but there can be seen transition between the three of blood cell crods and the primordial sex cord. (4×6)
(29) Gonad at 6 days of incubation, showing there is already a primordium of sex cord independent of germinal epithelium. (4×18)
(30) Gonad and Wolffian body at 5 days of incubation, showing the germinal epithelium of the gonadal primordium connected closely with the Wolffian body and there

can be seen transforation from Wolffian tubules and glomeruli into elements of the gonad at the fundamental region of the gonad. (2×6)

PLATE IV.

(31) The gonadal primordium and Wolffian body at 5 days of incubation, showing the primordium of sex cord already developed from the elements of Wolffian body. (4×6)

(32) Section of the testis at 14 days of incubation, showing the presence of blood layer covering the surface of testis. (4×6)

(33) Section of the testis at 15 days of incubation, showing the blood layer covering the whole surface of testis. (4×2)

(34) Section of testis at 21 days of incubation, showing that the blood layer can be seen, also at this stage, at a certain region of the gonadal surface. (4×1.8)

(35) Section of the testis at 10 days of incubations, showing that there are three sex cords in the middle portion of the figure, which merge in a seminiferous tubule by a process of fusion of three cords arranged vertically and closely to the other. The outermost part of the sex cords were covered with connective tissue cells showing transitions from blood cells. (4×6)

(36) Part of a seminiferous tubule of chick embryo at 21 days of incubation, the enlargement or growth of germ cells by the process of cell-fusion. (4×1.8)

PLATE V.

(37) A seminiferous tubule at 21 days of incubation, showing degeneration and fusion of the germ cells. (4×1.8)

(38) Comparison between the right and the left ovary in a chick embryo at 14 days of incubation, showing the right ovary with a defect in the blood layer on the surface, and the stroma including the glomeruli and Wolffian tubule well preserved in their original forms. On the contrary, the left ovary was covered by a thick cortical layer which shows transitions from the blood layer (right). (4×2)

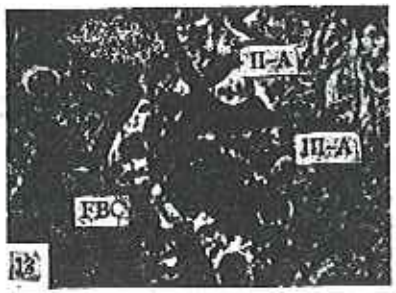
(39) Section of left ovary in a chick embryo at 9 days of incubation, showing the presence of the blood layer and the under-lying cortical cords are connected with the blood layer. (4×3)

(40) Right ovary from the same chick as shown in fig. 39 showing that there is neither blood layer nor cortical cord. And the stroma composed of the medullary cords and distended medullary cords showing transitions from the elements of the mesonephros. (4×3)

(41) Section of left ovary at 13 days of incubation, showing the cortical cords and its primordium already formed by condensation of elements of germinal epithelium which indicates transitions from "blood layer." There is almost entirely no mitotic figure. (4×6)

(42) Section of left ovary at 13 days of incubation, showing that certain region of germinal epithelium in this age of incubation, also, contains the cortical cords which have arisen from the blood layer. And some cortical cords detached from germinal epithelium are shown. (4×6)

(43) Section of left ovary at 14 days of incubation, showing the cluster of blood cells in the interstitial lumen of cortical cords which is beginning to differentiate into a primordium of cortical cord. (4×6)



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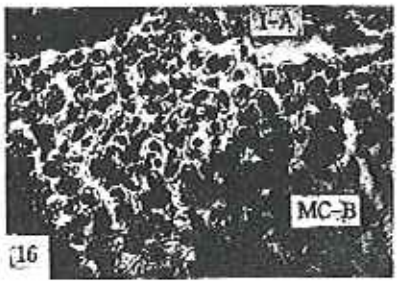

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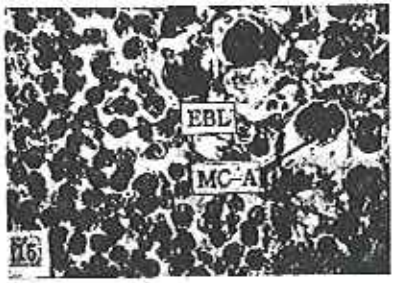

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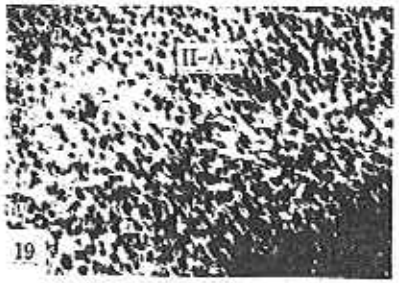

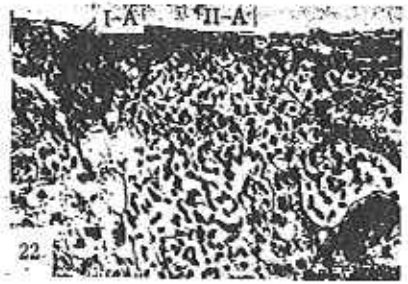

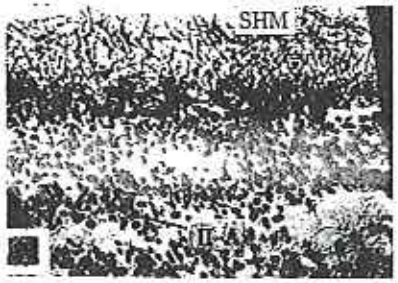

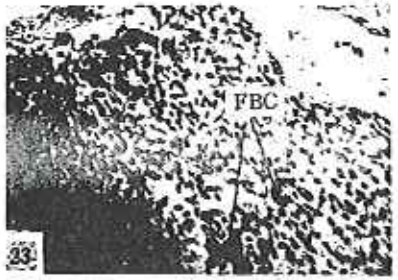

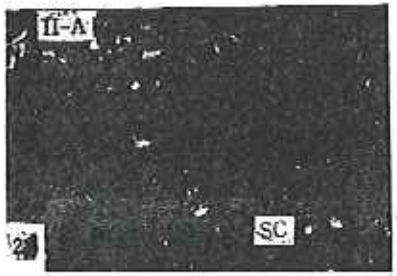

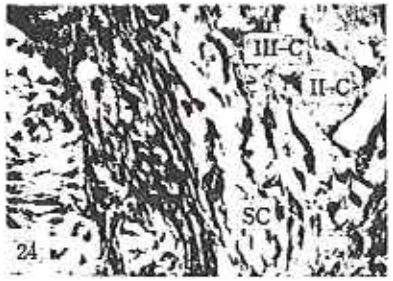

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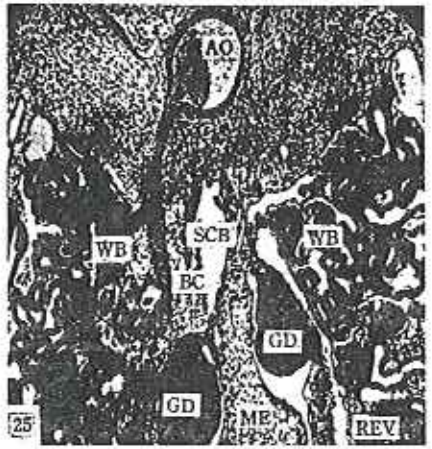

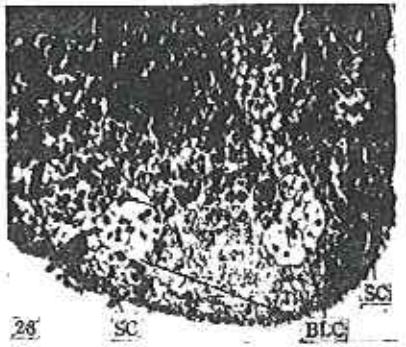

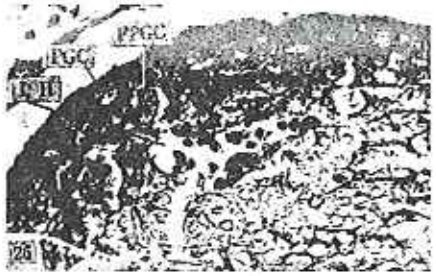

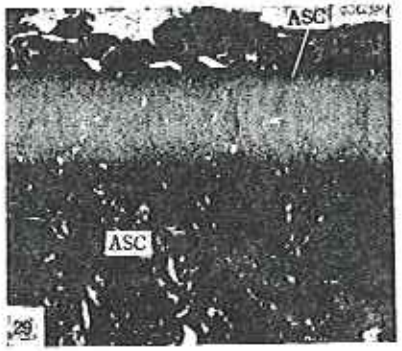

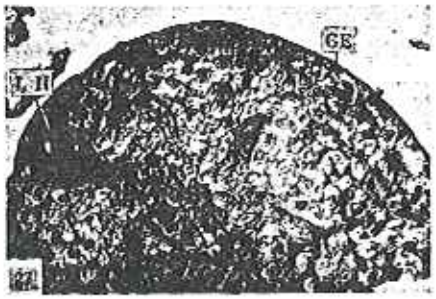

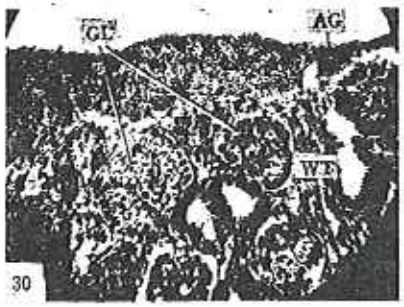

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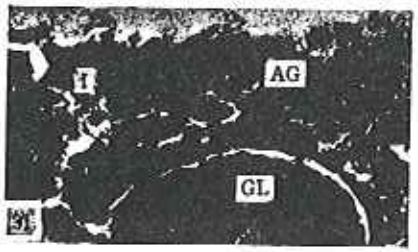

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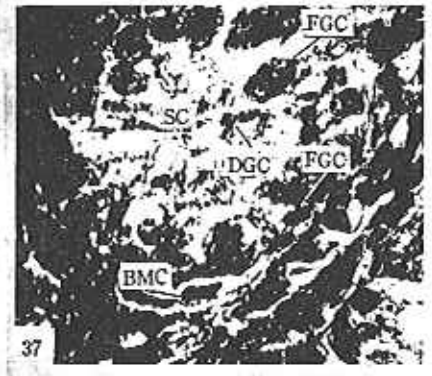

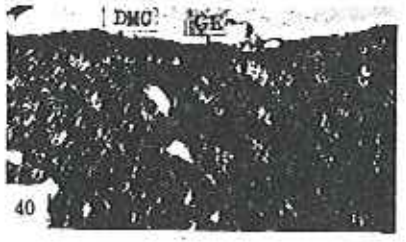

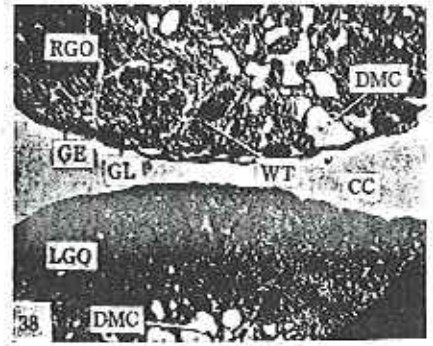

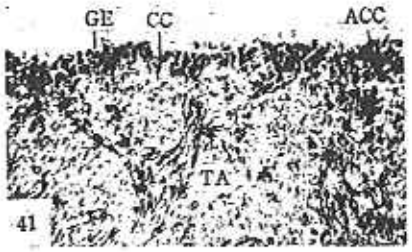

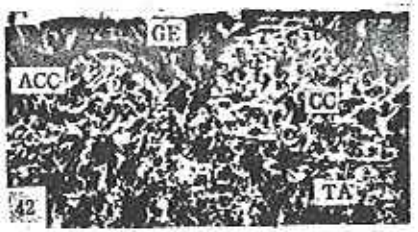

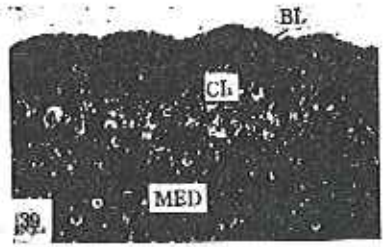

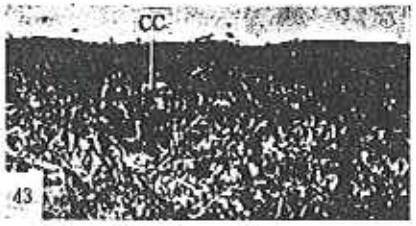

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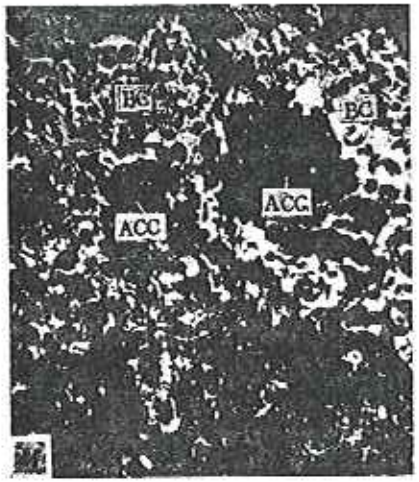

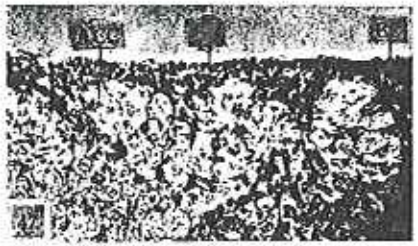

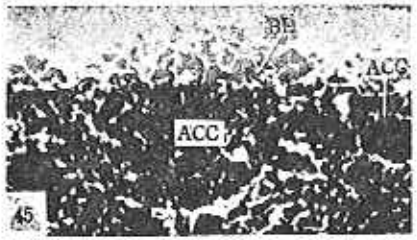

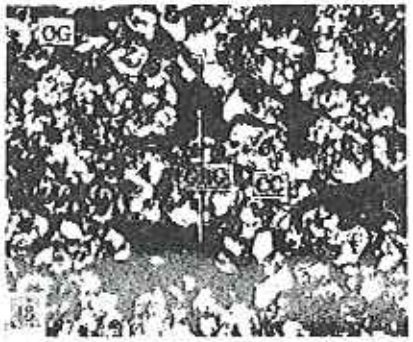

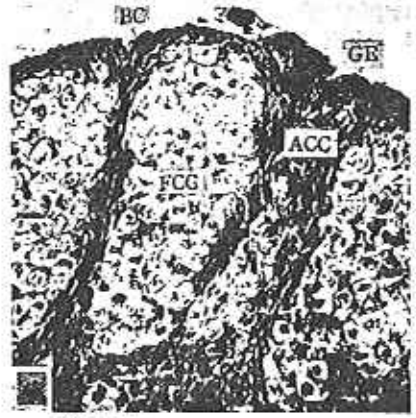

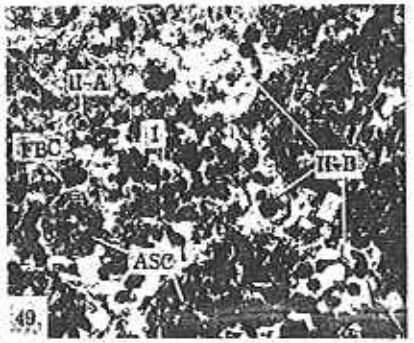

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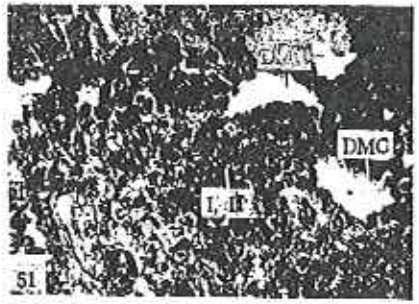

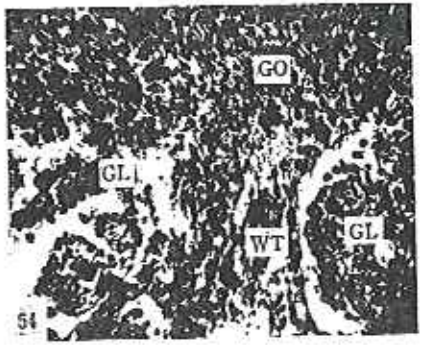

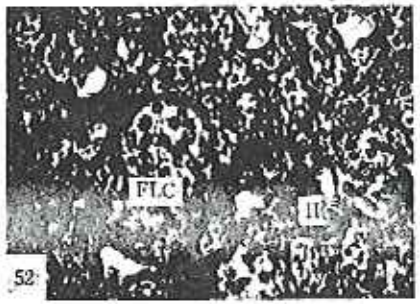

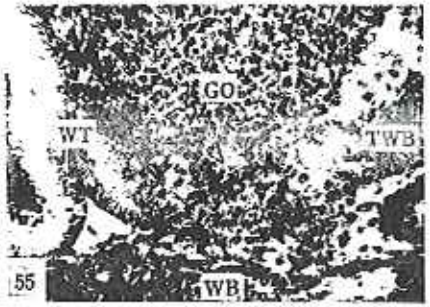

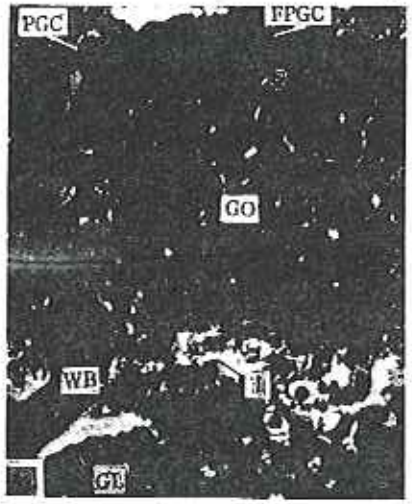

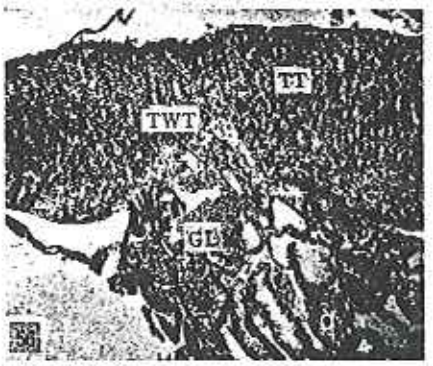

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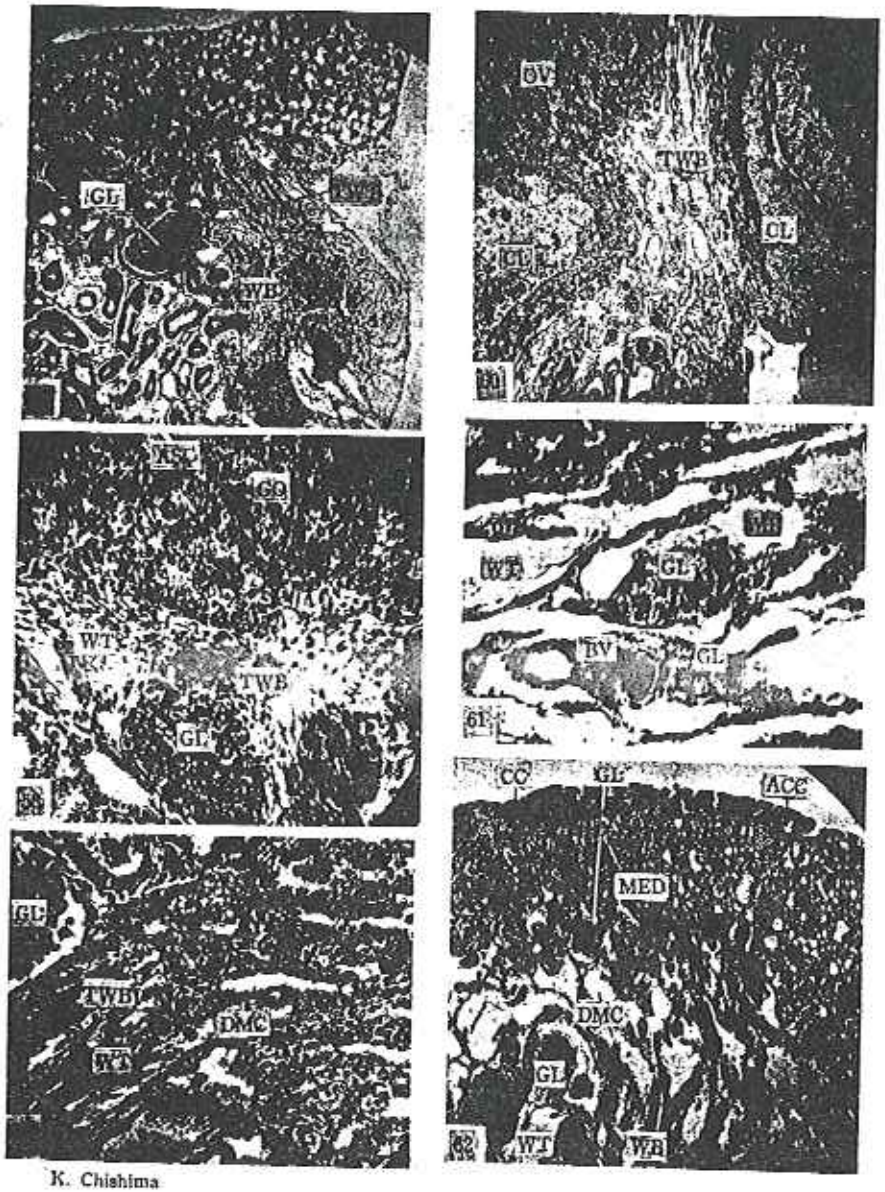

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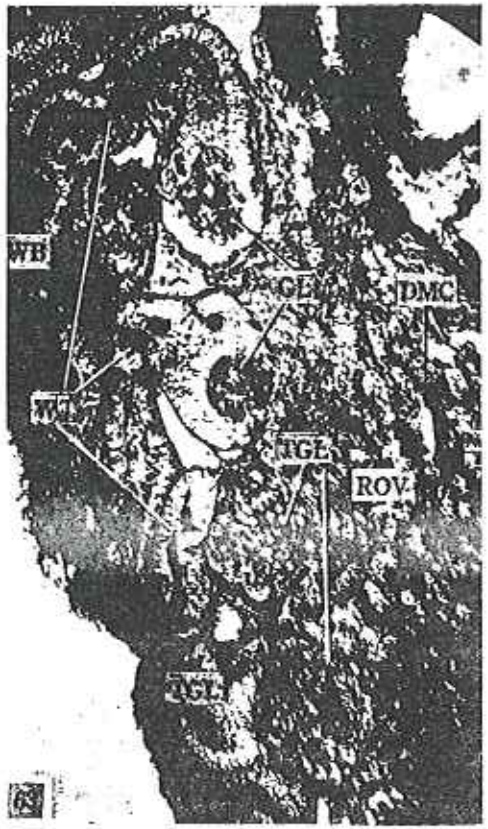

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PLATE VI.

(44) Tangential section of left ovary at 17 days of incubation, showing the formation of the primordium of cortical cord by the condensation of blood cells (type I-A, II-A cells). (4×6)

(45) A part of the cross section of left ovary at 21 days of incubation, showing the condensation of erythrocytes in the blood layer. (4×6)

(46) Cortical layer of left ovary at 21 days of incubation, showing the flow of blood cells on the surface of the ovary through the interstitial spaces between cortical cords. Then they give rise to new cortical cords. (4×6)

(47) Surface of left ovary at 21 days of incubation, showing the cells of blood layer, covering the surface of that ovary, are differentiating from the right side toward the left side. (4×6)

(48) Section of left ovary at 21 days of incubation. Showing blood cells, migrated into interstitial spaces between cortical cords. They are aggreagated and give rise to the cortical cord by self-differentiation. (4×6)

(49) Section of a part of testis at 14 days of incubation, showing second mode of formation of sex cord, that is to say, by condensation of blood cells (type I-A & II-A cells) which have migrated into the interstice of the testis. Mitotic figure can not be recognized in this field. (4×6)

(50) Section of the left ovary at 21 days of incubation, showing the so-called cluster of "fat-laden cells", and its transitions into distended medullary cord. (4×6)

PLATE VII.

(51) Section of left ovary at 21 days of incubation, showing differentiation from blood cells, migrated into the medullary space, into fixed elements of the medullary cord. (4×6)

(52) Medullary portion of left ovary at 21 days of incubation, showing the transitional phases from the clusters of blood cells into the clusters of "fat-laden cells". (4×6)

(53) Relationship between the gonad and Wolffian body at 6 days of incubation, showing the following; a) So-called primordial germ cell in the germinal epithelium arising as a result of cell-fusion, b) Transitions from the elements of mesonephros into that of the gonad, c) In the intermediate region between gonad and Wolffian body there are many extravascular erythrocytes scattered and mingled with mesenchymatous cells that show transition into the elements of the gonad. (4×1.8)

(54) The intermediate region between testis and Wolffian body at 8 days of incubation, showing transitions from the elements of Wolffian body into that of testis by breaking off from its original arrangement and by its rearrangement. (4×6).

(55) Testis and Wolffian body at 8 days of incubation, showing the Wolffian tubules, attached to the testis, about to be transformed into the elements of testis. (2×6)

(56) Testis and Wolffian body at 12 days of incubation, showing that, even in this age of embryo the sex cords also arise as a result of the differentiation of mesonephric elements. (2×2)

PLATE VIII.

(57) Testis and Wolffian body at 12 days of incubation, showing the transformation from glomeruli and Wolffian tubules into the elements of the testis. (2×2)

(58) Intermediate region between testis and Wolffian body at 18 days of incubation, showing transformation from mesonephros into gonadal elements, at first the elements of mesonephros breaking off from their original arrangement and later they form the sex cord by their rearrangement. (2×6)

(59) Intermediate portion between the left ovary and Wolffian body in chick embryo at 18 days of incubation, showing the Wolffian tubules of mesonephros (left side of the figure), maintaining its original form but about to be transformed into the distended medullary cord. (2×6)

(60) Part of the hilum of the left ovary in chick embryo at 21 days of incubation, showing the glomeruli and Wolffian tubules transforming into ovarian tissues at the region of hilum of ovary. (2×2)

(61) Highly magnified part of Fig. 60, showing the spaces, enveloped by the Wolffian tubules or by the wall of glomeruli, being transformed into the distended medullary cords. (4×6)

(62) Left ovary and left Wolffian body in chick embryo at 21 days of incubation, showing that there can be seen all transitional forms from blood layer (right side) to cortical layer (left side). And there are shown the rete cords, distended medullary cords and isolated medullary cords originating with mesonephric tissues. (2×2)

PLATE IX.

(63) Right ovary in chick embryo at 9 days of incubation, showing four glomeruli and Wolffian tubules are going to transform into ovarian elements. (4×3)

III THE ROLE OF ERYTHROCYTES AND PLATELETS IN THE BLOOD COAGULATION

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INTRODUCTION

The mechanism of blood clotting has been studied by many workers and the idea, that the blood platelets and fibrin are essential elements in blood coagulation, has been believed by most workers for nearly a century. Some recent investigators, who have been studying this mechanism from a chemical point of view, however, claim that the platelets are normally important participants in blood clotting, but are not always essential for that process. Moreover, there are many conflicting conceptions regarding the details of mechanism of blood coagulation.

These controversies in opinion, most probably, are due to the following reasons;—— (1) observations on the behaviour of platelets during blood coagulation are very few. Consequently the opinion regarding the relationship of the blood coagulation to platelets has remained, even by the present day, almost the same as it was in the days of Bizzozero[1], (2) the overlooking or negligence of the extremely labile properties of erythrocytes, (3) lack of investigations on the relationship between the origin of the platelets and the erythrocytes.

There is no worker, so far as I am aware, who has investigated the behaviour of erythrocytes in blood coagulation. The writer (2-11) previously noted that the erythrocytes are not highly differentiated cells, as has been believed, but are very young, undifferentiated cells with very wide differential potencies *in vivo* or *in vitro* and, further, the erythrocytes show many curious behaviours *in vitro*.

From point of view described above, the observations described in this paper were planned to re-examine the process of blood coagulation with special reference to the behaviour of formed elements in the blood, especially of erythrocytes.

MATERIALS AND METHODS

The animals used in this study were rabbits, horse, chicks, chick embryos, frogs, tadpoles, newts, snakes, occasionally human being, goat, mouse, and crucians etc.

Erythrocytes are so labile that some of them are destroyed immediately after the blood is shed, so their role in blood coagulation is apt to be overlooked. For that reason, I observed under a microscope the process of blood coagulation from the moment of bleeding out of capillaries, which were punctured with fine needle, until the blood is coagulated. These observations have been made on the tail of the tadpole and mesentery in anesthetized frogs and chicks.

For comparison, very thick or thin layered blood smears which were fixed in methanol and stained with Giemsa solution, were made. Some of the blood clot of horse and chick was fixed in 10 percent solution of formalin, imbedded in paraffin, and sections at 5-7 micra were cut and stained with Delafield hematoxylin and eosin. In some cases the clots were immersed in water for 24 hours, in order to cause erythrocytolysis, then the sections were made in the same manner as described above.

In certain cases the phase contrast microscope (Chiyoda) and electron microscope (Shimazu and Hidachi) were used.

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RESULTS AND DISCUSSION

The process of blood clotting may be divided for convenience, into the following three phases: (1) agglutination and destruction of erythrocytes, (2) formation of fibrin mass, (3) retraction of blood clots. These three phases, however, cannot be sharply separated from each other because they show continuity.

PHASE I AGGLUTINATION AND DESTRUCTION OF ERYTHROCYTES

(A) AGGLUTINATION OF ERYTHROCYTES The agglutination of erythrocytes begins when the blood becomes stagnant. This phenomenon is, most probably, due to the cytoplasmic extrusion of erythrocytes. In mammalian blood, erythrocytes agglutinate at random, but occasionally they are arranged in the so called "column formation" or "Rollen Bildung". In oviparous vertebrates, however, erythrocytes clump at random. This process occurs in wet blood preparations, for several minutes after the blood is shed. In this case if the blood is intercepted, with a cover slip and vaselin, from atmospheric air and allowed to stand quietly for several hours at room temperature, the erythrocytes gradually separate again. And some of them, which are suspended freely in plasma, then transform into spherocytes. The spherocytes preserved in stagnant condition are more fragile than fresh erythrocytes. (Fig. 2-6, 9, 12)

(B) DESTRUCTION AND FUSION OF ERYTHROCYTES Destruction of erythrocytes happens explosively when the blood is shed and the erythrocytes are exposed to the following factors, namely, (1) contact with atmospheric air, (2) contact with the surface of other substances, such as the slide glass etc, (3) stasis of blood, (4) mechanical trauma.

This process occurs most easily, at first, at peripheral zone of the shed blood. Actual sight of the destruction of erythrocytes is recognizable under a microscope by watching steadily the extravasated erythrocytes on the slide glass.

In this case the outline of erythrocytes suddenly becomes faint, when the erythrocyte is destroyed and pours out its cell content. The extruded cytoplasm of erythrocytes, if it comes into contact with atmospheric air, suddenly acquire neutrophilic (when cytoplasm is diluted with plasma) or basophilic staining properties. (when the cytoplasm held its thick condition.) (Fig. 1-8)

(a) **Destruction and fusion of erythrocytes in mammals.** In very thick blood smear preparations of mammals, there can often be seen certain areas, so-called "clump of agglutinated and disintegrated platelets" or "viscous metamorphosis of platelets". That area includes the following elements such as lymphocytoid elements, remnants of destroyed erythrocytes, few well preserved erythrocytes or light purplish granules, neutrophilic granulocytes or their degenerating forms, extruded or fragmented cytoplasm of erythrocytes and the transitional forms among these elements. (Fig. 1-3).

That area corresponds to the "field of leucocyte-formation" designated by the author[2-5], which is surrounded by agglutinated or fused masses of erythrocytes. It can not be denied that a small portion of these leucocytes and platelets included within the coagulated area may have origigated from those elements pre-existed in the circulating blood, but the majority of them may

be said to have originated from disintegrated erythrocytes *in loco*. This opinion may be supported by the following evidences: — (1) The writer is unable to confirm any evidence that platelets actively gather together by their own ameboid movement or by other forces. Into a certain center from other parts of the blood in a few minutes after the blood is shed. Moreover, it is logically impossible for platelets to gather rapidly to a certain center, because they are sticky in nature and have no ability such as a rapid and strong locomoting power to gather by pushing apart through the very narrow spaces among the closely arranged erythrocytes. On the contrary, it is recognizable that so-called "disintegrated platelets and other elements" included in the blood coagulation area are derived from the disintegration or cytoplasmic extrusion of erythrocytes.
(2) Blood clotting promoting factors correspond perfectly to the erythrocytes-disintegration-promoting factors described above. Tsai, Lee and Wu[35], Ham and Castle[18], Murphy and Shapiro[17], Cornish[36], Bauer[37], and Shu Chu Shen and Flemming[38], have stated that red cell fragility is increased by stasis of blood in vitro or in tissue capillaries, but Waller[39] and Mettler and Weaver[12] state that there are no increased fragility in blood removed from the cubital vein or varicose vein which are under a condition of stasis. However, it is significant that the red cell fragility is increased by stasis of blood in vitro or on a slide or even in the blood vessels by prolonged and sufficient stasis of blood.

Recently, Conley and Lalley[20] described that increasing the glass surface area in contact with plasma shortened the clotting time and crushed glass was more effective in shortening the clotting time of plasm.

(b) Discussion on the origin and behaviour of the blood platelets in mammals. It is important to make clear the origin and behaviour of mammalian platelets for the solution of the question regarding the mechnism of blood coagulation. Recently Tocantins[24] published a stimulating and widely quoted review on the blood platelets. According to him there are many scientists (Wlassow, '94; Bremer, '94; Arnold, '97; Muller, '98; Determan, '98; Maximow, '99; Schwalbe, '02) who maintained the view that the blood platelets came from disintegrated erythrocytes. However, antagonists of this view (Petrone, '97; Sacerdati, '00; Dominici, '00) claimed that view of Arnold and his adherents rested on artifacts from preparations of dried smear preparations. It may be sure that in most preparations, in a sense, platelet may be an artifact; however, many hematologists regard them as blood platelets even at the present day. Sometimes it is difficult to say whether a platelet on the preparation is pre-existed in circulating blood or it was produced explosively by extrusion of erythrocytic cytoplasm. Recently, Griffin and Muller[40] have reported as to the influence of exercise on the blood platelets count and they say "our experience in counting platelets has convinced us that the counts are unreliable unless they are made quickly after the sample is obtained and that the importance of meticulous technique can not be over emphasized". That is true, and it may be impossible to obtain, in strict sense, the true reliable count of platelets because it is the same impossible matter to expose erythrocytes to the destruction promoting factors mentioned above without avoiding the cytoplasmic extrusion or destruction of them. If the platelets are distinct and formed elements having certain proportional numbers to the erythrocytes, their count, even though they are very labile, would not be so variable. It is possible, really, that the numerical ratio of platelets and leucocytes to the erythrocytes may be changed by making a very thick layered blood smear preparation.

It is a widely accepted conception that the blood platelets are distinctly formed elements and they arise from the fragmentation of megakaryocytes in bone marrow. This conception may account for as an opinion that explains the partial source of platelets, but the most part of platelets which were observed in coagulated blood can not be referred to megakaryocytic origin.

Howell and Donahue[31] described that the lung is an important organ of platelets production. Jordan[27], however, denied this conception. It seems to me that the higher platelets count in the lung may be attributed to the fact that the erythrocytes in the lung capillaries contact most easily with atmospheric air so that the platelets-formation may be accelerated by means of cytoplasmic extrusion or disintegration of erythrocytes.

(c) Destruction and fusion of Erythrocytes in Oviparous Vertebrates. The factors underlying the destruction of erythrocytes in oviparous vertebrates are the same as in mammals, however, the manner of destruction is more easily recognized than in mammals. In the blood coagulation area of very thick blood smears, considerable numbers of erythrocytes disintegrate and give rise to a clumped mass which is undoubtedly homologous to the so-called "mass of disintegrated platelets" in mammals but the components of that mass differ from that of mammals. It is composed, mainly, of lymphocytoid elements or thromboblast, some of the remnant or granular fragments of destroyed erythrocytes and occasionally neutrophilic leucocytes, derived from extruded cytoplasm of erythrocytes. These elements are matted together with basophilic net-work, and surrounded by normal erythrocytes or disintegating erythrocytes with cytoplasmic matrix. These blood coagulating areas extend to surrounding zones.

Uncertainty exists concerning the concept that these thromboblasts aggregate rapidly into a certain area from other parts, on the contrary, there are many evidences that they are derived from budding, fission or denuclei of erythrocytes *in loco*.

It is of interest to note that in oviparous vertebrates the blood coagulation takes place, even in this phase without formation of fibrin. In general, erythrocytes of chick, frog, snake and newt are more fragile than those of mammals, so that this fact may be in correlation with the more rapid blood clotting in the former than in the latter. (Fig. 3-8)

(d) Origin and Behaviour of the lymphocytoid elements and Spindle cell in Oviparous Vertebrates. Tocantins[24] states that "the observations of Deetjen were supported by Deckhuysen and Koffsch. These concepts influenced Deckhuysen perhaps to regard the mammalian platelets as homologous to the nucleated spindle cells of invertebrates and oviparous animals, which behave, when blood is shed, very much like the mammalian platelets". Deetjen and Schidorfbauer described that the spindle cells of oviparous animals underwent changes similar to those of mammals during coagulation. (quoted by Tocantins).

If spindle cells, in oviparous vertbrates which have no megakaryocytes in bone marrow, are homologous with those of mammals, its origin can not be explained from Wright's point of view. Jordan[20] described that "the thrombocytes (in Urodele) producted a variable number of thromboplastids, by process of pseudopod separation or cytoplasmic fragmentation" The thromboplastids designated by Jordan perhaps correspond to my "lymphocytoid elements or thromboblasts derived from cytoplasmic extrusion of erythrocytes or from disintegrated erythrocytes in blood clotting or from cytoplasmic budding of erythrocytes in blood cell culture, as I[5,10] have already reported.

Werner[30], Pluja[31] and Ralph[32] observed the formation of erthrocytes by localized budding

of the cytoplasm of megaloblasts or of the megalocytes. Bostrœm[33] also found that the non-nucleated erythrocytes are formed by budding of cytoplasm from normoblasts. Such facts mentioned aobve, may be referred to a very strange matter for general investigators who have believed that the division of one cell into two daughter cells is the chief or only mode of proliefration of blood cells. However, it is a significant fact that the production of new cells by budding of erythrocytes is not a rare phenomenon. (Chishima[34])

PHASE II FORMATION OF FIBRIN IN BLOOD CLOTTING

It is generally believed that the blood clotting is due to the formation of a jelly by the deposition of protein material called fibrin which is contained in the plasma as fibrinogen, as described by MacDowall[35]. However, this process is not absolutely necessary for the first stage of blood coagulation in oviparous animals as mentioned above. Fibrin formation, however, at the second stage of blood coagulation is common in both mammals and oviparous vertbrates. But fibrin plays a less important role in the latter than in the former.

In fresh blood clots of mammals, occasionally, there can be seen very fine fibrin strands radiating from the mass of disintegrated erythrocytes (so-called clump of disintegrated blood platelets) and the minute granules (micron) suspended in plasma adhere to these fibrin strands, but this process is relatively rare and needs a relatively long time (about fifteen minutes or more). Thus I could not observe actually the very rapid process where the needle-like fibrin makes its appearance by a process resembling crystalization of minute particles suspended in plasma.

In rabbit, chick and snake, the large fibrin bands, several hundreds micra or more in dismeter, are demonstrable in a fresh blood clot which has received mechanical trauma, viz. by stirring with glass rod or mechanical compression and retraction. These fibrin bands also, clear and somewhat refractile in the fresh condition resembling the fine fibrin strand, but they often show very light blue or purplish staining properties after methanol fixation and staining with Giemsa solution. On the surface of these large fibrin bands almost always are seen the erythrocytes joined end to end, forming a strands and running parallel to the fibrin bands. The erythrocytic strand shows transitional phase into fibrin band, and well preserved or disintegrating erythrocytes or lymphocytoid elements occasionally lie within or on the periphery of these bands. These elements and disintegrated erythrocytes show transitions into the fibrinous or gelatinous mass in the clot. Consequently, the fibrin band or mass may most probably have originated from the fibrous or gelatinous metamorphosis of disintegrated erythrocytes.

It has been generally believed that there is no relationship between the fibrin formation and the metamorphosis of erythrocytes. This may, perhaps, be due to overlooking the following facts that the erythrocytes are so fragile that they set free their protoplasm by their disintegration and the protoplasm changes its staining property and colloidal state, and thus they rapidly become an elastic mass.

It is noteworthy that such a large fibrin band or dense fibrous mass does not occur in sections of the horse's blood clot when the blood is allowed to stand for 48 hours quietly in a glass tube without receiving any mechanical trauma. In these sections, the surface of the peripheral portion of the clot consist of a fine mesh of fibrin. The interstitial spaces of this fibrin mesh are filled with clear spherioid elements, which show transitional phases (in staining properties, size and shape,) from erythrocytes into the fibrin. From these facts the main part of fibrin network may be said to be derivatives of a fibrous metamorphosis or denaturation of extruded cytoplasm of erythrocytes or destroyed erythrocytes themselves by fixative agent. In the peripheral layer of the blood clot there are very few well preserved erythrocytes, however, as we examine the more inner part of the clot they become more numerous and the fibrin less. And at the inner most part of the clot there exist considerably large masses of agglutinated erythrocytes. (Fig. 13, 14)

These masses include small amount of the derivatives of disintegrated erythrocytes; however, these derivatives still retain their acidophilic property, owing to the interception of erythrocytes from exposure to atmospheric air or oxygen according to their situation within the clot.

Sections of chick's blood clot were made in the same manner as that for the horse mentioned above. In these section, the outer-most part of the clot was covered with a thin fibrous layer, staining a light brownish gray. This layer is often connected to thread-like elongated cytoplasm of erythrocytes, and occasionally contains the disintegrated erythrocytes, extruded nuclei from erythrocytes and polychromatic epherocytes.

The peripheral part near the fibrous layer contains following elements namely, polychromatic erythrocytes with eccentrically located pycnotic nuclei which have its perinuclear clear zones, extruded nuclei of erythrocytes resembling the small lymphocytes, fragments of erythrocytes, small amount of fine fibrin and clumps of polychromatic erythrocytes.

On approaching the more inner zone of the clot, normal or somewhat smaller erythrocytes grow more numerous and here and there are clumped masses of lymphocytoid elements contained within the polychromatic matrix. These clumps undoubtedly may be homologous with the masses of viscous metamorphosed erythrocytes in mammals. (Fig. 15, 16)

It is of interest that in the blood coagulation of chick, fibrin plays no important role even in the second stage in blood clotting, on the contrary, gelatinous metamorphosis of disintegrated erythrocytes plays the chief role in blood coagulation. Sections were made of horse blood clot which had been allowed to stand for 48 hoursand and later washed with water for 24 hours. In such a section almost all of the erythrocytes were hemolysed and only a fine fibrin net was remained.

The structure and components of this fibrin network resemble that of the peripheral zone of the horse blood clot mentioned above excepting that there are no well preserved erythrocytes. (Fig. 17, 18) The chick's blood clot treated with water in the same manner as that of horse's blood still preserves its fresh red colour.

The section of the clot, even though the outer-most part of it was covered with thin fibrous layer as in the case of the horse, showed that the outer zone of it is composed mainly, of lymphoid cells showing transitional phases from polychromatic erythrocytes. At the most inner part, most of erythrocytes have lost its eosinophilic property of cytoplasm, and transform into a clear cytoplasm connected or matted with brownish red staining matrix derived from extruded cytoplasm of erythrocytes. And here and there can be seen a brownish-red staining mass of disintegrated erythrocytes. On the contrary, there can not be observed the existence of a typical fibrin network. Some investigators may suppose that these clumps of lymphocytoid elements are derived from a rapid gathering of preexisting elements, however, there is no evidence indicating such a fact.

From the above facts the author can not escape from the view that the leading factor in

blood coagulation in the chick, even at the completely coagulated condition, is not the fibrin formation, but is the fusion and viscous metamorphosis or denaturation of extruded cytoplasm of erythrocytes or of disintegrated erythrocytes themselves.

In general, blood clotting propagate from the outer layer or certain clotting centers towards the adjoining inner layer or surrounding area, as if an autocatalytic reaction makes its advance.

Based on studies on the blood clotting mechanism from a chemical point of view, MORSON[15] says that "On the experimental side it appears that the chain reaction will proceed in the absence of whole platelets. This does not prove that material from platelets are not concerned when they are present" His description may be true and actually it may be impossible to obtain so-called platelets-substance free plasma, by ordinary means, because the extremely labile erythrocytes set free explosively their cytoplasmic contents, consequently it must be inferred that the blood plasma, circulating within living animals, changes rapidly its chemical composition after the blood is shed.

The architexture of the fibrin band or network within blood clots has hitherto been described only a very few in literature. But the results of my observations show that the alignment of fine fibrin in the horse blood clots, which have been allowed to stand, for long hours in a quiescent condition, are arranged irregularly, but when the clotting blood receives some external mechnical force the large fibrin bands run parallel to the direction of the force applied. This tendency resembles, somewhat, the observations of ZYCHA[17] in puncture wound of human skin.

It seems to me that the some portion or a large portion of the fine fibrin mesh in the sections of blood clots may be a residual product (artifact) of denaturated protein, contained in the exfruded cytoplasm and in the plasma, by fixative agent, because in fresh blood clot even of mammals, there can be seen only relatively few of fine fibrin.

PHASE III RETRACTION OF BLOOD CLOT

As is generally recognized the coagulated blood clot retracts and decreases its volume by squeezing out the serum.

QUICK et al.[16] have described that the greater the number of platelets the sooner the clot retraction begins and the smaller the clot and clot retraction is characterized by a relatively long latent period followed by an accelerated, phase and protracted completion. This observation may be true, but plasma coagulation, sometimes, may occur without fibrin formation even after sufficient time. This fact suggests that the plasma coagulation may be considered, in a sense, as a process homologous with the gelation of gelatin sol.

Biological meaning of blood clotting. There are many evidences that the fragmentation, fusion or disintegration of erythrocytes in blood clotting *in vivo* does not mean their ultimate degeneration, but, it is a first stage in the metamorphosis and differentiation of erythrocytes from which arise the new fixed tissues such as thrombi or connective tissue in wounds.

In other word, blood clotting plays not only an important role in blood stasis but also contribute to the healing of wounds by the formation of new tissues. The author will report further in details regarding this problem.

(1) Studies on the mechanism of blood clotting with special referrence to the behaviour of erythrocytes in two mammalians and six species of oviparous vertebrates were carried out by means of comparative researches on actual mechanism of blood clotting in living animals, *in vitro*, blood smear preparations and on the sections of blood clots. (2) This observation showed that agglutination, viscous metamorphosis and disintegration of erythrocytes are very important factors in blood coagulation. (3) The close parallel between the blood coagulation promoting factors and the accelerating factors of erythrocyte-destruction supports the opinion that the process of blood coagulation, has direct relation with disintegration of erythrocytes. (4) Most of the components of so-called "clumps of agglutinated and disintegraced platelets" in mammalian blood clot and the clumped mass composed, chiefly, of lymphocytoid elements in coagulated blood of oviparous vertebrates are mainly a product of disintegrated erythrocytes *in loco*. (5) Cell content extruded from disintegrated erythrocytes may change rapidly its characteristics especially their staining properties and colloidal state and become a jelly-like mass. (6) There are many evidences that a greater part of fibrin or fibrin mass may be derived from the viscous metamorphosis of erythrocyes or disintegrated erythrocytes themselves. (7) Fibrin strand plays a considerably important role in blood clotting in mammals but it may not be an essential element in oviparous vertebrates. Furthermore there are evidences that the fine fibrin mesh within the section of blood clot may be a residual product of denaturated protein produced by fixative agents.

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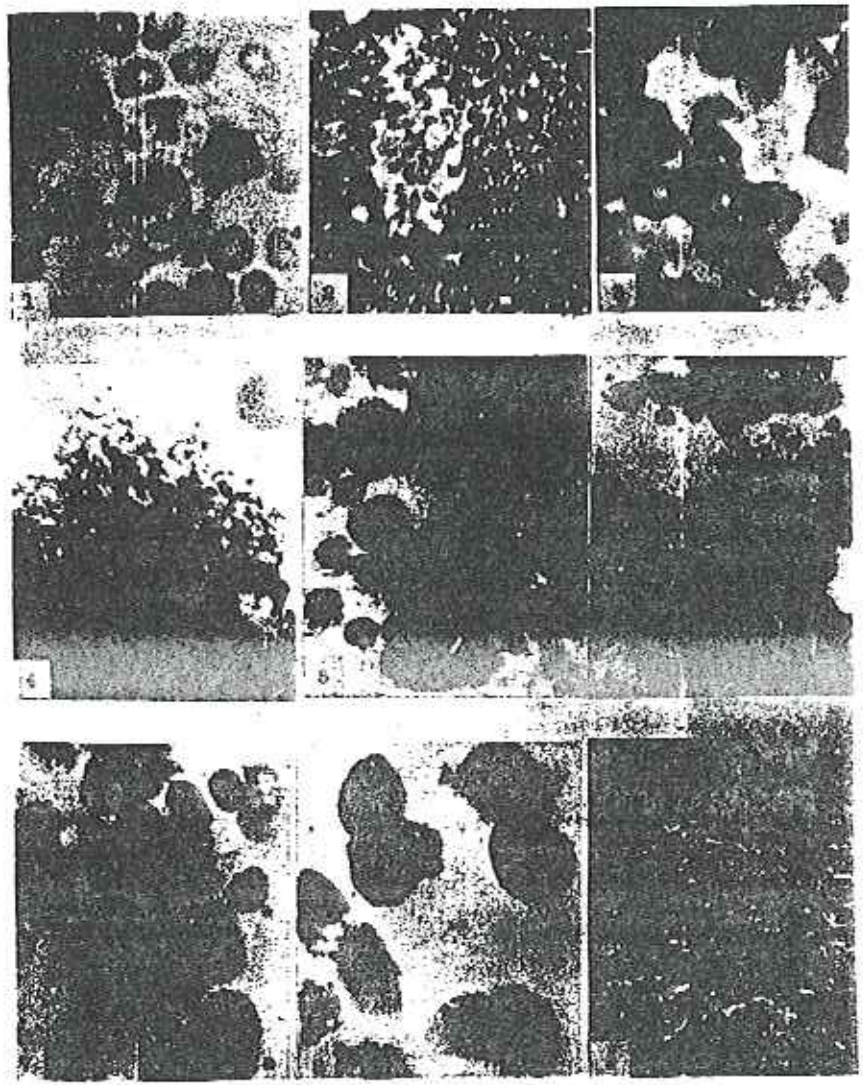
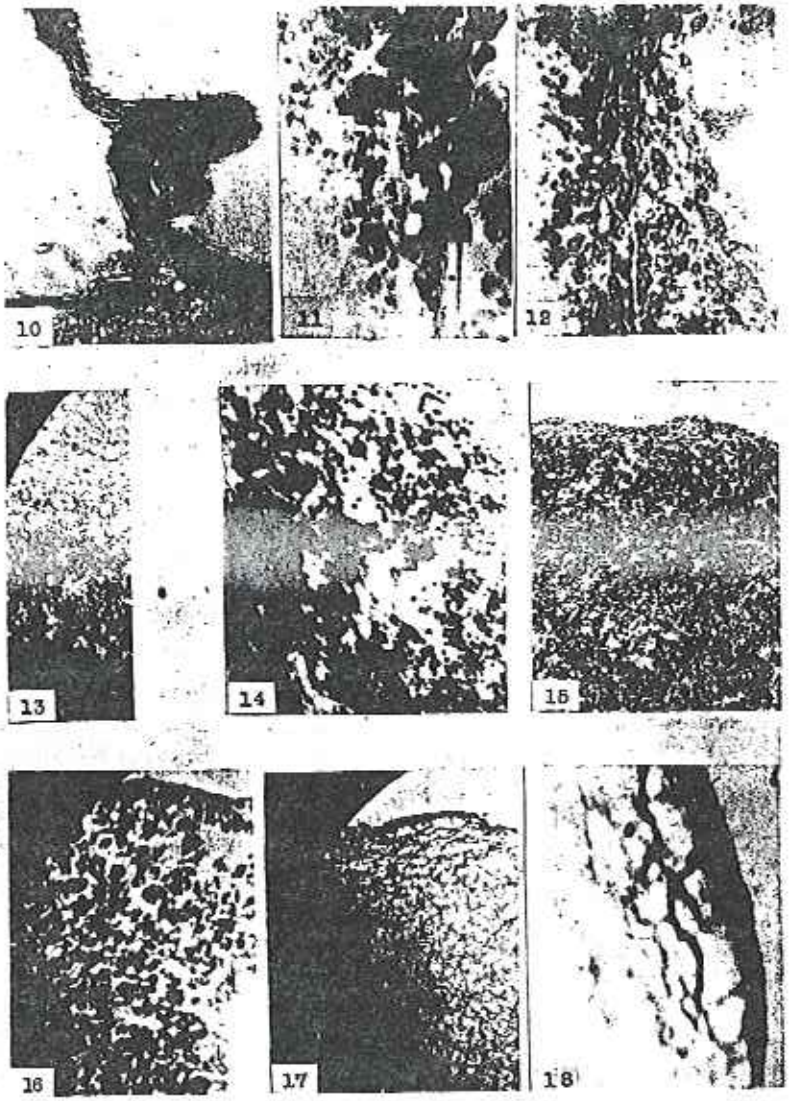
EXPLANATION OF FIGURES

(1) Blood smear preparation of rabbit showing a blood clotting center composed of so-called "agglutinated mass of platelets and leucocytes" which were produced by disintegration of erythrocytes (2) Blood clotting areas (light portion) on the blood smear preparation in the same material and treatment as illustrated in Fig. 1. (×600) (3) Higher magnification of a part of Fig. 2. These areas also composed of the same elements illustrated in Fig. 1. (×2000) (4) Electron microscopic photograph of the surface of an erythrocytes of domestic hen showing the granular particles derived from its disintegration. (×8000) (5–7) Blood coagulation areas on the slide glass. Showing the areas composed of small lymphocytoid elements (thromboblasts with narrow clear cytoplasm) and disintegrating erythrocytes with cytoplasmic network. (×700, ×500, ×1000) (8) Four disintegrating newt's erythrocytes with dark irregular outline are connected with lighter, discoidal cytoplasmic extrusion. (×1000) (9) Phase contrast microscopic photograph of the blood clotting area of duck's blood in fresh condition. Showing the mass of agglutinated and disintegrated erythrocytes (from down to middle portion) and normal erythrocytes (upper left side). (×600) (10) A large fibrin band formed by metamorphosis of erythrocytes in the snake's blood which had exposed to atomospheric air after three days culture (slide-cover method). (×500) (11–12) Showing the viscous metamorphosis of erythrocyses in snake's blood treated as in Fig. 10. Notice the linear arrangement, fusion and disintegration of erythrocytes which show transitional phases into fibrous band or mass. (×860, ×490) (13) Section from the horse blood clot which was allowed to stand quietly for 48 hours in a glass tube. The upper part is the surface and the lower part the inner part of the clot. ×150. (14) Higher magnification of a part of Fig. 13, (×500.) (15) Section from the chick blood clot which was treated in the same manner as Fig. 13, (×150.) (16) Higher magnification of a part of Fig. 15. Notice there is no fibrin. (×500) (17) Section from the horse's blood clot which had been allowed to stand for 48 hours and then immersed in water for 24 hours. (×500) (18) Higher magnification of a part of Fig. 17. ×500. Fig. 1, 2, 3, 5, 6, 7, 8, 10, 11 and 12 were stained with Giemsa's solution and Fig. 13–18 were stained with H. E.





Ⅳ STUDIES ON THE ORIGIN OF THE CANCER CELL *

By

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Introduction

The cancer problem has been studied by many investigators for many years from its clinical, experimental and biological point of view. However, the origin of cancer cell, that is the most fundamental problem of cancer research, has not yet been solved. Why so?

According to my opinion, it may, most probably, due to uncritical acceptance of scientists to the following three orthodox theories; the first, cell proliferates by means of cell division, especially by mitosis, the second, the erythrocyte is the most highly differentiated cell, and it can not be differentiated into any other kind of cell. Therefore, erythrocyte degenerates after finishing its function as a carrier of O_2 and CO_2, the third, as the blood capillary is lined with a thin layer of endothelial cells, the erythrocyte can not pass away through the capillary wall. However, it seems to me that the cancer problem may not be solved, so long as scientists are accepting uncritically to the above mentioned three orthodox theories. Because, as I have already pointed out (Chishima, '48–'61) the three orthodox principles described above must be re-examined from both practical and methodological point of view.

I have carried out histological studies on the human uterine cancer, and it was revealed that the origin of cancer cell is related closely to the blood vessel and blood cell. In this paper I will describe the results of my studies on the differentiation of erythrocyte in relation with the origin of cancer cell. For convenience sake, in the present paper, I will describe putting together with the results of histological studies on the cancer tissue and the discussion about the origin and the mechanism of multiplication in human cancer cell.

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Material and Method

The observations are based upon the study of histological sections of five human uterine carcinoma obtained from the Japan Red Cross Hospital (The Shinzhyuku Nisseki Sanin) by courtesy of Dr. T. Suzuki, the director of that hospital.

For histological studies 5-7 μ paraffin sections were prepared from tissues fixed in Bouin's fluid. These were stained by haematoxylin and eosin. In this paper, the material and method excepting those of the human carcinoma are omitted since those were already published on my papers and monographs (Chishima, '48–'61).

Observations and Discussion

(1) Orthodox view regarding the characteristics of cancer cell

The following views regard with the cancer cell are accepted by almost all of investigators (Anderson, '57; Needham, '50; Rudnick, '58; Sodeman, '56 and others) as a truth.

(i) Cancer cell increases in number, vigorously and homogeneously, by means of unrestricted cell division which is asymmetrical or atypical. So that, the cancer cell rarely shows typical mitotic figure.

(ii) Cancer cell is derived from a normal tissue cell, especially from epithelial elements through the influence of various cartinogenic factors such as different kinds of chemicals, physical or biological factors.

(iii) The cancer cells proliferate so vigorously that they crowd together and then invade into surrounding tissue, then they give rise to the so-called cancer nest (or cancerous nest).

(iv) The external appearance of cancer cell is approximately normal, in other words, it has no definite cell type. Varying in size and shape, it has no specific staining capacity. Yet it is defective, because it gives off certain chemicals that perturbs another cells of body.

(v) Cancer cell often spreads (metastasis) through lymph and blood circulation.

(2) Re-examination of the orthodox view regarding the mitotic cell division theory of cancer cell

(i) Is the mitosis a chief mechanism of increasing in number of somatic cells?

Virchow's doctorine, "Omnis cellula e cellula" has been generally believed as a golden rule in the biological and medical science. Accordingly, cancer investigators also agreed with the thesis, "the tremendous increasing in number of cancer cell is due, mainly, to their unrestricted mitotic cell division".

But, if investigator examines, thoroughly and conscientiously, the normal or pathological sections. I am sure, that there should have arisen a question about the orthodox view, the theory of mitotic cell division.

Because, in general, there can be found so few mitotic figures in various sections of a normal or pathological tissue, that we can hardly expect the quantitative coincidence between the actual increasing of cell number and the value of mitotic index.

As a matter of fact, O.B. Lepeshinskaya ('37–'55), a famous scientist in Soviet, and her daughter O.P. Lepeshinskaya have already published her revolutional finding that the erythroblast or mesenchymal elements can arise from living substances such as yolk sphere, egg albumen etc.

I have also found the same fact, independently of Lepeshinskaya's work. Furthermore, I have found, that the erythrocytes in several vertebrates can be differentiated into almost all of other kinds of somatic and germ cells (Chishima '48–'61). Moreover, I have published that the cellular elements in the lesion of wound or in the blastema of regenerating area are all derived from the differentiation of blood cells especially erythrocytes, and that, the erythropoietic center is not the bone marrow under the well nourished condition. However, under the starved conditions of animals (mammals. aves and amphibian) the cellular elements of bone marrow, several organs and tissues, especially fat tissue, reversely differentiate into erythroblastic leucocytes. (Chishima '54, '57, '58; Morishita '57 a, b, c.)

And, under the well nourished condition, erythroblast is newly formed from living substance, the digested food substances. So, I have designated this phenomenon as "Reversible differentiation between blood cells and fixed cells under the influence of different nutritional conditions". I have also presented an opinion that the blood cell and some other kinds of cells increase in number through budding (Chishima, '50, '58), sporulation (Chishima, '53, '54.

'58) or 2nd coacervation (Chishima, '53, '58) but these types of cell multiplication are not by mitosis.

(3) *A new opinion regarding the origin of cancer cell (Cancer cell is derived from erythrocyte)*

As has been mentioned above, we can hardly find the typical mitotic figure in the cancer tissue, though there are some of "the so-called atypical mitotic figures of cancer cells". And even, the so-called atipical mitotic figure shows no firm evidence that the figure gives rise to an actual cell division. Therefore, there is merely an unreliable evidence on the orthodox cell theory based on the mitosis.

On the contrary, in the cancer tissue, there can easily be seen various stages of the transitional phases from erythrocyte into cancer cell. (Figs. A, 1~11).

This transition, of course, is continuous, however, for convenience' sake, it may be divided as the following five stages;

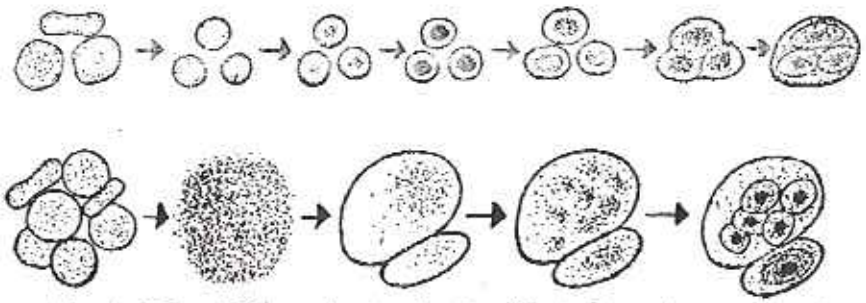

Fig. A. Schematic figure showing the transitional phases from erythrocytes into cancer cell; the upper row (isolated erythrocytes. type A), normal shaped erythrocytes→spherical erythrocytes→appearance of primordial nucleus→small lymphocytoid stage→mesenchymal elements→fusion of mesenchymal elements→polynucteated caucer cell. The lower row (type B) erythrocytes→blood monera→pre-cellular vacuoles→appearance of primordial nuclei→a polynuclear cancer cell and a cancer cell.

The 1st stage

common erythrocyte (eosinophilic) (Figs. A, 1).

The 2nd stage

The differentiation of erythrocyte due to (i) stagnation or stoppage of blood stream, and (ii) induction of cell-milieu (Figs. A, 1, 7).

Type (a). (Single or isolated erythrocyte)

gradually rounding up into a spherical one.

Type (b). (Aggregated erythrocytes)

Fused together and oecome a fused mass. (I have designated it as blood monera contained few number of leucocytes which are included, previously, in the circulating blood).

The 3rd stage

Type (a). The eosinophilic staining capacity gradually declines, and a faintly staining primordial nucleus takes its appearance in the cytoplasm. (Figs. A).

Type (b). The same as the type (a) (single or isolated erythrocyte), yet, the eosinophilic staining capacity, (that is correlated with haemoglobin content of protoplasm) is more intense than that of the type (a), and there appear several vacuoles (Pre-cellular vacuoles). (Figs. A, 2, 3, 9).

The 4th stage

Type (a). Becomes a small lymphocytoid element with thin layer of neutrophilic and then basophilic cytoplasm and a deep staining nucleus. This lymphocytoid element is, essentially, the same as the small lymphocyte or small round cell observed at imflammatory lesion. Cell at this stage can be referred to be a primordial or young stage of cancer cell. (Fig. A).

Type (b). Aggregated mass, the blood monera also show transitional phase the same as that of the (a), but the speed of differentiation is slower than the (a). So, the aggregated lymphoid elements take its appearance in a pre-cellular vacuole, so that they are surrounded by light eosinophillc or neutrophilic cytoptasm. Structure of whole mass is reticular, and shows here and there, clear and round spaces owing, probably, to the dissolved away of lipoid substances. (Figs. 2, 3, 9).

The 5th stage

Type (a). The cell, at this stage, corresponds to the socalled reticular cell or small cancer cell with somewhat lighter staining nucleus than that of the 4th stage. Cellular elements of this stage often show the sign of fusion with others and grow to be the middle or large type of cancer cell.

Type (b). At this stage the middle or large sized cancer cell with a bright and vesicular nucleus, and with one or more of eosinophilic (or basophilic) nucleolus appears. Of course, there can be recognized all transitional phases between this type of cancer cell and that of the 4th stage. (Figs. A, 3, 10).

In the above mentioned transitional stages from erythrocyte into cancer cell, the new cell formation (at the stage 3-5th), especially, the spontaneous arising of new nucleus or many of nuclei in an erythrocyte or in a mass of aggregated erythrocytes may referred to be an impossible matter to the many scientists who are confident of the orthodox cell theory. Even if the mechanism of DNA synthesis in the erythrocyte-protoplasm can not be explained by present-day biochemistry, spontaneous arising of nucleus is undoubted fact. On the detail of new cell formation I have already published (Chishima, '52, '53, a, b, '56, '58, '61).

There are many investigators who have observed the mitotic division of cancer cell through its tissue culture, but it should not be forgotten that the observations are not only under the artificial conditions (atomospheric pressure, strong light, temperature and artifical medium etc.) but also the mitotic figure of living cancer cell does not necessarily give rise to actual cell division. (Figs. 3, 10).

It seems to me that the so-called atypical mitotic figure of cancer cell may be misunderstanding of the new nuclei formation in an aggregated erythrocytic mass at the stage 3rd to 5th.

At the most early stage of cancer formation the transformation from normal epithelial elements into cancer cells may be possible by the action of carcinogenic factors, however, the normal epithelial cell also, originally, is a derivative of erythrocyte.

And at the growing stage of carcinoma, the most part of the increasing cellular elements in cancer tissue are derived from erythrocytes as has been described above.

These opinions may be substantiated, further, by the facts of which I will describe in the following chapter.

(4) *Intimate relationship between the pattern of a cancer nest and the shape of plexus of venous sinuses*

According to the widely accepted opinion, the cancer cell grows and proliferates so vigorously that the excessive cells penetrate into nearby tissue, and they give rise to a mass of cell, the so-called "cancer nest, or cancerous nest" by which the cancerous tumor is characterized histologically.

In general, cancer nest is not a sphere shaped cell-mass, but is an elongated cord, surrounded by interstitial tissue, and it is ramified as if a plant root or branch, therefore, it would rather

be suitable to designate it as "cancer cord" than "cancer nest" (Fig. 6). And the plexus or network of the cancer cords shows close resemblance to that of venous sinuses in spleen and in some other organs or of arterio-venous anastomosis.

Both the cord-like structure of the venous sinuses and the cancer nest (cord) are irregular in size (diameter) and in shape at various part. Moreover, they resemble in structure, that is, the wall of cords has not been necessarily provided with epithelial elements (Fig. 11), and within the cancer cord the erythrocytes are often scattered here and there, showing the transitions into the young cancer cells (Figs. 8~11). From these structural resemblance in these two kinds of cords and from the existence of the transitions from venous sinuses into cancer cord, we can not escape from the conclusion that the cancer nest (cord) is derived, most probably, from the venous sinuses (or some of arterio-venous anastomoses or from other circulation system) according to the stoppage of blood stream, and the differentiation of blood corpuscles incorporated in the vessel, and of the perivascular elements (leucocyte, or mesenchymal elements). And these elements are also the derivatives from the erythrocytes) (Figs. 4~6).

(5) *Significance of extravasated erythrocyte in the cancerous tissue (Open-type theory of capillary)*

It has long been believed that the capillary is lined with a thin layer of endothelial cells. Even the famous investigator of capillary A. Krogh ('28 Anatomy and physiology of capillaries) also accepted this orthodox opinion.

Extravascular lymphoid areas have been observed in parenchymal organs or nervous tissues of normal chickens, by Oakberg ('50), Oakberg and Lucas ('49) and others, but they had overlooked that those lymphocytes are the derivatives from erythrocytes.

But according to the results of my observations on the capillary in living animal (Amphibian larvae) and in sectioned materials from several kinds of vertebrates it was revealed that the capillary is not necessarily a closed type, while it often shows an open type in the normal and pathological tissues, especially in an inflammatory lesion.

Carcinoma can be referred to be a kind of chronic inflammation, so there can be seen an extraordinarily abundant blood supply in the tumor. Furthermore, it is a common matter that there can

be found the vast number of extravasated erythrocytes which mingle and directly contact with young cancer cells (Figs. 7, 9). In my view, the vascular endothelial cell is derived, at the early stage of capillary formation, from erythrocyte which is adhered and is pressed, physically, to the surface of intercellular tissue space. And some of the membraneous part of capillary wall may be originated from a denaturated serum globulin. Therefore, the newly formed capillary, venous sinuses or arterio-venous anastomosis are nothing but a kind of a pond or puddle of stagnated blood, though aged one has its endothelial lining.

In the cancer tissue there can be found many of the blood pond, the venos sinuses without endothelial lining (Fig. 11). By the open type theory of capillary, the metastasis of cancer cell can be explained without difficulty. Existence of extravasated erythrocytes in the cancer tissue means neither a result of technical error nor a result of passing out through the capillary wall, but it is an ordinary observable true and natural fact.

(6) *Is the cancer cell derived from epithelial element?*

It is a common sense accepted widely by many pathologists that the cancer cell is derived from epithelial element while that of sarcoma is originated from non-epithelial element. However, it is also a well-known fact that there is carcinosarcoma. This fact indicates that there is, in a strict sense, no decisive distinction between the carcima and the sarcoma.

In my view, it is rather a matter of course that the both epithelial cell and non-epithelia cell are derivatives from erythrocytes under the different cellular circumstances.

The orthodox theory, the epithelial origin of cancer cell, is presumably, due to the following facts that the carcinoma makes its appearance through the transformation from vascular system including both of its contents (blood cells) and perivascular elements (lymphocyte and other kind of leukocyte), furthermore, these elements can be transformed into the epitheloid shape by physical pressure of each other or from other elements, especially, the elements of blood vessel wall (intima, media and adventitial elements) are, originally, epitheloid in appearance.

However, there is no firm evidence that all of cancer cells are descendant of epithelial elements, through the mitosis. On the contrary, there can be shown clear evidence of transitions from

erythrocyte into cancer cell.

(7) *Summary*

The origin of cancer cell in human uterine carcinoma was investigated, mainly, on the ordinary sectioned materials stained with haematoxylin and eosin. The results obtained are described as follows;

(i) The new cell theory, presented by O. B. Lepeshinskaya and O. P. Lepeshinskaya is true, so that the orthodox cell theory must be re-examined fundamentally.

(ii) As the typical mitotic figure of cancer cell is so rare that the main factor of the vigorous increasing in number of cancer cell may not depend on the result of mitotic cell division of cancer cell.

And the so-called atypical mitotic figures observed in cancer cells also show no firm evidence that the figure is a factor by which the proliferation of cancer cell necessarily bring about. On the contrary, in the cancer tissue, there can easily be seen every transitional stage from erythrocyte into cancer cell.

(iii) The writer classified the transitional phase into five stages, for convenience' sake. And each stage is further divided into two groups, the differentiation of (a) single or isolated erythrocyte, and the (b) aggregated or fused mass of erythrocyte. It is a noteworthy fact that a nucleus or nuclei take its appearance in a erythrocyte or in a fused mass of erythrocytes, and it then show transitions into cancer cell through a stage of the small lymphocytoid element and the primordial cancer cell.

(iv) The so-called cancer nest is not a single, isolated, sphere shaped cell-mass, but it is an elongated cord-like one in structure resembling closely with the pattern or plexus of the venous sinuses or arterio-venous anastomosis, moreover, there can be recognized the transitional phases between them.

(v) The capillary system in the cancer tissue does not necessarily a closed type, rather it is an open type system. So that many of extravasated erythrocytes can be found in the cancer tissue. Furthermore, they show transitions into cancer cell through intermediate phases described above.

(vi) The most widely accepted opinion that the cancer cell is an epithelial origin has not been confirmed in the present observation. And there can hardly be seen any evidence of continuation

from epithelial element into cancer cell through mitotic cell division. From all the evidences described above, I can not escape the conclusion that the most of cancer cells are derived from a result of differentiation of erythrocytes.

The present author wishes to express his gratitude to Dr. T. Suzuki for kindly furnishing the material.

Additional Note

In order to solve, not only the cancer problem but also other difficult matter in the biological or medical science, I think, it is an important matter that the methodology of science, the principle of thinking (logic), must be changed from the formal logic accepted traditionally into a new dialectic synthesized both of mind and material.

As I have already discussed on this problem in another paper (Chishima.·'61.) it was omitted in the present paper.

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Explanation of Figures

Figures 1 to 12, Photomicrographs from human uterine carcinoma. Paraffin sections were stained with haematoxylin and eosin.

Fig. 1. Section of carcinoma showing the transitional phase (C) from erythrocytes (A) in a venous sinuses into a fused and degenerated mass of erythrocytes, "the blood monera" (B). (×850).

Fig. 2. An area of a section of carcinoma showing the appearance of several light areas, the "pre-cellular vacuoles" (A) (designated by present writer). And within the vacuole, then there appears a new nucleus (B) (or New nuclei), and it further shows the following transition; lymphocytoid element (C) mesenchymal element (D) young cancer cell (E). (×850)

Fig. 3. Section of carcinoma showing about the same stage shown in Fig. 2. Notice a large polynuclear cancer cell (A) developed in a vacuole in which several nuclei were synthetized. (×850).

Fig. 4. Section of carcinoma showing a young cancer nests (A) and a primordial cancer nest (B) being transformed from a blood vessel (C) or a venous sinuses (D). (×300)

Fig. 5. Section of carcinoma showing the further differentiated stage than the Fig. 4. There can be seen two typical cancer nests (A). (×300).

Fig. 6. Section of carcinoma showing the direct connection of a cancer nest (A) with a venous sinuses (B) or with a blood vessel (C) plugged with blood corpuscles. Notice that the cancer nest look, rather, like an elongated cord (cancer-cord). (×300).

Fig. 7. Section of carcinoma showing the transitions from newly formed lymphoid elements (B) in the blood monera (A) into young cancer cell (D) through the intermediate stage (the mesenchymal elements (C)). (×850).

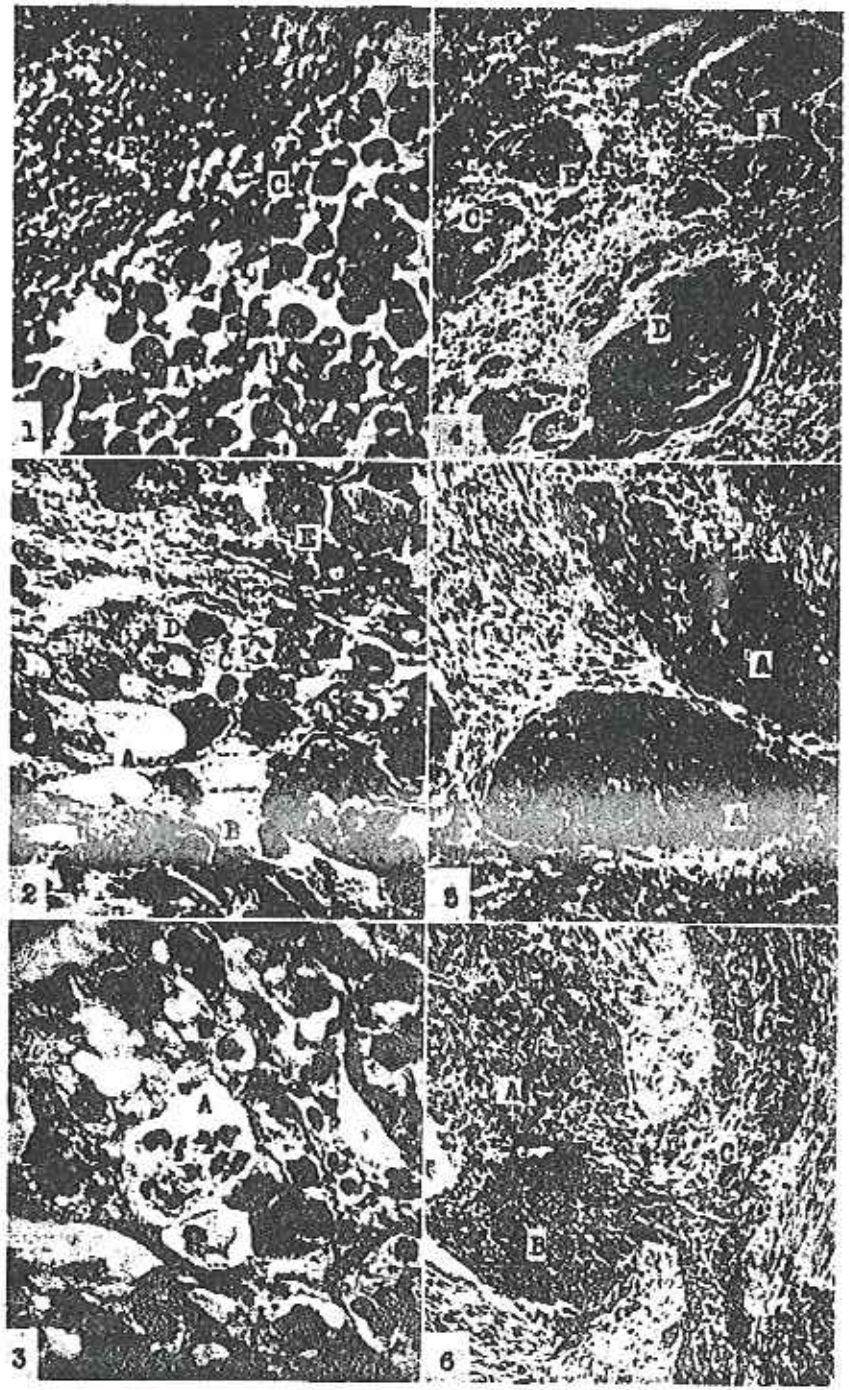
Fig. 8. Section of carcinoma showing an oval shaped oblique section of an old venous sinuses (A) in which can be seen the following transitions, from a blood monera (B) into mesenehpmal elements (C). (×850).

Fig. 9. Section of carcinoma showing an oblique section of an old venous sinuses of which circulation apparently had stopped completely, for there are several masses of blood monera (A) and every phase of new formation of lymphocytoid nuclei in the blood monera. (×850).

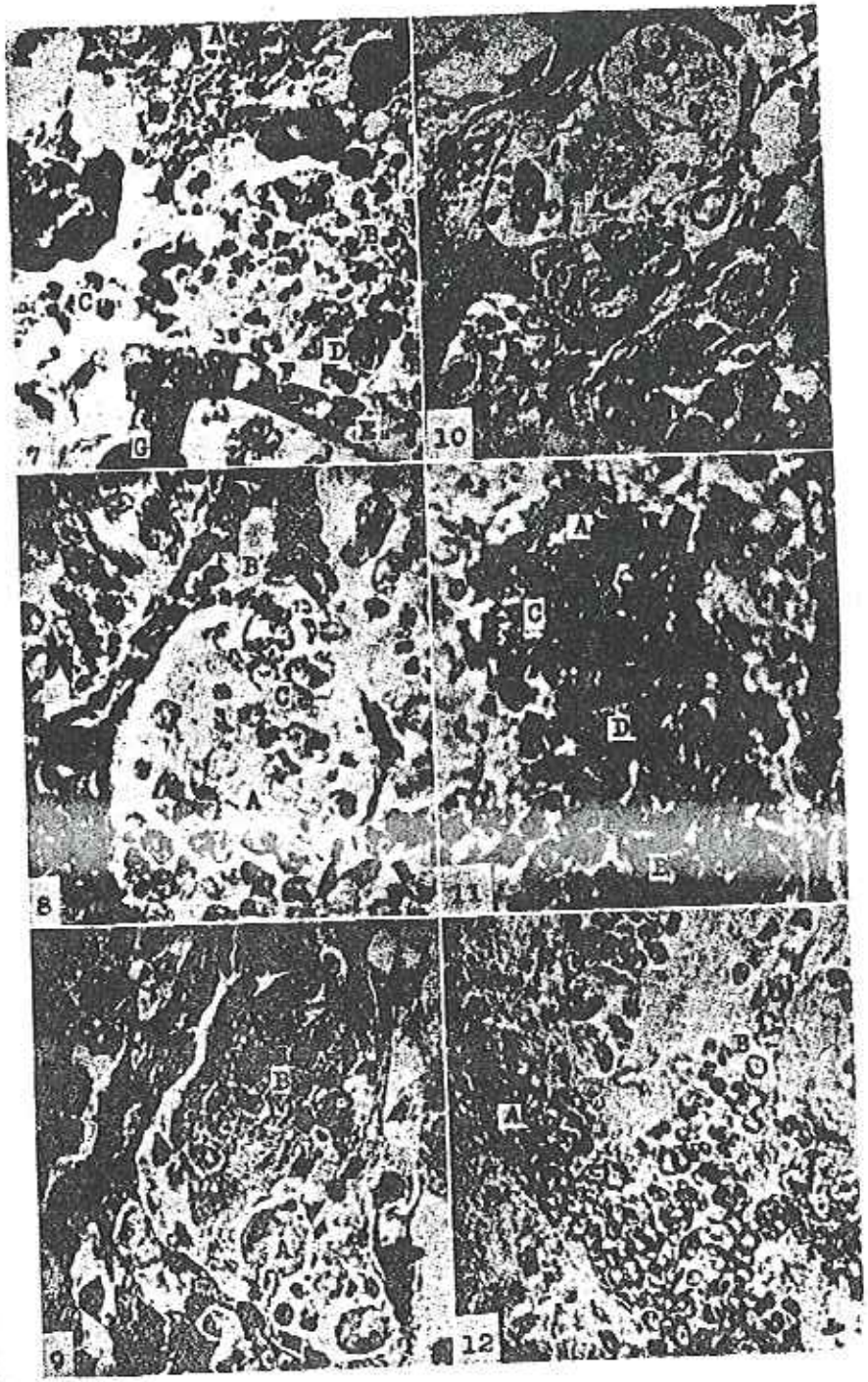
Fig. 10. Section of carcinoma showing an old venous sinuses in which the blood monera (A) polynuclear cancer cell (B) and an intermediate stage between them can clearly be seen.

Fig. 11. Section of carcinoma showing an early stage in formation of cancer nest from a venous sinuses plugged with its contents (darkly stained area) (A). Notice that the sinuses have no endothelial lining, and intrasinuses erythrocytes (B) show transitions into an orange colored cerroid-like substance (C) mesenchymal elements or young cancer cells (D). (×850).

Fig. 12. Section of carcinoma showing an open-type capillary without endothelial lining. From which many erythrocytes spreading out and scattered into surrounding tissues. (×850).



K. Chishima



K. Chishima

V Relation between the Origin of the Ovum and the Degenerating Differentiation of the Blood Cells in Amphibia

Kikuo CHISHIMA*

The origin of Oogonia and follicle-growth in the ovary of frog (Rana japonica G.) was investigated on the ordinary section materials stained with H. and E..

The results obtained are described, in brief, as follows :

Results

(1) The so-called primordial germ cell laying in the inner surface of mesonephros of frog's larvae at 6 days after hatching, showed the following transition from erythrocytes; erythrocytes→small lymphoid elements→mesenchyme cells→fusion of them→young primordial germ cell (Fig.1). The outline of primordial germ cell is, at first, not clear, but afterward it becomes a typical one.

(2) It is clear that the ovarian surface of adult frog is generally drained with abundant blood (Figs. 2. 3). And it can often be observed that the blood cells pouring into a triangle or wedge-shaped area surrounded by ovarian theca and edges of two follicles (Figs. 2. 3. 10. 11). That area (venous sinusoid) is composed of the elements showing the following transition from erythrocytes; erythrocytes→small lymphoid elements→mesenchyme cells. It is of great importance that these mesenchymatous elements show a clear trasitinal phases into Oogonia through the fusion of them (Fig. 3). And there can be found no reliable evidence of mitotic proliferation of the Oogonia, or of the continuity of definitive Oogonia from primordial germ cell. It is evry quetionable, rather it may almost be impossible, that the Oogonia can be traced so far back to the early embryonic stages. From the above stated facts I can not escape from the following interpretation that the definitive Oogonia arises, rather, *de novo* and *in situ*, frum the derivatives (small lymphocytoid clements and mesechyme cells) of erythrocytes by means of their fusion and degenerating differentiation. But it is highly improbable that the Oogonia is a progeny of primordial germ cell.

(3) A certain investigator (Stärk'55) has interpreted the small lymphocytoid elements with pycnotic nuclei in the urodel's ovary, as a degenrating germ cells. But, so far as present material is concerned the lymphoid elements in the ovary, especially in germinal epithelium, is only a first step of differential phase from erythrocyte into mesenchyme cell (Figs. 2. 10. 11. 17).

Johnston ('51) has stated the resemblance between the primary germ cell and the primary blood cell in teleost, but she has made a discrimination the blood cell from primary germ cell by relative size of nucleus. However, it is the common fact that the size or volume of nucleus is by no means constant through the all life cycle of a cell. Thus it shoud be emphasized that we must catch a morphological feature of ovarian elements as a phase of a dynamic process (differentiation)

(4) The modes of growth and yolk-accumulation of the growing follicle of the frog can be divided into the following three groups as I ('48.'52.'53) have already reported on the follicle-growth of hen and rabbit. (i) The yolk-formation through the degeneration of the wall of blood vessel and the blood cells contained within the vessel (Figs. 4. 6. 8. 15—20). Grodzinski (39,50,53) has carried out the extensive studies on the yolk-formation in hen and turtle, but he has not stated on the relationship between the yolk-formation and the blood cell-degeneration. (ii) The follicle growths by the fusion of adjacent one or more of follicles by a dissolution of follicular wall (Figs. 5 . 12–14. 18). (iii) The yolk-formation through the convertment of follicular epithelium into yolk -substance. And the epithelial cells (connective tissue cells) are continuously replenished with the transformation from erythrocytes brought there (Figs, 2. 10—12). It is also illustrable that the erythrocytes are pouring, directly, onto the yong follicle throgh the open end of capillaries. And at where they are degenerated, and then they are converted into yolk material (Figs. 10—12).

(5) The so-called nucleus or germinal vesicle in the Oogonia or the youngest follicle, in general, can be seen as a light staining and a lobulated one at first (Figs. 1. 3).

This characteristic feature of nucleus is due to the fusion of mesenchyme cells, so then it becomes gradually to an ovoid or potato-shape with relatively smooth outline (Figs. 7. 18). However, (According to the growth of folli cle and with accumulation of yolk material) the outline of nucleus becomes more and more obscure, having no definite border to the peripheral cytoplasm(Figs. 4, 7, 18). And the nucleus of large follicle contains hardly basochromatin substance. On the contrary, it includes a large number of acidophilic or polychromatic granules (Fig. 18).

Some worker (Duryee '50) has described the existence of the so-called "lateral loop chromosome" in the living ovum of amphibia. But on the present material and method, I could not find such a characteristic chromosomes as has been described by Duryee. From the above described facts, it is in very question that the germinal vesicle of frog ovum is a typical nucleus homologous with that of ordinary cell.

(6) It is an important fact that the melanin pigment locarized in the surface layer of growing follicle or in the other site show transition from the follicle cell derived from erythrocyte, and then it show, further, transitions into yolk material, though I have not yet analysed its chemical mechanism (Figs. 15. 19—22).

(7) Therefore, it is concluded that the present investigation does not support the germ-plasm theory of Weismann, but agrees with Waldyer's view which claims the somatic origin of germ cell.

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Explanation of Figures

Abbreviations

bv……blood vessel
dbv……degenerating blood vessel
der……degenerating erythrocyte
dmc……degenerating mesenchyme cell
egc……early stge of primordial germ cell
er……erythrocyte
fc……follicle cell
fo……fusion of ovum
me……mesenchyme cell
mp……melanin pigment
n……nucleus of ovum
og…… Oogonia
ov……ovum
se……small lymphocytoid element
vs……venous sinusoid
yf……young follicle
ys……yolk sphere

All microphotographs excepting figs. 19 & 20 are taken from frog's ovaries.

(1) Section of primordium of gonad with mesonephros from a fro's larvae at 6 days after hatching. Observe that the following transition of the elements in the gonad primorium; erythrocyte→small lymphocytoid elements→mesenchyme cells→fusion of m.c→early stage of primordial germ cell.

(2) Part of a section of an adult frog's ovary showing a triangle area, the venous sinusoid, on the ovarian surface.

(3) A magnified part of fig. 2. Notice that there is the same transition from derivatives of erythrocytes (small lymphocytoid element) into Oogonia as shown in fig.1. It is also clear that there is no sign of mitotic proliferation of the elements of germinal epithelium.

(4) Section of ovarian surface from adult one. Notice that the most outer layer of ovary is occupied by vascular system, and there can be seen transition from mesenchymatous elements into Oogonia and young follicle.

(5) A portion of follicle surface shows that the four young ova laying on the two large follicles are going to fusion with underlaying larger one.

(6) A portion of ovarian theca (the vascular area). Observe, that a young developing follicle in degenerating blood vessel (left).

(7) Part of a section of an ovary shows that three young follicles (upper part) are laying side by side and they show transition from a mass of mesenchyme cells (left) derived from blood cells. A nucleus of large follicle has no clear border.

(8) Section of an ovarian surface. observe that an ovum is developing within a degenerating blood vessel.

(9) Part of a section of an ovarian surface shows that a large blood vessel and its contents are degenerate. Most probably, that part then may becomes to a follicle.

(10, 11) Part of an ovary, showing a new ovarian follicle-formation at a inter follicular space. From the open ends of the capillaries the erythrocytes are poured onto the surface of growing follicles. And there can be seen clear transition from erythrocytes into yolk substance through small lymphocytoid or mesenchyme cell stage.

(12) Part of ovarian theca. Notice that the both of ovarian theca and the germinal epithelium ara composed of a capillary and blood cells. Moreover it is clear that the erythrocytes contact directly with a growing follicle and the degenerating erythrocyte show trasition into the cytoplasm of the follicle.

(13) Part of a Section of an ovary. Observe that there is a transition from the contents of venous sinusoid into mesenchyme cells. And the young follicles which arose *de novo* are going to fusion with the larger one.

(14) Part of a section of an ovary showing the fusion of two follicles.

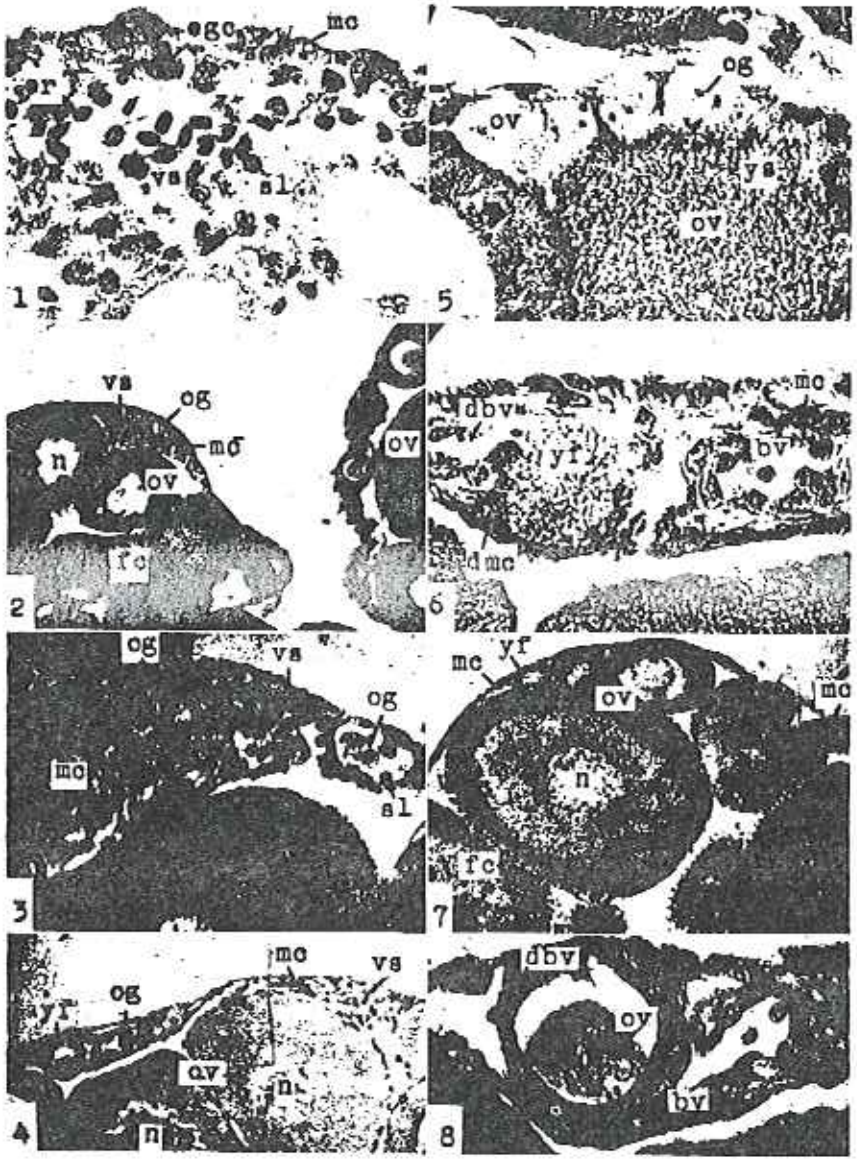
(15, 16) Part of a section of an ovary near to the mesomovarium. Notice that the ends of blood vessels are swollen, and these swollen parts show transition into young follicles.

(17) Part of a section of a degenerating blood vessel in an ovary.

(18) Part of a section of an ovary showing that the follicles are growing to fusion with others.

(19, 20) Part of a section of an ovarian part near to the mesoovarium of a snake (Elaphe) showing that the degenerating blood vessel is giving rise to a primordum of follicle.

(21, 22) Part of sectins of the surface of the vigorously growing follicles. Notice that the melanin pigment granules derived from the degenerated erythrocytes or from its derivatives show transition into yolk-spheres.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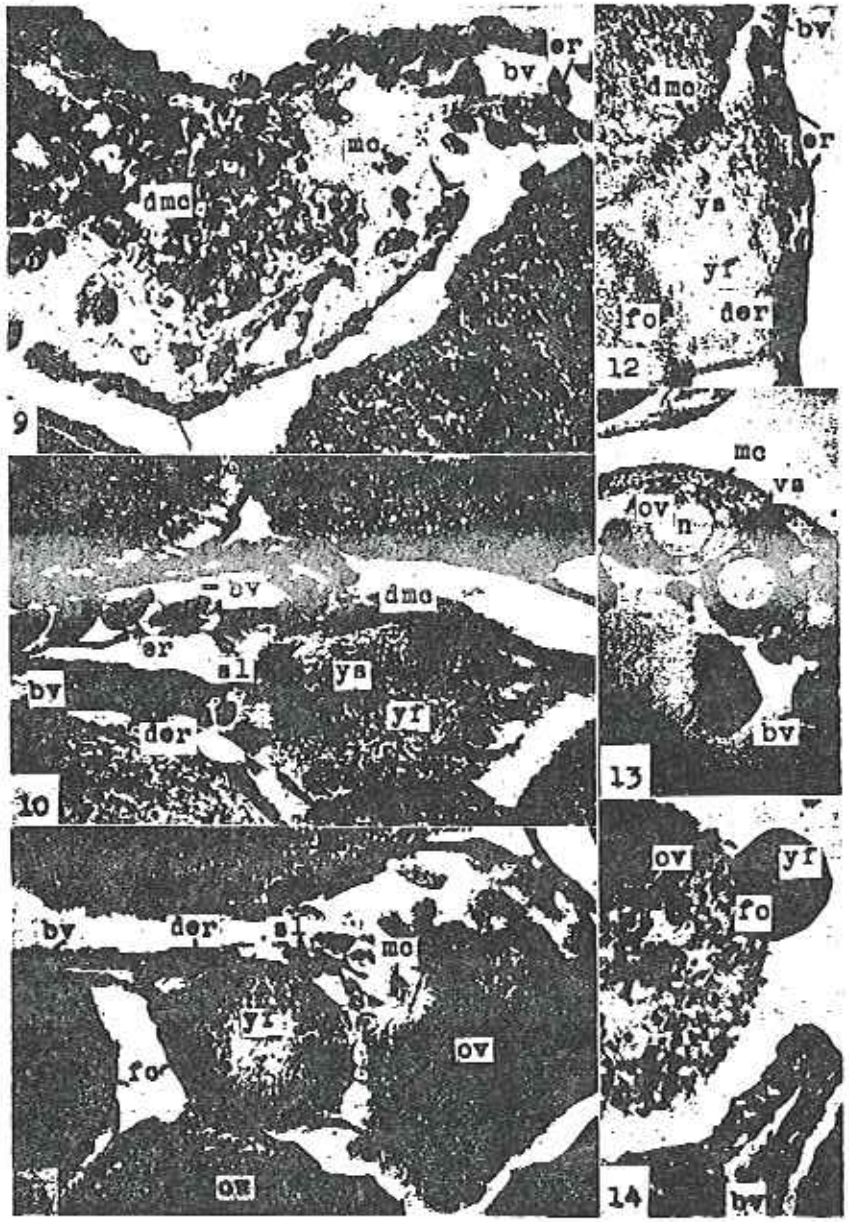
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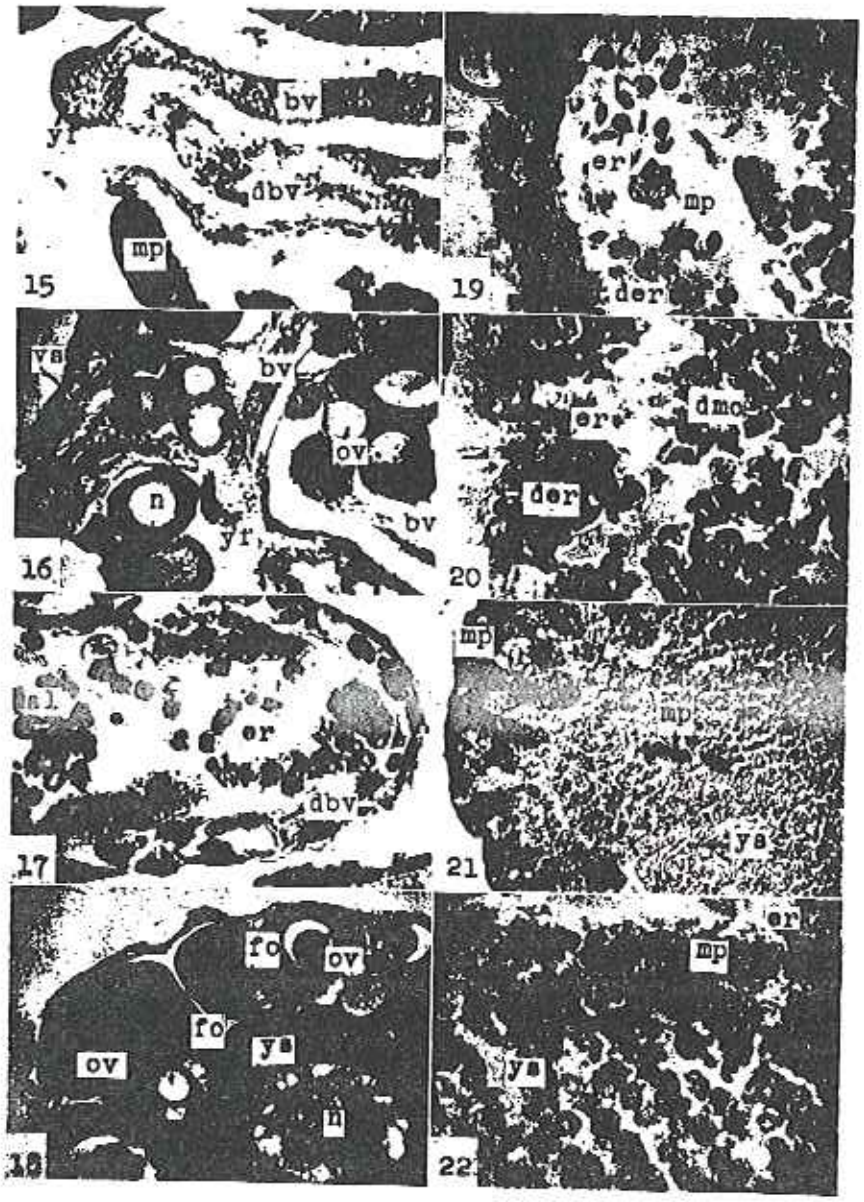

VI On the Differentiation and De-differentiation of Erythrocytes into Ovarian Elements, and on the Re-differentiation of Yolk material into Erythrocytes in Chickens and Rabbits.

(In English with 38 microphotographs),
Research Bull. Facult. Agric. Gifu Univ. no. 2, pp. 125-145. 1953.

Summary

(1) Observations concerning the follicle growth and the yolk formation in the ovaries of chick, hen and rabbit are presented here. Emphasis has been placed on the following accounts that have not yet been described, or have been misinterpreted; (i) erythrocytes differentiate into ovarian elements which then de-differentiate into yolk material, (ii) erythrocytes in blood island of chick embryo arise spontaneously as a resultant of the re-differentiation of yolk spheres, (iii) the growth of ovary or ovarian follicle is not due, exclusively, to the mitotic proliferation of the pre-existed ovarian elements as has been generally accepted.

(2) Vascular systems of the growing and mature follicle-walls in laying hens showed the following characteristics; (i) strikingly disproportional development of venous system to arterial system, (ii) blood stream of venous system shows significantly the sign of physiological stagnation or stoppage, and the blood cells included in it often show physiological stagnation or stoppage, and the blood cells included in it often show transition into yolk material through lymphoid or mesenchymal stage (iii) open type vascular system can be seen, commonly, and the extravascular erythrocytes also show transitions into yolk material.

(3) Ovum and primary follicles in chick and rabbit show transitions from an aggregated and fused mass of lymphoid or mesenchymal elements which further show transitions from erythrocytes.

(4) Three possible modes of follicle growth in chickens and hens are recognized. First, the fusion of small follicles into one. Second, de-differentiation from elements of the follicle-wall, (including blood cells, vessel-wall, connective tissue cells and granulosa cells etc.) into yolk materials. Third, yolk formation from blood and blood cells poured directly into follicular cavity through the open end of cappilary, but this mode is rather less important than the former two modes.

(5) The mode of growth of the ovarian follicle in adult rabbits is the same, in essential points, as that of hens. But the differential process of erythrocytes in the rabbit differs from that of the hen. In chick and hen an erythrocytes differentiate, at first, into a small lymphoid elements and then it transform further, into all kinds of ovarian elements, and at last, into yolk material. While a non-nucleated erythrocyte of rabbit does not transform

directly into a small lymphoid element but it transforms through "the TAEC phenomenon of erythrocytes". That is to say, several numbers of erythrocytes come together and fuse into a homogeneous, eosinophillic, monera-like substance. In this mass, then, appear several vacuoles. Accompanying the decrease in eosinophillic staining capacity of the mass, these vacuoles acquire basophilic staining capacity and at last they become lymphoid or mesenchymal elements. Subsequent behaviour of these lymphoid elements in rabbit's ovary is the same as that of hen.

(6) Mitotic figures of the ovarian elements in chick, hen and rabbit are so extremely rare that the mitotic proliferation of ovarian elements can not be considered as a main factor of the extraordinarily rapid growth of the ovary and its follicular elements. On the contrary, there are sufficient evidences that the ovarian follicle may grow at the cost of all kinds of the ovarian elements derived from erythrocytes.

(7) Erythrocytes in blood island of chick embryo newly arise from yolk material through the re-differentiation process, the second coacervation of the yolk material, but neither by means of mitosis nor amitosis. Consequently, the possibility of the reversal differentiation between erythrocyte and yolk-sphere can be concluded.

(8) The origin and the fate of erythrocytes in the ovary were discussed.

i **Relation between the Vas deferens and the Spermatogenenesis in the Insects.** (in Japanese with English résumé, 9 microphotographs. Cooperation with T. Hosono) Research Bull. Facult. Agric. Gifu Univ. no. 5. pp. 215-219. 1955.

Summary

Studies were carried out on the formation of the sperm bundles in the vas deferens of silk worm and grass hopper. The results may be summarized as follows:

(1) Vas deferens gets its growth by transformation from fat tissue, blood cells and degenerating trachea adhering on the peripheral part of vas deferens. While there can hardly be seen the evidence of mitotic proliferation of the elements of vas deferens.

(2) There was found the following series of the transitions from the epithelial cells of vas deferens into the sperm bundles; enlargement and elongation of the epithelial cells→polychromatic granules or homogeneous substance→ sperm bundles. On the other hand, there can also be seen transitions from a spermatozoa-like-cell-mass involved in the vas deferens into sperm bundles.

(3) The sperm bundles are already found in the vas deferens of silk worm at 5th instar stage, at that stage the sperm bundles in the testis show no sign of going down into the lumen of vas deferens. The evidence of descending features of the testicular sperm bundle into the vas deferens could not be found even at pupal or adult stage of the insects observed. Therefore, we can not escape from the conclusion that the formation of sperm bundle can occur within the vas deferens, independently of testis, though it is possibly influenced by the induction of testis.

ii **Relation between the Origin of the so-called Primordial Germ Cell and Differentiation of Blood Cells in Chick Embryos.** (in Japanese) Kagaku (The Science) vol. 18, no. 3, pp. 130-131, 1948.

iii **Histogenesis of Gonads and Differentiation of Blood Cells in Chick Embryos.** (in Japanese) Tikusan no Kenkyu (Studies on Zootechny) vol. 2, no. 7, pp. 206-209, 1948.

iv **Relation between the Formation of Spermatogonia and Differentiation of Fat body derived from Blood Cells in Silk Worm and Grasshopper.** 24th Annual Meeting of Japanese Zoological Association, 1953 (at Kyoto Univ.); Kagaku (Science. Iwanami) vol. 24, no. 7, pp. 369-370, 1954.

Ⅶ ENGLISH SUMMARY
of
Basis of Neo-Biology
Volume II*
——The Origin of Life, Cells and Blood Corpuscles——

(A separate volume in Japanese, 490 pages, 200 figures)

by

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Contents

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1958

Summary and Conclusion

It is said that we are now living in an epoch of atomic energy or in a cosmic age which was brought about in the first half of the twentieth century, by revolutional advance of physical technique.

But we hope and expect that in the forthcoming latter half of our century there would occur not less revolutional discoveries on the principle and practice of life than that of physics. And, as a matter of course, those discoveries should be favourable not only for the promotion of the human happiness but also for the establishment of world peace.

As a matter of fact, some of the leading scientists, namely, Meyer, Bertalanffy, Bernal, Lysenko, Lepeshinskaya and the others have pointed out that the basic priciples of biology are now confronted by a turning point.

In November 1859 appeared Darwin's great book, the "Origin of Species." It shows that the higher forms are evolved from simpler and lower ones. Despite the great value and stimulating character of his theory, Darwin himself, in his day, was unable to find the origin of unicellular microorganism. Hence, on this point, Darwin does not probe a matter of bottom, viz., the origin of the lowest microorganism.

That situation of Darwinism has been continued to the present time. So, according to the modern biological thought, all living beings, even the lowest microorganism, can arise only from others like themselves, so they can not emerge on our Earth at the present time.

In other words, this orthodox view held the conception that even the lowest microorganisms, such as bacteria living at the present day are descendants of pre-existed ones, and they have remained as they were since innumerable years ago, and would remain, as they are, till far off future. As to why some bacteria or amoebas have evolved to higher forms, while some others remain as they were in the geological age, current biology gives us no information whatever.

Consequently, the contemporary biological thought on the origin of life is, in essence, a preformation theory. True biologists, therefore, can not and must not

overlook the contradictory idea between Darwinism (Origin of Species) and the preformation theory as to the origin of life (microorganism and cell).

Hence, this volume was written with the purpose of the re-examination of the two fundamental laws of biology, the laws represented by the following thesis "Omnis vivum e vivo (Pasteur), and "Omnis cellula e cellula (Virchow).

In this volume, then, I have described and discussed, on the subject, from point of view which was secured, mainly, by my own works as to the origin of bacteria, cell and blood corpuscles, and on the related matters. By the way of summary, I should like to state the outline of my opinion.

(1) Spontaneous generation of bacteria

Ever since Pasteur defeated Pouchet, the negation of spontaneous generation has been accepted by the modern biologists as a firmly established truth, though they can cite no firm evidence under the natural conditions.

However, I have confirmed the following facts, by means of continuous observation under microscope, that some kinds of bacteria can emerge, *de novo*, in the putrefaction-process of such organic substances as the blood of amphibia and aves, or of several organic substances.

During the putrefaction-process of blood, at first, the minute particles or cellular debris in the blood show transitions into saprogenous bacteria and then a number of bacteria arise within the cytoplasmic extrusion from erythrocyte, and, at last, the bacteria appear within the leucocytes or erythrocytes themselves, notwithstanding the fact that there is no sign of invasion of bacteria from outside into their cytoplasm. In this case, there arise, *de novo*, many of granules, the bacterial anlage, in the cytoplasm, and then in the degenerating nucleus, and, at last, these blood cells become, *in situ*, a mass of bacteria. The individual bacterium, then, detaches gradually from that mass, gets free, and then swims away.

The so-called "division of bacillus," due, most probably, to the misunderstanding about the detachment or separation of individual bacterium which emerged, as a mass, in the cell or in the organic substances at the same time.

(2) New formation of blood corpuscles.

Virchow's thesis "Omnis cellula e cellula" has been accepted by the majority of

the modern biologists as a fundamental law of biology. But, O. B. Lepeshinskaja ('37-'55) has already published her revolutional finding that the erythroblast or some other cells can arise from living substances (yolk sphere, egg albumen etc.), and I ('49~'58) have also found, independently of Lepeshinskaja's finding, the same fact. The above-described opinion of ours, however, does not imply that we deny entirely the cell division.

Though we can observe, in relatively few cases, the so-called mitotic figure, yet it does not necessarily produce two daughter cells and, moreover, there are many evidences that the cells can arise from organic substances which have no cellular structure. So that it must be obvious to true scientists that the role played by the so-called mitosis, in increasing in number of cells, has been overestimated by cytologists.

(3) New formation of the sponge cell from an aggregated mass of zoochlorellas

This author found that the sponge cell and amoeboid leucocyte in the fresh water sponge (*Spongilla semispongilla* Annandale sp.) are formed, *de novo*, from an aggregated mass of a lot of *zoochlorellas*, the unicellular green algae. These algae included in the sponge cell, hitherto have been considered it as intracellular symbionts, But, according to my observation, the sponge cell shows neither any sign of phagocytic ingestion of zoochlorella nor cell division. While there can be seen clear transitions from an aggregated mass of *zoochlorellas* into a sponge cell, and then, it differentiates into an amoeboid leucocyte. So that the major portion of a sponge is composed of the aggregated or isolated *zoochlorellas*. It is said that the sponge is "a living hotel". So in my material too, it gives harbour to other many kinds of microorganisms (bacteria, algae, diatoms, ciliates and the others). Therefore, we can say that, so far as, the present material, the fresh water sponge, is concerned, the sponge may be classified rather to plant kingdom than animal.

(4) Evolution of bacteria through the second coacervation

It has been supposed that every microorganism proliferates and continues its existence by means of cell division. But according to my opinion it is not in all. That is to say, the results of my own observations show that some of saprogenous

bacteria, on the surface of foul water or on the putrefying substance, aggregate and agglutinate and then they form a mass, it further differentiates, in accordance with its different circumstances, into a certain kind of fungus, spore, alga, infusoria or amoeba etc. by means of the second coacervation-process (designated by me. It means the new formation of a cell from a mass of lower microorganisms). Some kind of earthworm's blood cell filled with a number of fungi-like inclusion also may be included in the same category.

(5) Intracellular symbionts and the new formation of cells.

It is a well-known fact that the mycetocytes found in a certain kind of insects and other animals or plants include many bacteria or yeasts in their cytoplasm, and these microorganisms are, generally, considered as symbionts.

But, according to my observations described above (3 and 4) the so-called intracellular symbionts can not be considered as mere symbiotic microorganisms, but it may rather be referred to an aggregated mass of microorganisms, and from that mass then arises a new cell element through the second coacervation (aggregation, fusion, organization, and differentiation) of it. No evidence has yet been shown, by any investigators, that these microorganisms had been phagocytized by prexisting phagocyte (mycetocyte).

(6) New formation of erythrocytes in the digestive tract

According to my opinion the erythrocytes in higher animals are a basal element from which almost all kinds of cells are derived, while there can be found no firm evidence that erythrocytes proliferate in the normal bone marrow (Chishima '53 b, '54 b).

The author agrees with the opinion presented by Duran-Jorda ('47~'51) who claims that the bone marrow of higher animals is not a site of erythropoiesis, but erythrocytes are produced, *in situ*, within the granular leucocyte in the intestinal wall. And further, I have, found (Chishima '53 b, '55 a) that the nucleated erythrocytes in aves and amphibia show transitions from the cell in lamina propria of the intestinal villi, on the other hand, primordium of non-nucleated mammalian erythrocytes make its appearance, *de novo*, in a vesicular and large cell located in the lamina propria by means of sporulation-like process. The surface of

intestinal villi, under the well-fed condition, is covered with a layer of digested food (food monera), the homogeneous layer, which is generally apt to be misunderstood as a degenerating epithelium of villi. But, my experiment results secured by the feeding of colloidal carbon show that the layer, under the normal condition, is not a degenerating one but is a reverse case. In this homogeneous layer then arise, *de novo*, several numbers of lymphocytoid elements, which transform into the epithelial cells of villi, and further into spindle cells in the frog, or into normoblasts (or globular leucocytes) in the lamina propria of birds and mammals.

(7) Erythropoiesis in yolk sack and placenta.

Erythropoiesis in yolk sack (bird) and placenta (mammal) is the same in principle with that in the intestinal canal, though the materials for hemopoiesis differ in each case. Namely, the basic material of the yolk-sack-hemopoiesis is the yolk spheres, and that of placenta is the maternal blood.

In this case, it must not be overlooked that the both of placenta and yolk sack belong, ontogenetically, to the digestive system. From this point of view, the ontogenetical transition of the erythropoietic center from yolk sack or placenta into digestive tract may be considered as a very natural route. My opinion mentioned above further may be substantiated by phylogenetical transition of hemopoietic center. On the contrary, there is no reliable evidence substantiating the theory of intramedullary erythropoiesis. In addition to it, the orthodox view involves a theoretical jumping as to the transition of hemopoietic center.

(8) In this volume, the author described, further, on the following problems;

(i) The transformation from non-pathogenic bacteria into some of pathogenic ones, may be quite within the bounds of possibility.

ii) Mitochondria, possibly, may regard as a phylogenical or ontogenical vestiges derived from a bacteria-mass from which ancestral cell might be developed.

(iii) The author laid great stress on the intimate relationship between intracellular symbionts in the digestive canal of insects and new formation of cell.

In conclusion I should like to say that, for the question "Does life arise now, at the present time on our Earth?", "Does cell arise *de novo*, from some organic

matters having no cellular structure?" I can answer with confidence "Yes, it undoubtedly does." That is to say, under suitable conditions, some of bacteria, protozoa, and blood corpuscles emerge, *de novo*, from some organic substances with no cellular structure, and there can be shown more surprising facts that some type of cells are derived from an aggregated mass composed of a large number of bacteria or of lower microorganisms including the so-called intracellular symbionts.

My theory described above is so antagonistic to the orthodox view that the present author must expect many oppositions. However, the skeleton of my theory would not be broken by any other investigators.

So far as the origin of bacterium, cell or blood corpuscle is concerned, the adherents of Pasteur's doctrine and of Virchow's view should return, shortly after or in the near future, to the idea of Pouchet and Aristotle.

So bleibe denn die Sonne mir im Rücken!

Der Wassersturz, das Felsenriff durchbrausend,

Ihn schau' ich an mit wachsendem Entzücken.

Von Sturz zu Sturzen wälzt er jetzt in tausend,

Dann aber tausend Strömen sich ergiessend,

Hoch in die Lüft Schaum an Schäume sausend.

Allein wie herrlich, disem Sturm erspriessend,

Wölbt sich des bunten Bogens Wechseldauer,

Bald rein gezeichnet, bald in Luft zerfriessend,

Umher verbreitend duftig kühle Schauer.

Der spiegelt ab das menschliche Bestreben.

Ihm sinne nach, und du begreifst genauer:

Am farbigen Abglanz haben wir das Leben.

——J. W. von Goethe——

VIII A Retrospective Bird's-eye View on My Biological Research Works for the Last Forty Years ※

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This paper is written retrospectively about a general outline of my research works carried out for the last forty years because I am to retire from professorship under the age limit of Gifu University at the end of March next year.

The majority of my works are concerned directly or indirectly with the basic principles of biological sciences.

Results of my research works were already published on the five monographs, seventy-six papers in Japanese and on the twelve papers in English.

For convenience sake, my researches may be separated into the following two chronological periods, the first stage (1924-1940 : from the prewar days to the beginning of the world War Ⅱ) and the second stage (1940-1962 : from the latter period of the war time to the present time).

(A) **Research works in the first stage** (1929-1940)

Leading themes of my works in this stge are as follows;

(1) Physiological and mathematical analysis of the wavy curves obtained from the experiments on the temperature effect upon the velocity of spiral movement or upon the asphixiation of aquatic insect, *Eletes Stictieus L.*

(2) Studies on the origin and mechanism of the asymmetrical, wavy and spiral patterns of the chalazae, yolk, albumen, shell and shell membrane of dird's eggs, and those of hen's oviduct, dried film of egg albumen or of mammalian serum.

(3) From the foregoing works I have pointed out that all of schematic illustrations of bird's egg structure which have long been accepted by ornithologists and embryologists must be corrected.

In addition to this I have reported the morphological anafogy between the patterns of cracks or wrinkles appeared on the dried gel of egg albumen or of the serum and the structural patterns of hardened body surfaces in several kinds of animals.

In short, the most part of my works in this period was concentrated to research for the asymmetrical, periodical, and spiral tendencies in the structure, physiological phenomena and the movement in the insedts and in the bird's eggs

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etc.

(B) **Research works in the second period** (1940-1962)

My own themes presented in this stage markedly differ from those of the first stage. So I have published the following heterodox opnions which are so antagonistic to the basic, orthodox principles of biological sciences that it seems at first sight the most unlikely to be true for general biologists, especially for the adhererents of orthodox doctorines.

(1) Germ cells in chick embryo and the adult animals of several vertebrates are derived from the differntiation of erythroctes. (Theory Ⅰ. Germ cell is derived from blood cell) The functional spermatozoa are be formed in the testis but they are formed at epididymis in the aves and mammals, and at the basal portion of vas deferens in the insects (Theroy Ⅱ. Spermatogenesis in epididymis or vas deferens)

(2) Erythrocyte-poiesis takes place, at first, on the blood island of yolk sack in the embryos of aves and amphibia, and the erythrocytes arise *de novo* from aggregated masses of yolkspheres through mesenchymal stage. In the embryo of mammal the erythrocytes arise from maternal blood monera (fused mass of blood corpuscles) in the villie of placenta. After the hatching or birth the erythropoietic center translocate into digestive tracts, but not bone marrow in vetebrates as has been believed. In this case, erythrocytes are formed, *de novo*, from food monera (digested food substances Theory Ⅲ. Erythrocyte-poiesis in the digestive tract).

I have also found the new formation of several kinds of cells, namely, (i) all kinds of embryonal cellular elements in amphibia are formed from yolkspheres through mesenchymal stage, (ii) primordia of amoeba and paramecium from bacteria-mass, (iii) cellular elemeuts of fresh water sponge from aggregated zoochlorellas. In all these cases the newly formed cells arise by means of AFD process (Aggregation, Fusion and Differentiation) of living substance (yolkspheres) or microorganisms (bacteria or Zoochlorellas), but not by mitotic proliferation. (Theory Ⅳ. New formation of cell by means of AFD process).

On the other hand, erythrocytes give rise to small lymphocytes or eosinophilic granular leucocytes by means of its differentiation, budding or

extrusion of its cytoplasmic contents. (Theory Ⅴ. Leucocytes are formed from erythrocytes)

(3) Under the well-fed condition the erythrocytes of vertebrates also differentiate into almost all kinds of somatic cells or tissues, namely, the cellular or non-cellular elements of muscle, connective tissue, bone, cartilage, bonemarrow, epithel, nervous tissue including brain and spinal cord, liver, pancreas, kidney, spleen, gonad and all endocrine organs and leucocytes (Theory Ⅵ. Erythrocyte with wide differentiation-capacity differentiate into almost all somatic cells under the well-fed conditions).

Cellular elements of blastema in wound-healing or regeneration also are derived mainly from erythrocytes through their differerntiation. (Theory Ⅶ. Regeneration or wound healing is due, mainly, to the differentiation of erythrocytes).

On the contrary, under starved condition all the fixed cellular elements or tissues reversely re-differentiate into hemocytoblasts or erythrocytes. (Theory Ⅷ. Reversible differentiation between blood cells and fixed elements under the influence of nutritional conditions).

(4) Negation of the inheritance of acquired characters has long been believed as a truth by adherents of Morganism, but its theoretical and practical basis has apparently been shaken by my findings (my theory Ⅰ-Ⅷ).

Moreover, gene mutation theory involves many inconsistency. Therefore, from logical point of view, possibility of inheritance of acquired character should not be denied. (Theory Ⅸ. Confirmation of the inheritance of acquired character).

(5) Though the Pasteur and Oparin's view, the negation of spontaneous generation of bacteria has been accepted by all scientists, this account is at variance with my findings that the putrefactive bacteria arise, *de novo*, within decomposing erythrocyte without invading spores or preexisting bacteria into it. The lowest form of living things undoubtedly emerge spontaneously from decomposing organic matters on this earth at present time. (Theory Ⅹ. Spontaneous generation of bacteria and other microorganisms takes place on this earth at the present time).

(6) I have pointed out the following blind spots and distortions about evolution theory, namely (i) Darwinism, until now, has not explained the origin of unicellular micro-organisms such as bacteria or amoeba, (ii) overestimation of the role "struggle for existence" (the stronger preys upon the weaker) as a factor of evolution, and disregard for the important meaning of symbiosis or mutual aid (AFD process) as a most important factor of evoltion in the microorganismic world, (iii) overestimation of the role of "mutation" as a factor of evolution, and negation of the inheritance of acquired characters of which negation is an irrational conception, (iv) present day evolutionists neglect the dialectical structure of evolution, while, according to my opinion, evolution always accompanies devolution and the course of evolution is rather wavy than rectilineal one.

(7) From the foregoing accounts obtained by my own research I can not escape from the following conclusion that the five basic principles of orthodox biological sciences must de re-examined. It is also evident to me that the confusions and inconsistencies involved in the basic principles of orthdox biological sciences are due, most probably, to (i) the way of thinking, the formal logic which has been accepted by majority of scientists, (ii) uncritical acceptance with the orthodox cell theory of which basic law is "omnis cellula e cellula", (iii) erythroytes are so highly differentiated cells that they have no difierentiation capacity. The results of my own researches, however, differ from those conceptions mentioned above (i-iii).

(8) Thus my opinions are entirely antagonistic to the orthdox point ot view, Perhaps many scientists may feel strange to my theories, but, my accounts from my own experiments and observations are itesms for which I clearly assume personal responsibility. And I hope and confidently believe that the time will come in the near or distant fututre when my heterodox theories will gain general acceptance though it may appear strange now. And my studies on the spiral tendency in the life phenomena will last for my whole life as a life work.

In closing, I should like to acknowledge to my seniors, colleagues and co-workers through whose encouragement and supporing to my reserch works I could conitnue my research course for forty years long, though my course was never a smooth road but was a thorny way.

[IX] DIFFERENTIATING POTENCY OF ERYTHROCYTES IN THE VERTEBRATES

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The erythrocyte has been regarded as so highly differentiated a cell that it can not be differentiated into any other kind of cells, and as the element which undergoes fragmentation and then eliminates from circulation after fullfilling its function as a gas transportor.

The following results, however, were obtained by my studies on the behaviors and functions of erythrocytes in amphibia, birds and mammals by means of histological sections and phases microscopic observations of living erythrocytes.

Mitotic indices in muscle, adipose tissue, liver, brain, bone marrow, ovary and Wolffian body in the normal subjects showed extremery low value.

Furthermore, sometimes the mitotic figure does not necessarily follow the cell division. On the contrary, extravascular erythrocytes scattering in the interstices of various tissues can easily be seen. And these erythrocytes show transitions, depending upon their cellular environment, respectively, into each cellular element of various tissues including the tissues of regeneration and wound healing, through the transitional phases (disintegrated erythrocytes-mass or isolated spherical erythrocytes → Synthesis of DNA → lymphocytoid or mesenchymal cells). These facts suggest that the mitosis may not be considered as an only mechanism of cell-proliferation in the various tissues. Consequently, so far as the present studies are concerned, it may be said that the erythrocytes endowed with pluripotency, play an important role as a supplying source of cellular elements of various tissues. Moreover, the following methods of cellproliferation were found; (a) Cytoplasm-extrusion from erythrocytes. (b) Budding-like process of erythrocytes in the depleted or starved bone marrow. (c) Reverse differentiation from adipose tissue into blood cell under the starved condition. (d) New-formation of blastomeres and embryonal cells by means of aggregation, fusion and the differentiation of yolkspheres in amphibian embryo. Though the observations and interpretation mentioned above may be seen peculiar, I intend to demonstrate and explain it by the aid of microphotographic slides.

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(C) Abstrcts of the Papers

The Origin, Behaviour and the Differentiation of Blood Corpuscles and Certain Other Cells. [I]*

The Abstracts of the Author's Works published in the Last Eleven Years (1948~1958)

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Introduction

The present day of specialization and mechanism has a tendency to demand that investigator's attention should be limited to physico-chemical analysis of alimited subject. The unfortunate result of this demand is to bring forth a tendency to neglect the reconsideratin on the blind spots included in the basic problems on the borderline field of biological sciences, and it makes investigators compete, each other, in ultra-advanced technique. Hence, the problems regarding the origin, behaviours and differentiating potencies of erythrocyte or microorganisms, and the problems regarding the biologic retationships between the cell-formation and differentiation of intracellular symbiotic microorganisms are almost sorely neglected by the most of investigators excepting a few investigators who have published very valuable works on these problems.

This tendency of biological sciences may, most probably, due, primarily, to an uncritical acceptance to the orthodox cell theory, the Virchow's doctorine on the cell-proliferation.

So that, though there has been accumulated a very large amount of papers bearing on the hemopoiesis and the behaviour of blood cells in several kinds of animals and human, the opinions about the following problems have not yet been arrived at a complete agreement; (i) the origin and the mode of proliferation of the so-called hemocytoblast, (ii) the mode of denucleation and maturation process of mammalian normoblast, (iii) origin and fate of yellow bone marrow, (iv) fate of bone marrow elements (v) the relationship between blood platelets-formation and megakaryocyte, (vi) relation between the blood coagulation and erythrocytes, (vii) the behaviour of living erythrocytes *in vitro* or *in vivo*, (viii) ultimate fate of erythrocytes, (ix) relation between erythrocyte and leucocytes. While O. B' Lepeshinskaya ('37-'55) has published an epoch-making finding that the cell arises from living substances, (the yolk

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spheres etc.), and she declared bravely, "Virchow's thesis is an erroneous one". Bostrōm ('48) and Duran-Jorda ('47~'51) also have presented respectively a new theory as to the erythropoiesis. Though these important findings have already been reported, the famous aphorism of Virchow, "Omnis cellula e cellula" has been believed, even at the present day, as a most firmly established basic principle of biological sciences.

And it is also the most widely accepted opinion that (i) the erythrocyte is the most highly differentiated cell, and it proliferates by mitotic division of hemocytoblast in the bone marrow of vertebrata, (ii) major function of the erythrocyte is to carry O_2 into tissue and bring away CO_2 from tissue, (iii) the erythrocyte has no differentiating potency into any other kind of cell, accordingly, the erythrocyte degenerates, within about 115~120 days, after it has finished its function as a carrier of O_2 and CO_2.

However, as I have already published, the results of my studies on the blood cells are opposed to the orthodox view, that is to say, (i) the hemocytoblasts arise, *de novo*, from certain organic substances, such as yolk spheres (in the villi of embryonic yolk sac), monera-like substance derived from erythrocytes (in the villi of mammalian placenta) or from digested substance (in the villi of intestine)(ii) under the well nourished condition of animals, the site of erthropoiesis in postnatal life is not in the bone marrow as has been believed, but is in intestinal villi, (iii) there is a reversible differentiation between erythrocytes and the bone marrow elements including fatty tissue. (iv) red blood corpuscles have so extensive differentiating capacity that they differentiate into leucocytes, germ cells and almost all kinds of somatic cells under normal condition, but under the starved condition of organisms the fixed cells or fatty tissue redifferentiate reversely into the blood cells. (v) the reversible differentiations between erythrocytes and fixed elements are induced by, not only nutritional differences but by a different stage of ontogeny, e. g, in the maternal ovary the erythrocytes differentiate into the yolk spheres but in the developing egg or in embryo the yolk spheres redifferentiate into blastomeres, or all kinds of embryonic cells. (vi) the living erythrocytes show, *in vitro* or *in vivo* a certain extraordinary behaviour.

Strange as it may seemas to the origin of life, I have confirmed an important fact that certain kinds of bacteria arise spontaneously, on our earth even at the present day, from putrefying organic matter.

Furthermore, I have found that the cells (including leucocyte) of fresh water sponge are derived, *de novo*, from an aggregation of zoochlorella (the so-called intracellular symbionts of fresh water sponge) by means of their

fusion and organization (coacervation-like process).

In this paper I will describe the abstracts of my works published in certain Journals and in three monographs.

Part One. Papers published by the Present Author.

[A] The Behavior of Erythrocytes and the Formation of Leucocytes from Erythrocytes.

I. Relation between the "Cytoplasm-Extrusion of Erythrocytes" and the Formation of Blood Platelets or Several Kinds of Leucocytes in Mammals. (a) Behaviour of Erythrocytes and Formation of Blood Platelets, Lymphocytes and Monocytes in Blood Cell-Cultures or on the Blood Smear Preparations.

(In Japanese with English résumé, 29 figures) The Dobutsugaku Zassi (The Zoological Magazine) Vol. 58, no. 12, pp. 9-12, 1949.

Summary

Studies were made on the behaviour of mammalian erythrocytes in adult rabbit, mouse and goat by means of comparative studies using several kinds of methods such as blood cell-culture with super-vital dye in vitro, or fresh blood smears, mechanical destruction of erythrocytes and some of other experimental procedures. The result of this investigation may be summarized as follows.

Shortly after the cultivation of blood cells the flagellum-or cilium-like processes (or spicules) often arise on the surface of an erythrocyte. These cilia make their appearance by extrusion of cytoplasm out of erythrocyte or in some cases by adhering and fusion of the microns which were suspended in serum. The erthrocytes then change their forms resembling suger-plum, cap or bell-shape or constricted forms and at last they transform into spherical form (spherocytes).

Through the processes of these transformations erythrocytes show continuous rhythmic and contracting movement of their outer surface and some of spherocytes show active rotating motion.

Mammalian erythrocytes extrude their cytoplasm through their cell-walls, during their cultivation or on the smear preparation. These extruded cytoplasm then become leucocytes. The present author defines this phenomenon as "cytoplasm-extrusion of erythrocytes".

This phenomenon may be classified as following four types in accordance

with the manner of extrusion and with the circumstances of the extruded substances; (i) diffusion of extruded cytoplasm which have been poured out of the whole surface of erythrocytes into the medium (serum), (ii) formation of blood platelets by extruded cytoplasm, (iii) formation of lymphocytes, monocytes or neutrophilic leucocytes by means of fusion and differentiation of cytoplasmic substance which was produced by mutual extrusion from several number of erythrocytes, (iv) extrusion of microlymphocytes, yet, this type is relatively rare. Formation of such different kinds of leucocytes by the same cytoplasmic substance shows close connection with relative amount of ertxuded cytoplasm to the dimension of an area surrounded with erythrocytes. Furthermore it may have some relationship to the age of erythrocyte or of individual animal or to the difference in time elapsed after the cytoplasm-extrusion.

II. Relation between the Cytoplasm-Extrusion of Erythrocytes and the Formation of Blood Platelets or Several Kinds of Leucocytes in Mammals, (b) Formation of Neutrophilic Leucocytes and the "Field of Leucocyte-Formation" in Blood Cell Culture or Blood Smear Preparations.

(In Japanes with English résumé, 3 microphotographs.). Ibid vol. 59, no. 1, pp. 13-16, 1950.

Summary

The materials and methods used in these studies were the same as that of my previous report. In this paper the results of my studies on the formation of neutrophilic leucocytes, "field of leucocyte-formation" and some of other experimental results in blood cell cultures and blood-smear preparation are presented. The phenomenon of cytoplasm-extrusion of erythrocyte in mammal takes place in somewhat slighter degree than that of bird, reptile or amphibian, and this phenomenon has important effect upon the blood coagulation so that it correlates to the difference of the rapidity of blood coagulation between mammal and bird. In the blood-cell culture or on the thick blood layer of the smear preparation there can be seen several sizes of "leucocyte-formation areas", which are made up of following elements: (i) lymphocytes and monocytes transformed from extruded cytoplasm, (ii) extruded cytoplasm itself, (iii) transitional forms (proleucocytes) between extruded cytopasm and leucocytes, (iv) remnants of erythrocytes or transitional forms from these remnants into leucocytes.

These areas consist of the fused mass of above mentioned elements varying in number from 5 or 6 to several hundreds of them.

Of the same blood of an animal, the numerical ratio of leucocytes to erythrocytes on the blood-smear preparation can be altered by means of giving the mechanical actions such as friction or pressure against the erythrocytes

at the time of smearing and by changing the thickness of blood layer on the preparation. From these facts it can not be ascertained that the blood-figure of the blood smear preparation always indicate the same as that of circulating blood in living animals.

It can be shown that (i) in blood cell-culture, the erythrocytes become fewer and leucocytes become more numerous after 24 to 48 hours, while, (ii) there can hardly be seen mitotic figures of either erythrocytes or of leucocytes. Moreover, spherical erythrocytes themselves show a sigh of transitional staining character into small lymphocytes, (iii) the extruding condition of cytoplasm out of erythrocytes can be actually recognized by continuous watching of living erythrocytes, (iv) on blood-smear preparation, there are many of tansitional forms from the extruded cytoplasm into the leucocytes or blood platelets and there are many of connected conditions between these elements (leucocytes, blood plateletes or extuded cytoplasm) and erythrocytes.

It was discussed that the so-called phenomena of "pinocytosis" defined by Ropes, and of the "erythrocytes engorged into macrophage by phagocytosis" probably correspond to a certain differential stage of "a mass of extrued cytoplasm including erythrocytes" accompanied by the differentiation of erythrocytes.

The phenomenon of the "cytoplasm-extrusion of erythrocytes" has close connections with the following factors: (i) change of environmental factors of blood cells and stop of blood current by pouring out the blood, (ii) adhering of erythrocytes to the foreign bodies, (iii) mechanical action against erythrocytes, (iv) thickness of blood layer smeared on the slide glass. From these points of view, the mammalian erythrocyte might be looked upon as a very primitive and undifferentiated elements, of which nuclear substance has not yet made its appearance.

III. The Behaviour of Blood Cells in the Capillaries in Living Amphibian Larvae. (In Japanese with English résumé, 6 figures) Ibid. vol. 59, no. 5, 1950.

Summary

(1) There are 2 types of capillaries in the living tadpole's tail: (i) the so-called arteriorvenular bridges which convey continuous flow of blood and (ii) the blocked capillary (it corresponds to the "true capillary "defined by Zweifach or the "lymphatics" defined by Speidel) which is connected with A-V bridge and its blood current being interrupted or stagnant and which contains a stagnant column of blood or small number of blood cells.

(2) The blood current of the capillary often reverses in direction or changes its route by the formation of physiological or artificial embolism or by giving

a temporary pressure on the blood vessels.

(3) There can be seen only two kinds of blood cells, namely erythrocytes and lymphocytes in circulating blood and the ratio of these two kinds of blood cells show considerable variation according to the parts of the capillaries, and the writer could not recognize such a peculiar, connected condition of erythrocytes with leucocytes as often seen on blood smear preparation.

(4) In the blocked blood vessel, the erythrocytes often extrude their cytoplasm and fuse each other and differentiate into the condition that hitherto has been referred to be "a macrophage engorged erythrocytes in lymphatics"

(5) There can be seen no identity of blood-figures between the flowing blood in living tadpole and that of the blood smear preparation in a same individual. This difference is due to the explosive cytoplasm-extrusion of erythrocytes, so that we can not escape perfectly from this phenomenon occuring in blood smear preparation so far as our present smearing technique is concerned.

(6) The erythrocytes and lymphocytes in the continuous flowing blood sometimes adhere on the inner surface of the vessel and then, not only the lymphocytes but also the erythrocytes, wandering out to the exterior of the vessel through the vessel-wall. And in this case the wandered out erythrocytes already have lost their erythrocytic characteristics and change into leucocytes.

(7) The blood cells in the stagnant or blocked capillaries also exhibit the same behaviours as that of the above described case of the flowing blood.

IV. New formation of Cell by Budding of Erythrocyte in Aves and Amphibia. (In Japanese, 4 microphotographs,) Ibid. vol. 20, no. 10. pp. 476-477: vol. 20, no. 11, pp. 522-523, 1950.

Living erythrocytes in the wet preparations of the whole blood of aves, reptiles, amphibia and fishes were observed by phase contrast microscope at room temperature. And it was found that the erythrocyte gradually protrudes a clear blister-like bud on the one side of erythrocyte by its cytoplasmic budding. After 75 hours, there appears in the bud a nuclear structure which shows positive Feulgen reaction. From the results of this observation it can be said that the living erythrocyte, in vitro, produces new cell by its budding but neither by mitosis nor amitosis.

V. On the Behavior and Differentiation of the Erythrocytes of the Frogs in vitro. (In Japanese), Acta Anatomica Nipponica, vol. 26, no. 2, pp. 39-40, 1951.

VI. Differentiation from non-nucleated Red Blood Corpuscles into Lymphocytes. Essentials of the 57th General Meeting of the Nippon Kaibo Gakkai

(Societas Anatomica Nipponica) 1952. and Zikkenseibutsugakuho, vol. 2, no. 1, 1952.

VII. Budding Phenomenon of the Amphibian Erythrocyte. Acta Anatomica Nipponica vol. 32, no. 6, pp. 651-652.

[B] New Formation of Cells and Blood Corpuscles from certain Organic Matter or from Microorganismic Symbionts by means of 2nd Coacervation

I. On the New-formation, Differentiation and Mitosis of the Cells—— O. B. Lepeshinskaya's Theory and My Views on the Blood Cells. ——(In Japanese, 24 microphotographs) The Seibutsukagaku (The Biological Science, Iwanami) vol. 4, no. 1, pp. 20-28, 1952.

II. The Cleavage of the Amphibian's Egg, and the York Sphere's Role played with the Increasing in Number of Embryonic Cells. ——Re-examination of the Cell Theory—— (In Japanese with English résumé, 21 microphotographs) Research Bulletin of the Faculty of Agr. Gifu University, no. 6. pp. 268-278, 1956.

Summary

Some investigations were made on the relation between the mechanism of increasing in number of blastomeres during the cleavage and on the new-formation of cells from the yolkspheres of developing egg of amphibia, *Bufo vulgaris, Rana japanica G.* and *Rhacophorus reinwardti* by means of ordinary sections and of some histochemical techniques.

The results of the investigations may be summarized as fallows:

(1) The blastomere-formation at an early stage of development is performed by the formation of a segmentation plane accompanied with melanin granules. But the nuclei of these blastomeres do not show typical nuclear structure and behaviour, e. i., (i) negative Feulgen reaction, (ii) show no typical phases of mitotic division, (iii) each of these nuclei is composed of clear vacuole surrouneded by melanin granules which are continuous and mingled with peripheral yolkspheres. Therefore, the writer designates it as a"melaninous primordial nucleus".

(2) Gastrulation is not an actual invagination of ectodermal cells, but is an invasion of cleavage line accompanied melanin granules. Though there is no celluar structure at first, then there appear clear transitional phases from yolksperes situated around the melanin (cleavage plane) into the elements of entoderm and of dorso or ventral lip of blastopore. Therefore, it seems

probable that the melanin pigment derived from yolkspheres plays an important role in the development or differentiation of embryonic tissues and it seems to bear a certain relation to the organizer.

(3) There can be fouund no sound evidence of the orthodox view which interprets the small and numerous blastomeres appearing at the blsatula stage, as a descendants derived, by mitotic division, from a giant blastomere of the morula stage. On the contrary there is the following clear series of transition: scattered yolkspheres → aggregationa of certain number of yolkspheres → formation of melanin granules from yolkspheres and yolklysis →appearance of melanin in the yolksphere-mass→small blastomere with faint basophilic nucleus and with cytoplasm contained yolkspheres (the so-called yolk-cell)→ ordinary embryonic cell with a nucleus which holds DNA, and with cytoplasm contained melanin granules.

(4) At the early developmental stage of the embryo there can be found, sometimes, the blastomere with double nuclei or lobed nucleus. These blastomeres seem to be at a stage of new-formation of nucleus in the yolksphere-mass, but they may not be a phase of amitosis, since there can be seen no typical phases of amitosis.

(5) Yolkspheres of developing frog's egg should be considered as a ground substance, from which the embryonic cells may be formed, rather than a nurient substance for the cell-growth.

The gelatinous layer surrounding the egg surface shows the following series of the transitions into ectodermal elements: Gelatin layer → the melanin granules on the egg surface → yolkspheres → ectodrmal element with melaniferous primordial nucleus. The above mentioned process is supported further by the fo llowing facts that, (i) the erythrocytes on the surface of growing ovum in adult frog, transform into yolkspheres through melanin pigment-stage, (II) newly hatched larvae still has a large amount of yolk as much as that of egg at early developmental stage.

(6) It is inevitable that the results of the present studies should lead to a re-examination of germ-layer theory, which claims that the increase in number of several kinds of embryonic cells is due, entirely, to the mitotic division of respective blastomeres belonging to a certain germ-layer. On the contrary, there can, always, be found a sound evidence of new-formation of embryonic cells from yolkspheres.

(7) The relation between the orthodox view on the cleavage and Lepeshinskaya and my opinion as to the "new-formation of cell from living substance was discussed.

III. New formation of Blastomeres from the Yolk Spheres in the Amphibian

Larvae. (in Japanese, 13 microphotographs) The Seibutsukagaku, (The Biological Science, Iwanami) vol. 8, no. 3, pp. 118-125, 1956.

IV. An Agreed or Divergent Points in Opinion between O.B. Lepeshinskaya and K.Chishima as to the New-formation of Cell from Organic Matter. (in Japanese) Soviet Igaku, vol. 4, no. 2, 1956: and Kokuminnokagaku (National Science) Feb. pp. 51-58, 1956.

V. New-formation of Cells (including an Amoeboid Leucocyte) in the Fresh Water Sponge by means of Aggregation and Differentiation of Unicellular Green Alagae, Zoochlorellas. (Published at the Sixth Annual Meeting of Nippon Haematological Association-TōKai bloc, Dec. 1957, and at the Fifth Annual meeting of Nippon Ecological Association, April, 1958.

VI. Symbiosis between Human Beings and Microorganisms. Academia (Nippon Gakujyutsusinpo) (in Iapanese, 4 microphotographs) no. 32-34, 1958.

VII. Divergent Points in Opinion with A.I. Oparin and me as to the Origin of Life (in Japanese) Ibid no. 29, pp. 20-25, 1958, and Soviet Igaku, vol. 5, no. 2, pp. 82-83, 1957.

[C] Re-examination of the Orthodox Theory as to the Intramedullary Haematopoiesis.

I. Reversible Differentiation between the Erythrocytes and the Bone Marrow Elements or Bone under Normal or Starved Conditions. (in English with 19 microphotgoraphs) Okajimas Folia Anatomica Japonica, Band 25, Heft 3, pp. 119-148.

Summary

Differentiation, de-differentiation and re-differentiation of the bone marrow elements including the blood cells and yellow marrow of oviparous animals (chick embryos, chicken and adult fowls) and mammals (rabbit, goats, dogs, cats and guinea-pigs) under the well fed and the starved conditions were studied by means of examination on the imprinting, sections and culture of the bone marrow. The results obtained are summarized as follows.

(*A*) *Differentiation from blood cells into the bone marrow elements under normal conditions.*

(1) Erythrocytes in the marrow of the oviparous animals often show transition into small lymphoid elements (meta-lymphocyte) through spherocyte stage, while the mammalian erythroytes generally form a fused mass ("Erythrocyte-monera) in which small lymphoid elements arise *de novo* through the

"sporulation and coacervation process" (new synthesis of chromatin substance in the F. E. monera).

(2) Mammalian "Meta-lymphocytes" are not always equal in size owing to the amounts of erythrocytes contributed to their formation.

(3) Mitotic figure of the "meta-lymphocytes" in the marrow is hard to find, while the "meta-lymphocytes"show the evidence of further differentiation into monocytes, promyelocytes or into myelocytes. Therefore, the "meta-lymphocytes" should be discriminated from "pro-lymphocyte" which is a stem cell of erythrocyte and are found in the depleted bone marrow only.

(4) Isolated erythrocytes in the marrow often extrude its cytoplasm and the both the extruded portion and the residual portion transform into two connected lymphocytes resembling an amitotic figure of a lymphocyte. This type (connected or budding type of cell) is seen in the marrow film of chick embryos and young chickens. The extruded portion of the erythrocyte show transition into eosinophilic granulocyte. However, in birds and especially in mammals, two or several erythrocytes extrude co-operatively their cytoplasmic content and form a neutrophilic granulocyte. And those erythrocytes eventually contribute themselves for the farmation of myeloid elements.

(5) The neutrophilic granulocyte then transform into eosinophilic and then into basophilic granular myeloid element. There can not be drawn a sharp distinctive line among the three kinds (neutro, acido, and basophile) of granulocytic series, or even between non-granular and granular myeloid elements.

(6) There is no firm evidence that the so-called macrophage belongs to a specific cell lineage, but it seems most probable that it is a derivative from fused blood cells irritated by foreign substance induced into the blood.

(7) Polynuclear giant cells in chicken marrow and megakaryocytes in mammalian bone marrow also have no special stem cell, but are the derivative from fused blood cells. Mammalian blood platelets hardly show the evidence of their megakaryocytic origin, but show a transition from the extruded cytoplasm or debris of erythrocyte.

(8) Yellow bone marrow composed of fat cells and fatty drops having no cellular structure, is a resultant from fatty degeneration of granular myeloid elements including macrophage and megakaryocyte, and sometimes, from that of erythrocytes or non-granular myeloid elements.

(9) Capillary system, including venous sinusoid in the bone marrow, are, in general, open type, and it is not uncommon that there can be found collapsed or degenerating arteries.

(B) Erythropoiesis, the reverse differentiation from the yellow bone marrow into blood cells under starved conditions.

(10) Under inanition or malnutrition, the fatty tissue in the bone marrow decreases in amount, however, it does not dissolve away. While the fat drop transforms into a neutrophilic or polychromatic cloudy substance ("fat monera") in which arise *de novo* several scores of mesenchymatous hemocytoblasts, which show, further, transitions into basophilic normoblasts and then into orthochromatic normoblasts.

(11) Avian orthochromatic normoblasts with somewhat granulocytic nature maturates usually into a nucleated erythrocyte. However, mammalian normoblast is, in general, several times larger in volume than the erythrocyte, so that one normoblast gives rise to several or more of non-nucleated sphere erythrocytes. During the maturation of normoblast, the nucleus swells and diffuses into the cytoplasm and transforms into acidophilic cytoplasm, from which emerge spontaneously several erythrocytes by "budding" (at periphery) and by sporulation (at interior part) of the cytoplasm.

(12) A characteristic cell, "erythromyelocyte" found in the depleted bone marrow of cocks under prolonged starvation. The cell resembles the promyelocyte but it contain 1-3 spherocytes or its primordium. These elements may be considered as a transitional form of the reverse differentiation from the myeloid element into erythrocytes under starved condition.

(13) Not only the yellow bone marrow but also the marrow elements show reverse differentiation into normoblasts, eosinophilic granulocyte or directly into erythrocytes.

II. A New theory on Erythropoiesis in the Digestive Tract. Japanese Medical Journal (in Japanese) (Nippon Ijisinpō), no. 1584, pp. 105-106)

III. On the Blind Spots of the Intramedullary Haematopoietic Theory and on My Opinion as to Erythropoiesis is the Digestive Tract. (in Japanese) The Sogoigaku (The Synthetic medicine) vol. 12, no. 4, pp. 299-309, 1955.

[D] Differentiation from the Erythrocytes into Germ Cells and Gonad's Elements in Vertebrates and Invertebrates, and Reversible Differentiation between the Erythrocytes and the Germ Cells

I. Studies on the Relationship Between the Histogenesis of the Gonads and the Differentiation of the Blood Cells in the Chick Embryos. (in English) with 63 microphotographs. Okajimas Folia Anatomica Japonica Bd. 24. Heft 3 pp. 149-186, 1952.

Summary

(1) In this paper the origin of the so-called primordial germ cell and the

histogenesis of the gonad in chick embryo are described with special reference to the differentiation of erythrocytes.

(2) Erythrocytes in the gonad of chick embryos show transitional phases into several kinds of formed elements of the gonad, such as small lymphoid cell, "fat-laden cell", fibroblast or connective tissue cell, eosinophilic granulocyte and mesenchymal cell-B, according to the cellular environment, where the erythrocytes are localized or remain stagnant.

(3) The differential potencies of erythrocyte were recognized by results of the following experiments, viz. (i) blood cell culture, (ii) gonad implantation and (iii) experimental wounds and their healing in the testis of newly hatched chickens.

(4) Gonad primordium in early embryonal stage directly contacts with erythrocytes contained in the subcardinal vein and revehent vein, and some of these erythocytes adhere to the gonad and show transitional phases into gonadal elements.

(5) The so-called primordial germ cells with very low mitotic index value show no reliable evidence of genetic continuity with oögonia or spermatogonia; on the contrary, they arise, most probably, from the incorporation (fusion) with many of the mesenchymal elements of the germinal epithelium *in situ*, and from the germinal epithelial cells showing transitions from the erythrocytes.

(6) There are three different modes, through which the sex-cord may be formed. The first is from the germinal epithelium-cell which is a derivative from the blood layer (after 8-9 day of incubation). In this case there can be found no evidence of local intensive proliferation of the epithelial elements, on the contrary, migration and differentiation of erythrocytes can be clearly demonstrable. The second mode is formation from the condensation and differentiation of erythrocytes migrated into the interstices of the gonad. The third mode is the transformation and rearrangement of mesonephric elements.

(7) Asymmetrical development of the embryonic gonad in the female chick is due to the lack of the first and second modes of sex-cord formation of the right ovary. The right ovary, consequently retrogress by hatching time, according to the degeneration of the right mesonephros.

(8) There is no evidence that the oögonia situated in the ovigerous layer of the left ovary are produced by mitotic proliferation of their own kind. Their characteristic nuclear features resembling the prophase of mitosis, are not true mitotic figures.

(9) The so-calld "cluster of fat-laden cells"shows transitional phases from cluster of erythrocytes that have migrated into the interstices of the medullar

region.

(10) Distended medullary cords are derived from the degeneration of the medullary cords and "cluster of fat-laden ells",

(11) Rete cords are transitional portions from the mesonephric tissues into the gonad.

II. On the Differentiation and De-differentiation from the Erythrocytes into Ovarian Elements, and on the Re-differentiation from Yolk material into Erythrocytes in Chickens and Rabbits (in English with 38 microphotographs), Research Bull. Facult. Agric. Gifu Univ. no. 2, pp. 125-145. 1953.

Summary

(1) Observations concerning the follicle growth and the yolk formation in the ovaries of chick, hen and rabbit are presented here. Emphasis has been placed on the following accounts that have not yet been described, or have been misinterpreted; (i) erythrocytes differentiate into ovarian elements which then de-differentiate into yolk material, (ii) erythrocytes in blood island of chick embryo arise spontaneously as a resultant of the re-differentiation of yolk spheres, (iii) the growth of ovary or ovarian follicle is not due, exclusively, to the mitotic proliferation of the pre-existed ovarian elements as has been generally accepted.

(2) Vascular systems of the growing and mature follicle-walls in laying hens showed the following characteristics; (i) strikingly disproportional development of venous system to arterial system, (ii) blood stream of venous system shows significantly the sign of physiological stagnation or stoppage, and the blood cells included in it often show physiological stagnation or stoppage, and the blood cells included in it often show transition into yolk material through lymphoid or mesenchymal stage (iii) open type vascular system can be seen, commonly, and the extravascular erythrocytes also show transitions into yolk material.

(3) Ovum and primary follicles in chick and rabbit show transitions from an aggregated and fused mass of lymphoid or mesenchymal elements which further show transitions from erythrocytes.

(4) Three possible modes of follicle growth in chickens and hens are recognized. First, the fusion of small follicles into one. Second, de-differentiation from elements of the follicle-wall, (including blood cells, vessel-wall, connective tissue cells and granulosa cells etc.) into yolk materials. Third, yolk formation from blood and blood cells poured directly into follicular cavity through the open end of cappilary, but this mode is rather less important than the former two modes.

(5) The mode of growth of the ovarian follicle in adult rabbits is the same, in essential points, as that of hens. But the differential process of erythrocytes in the rabbit differs from that of the hen. In chick and hen an erythrocytes differentiate, at first, into a small lymphoid elements and then it transform further, into all kinds of ovarian elements, and at last, into yolk material. While a non-nucleated erythrocyte of rabbit does not transform directly into a small lymphoid element but it transforms through "the TAEC phenomenon of erythrocytes". That is to say, several numbers of erythrocytes come together and fuse into a homogeneous, eosinophillic, monera-like substance. In this mass, then, appear several vacuoles. Accompanying the decrease in eosinophillic staining capacity of the mass, these vacuoles acquire basophilic staining capacity and at last they become lymphoid or mesenchymal elements. Subsequent behaviour of these lymphoid elements in rabbit's ovary is the same as that of hen.

(6) Mitotic figures of the ovarian elements in chick, hen and rabbit are so extremely rare that the mitotic proliferation of ovarian elements can not be considered as a main factor of the extraordinarily rapid growth of the ovary and its follicular elements. On the contrary, there are sufficient evidences that the ovarian follicle may grow at the cost of all kinds of the ovarian elements derived from erythrocytes.

(7) Erythrocytes in blood island of chick embryo newly arise from yolk material through the re-differentiation process, the second coacervation of the yolk material, but neither by means of mitosis nor amitosis. Consequently, the possibility of the reversal differentiation between erythrocyte and yolk-sphere can be concluded.

(8) The origin and the fate of erythrocytes in the ovary were discussed.

Relation between the Vas deferens and the Spermatogenenesis in the Insects (in Japanese with English résumé, 9 microphotographs, Cooperation with T. Hosono) Research Bull. Facult. Agric. Gifu Univ. no. 5. pp. 215-219, 1955.

Summary

Studies were carried out on the formation of the sperm bundles in the vas deferens of silk worm and grass hopper. The results may be summarized as follows;

(1) Vas deferens gets its growth by transformation from fat tissue, blood cells and degenerating trachea adhering on the peripheral part of vas deferens. While there can hardly be seen the evidence of mitotic proliferation of the elements of vas deferens.

(2) There was found the following series of the transitions from the epithelial cells of vas deferens into the sperm bundles; enlargement and elongation of the epithelial cells→polychromatic granules or homogeneous substance→ sperm bundles. On the other hand, there can also be seen transitions from a spermatozoa-like-cell-mass involved in the vas deferens into sperm bundles.

(3) The sperm bundles are already found in the vas deferens of silk worm at 5th instar stage, at that stage the sperm bundles in the testis show no sign of going down into the lumen of vas deferens. The evidence of descending features of the testicular sperm bundle into the vas deferens could not be found even at pupal or adult stage of the insects observed. Therefore, we can not escape from the conclusion that the formation of sperm bundle can occur within the vas deferens, independently of testis, though it is possibly influenced by the induction of testis.

IV. Relation between the Origin of the Ovum and the Degenerating Differentiation of the Blood Cells in the Amphibia (in English with 22 microphotographs) Research Bull. Facult. Agric. Gifu Univ. no. 7. pp. 205-211. 1956

Summary

The origin of Oögonia and follicle-growth in the ovary of frog (*Rana japonica* G.) was investigated on the ordinary sectioned materials stained with H. and E.

The results obtained are described, in brief, as follows:

(1) The so-called primordial germ cell laying in the inner surface of mesone-

※phros of frog's larvae at 6 days after hatching, showed the following transition from erythrocytes; erythrocytes→small lymphoid elements→mesenchyme cells→ fusion of them→young primordial germ cell. The outline of primordial germ cell is, at first, not clear, but afterward it becomes a typical one.

(2) It is clear that the ovarian surface of adult frog is generally drained with abundant blood. And it can often be observed that the blood cells pouring into a triangle or wedge-shaped area surrounded by ovarian theca and edges of two follicles. That area (venous sinusoid) is composed of the elements showing the following transition from erythrocytes; eryrocytes→small lymphoid elements →mesenchyme cells. It is of great importance that these mesenchymatous elements show a clear trasitional phases into Oögonia through the fusion of them. And there can be found no reliable evidence of mitotic proliferation of the Oögonia, or of the continuity of definitive Oögonia from primordial germ cell. It is very questionable, rather it may almost be impossible, that the Oögonia can be traced so far back to the early embryonic stages. From the above-stated facts, I can not escape from the following interpretation that the definitive Oögonia' arises, rather, *de novo* and *in situ*, from the derivatives of erythrocytes (small lymphocytoid elements and mesechyme cells) by means of their fusion and degenerating differentiation. But it is higly improbable that the Oögonia is a progeny of primordial germ cell.

(3) A certain investigator (Stärk'55) has interpreted the small lymphocytoid elements with pycnotic nuclei in tle urodel's ovary, as a degenerating germ cells. But, so far as the present material is concerned the lymphoid elements in the ovary, especially in germinal epithelium, is only a first step of differential phase from erythrocyte into mesenchyme cell.

Johnston ('51) has stated the resemblance between the primary germ cell and the primary blood cell in teleost, but she has discriminate the blood cell from primary germ cell by relative size of nucleus. However, it is the common fact that the size or volume of nucleus is by no means constant through the all life cycle of a cell. Thus it shoud be emphasized that we must catch a morphological feature of ovarian elements as a phase of a dynamic process (differentiation).

(4) The modes of growth and yolk-accumulation of the growing follicle of the frog can be divided into the following three groups as I ('48, '52, '53) have already reported on the follicle-growth of hen and rabbit. (1) The yolk-formation through the degenration of the wall of blood vessel and the blood cells contained within the vessel. Grodzinski ('39, '50, '53) has carried out the extensive studies on the yolk-formation in hen and turtle, but he has not stated on the relationship between the yolk-formation and the blood cell-degeneration. (ii) The follicle growths by the fusion of adjacent one or more of follicles by a dissolution of follicular wall. (iii) The yolk-formation through the convertment of follicular epithelium into yolksubstance. And the epithelial cells (connective tissue cells) are continuously replenished with the transformation from erythrocytes brought there. It can also be illustrated that the erythrocytes are pouring, directly, into the young follicle through the open end of capillaries. At there they are degenerated, and then they are converted into yolk material.

(5) The so-called nucleus or germinal vesicle in the Oögonia or the youngest follicle, in general, can be seen as a light staining and a lobulated one at first.

This characteristic feature of nucleus is due to the fusion of mesenchyme cells, so then it becomes gradually to an ovoid or potato-shape with relatively smooth outline. However, according to the growth of follicle and with accumulation of yolk material the outline of nucleus becomes more and more obscure, and shows no definite border lines to the peripheral cytoplasm. And the nucleus of large follicle contains hardly true basochromatin substance. On the contrary, it includes a large number of acidophilic or polychromatic granules.

Some worker (Duryee '50) has described the existence of the so-called "lateral loop chromosome" in the living ovum of amphibia. But on the present material and method, I could not consider such a substance as true chromosomes as has been believed by Duryee. From the above described facts, it is in very question that the germinal vesicle of frog ovum is a typical nucleus homologous with that of ordinary cell.

(6) It is an important fact that the melanin pigment localized in the surface layer of growing follicle or in the other site show transition from the follicle cell derived from erythrocyte, and then it shows, further transitions into yolk material, though I have not yet analysed its chemical mechanism

(7) Therefore, it is concluded that the present investigation does not support the germ-plasm theory of Weismann, but agrees with Waldyer's view who claims the somatic origin of germ cell.

V. Relation between the Origin of the so-called Primordial Germ Cell and Differentiation of Blood Cells in the Chick Embryos (in Japanese) Kagaku (The Science) vol. 18, no. 3, pp. 130-131, 1948.

VI. Histogenesis of Gonads and Differentiation of Blood Cells in Chick Embryos (in Japanese) Tikusan no Kenkyu (Studies on Zootechny) vol. 2, no. 7, pp. 206-209, 1948.

VII. Relation between the Formation of Spermatogonia and the Differentiation of Fat body derived from Blood Cells in the Silk Worm and Grasshopper. 24th Annual Meeting of Japanese Zoological Association, 1953 (at Kyoto Univ.); Kagaku (Science, Iwanami) vol. 24, no. 7, pp. 369-370, 1954.

VIII. A New Theory regarding Heredity, Blood Cells and Germ Cells (in Japanese). Academia (Nippon Gakujyutsusinpo), no. 28, pp. 14-19, 1957.

[E] Differentiation from Erythrocytes into Various Kind of Somatic Cells, and Reversible Differentiation between The Erythrocytes and Somatic Cells

I. Relation between the Formation of Muscle-Fiber and the Differentiation of Blood Cells in the Amphibian Larvae (in Japanese), Igaku to Seibutsugaku (The Medicine and Biology) vol. 16, no. 2, pp. 74-78, 1950.

II. Relation between the Formation of Pigment Cell and the Differentiation of Blood Cells in Amphibian Larvae. (in Japanese with English résumé), Kaibogaku Zassi (Acta Anatomica Nipponica) vol. 25, no. 1, pp. 33-38, 1950.

Summary

(1) In many cases, the pigment cells localized along the blood vessel, especially on the branching point of the vessels in tadpole's tail, and they show very close connection with the blood cells wandered out of the vessels.

(2) In the cytoplasm of the wandered-out blood cells, at first, appear some of yellowish granules and these granules, then, increase in number and in degree of darkness and at last transform into melanin granules. This process also occurs later in the nucleus. The fuse of pigment cells in each other is the most common event in tadpole.

(3) Melanin granules often make their appearance in interstices among epithelial cells and then they connect with the projection of pigment cell. Full grown pigment cells, generally, have no nuclei, so that there can be seen no mitotic figures of them.

(4) The melanin granules often appear in the muscle, epithelium, meninx, nerve cells and sometimes they make their appearance in erythrocytes which are included in the blocked vessels.

(5) The walls of physiologically blocked-vessels become distended and then the wall disappears, so that the blood cells included in it are scattered into neighboring epithelial tissue and *in situ* they show every transitional phases into the epithelial cells.

(6) There can be seen many of the evidences that the macrophage, neutrophile, eosinophile, connective tissue cell and epithelial cell, etc. are undoubtedly the derivatives of blood cells, especially of erythrocytes. So the present author believes, that the erythrocytes are very immature cells with multiple developmental potentialities and it may be regarded as one of the most fundamental elements of the various kinds of cells in tadpole.

III. Relation between the Histogenesis of the Wolffian Body and the Differentiation of Blood Cells in the Chick Embryos, (in English with 6 microphotographs).

Okajimas Folia Anatomica Japonica, Band 23, Heft 6, pp. 337-350, 1951.

Summary

Studies on the histogenesis of Wolffian body and the differentiation of erythrocytes in chick embryos have been carried out. The results obtained in these studies may be summarized as follows:—

(1) Erythrocytes in the Wolffian body in chick embryo show transitional phases into the several sorts of elemets of Wolffian body, such as small lymphoid cell, the cell resembling fat-laden cell, fibroblast or connective tissue cell, eosinophilic granulocyte and mesenchymal cell.

(2) The primordium of glomeruli begins its development by condensation of erythrocytes, but thereafter the erythrocytes included in that anlage gradually decrease in number, on the contrary, the small lymphoid cells increase in number and there can be seen transitional forms between these two elements.

(3) The development of Wolffian tubules begins, at first, by condensation of the so-called nephrogenous tissue cells, but then they are formed from condensed mass of erythrocytes by means of the differentiation of erythrocytes, and formation of lumen in the center of the mass and fusing together with these masses, thus the cord like tubule may be formed.

(4) Formation of Wolffian tubules of chick embryos does not cease on the 10 to 11 days of incubation, but continues to the later embryonic stage.

(5) The mitotic indices in glomerull, Wolffian tubules and intertubular region are so small in value that mitosis can hardly be considered as a chief factor of vast increase of cells in these tissues, on the contrary, there are many evidences that the leading factor of increasing in number of cells in developing mesonephros rests on the basis of the migration, and differentiation of erythrocytes into the fixed elements of Wolffian body.

(6) The experimental results show that the erythrocytes actually differentiate into small lymphoid cells in Wolffian bodies which were transplanted into chorio-allantoic cavity or received certain treatment.

IV. Reversible Differentiation between the Hepatic Cells and the Blood Cells under the Well-fed or the Starved Conditions. (in Japanese with English résume 15 microphotographs), Research Bull. Facult. Agric. Gifu Univ. no. 5. pp. 203-214. 1955.

Summary

The behaviour of the hepatic cells during the growth or the de-growth of liver in mammals (rabbits, goats, cats, and dogs), birds (chickens) and amphibia (frogs) under well-fed or starved conditions were studied by means of comparative examination of serial sections (H. E. staining) of normal and treated livers (intravenous injection of colloidal carbon). The result obtained can be summarized as follows:—

(1) The surface of the venous sinusoid of the liver is, if any, not invariably covered with endothelial cells. So that hepatic cell often contacts directly with erythrocytes. Therefore, it can be said that the capillary system of liver is rather an open type.

(2) It was found in the venous sinusoid that the erythrocytes under the well fed conditions show transitions into hepatic cells. The differential process in the bird is as follows: erythrocyte → small lymphocyte→endothelial cell or Kupffer cell→young hepatic cell→hepatic cell, while, in mammals, aggregated mass composed of several or more of erythrocytes→an eosinophilic fused mass of erythrocytes (monera-like substance), but it then acquires polychromatic staining ability→small lymphoid nuclei, poly or polymorph nuclei or the so-called macrophage (hemophage) phagocytosed the erythrocytes, arise spontaneously in the mass→transform into young hepatic cell or cells according to the size of the mass→hepatic cell or hepatic cells.

(3) The blood cell-cords localized in the interstices of the hepatic cords often show transitions into hepatic cords through the same way as described above. Such differentiation of erythrocytes may start, most probably, by the physiological stagnation or stoppage of the blood current within the venous sinusoid. Evidences favouring the view also were obtained by, i) experimental results of intravenous injection of colloidal carbon, ii) cell culture of hepatic cells mixed with erythrocytes, and by iii) centripetal pattern of hepatic cords toward the central vein.

(4) The large syncytial macrophage seems to be a reaction type of the blood cells to the irritative foreign substance (carbon etc.), and the macrophage storing carbon particles does not show normal differentiation into hepatic cell. Thus, after their degeneration, the released carbon particles aggregate together with others and become larger mass, and they remain in the hepatic parenchyme as long as four months or more.

(5) There can hardly be seen the typical mitotic figure of hepatic cells in the postnatal normal liver. And the evidence favouring the orthodox view that the growth of liver is due mainly to i) increase in size of individual hepatic cell, or ii) increase in relative volume of interstitial tissue of liver has not confirmed.

(6) It is a generally accepted opinion that the erythrocytes in the liver are often phagocytosed by hemophage (or macrophage), however, it may not be a true phagocytosis, but is only an intermediate differential stage from a fused blood cell-mass into hepatic cell or hepatic cells.

(7) Melanin-like pigment resembling the so-called cerroid substance was often found in the liver elements of animals observed, especially in the frog. Such pigment-formation may be closely related both to the degeneration and re-differentiation of erythrocytes.

(8) Under the starved or under-fed conditions the liver decreases in size owing, in parts, to the degeneration and atrophy of the cells, and in the other parts to the decrease in number of blood cells which are, in my view, the ground substance of hepatic cell-formation. And under the starved conditions the hepatic cells show the following order of reverse transitions into erythrocytes: in mammals, increase in polychromatic staining capacity of cytoplasm of hepatic cell →normoblast-like cell →several or more of non-nucleated erythrocytes through budding and sporulation-like process; (in birds and amphibia) elongation of cell →spindle cell→nucleated erythrocyte.

(9) The view that the bile is secreted from hepatic cells is not valid. The result of present studies shows that the bile may be derived from the degenerated hepatic cells itself.

(10) The so-called "erythropoiesis in the embryonic liver" is, most probably, a misinterpretation of the "differentiation from erythrocytes into hepatic cell (my view)". At the most early stage of liver formation in frog embryo, the hepatic cells arise undoubtedly from yolk spheres through mesenchymal stage.

(11) The possibility of reversible differentiation between erythrocytes and cellular elements of the liver was discussed.

V. Relation between the Histogenesis and the Differentiating Capacity of Yolk-Spheres in the Amphibian Embryos and Tadpoles ——Re-examination of the Germ-layer theory ——(Cooperation with T. Hosono). (in Japanese with English résumé, 21 microphotographs). Research Bull. Facult. Agric. Gifu Univ. no. 6, pp. 279-288, 1956.

Summary

Studies were carried on the relations between the histogenesis and the differentiating capacity of yolk-spheres of developing eggs in Amphibia, *Bufo vulgaris*, *Rana Japonica G*, and *Rhacophorus reinwardti* by means of ordinal sections and some of histochemical techniques. The results obtained may be summarized as follows:

(1) O. B. Lepeshinskaya ('36) already has found the new formation of ery-