Dept. of Anat. (Director: Prof. T. SANO), Tokyo Med. College, Higashiokubo,
Shinjuku-ku, Tokyo.

Studies on Blood Vascular Silver-Blackened Fibers (SF, BSF) Related to Heart Beat and Vascular Phenomenon in Vertebrates.

IV. Electron-microscopical Structure of Silver-blackened Fibers (SF) of the heart.

脊椎動物の心働ならびに血管現象に関する血管系銀黒線維 (SF, BSF)
についての研究.

IV. 心臓の銀黒線維 (SF) の電子顕微鏡的構造について.

Tsuyoshi SANO, Gundai KAWAHARA and Ichiro KASUYA.

佐野 幹, 川原 群大 と 糟谷 一朗.

(Received October 7, 1965.)

The authors and their associates have carried out a series of studies on blood-vascular silver-blackened fibers (BSF) related to heart beat and the vascular phenomenon in the vertebrate. They have published many papers on the results of these studies since 1954.

The silver-blackened fibers (BSF) of the heart are regarded as the principal root of the fibers mentioned above. They have been dealt with in the following papers: Studies on the course of running, location of nodular formation, and histogenesis of SF, as compared with the conventional impulse-conducting system (1959); a method of demonstration (detection) of SF, BSF and the mechanism of silver staining by this method (1961); histochemical studies on SF (1961); and the relationship between erythrocytes and the fine architectonics of SF, in cardiac muscle and BSF of blood capillaries in cardiac muscle (1962). No reports have yet been made, however, on the ultramicrostructure of the fibers in question.

I. Materials and Methods.

1. Materials.

The materials used were fresh tissue blocks collected from the hearts of a human fetus and embryos of chicken (*Gallus gallus domesticus*), eels (*Anguira japonica*), cattle (*Bos taurus* var. *domesticus*) and man, and the heart of a dog (*Canis familiaris* var. *japonicus*) which had been removed while beating in a regular state. These blocks of cardiac tissue were treated with various fixatives according to the different methods of investigation.

2. Methods.

In the present studies, light- and electron-microscopic observations were made also on smears of cardiac tissue at the early stage of embryonic life. In particular, the electron-microscopic structure of cardiac tissue treated with the silver-staining method for tissue blocks (KAWAHARA's silver-staining method NO. 4 made public in 1952—1961) was investigated.

The characteristics of this silver method consist in preventing any substance in a tissue from dissolving in water or a chemical solution and in facilitating the preparation of serial sections for light microscopy and ultra-thin sections for electron microscopy.

1. A fixing agent (to be called the weak fixative) is prepared by mixing 7 cc of three- to fourfold dilution of the original fluid of formalin and 1 cc of 0.3—0.5 % solution of potassium bromide. Another fixing agent (to be called the strong fixative) is prepared by mixing 8 cc of three- to fourfold dilution of the original fluid of formalin and 2 cc of 0.4—0.7 % aqueous solution of potassium bromide. A material is fixed with the weak fixative (in cool conditions) for more than 7—12 days. After that, it is fixed again, for more than 24 hours, with the strong fixative (which has been boiled after mixing its two components).

2. After that, the material, as is in the form of tissue fragment, is dipped into distilled water for 10—15 minutes and then moved into ammonia silver solution to which pyridine has been added, for more than one hour. The heating method is used. Preparation of the ammonia silver solution containing pyridine: To 50 cc of 8—10 % solution of silver nitrate, 50—70 drops of 30 % solution of caustic potash are added. To this mixture are added 350 cc of distilled water while sufficient shaking and then 32 % ammonia water so that a small amount of fine silver particles may remain in the resulting solution. Then 50—60 cc of tap water and 30—50 drops of pyridine are added to the mixture.

3. The tissue fragment is washed with water for more than 2 minutes and placed in neutral formalin solution, at fourfold dilution of the original fluid, for 10—60 minutes so that reduction might be carried out during this period.

4. After washed thoroughly with water, the tissue fragment is dipped into more than two thousandfold dilution of gold chloride solution (to which has been added glacial acetic acid) for 24—30 hours and then dipped into 5 % solution of sodium thiosulfate. It is passed through a series of alcohol for the purpose of dehydration and embedded in paraffin or celloidin.

The step in which glacial acetic acid is used with gold chloride has already been employed generally. In this method of silver staining, a solution capable of adjusting the color of gold staining is used, in addition to gold chloride (to which are added some drops of glacial acetic acid), as gold staining solution. The solution capable of adjusting the color of gold staining is composed of 800 cc of water, 12 g of ammonium thiocyanate, 25 cc of 2 % solution of gold chloride, and 3 g of sodium hyposulfite. At the time of use, a small amount of glacial acetic acid is dropped to this solution so that the final quantity of the solution may become 1,200 cc.

The time required for silver staining is adjusted according to the size of material. The standard size of tissue fragment generally employed is 5 mm cube. It is essential for complete silver staining of a whole tissue fragment to adjust the time required for each step of the silver staining method.

The results obtained from the electron-microscopic examination were discussed in comparison with those from the optical-microscopic examination. Such being the case, fresh materials had been fixed in osmium tetroxide solution and KAWAHARA's weak fluid. All the fixed materials and tissue blocks stained with silver were embedded and made to ultrathin sections in a regular manner.

II. Results.

In accordance with the methods mentioned above, the authors investigated SF distributed in cardiac muscle. The histogenesis of these fibers, as represented by the picture of particles, and the minute architectonic of peripheral fibers (the electron-microscopic structure of the silver-blackened picture of the fibers) were examined with the following results.

a) *Heart of chick embryo.*

In the heart of a chick embryo at the early stage (25 to 42 hours) of incubation, SF are seen as chain-like fibrillar structures consisting of fine proteinic particles, which have been blackened with silver, and located in such interstices as filled with cardiac musculature in a later stage of development. This finding has already been described in part I of this series of studies.

Portions of the heart of chick embryo containing interstices to be filled with cardiac musculature in a later stage of development were harvested by the aid of a microscope. They were fixed with osmium tetroxide gas, shadowed with chromium, and examined for the presence of particles forming fibers called SF by electron microscopy. As a result, the following pictures were observed.

Fig. 1 is an electron photomicrograph of particles in the portion of a 38-hour-old chick embryo where myocardium is to be formed later. The particles shown in the photograph are those of the fiber system developing in the interstices of cells and have an electron density not so high.

These fine particles form chains or clumps eventually presenting a fibrous or reticular appearance. In this stage, however, they have not distinctly formed fibers as yet.

As already reported, the fibrous substance formed by the aggregation and combination of these particles can be detected by deposit of silver particles and represented as a blackened picture which is visible by light microscopy. The picture of this substance is not clear when the dye adsorption method is applied (1959). The outline

Fig. 1. Heart of a 38-hour-old chick embryo. Fixed with osmium tetroxide gas. Electron photomicrograph of particles (indicated by arrows) forming silver-blackened fibers in the portion of the embryo where the cardiac muscle is to be formed later. In this stage, the particles are gathering and combining to form less conspicuous fibers. The particles are arranged irregularly, although they show a general tendency to be arranged in a longitudinal direction. No periodical structure is observed between the particles combined with one another. $\times 25,000$

of this substance, as observed by the electron microscope, was already given in our report dealing with the method of demonstrating the fibers and the mechanism of silver staining (1961).

The fibrous substance that develops in the interstices of cells of an embryo in which no cardiac muscle has been formed as yet and that can be detected in a smear preparation by the electron microscope, has not such periodical structure as possessed by the collagenous fibril. Furthermore, this substance is divided into collagenous fibrils and fibers of the muscular system according to the difference in electron density. It seems to have a structure similar to that of a muscular filament which is composed of actin and myosin. The substance is assumed to belong to the synthetic

Fig. 2. Heart of a 75-hour-old chick embryo. Fixed in osmium tetroxide. Not stained. The main portion of a thick fiber is observed in a smear prepared from a region of the embryo where the cardiac muscle has been formed. $\times 300$

system of amino acid.

Figs. 2—7 indicate the process of formation of this fibrous substance in the heart of a 75-hour-old chick embryo with the lapse of time. This substance is formed gradually in the cardiac muscle tissue.

In the 38-hour-old chick embryo, the fibrous substance appears in a form of chain of fine particles, showing that it is in the course of fiber formation (indicated by arrows in Figs. 3—7).

When ultra-thin sections fixed with osmium tetroxide are examined in the dark field by the light microscope, it is not easy to observe minute particles in the sections. This is because the cardiac muscle formation has already begun in those sections.

Fig. 2 shows a slightly thick fiber formed in the portion of the embryo where the cardiac muscle is to develop later.

Fig. 3 shows a part of the heart in a 75-hour-old chick embryo treated by the same method of collection as the material shown in Fig. 1. In this electron photomicrograph, it is easily observed that the fiber-forming particles recognized in the 38-hour-old embryo have formed a systematic fiber in the portion where the cardiac muscle is to develop later.

Thus it seems to be the most effective method for the morphological examination of the fibers contained in the structure of the myocardium to observe ultra-thin sections prepared from a tissue block which has been blackened with silver by KAWAHARA's silver staining method No. 4.

Fig. 3. Heart of a 75-hour-old chick embryo. Fixed in osmium tetroxide. Electron photomicrograph. This picture shows the formation of fibers from minute fibrous particles in a thin layer of cardiac muscle. The arrows indicate the peripheral portion of the fibers less than 0.3μ in diameter. $\times 10,000$

Fig. 4 shows a fiber formed by particles in the immature cardiac muscle tissue. SANO and KAWAHARA (1959) studied, by light microscopy, the morphology of this fiber in the heart of 66 to 85-hour-old chick embryos, by preparing silver-blackened

Fig. 4. Heart of a 75-hour-old chick embryo. Light photomicrograph of an ultra-thin section stained by KAWAHARA's silver staining method No. 4. A silver-blackened fibers (indicated by arrows) are shown in regions of the embryo where the cardiac muscle has been formed. The figure of these fibers is discontinuous in an ultrathin section. $\times 300$

sections. They found that the architectonic of the fiber was very delicate. Ultra-thin sections are inconvenient for a systematic observation of that fiber, since it winds and twists to some extent and appears discontinuous in an ultra-thin section. These sections, however, are useful, as they present the morphology of terminal fibers clearly in the portion of the embryo where the cardiac muscle has been formed.

Consequently, ultra-thin sections were prepared, taking the light photomicro-

graph of silver-blackened fibers into consideration, and examined by the electron microscope. Figs. 5 to 7 are electron photomicrographs taken in this examination.

Fig. 5 is a silver-stained electron-photomicrograph which shows peripheral fibers of silver-blackened fibers (SF) in the structure of cardiac muscle (indicated by arrows),

Fig. 5. Heart of a 75-hour-old chick embryo. Electron photomicrograph of an ultra-thin section stained by KAWAHARA's silver staining method No. 4. Part of the fibers running in the myocardium is demonstrated by the deposition of silver-salt particles of high electron density. This picture indicates that metallic silver particles are arranged along the long axis of the fiber. $\times 30,000$

and minute fibers and formation of fine networks of these fibers on the surface of the cardiac muscle. The fibers of this system, which themselves are of low electron density, stand out by the deposition of silver particles on them. Since they twist and wind already in this stage, it is difficult to grasp the whole picture of them in an ultra-thin section.

Fig. 6 is an electron photomicrograph of silver-blackened peripheral fibers (indicated by arrows) exhibited as the deposit of particles of silver salt.

Fig. 7 is an electron photomicrograph taken from a slightly thick silver-blackened

Fig. 6. Heart of a 75-hour-old chick embryo. Electron photomicrograph of an ultra-thin section stained by KAWAHARA's silver staining method No. 4. This picture shows the peripheral portion of the fibers distributed in the myocardium. Those fibers which are less than 0.025μ in diameter can be seen by the arrangement of metallic silver particles deposited on them. $\times 30,000$

section. The delicate way in which a fine network has been formed by terminal fibers 0.010 to 0.025μ in diameter is represented by silver-blackened particles gathering into chains (indicated by arrows).

Fig. 7. Heart of a 75-hour-old chick embryo. Electron photomicrograph of an ultra-thin section stained by KAWAHARA's silver staining method No. 4. Peripheral fibers, especially those less than 0.03μ in diameter, can be seen to form a fine network of beads of metallic silver particles. $\times 30,000$

8

9

Fig. 8. Heart of a 7-day-old chick embryo. Stained by KAWAHARA's silver staining method No. 4. This picture shows the continuous course of running of main-trunk fibers in a relatively thick silver-blackened tissue section. The fibers have already completed their systematic distribution and are seen to twist and wind in this section. $\times 300$

Fig. 9. Heart of a 7-day-old chick embryo. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrograph. Fibers less than 0.1μ in diameter (arrows) can be seen by the deposition of metallic silver particles on them, forming a fine network on the sarcolemma. $\times 12,000$

Fig. 10. Heart of a 10-day-old chick embryo. Stained by KAWAHARA's silver staining method No. 4. This picture shows silver-blackened fibers which appear discontinuous in such a thin section. In this stage these fibers are of essentially the same morphology as those of the adult subject. $\times 300$

Figs. 8 to 11 (Figs. 8 and 9 being of a 7-day-old, Fig. 10 of a 10-day-old and Fig. 11 of a 14-day-old chick embryo) show the pictures observed in the heart in the sequence of development. Of them, Figs. 8, 10 and 11 are light photomicrographs of silver-blackened tissue sections (0.5 to $1.0\ \mu$ in thickness). The fibers have come to twist and wind extremely by this stage. These pictures indicate that the fibers in this stage become discontinuous in a section which is not sufficiently thick. Therefore, it is impossible to study the distribution of the fibers systematically in an ultra-thin section. Such section is used mostly for the observation of the terminal portions of these fibers.

Fig. 8 was taken from a relatively thick section. In this picture, it is easy to investigate the course of twisting and winding fibers (indicated by arrows). Fig. 9 is an electron photomicrograph. In this picture, silver-blackened fibers less than $0.1\ \mu$ in diameter are visible by the deposition of silver particles on them. The network of the beads of particles has been formed only on the sarcolemma.

Fig. 11. Heart of a 14-day-old chick embryo. Stained by KAWAHARA's silver staining method No. 4. This picture, as well as Fig. 10, shows discontinuous fibers of the silver-blackened cardiac tissue. It is suggested from this picture that a section of sufficient thickness is necessary for the observation of systematic distribution of these fibers. This section is useful, however, to study the terminal portions of the fibers and their relationship to cells, as indicated by arrows. $\times 300$

b) *Cardiac muscle of human fetus.*

Description has already been made on the light-microscopic architectonic of the silver-blackened fibers distributed in the cardiac muscle of the human fetus. Accord-

Fig. 12. Heart of a human fetus. Stained by KAWAHARA's silver staining method No. 4. This light-microscopic picture shows the main trunks and their branches of silver-blackened fibers in a quasi-ultra-thin section from the silver-blackened cardiac tissue. $\times 300$

Fig. 13. Heart of a human fetus. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrograph. The arrows indicate the intertwining peripheral portions of silver-blackened fibers and the formation of terminal networks of them. $\times 12,000$

Fig. 14. Heart of a human fetus. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrograph. This picture presents a high-power magnification of part of the peripheral fibers shown in photograph 13. No periodical structure is recognized in these fibers. $\times 30,000$

ingly, in the present paper, the electron-microscopic picture of these fibers is mentioned in comparison with the light-microscopic findings of them.

Fig. 12 is the light photomicrograph of a relatively thin section. It is easy to investigate the distribution of these fibers rather systematically in this section. As only discontinuous appearance of these fibers is observed in an ultra-thin sections, electron microscopic studies were carried out with relatively thick sections (quasi-ultra-thin sections), as shown in Figs. 13 and 14.

Fig. 13 is a light photomicrograph showing the peripheral portions of fibers less than $0.5\ \mu$ in diameter. These fibers are represented as chains of metallic silver particles. Intertwining of fibrils and terminal networks can be seen. Fig. 14 is an electron photomicrograph where part of Fig. 13 is exhibited at high-power magnification. Fibers less than $0.5\ \mu$ in diameter distributed in the sarcolemma are shown by the deposition of silver particles on them.

c) *Cardiac Muscle of the eel.*

Silver-blackened fibers distributed in the cardiac muscle of the eel were studied. The structure and endings of these fibers are shown in Fig. 15 (light photomicrograph) and Figs. 16 to 20 (electron photomicrographs).

Fig. 15 was taken from a relatively thin silver-blackened section. Silver-blackened

15

16

Fig. 15. Cardiac muscle of an eel. Stained by KAWAHARA's silver staining method No. 4. The silver-blackened fibers in this relatively thick section exhibits only the discontinuous appearance. $\times 300$

Fig. 16. Cardiac muscle of an eel. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrograph of silver-blackened specimen. This picture shows the discontinuous appearance of fibers presented as beads of silver particles. $\times 12,000$

fibers, as indicated by arrows, twist and wind. This section, as well as others, fails to show the systematic distribution of the main trunks of these fibers, exhibiting only the dispersed distribution of the discontinuous fibers. For the observation of the terminal portions of the fibers and their relationship to cells, it is not necessary to use so thick a section.

The terminal structure of these fibers, which is difficult to observe by light microscopy, could be studied more in detail by the electron-microscopic observation of an ultra-thin section from the same tissue block as used for the light microscopy.

Fig. 16 shows the discontinuous appearance of peripheral fibers less than $0.25\ \mu$ in diameter in the myocardium. This picture also indicates that the terminal fiber is composed of minute particles of silver salt. It is mainly fibers approximately $0.025\ \mu$ in diameter that chains of silver particles deposited on.

Fig. 17 shows that the terminal portions of these fibers, as indicated by arrows, twist and wind conspicuously.

Figs. 18 and 19 are electron photomicrographs represented by metallic silver particles of high electron density. They show the dispersed (discontinuous) appearance of those fibers which finally form a fine network on the sarcolemma.

When these fibers distributed in the cardiac muscle were not blackened with silver and examined by the electron microscope, it is impossible to observe the distribution and running of these fibers so clearly as shown in the silver-blackened ultra-thin section.

Fig. 20 was taken from a section of the cardiac muscle fixed in the weak fluid of KAWAHARA. As indicated by arrows, these fibers are presented as a continuation of particles in the electron photomicrograph of mild contrast.

Fig. 17. Cardiac muscle of an eel. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrograph of silver-blackened cardiac muscle. The portions indicated by white arrows show that the discontinuous picture of the main trunks of these fibers has been made visible by metallic silver particles deposited on them. In the portion indicated by black arrows, there are fine twisting and winding peripheral fibrils (less than $0.025\ \mu$ in diameter). The continuous appearance of these fibers has been made possible in this photograph, which was taken from one of the sections cut in parallel with the plane on which these fibers were laid
 $\times 30,000$

Figs. 18 and 19. Cardiac muscle of an eel. Stained by KAWAHARA's silver staining method No. 4. Electron Photomicrographs of silver-blackened tissue. These pictures show the deposition of silver-salt particles on those fibers which are in the sarcolemma of the myocardium. Chains of these particles have formed terminal net works. $\times 30,000$

Fig. 20. Cardiac muscle of an eel. Fixed in KAWAHARA's weak fluid. Chrome shadowing. Electron photomicrograph of an ultra-thin section. This picture does not show the appearance of such distinct fibers as those of the silver-blackened cardiac muscle, but the presence of particles forming fibers, as indicated by arrows. These fibers are composed of a substance different from that of the cardiac muscle which has a periodical structure. $\times 30,000$

d) *Bovine cardiac muscle.*

The bovine cardiac muscle blackened with silver was examined by the electron

microscope. As a result, it has been demonstrated that the fibers less than 0.02μ in diameter are of low electron density also in cattle, and that they can be made visible only when silver-salt particles are deposited on them.

Fig. 21 is a light photomicrograph taken from a thin section. It shows that only

Fig. 21. Cardiac muscle of a cow. Stained by KAWAHARA's silver staining method No. 4. This picture shows the dispersed distribution of the terminal portions of the fibers, as presented in a thin section. $\times 300$

22

23

Figs. 22 and 23. Cardiac muscle of a cow. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrographs of silver-blackened fibers. This pictures shows the state of formation of fibrous terminal networks by independent or beaded metallic silver particles. $\times 30,000$

the terminal portions of these fibers come in contact with the sarcolemma. The main trunks of these fibers are discontinuous in this picture.

Then the terminal portions of the fibers were observed electron-microscopically in one of the ultra-thin sections cut in parallel with, and very close to, the plane on which the light-microscopic section had been prepared (Figs. 22 to 24).

Fig. 24. Cardiac muscle of a cow. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrograph of the terminal portions of silver-blackened fibers. This picture shows the branching terminal portions of fibers of low electron density in the sarcolemma, and terminal networks formed by silver particles deposited on the fibers. $\times 30,000$

Figs. 23 and 24 show structures consisting of spot-like particles. They are electron-microscopic views of silver-salt particles deposited on the fibers. Terminal fibrous networks formed by chains of particles deposited on the fibers are also seen. These networks are found exclusively in the sarcolemma. Their fibrous structure, confirmed by the deposition of metallic silver particles, indicates clearly that they are composed of some other substance than that constituting the muscle filament.

Particularly in Fig. 24, silver particles deposited on the fibers and particles forming the fibers are connected with one another into chains. These chains build terminal apparatuses (0.02μ in diameter), with branches given off at their tips, on the surface of the sarcolemma. Furthermore, fiber-forming particles are scattered in the picture. They are related to the building of fine networks.

e) *Cardiac muscle of the dog.*

Fig. 25. Cardiac muscle of a dog. Stained by KAWAHARA's silver staining method No. 4. This picture shows a discontinuous appearance of the peripheral portions of silver-blackened fibers. $\times 300$

Fig. 26. Cardiac muscle of a dog. Stained by KAWAHARA's silver staining method No. 4. Electron photomicrograph of silver-blackened fibers of the cardiac muscle. The peripheral portions of these fibers have been made clear by the deposition of silver-salt particles of high electron density. The intertwining of peripheral fibers distributed in the sarcolemma, is shown in the picture. $\times 30,000$

The silver-blackened fibers of dog cardiac muscle, when examined by the light microscope, show a discontinuous appearance even in a relatively thick section selected from among those prepared by the method of cutting ultra-thin sections. Therefore, it is impossible to study the systematic arrangement of the main trunks of these fibers in ultra-thin sections.

In Fig. 26, fibers most of which are 0.1μ in diameter have become visible by the deposition of metallic silver. Silver particles have deposited on these fibers in a homogeneous manner. The course of running of winding fibers is shown without interruption in this picture.

There is also a tendency that these fibers are distributed exclusively in the sarcolemma. The arrangement of particles on these fibers exhibits no periodical structure of the fibers.

III. Discussion.

The authors studied the fibers in question using silver-staining methods which they had devised and found some different points of these fibers from collagenous, lattice, and nerve fibers by light-microscopy. The results obtained were published in the following papers: SANO and KAWAHARA (1959), SANO and KAWAHARA (1961, 1962), and SANO, KAWAHARA, HIROKI, KIDO and YOKOTA (1961). All these papers indicated that our silver-blackened fibers (BSF), the presence of which had been demonstrated by our silver-staining methods, Nos. 4 and 5, are completely different from collagenous and lattice fibers. In the present investigation, those fibers (BSF), were examined by the electron microscope.

1. Findings of SF in the heart at early embryonic life.

In the heart at the early stage of embryonic life where no cardiac muscle has been developed as yet, the ultra-microscopic structure of these fibers can be observed, by virtue of blackening with silver, in the interstices where the cardiac muscle is to develop in a later stage.

The fibers in question did not show such basic periodicity as observed in the collagenous fibrils. Moreover, it has been known that none of lattice or reticular fibers, which are stainable by the silver blackening methods of BIELSCHOWSKY and PAP, appear in this stage of development. Accordingly, it is certain that the fibers stained with silver by our method in that stage are neither lattice nor reticular fibers.

The structure of our fibers is represented as chains made of connected fine proteinic particles of low electron density. These fibers do not exhibit such periodical structure as that of the muscle filaments or that of the collagenous fibrils.

In the stage of development, it is not easy to detect any fibrous substance formed by the connection of particles of the nature mentioned above in the cardiac muscle, unless the silver-blackening method is used. Therefore, these fibers were able to be studied only by applying the silver-blackening method to tissue blocks.

2. *Findings on SF in the cardiac muscle treated by our silver-blackening method.*

The cardiac tissue was blackened with silver by the silver-blackening method. Then it was made to ultra-thin sections, which were examined microscopically, much importance being attached to the observation on silver-salt particles. When observed in an ultra-thin section, these fibers are discontinuous. Accordingly, it is difficult to study the state of systematic distribution of these fibers in any electron photomicrograph. Such being the case, the ultra-microscopic structures of terminal fibers and their endings were investigated in detail in the present study.

When examined in ultra-thin sections, these fibers are represented by silver-salt particles of high electron density deposited on them, and a structure made of particles of low electron density. Silver-blackened particles form a conspicuous chain-like structure in the main trunks of these fibers in particular. They are dispersed or arranged in dotted lines in portions close to their endings. On the surface of the sarcolemma of the cardiac muscle, these give rise to ultra-microscopic structures which consist of branches given off by the terminal portions of the fibers or which are formed by fine networks of branches (meshes being approximately 0.010 to 0.025μ in diameter).

In the pictures of the fibers described above there can be recognized no such periodical structure as observed in the striated muscle fibers (muscle filaments) and collagenous fibrils. This is a point at which the SF are distinguished from collagenous fibrils. Since the interstices between the particles forming the fibers are narrow, it is presumed that there may be no other substance sandwiched between the particles and giving a periodical structure to the fibers.

3. *Electron-microscopic theory for the silver-blackening method.*

The picture of our fibers with silver-blackened particles adsorbed on them resembles the electron photomicrograph of an ultra-thin section of silver-blackened reticular fibers. This resemblance suggests that silver-salt particles may be in the state of adsorption in the interstices of the architectonic of proteinic minute particles. It is asserted that the principle of the silver-blackening method is based on physicochemical proof as well as on optical findings.

When the silver-blackening method is applied to SF, erythrocytes, cardiac muscle (actin and myosin of striated muscle fibers), and collagenous fibrils for light

microscopy, they are arranged in the following order so far as the tinctorial property is concerned: silver-blackened fibers (SF, BSF) = erythrocytes > cardiac muscle > lattice fibers = reticulin fibers > collagenous fibrils. The four histological specimens are represented in the color tone of blackish purple, light purple, reddish purple, and red, respectively, when treated with the silver-blackening method.

Moreover, the particles forming these fibers are blackened more intensively with silver than the substance constituting the muscle filament. It can be presumed that the substance constituting these fibers may be a proteinic (polypeptide) substance. This presumption is supported by the results of silver staining and electron microscopy, which have clarified the system to which these fibers belong and which is similar to that of the proteinic substance mentioned above, the degree of electron density, and the state of architectonic of the particles forming the fibers.

IV. Summary.

A specific type of fibers distributed in the cardiac muscle, which can be demonstrated by KAWAHARA's silver staining method No. 4 (1952), were examined by the electron microscope to clarify their fine structure, the process of fiber formation, and their terminations.

1. In the heart at an early stage of embryonic life where no cardiac muscle has been developed as yet, fiber-forming particles of low electron density were connected with one another into chains in the interstices of the cardiac tissue observed in electron photo-micrographs. No reticular fibers appear in this stage of development (methods of BIELSCHOWSKY and PAP). When examined by the electron microscope, none of the fibers in question exhibited such basic periodicity as observed in the collagenous fibrils. Therefore, it may well be that those fibers are different from collagenous and reticular fibers.

2. In the stage of development of the cardiac muscle (in embryos and adults), it is difficult to confirm the presence of the fibers even by electron microscopy. When the silver-blackening method is applied to the cardiac muscle, however, the peripheral and terminal portions of the fibers in the architectonic of the cardiac muscle can be recognized even in this stage.

3. These fibers are of low electron density. When silver-treated, they are recognized as beads of silver particles deposited in the interstices of the cardiac tissue.

4. The terminations of the fibers are mostly located in the sarcolemma and are represented by a fine network or branching structure, both of which are formed by chains of silver particles of high electron density and a fiber-forming substance of low electron density.

5. In ultra-thin sections, the trunks of our fibers appear only discontinuous. The silver particles deposited on the fibers are arranged chiefly along the long axis of the fibers. The intervals between silver particles deposited on these fibers were relatively narrow.

6. None of the fibers in question showed periodic structure as in the collagenous fibrils. They were also distinguished from lattice fibers by using our silver staining

method. By employing our silver staining method, the fibers in question were observed in the myocardium of the heart in the earliest stage of embryonic life.

内 容 自 抄

脊椎動物の心臓と血管現象の形態学的一環研究として、血管系に検出される銀黒線維を著者らの銀染第4法で追究中の筆者らは、すでに心筋の銀黒線維微細構築の所見を第3報(1962)で詳述したので、今回は心筋に分布する銀黒線維の粒子構成、線維形成ならびに終末装置の超微細構造を電顕的に調べた結果を報告する。

1. 心筋がまだ形成されず、また、膠原原線維や網質線維が現われない胎生初期の心臓では、その組織の空隙部に、電子密度の低い粒子の線維状連鎖が見られ、これは蛋白性微粒子の連鎖像らしく、そして筋フィラメントや膠原原線維のような周期構造は示さない。

2. 心筋構成初期には、銀黒法を用いない限り、このような粒子結合の線維様物質の検出は出来ない。

3. 銀黒法を施した心筋の超薄切片では、本線維は電子密度の高い銀粒子の沈着像と電子密度の低い粒子構造から成り、銀粒子の連鎖は、特に主幹線維に強く、線維の終末遠位では散在性もしくは点状性の連鎖になる。

4. 心筋線維の筋鞘の表面では、本線維の終末枝の分枝と細網形成(遠位では $0.01-0.025\mu$ の網目)により超微細の終末装置がえられる。

5. 超薄切片で見ると、主幹線維の形像は断続的に現われるが、終末装置の観察は容易である。

6. 本線維内の銀沈着の電顕像は膠原原線維のものに似るが、よく見ると、本線維の粒子はおもに長軸の方向に排列する。またそれが周期構造を示さないこと、その粒子の間隙が比較的狭いこと、構造が単調であることなどからみると、この線維は膠原原線維、網質線維、膠原線維と本質的に相異することが明らかである。

7. 銀粒子が吸着するところは、蛋白性と見られる微粒子の連鎖の間隙であることが明確で、その吸着模様は膠原原線維の場合と同様である。

以上のように、本線維の銀黒化したものでは電子顕微鏡内で電子密度の高い銀粒子像とその低い線維粒子の連鎖との組み合わせとして現われ、その線維の末梢には超微細終末網が構成されている。

かくて、本線維は光学顕微鏡の所見(既報)から推測されていたように、膠原原線維、網質線維、膠原線維と本質的に異なる線維系であることが確められた。

References.

Ashley, C. A. et al.: J. exp. Med. 94 (1951). — Azumi, G. and A. Tomita: (Jap.) Med.

and Biol. 33 (1954). — **Fuziwara, T.** and **E. Chiuma** : J. Electronmicrosc. 1 (1953). — **Fuziwara, T.** : Proc. Reg. Conf. Elec. Microsc. Asia and Oceania. 1965. — **Gross, J.** and **F. O. Schmitt** : J. exp. Med. 88 (1948). — **Hanson, J.** and **H. E. Huxley** : Symp. Soc. exp. Biol. 9 (1955). — Symp. Soc. exp. Biol. 9 (1955). — **Haruyama, H., G. Kawahara,** and **T. Sano** : (Jap.) Bull. Tokyo Med. Coll. 17, Suppl. (1959). — **Hatakeyama, T., G. Kawahara,** and **T. Sano**, : (Jap.) Proc. Kanto District Division of Jap. Anat. Soc. 1954. — **Hodge, A. G.** : J. biophys. biochem. Cytol. 2 (1956). — **Huxley, H. E.** : Biophys. biochem. Acta 12 (1953). — J. biophys. biochem. Cytol. 3 (1957). — **Kawahara, G.** : (Jap.) Proc. Med. Soc. of Tokyo Med. Coll. 1954. — **Kawahara, G.** and **T. Sano** : (Jap.) Proc. Med. Soc. of Tokyo Med. Coll. 1956. — (Jap.) Proc. Jap. Anat. Soc. 1957. — **Kazikawa, K.** : Juzenkai Med. J. 59 (1957). — **Matsuda, M., T. Hatakeyama,** and **G. Kawahara** : (Jap.) Proc. Jap. Anat. Soc. 1956. — **Roza, G., A. Szent-Györgyi** and **R. W. G. Wyckoff** : Exp. Cell Res. 1 (1950). — **Sano, T.** : (Jap.) Proc. Med. Soc. of Tokyo Med. Coll. 1957. — **Sano, T.** and **G. Kawahara**, : (Jap.) Bull. Tokyo Med. Coll. 17, Suppl. (1959). — (Jap.) Bull. Tokyo Med. Coll., 19, Suppl. (1961). — Arch. histol. jap. 22 (1962). — **Sano, T., G. Kawahara, T. Hiroki, Y. Kido** and **M. Yokata** : (Jap.) Bull. Tokyo Med. Coll. 19, Suppl. (1961). — **Seki, M.** : Arch. histol. jap. 7 (1954). — **Shinohara, M.** and **G. Kawahara**, : (Jap.) Proc. Med. Soc. of Tokyo Med. Coll. 1954. — **Shinohara, T., M. Matsuda,** and **G. Kawahara** : (Jap.) Proc. Kanto District Division of Jap. Anat. Soc. 1955. — **Spiro, D.** : Exp. Cell Res., 10 (1956). — J. biophys. biochem. Cytol. 2 (1956). — **Szent-Györgyi, A.** : Chemistry of muscular contraction. New York, Academic press, 1951. — **Tomita, A.** Osaka Univ. Med. J. 7 (1955).