Microcinematographic Observation on the Behavior of the Connective Tissue Cells in the Spread and Cultured Materials

Gako JIMBO, Kazuo SODA, Katsu KAMEDA,
Toshio KANTANI, Moichi FUJIWARA, Tadao KUBO,
Shiro KAMEI and Kazuko KIMURA
Department of Anatomy (Director: Prof. Gako Jimbo, M. D.), Yamaguchi Medical School, Ube, Japan
(Received July 29, 1963)

CONTENT

I. INTRODUCTION

Tissue culture originated in Harrison's experiment in 1907 when he succeeded in developing isolated nerve fibers in the lymph from a frog. Later the efforts of Carrel, Burrow, Ebling and Fischer and others established the foundation of the methods of culturing. In addition, as the result of the study of a great number of the investigators, the culture of any tissue or any cells of a tumor of various kinds of animals or a human body is within possibility.

There is no evidence, however, the cells or tissue in culture, which lie under a different environment, grow in just the same way as those would if they remained in the living body. Hence the data collected through the study of the tissue culture are not necessarily applicable to those in the living body. Tissue culture has cer-

tainly many advantages. For example, any tissue or cells can be observed in the living conditions, especially by means of time lapse cinematography, even the slow movements of the cytological details in tissue culture can be investigated in a mechanically accerated form, which hardly could be imitated in any other fields where only dead tissues are dealt with. On the other hand, phase contrast microscopy requires, from the optical point of view, a thin spread with a sufficient flat surface, whereas tissue culture requires thick plasma clot and a glass instrument admitting air space for oxygenation. These, indeed, are far from easy in handling. Furthermore, the advance of culture along its procedure from the cover slip culture, flask culture, then to the roller-tube culture, brings about, in the culture equipment, changes in form——from the hollow-ground microscope slide, Carrel's flasks, and again to the test tube, causing their handling still more difficult, where the oil immersion technique in observation seems next to impossibility. Hence the reports on the microscopic observation utilizing the technique at home and abroard are few and far between. Among what few have been published recently are: Frederic and Chevremont: The Movement of Mitochondria, Makino: Nuclear Division of Karyokinesis, Nakai: Nerve Cell, Barski, Robineaux, Endo respectively: Poliovirus, Kaneda: Agranulocyte, Okano: The Influence of Poliovirus upon the Cultured Cells, Kawai and Yashima: On Cell Division, Hashimoto: On Culture of Epidermis, and Fujinami: On Cancer Cell Division. Every one of the above was observed either with the aid of low magnification microscopy or as a thin layer of spread material. None of them, therefore, is reported to have taken a recourse to an oil immersion microscopy based on such a roller-tube culture after the roller-tube method.

Yodoi (1951), however, invented a metal culture observing apparatus utilizing a condenser of long focal length (manufactured by the Olympus Co.), with its fitting metal fixture and a heating device attached to it. By means of this instrument he adopted a hanging-drop culture which admitted a sufficient air space and continued his observation for a long period of time. The metal culture apparatus which Yodoi worked out consisted of a metal part, its main body and a glass disk 4.5 cm in diameter or a metal small disk 9 mm in diameter which was only to be used when a high power of magnification was applied.

The metal part consisted of two divisions, the upper one occupying the culture-room, the lower was the moval section and setting up the border between them; there was a glass disk which was to be fitted in from below and sealed up with paraffin. The top of the culture room was all made of metal with a hole in the center and the lower part demarcated with the glass-disk mentioned above.

Jimbo, G. and his co-workers: Kazuo Soda, Katsu Kameda, Toshio Kantani, Moichi Fujiwara, Tadao Kubo, Shiro Kamei, Kazuko Kimura investigated the behavior of connective tissue cells making use of revised Dr. Yodoi's tissue culture apparatus.

II. MATERIAL AND METHOD

(a) Metal Culture Apparatus

Without using the condenser of long focal length, we, co-workers, improved the apparatus which Yodoi worked out; that is, getting rid of the movable part, leaving the culture-room alone and making it as thin as possible.

As Figure 1, 2, 3 shows, the top part was made of metal with a hole in the center and the lower part of the disk. The diameter of the hole for specimen was 1.4 cm. The metal part was made of aluminium which was given a finishing touch with alumite; whereas the culture-room, at just right moment of cooling period after the dry heat sterilization, was applied with paraffin. Further, on the side wall of the metal part was made a small hole 1 mm in diameter (Fig. 2), through which nutrious or medicinal fluids for study were injected by a syringe. After the work was done, it was also covered with paraffin.

(b) Spread Materials

After a mouse was killed, its back subcutaneous connective tissue was out off and spread on a cover slip as soon as possible and the cover slip was inverted over a slide in which there was a hollow ground filled with a culture fluid——a mixture of tyrode's solution, beef serum and chick embryo extract at 7 : 2 : 1 and the fibrocytes and histiocytes were observed by phase contrast microcinematography.

(c) Cultured Materials

(1) How to Prepare Embryonic Juice.

Some eggs which had been placed in the incubator for from 7 to 9 days were taken up and turned into chick embryo extract, which was frozen and fused twice, precipitated by centrifugalization, then kept in a dark cool place.

(2) How to Accompish Culture.

(a) Method of Plasma Clot Utilization

The cultivation was carried on in hood which had been sterilized with a sterilizing lamp. On the cover glass we let fall a drop of chick embryonic juice and a drop of plasma, the mixture of which was spread thinly and turned into a plasma clot. The thinly sliced bits of tissue were arranged in 2 mm square over the clot and the cover glass with the clot on was inverted over the culture apparatus mentioned above.

(b) Method of Enzyme Utilization

In this culture bits of tissue were treated with trypsin. As the culture fluid, the

mixture of L. A. solution and beef serum at the rate of 8 : 2 was used. Since trypsin is subjected to hinderance of its activity by Ca^{++} and Mg^{++}, 0.25 % of tyrode's solution which was got rid of Mg^{++} was used. First to the warmed trypsin as much as 37°C. the bits of tissue which was washed and thinly sliced in L. A. solution was added. This mixture was repeatedly sqeezed out by absorption by means of a pippet for 5 minutes. This solution was centrifuged, rotating 1500 times in about 3 minutes. After the upper clear fluid was removed, the rest which was in the same way digested twice with L. A. solution was added to the culture fluid, which was made into a uniform floating fluid. Then it was placed in the culture implement, sealed and laid still in the incubator 37°C. for about 20 hours. The cells which were fixed on the cover glass were observed by phase contrast microscopy, the details being put on record.

(3) Photography

Photographing was carried on with the aid of phase contrast microscopy in the thermotic-room at 38°C. (Fig. 4). A still photograph was taken automatically by motion picture cameras with its timer at the interval of 8 seconds after the time lapse cinematography.

III. SPREAD MATERIALS

The connective tissue cells shown here were survivals of the cells of living tissue, not those which started anew in the culture. The authors, however, consider, since they had stromata such as fibers, that they were more natural than the cultured cells.

(a) Forms and Movement of Normal Histiocytes in the Subcutaneous Connective Tissue

Many of the cells were similarly circular in form and the borders of the cytoplasms were clearly serrated. The nuclei were somewhat one-sided. In the spacious protoplasm minute granules of intermediate brightness were scattered and several vacuoles varying in size were visible congested, chiefly toward the borders of the cytoplasms. Many of their mitochondria were short rods or dots, giving an impression of somewhat hardness. Though their distribution was somewhat denser around the nuclei, they were almost evenly scattered in the cytoplasm. The nuclear membrane was high in refractivity and clear. The nuclei were ovoid or kidney-shaped in form. Either of them, however, slight in karyolobism and rather flat. The density of the substances forming the network of the cell nucleus was high, hence their refractivity was high. The network was rather sparce and a round nucleolus of intermediate brightness was seen in it (Fig. 5).

As to the movement of the histiocytes, their pseudopodia and processes were divergent in form ——a club-shaped, needle-pointed, serrated, membranous, antenna-like, or bubble-like and they were slow in motion. When they were observed for a long period of time, their expansion and contraction, stirring or appearance and disappearance could be seen. Further, the processes of these pseudopodia proved sometimes of one kind, but many of them were of various kinds mixed. As they moved little by little the transformation of the cytoplasm was observed. And granules in the cytoplasm, too, were observed to move, though very slowly.

(b) Forms and Movement of a Normal Fibrocyte in the Subcutaneous Connective Tissue

The cytoplasm which was long oval in shape, sent out a branch-like processes before and behind. In the cytoplasm, slight large uniform granules of intermediate brightness were seen. Their distribution was rather denser toward the border of the cytoplasm and in the processes, but they were scarce around the nucleus. Mitochondria were in groups, scattering here and there in the cytoplasm, though not necessarily dense around the nucleus. Their forms were also large and various in shape——from large dots, rods to filaments. The nucleus was rather strong in brightness and the nuclear membranes were clear and flatter. The nucleoles from 5 to 6 were scattered in the nucleus, strong in brightness and seemed floating over the surrounding substance forming the network of the nucleus (Fig. 6).

(c) Addition of the Drugs

Removing a bit of the subcutaneous connective tissue from a mouse and spreading it on a coverglass, the authors placed the coverglass inverted on the slide-glass with the hole which contained the culture medium (a mixed fluid of an extract of chick embryonic tissue, tyrode's solution and hen blood serum at the ratio of 1 : 7 : 2). They added cortisone (10 γ per medium 1 cc) to it and investigated it by the aid of a 16 m/m apparatus of film production and found fibrocytes with constricted processes having a round head looking as if it were a snake's head (Fig. 7) and the protoplasms contracted and roundish, making a movement as if they were twisting themselves.

Next the authors added butter yellow (10 γ per medium 1 cc) to it and investigated it by the aid of a 16 m/m apparatus of film production and found the fibrocytes somewhat contracted with a round process looking as if it were an adballoon flying in the sky (Fig. 8) and that the round processes were brisk in motion. Mitochondria were hardly seen, but round fat bodies were observed.

In the series of this experiment, however, no bubbling was seen at all.

(d) Applying the Drug on the Skin of Animals

After applying the solution of 1% butter yellow olive-oil on the back of a mouse once every day for a week running, the authors removed a bit of the subcutaneous connective tissue of the back, spread it out on the cover glass. The cover glass was placed inverted on the slide-glass with the hole which contained the culture medium (a mixed fluid of a extract of chick embryonic tissue, tyrode's solution and hen blood serum at the ratio of 1 : 7 : 2). The authors investigated it by the aid of a 16 m/m apparatus of film production and observed distinctly that out of the fibrocytes there appeared and disappeared briskly large membraneous pseudopodia like leukocytes (Fig. 9).

IV. CULTURED CELLS

(a) Types of Cellular Movements

The fibrocytes which grew in the culture of chick heart tissue, after about 24 hours' culturing, sent out vigorously new-growths of cells around the chick heart tissue. Near the plasma clot was seen a broad layer of proliferation zone. The fibrocytes were extended extremely slender, ranging in a row lengthwise; they ran on and on outward and toward the periphery. As the result, the culture tissues generally assumed an efficient radial cell-arrangement (Fig. 10). Many of the wandering cells were scattered around the most exterior periphery, leaving spaces among one another and most energetic ones extended so much that their processes were moving nearly within the reach of one another. Hence the proliferation zone of the latter expanded exceedingly.

The fibrocytes in their movement formed processes ahead. The shape of the processes were variable: Some were a broom or fatsia-japonicalike, possessing branches (Fig. 11) and some were like a knob of a Japanese bridge post or the tip of a writing-brush in form, and but few were of spindle-shape which is common in many of these types of cells and others which were curious and slender ones looking as if, stretching their arms extremely, they had been chasing game. Of course, these processes might be taken for making on scouting service, seeking nourishment or not few of them might be for making contact with one another. And they were most varying in length, too, —— from very short ones which were sent out across the intercellular spaces to such long and slender ones which looked like tapes stretched between two cells, pulling against each other (Fig. 12). In course of time, these slender tape-like processes snapped as an elastic band does. While the cells were moving, they changed in form. When some other cells approached, they made their protoplasm stretch out showing apparently signs of capturing them. But instead of surrounding them, they contracted the lengthened protoplasms again.

(b) The Movement of the Granular Substances in the Cell Body

As to the movement of the granular substances in the cell body, the granules in its processes, as Canti observed by means of dark field microscopy, were making a reciprocating movement; whereas the ones which swarmed around the nucleus showed nothing but a simple gregarious movement as if they had been crowding together (Fig. 13). Among those which swarmed around the nucleus, however, though few in number, were some which were going into the processes or some which were making a reciprocal movement, only the difference between them being in the distance of their locomotion or in the amplitude thereof. All these granules are to be considered substantially as one and the same. Their movements were regarded to differ according to the positions and environment they were placed. And all the granules with their respective movements seem to act quite independently; their movements are regarded to be self-operating. Further in the cell bodies which were brisk in motion there were found numerous granules and their movements in reciprocation, too, were brisk. And the number of these granules are considered to have a close relationship with the growth and proliferation of the cell body. These granules showed a sensitive reaction and as the living activity degenerated, first they became slow in motion till they ceased to move.

(c) The Vacuoles in the Cell Body

After about 5 days in tissue culture there appeared a lot of speherical vacuoles in the cytoplasms, with images of an acute degeneration (Fig. 12). With the lapse of time these vacuoles gradually increased in size. The granules which became honey-combed or reticular were thrust away to around the vacuoles, assuming a ring-like formation. After about 8 days in tissue culture, the vacuoles became larger and larger and fused into giant vacuoles in the cytoplasms.

(d) Bubbling

(1) Observation of Fibrocytes after the Application of Cortisone.

a) The observation of fibrocytes after the application of cortisone $1/10\,\gamma$.

On the surface of cells there appeared and disappeared processes as if they had been first thrust out. The bubbling of this sort was certain to retract and never discharge concrete objects. The bubbles of this sort which appeared and disappeared out of the cells were a few in number and slow in motion. The time of appearing of a single bubble was 66.4 seconds (Fig. 13).

b) The observation of fibrocytes after the application of cortisone $1\,\gamma$.

Outside one cell not a few of the bubbles which were fast in motion appeared and disappeared. The time of appearing of one bubble was 48.0 seconds.

c) The observation of fibrocytes after the application of cortisone 10 γ.

The bubbling phenomenon was most active and its period was short. The bubbles appeared compactly around the whole circumference. The time of appearing of a bubble was 30.4 seconds on the average. What was specially noteworthy was that there also appeared bubblings just like a firerocket displayed. The time of appearance of a bubble of that sort was 192 seconds (Fig. 14).

(2) Observation of Glucuronic Acid-Administered Fibrocytes.

(A. 1) Observation of the fibrocytes immediately after the application of glucuronic acid 1 mg.

Their cytoplasms contracted, the processes remaining rodlike and moving (Fig. 15). The granules gathered around the nucleus and their motions were not active.

(A. 2) Observation of the fibrocytes 1 day after the application of glucuronic acid 1 mg.

On the surface of the cells the cellular processes made frequent appearances as if they had been fists thrusted out. These "bubbles" were certain to retract and once separated, they never discharged any concrete objects. "Bubbles" pretty short in period were observed. The time of the appearance of a "bubbles" was 32 seconds and many vacuoles were seen, too. The granules gathered around the nucleus and their motions were not active.

(A. 3) Observation of the fibrocytes 2 days after the application of glucuronic acid 1 mg.

"Bubbles" rather short in period were observed. The time of the appearance of one "bubble" was 40 seconds. Besides, some "bubbles" looking like sky-rockets were observed, too. The time of the appearance of these "bubbles" was 40 seconds.

Plenty of large bright vacuoles were seen in the protoplasm.

(B. 1) Observation of the fibrocytes immediately after the application of glucuronic acid 1/10 mg.

The bubbling came and went slowly. The time of appearance of one "bubble" was 56 seconds (Fig. 16).

(B. 2) Observation of the fibrocytes 1 day or 2 days after the application of glucuronic acid 1/10 mg.

Granules swarmed around the nucleus and their motions were active, but no "bubbling" was observed.

(C. 1) Observation of the fibrocytes immediately after the application of glucuronic acid 1/100 mg.

Granules swarmed around the nucleus and their motions were active. No "bubbling" was observed.

(C. 2) Observation of the fibrocytes 1 day or 2 days after the application of glucuronic acid 1/100 mg.

Granules swarmed around the nucleus and for the rest, they remained almost normal.

(D. 1) Observation of the fibrocytes immediately after the application of glucuronic acid 1/1000 mg.

Granules gathered around the nucleus and their motions were fairly active.

(D. 2) 1 day or 2 days after the application of glucuronic acid 1/1000 mg.

Granules gathered around the nucleus and their motions were fairly active.

(E. 1) In the case where glucuronic acid 1/10 γ was administered.

No "bubbling" was observed and the motions of the cells were normal.

(3) Observation of the Fibrocytes after the Application of Thioctan.

(A. 1) Observation of the fibrocytes immediately after the application of thioctan 1 mg.

Large "bubbles" appeared slowly. The motions of the granules were active. The time of the appearance of one "bubble" was 72 seconds.

(A. 2) Observation of the fibrocytes 1 day after the application of thioctan 1 mg.

Small "bubbles" short in period appeared. The time of the appearance of one "bubble" was 24 seconds.

(B. 1) Observation of the fibrocytes immediately after the application of thioctan 1/10 mg.

"Bubbles" short in period appeared. The time of the appearance of one "bubble" was 32 seconds.

(B. 2) Observation of the fibrocytes 2 days after the application of thioctan 1/10 mg.

"Bubbles" short in period appeared and disappeared. The time of the appearance of one bubble was 16 seconds. Extremely many vacuoles were seen.

(C. 1) Observation of the fibrocytes 1 hour after the application of thioctan 1/100 mg.

Every condition of the cells was the same as in the normal case.

(C. 2) Observation of the fibrocytes 3 hours after the application of thioctan 1/100 mg.

Large "bubbles" appeared and disappeared quickly. The time of the appearance of one "bubble" was 48 seconds.

(C. 3) Observation of the fibrocytes 1 day after the application of thioctan 1/100 mg.

All the fibrocytes cultured were found dead.

(D. 1) Observation of the fibrocytes 1 hour after the application of thioctan 1/1000 mg.

The "bubbling" appeared quickly. The time of the appearance of one "bubble" was 48 seconds.

(D. 2) Observation of the fibrocytes 1 day after the application of thioctan 1/1000 mg.

The "bubble" was seen. The time of the appearance of one "bubble" was 40 seconds.

(E. 1) Observation of the fibrocytes 2 hours after the application of thioctan 1/10 γ.

Granules swarmed around the nucleus. These granules did not move very actively. Neither "bubbling" nor vacuole was observed.

(F. 1) Observation of the fibrocytes 2 days after the application of thioctan 1/100 γ.

They were normal.

(F. 2) Observation of the fibrocytes 2 days after the application of thioctan 1/1000 γ.

Granules swarmed around the nucleus.

(G. 1) Observation of the fibrocytes 2 hours after the application of thioctan 1/10000 γ.

Granules swarmed around the nucleus.

(G. 2) Observation of the fibrocytes 1 day after the application of thioctan 1/10000 γ.

Granules swarmed around the nucleus.

(4) Observation of the Fibrocytes after the Application of Neston.

(A. 1) Observation of the fibrocytes 2 hours after the application of Neston 1 mg.

No "bubbling" was observed. Granules swarmed around the nucleus. The motions of the granules were inactive; they did not move much.

(A. 2) Observation of the fibrocytes 1 day after the application of Neston 1 mg.

The cells actively sent out pseudopodium. Large "bubbles" appeared and disappeared. The time of the appearance of one "bubble" was 40 seconds.

(A. 3) Observation of the fibrocytes 2 days after the application of Neston 1 mg.

Many of small "bubbles" quickly came and went. The granules moved but a little. Extremely many round vacuoles of various sizes were observed, but they did not move.

(A. 4) Observation of the fibrocytes 4 days after the application of Neston 1 mg.

Many vacuoles were observed. The granules swarmed around the nucleus and all of the cells were found dead.

(B. 1) Observation of the fibrocytes 2 hours after the application of Neston 1/10 mg.

Granules swarmed around the nucleus. Neither "bubbling" nor vacuole was yet observed.

(B. 2) Observation of the fibrocytes 1 day after the application of Neston 1/10 mg.

A few "bubbles" were observed. Granules swarmed around the nucleus. No vacuole was seen.

(B. 3) Observation of the fibrocytes 2 days after the application of Neston 1/10 mg.

A small number of vacuoles were observed in the protoplasm and all the cells were dead.

(C. 1) Observation of the fibrocytes 3 hours after the application of Neston 1/100 mg.

Large "bubbles" appeared and disappeared. The time of the appearance of one "bubble" was 40 seconds. Granules swarmed around the nucleus. No vacuole was observed.

(C. 2) 1 day after the application of Neston 1/100 mg.

Granules swarmed around the nucleus. The granules moved a good deal. No. "bubbling" was observed.

(C. 3) 2 days after the application of Neston 1/100 mg.

All the cells were dead.

(D. 1) 1 hour after the application of Neston 1/1000 mg.

The granules were normal. Neither "bubbling" nor vacuole was observed.

(D. 2) 1 day after the application of Neston 1/1000 mg.

Granules swarmed around the nucleus and moved a good deal.

(D. 3) 2 days after the application of Neston 1/1000 mg.

Ditto.

(D. 4) 3 days after the application of Neston 1/1000 mg.

The granules swarmed around the nucleus, but their motions got inactive.

(D. 5) 4 days after the application of Neston 1/1000 mg.

The fibrocytes were dead.

(E. 1) 3 hours after the application of Neston 1/10 γ.

The fibrocytes were normal.

(E. 2) 1 day after the application of Neston 1/10 γ.

Pseudopodia were formed vigorously.

(F. 1) 3 hours after the application of Neston 1/100 γ.

Granules densely swarmed on one side of the nucleus. Their motions were not active.

V. CONCLUSION

Stated in the following are the findings of the time lapse microcinematographic observations on the behavior of the connective tissue cells in the spread and cultured materials.

(1) Removing a bit of the subcutaneous connective tissue from a mouse and spreading it on a coverglass, the authors placed the coverglass inverted on the slide-glass with the hole which contained the culture medium (a mixed fluid of an extract of chick embryonic tissue, tyrode's solution and hen blood serum at the ratio of 1 : 7 : 2). They added cortisone to it and investigated it by the aid of a 16 m/m apparatus of film production and found both histiocytes and fibrocytes with constricted processes having a round head looking as if a snake's head and the protoplasms contracted and roundish, making a movement as if they were twisting themselves.

(2) The authors added butter yellow and found the fibrocytes somewhat contracted with a round process looking as if it were an adballon flying in the sky and that the round processes were briskly in motion.

(3) After applying the solution of 1 % butter yellow olive-oil on the back of a mouse once every day for a week running, the authors removed a bit of the subcutaneous connective tissue of the back, spread it out on the cover glass. The cover glass was placed inverted on the slide-glass with the hole which contained the culture medium (a mixed fluid of an extract of chick embryonic tissue, tyrode's solution and hen blood serum at the ratio of 1 : 7 : 2). The authors investigated it by the

aid of a 16 m/m apparatus of film production and observed distinctly that out of the fibrocytes there appeared and disappeared briskly large membraneous pseudopodia like leukocytes.

(4) The fibrocytes which were grown out of the cultured chick embryonic heart tissue were applied with cortisone. When cortisone 1/10 γ was applied, a small number of bubbles slowly appeared and the time of appearing a bubble was 66.4 second. Next when applied with cortisone 1 γ, the phenomenon of bubbling was the most active and the period short and compact around the cells there appeared bubbles. The time of appearing of a bubble was 30.4 second on the average. What was specially noteworthy was that there also appeared bubblings just like a firerocket displayed. The time of appearance of one bubble in this case was 192 seconds.

(5) The fibrocytes which were given rise to by the culture of the chick embryonic liver were administered glucuronic acid, thioctan or Neston and by the aid of the apparatus of film production of phase-contrast-microscopy, they were recorded and investigated. While the cells were energetic, the granules therein were scattered through the length and breadth of the cells and their motions were active. When the cells became less energetic, "bubbling" made frequent appearances, the motions of the granules grew inactive and some vacuoles were observed. 1) One day after glucuronic acid 1 mg was applied, "bubbling" appeared and continued 2 days. Besides, no cell was found dead. Although "bubbling" appeared immediately after the application of the grucuronic acid 1/10 mg, no "bubbling" was observed when more than 1 day elapsed after its application. Even when the acid diluted less than 1/100 mg was added, no "bubbling" was observed, either. 2) When thioctic acid 1 mg, 1/10 mg, 1/100 mg, or 1/1000 mg was applied, immediately after the application, 1 day later or even 2 days later the "bulling" was observed, but the acid diluted less than 1/10 was applied, no "bubbling" was observed. 3) When Neston 1 mg., 1/10 mg, or 1/100 mg was applied, the "bubbling" appeared, but 2 days or 4 days after the application all the cells were found dead. However, when Neston diluted less than 1/1000 mg was added, no "bubbling" was observed.

(6) The fibrocytes with the plasma clot as the center were arranged in a radial cell-arrangement. The movements of granules in the cytoplasms of the fibrocyte can be broadly divided into two—one group of great reciprocating movement and the other of small gregarious movement. Where there were many granules which performed reciprocating movements, the cell bodies themselves were brisk in motion.

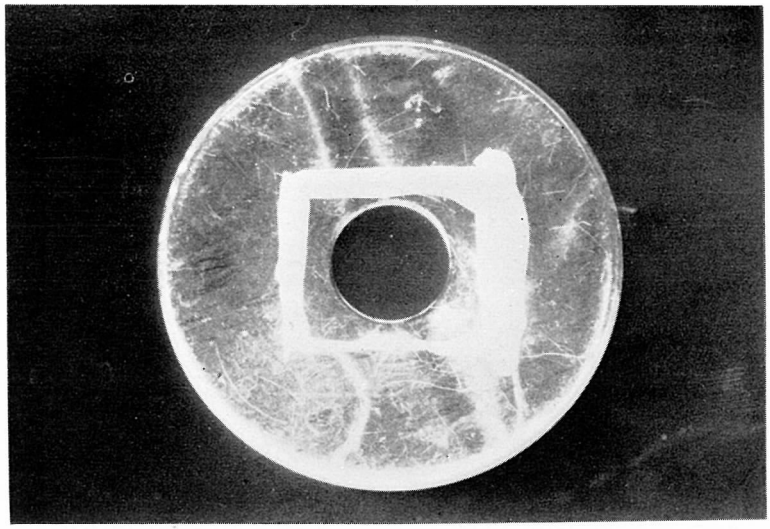
VI. REFERENCES

1. Barski, G., Robineaux, R. & Endo, M.: *Ann. New York Acad. Sci.*, **61** : 899, 1955.
2. Canti: *Archiv. f. exp. Zellforsch.*, **8** : 133, 1926.

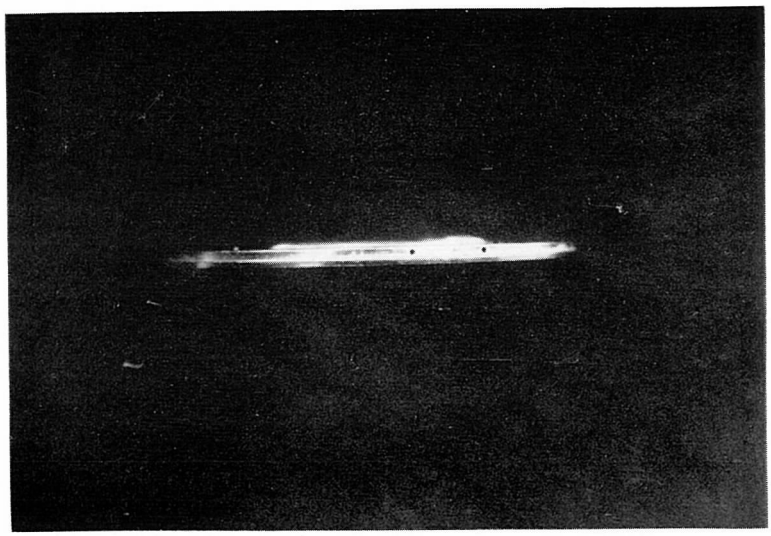
3. Fujinami, T.: *Jap. J. Dermat. and Venereaol.*, **6** : 111, 1956.
4. Frederic, J. M. & Chevremont: *Arch. Biol.*, **63** : 109, 1952.
5. Hashimoto, Y.: *Jap. J. Dermat.*, **68** : 155, 1958.
6. Hashimoto, Y.: *J. Showa med. Ass.*, **18** : 715, 1958.
7. Kamura, J.: *J. Okayama med. Ass.*, **72** : 845, 1960.
8. Kaneda, H.: *Acta anat. jap.*, **30** : 68, 1955.
9. Kasahara, T.: *J. Showa med. Ass.*, **20** : 119, 1960.
10. Kawai, K. and Yajima, T.: *Trans. Soc. path. Jap.*, **46** : 373, 1957.
11. Makino, S.: *Int. Rev. Cytol.*, **6** : 25, 1957.
12. Nakai, J.: *Am. J. Anat.*, **9** : 81, 1956.
13. Okano, K.: *Acta pathol. Jap.*, **6** : 415, 1956.
14. Yamachika, Y.: *J. Okayama med Ass.*, **70** : 3145, 1958.
15. Yodoi, T.: *Handai Isi* (Medical Journal of Osaka University Japanese Edition), **8** : 301, 1956.
16. Yodoi, T.: *Handai Isi* (Medical Journal of Osaka University Japanese Edition), **10** : 2253, 1958.

EXPLANATION OF PLATE FIGURES

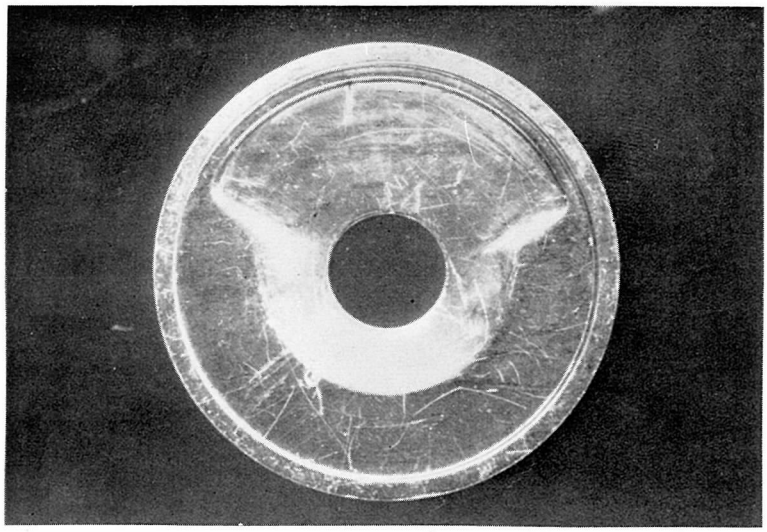
Fig. 1. Revised Yodoi's tissure culture apparatus. (dorsal surface)
Fig. 2. Revised Yodoi's tissure culture apparatus. (side surface)
Fig. 3. Revised Yodoi's tissure culture apparatus. (abdominal surface)
Fig. 4. Thermotic room.
Fig. 5. Normal fibrocytes in the spread material.
Fig. 6. Normal histiocytes in the spread material.
Fig. 7. Fibrocytes with constricted processes having a round head looking as if it were a snake's head.
Fig. 8. Fibrocytes some what contracted with a round process looking as if it were an adballoon flying in the sky.
Fig. 9. Out of the fibrocytes there appeared and disappeared briskly large membraneous pseudopodia.
Fig. 10. The migrating zone of fibrocytes.
Fig. 11. The shapes of the processes were broom like.
Fig. 12. Processes were most varying in length—from very short ones which were sent out across the intercellar spaces to such long and slender ones.
Fig. 13. The swarming granules images in the nuclei and processes of fibrocytes.
Fig. 14. Granules and vacuoles in the cell body.
Fig. 15. Fibrocytes after the application of cortisone 1/10 γ. Out side one cell not a few of the bubbles were appeared and disappeared.
Fig. 16. Fibrocytes after the application of cortisone 10 γ. Bubbles just like a fire rocket were appeared and disappeared.
Fig. 17. Fibrocytes immediately after the application of glucuronic acid 1 mg. The cytoplasm contracted, their processes remaining rod like and moving.
Fig. 18. Fibrocytes immediately after the application of glucuronic acid 1/10 mg. Bubbles were observed.



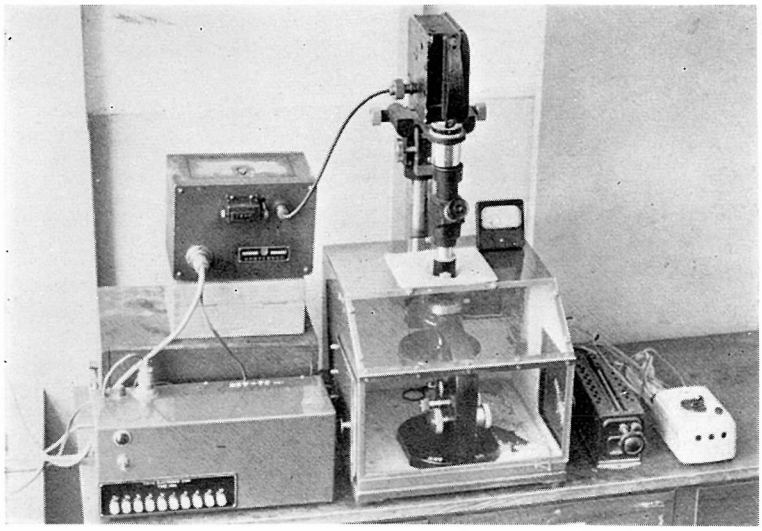
1



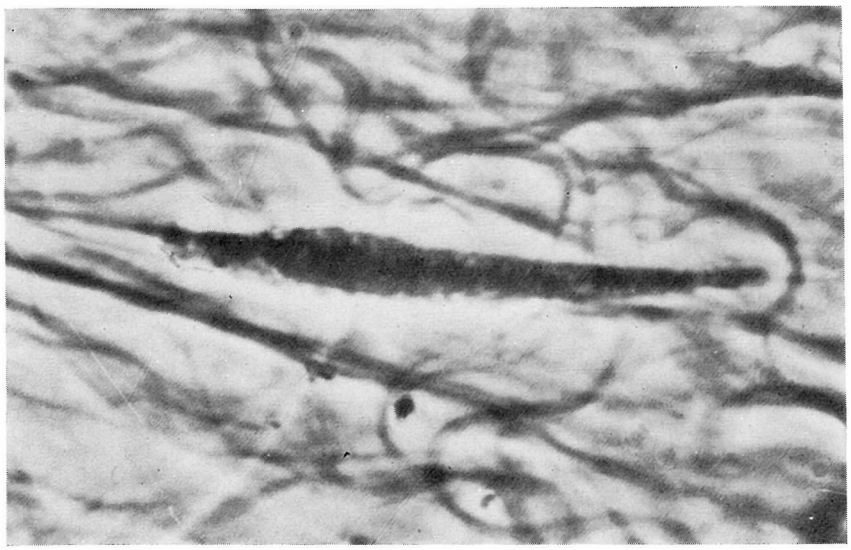
2



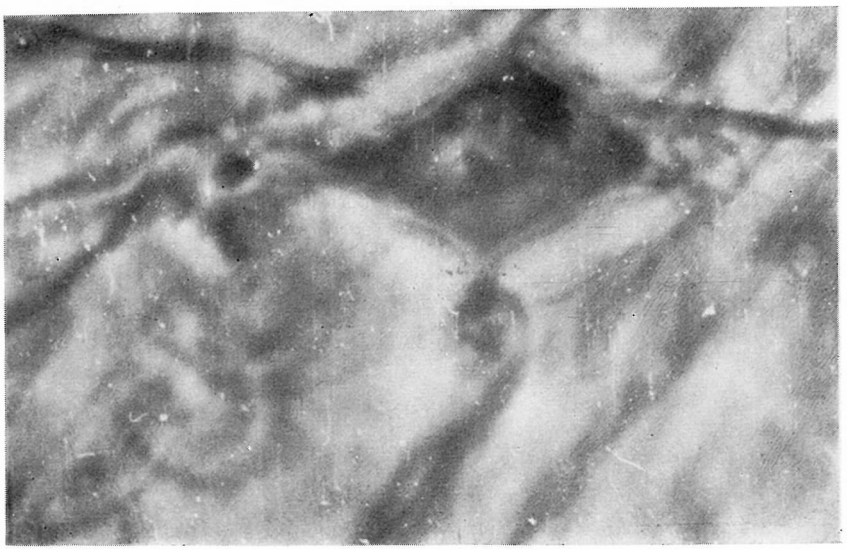
3



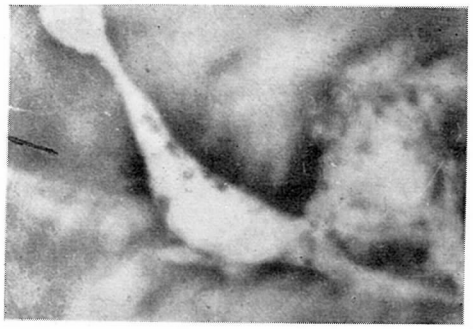
4



5

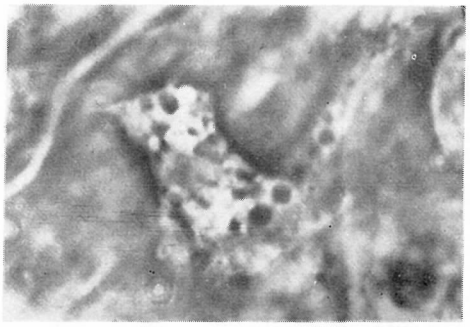


6

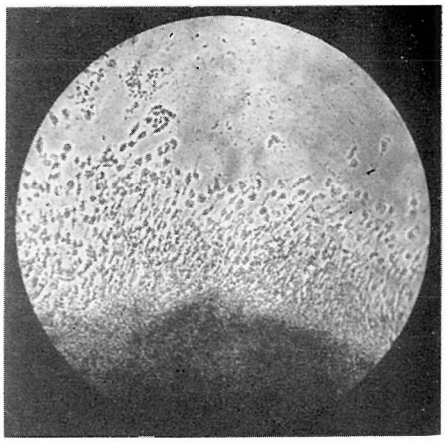


7

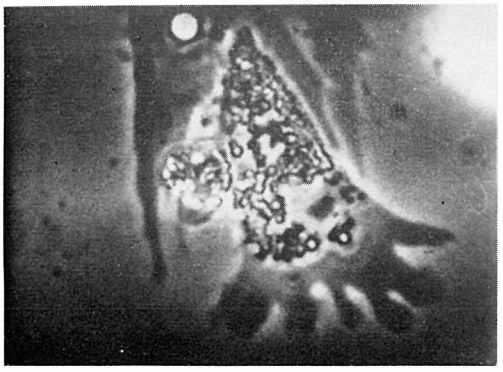
8



9

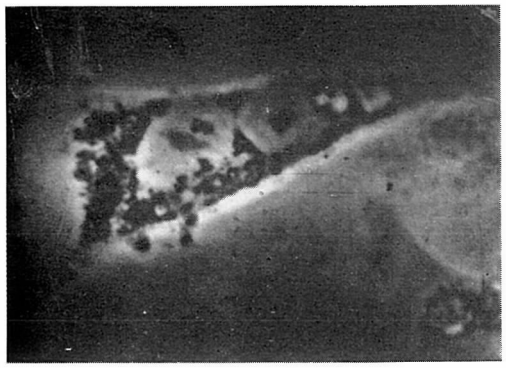


10

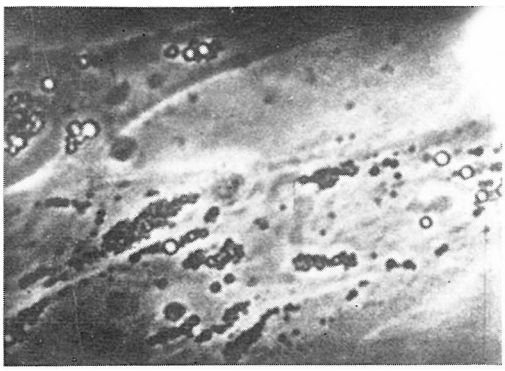


11

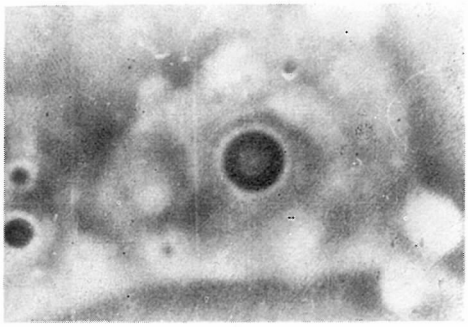
12



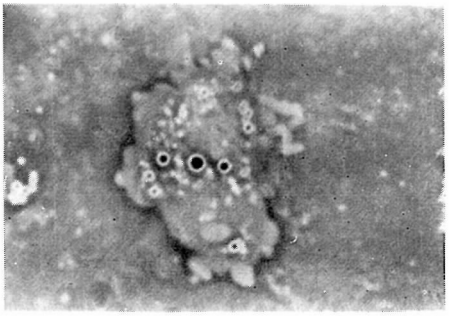
13



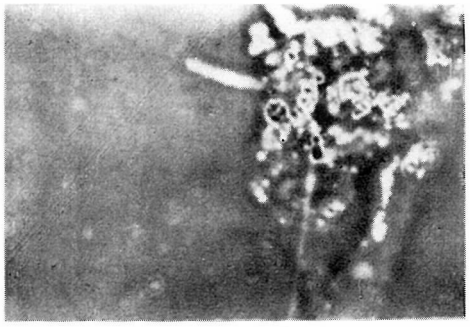
14



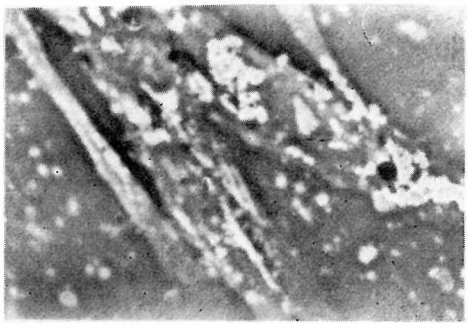
15



16



17



18