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The Milk-spots, "Tâches Laitieuses" in Omentum. Histological and Biological Studies Made Especially on Their Mesothelial Cell-layer and Histiocytes.

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The great omentum requires further investigation, and the most interesting point hinges upon the study of its milk-spots, "tâches laiteuses."

The study of the "vital staining" has been markedly improved, and it has thrown some light upon the origin of the milk-spots. However, there still remain many questions. In this paper, it is intended to deal further with the subject of the milk-spots from the point of the histology and biology.

I. Method; especially on the "Silver chloride method" newly discovered by the author.

The rabbit was used for this experiment. The animal was sacrificed by bleeding from the carotid artery. The abdominal cavity was opened. The omentum was totally removed and placed in ten per cent solution of formalin for one day. It was washed thoroughly in running water and examined its extended specimen as well as its paraffin serial sections stained with Delafield's hematoxylin-eosin.

Various modifications have been devised by the others, since the method of silver impregnation was first attempted by v. Recklinghausen in 1860, but none of them proved to be free of artifacts, and one is apt to misinterpret such a resulting artifact. However, any misinterpretations of that kind are avoidable, if the comparative study be made on the results from the following three different methods.

1. Vital silver impregnation:—After fixed a rabbit on its back on a table, about 300 to 400 c.cm. of 0.1 per cent solution of silver nitrate was injected into the abdominal cavity. About forty minutes later, it was sacrificed by bleeding. The abdominal cavity was opened. The omentum was totally removed and after it was placed in a quite sufficient amount of ten per cent solution of formalin for a day, was washed in running water for a day, keeping always in the dark. Then the omentum exposed in the diffuse sunlight, until it had become brown in color. When the technique has been perfectly done, the cement substance of the peritoneal mesothelium will be seen as a distinct line. The colouring by the silver nitrate on the protoplasm of the mesothelial cell is very light and consequently it does not affect the subsequent staining with hematoxylin-eosin.

2. Supravital silver impregnation:—The same method as that of vital impregnation was used, except the silver nitrate injection was made after the animal was sacrificed by air-embolus.

3. Impregnation on the excised omentum:—As a generally used silver impregnation stains the cement-line very poorly and irregularly, and particularly is it so in

the case of the cement-line of the milk-spots which are minutely and complicately constructed, the author finally discovered a new method, "silver chloride method," after many failures. The method as follows:—

(1) The removed omentum is divided into several pieces of a suitable size and rinsed in normal saline solution for about ten minutes. By this treatment the section will take a sufficient amount of NaCl.

(2) The solution is then substituted with 0.1 per cent aqueous solution of silver nitrate, so that the milky cloudiness of silver chloride appears around the section.

(3) It is then incubated at 40°C. for about forty minutes.

(4) Transferred in ten per cent solution of formalin.

(5) Thoroughly washed in tapped water.

(6) Reduced by diffuse sunlight.

(7) Differentiated by one per cent solution of aqua ammoniac for a few minutes.

(8) Washed in water and stained with hematoxylin.

The specimen thus obtained shows its cement-substance as dark lines brown to black in color.

Appendix:—Origin of the cement-line.

The definite conclusion as to the origin of cement-substance of pavement cell has not yet been reached. The theory hitherto mentioned is as follows:—The cement-substance is a semi-fluid and occupies the inter cellular space of the pavement cells. Combining with silver nitrate the albumin in the cement-substance forms an insoluble substance, viz., the silver albuminate, which is the substance reduced to brownish color in the sunlight.

By the vital silver impregnation in my experiments, the abdominal cavity is always filled with quite sufficient amount of milky fluid and the peritoneal surface also became opaque in color. Such milky coloration of the solution injected and of the cement-substance always parallels; namely, when the former was lightly colored, the latter was also colored lightly and when the former was deeply colored, the latter was also in deep color.

The above rabbit was substituted with frog and treated with 0.1 per cent solution of silver nitrate similarly as above-mentioned. On this occasion, no milky color could be seen either in the injected solution or on the peritoneal surface and so the cement-substance was not reduced to brown in color. However, if 0.2 per cent instead of 0.1 per cent solution of silver nitrate was used, milky color could be seen in the injected solution as well as on the peritoneal surface, as in the above rabbit's case.

Considering from this fact, it may be definitely said that the impregnation with silver nitrate of the cement-substance is principally due to an intercellular precipitating of the silver chloride which is chemically composed of the sodium chloride exist in the semi-fluidy matrix and of the injected silver nitrate. From this presumption,

the above-mentioned "silver chloride method" was here brought forth and experimentally assured that this view is quite right.

Achard and Aynaud described that the sodium chloride in the serous membrane plays an important rôle on the impregnation. They confirmed that the impregnation on the cement-substance cannot be obtained, when the sodium chloride is removed from the tissue by either Glauber's salt (sodium-sulphate) or sugar-solution. This fact furthermore affirms that the above view is correct.

II. The milk-spots.

1. Gross finding:—

Removing the great omentum as a whole from the great curvature of the stomach, gastro-splenic omentum, gastro-colic omentum and the hepato-colic ligamentum, it was stretched on the slide. There is a main trunk of blood-vessels parallel with and two or three centimeters apart from the gastro-epiploica. In its course branches are derived and distributed to the main part of the omentum.

It is convenient to observe the great omentum in two parts, the upper small and the lower large, dividing along the main trunk of the blood-vessels. The upper small has a few net-meshes and the milk-spots are finely constructed similarly as in the case of mesentery. On the contrary, the lower large has many net-meshes and the milk-spots are numerous and in various sizes. This lower large part is of very importance from both the physiological and pathological stand points.

In the young age, the milk-spots appear mostly as groups of so called adventitia-cells (by Marchand) along this main trunk of the blood-vessels. The fatty tissue is very scanty. However, in the adult age, the fatty tissue mostly substitutes for the groups of the adventitia-cells and consequently, the milk-spots existing only along the distal branches of the blood-vessels are well developed and they were named "primary milk-spots" by Renaut. The typical primary milk-spots are sharply differentiated from the circumference and some possess spherical tufts of blood-capillaries. The milk-spots existed in such an area where is not supplied with the blood-capillary were specially called "secondary milk-spots" by Renaut and they are mostly occupied the lower middle part of the omentum.

It must not be disregarded that the development of the milk spots is sometimes very indefinite in the same species in nearly the same age.

2. Histological appearance:—

The great omentum is consisted of four layers; two outer and two inner lamellae. The former belong to the wall of the greater sac of the peritoneal cavity and latter, to that of the lesser sac of the same cavity. The area between the outer and inner lamellae is occupied by scattered cell-elements that in places group in situ. These groups or islets are the so called "tâches laiteuses."

There have been many investigations on the main cell-element of the milk-spots and several literatures of them will be quoted here.

In 1863, v. Recklinghausen described on the cloudy spots of the omentum of a rabbit which consisted of the connective tissue-cells in various shapes. In 1877, the cloudy spots were furthermore investigated by Ranvier and he named them "tâches laiteuses" or "milk-spots." He regarded that the cell-element of the milk-spots is one of the connective tissue-cells—the "clasmatocyte"—by him. "It was characterized by its clasmatosis-phenomenon, viz., the protoplasm with many granulae is devided into several pieces." Renaut 1893 opposed the Ranvier's opinion. Considering the morphology and evolution of the cell-element of the milk-spots, he stated that it belongs to one of lymphocytes. "Wandering into the omentum and resting there, the lymphocyte takes part of the formation of the connective tissue-cell of the omentum." "With the consideration that this lymphocyte has a secretary function just like the other connective tissue-cells, he named it "cellules rhagiocrines." Marchand 1901 emphasized that the milk-spots are consisted of some connective tissue cells, and that they are quite similar to the adventitia-cell named by him. Maximow 1902 and Tschaschin 1913 viewed that the cell-element of the milk-spots is one of the histogenic wandering cells, resting in that region, but by a inflammatory irritation it wanders freely, returning to it fundamental nature. It is what is called Maximow's "resting wandering cell." Schwarz 1905 said that the milk-spots are consisted of the mononuclear wandering leucocytes. Weidenreich 1911 regarded that the omentum is one of lymphatic apparatus and therefore it is similar to the reticulum of the lymphatic node, and accordingly the tâches laiteuses are corresponded to the secondary nodules.

Although there had been many investigations on the main cell-element of the milk-spots, it has not been dissolved, untill, recently, based upon the studies of the vital staining of Goldmann, Kiyono, Pappenheim and Fukushi, a definite conclusion was reached. Kiyono emphasized that the milk-spots are consisted mostly of the so called "histiocytes" (by Kiyono), positive by vital staining, and partly of those cells as lymphocytes, plasmacytes, and a few polymorphonuclear leucocytes, eosinophilic leucocytes those that are negative by vital staining. He pointed out that without doubt a part of Ranvier's clasmatocytes and Renaut's cellules rhagiocrines, most of Marchand's adventitia-cells and all of Maximow's resting wandering cells belong to the histiocyte.

Notice: An article, entitled "the development of the milk-spots in great omentum" by the author is to be published later and in which on the cell-element of the milk-spots will be discussed more in detail.

III. Morphological observation on the mesothelial cell of the milk-spots.

In 1860, the silver impregnation was first discovered by v. Recklinghausen and three years later he published a famous article "On the Fat-absorption." For nearly six decades since that time, there had been many investigations upon the peritoneal mesothelium. The studies, however, as that of v. Recklinghausen, have been limited mostly to the peritoneal surface of the diaphragm, and attention has not been paid much to the mesothelial cells in the great omentum, in the mesentery, and particularly in the peritoneal surface of the milk-spots.

Ranvier and Schwarz said that the milk-spots are covered always with the continuation of a mesothelial cell-layer. In an article "On the stomata of serous cavities" Walter described, "Diese Gebilde (milchflecke) stehen mit dem Thema "Stomata" in kleiner direkter Beziehung."

In the extended specimen of the omentum treated with the solution of silver nitrate, it is found that the mesothelial layer is generally consisted of large flattened polygonal cells arranged each other in close apposition like a tiled pavement. The cement-line between the mesothelial cells is generally finer, and its net-work is more irregular than that in the case of mesentery. The thickness of the cement-line is definite and either the so called "stomata" or "stigmata" cannot be found in a normal condition. The mesothelial cell on the surface of the milk-spots is quite smaller than that on the other places where there is no milk-spot existed. The former cell is nearly from one half to one fifth in diameter as small as the latter one. Such mesothelial cells on the milk-spot, especially those of the outer lamella are very irregular in shape and accordingly the cement-lines are indefinite and make an irregular net-work. It is noticeable that among these mesothelial cells there are seen many apertures, the small rounded inter-cellular spaces, and some peculiar cells that look like epithelioid cells.

The aperture is generally round in shape and its size ranges from three to ten microns in diameter. These apertures are stained relatively light brown in color but deeper than the protoplasm of the mesothelial cell and they are encircled distinctly with a line which stains similar to the cement-substance, but very small apertures represent as deep brown puncta. It is of much interest that some apertures have a marked refractive appearance and that some large ones possess in their subjacent area nuclei of cells which are rich in chromatin substance. These nuclei can scarcely be found in an extended specimen, but are shown clearly in the section embedded in

paraffin.

The epithelioid cells are limited to the surface of the milk-spots, some are scattered and some are grouped. These cells range from seven to twenty microns in diameter and are mostly round or oval in shape, rarely triangular or stellated. The nuclei are rich in chromatin substance and mostly in oval or kidney-shape, rarely in horse-shoe-shape and are smaller than those of the mesothelial cells. The protoplasts are stained in light brown by silver nitrate and some refract light strongly. Sometimes giant cells with two nuclei are seen.

From the above view two questions will arise.

(1) Are the apertures on the surface of the milk-spots same with the so called "stomata" which are a subject under consideration among many authorities?

(2) What are the epithelioid cells that exist among the mesothelial cells of the milk-spots?

These questions will be discussed in the following investigations.

IV. Are the aperture of the milk-spots same with the so called "stomata"?

1. Literature.

It was considered by Galen, Hippokrates and etc. that the endings of lymph- and blood-vessels have freely opened apertures. In 1863 v. Recklinghausen further investigated on this subject from his study on the absorption of fat. Pouring some amount of milk on the peritoneal surface of the removed diaphragm of a rabbit, he microscopically observed that the fat-droplets were accumulating to several points and flowing into subjacent lymph vessels in whirls. When the milk was substituted with the solution of silver nitrate, the cement-substance gradually came in sight. He found the sites of the whirls covered with small mesothelial cells, and that there were round swellings on the cement-line, especially on their cross points. Some swellings were of circular in shape. He called these swellings "Öffnungs." "The enlarged "opening" was larger than the largest milk droplet." Since he announced the above opinion there appeared various investigations on the stomata of the serous membranes.

Considered from v. Recklinghausen's opinion, the stomata must be a freely opened aperture of communicating with a lymphatic vessel and of possessing an absorptive function. Later, however, the swellings of the cement-line have been generally called

"stomata" in wider sense, and its absorptive function has been disregarded.

Oedemansson, Dybkowsky, Lavdowsky, Klein and Burdon-Sanderson, Ranvier, Skworzow, Toldt, and Norris agreed with the v. Recklinghausen's theory, though there are a little differences in opinions. But Auerbach, Frey, Afonassiew, Walther, Tourneux, Foà, Arnold, Rajewsky, Bizzozero, Grasser, Fleiner, Kolossow, Muscatello, Sulzer, Ussow, Walter and Y. Tanaka denied the above view.

The data of the latter investigators will be described briefly. (1) The existence of the so called "stomata" is indefinite on the peritoneum and has no definite situation in the mesothelial cell-layer, and is sometimes detected in the cytoplasm (Afonassiew, etc.). (2) There is a marked difference in the size and in the number of the so called stomata in the specimens selected from nearly the same parts of the peritoneum of the animals in the same species and of nearly the same ages (Kolossow, Muscatello). (3) When the technique of the silver impregnation has been very carefully done, especially to avoid an over-stretching, no stomata can be seen in situ (Muscatello, Kolossow, etc.).

Based upon the above facts, the latter authorities agreed that the v. Recklinghausen's stomata are nothing but an artificial product and accordingly they are not normally existed. However some investigators occasionally found a few stomata even though the silver impregnation had been perfectly done. With regard to its occurrence, Muscatello illustrated that such stomata are made by the wandering cells which emigrate into the peritoneal cavity, penetrating the mesothelial cell-layer and thus temporarily making many minute openings in situ. Still Kolossow and Walter pointed out that with the respiratory movement of the animal, the mesothelial cell-layers, above all, those of the diaphragm and of the intercostal spaces expand and contract, and consequently the mesothelial cells, especially on the cross points of the cement-line disunited. They also deny the communication of the stomata with the subjacent lymph-vessel.

Comparison of the peritoneal surface of the milk-spots with that of the diaphragm:—

It is not useless to compare the peritoneal surface of the milk-spots with that of the diaphragm, because the stomata of the serous membranes have been mainly investigated on the diaphragm.

The material were selected from the animals treated with the vital or supravital silver-impregnation. The special staining of the diaphragm was as follows:—The animal was sacrificed by bleeding. The table on which the animal was fastened was arranged so as to keep the diaphragm always in the horizontal position. After the abdominal organs were totally removed 0.1 per cent solution of silver nitrate poured on the surface of the diaphragm and left it in the dark. After ten to fifteen minutes the above solution was substituted with ten per cent

solution of formalin, thus fixing the diaphragm in situ. Thereafter, the diaphragm was totally removed and reduced in the similar method as previously described. The removed diaphragm was used partly for the celloidin section and partly for the extended specimen. The latter specimen was made transparent with the mixed solution of the equal parts of glycerin and acetic acid.

Histological appearance:—

The surface of the diaphragm is covered with a mesothelium consisted of pavement cells closely attached each other. It is noticeable that the cells which occupy the spaces in the radiated tendon-fasciculi considerably decrease their size, but their shapes remain unchanged.

Ranvier discovered the deposition of many small rounded cells in the peritoneal surface of the diaphragm, particularly in the spaces in the radiated tendon-fasciculi and he named it "lymph-fountain." Later, the Ranvier's lymph-fountain was called "proliferating center" by Teurneux and Hermann. Bizzozero and Muscatello discovered some leucocytes among the mesothelial cells on the peritoneal surface of the diaphragm. According to Walter and others, there are a few apertures among the mesothelial cells which occupy the spaces in the radiated tendon fasciculi.

In my normal materials, no aperture, lymph-fountain nor proliferating center can be seen on the peritoneal surface of the diaphragm, but it is noticeable that a few epithelioid cells which resemble to those of the milk-spots are detected among the mesothelial cells, particularly in the spaces in the radiated tendon-fasciculi. Still, a few small rounded swellings are detected on the cross points of the cement-lines of the peritoneal layer in these spaces.

The further details of lymph-fountain and of the proliferating-centre will be written in a later paper.

2. Is the aperture of the milk-spots an artifact or not?

It has hitherto been viewed by many authorities that a certain artificial products of the cement-line due to the silver impregnation were frequently mistaken for the stomata. Such misconception is, however, easily avoidable by above-mentioned three methods of silver impregnation. The cement substance is less distinctly stained by supravital impregnation than by vital impregnation but by either method no abnormality can be seen in both the cement-line and the aperture. The cement-line impregnated after the omentum was excised is generally thicker than that treated by either vital or supravital impregnation. On this occasion, some small swellings colored in dark brown frequently appear on the cross-points of the cement-lines, but none appear on the vital impregnation, so that they are nothing but artificial product.

The so called stomata are not yet definitely concluded and its appearance as well as its location is very uncertain. But, the aperture what is meant by the author are localized definitely in the mesothelial cell-layer of the milk-spots. The apertures in the milk-spots are more numerous on the matured animal than on the younger one and the more the milk-spots become larger and the more their cells increase, the more the aperture usually increase. No typical aperture is detected in the newly born. The

so called stomata seem to select their locations mostly at the cross points of the cement-line, but the apertures in the milk-spots have not such definite choice.

In consideration with these facts, it is sure that the apertures in the milk-spots are not artifacts, but pre-existing figure.

3. Relation between the aperture and the basal-membrane of the omentum.

The basal-membrane, the Bizzozero's "membrane limitans" on which the mesothelial cells are situated has been described by Wodd, Bizzozero, Muscatello and others. According to Bizzozero, the "membrana limitans" is a very thin, homogeneous membrane, but only on the central tendon of diaphragm it has numerous openings which range in size from four to sixteen microns. No literature has hitherto been found as to the basal-membrane of the great omentum. This is because, the author believes, the basal-membrane, being existed in such a thin membrane as the omentum, has made one very hard to investigate on.

In my investigation of the membrane limitans the great omentum was placed in the tapped water for twenty hours and then the "silver chloride method" was applied. It was examined its extended specimen.

It was found microscopically that the mesothelial cell-layer had been removed from the peritoneal surface and a smooth basal-membrane underneath appeared. Such a basal membrane of the great omentum is homogeneous, but the membrane on the milk-spots has many round or oval-shaped spots which stain deep brown in color. These brown spots are five to fifteen microns in diameter. They have dark brown rings and are well-defined from the circumference.

There are no data to indicate that whether or not these brown spots are the openings of the basal membrane and connect with the apertures in the mesothelial cell-layer of the milk-spots. However there may be some connection between these brown spots and apertures, as both of them have almost similar behavior on staining with silver nitrate.

4. Relation between the aperture and the subjacent lymphvessel.

If the aperture in the milk-spots belong to the so called stomata as v. Recklinghausen maintained, they must be connected with the subjacent lymphvessel. To investigate this subject, either five per cent suspension of carmine in normal saline solution or the Indian ink was injected into the peritoneal cavity.

Case 1. On a female rabbit of 1380 gms. body-weight, one c.cm. of the carmine suspension was injected into the peritoneal cavity. One hour later the animal was killed by bleeding.

The removed omentum was partly used for the "silver chloride method," and partly for the serial section embedded in paraffin.

Case 2. On female rabbit of 1340 gms. body-weight, 1.5 c.cm. of the carmin suspension was injected into

the peritoneal cavity. Thirty minutes later, 320 c.cm. of 0.1 per cent solution of silver nitrate was followed. Forty minutes later the second injection the animal was killed by bleeding.

Gross finding:—

The great omentum in both cases, especially the milk-spots stained very poorly in pink color, but the peritoneal layer of the diaphragm and others did not macroscopically stain.

Histological appearance:—

In the serial section (case 1), the fine carmine-masses invaded almost all of the epithelioid cells of the milk-spots. In both the extended specimens treated with silver nitrate, the epithelioid cells were differentiated distinctly with the cement-line and these cells mostly phagocytosed the carmine-masses. However, in the mesothelial cells and in the intercellular substance, no carmine-masses were found.

Case 3. On a female rabbit of 1420 gms. body-weight, ten c.cm. of the carmine-suspension was administered into the peritoneal cavity. Thirty minutes later 330 c.cm. of 0.1 per cent solution of silver nitrate was also injected. Forty minutes after the second administration the animal was killed by bleeding.

Case 4. On a male rabbit of 1355 gms. body-weight, ten c.cm. of the carmin-esuspension was injected into the peritoneal cavity. One hour later the animal was killed by bleeding. The removed omentum was partly used for the "silver chloride method," and partly for the serial section embedded in paraffin.

Gross finding:—

The milk-spots were saturated with abundant carmine-masses and the rest of the omentum also stained deep in carmine-red.

Histological appearance:—

In the extended specimen, the epithelioid cells were so extremely filled with the fine carmine-masses that their nuclei could not be differentiated. Some apertures being filled with carmine-masses enlarged in various grades. The cement-substance in the milk-spots also were filled with the fine carmine-masses, and the mesothelial cells of the omentum did not escape from the invasion of carmine-masses. The cement-line of the omentum was very irregularly reduced and deformed; some of which were thickened, damaged or obscured.

In the vertical serial section, the mesothelial cell-layer showed a diffuse line of fine carmin-masses on its surface but not within its protoplasm. Although, this line slightly sank into the intercellular spaces, the spot did not in any respects look like opening in which free carmine-masses were contained. The macrophages situated just beneath the mesothelial cell-layer markedly phagocytosed and some of them wedged their cytoplasmic processes filled with carmine-masses into the intercellular spaces of the mesothelial cell-layer. Some macrophages connected tangentially with the intercellular

spaces, so that their protoplasts partly appeared on the peritoneal surface through the intercellular spaces. There were considerably many carmine-masses on the mesothelial cell-layer and in the intercellular substance, but freely existed carmine-masses could scarcely be found in the deeper area, consequently these masses seemed hardly to find their ways through the lymph-vessels to the deeper area. The mesothelial surface of some milk-spots, particularly the outer lamellae of the omentum, were covered with a thick layer of the fine carmine-masses. In such figures, the difference between the epithelioid cells and the mesothelial cells was frequently quite hard.

Case 5. As a control. On a female rabbit of 1530 gms. body weight, five c.cm. of the Indian ink was filtered, sterilized and injected into the peritoneal cavity. One hour later the animal was killed by bleeding.

The removed omentum was partly used for the extended specimen and partly used for the serial section embedded in paraffin.

This case showed no particular appearance compared with those of above cases treated with carmine-suspension.

It is concluded from the above facts that the aperture in the milk-spots has no connection with lymph-vessels, but such an aperture is wedged by the process or by the cytoplasm of the subjacent macrophage.

Comparison with the peritoneal surface of the diaphragm:—

Cases 1 and 2. Macroscopically the peritoneal surface of the diaphragm showed no carmine-red. In the extended specimen treated with the mixed solution of glycerin and acetic acid, the epithelioid cells with variable amount of minute carmine-masses were sometimes found on the peritoneal surface of the central-tendon. In the section, the free carmine-masses in the deeper area of the diaphragm were also scarce.

Cases 3, 4 and 5. In the peritoneal surface of the central-tendon, many radiated lines colored carmine-red or black and corresponded to the spaces in the radiated tendon-fasciculi were seen. In the extended specimen, quite a large amount of carmine-masses or Indian ink-granulae were found in the spaces of the radiated tendon-fasciculi and continued to the subjacent lymphvessels which were in irregular net-work. In the section, the peritoneal surface of the central-tendon was regularly waved; the troughs of the waves just fit to the spaces in the radiated tendon-fasciculi. Such spaces were communicated with the lymphvessels situated under the mesothelial cell-layer of the pleural surface of diaphragm and usually contained many carmine-masses. On account of some of these spaces filled with the carmine-masses or Indian ink-granulae, the peritoneal layer consisted of mesothelial cells was very much obscured in places. In the large amount of free carmine-masses or the Indian ink-granulae, several macrophages were found, but the boundaries of which were generally very much obscured.

V. What are the epithelioid cells of the milk-spots?

1. General histological appearance.

The epithelioid cell and the mesothelial cell must be clearly differentiated as there

are differences between the two in the shapes and in the staining.

It is not very hard to find out the epithelioid cell in the specimen stained with hematoxylin-eosin. In this specimen, the protoplasm of the mesothelial cell does not stain with hematoxylin-eosin and so its boundary is very obscured. The nucleus of the latter is large and oval in shape, poor in chromatin substance and distinctly shows one or two nucleoli. On the contrary, the epithelioid cell has a distinct boundary and is mostly round or oval in shape. Its protoplasm, being amphophilic, stained with both hematoxylin and eosin, and therefore appears violet-red in color. Its nucleus is of oval or kidney-shaped, rich in chromatin-substance and smaller than that of the mesothelial cell. Thus, the epithelioid cell is apparently differentiated from the mesothelial cell. Still, the nucleus of the epithelioid cell is larger than that of the lymphocyte and has less chromatin.

In the vertical serial section, of the milk-spots, the epithelioid cell of mostly lens-shaped is slightly protruded from the mesothelial surface and shows characteristic staining. The cells are not always situated exactly in the mesothelial cell-layer, and their protoplasmic processes either appeared on the peritoneal surface or are partly exposed in the peritoneal cavity. There is a peculiar phenomenon in which the epithelioid cell caps on the mesothelial cell, and sometimes causes the surface of the latter slightly subside. A narrow pellucid zone is shown between the two. Such a cell sometimes is artificially displaced from its normal position and leaves depression on the peritoneal surface (see Fig. 7). The nucleus of the epithelioid cell is mostly situated basally and when the nucleus is of kidney-shaped its convex side usually faces to the periphery (see Fig. 9).

Notice: Ranvier discovered some lymphocytes among the mesothelial cells in the omentum of normal rabbit impregnated with silver nitrate. It may be suggested that his material is not quite normal, because he described, "..... Beim Kaninchen besonders sind sie (lymphzellen) zuweilen so zahlreich, dass die Peritonealflüssigkeit dadurch milchig getrübt wird." He still said, "Beinahe bei allen Kaninchen sind im grossen Netz isolierte Cysticerken vorhanden." In my material, however, such lymphocytes could not be detected.

2. Where does the epithelioid cell belong?

The epithelioid cell has a round or oval-shaped nucleus which is frequently depressed in one side and relatively rich in chromatin substance. Its protoplasm is ampho- or basophilic. From these facts the epithelioid cell must be a kind of histiocyte. To investigate this subject the vital staining was first applicated.

Four per cent solution of lithium-carmin was injected by graded dosis once a day into the auricular vein, the average dosis being about four c.cm. per Kilo of body weight. The injection was continued six or seven times and 24 hours later from the last administration the animal was killed by bleeding. The tissue was preserved in

ten per cent solution of formalin in the dark for one or two days. It was then thoroughly washed in tap water and stained with hematoxylin.

Case 1. A female rabbit of 1725 gms. body-weight. The total amount of the carmine-solution injected was estimated 45.5 c.cm. in seven days.

Case 2. A male rabbit of 1630 gms. body-weight. The total amount of the carmine-solution injected was estimated 41 c.cm. in six days.

Case 3. A female rabbit of 1400 gms. body-weight. The total amount of the carmine-solution injected was estimated 40 c.cm. in seven days.

Case 4. A female rabbit of 1250 gms. body-weight. The total amount of the carmine-solution injected was estimated 31 c.cm. in six days and 24 hours after the last injection 350 c.cm. of 0.1 per cent solution of silver nitrate was administered into the peritoneal cavity and one hour later killed by bleeding.

Case 5. A female rabbit of 1420 gms. body-weight. The total amount of the carmine-solution injected was estimated 40 c.cm. in seven days and then it was similarly treated as above.

Case 6. A male rabbit of 2600 gms. body-weight. The total amount of the carmine-solution injected was estimated 60.5 c.cm. in seven days. 30 minutes later the last injection the animal died and about 20 minutes later 500 c.cm. of 0.1 per cent solution of silver nitrate was administered into the peritoneal cavity for the supravital impregnation.

Case 7. A female rabbit of 1860 gms. body-weight. The total amount of the carmine-solution injected was estimated 40 c.cm. in six days and then similarly treated as case 4.

The results of the above experiments will be summarized as follows:—

Cases 1, 2 and 3. In the extended specimen, some cells in the milk-spots strongly positive with the vital staining are irregularly shaped and their protoplasmic processes are extended in various directions. Each cell contains a flattened nucleus rich in chromatin substance. The protoplasm of these cells is hardly differentiated from the circumference but its boundary is scarcely recognized by an arrangement of the carmine-granulae. On the surface of the milk-spots, there are round or oval-shaped cells and their protoplasm is slightly basophilic, and consequently the boundary of the cells is well-defined. These round cells generally have less carmine granulae than the above cells and their nuclei are of oval or kidney-shaped rich in chromatin substance.

From these observations, it is ascertained that the two kinds of cells above-mentioned belong to the histiocyte by Kiyono. The one which is strongly positive with vital staining belongs to the histiocytic cell or so called clasmatocyte by Ranvier and the other which is weakly positive with the vital staining belongs to the histiocyte. The latter is quite the same with the epithelioid cell found by the author in the specimen treated with silver nitrate.

In the serial sections, almost all of the epithelioid cells are readily made out by their characteristic staining, shapes and situations. They are weakly positive with the

vital staining, but some of them which are partly protruded to the peritoneal cavity are infrequently negative. The histiocytic cells, however, are strongly positive with the vital staining and situated in the deeper layer of the milk-spots. These cells are especially, found more frequently in the peripheral than in the central area of the milk-spots. Some histiocytic cells just beneath the mesothelial cell-layer, make, sometimes, a row parallel to the latter, and some others situated diagonally to the latter extend their processes between the mesothelial cells.

Cases 4, 5, 6 and 7. In the extended specimen, the epithelioid cells contain some carmine-granulae in their protoplasm, but it is quite difficult to detect, because the protoplasm has been stained in brownish color by silver impregnation. The apertures in the mesothelial cell-layer infrequently show a few carmine-granulae.

Thus it is confirmed that the epithelioid cells are mostly positive with the vital staining but rarely negative. The negative one can not be distinguished from the histiocyte, because it is partly protruded to the peritoneal cavity and therefore is not sufficiently supplied with the carmine-solution introduced into the blood circulation. This will be illustrated by the following experiment.

Case 8. A female rabbit of 1390 gms. body-weight. Six c.cm. of two per cent solution of lithium-carmine was injected into the peritoneal cavity and 24 hours later it was killed by bleeding.

In the abdominal cavity, a small amount of viscous exudate was found. The great omentum was stained deep in red with carmine-solution and its lower parts were strongly adhered together.

Case 9. A male rabbit of 1300 gms. body-weight. Three c.cm. of the above solution was administered and then treated as above.

In the abdominal cavity a small amount of exudate was found. The omentum was stained in deep carmine-red. But on this occasion the adhesion of the lower parts of omentum was not so strong as that of case 8.

Case 10. A male rabbit of 1320 gms. body-weight. In this case the quantity of the carmine-solution was diminished to 2.5 c.cm.

The intra-abdominal appearance was almost similar to that of the above case, but the adhesion of the omentum was less marked than that of case 9.

Case 11. A female rabbit of 1540 gms. body-weight. Two c.cm. of the solution was used in this case. The great omentum tinged pink, but the adhesion existed very slightly.

Case 12. A male rabbit of 1250 gms. body-weight. 1.5 c.cm. of the solution was used in this case.

Only the milk-spots in the omentum appeared in pink color, the rest being very poorly stained.

Case 13. A female rabbit of 1580 gms. body-weight. Dosis ditto.

The intra-abdominal appearance was similar to that of the above case.

Case 14. A female rabbit of 1400 gms. body-weight. Dosis ditto.

24 hours later, the vital impregnation with 350 c.cm. of 0.1 per cent silver nitrate solution was done.

Case 15. A female rabbit of 1460 gms. body-weight. It was quite similarly treated as above.

It is well known that the carmine-solution considerably irritates the peritoneal tissue therefore the cases 1 and 2 were not proper on this subject, as they were ad-

ministered too much carmine. But the others, especially cases 12 to 15 treated with quite a small amount of the solution were not so much influenced by the carmine-irritation.

In the specimen treated with the silver nitrate, the epithelioid cells on the milk-spots showed many carmine-granulae and they were well-defined from the mesothelial cells by the cement-line.

The apertures in the mesothelial cell-layer of the milk-spots tended to be enlarged and generally showed carmine-granulae to some extent. Some apertures especially the enlarged ones accompanied the nuclei rich in chromatin substance. The cement-lines in this cases especially those which were strongly irritated by the carmine-solution were irregularly thickened, obscured or disappeared.

In the vertical serial sections, the epithelioid cells were markedly filled with the carmine-granulae, generally enlarged spherically and nearly detaching from the mesothelial cell-layer in large number. On this occasion, the most of the histiocytic cells in the milk-spots were superficially situated and some of those which existed just beneath the mesothelial cell-layer protruded their protoplasmic processes partly through the intercellular spaces.

In consideration with the results of the above two experiments, it may be concluded that the epithelioid cell belongs to the histiocyte. The experiment of Chapter IV, 4 in which the epithelioid cell shows a remarkable phagocytotic ability endorses this opinion.

Comparison with the peritoneal surface of the diaphragm:—

Cases in which the carmine-solution was intravenously injected:—In the extended specimen treated with the mixed solution of glycerin and acetic acid, the epithelioid cells on the peritoneal surface usually possessed a few carmine-granulae in the protoplasm.

Cases in which the carmine-solution was administered into the peritoneal cavity:—

Cases 8 and 9. In the extended specimen, the spaces in the radiated tendon-fasciculi were filled with the histiocytic wandering cells with many carmine-granulae.

Cases 12 to 15. In the extended specimen, the epithelioid cells were numerous and had many carmine-granulae in their protoplasm.

VI. The relation between the aperture and epithelioid cell on the milk-spots.

There are normally many cell-elements in the peritoneal cavity and they mostly belong to the histiocyte. It is generally acknowledged that the most of these histio-

cytes are derived from the milk-spots. The histiocytic cells in the milk-spots becoming round, shortening their protoplasmic processes, always wander into the peritoneal cavity and this action is caused by the physiologic stimulation.

From this fact it may be considered that there is a certain connection between the aperture and the epithelioid cell—the histiocyte which is derived from the milk-spots.

Muscattello described that in the specimen of great omentum and gastrohepatic ligamentum impregnated with silver nitrate, there can be seen many lymph-follicles (the milk-spots) covered on both sides with mesothelial cells and that the mesothelial cells but on one side do not connect each other. From this fact he viewed that the lymph-follicles in the great omentum and gastro-hepatic ligamentum will probably supply the leucocytes to the peritoneal cavity like those of the intestinal wall supplying the leucocytes to the intestinal canal. Although, this Muscattello's view quite agrees with mine, it is regrettable that he has not mentioned as to the intercellular spaces existed in the mesothelial cell-layer.

Chapter III, IV and V are summarized as follows:—

1. Some of the apertures and the epithelioid cells on the milk-spots similarly have a remarkable refractive appearance and consequently these two may be standing in a certain relation. The wandering cell, as Arnold already discovered, usually refract ray of light especially when it penetrating a small space in the epithelial cell-layer more distinctly than it is at rest.

2. In the cases of Chapter IV, 4 and those of Chapter V, 2, some of the apertures also show minute carmine-masses and carmine-granulae respectively and in the vertical sections of the milk-spots these apertures are wedged by the protoplasmic processes of the subjacent histiocytic cells with carmine-masses or carmine-granulae. These histiocytic cells and the epithelioid cells (the wandering histiocytes) may have a certain relation.

3. Some of the large apertures normally have in their subjacent parts the nuclei rich in chromatin substance. This is a transitional form of the aperture—the intercellular spaces in the mesothelial cell-layer wedged by the protoplasmic process of histiocyte—, to the epithelioid cells—the histiocyte existed among the mesothelial cells—, on the surface of the milk-spots.

From three facts, the relation between the aperture and the epithelioid cell has thrown some light and will become clearer by the following experiment:—

The carmine-vital staining was done along with irritations in various ways into the peritoneal cavity.

Case 1. A male rabbit of 1500 gms. body-weight. 1st day, 5.0 c.cm. of 4 per cent solution of lithium-carmin (intravenously) + 0.5 c.cm. of the sterilized olive-oil (intraperitoneally). 2nd day, 6 c.cm. of the carmine-solution (intravenously). 3rd day, 6 c.cm. of the carmine-solution (intravenously) + 0.5 c.cm. of the olive-oil (intraperitoneally). 4th day, 6.5 c.cm. of the carmine-solution (intravenously). 5th day, 7 c.cm. of the carmine-solution (intravenously) + 0.5 c.cm. of the olive-oil (intraperitoneally). 6th day, 7 c.cm. of the carmine-solution (intravenously). 7th day, ditto. The total amount of the carmine-solution and olive-oil was estimated 44.5 c.cm. and 1.5 c.cm. respectively.

24 hours after the last injection 350 c.cm. of 0.1 per cent solution of silver nitrate was injected into the peritoneal cavity and one hour later the animal killed by bleeding.

Case 2. A female rabbit of 2160 gms. body-weight. 1st day, 6 c.cm. of the carmine-solution (intravenously) + 0.5 c.cm. of the olive-oil (intraperitoneally). 2nd day, 7 c.cm. of the carmine-solution (intravenously). 3rd day, 7 c.cm. of the carmine-solution (intravenously) + 0.5 c.cm. of the olive-oil (intraperitoneally). 4th day, 8 c.cm. of the carmine-solution (intravenously). 5th day, 8 c.cm. of the carmine-solution (intravenously) + 0.5 c.cm. of the olive-oil (intraperitoneally). 6th day, 8.5 c.cm. of the carmine-solution (intravenously). 7th day, ditto. The total amount of the carmine-solution and the olive-oil was estimated 53 c.cm. and 1.5 c.cm. respectively.

24 hours after the last injection the animal was killed by bleeding.

In the abdominal cavity many minute oil-droplets could be found and the omentum was slightly opaque.

Case 3. A female rabbit of 1530 gms. body-weight. The carmine-solution was intravenously injected once a day and its total amount was estimated 48.5 in seven days. The last injection was followed by 10 c.cm. of the sterilized normal saline-solution into the peritoneal cavity. 24 hours after the last administration the animal was killed by bleeding.

Case 4. A female rabbit of 1730 gms. body-weight. The carmine solution intravenously injected was estimated 50 c.cm. in seven days. On the 8th day, 10 c.cm. of the normal saline solution was intraperitoneally injected, and two hours later the last administration the animal was killed by bleeding.

The removed omentum was used partly for the "silver chloride method" and partly for hematoxylin-eosin-staining.

Case 5. A female rabbit of 1650 gms. body-weight. The carmine-solution intravenously injected was estimated 48.5 c.cm. in seven days and the last injection was followed by 10 c.cm. of the normal saline solution into the peritoneal cavity. On the 8th day 350 c.cm. of 1 per cent solution of silver nitrate was injected intraperitoneally, and 40 minutes later it was killed by bleeding.

Histological appearance:—

Case 1. In the extended specimen impregnated with silver nitrate, both the epithelioid cells and apertures considerably increased in number and were generally enlarged. Both of them had some carmine-granulae which were generally indistinct by the influence of the solution of silver nitrate. Some of the most enlarged apertures had, in their subjacent area, the nuclei rich in chromatin substance. The cement-line of the milk-spot was variously damaged, in parts either obscured or disappeared. A few polymorphonuclear leucocytes existed in the intercellular spaces in the mesothelial cell-layer.

Case 2. In the serial sections, the milk-spots were generally swollen edematous-

ly and the blood- and lymph-vessels were dilated. The epithelioid cells, the histiocytic wandering cells on the milk-spots especially the enlarged ones which have two nuclei tended to increase in number. Some of these epithelioid cells, particularly, enlarged ones were situated just on the surface of the milk-spots, and some in the deeper area showed their protoplasm partly on the peritoneal surface. Some of the epithelioid cells had a few and others had many carmine-granulae in their protoplasm. Histiocytic cells in the deeper area of the milk-spots which were very irregularly shaped and possessed many carmine-granulae in the normal condition, had almost disappeared and they were substituted with the histiocytic wandering cells which were resembled to the epithelioid.

In this case, there appeared many giant cells consisted of from four to seventeen nuclei. Such giant cells were of from twenty to thirty microns in diameter and clearly distinguished from the circumference and negative with the vital staining. The nuclei of the giant cells were of about seven microns in diameter and rich in chromatin-substance more than that of the histiocyte. These giant cells like those of the epithelioid cells were situated partly on the milk-spots and partly in deeper area. There were some polymorphonuclear leucocytes in the milk-spots and some of them existed in the intercellular spaces of the mesothelial cell-layer.

Case 3. In the serial sections, the epithelioid cells as well as the polymorphonuclear leucocytes in the milk-spots were more abundantly existed than those of case 2. The other findings were similar to those of case 2, with an exception of the giant cells which could not be seen in this case.

The appearances of cases 1, 2 and 3 were similar to those of the cases in which the carmine-solution was administered into the peritoneal cavity (compare Chapter V, 2), but with an exception that the carmine-granulae in the former epithelioid cells were less than those of the latter ones.

Cases 4 and 5. In the extended specimen treated with the "silver nitrate, both the epithelioid cells and apertures increased in number and contained carmine-granulae in a varied degree. In the serial sections, the general epithelioid cells as well as some epithelioid cells partly protruded from the mesothelial surface which are both naturally weak-positive with carmine vital staining, exceptionally showed quite a many carmine-granulae in their protoplasm. Many histiocytes derived from the histiocytic cells in the deeper area of the milk-spots were situated just beneath the mesothelial cell-layer and send their protoplasm into the intercellular spaces of the layer. Some of these wandering cells were swollen spherically and most of them were tangentially

adhered to enlarged intercellular spaces of the mesothelial cell-layer. It is very interesting to observe a flattened histiocytic cell situated exactly between the mesothelial cells, though not frequent, and also looks like the epithelioid. This cell was obviously differentiated from a normal epithelioid cell by its shape and staining characteristic: the protoplasm of the former showed more carmine-granulae than the latter's. The former not being basophilic, contrarily to the latter, the arrangement of the carmine-granulae was the only guidance to find out the cell-boundary. In other words, the appearance of this new epithelioid cell was quite resembled to that of the histiocytic cell in the milk-spots. Still, the nucleus of the new epithelioid cell mostly occupied the periphery of the protoplasm and was depressed, always facing its depressed side to the center of the cell, contrarily to the normal epithelioid cell (compare Chapter V, 1). By the above morphological differences, two kinds of the epithelioid cells could be well differentiated and consequently they probably have a different function of which the following chapter will illustrate.

In summarizing above experiments, it was concluded that both the aperture and epithelioid cell in the milk-spots are represented by the wandering histiocytes which are passing through the mesothelial cell-layer, viz:—

(1) The aperture. The each histiocyte which is going to begin or finish its penetrating through the mesothelial cell-layer shows its protoplasmic process only in the mesothelial intercellular space and its nucleus is seen in the deeper area of the milk-spots, so that in the extended specimen treated with silver nitrate, the nucleus can not be found in this intercellular space. This figure is the aperture by the author and it is a small round intercellular space in the mesothelial cell-layer. (2) The epithelioid cell. In some period, the histiocytic cell is totally wedged into the intercellular space and consequently its nucleus exist in situ. This is represented as a round cell encircled by the cement-line on the milk-spots, namely, this is the epithelioid cell by the author. (3) The transitional between the above two figures. When the peritoneum is experimentally irritated, a transitional form between the above two figures distinctly appeared, namely, the irritated histiocyte of spherically shaped is nearly tangentially connected with the space exists in the mesothelial cell-layer, this is an enlarged aperture where a nucleus can easily be found in its subjacent area.

Comparison with the peritoneal surface of the diaphragm:—

Cases 1 and 2. In the extended specimen treated with silver nitrate and next with the mixed solution of glycerin and acetic acid, many mesothelial cells on the peritoneal surface of the central tendon, especially on the spaces which occupy in the radiated tendon-fasciculi, were exfoliated. Still, on this spaces many small rounded cells were accumulated in several spots and some of these cells possessed many carmine-granulae. The cement-

line was irregularly thickened, and many round spots of dark brownish color appeared on the cross points of the lines.

Cases 4 and 5. In the extended specimen, several brown spots were situated on the cross points of the cement-lines. Almost all of the mesothelial cells coalesced closely.

Summary of the comparison with the peritoneal surface of the diaphragm :—

Normally, a few epithelioid cells occupy the intercellular spaces of the mesothelial cell-layer which covers the spaces in the radiated tendon-fasciculi of the diaphragm and it may be considered that these epithelioid cells also belong to the histiocyte. What reported by Muscatello as the leucocytes among the mesothelial cells of the peritoneal surface of the central tendon on a normal rabbit are also to be considered nothing but the epithelioid cells (by the author).

Such epithelioid cells generally and also the small brown spots (the so called stigmata by Arnold) on the cross points of the cement-lines to some extent increase in number by a certain irritation produced in the peritoneal cavity.

Appendix :—

The milk-spots in mesentery.

It is not so infrequently to observe small milk-spots in mesentery of rabbit, as already described by Ranvier and Walter. The investigation of this milk-spots in mesentery has been hitherto overlooked, but much attention should be paid to as they play an important rôle similarly to those of the great omentum. Since March 1922, I have collected 100 cases out of which obtained 20 positive cases of the milk-spots. These milk-spots are oval or round in shape and they range in size from 0.05 to 0.2 m.m. in diameter.

In the extended specimen impregnated with silver nitrate, the milk-spots are swelled on either surface of mesentery and its peritoneal cover of each swelling is consisted of small irregularly shaped mesothelial cells while the opposite side is consisted of ordinary mesothelial cells. The cement-lines on these milk-spots are generally thicker than those on the other area and form an irregular net-work, as those of the milk-spots of the omentum. Still, the cement-lines possess several round spots stained dark brown in color. But those large ones encircled with cement-lines that could be seen in the omentum are not present. A few epithelioid cells are sometimes situated in the mesothelial cell-layer on the milk-spots. The existence of the milk-spots of the mesentery is uncertain, and I could not unfortunately find the milk-spots in the specimen with the carmine-vital staining. And therefore I am not sure whether or not the epithelioid cell is positive with the carmine-vital staining. However, it may be suggested that the epithelioid cells of the mesentery also belong to the histiocyte, as their shape and appearance are quite similar to those of the epithelioid cells on the milk-spots of the omentum.

(To be continued)