Resumen por los autores, Eliot R. Clark y Eleanor Linton Clark.

La reacción de las células de la cola del renacuajo hacia la inyección de aceite de croton, en el animal vivo.

Los autores han inyectado glóbulos microscópicos de aceite de croton diluido en aceite de parafina, en la expansión caudal de larvas de *Hyla*, observando en el animal vivo la "inflamación aséptica" resultante. El punto estudiado especialmente es la cuestión de si, durante la inflamación, las células de tipo definido permanecen como elementos específicos o si se transforman en otros tipos celulares. Primeramente se obtuvo una idea general de los procesos que tienen lugar, estudiándose después cuidadosamente los diferentes tipos de células. Se ha observado que los leucocitos y células emigrantes del tejido caminan hacia el aceite de croton, pero no llegan a ponerse en contacto con él, permaneciendo a una corta distancia y haciéndose estacionarios al mismo tiempo que producen en su superficie apéndices puntiagudos que les asemejan a las células pequeñas del tejido conjuntivo. Sin embargo, no se transforman en células conjuntivas. Por el contrario, mas tarde se hacen esféricos y después de varias horas o aun dias, vuelven presentar su actividad amiboide y emigran, habiendo sido eliminado el aceite durante este tiempo. Las células conjuntivas cercanas a las gotas de aceite se transforman en elementos granulares, se hinchan y llenan de vacuolas y algunas de ellas mueren. La mayor parte de ellas sanan después de la eliminación del aceite y vuelven a adquirir la facultad de moverse. Los capilares sanguíneos presentan vacuolización endotelial y retracción, a las que sigue nuevo crecimiento. El endotelio linfático se comporta de un modo semejante. Todos los tipos celulares permanecen específicos. Las células emigrantes no se forman a expensas del endotelio o de las células conjuntivas, ni tampoco originan ninguno de estos tejidos. Las células conjuntivas y los endotelios sanguíneo y linfático también se conservan específicos, y ni se convierten en otros tipos celulares ni se derivan de ellos.

REACTIONS OF CELLS IN THE TAIL OF AMPHIBIAN LARVAE TO INJECTED CROTON OIL (ASEPTIC INFLAMMATION)

ELIOT R. CLARK AND ELEANOR LINTON CLARK

Department of Anatomy, University of Missouri

FIFTEEN FIGURES

INTRODUCTION

For a number of years the authors have been engaged in a study of the growth and reactive power of connective-tissue cells, wandering cells, and of blood-vessels and lymphatic endothelium. These cells and tissues are conceded by most investigators to have a common origin ultimately in a primitive mesenchyme cell, but there is no such unanimity of opinion as to the manner in which they differentiate, and there has been a great deal of controversy in regard to the degree to which they become specific in the adult organism. In former studies of the normal growth of these cells and tissues and of their reaction to injected substances, such as paraffin oil, carbon granules, olive oil, yolk of egg, etc., in the transparent tails of living Amphibian larvae, we found that the individual cells and tissues remained specific in their growth and in their response toward outside stimuli (E. R. Clark, '09, '12, '16; E. R. and E. L. Clark, '17). The question of the specificity of tissues has been raised most frequently, with respect to this very group of cells and especially during the process of inflammation in which they play such a prominent rôle. We therefore decided to produce a micro-inflammation in the transparent fin of an Amphibian larva and to study the reaction of these different cells and tissues toward an injurious agent by continuous observation of the living.

Hyla larvae were used almost exclusively in these studies. These diminutive tadpoles have very few pigment cells and their

connective-tissue cells are fewer in number than in the larvae of frogs and toads. They are therefore wonderfully transparent and the individual cells may be followed readily. As was previously mentioned (E. R. Clark, '12), this form is much easier to anaesthetize than the larger larvae of the Anura, it responds more quickly to chloretone and remains motionless for longer periods without deleterious effects on the circulation.

The irritating substance selected for these experiments was croton oil. The undiluted oil was injected in a few larvae, but, in the majority of cases, 10 per cent croton oil (made of one part croton oil to nine parts paraffin oil) was employed. The mixture was sterilized before injection.

The tadpoles were anaesthetized in chloretone 1 to 3000, made up in pond water. Under the binocular microscope, small globules of croton oil were injected into the fin by means of small glass cannulae. The larvae were then transferred immediately to the observation chamber, previously described (E. R. Clark, '12) and examined under the compound microscope in 1 to 4000 chloretone. With the microscope tube horizontal and a drawing-board placed beneath, at an angle of 45 degrees, and the use of a Leitz drawing eye-piece (no. 112), it was possible to make consecutive sketches of individual cells and to record the changes which occurred. In some cases, the region was watched and frequent records made over periods of six to twelve hours, after which the larvae were returned to fresh water overnight, and the region again observed on the following day. In other cases, especially where it was desirable to follow individual rapidly moving and changing cells, the region was kept under continuous observation for twenty-four hours or more.

The cells and tissues present in the transparent fin, before the production of an injury, are the following:

1. Stellate connective-tissue cells.
2. Wandering cells: *a*) pigmented; *b*) non-pigmented.
3. Blood capillaries lined with a single layer of endothelium.
4. Lymphatic capillaries, also lined with endothelium.
5. Blood-cells—red blood-cells and leucocytes inside the vessels.

6. Stationary pigment cells.

7. Nerve cells.

8. Epidermal cells, covering the surface of the fin.

Of these cells, the connective-tissue cells, the wandering cells (including migrated leucocytes), and the endothelial cells lining the blood-vessels and lymphatics were the ones observed particularly. These are the cells which had formerly been studied in the normal larvae and in the experiments in which various foreign substances had been injected into the fin. The character, behavior, and growth of the blood-vessels and lymphatics in the normal tail have been described in detail in earlier papers. The normal growth of connective-tissue cells has also been studied intensively (E. R. Clark, '12). During the course of the present study records were also made of the wandering cells of the tissues in normal uninjured larvae. Consecutive records of these cells showed that they are constantly moving through the tissues, wandering around between the connective-tissue cells and coming back again to their starting-point. Each cell seems to have a more or less definite route of its own.

The blood-cells of Amphibia have been described by Maximow ('10) and by Friedsohn ('10). The latter divides the leucocytes into the following classes:

a. Leucocytes without granules.

1. Small leucocytes.

2. Large leucocytes.

3. Polymorphonuclear leucocytes.

4. Small oval cells with oval nuclei.
(spindle cells).

b. Leucocytes with granules.

1. Eosinophile leucocytes.

2. Mast leucocytes (basophile).

3. Pigment leucocytes (not present in mammals).

GENERAL PICTURE OF THE REACTION TO CROTON OIL

Immediately after the injection of a small globule of croton oil, a decided change was noticeable in the fin near the site of injury. The whole region became more opaque than other portions of the tail. This opacity is due to a granular appearance of the epidermal cells and connective-tissue cells of the neighborhood. Higher magnification shows that their 'granular' appearance is caused by the formation of vacuoles in the cells. An opaque 'granular' zone extends for a distance around the globule. This zone is wider proximally, toward the muscle, than it is in the region distally between the globule and the fin margin, evidently corresponding to the direction of flow of the currents of fluid in the tissue spaces. The processes of the connective-tissue cells in this zone are drawn in, the cell bodies become swollen and vacuolated, and the nuclei become visible. The endothelial cells of the lymphatic capillaries within this zone also become swollen and vacuolated and their nuclei show up plainly.

Fifteen or twenty minutes after the injection a distortion of the fin at the injured region may be observed. Most frequently this takes the form of a bend or fold in the fin. At other times, particularly with the stronger concentrations of croton oil, a blister forms around the globule, which usually disappears within a few hours.

In the main longitudinal blood-vessels of the tail and in the larger vessels situated near the muscle edge, the circulatory changes, described by early observers of inflammation, can be followed—the increased blood flow, followed after a half hour or more by a slowing of the current and a tendency of the leucocytes to stick to the vessel wall. In the capillary loops of the fin these changes are not so evident, owing to the narrowness of the vessels which permits the passage of only one corpuscle at a time. In such a capillary within the 'granular area,' the circulation usually ceases within an hour or two after the injection of croton oil. Wandering cells in the neighborhood are attracted toward the injected substance.

An hour after the injection, the diapedesis of leucocytes has commenced and continues for as long a time as the croton oil remains in the fin. The leucocytes come through the walls of blood-vessels situated outside the granular area. They make their way rapidly through the tissue spaces toward the croton oil, moving past many of the vacuolated mesenchyme cells. However, these leucocytes do not reach the globule, but instead they become stationary at a short distance from the croton oil. Here they send out numerous fine processes until they appear to be covered with spicules and resemble sea-urchins. New leucocytes approach, and they, too, become stationary and send out processes until a thicket of such cells is formed (figs. 1 and 2). The fine processes change shape rapidly at first, but after an hour or two they become more stable, larger, and fewer in number, and the migrated leucocytes then resemble small connective-tissue cells (fig. 6).

Outside this newly formed wall of sessile leucocytes, the connective-tissue cells and endothelial cells, which had possessed the opaque granular appearance characteristic of injury, become clear again and regain their normal contours and refractility. From now on the new leucocytes which approach the area of inflammation move in nearer to the globule before becoming stationary.

In the meantime, numbers of the large leucocytes containing brown pigment have arrived on the scene, and these cells, apparently a more resistant type than the ordinary clear leucocytes, move in very close to the oil globule, even flattening out on its surface.

The epidermal cells, directly over the globule, become heaped up. This apparent increase in the epidermal cells is due to the drawing together of cells of the vicinity rather than to a proliferation.

The clear leucocytes continue to migrate into the region, to become stationary, and to send out processes. By this means, the area involved in the inflammation becomes more and more circumscribed. The globule finally shifts its position toward the surface of the fin, probably owing to the accumulation of

fluid, and is finally excluded. The heap of epidermal cells covering the globule is apparently thrown off at the same time.

As soon as the globule is extruded, the connective-tissue cells recover their normal shape and the circulation in the blood-vessels of the injured region is restored. The stationary leu-

Figs. 1 to 5 A series of sketches showing the general pictures of an inflammation produced by injecting a drop of croton oil (10 per cent in paraffin oil) into the dorsal fin of a *Hyla* larva. The connective-tissue cells are shown in solid black, the pigmented leucocytes are dotted, and wandering cells in outline. $\times 133$.

Fig. 1 Sketch started May 30th at 10:50 A.M., immediately after the injection of croton oil. The broken line indicates the extent of the injured area as evidenced by the granulation of the connective-tissue cells. The paths of some of the migrating leucocytes are indicated by dotted lines and arrows. X marks the location of a sessile leucocyte at 12:15 P.M. *Lym.*, lymphatic capillary; *B.V.*, blood-vessel; *O.G.*, oil globule.

cocytes, which had assumed the form of small connective-tissue cells, now draw in their processes and resume their normal amoeboid shape. However, within an hour or two, all of the leucocytes of the region have become motionless again, but with a spherical form (fig. 3).

Within a few hours after the extrusion of the globule of croton oil, the opaque area which had occupied the region of the croton oil begins to clear up. The pigmented leucocytes, which had crowded in close to the croton oil, now scatter in all directions and wander wildly and rapidly through the tissue spaces away from the region. After this scattering of the pigmented leucocytes, the area once occupied by the croton oil, is very thin,

Fig. 2 Sketch of the same region as in figure 1, begun at 12:30 P.M., May 30th, showing the wall of leucocytes, with processes, which has formed at a short distance from the oil globule.

and there may even be a hole through the tail at this point. However, a small opaque clump, consisting of cell debris surrounded by pigmented leucocytes, remains in the fin and is not disposed of for several days.

During the first twelve hours or more after the extrusion of the croton oil the clear leucocytes in this part of the fin remain motionless and spherical. During this period new leucocytes were seen to come out from the blood-vessels and to approach the site of injury. However, when they reached the region

occupied by the other leucocytes, they too became round and motionless like the others.

Soon after the extrusion of the oil globule, the connective-tissue cells on the border of this torn area have all recovered their normal stellate form and refractile appearance. They begin to change shape and to wander in toward the injured area. During this stage the processes of all the mesenchyme cells of

Fig. 3 Sketch of the same region at 6:30 P.M., May 30th. The oil globule was extruded at 3:20 P. M. A mass of opaque material is present at the site of injection. The leucocytes have all withdrawn their processes and become spherical. Several red blood-cells (*r.b.c.*) have escaped from the blood-vessel at the right of the field.

the vicinity radiate toward the site of injury. During the succeeding day the connective-tissue cells continue to move in and fill up the gap in the tissue left by the extrusion of the croton oil.

The final repair of the injury and return of the fin to normal consumes several days. Figures 4 and 5 show successive stages in this process. With the exception of one or two connective-tissue cells, which showed an extreme degree of injury and which

finally perished, and also of the epidermal cells which were lost at the time when the globule was extruded, there was no disintegration of cells in the inflamed area. The mesenchyme cells recovered completely. The leucocytes, after remaining for twelve hours or more in the motionless and rounded condition, gradually migrated away from the region. Some of them

Fig. 4 Sketch of the same region made June 1st, two days after the injection of croton oil. The opaque mass is much smaller and has shifted nearer to the fin margin. A number of clear leucocytes are scattered through this region. Several pigmented leucocytes are present, most of which have wandered out from the opaque mass at the site of injury.

wandered off through the tissues, others entered blood-vessels or lymphatics. Two days after the injury, a number of these rounded leucocytes were still present in the region, and even after five days their number still exceeded that of the wandering cells present in adjoining regions.

The small mass of debris remaining after the extrusion of the globule gradually diminishes in size and, at the same time, it is forced nearer and nearer the fin margin. During this process it is surrounded by some pigmented leucocytes, which appear to

ingest it. A week after the production of such an injury, the only signs of reaction remaining are a slight irregularity in the edge of the fin, the radiation of the processes of near-by connective-tissue cells toward this defect, and the presence of more than the usual number of pigmented leucocytes (fig. 5).

Fig. 5 Same region one week after the injection of croton oil. The injury is almost repaired, only a slight irregularity in the edge of the fin is noticeable, toward which the processes of the connective-tissue cells radiate. A slightly greater number of wandering cells than in adjoining regions is still present.

The general picture of a micro-inflammation, illustrated in figures 1 to 5, was obtained by watching the same field without interruption for twenty-four hours or more after the injection of a globule of croton oil. During the succeeding days, the region was observed once or twice daily and a record made. In other specimens, a certain type of cell or tissue was selected and watched intensively during the course of the inflammation. These will be described separately.

LEUCOCYTES

1. *Clear leucocytes*

This is a large group of cells, which includes wandering cells of the tissues and the leucocytes which migrate from the blood-vessels and lymphatics, without regard to their staining reactions. Such cells were included in one group in the present study for the reason that, in the living animal, their morphological characteristics are the same, as is also their behavior toward the croton oil.

All wandering cells in the vicinity of the injected globule make their way toward the croton oil soon after the injection. At the end of an hour, the leucocytes in the blood-vessels just outside the 'granular zone' become adherent to the walls of the vessels and move directly toward the site of injury. This movement and the change in shape of these migrating leucocytes take place so rapidly that it is necessary to follow individual cells continuously. In some cases, leucocytes which had already migrated from blood-vessels were observed to crawl from the tissue into a lymphatic capillary and then to proceed rapidly down toward the tip by amoeboid movement. On reaching the tip of the lymphatic capillary, the leucocytes were seen to crawl out through the wall again. This observation was repeated several times in different larvae and in some cases several leucocytes in succession were observed to follow each other into a lymphatic, down to the tip and out again. By this means, the leucocytes reached the site of injury by a much more direct and speedy route, since they were not forced to make their way in and out among the fine processes of the connective tissue cells (fig. 14).

Figures 1 and 2 give the general picture of the migration of the leucocytes and the formation of a ring of sessile leucocytes with processes at a short distance from the globule. In some specimens individual cells were watched consecutively for a number of hours. The series of sketches in figure 6 shows the changes which occur in a leucocyte which has migrated from a blood-vessel and has become stationary after approaching within

a short distance of the croton oil. The first processes sent out are fine and hair-like. These streamers are withdrawn and sent out again, thus changing the contour of the cell frequently. After a few hours these processes become reduced in number,

Fig. 6 Series showing changes in a single leucocyte which has emigrated from a blood-vessel and become stationary near the site of injection. 21×3 .

Fig. 7 Series showing the changes undergone by a sessile leucocyte, situated near the injected site, for a time before and after the extrusion of the croton oil. At 11:45 the oil globule was very superficial and the connective-tissue cells of the region had begun to show signs of recovery. At 1:25 many of the nearby leucocytes were amoeboid. At 2:30 all the clear leucocytes had rounded up. The pigmented leucocytes were wandering around actively. The connective-tissue cells were changing and moving toward the site of injury. At 4:00 the clear leucocytes of the region were still motionless and spherical. $\times 133$.

larger, and more stable, and the cell then resembles a small immature connective-tissue cell.

Metchnikoff ('93), in his account of inflammation in the tadpole's tail following a burn with silver nitrate, described the

transformation of polymorphonuclear leucocytes into connective-tissue cells. His description of this process and his illustrations show clearly that he was dealing with the same phenomenon described here, although croton oil is apparently a much more powerful irritant than silver nitrate, since the leucocytes migrated in much greater numbers in our experiments. In our first observations of the reaction to croton oil, we studied the region intensively for six or eight hours after injection of the irritant, returning the specimen to fresh water overnight. And, in this case, we followed many individual leucocytes from the blood-vessels during their wanderings through the tissue and through the stages illustrated in figure 6, and we were practically convinced that leucocytes may become transformed into connective-tissue cells. However, in subsequent studies in which certain of these cells were followed without interruption for twenty-four to thirty hours, we found that, at the time of the extrusion of the croton oil, these same stationary cells all withdraw their processes and resume their normal amoeboid form (fig. 7).

An hour or two later, the same cells again become stationary, but this time they have a perfectly spherical form and remain in this rounded condition for several hours—some of them for several days, in spite of the fact that the pigmented leucocytes and even the stellate connective-tissue cells of the same region are moving and changing their shape constantly. This rounding up of the leucocytes takes place regardless of whether the tail is edematous or not (determined by measurement with the fine-adjustment screw). Moreover, simultaneous observations and records showed that, at the very moment when all of these leucocytes near the injured area were motionless and rounded, the wandering cells of the uninjured region were moving around through the tissue in a normal manner. Figure 7 shows in detail the changes which an individual leucocyte underwent shortly before and for some time after the extrusion of a globule of croton oil.

The wandering cells present in the tissue before the injection reacted in exactly the same manner as the leucocytes from the

blood-vessels. In cases in which wandering cells were situated very near the point of injection, they stopped their amoeboid movement immediately after the injection and sent out fine processes.

Several specimens were fixed in Bouin's fluid and stained with Ehrlich's haematoxylin and counterstained with eosin, orange G, and aurantia, and the whole tail mounted in balsam (fig. 13). These specimens showed leucocytes in stages of migration or in the stage in which they were covered with fine processes. In these specimens the polymorphonuclear leucocytes predominated, but mononuclear cells, with an oval nucleus eccentrically placed, and a few cells resembling lymphocytes were also present. The behavior of all the non-pigmented leucocytes was the same regardless of the kind of nuclei they possessed.

The reaction of leucocytes and wandering cells toward this injurious substance differs from their behavior toward the materials injected in former experiments. With sterile paraffin oil there was very little migration of leucocytes. Wandering cells of the tissue moved toward the globule, sometimes flattening out on its surface and then moving away again. The paraffin oil remained in the tissue without occasioning any disturbance, and after the first day or two no cells reacted toward it in any observable way. When carbon and carmine granules, previously sterilized, were injected into the fin, they were phagocytized by wandering cells which migrated toward them. In the case of injected fat (in the form of olive oil, oleic acid, cream, and yolk of egg) leucocytes migrated from the blood-vessels, going directly toward the injected substances and actively engulfing small globules of fat. In this case only part of the wandering cells were attracted toward the injected substances.

Any explanation of the exact nature of the reaction of these leucocytes and wandering cells toward an injurious agent such as croton oil would be mere speculation. However, the observations yielded abundant evidence that the formation of this barrier of stationary leucocytes with processes has a neutralizing effect on the oil and forms an efficient method of localizing the injury. It was noted in every case that connective-tissue cells,

which had previously shown all the signs of injury, cleared up and regained their normal appearance as soon as a ring of these sessile leucocytes with processes had formed between them and the globule of croton oil. Moreover, in contrast with the appearance just described, we observed one case of inflammation in a very young tadpole in which only a few wandering cells were present in the tissue spaces and only an occasional white blood-cell in the blood-vessel. In this case the granular area was very extensive. Connective-tissue cells and endothelial cells became swollen and vacuolated and the circulation in the blood capillaries stopped in a region three or four times as large as the one involved in the case of a similar injury in an older tadpole in which the migration of leucocytes had been abundant.

In the later stages, after the extrusion of the globule, these clear leucocytes were seen to act as phagocytes, picking up extruded red blood-cells.

2. Pigmented leucocytes

These cells are normally present in the tissue spaces of the fin of Amphibian larvae, and they are also found occasionally inside blood-vessels and lymphatics. They are relatively large cells and contain brown and black pigment granules of varying sizes. Their origin and their relation, if any, to the large branching chromatophores of tadpoles have not been studied. In stained specimens they may be seen to possess a single nucleus, usually round or oval in shape. Maximow and Friedsohn consider the pigmented leucocytes to be a special class of cells without any homologue among mammalian blood-cells.

The pigmented leucocytes are strongly attracted toward the croton oil, and those located near the site of injury wander toward it soon after injection. These cells are remarkably resistant to injury. They wander in through the 'granular area,' past the line at which all the non-pigmented leucocytes have stopped, and make their way directly into the opaque region immediately surrounding the croton oil, and even flatten out directly against the oil globule. In some cases pigmented

leucocytes were found containing small refractile globules of oil and, in one instance, such a pigmented cell was followed as it approached a minute droplet of oil and proceeded to ingest it.

These cells continue to surround the globule of croton oil during its sojourn in the fin, and they occupy the site of injection until after the globule has been extruded. Two or three hours after the extrusion, they begin to scatter in all directions and to wander away from the scene of the injury. The clearing up of the opaque area which occurs at this time is chiefly due to the dispersal of these pigmented leucocytes. Most of them wander away through the tissues, while some of them were observed to crawl through the walls of the neighboring lymphatic vessels. A number of pigmented leucocytes remain at the site of injury during the succeeding days and appear to be instrumental in the disposal of the small mass of debris which remains in the tail after the extrusion of the globule. This opaque material gradually diminishes in amount during the next few days and, at the end of a week, the place of injury is marked only by one or two of these pigmented leucocytes.

Like other leucocytes, these pigmented cells were also seen to act as phagocytes of extravasated red blood-cells.

3. The connective-tissue cells

The general picture of the reaction of these cells toward the croton oil has already been given. In one specimen this class of cells was followed with particular care. In this case, a record was made, before injection, of a selected portion of the tail and every mesenchyme cell in the region was drawn. By using two pigment cells as markers, it was possible to insert the globule of croton oil in the exact position desired. On examining this region immediately afterward in the observation chamber it was found that the globule had been inserted without disturbing any of the cells of the tail. The connective-tissue cells were numbered and observed continuously for twelve hours. On account of the mildness of the inflammation produced in this specimen, it was possible to follow individual cells throughout the process.

Different types or degrees of reaction of connective-tissue cells toward the croton oil were noted. These depended on the location of the cells with relation to the globule and on the time elapsing after the injection. The first type—mild injury—appeared immediately after the injection in cells surrounding the globule. Later, this type persisted in the cells situated between the globule and the margin of the fin, in the cells of the middle layer located on the outskirts of the 'granular zone.' In the mild type of injury the processes of the cells become shorter, thicker, and more highly refractile and stand out straight and stiff (fig. 8 and fig. 10, B). In the case of the cells distal to the croton-oil globule, connections between neighboring cells are maintained and the shortening and stiffening of the connecting processes may be instrumental in causing the puckering of the tail which occurs in this region soon after injection (fig. 8).

A more severe type of injury begins to appear about twenty minutes after the injection in the connective-tissue cells of the middle layer situated nearest the globule, and later spreads to other more distant cells. It is this type of reaction which gives the characteristic granular appearance to the area around the globule and especially centrally to it. This type of injury is illustrated in figure 9, C, and figure 10, C. The cell processes are greatly shortened, the cell body becomes thicker and vacuolated, and the nucleus shows plainly. The vacuoles give the cell a honeycombed appearance, and they may be so large and numerous as to indent the nucleus. Many of the vacuoles contain specks in Brownian movement. This rounding up and vacuolization of the connective-tissue cells spreads to the cells farther away from and more proximal to the globule, and also to the superficial layer of connective-tissue cells in the vicinity of the globule.

The third type of injury may be observed two or three hours after the injection in those connective-tissue cells located in close proximity to the croton oil. In these cells, one of which is shown in figure 10, D, the processes are shortened until the cell, in some cases, resembles a round ball. The cell proptolasm is practically filled with vacuoles and the outlines are hazy.

Fig. 8 Shows a group of connective-tissue cells before (A) and immediately after (B) the injection of croton oil. The globule (O. G.) was inserted proximally to these cells. The position later occupied by the oil globule is dotted in the first sketch. The same cells are shown in both drawings (see numbers). The cell processes become shorter and thicker and the whole region shrinks. The increase in anastomoses between the processes of the different cells shown in the sketch made after the injection is apparent rather than real, since in the normal tadpole many of the branches of the processes are too fine to distinguish without the oil immersion. $\times 400$.

9

10

Fig. 9 Series illustrating the changes in a single connective-tissue cell situated near the globule of croton oil. Illustrates serious injury and complete recovery. A. Sketch made June 4th, 1 P.M., just before injection. B. Sketch made June 4th, 2:45 P.M., one-half hour after injection of croton oil. Processes shortened, nucleus visible, vacuoles present. C. Sketch made June 4th at 4 P.M., processes mostly withdrawn, more vacuoles present. D. Sketch made June 6th, 2:30 P.M. about twenty-five hours after the extrusion of the oil globule. Cell has recovered—processes radiate toward the site of injury. E. Sketch made June 7th, 4 P.M., three days after the injection of croton oil and two days after the extrusion of the globule. Cell has completely regained its normal appearance. A, $\times 200$ =B-E, $\times 300$.

Fig. 10 Series of sketches of another connective-tissue cell situated very near to the site of injection. Illustrates extreme grade of injury. This is one of the few examples observed in which the cell did not recover. A. Sketch made June 4th, 1 P.M., before injection of croton oil. B. June 4th, 2:30 P.M., fifteen minutes after the injection. Processes shorter and thicker. Nucleus visible. C. Sketch made June 4th, 4:30 P.M. Processes withdrawn, vacuolization. D. Sketch made June 5th, 9:40 P.M. About ten hours after extrusion of the oil globule. Practically all the other connective-tissue cells of the region have recovered. This cell is a thin-walled sac containing vacuoles or granules. Nucleus does not show. On the following day (June 6) this cell was still present as a shadowy outline containing a few granules in Brownian movement. On June 7th no trace of it could be found. A, $\times 200$; B, C, D, $\times 300$.

The vacuolization of the last two types has been described recently by Lewis ('19) in studies of the "degeneration granules and vacuoles" of cells in tissue cultures. In our studies it is noteworthy that cells showing such marked 'degenerative' changes could recover so rapidly and completely. As soon as the ring of sessile leucocytes had formed, the vacuolated connective-tissue cells, outside of this barrier, became clear and refractile, the cell bodies became slender and were occupied solely by the nucleus, and the long branching processes again extended to their normal length. After the extrusion of the croton oil, practically all of the connective-tissue cells returned to normal and their recovery was amazingly rapid. Only one or two cells were lost and these were always cells which had previously shown the most extreme type of injury. One of these cells, watched continuously, became paler and paler and finally remained as an outer rim with a few granules inside before disappearing entirely (fig. 10, D). On the other hand, a number of cells which had shown the most extreme type of reaction toward the irritant were followed throughout the inflammation and were seen to recover completely soon after the extrusion of the oil (fig. 9).

This vacuolization and rounding up of the connective-tissue cells, in contrast to the behavior of the leucocytes, is a purely passive reaction. However, in the later stages of the process, after the extrusion of the croton oil, these cells play a very active part. They wander actively toward the gap in the tissue left by the extrusion of the oil, and some of them disappear from view temporarily, in the opaque regions at the site of the injury. During this period of migration, the processes of the connective-tissue cells radiate towards the injured region. The ends toward the gap are brush-like in appearance, while the opposite ends are rounded (fig. 11). One of the authors (E. R. Clark, '12) had previously studied the connective-tissue cells in the normal tadpole, and found, by observing the same cells for several weeks, that they are not fixed, but constantly change their position, in most cases gradually wandering in the direction of the fin margin. However, after the extrusion of the oil the con-

nective-tissue cells move much more rapidly, changing their shape and position to such an extent that continuous observation is necessary in order to follow individual cells. Thus, in figure 12, a cell is shown which moved in three hours as great a distance as a connective-tissue cell would wander in as many days in normal growth. (Compare with fig. B, in article in *Journal of Anatomy*, 1912, vol. 13, p. 368.)

Fig. 11 Drawing to illustrate radial arrangement of connective-tissue cells and their movement toward the croton oil, after the croton oil has been moved toward the surface of the fin and its irritating action decreased. Croton oil injected twenty-one hours before the drawing was made. The broken lines and arrows indicate the direction and approximate distance of movement of the connective-tissue cells during eleven hours. No leucocytes shown. Cells *a*, *b*, *g* and most others near the globule show the absence of long processes characteristic of the more rapidly moving cell. Compare with the normal cells *x* and *y* and others at the right of the drawing. *C*, *d*, and *e* are cells injured by the croton oil. Cells *d* are vacuolated; in cells *c* and *e* the walls of the vacuoles have broken down, leaving granules suspended in fluid. Cell *C* is one of the stages of the cell shown in figure 10. Cells *g* and *h* are the same cells as are shown in figure 12. *ch*, two fixed chromatophores; *b. v.*, blood-vessel; *lym*, lymphatics. $\times 200$.

THE BLOOD-VESSELS

The blood-vessels situated in the area in which the connective-tissue cells became vacuolated showed definite signs of injury. The endothelial cells became vacuolated and the nuclei became more prominent within a few minutes after the injection. The vessels became constricted and the circulation ceased about a half-hour later. At some points these vessels constricted until they consisted of a solid hyalin thread. At other points they still

Fig. 12 Drawings showing three successive stages in the movement of the two cells *g* and *h*, which are shown in the first stage (10:30 A.M.) in figure 11. The marked reduction in length of processes during this (relatively) rapid movement is particularly noticeable in the last stage (11:30 P.M. and 10 P.M., respectively). O. G., oil globule. $\times 320$.

contained red blood-cells and leucocytes. There was no migration of white blood-cells from the vessels within the 'granular' area. Outside this region, the blood-vessels showed the typical changes of inflammation, acceleration in the flow followed after about an hour by a slowing of the current and diapedesis of leucocytes. Inside the 'granular' area the frequent occurrence of hemorrhage gave further evidence of injury to the vessel wall. And twenty-four hours or more after the injury, a retraction of one or more of these blood-vessel sprouts or loops located within

this 'granular area' was always observed. On the contrary, the vessels just outside this zone usually showed a tendency to send out new sprouts during this period. A day or two after the extrusion of the globule during the stage of repair, the vessels of the injured area began to send out new sprouts and formed new loops once more.

The reaction of the blood-vessel endothelium toward the croton oil appeared to be entirely passive. In a number of larvae records were kept of the nuclei of the blood-vessels both inside and outside the injured area, but no unusual changes were noted. There was no sign of any proliferation of endothelial nuclei to form wandering cells or endothelial leucocytes. Occasionally leucocytes were observed to flatten out on the interior of the wall of a blood capillary and to pass through the wall sideways. During this process there were moments at which such a leucocyte greatly resembled a nucleus protruding into the lumen of the vessel, and a little later it appeared very much like a nucleus detaching itself from the wall. However, in the consecutive observations of living specimens it was always possible to follow the individual endothelial nuclei and to observe that the phenomenon consisted of the emigration of a leucocyte through the wall, rather than the formation of a leucocyte from an endothelial cell.

THE LYMPHATICS

The lymphatic endothelium plays both an active and a passive part in the inflammation set up by the presence of a drop of croton oil. The lymph capillaries within the 'granular area' show the changes characteristic of injury displayed by the connective tissue and epithelial cells. The areas surrounding the nuclei of the lymphatic became swollen, opaque, and vacuolated, particularly toward the tip. Such vessels usually retracted at about the time of the extrusion of the oil. A day or two later they sent out new sprouts into the regeneration area. The vessels outside the 'granular area' showed the fine processes characteristic of functional activity (E. R. Clark, '09) and even continued to increase in length during the period of active inflam-

mation. Such growth on the part of blood-vessels and lymphatics, however, did not appear to be in excess of the normal rate of growth.

The manner in which lymphatic capillaries served as paths by which the migrating leucocytes were enabled to reach the

Fig. 13 Sketches of cells from a fixed specimen. Five hours after the injection of croton oil, this larva was examined and found to be in the stage in which a ring of sessile leucocytes was in process of formation. The tadpole was fixed in Bouin's fluid, the whole tail was stained in alum haematoxylin, counterstained in eosin, orange G, and aurantia, dehydrated, cleared in xylol, mounted in damar. The sketches were made with the oil-immersion lens. A. Amoeboid leucocyte. B and C. Sessile leucocytes with processes. D. Normal connective-tissue cell outside injured area. E. Connective-tissue cell near the croton-oil globule. F. Connective-tissue cell in close proximity to the croton oil, showing extreme type of injury (chromatin scattered through the cell). $\times 600$.

scene of action more quickly has already been described. There are steps in the migration of such a leucocyte through the wall of a lymph-vessel in which it is easy to be deceived into the belief that the proliferation of an endothelial leucocyte is taking place, for the leucocyte often comes out of the vessel near the point where a nucleus is located. But continuous observation

of a lymphatic capillary near the site of injury shows that such leucocytes from the tissue wander through the wall into the lymphatic, move down the vessel, and crawl out again at the tip, nearer the globule of croton oil (fig. 14).

Fig. 14 1) Illustrates the migration of a leucocyte from the tissue into a lymphatic capillary, down toward the tip nearer the site of injection, and out again. Lymphatic sketched at 2:30 P.M. A, B, and C are three different leucocytes. The arrows and dotted lines show the respective paths which they followed. $\times 213$. 2) shows the appearance of a part of the lymphatic near the tip at 3:25 P.M., at the moment when a leucocyte (A) is in the act of emerging and illustrates the deceptive appearance of endothelial proliferation which accompanies the phenomenon. As seen in sketch (1), leucocyte A entered the lymphatic higher up at 3:05, and at 3:30 it was again outside the vessel. $\times 213$.

As in the case of the blood-vessels, we kept records of the lymph-vessels and of their endothelial nuclei, both inside and outside the 'granular area,' in several different larvae. The lymphatic sprouts within the 'granular' zone, which had become vacuolated while the croton oil was present, often retracted,

while those outside the area continued to send out fine processes and to grow in a normal manner, but in no case do we find any evidence of endothelial proliferation to form wandering cells.

In a number of cases, hemorrhages occurred from blood capillaries located near the croton oil. The extravasated red blood corpuscles were taken up by the lymphatic vessels, as well as by leucocytes of both the pigmented and clear varieties. The method by which the occasional extruded red blood-cell found in the normal tail is taken up has been described by one of the authors (E. R. Clark, '09). In this case the process was the same, but on a much larger scale. Instead of one sprout growing toward a single blood-cell, a number of processes appeared on both sides of the lymphatic and, by means of these, several red blood corpuscles were picked up and simultaneously taken into the lumen of the vessel, where they were seen to move along the lymph capillary to the main longitudinal lymphatic. After two or three days, such an area would be almost clear of blood-cells, and the majority of these would have been salvaged by the activity of the lymphatics.

In a number of larvae an edema developed in the injured region. This usually occurred soon after the extrusion of the globule, although it was sometimes noted on the following day. The edema may be demonstrated by measuring the thickness of the tail by means of the fine-adjustment screw. The appearance of the tail is altered in such areas; the region becomes clearer, the connective-tissue cells more widely separated, and all the leucocytes, including the pigmented ones, round up into spheres. The lymphatic sprouts of the area become markedly distended at the tip. This enlargement is evidently due to an increase in the fluid content of the vessel, for the endothelium is represented merely by a thin line. This differs from the enlargement of the lymphatic tips which occurs soon after the introduction of the croton oil and which is due to a swelling of the endothelial cells proper. Figure 15 shows successive records of a lymphatic sprout in an inflammation area which has become edematous after the extrusion of the globule of croton oil. Comparison of the different drawings with the measurements of this

region of the tail recorded at intervals shows that the lymphatic capillaries expand with an increase in the thickness of the tail and contract again when the tail resumes its normal dimensions. Absorption through the lymphatic is evidently active during inflammation and appears to increase with an increase in the amount of fluid present in the tissue outside.

Fig. 15 Sketches showing the reactions of a lymphatic sprout within the area affected by the injected croton oil. In this specimen an edema developed after the extrusion of the globule. Records of the thickness of the tail at the point under observation were made by means of the fine-adjustment screw. A. Sketch made May 29th, 12:15 P.M., immediately after the injection. Thickness of the tail, 55 μ . B. 3 P.M. Wall of the lymphatic swollen and granular at the tip. Thickness of the tail, 66 μ . C. Lymphatic at 5:15 P.M. (globule extruded at 3:45 P.M.). Endothelial wall of lymphatic smooth and thin, lumen enlarged. Thickness of tail, 100 μ . D. 6 P.M. Lymphatic not so wide, but still enlarged at the tip. Thickness of tail, 74 μ . E. May 30th, 10 A.M. Lymphatic capillary is normal in size and contour, and has retracted. Thickness of tail 57 μ . $\times 178$.

DISCUSSION

The subject of inflammation has been studied so extensively and the literature is so voluminous that it has seemed inadvisable to attempt an extensive review in this paper, and we shall merely indicate briefly some of the divergent views. Many of the early studies of Cohnhein, Metchnikoff, and Arnold were made on living material, and the general features of inflammation and many of the specific details, as discovered by them, are accepted by most pathologists. With regard, however, to the derivation and possible transformation of the cells taking part in inflammation, the widest difference of opinion may be found—among both pathologists and anatomists. The question as to the specificity of this group of cells, in normal growth, has not yet been settled by anatomists. On the side of non-specificity are found Maximow, Danchakoff, Weidenreich, and particularly Mollier ('11), who, although their studies have been confined mainly to early stages of embryonic development, believe, to a greater or less extent, in the interchangeability of endothelial cells, leucocytes, mesenchyme, and reticulum. Schulte ('14) has gathered together data and opinions which apparently support this view and presented them in the form of a plausible though not entirely convincing argument. The specificity of the cells of the group has been advocated particularly by Minot, Marchand, MacCallum, and Mall and his coworkers—Sabin, Evans, E. R. Clark—although Mall apparently recognizes an exception in the 'reticulo-endothelial' cells of the spleen, liver and endocardium, and Evans ('14) in the formation of free phagocytic cells from the Küpffer cells of the liver, following injections of tubercle bacilli.

In studies made on inflammation, the various views as to the possible transformation of one type of cell into another have been supported by the following: *a*) connective-tissue cells may be derived from leucocytes: Ribbert ('90), Metchnikoff ('93), Maximow ('02-03); *b*) endothelial cells from leucocytes: Ribbert ('90); *c*) connective-tissue cells from endothelium: Ribbert ('90), Maximow ('05), in intense inflammation; *d*) endothelial cells

from connective-tissue cells: Minervini ('11); *e*) leucocytes from endothelial cells: Cornil and Ranvier ('80) from lymphatic endothelium, Beattie ('02), Evans ('14), from Küpffer cells in the liver, Marchand ('13) and MacCallum ('16), possibly in special organs, Mallory ('14); *f*) leucocytes from connective tissue or reticulum: Herbert ('01), Downey ('12) in lymph glands. It is to be noted that the most of these studies have been made upon preserved material, in which deductions as to cell transformations are notoriously unreliable.

In our investigation the cells were watched in the living animal, in a region in which it is possible to follow the changes of individual cells throughout the process of the tissue reaction to an injurious chemical agent. We were unable to find the slightest evidence for the transformation of a cell of one type into a cell of another type. Blood-vessel and lymphatic endothelial cells maintained their identity and did not give rise to wandering cells, nor was new endothelium formed by any other way than by the sprouting of preexisting endothelium. Leucocytes were not formed from connective tissue nor endothelium nor were they transformed into connective-tissue cells or endothelial cells. Connective-tissue cells maintained their identity completely throughout the entire process. *This entire group of cells, then, remained specific, in an aseptic inflammation, in the transparent fin expansion of the tadpole's tail, produced by the injection of croton oil.*

There were a number of appearances produced in the reaction of the various tissues which were so suggestive of possible transformations from one type to another that we were forced to make long continuous observations before arriving at definite conclusions. During the first season in which we studied this problem, we were nearly convinced that the leucocytes, after becoming sessile and sending out their processes, were actually transformed into connective-tissue cells. It was exactly this picture which led Metchnikoff to conclude that such a transformation takes place. However, since we had been unable to follow the same cells through the entire period, we postponed publication of our results, until we might have another tadpole

season, in order, chiefly, to follow the same leucocytes throughout the process. This we succeeded in doing, and found in every instance that the leucocytes revert, after the extrusion of the oil, to typical rounded or amoeboid leucocytes. We feel morally certain that had we seen these leucocytes in sections, we should have labelled them connective-tissue cells.

Again, when leucocytes were first seen emerging from lymphatic capillaries, they often gave the appearance of pulling away from the endothelial cells—an interpretation which might very readily be made from sections. However, continuous observation of lymphatic capillaries showed that the leucocytes always entered the lymphatic vessel, from the outside, that they moved a variable distance along the lumen and then passed out through the endothelial wall. In their movements among the connective-tissue cells, again, leucocytes frequently pass over them so closely that only continuous observation shows that they are not being formed in some way by the connective-tissue cells.

While we hesitate to generalize from a set of observations restricted to a single species of a lower vertebrate, still our results are so definite and so positive that it seems fair, at least, to advance the hypothesis that similar tissues in other animals react similarly. Certainly, one must be very skeptical toward conclusions as to cell transformations which are based on studies of fixed material, especially when such studies yield such a variety of conflicting views.

One matter remains to be cleared up by further studies, namely, the possibility of the transformations of one type of leucocyte into another. Owing to the great difficulty in seeing the outlines of the nuclei in the cells studied, we did not attempt to distinguish, in the living, between the different types of amoeboid cells, except for the pigment cells, and our studies of total mounts of fixed specimens are still incomplete. Some investigators hold that polymorphonuclear leucocytes may be transformed into mononuclear cells (Janowski, Metchnikoff). A suspicious fact in this connection is the oft-repeated observation that, early in inflammation, polymorphonuclears predom-

inate, while mononuclears are much more numerous later. This is explained by many as due to the destruction of polymorphonuclears. In our study we saw very little, if any, destruction of leucocytes. Moreover, the rounding up of all non-pigmented leucocytes after the extrusion of croton oil and retention of this shape for several days has led us to wonder whether in this phase the nuclei also round up. This point we hope to take up in future studies.

SUMMARY

The reactions and changes undergone by the cells in the tail of Amphibian larvae in response to injections of minute globules of croton oil are as follows:

1. Connective-tissue cells manifest all grades of injury, depending upon their nearness to the oil. In the nearest cells the branched processes are withdrawn, the body of the cell becomes much swollen and vacuolated, and the nucleus becomes easily visible. Later, the vacuoles run together and the cell becomes a delicately walled sac, containing a few granules suspended in fluid. Later, the outlines of the cell are lost completely. Complete destruction, however, is limited to a very few cells. A little further away, cells become swollen and vacuolated, processes become much shorter, but are not retracted entirely, and the nucleus becomes visible, and may be indented by vacuoles. Such cells may recover completely—the vacuoles disappear, the cell becomes smaller, the nucleus less distinct, new processes are sent out and movement of the cell resumed. Still further away, the cells may show merely slight swelling with granulation. The cells on the border zone of the area affected react by moving toward the oil—at a rate much faster than the normal rate of movement of these cells. In this movement, the number and length of their processes are much reduced, until they resemble the typical filoblast.

2. *a*) Non-pigmented leucocytes (including the wandering cells outside the blood-vessels) respond immediately by moving toward the globule of croton oil. When about half-way between the outer limits of the affected connective-tissue cells and the oil,

they become stationary and send out many fine processes. These processes are withdrawn and renewed continuously, though tending to become more and more stable. At this stage they resemble very closely small connective-tissue cells. They are not transformed into connective-tissue cells, however, for eventually—probably corresponding to a diminution in the intensity of the croton-oil action—the processes are retracted and the leucocytes resume amoeboid powers. After the oil has been extruded, the leucocytes remain stationary for several days in a spherical condition, after which they scatter through the tissues. Wandering cells free in the tissue spaces and leucocytes which emigrate from the blood-vessels do not differ in their reaction. Leucocytes may enter lymphatic vessels and emerge again, but we saw no evidence whatever of the origin of leucocytes from lymphatic or from blood-vascular endothelium.

b) Pigmented leucocytes move toward the croton-oil globule and are more resistant to its action than the other leucocytes, for they may even come in contact with the oil globule without showing any diminution in their motility.

3. Blood capillaries near the globule show thickening and vacuolization of the endothelium, and, later, narrowing of the lumen, with, in many cases, retraction of endothelium. Extravasation may occur through the injured part of the wall.

4. Lymphatic capillaries near the globule also show vacuolization of the endothelium, and their lumen may become much distended, particularly in case an edema develops. The injured capillary may be retracted, but may grow out again later after the oil globule has been extruded.

5. Throughout the inflammatory reaction, each type of cell—leucocyte, connective-tissue cell, blood-vessel endothelium, and lymphatic endothelium—maintains its specificity, and there is no evidence for the transformation of one type of cell into another.

BIBLIOGRAPHY

- ARNOLD, J. 1893 Altes und neues über Wanderzellen insbesondere deren Herkunft und Umwandlungen. *Virch. Arch.*, Bd. 132, S. 502.
- BARDENHEUER, F. 1891 Über die histologischen Vorgänge bei der durch Terpentins hervorgerufenen Entzündung im Unterhautzellgewebe. *Ziegler's Beiträge*, Bd. 10, S. 394.
- BEATTIE, J. M. 1902 The cells of inflammatory exudations, etc. *Journal of Path. and Bact.*, vol. 7, p. 129.
- CLARK, E. R. 1909 Observations on living growing lymphatics in the tail of the frog larva. *Anat. Rec.*, vol. 3, no. 4, p. 183.
1912 Further observations on living growing lymphatics: their relation to the mesenchyme cells. *Am. Jour. Anat.*, vol. 13, p. 351.
1916 A study of the reaction of the mesenchymic cells in the tadpole's tail toward injected oil globules. *Anat. Rec.* vol. 11, p. 1.
- CLARK, E. R., AND CLARK, E. L. 1917 A study of the reaction of lymphatic endothelium and of leucocytes, in the tadpole's tail, toward injected fat. *Amer. Jour. Anat.*, vol. 21, p. 421.
- CORNIL AND RANVIER 1880 *Pathological histology* (transl. by Shakespeare and Simes), chap. 3.
- DOWNEY, H. AND WEIDENREICH, F. 1912 Über die Bildung der Lymphocyten in Lymphdrüsen und Milz. *Archiv. für mikr. Anat.*, Bd. 80, S. 306.
- EVANS, H. M., BOWMAN, F. B., AND WINTERITZ, M. C. 1914 An experimental study of the histogenesis of the miliary tubercle in vitally stained rabbits. *Jour. of Experim. Med.*, vol. 19, p. 283.
- EVANS, H. M. 1912 The development of the vascular system. *Human Embryology*, ed. by Keibel and Mall, vol. 2, p. 570.
- FRIEDSOHN, A. 1910 Zur Morphologie des Amphibien Blutes, etc., *Arch. f. mikr. Anat.*, Bd. 75, S. 435.
- HERBERT 1901 The young plasma cell or lymphocyte in chronic inflammation. *Jour. of Path. and Bact.* vol. 7, p. 90.
- JANOWSKI, W. 1895 Zur Morphologie des Eiters verschiedenen Ursprungs. *Archiv. f. experim. Path. u. Pharm.*, Bd. 36, S. 8 (reviewed by Marchand).
- LEWIS, W. H. 1919 Degeneration granules and vacuoles in the fibroblasts of chick embryos cultivated in vitro. *Bull. of the Johns Hopkins Hospital*, vol. 30, p. 81.
- MACCALLUM, W. G. 1903 Relations between lymphatics and connective tissue. *Johns Hopkins Hosp. Bull.*, 1903, vol. 14, p. 142.
1916 A textbook of pathology. W. B. Saunders Co., chap. 9.
- MALL, F. P. 1912 On the development of the human heart. *Am. Jour. Anat.*, vol. 13, p. 249.
- MALLODY, F. B. 1914 *The principles of pathological histology*. Philadelphia, W. B. Saunders Co.
- MARCHAND, F. 1913 Über die Herkunft der Leukocyten und ihre Schicksale bei der Entzündung. *Verhandl. d. deutsch. Pathol. Gesellsch.*
- MAXIMOW, A. 1902 Experimentelle Untersuchungen über die entzündliche Neubildung von Bindegewebe. *Ziegler's Beiträge*. Supplement, Bd. 5.

- MAXIMOW, A. 1903 Entstellung, Struktur, und Veränderung des Narbengewebes. Ziegler's Beiträge, Bd. 34, 1903.
 1905 Beiträge zur Histologie der eiterigen Entzündung. Ziegler's Beiträge, Bd. 38, S. 301.
 1910 Ueber embryonale Entwicklung der Blutzellen bei Selachiern und Amphibien. Verhandl. der Anat. Gesellschaft. Fischer, Jena.
- METCHNIKOFF, E. 1893 Leçons sur la pathologie comparée de l'inflammation. Paris, 7th Leçon, p. 111.
- MINERVINI, R. 1911 Über die Neubildung von Blutgefässen. Virch. Arch., Bd. 204, S. 75.
- MINOT, C. S. 1912 The development of the blood. In Human Embryology, ed. by Keibel and Mall, vol. 2, p. 498.
- MOLLIER, S. 1911 Über den Bau der capillaren Milzvenen (Milzsinus). Archiv. für mikrosk. Anat. und Entw., Bd. 76, S. 608.
- RIBBERT 1890 Über die Betheilung der Leukocyten an der Neubildung des Bindegewebes. Centrabl. f. allgem Path. u. path. Anat., Bd. 1, S. 667.
- SABIN, F. R. 1913 The origin and development of the lymphatic system. Johns Hopkins Hospital Reports, Monogr. new series, no. 5.
- SCHULTE, H. VON W. 1914 Early stages of vasculogenesis in the cat. Memoirs of The Wistar Institute of Anatomy and Biology, no. 3.
- ZIEGLER, E. 1905 Lehrbuch der allgemeinen Pathologie und der pathol. Anat., 11th ed., G. Fischer, Jena, S. 368.