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Microscopical Anatomy of the Involution of the Branchial Bars of Amphibia.

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Microscopical Anatomy of the Involution of the Branchial Bars of Amphibia.

Preliminary Note.

By
A. Grotans.

Contents.

The degeneration of tissues and organs has long been known in pathological histology. Pathologists consider various diseases and the ageing of the organism as causes of the degeneration of the tissues of the human body. Physiological reduction is of a quite different nature. Its result is the disappearance not only of certain microscopical elements of the tissues and of certain organs, but even of entire parts of the body. The metamorphosis is accompanied by complicate histological as well as physiological, chemical. and physical processes, as a result of which certain organs or parts of the body become liquified and serve as nutriment or even as material for the building up of new organs. Physiological reduction is very frequent in the animal kingdom.

The aim of the present paper is also to describe the processes that take place during the reduction of the gills of several amphibia. A survey and a list of the literature pertaining to the subject will be found in my projected detailed publication.

I avail myself of the opportunity of expresseing my sincere thanks to the Director of the Institute of Comparative Anatomy and Experimental Zoology — Professor Dr. *N. G. Lebedinsky* for his kind permission to work in the Institute during the time of the investigation and to use the instruments, the library, and the aquarium. My thanks are also due to the Principal of the Department of Zoophysiology, Associated Professor *L. Āboliņš*, for his kind assistance with the accessories of microphotography.

I Materials and Methods.

Macroscopical observations were effected over a period of 2½ years upon 12 white and 5 dark pigmented axolotls. During these macroscopical observations the animals were narcotised in water containing 1 p. c. of sulphuric ether. 22 axolotls *(Amblystoma mexicanum Cope)* were used for histological investigation — 17 white specimens and 5 dark pigmented ones.

The materials for the investigation of the newt larvae *(Triton cristatus Laur.)* were collected in 1924 and 1926, between the 15th and the 20th of August, and in 1931, between the 27th and the 30th of July. The materials of 1924 and 1926 were received from the Institute already fixed, but in 1931 live animals were obtained. The gills of 25 animals were used for histological research.

The triton larvae and the axolotl materials were fixed in Bouin's and in *Zenker's* fluid and in a formalin solution.

Two protei *(Proteus anguineus Laur.)* and nine salamander larvae *(Salamandra maculosa Laur.)* were obtained from the Institute already fixed. The gills of all the animals mentioned were used for histological research.

The series of paraffin slides were stained with eosin and haematoxylin and according to *Mallory's* method. I prepared longitudinal sections of the branchial bars (dorso-ventral direction) and also cross sections. The sections were 3—6 μ in thickness.

II Histology of the Reduced Branchial Bars of the Axolotl *(Amblystoma mexicanum Cope)*

Two rates of speed were observed in the reduction of the branchial bars of the axolotl: in some animals the reduction progresses slowly, with long interruptions, during which the processes of reduction stop, and in these cases the size of the gills diminishes by not more than one half; in other cases on the contrary the reduction is rapid: after 2—4 weeks already from what were well-formed gills only small rudimentary formations remain.

In a histological sense also both reduction processes differ: the slow reduction is connected with the inner processes in the tissues while in the speedy reduction a conspicuous part is played by the separation and the outward-bound migration of the elements of the cells.

Earlier histological investigations of the involution of amphibia were mostly concerned with the tail of the tadpole. Such are the works of *Barfurth* (1877), *Loos* (1889), *Noeltzel* (1895), *Saguschi* (1915), *Dennert* (1924), and others. Only *Kornfeld* (1914) in one of his works has given a skort account of the reduction of the gill lamellae of the salamander. The involution of the gill lamellae of the axolotl has been described by *Grotans* (1934).

During my investigations I observed the involution processes in the skin, (epidermis and cutis), the musculature, the blood-vessels, and the connective tissue.

1. Reduction of the Skin (Epidermis and Cutis).

Similarly to the reduction of the gill lamellae that of the epidermis and the cutis of the branchial bars proceeds mostly from their distal to the proximal end, but it occurs also, though to a lesser degree, on the entire ventral part of the bar and to some extent on the sides, while in the dorsal part the involution is slower (fig. 1, 2, and 3). Besides, the speed of the involution of the skin of the bar may be quickened in certain portions of the ventral or the lateral parts of the bar while it is less pronounced in the remaining part of the gills.

The first indications of the beginning reduction of the skin appear in the basal cells of the epidermis (str. cylindricum and str. germinativum) and in the basal membrane. I observed similar processes in the reduction of the gill lamellae of the axolotl. *Loos*, *Noetzel*, and *Dennert* have observed the same, though in a lesser degree in the involution process of the tailfold in the larvae of amphibia. The thin, smooth-surfaced, seemingly homogeneous basal membrane of the branchial bar does not keep its former structure, it becomes looser in consistency and its fibry structure is easily discernible.

At the same time the epidermis, especially in its basal part, becomes also looser and the intercellular cavities become larger. The cells in process of reduction grow smaller in size, and are connected with one another by bridges of protoplasm. As the cells become still smaller, the connecting branches of protoplasm between the adjacent cells break and the cells freely migrate in a basal direction, i. e. into the jelly tissue. The nuclei that have reached the connective tissue at first retain reduced remnants of protoplasm and of cell-membranes. The intercellular passages still serve to evacuate the products of the involution of the liquified epidermis. So long as the cutis still forms a compact membrane separating the cutis from the epidermis, the removal of the products of the reduction of the epidermis into the jelly tissue is impeded. These products are removed into the jelly tissue in smaller portions through the spaces between the looser fibres. In the separate slides it was possible to discover the connexion of the intercellular spaces with the cavities and the crevices in the jelly tissue. Besides this, branches of many cells of the basal epidermis insert themselves into the basal membrane and a connexion of those branches with the fibres of the connective tissue is possible. This indicates that the epidermis and the connective tissue are no longer strictly limited and that a removal of the products of the involution of the epithelium into the jelly tissue is possible. A similar connexion of the epidermis with the connective tissue has also been observed by *Loos* and *Dennert* in regard to the tailfold of amphibia.

On the other hand the basal membrane also puts forward wedge-shaped excrescences into the basal cell-layer of the epidermis (stratum cylindricum). In normal gills such excrescences are rarer and smaller in size.

The reduction progressing, the basal membrane becomes still looser and in places it is already plainly divided into fibres which are located one beside the other. As the process goes on, in certain places the basal membrane entirely disappears (fig. 2 and 3). This especially applies to the ventral parts of the branchial bar. In the dorsal parts of the branchial bar the reduction of the basal membrane is much retarded and frequently the involution shows it — self only by the basal membrane becoming looser in consistency.

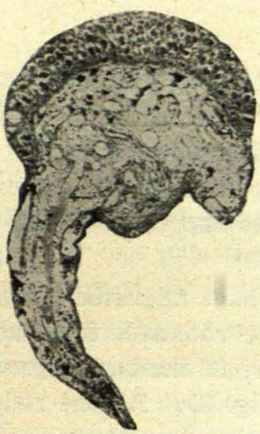

Fig. 1. *Amblystoma mexicanum.* Cross section × 85 of the distal end of the branchial bar at the beginning of the reduction. The dorsal epidermis is thick enough. The lateral blood-vessel of the lamella has joined the blood-vessels of the bar. The blood-vessels of the bar are considerably reduced.

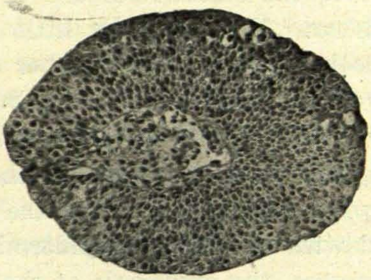

Fig. 2. *Amblystoma.* Cross section × 260 of the distal end of the bar at a later stage of reduction. The blood-vessels are completely reduced. Migration of the epidermal cells into the jelly tissue.

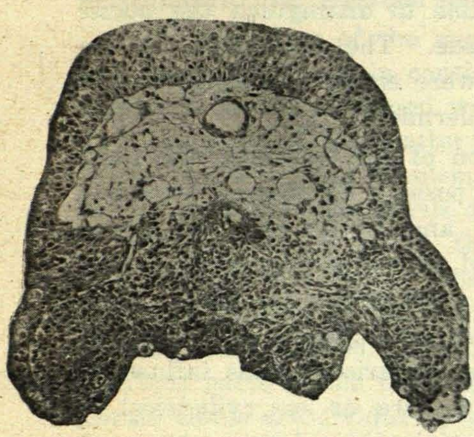

Fig. 3. *Amblystoma.* Cross section × 260 of the middle part of the bar at a later stage of reduction. In the ventral part increased speed of the reduction of the epidermis and the basal membrane. Blood-vessels considerably reduced.

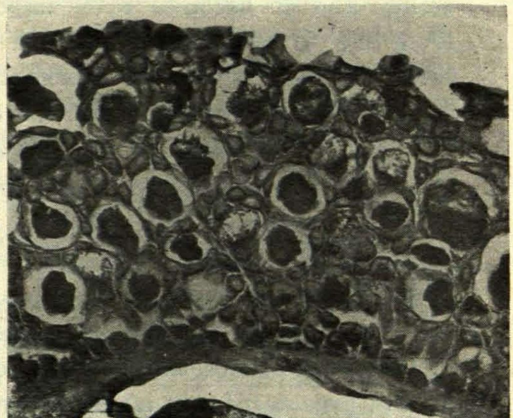

Fig. 4. *Amblystoma.* Cross section × 1010 of the middle part of the bar at a later stage of reduction. Pronounced reduction of the epidermis. Large light spots appear in the place of the reduced *Leydig* glandular cells. Outward-bound migration and migration into the jelly tissue of the products of the reduction of the epidermis. The basal membrane is fibrous and contains some nuclei of epidermal cells. A wide cavity along the basal membrane evidently receives the products of the reduction of the epidermis.

In connexion with the indicated reduction processes an uneven boundary appears between the epidermis and the corium in the place of the smooth surface of the basal membrane of the branchial bar. Thus little by little a strongly undulated basal membrane is formed. The number of wrinkles increases in the direction of the distal end of the bar.

In the place of the reduced cells that have migrated either into the jelly tissue or outwards there appear roundish free intercellular spaces (fig. 4). Thus the distal part of the bar is gradually filled with the reduced epidermis cells (fig. 2). As the reduction progresses, the epidermis of the bar sometimes considerably grows in thickness.

In those places of the bar where the basal membrane has disappeared the products of the reduction of the epidermis move unimpeded into the connective tissue. There they remain for some time and cause the boundary between the epidermis and the connective tissue to become hardly discernible (fig. 3). The nuclei are ordinarily quite bare or sheathed to a small extent with a thin layer of protoplasm. Later on the discernibleness of tissue diminishes and it becomes even impossible to distinguish the nuclei from the surrounding connective tissue. The distal ends of the branchial bars of several specimens were entirely occupied by the elements of the involution of the epidermis.

By the processes of the reduction of the epidermis the form of the cells is also changed. It was possible to observe prismatic cells, as already described by *Loos* and *Dennert,* with long narrow nuclei. These cells are placed with their longitudinal axes perpendicular to the basal membrane. Yet within the limits of my materials I could not establish whether, as a general characteristic in the reduction of the gills, the cells of various forms influenced by the process of involution assume a more or less cylindrical or prismatical form. Very often they retain their former shape remaining cubic or even round (fig. 2, 3, and 4).

In the exceedingly speedy reduction of the gills in several specimens a prominent part was played by the outward-bound migration of the cells of the outer layers, either as a migration of separate cells (fig. 4), or in groups. *Loos,* too, emphasises the migration outwards of the epidermal cells in the involution of the tailfold in larvae of amphibia, yet he does not attach to this phenomenon the proper importance in the process of reduction.

A complete reduction of the epidermis is possible and in this case the branchial bars are externally sheathed by the basal membrane only (fig. 5).

Most frequently the reduction of the bars may be observed at their distal ends, where there is no musculature. In the incomplete reduction of the gills the involution of the bars ordinarily ends when the part of the bar not provided with musculature is reduced.

The involution of the gills goes on without interruption until at a certain moment the process of reduction comes to a complete standstill. I did not observe any fluctuations in the speed during the whole process of reduction.

2. The Reduction of the Musculature.

As the involution of the gills begins at the distal end of the branchial bar, but the muscular fibres reach only the last third or quarter of the bar no changes are yet to be observed in the musculature during the first stages of reduction. In most animals the reduction is accelerated in those parts of the gills that are free of musculature, and retarded in those containing muscular fibres.

The reduction of the musculature begins by the muscular fibres becoming looser in consistency, the muscular fibrils being no longer so closely connected with one another. I could observe the disappearance of the matter connecting the muscular fibres and fibrils (sarcoplasma, sarcolemma, and the inner perimysium) while at the same time the fibrils did not change their relative position. As the reduction advances, the discernibleness of the muscular fibres diminishes rapidly. These latter are very likely to undergo chemical changes as the staining and the refraction of the rays of light are increased. The boundaries of the anisotropic and the isotropic parts of the fibres lose their former distinctness. The separate fibres are divided by intermediate spaces, which shows that the bulk of the muscles has become smaller. The indicated characteristics are more pronounced at the distal end of the bar and decrease towards the basal end; therefore it must be presumed that the reduction of the muscles, too, begins first of all at the free distal ends and progresses in the basal direction. The free spaces appearing with the progress of the reduction of the muscles are filled little by little by connective tissue. In my preparations I could not observe any active influence of the leucocytes in the reduction process of the musculature.

3. The Reduction of the Blood-vessels.

The musculature and the basal membrane of the branchial bar must be counted among the most durable components, as they keep their structure longest and do not submit to the processes of resorption. The system of blood-vessels, on the contrary, is the first to be influenced by the more important or smaller processes of the gill reduction. *Loos* also places the blood-vessels in the tails of larvae of amphibia among the tissues most easily reduced. The reduction of the blood-vessels shows itself first of all in the perceptible lessening of the quantity of the morphological blood elements and even in the total absence of these elements in the blood-vessels of the distal parts of the bar (fig. 1, 2, and 3). Yet the walls of the blood-vessels, though containing no blood corpuscles, do not shrink, but retain their former shape. Also from the outside it is possible to observe the absence of the morphological blood elements in the gill lamellae and bars. The lamellae which hitherto were pink now, at the beginning of the process of reduction, turn light, almost white. Through the epidermis of the bar pink-coloured blood-vessels are also no longer discernible.

Very soon it becomes possible to observe the reduction of the walls of the blood-vessels. The epithelial cells lining the inner walls of the blood-vessels become detached and migrate into the blood-vessels; the inner oblong and the outer layer of circular musculature become looser in consistency and vacuoles appear between the muscular fibres. The involuting epidermal cells migrate into the loose walls of the blood-vessels and also into the blood-vessels themselves. The reduction of the walls of the latter progresses, they become more and more loose until they entirely blend with the surrounding tissues — the elements of the epidermis and the connective tissue.

4. The Reduction of the Inner Connective Tissue.

At the very beginning of the involution of the connective tissue its loose fibres become less distinct. These, as seen in the preparations, to a great extent blend with the jelly tissue. As the branchial bars grow less in size, the nuclei of the connective tissue as well as the fibres of the loose connective tissue draw nearer to one another, and the jelly tissue, transparent and poor in cell elements before the reduction, is now filled with cell nuclei and fibres and loses its transparency (fig. 5 and 6). The fibres of the loose

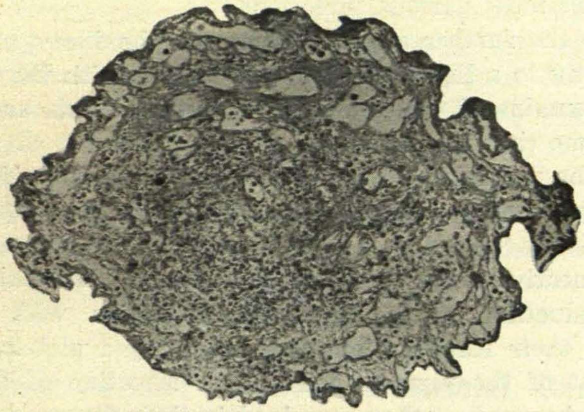

Fig. 5. *Amblystoma.* Cross section × 260 of the basal part of the bar at the last stage of reduction. The epidermis is considerably reduced. The (usually transparent) jelly tissue is filled with the products of the reduction of the epidermis and the musculature.

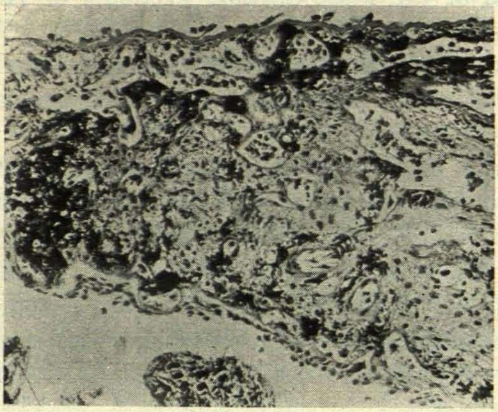

Fig. 6. *Amblystoma.* Longitudinal section × 630 of the distal part of the bar at the last stage of reduction. The epidermis is considerably reduced. The jelly tissue is filled with the products of the reduction of the epidermis and the musculature. At the distal end of the bar there is much pigment which has gathered there owing to the blending of the pigment of the chromatophore cells from the reduced part of the bar.

connective tissue are no longer located in a certain order, but extend in the most various directions.

During the further stage of reduction the fibres of connective tissue passing to a liquid state entirely blend with the jelly tissue, while the remains of the nuclei of the reduced cells are still to be seen for some time in the jelly tissue.

With the reduction of the connective tissue there form in this tissue many crevices and cavities filled with the liquid derived from the tissues (fig. 4, 5, and 6); they contain elements of reduction of connective tissue, blood-vessels, muscles, and skin.

The reductions of the pigment-cells begins with these cells drawing in their ramifications and assuming a globular shape. A displacement of the pigment-cells in the direction of the bar axis I have not observed — they remained in their former position, i. e. near the basal membrane. With the shrinking of the bar the quantity of pigment increases at the end of the bar and partially also round it: the pigment that was situated on a bar perhaps twice as long now concentrates especially at the distal end and the adjoining portion of the bar (fig. 6). This explains the increased colouring of the gills in process of reduction — especially at their distal ends — of the dark pigmented axolotl. I have not observed any further transformation of the pigment. The latter must be regarded as one of the most durable products of reduction of the involuting tissues.

Looking over the whole reduction process in the branchial bars of the axolotl and within the limits of my investigations I can state the following:

Independently of the consideration whether the cause of the reduction of the gills of the axolotl lies in the environment, i. e. the outer conditions of life, or in the metamorphosis of the animal, the process of reduction is in the first place connected with the involution of the blood-vessels which is soon accompanied by the reduction of the epidermis and the cutis. The further development of the process shows itself in the connective tissue and the musculature.

The common feature of the reduction of the skin, the musculature, the blood-vessels, and the connective tissue is that first of all the connective matter of all these tissues is reduced to a liquid state. This matter joins all the elements of these tissues into one whole, and as it disappears the tissues become loose in consistency. At the same time changes in the structure of the elements of the tissues can be observed: the nucleus offers a greater resistance to the processes of reduction, while in the other part of the cell the processes of dissociation are more rapid. Thus the separate tissue cells belonging to different germinal layers return again to the indifferent (primitive) condition. The reduction of the gills is not a simple degeneration of organs, but-if we do not take into account a number of cells migrating in an outward direction — it is a resorption in the truest sense of the word. The part played by the leucocytes in the process of reduction of the gills is quite insignificant; the liquifiers of the products of reduction are the fluids of the body and the blood.

III Histology of the Reduced Branchial Bars of the Proteus *(Proteus anguineus Laur.)*.

My observations are based on materials obtained from two specimens only and therefore cannot be generalised to such an extent as to allow of my speaking of the reduction of the gills of the proteus. I regard the following only as a description of the reduction of the gills of separate animals.

The involution of the gills begins with changes in the epidermis and in the basal membrane. In the basal layers of epidermal cells the reduction is more intensive than in the upper ones. At the beginning of the reduction the epidermis cells do not show any change in size, but the protoplasm stains lighter. Later on the nucleus and the protoplasm grow less in size, and the reduced cells appear located in a light spot. At a still later stage of reduction of the cell the protoplasm is no longer discernible. An outward-bound migration of the cells was not frequently observed. Simultaneously with the reduction processes in the epidermis the basal membrane becomes loose in consistency and undulated. Yet, though the basal membrane becomes undulated its wrinkles are quite insignificant when compared with those in the gills of the axolotls. The removal of the products of the involution of the epidermis into the connective tissue is slight. The diminution of discernibleness of tissue progresses much faster than in the axolotls.

Though the progress of the reduction processes in the epidermis was intensive and the walls of the cells of the blood-vessels, too, became looser in consistency, yet the blood-vessels were filled with blood even to the distal ends of the gills. As already stated, quite the opposite had been observed in the axolotls, excepting one specimen of these latter, in which the reduction of the epidermis had already begun at the distal end of the bar while the morphological blood elements still filled the blood-vessels.

Formations of crevices and cavities in the connective tissue are also observed in the proteus. The reduction of the musculature resembles that described in the axolotls.

At the completion of the involution of the gills the epidermis is entirely reduced, the surface of the gills is markedly undulated, and the blood-vessels are reduced. The transparent jelly tissue in the bar is filled with the fibres of the loose connective tissue that have gathered there from the entire bar. In the connective tissue there can now be seen many crevices and cavities filled with the liquid and the products of the reduced tissues.

Thus the migration outwards of cellswas insignificant also in the reduction of the gills of the protei, and therefore the process of involution must be regarded as resorption.

IV Histology of the Reduced Branchial Bars of the Newt Larva *(Triton cristatus Laur.)*.

The changes observed during the reduction in the walls of the blood-vessels in the musculature, in the elements of the connective tissue, and in the separate cells of the epidermis on the whole resemble the changes observed in the axolotls and the protei. On the contrary, the initial moment, the rate of speed, and the duration of the involution of the tissues (the epidermis, the muscles, and the connective tissue) of the triton are different from those observed in both above-mentioned animals.

The reduction begins in the epidermal cells and very soon the entire epidermis becomes loose in consistency. This looseness becomes especially evident if we compare the epidermis of the triton with the involuting surface-skin of the axolotl and of the proteus. Owing to the loose consistency of the epidermis of the triton an outward-bound migration of the cells can also be observed. The migration of the products of the reduction of the epidermis into the connective tissue was twofold: in some animals it was reinforced, in others to a great extent or even exceedingly limited. It was also impossible to notice a definite connexion between the reduction of the blood-vessels and that of the epidermis, the initial moment and the speed being different in the separate individuals. Though the walls of the blood-vessels were loose in consistency and the involution of the epidermis was almost completed, the blood-vessels of some animals still contained many blood-corpuscles. In other specimens the blood-vessels were completely reduced, especially at the distal parts of the bars, while the involution of the epidermis had just begun. In connexion with the transformation of the reduced tissues of the bar into a non-differentiated liquid state a great number of crevices and cavities formed in the connective tissue of the bar — they served to receive the liquified tissues and to transfer them into the body. The reduction of the musculature is slow. At a moment when the other tissues were considerably reduced the musculature showed no changes. In the ends of the branchial bars of several tritons an intensification of the colouring appeared, similar to that described in the axolotl. In the advanced stages of the gill involution the transparent jelly-tissue was filled with the products of the reduction of the tissues of the bar: epidermal cells, fibres of muscles and of loose connective tissue, and chromophores. Besides this I could observe that the reduction of the epidermis of the tritons is also not uniform in the whole bar and that in one part of the bar the involution may be accelerated while it is retarded in others. A complete reduction of the epidermis is also possible; in this case the bar is covered at the outside by the basal membrane. In the reduction of the gills of the triton there is no such progressiveness as in the gill involution of the axolotl. Strongly reduced bars were observed, but at the same time the length of the lamellae was not yet perceptibly changed. The outward-bound migration of tissues plays a more important part in the reduction of the gills of the tritons than in that of the axolotls and the protei.

V Histology of the Reduced Branchial Bars of the Salamander Larva *(Salamandra maculosa Laur.)*.

The reduction of the gills beginning, the epidermis becomes loose in consistency at the ends of the lamellae and of the branchial bar as well as on the ventral parts. At that moment the epidermis of the dorsal part shows as yet no sign of reduction. The speed of the reduction of the gills of the salamander is similar to that of the reduction of the gills of the proteus and the axolotl. Such an intensity of the reduction process as in tritons I have not observed in salamanders. Similtaneously with the travelling of the reduced epidermis cells into the connective tissue there was also an outward-bound migration.

In connexion with the retarded removal of the products of the reduction of the epidermis into the connective tissue the blood-vessels, too, are for a considerable time not filled with reduced epidermis cells and retain their former shape. The morphological elements in the blood-vessels are rare. The diminishing of the number of blood-corpuscles in the involuting blood-vessels has also been described by *Kornfeld* (1914).

The progress of the reduction of the musculature is very similar to that in the three animals first described. The musculature of the salamander must also be regarded as one of the gill tissues with late reduction.

At the later stages of the reduction a complete involution of the epidermis of the branchial bars can be observed. The bar is covered at the outside by the basal membrane and by only a few much reduced cells. In the jelly tissue of the bar there are many fibres of loose connective tissue. At this moment the musculature is yet relatively slightly reduced.

In the reduction of the gills of the salamander the outward-bound migration is less than in the case of the gills of the triton.

The initial moment, the rate of speed, and the duration of the involution of the tissues of the gills (epithelium, muscles, and connective tissue) of the salamander resemble the similar processes in the proteus and the axolotl, as the reduction first of all expresses itself in the involution of the blood-vessels, soon followed by that of the epidermis and the cutis and, last of all, by that of the inner connective tissue and the musculature.

VI Summary.

In the reduction of the gills of the axolotl, the proteus, the triton, and the salamander, the involution processes of the different elements of the tissues, as for instance the separate epidermis cells, the muscular fibres, the blood-vessels, and the connective tissue, are quite similar in their principal features. At the conclusion of the process many of the cells of the gills pass into an indifferentiated condition.

The initial moment of the involution of the tissues of the branchial bars (epithelium, muscles, and connective tissue) of the animals mentioned, the speed, and the duration of these processes are different.

The involution of the gills of the axolotl starts with the reduction of the blood-vessels which is soon accompanied by the reduction of the skin (epidermis and cutis); last follows the reduction of the inner connective tissue and the musculature.

The process of the reduction of the gills of the proteus, the triton, and the salamander begins mostly with the reduction of the skin, while for a certain time — differing for the separate individuals — an involution of the blood-vessels is not observable.

The speed of the reduction of the skin is greater in the axolotls and the tritons and less in the protei and the salamanders.

In all four animals mentioned the epidermis and the blood-vessels are reduced most rapidly, whereas the reduction of the connective tissue and of the muscles is slow. A complete reduction of the epidermis could be established in all these animals.

The reduction of the gills of the axolotl, the proteus, the triton, and the salamander is not a simple degeneration of the organs, it is — apart from some outward-migrating cells — a resorption in the truest sense of the word. The number of outward-migrating cells is greatest in the triton and smallest in the axolotl.

I could not observe that the leucocytes played any active part in the involution of the musculature of the animals mentioned.

(No Latvijas Universitātes salīdzināmās anatomijas un eksperimentālās zooloģijas institūta, Rīgā. Direktors: N. G. Lebedinsky.)

Abinieku žaunu kātu involūcijas mikroskopiskā anatomija.

Iepriekšējs ziņojums.

A. Grotans.

Aksolotlu, proteju, tritonu un salamandru žaunu redukcijā dažādu audu elementu, piem.: atsevišķu epiderma šūnu, muskuļu šķiedru, asinsvadu un savienotājaudu elementu atpakaļattīstības norises pamatvilcienos vienādas. Procesa noslēgumā daudzas no to šūnām pāriet nediferencētā stāvoklī.

Minēto dzīvnieku žaunu kātiņu audu (epitēla, muskuļu un savienotājaudu) atpakaļattīstības iesākšanās laiks, temps un ilgums ir dažādi.

Aksolotlu žaunu involūcija iesākas ar asinsvadu atpakaļattīstību, kam drīz pievienojas ādas (epiderma un cutis) redukcija un beidzot process norit arī savienotājaudos un muskulātūrā.

Proteju, tritonu un salamandru žaunu redukcijas process pa lielākai daļai sākas ar ādas redukciju, kamēr asinsvadu involūcija kādu laiku vēl (kas atsevišķiem individiem dažāds) nav vērojama.

Ādas redukcijas ātrums vislielāks aksolotliem un tritoniem, bet mazāks protejiem un salamandrām. Visiem četriem minētiem dzīvniekiem visātrāk reducējas epiderms un asinsvadi, turpretim savienotājaudu un muskuļu redukcija norit lēni. Varēju konstatēt visiem viņiem pilnīgu epiderma redukciju.

Aksolotlu, proteju, tritonu un salamandru žaunu redukcija nav vienkārša organu deģenerācija, bet gan, ja atskaita dažas uz āriem migrējušas šūnas, rezorpcija visīstākajā vārda nozīmē. Uz āriem migrējušo epiderma šūnu skaits vislielāks tritoniem, bet vismazāks — aksolotliem.

Minēto dzīvnieku muskuļu involūcijā aktīvu leikocītu darbību netiku novērojis.