On Acid Phosphatase During Lens Regeneration in Triturus Pyrrhogaster (BOIE).

Recently, attempts have been made to study the regenerative process in so-called WOLFFian lens regeneration not only from the morphological standpoint but also from the histochemical aspect. Among such studies, SAWANO (1942) has worked on the changes in glycogen of the lens and eye during lens regeneration, MIKAMI and NISHIMURA (1944) have studied the changes in fluorescent lipid in the retina, PÓSAŁAKY, KELEMEN, TÓRÖ and NÁNÁSY (1951) have investigated the behavior of alkaline phosphatase of the retina and iris, while TAKATA (1952) engaged in the research on the condition of RNA in the lens. However, there is as yet no research on acid phosphatase. Therefore, I have studied the state of acid phosphatase in the lens during the regenerative process.

I. Material and Method.

The test material consisted of adult Triturus pyrrhogaster (BOIE) captured in the suburbs of Nagasaki City and kept in a tank containing tap water for at least half a year after removal of both lens, these experimental animals were in a water tank at 24—27 degrees C. and sacrificed by decapitation on the 9th, 10th, 11th, 12th, 13th, 14th, 18th, 21st and 35th day after the operation, respectively. They were immediately fixed in a mixture containing equal amount of absolute aceton and alcohol cooled to 2 degrees C. 30 minutes later, only the eye ball was enucleated from the head and fixed for 15 hours in a fresh solution of the above mixture. This material after treatment with toluene was embedded in paraffin at 55 degrees C. for 40 minutes and serial sections 8 microns in thickness were prepared. Next, following deparaffinization with toluene, they were incubated for 20 hours in a substrate prepared by GOMORI's improved technique (1950), the acid phosphatase activity was demonstrated and after stain of the nucleus was done by the modified method of LILLIE (1953). Tests on the substrate showed that at pH 6 the reaction of the cytoplasm is the greatest and therefore this value was selected as the optimum pH. As controls, specimen inactivated by heating at 90 degrees C. for 5 minutes were incubated at the same time, or were incubated in a mixture from which beta-glycerophosphate had been removed. This had been done in order to avoid errors due to the non-specific reaction which is said to readily occur in this lead sulphide method. Also, so that the enzyme activity in each stage of regeneration may be more accurately compared, the various procedures subsequent to fixation of the
material were carried out at the same time, under the same conditions and by the same method.

II. Observations.

a) Regenerate 9 or 10 days after lentectomy.

Nine or ten days after lentectomy, depigmentation, cellular multiplication and the formation of a space between the outer and inner layers of the epithelium occur in the pigment epithelium at the mid-dorsal area of the iris which is the anlage for regenerated lens, and thus the early regenerate is formed in the form of a mild elevation on the margin of the iris.

In this regenerate, moderate acid phosphatase activity is noted in the perinuclear cytoplasm but the reaction of the nucleus is generally weak. Because of the reaction in the pigment granules of the surrounding epithelium and the capillary walls of the iris stroma, almost the entire iris is dark brown, but since the color of the regenerate is somewhat light, it can be distinguished (Fig. 1).

In the inactivated control specimen, the regenerate is completely negative and only the nuclei stained with safranin are noted (Fig. 2).

b) Regenerate 11 or 12 days after lentectomy.

The regenerate in this stage due to cellular multiplication and increase in the space between the outer and inner layers of the epithelium has developed to a lens vesicle with a large cavity which swells out from the margin of the iris. The cavity of this lens vesicle is connected to the space between the outer and inner layers of the epithelium and frequently contains degenerated pigment cells or pigment masses. The epithelium of the lens vesicle is composed of a single layer of low cylindrical cells and in advanced cases the height of the cells on the posterior wall is somewhat greater than that on the anterior wall.

Fig. 1. Acid phosphatase activity in regenerate 10 days after lentectomy. Moderate reaction is observed in the cytoplasm of the whole regenerate. GOMORI’s improved technique. × 175

Fig. 2. Control specimen inactivated by heating at 90°C for 5 minutes. The nuclei are slightly stained with safranin. × 175
Throughout the entire regenerate, the perinuclear cytoplasm demonstrates moderate phosphatase activity but the activity of the nucleus generally is weak. Some cells show a particularly strong reaction while in other the reaction is markedly weak. In general it appears that cells on the iris margin show stronger activity than those on the opposite side. Furthermore, no difference in activity could be demonstrated between cells on the posterior wall which were increasing in height and cells on the anterior wall (Fig. 3).

Inactivated control specimen were completely negative. (Fig. 4)

c) Regenerate 13 or 14 days after lentectomy.

In this stage, the multiplication of cells on the anterior wall of the regenerate and differentiation of cells on the posterior wall to fiber cells is most active and the regenerate shows a conspicuous increase in size in a short time. That is, on the anterior wall of the regenerate, cells become rather flat and begin to differentiate into cuboidal lens epithelium. On the posterior wall, the cells are markedly elongated and differentiate to fiber cells and form a ridge like elevation which protrudes into the lens cavity.

The epithelial cells of the anterior wall of the lens show moderate phosphatase activity in the nucleus and adjacent cytoplasm. In the fiber cells of the posterior wall, the activity is greatest at the posterior surface, particularly the potential posterior pole of the lens, and the nucleus also demonstrates a considerable reaction. In areas in front of this, that is toward the apical region, there is a gradual decrease in activity. Cells in the early stage of differentiation to fiber cells near the transitional zone between epithelial cells and fiber cells show moderate activity in the nucleus and surrounding cytoplasm but in addition the entire cell shows mild diffuse activity. However, in cells at the center in which the differentiation is advanced
the activity is limited to the nucleus and the infranuclear and perinuclear regions with hardly any reaction in areas above. The activity seems to be somewhat greater in the epithelium and fiber cells on the side of the iris than on the opposite side (Fig. 5, 7).

Fig. 5. Acid phosphatase activity in regenerate 13 days after lentectomy. In the cells which the differentiation to fiber cells advanced, reaction is decreased from apical part. GOMORI's improved technique. ×155

Fig. 6. Control specimen inactivated by heating at 90°C for 5 minutes. The nuclei are slightly stained with safranin. ×155

Fig. 7. Acid phosphatase activity in regenerate 14 days after lentectomy. Deposits of activity in infranuclear and perinuclear regions of the fiber cells and about the posterior pole of the lens are observed. GOMORI's improved technique. ×175
Inactivated control specimen were negative (Fig. 6).

d) Regenerate 18 days after lentectomy.

The lens vesicle shows marked increase in size due to multiplication of epithelial cells and progress in their differentiation to so-called secondary fiber. The lens cavity becomes even smaller because of the lens fiber mass formed by the concentrical accumulation of fiber cells on the posterior wall. Nuclei near the center of this fiber mass have already begun to degenerate.

The phosphatase activity is generally decreased. The nucleus and surrounding cytoplasm of epithelial cells show a reaction slightly less than moderate. In fiber cells, the part of the cytoplasm and nuclei near the posterior surface, in particular the potential posterior pole of the lens, show moderate activity and the reaction gradually decreases in areas further anterior. In nuclei in which degeneration is advanced or apical part of cells, the reaction is negative. The epithelium and fiber cells on the side of the iris show somewhat greater activity than that on the opposite side (Fig. 8).

Inactivated control specimen are negative (Fig. 9).

e) Regenerate 21 days after lentectomy.

Epithelial proliferation and differentiation to fiber cells further progresses and the lens vesicle is further increased in size. The height of epithelial cells are somewhat lower and become flat epithelium. Fiber cells near the center have lost their nucleus and become fiber. Thus the nucleus of the lens has developed but a small cavity still remains.

There is further decrease in phosphatase activity and in general the nucleus and
perinuclear cytoplasm of epithelial cells demonstrate mild activity, but in some cells there is moderate reaction and the condition is not uniform. The great majority of fiber cells are negative, with a moderate reaction demonstrable only in the part of the cytoplasm and nucleus at the posterior surface, in particular at the marginal area centered about the potential posterior pole of the lens. A weak reaction can also be seen in the nucleus of cells nearby. Also, the reaction appeared to be somewhat stronger in the epithelium on the side of the iris than on the opposite side (Fig. 10).

Inactivated control specimen were negative (Fig. 11).

![Fig. 10. Acid phosphatase activity in regenerate 21 days after lentectomy. Reaction is further decreased and moderate activity is observed only in some epithelial cells and in fiber cells at the posterior surface of the lens. GOMORI's improved technique. ×120](image1)

![Fig. 11. Control specimen inactivated by heating at 90°C for 5 minutes. The nuclei are slightly stained with safranin. ×120](image2)

f) Regenerate 35 days after lentectomy.

In the regenerate, mitotic figures become rare, while fiber cells which have completely become fiber except at the equatorial zone are attached to the posterior surface of the anterior wall and the cavity is completely filled. Morphologically, it has almost reached the state of completion. Also, the size is increased and extends to the opposite side of the pupillary zone, that is the lower margin of the iris. However, communication with the upper margin of the iris is not lost yet.

A trace of phosphatase activity is noted in the nucleus and perinuclear cytoplasm of the epithelium while the lens fiber is almost negative (Fig. 12).

Inactivated control specimen are negative (Fig. 13).

The lens capsule was negative in all stages. In the retina moderate activity was noted primarily in the inner and outer plexiform layers and mild reaction was noted in the sensory epithelial layer and ganglion cell layer, but changes in the activity in the different stages could not be demonstrated.
III. Discussion.

Observation of the state of acid phosphatase of the lens in the regenerative process showed moderate activity in the perinuclear cytoplasm in the early regenerate with mild reaction in the nucleus (10 to 12 days after lentectomy). In the course of differentiation to fiber cells, fiber cells in the developmental stage of differentiation showed moderate activity in the nucleus and perinuclear cytoplasm as well as mild, diffuse reaction throughout the entire cell, but in fiber cells in which differentiation was advanced, the reaction is gradually limited to the nucleus and surrounding area, particularly the infranuclear region, and is hardly noted in areas above. On the other hand, the epithelial cells in this stage first show a moderate activity, but with advance in differentiation it gradually decreased (13 to 18 days after lentectomy). When differentiation progresses further and lens fiber increase with loss of nucleus, the activity in fiber cells is limited to a small area at the posterior surface of the lens about the posterior pole of the lens and finally disappears when lens fiber is completed. On the other hand, the activity of the epithelial cell continues to decrease, but even in the terminal stage a weak activity still remained (21 to 35 days after lentectomy).

In literature results of histochemical studies on phosphatase in the lens have been presented as follows: MCKAY, ADAMS, HERTIG and DANZIGER (1955, 1957) reported that in human fetus measuring 5 mm in body length alkaline phosphatase (pH 9) was weak positive (+) and acid phosphatase (pH 5) was negative (−) while in 6 mm and 7 mm fetus alkaline phosphatase (pH 9.4) was negative (−) and acid phosphatase (pH 5) was weak positive (+). In the observation of unrolled...
preparation of lens capsule and epithelium removed together from the lens of adult rat by BROLIN and NORDSTRÖM (1952), alkaline phosphatase (pH 9.4) and acid phosphatase (pH 4.7) activity were high in the border zone (midway between the anterior pole and equatorial zone), central zone (near the anterior pole) and the transitional zone (equatorial zone) in the order given and the reaction of each was particularly marked in the nucleus and weak in the cytoplasm. MIURA (1957) has reported that in his study of rabbit fetus (12 day fetus) acid phosphatase (pH 4.7) is weak positive in the nucleus of lens epithelium and positive in the nucleus of lens fiber while they both become negative in the terminal stage of fetal life. Among these reports the findings for rat and rabbit are somewhat similar to mine for newt, but in my case no difference by region could be demonstrated in the lens epithelium. Also, in newt marked reaction was noted not only in the cell nucleus but cytoplasm, but this is thought to be due to the fact that the pH of the substrate used in this study was relatively on weak acid side (pH 6) as reported by TAUE (1955).

POSALAKY, KELEMEN, TÖRÖ and NÁNÁSY (1951) have observed that in the regenerative process of lens the alkaline phosphatase of the retina and iris increases particularly at time of differentiation to lens fiber but that the lens itself hardly contains any enzyme, and reported that a certain process is working in the retina during the regenerative process of lens in which alkaline phosphatase plays an important role. However, in my study of acid phosphatase, a marked change in the enzyme activity was noted accompanying the regenerative process in the lens itself, but such a condition could not be confirmed in the retina. This may be due to the essential difference between the acid and alkaline enzyme and the difference in their biological role as mentioned by GOMORI (1941), DEANE and DEMPSEY (1945), THOMSEN (1955), MCKAY, ADAMS, HERTIG and DANZIGER (1958).

The results of my research, as described above, showed the reaction of the epithelium and fiber cells on the side of the iris to be slightly stronger than that on the opposite side in the lens vesicle on the 11th to 21st day. Also, in lens vesicle in which differentiation to fiber cells was slightly advanced (13th to 21st day) there is a tendency for the strong reaction to be concentrated concentrically about the potential posterior pole of the lens. These phenomena may possibly be due to diffusion and resorption from other tissues with high lead phosphate activity which frequently occurs in this lead sulphide technique, and for this reason the reaction was attempted on only the regenerate by removing the iris and retina or by reducing the incubation time to 10 hours, but the same results were obtained. Therefore, this is not considered to be a non-specific reaction. Rather, it is felt that since the deposit of high activity is present only in the nucleus, infranuclear and perinuclear regions of fiber cells, marked reaction had appeared in the potential posterior pole of the lens because the basis of the cells are concentrated here. The study by DEANE and DEMPSEY (1945) on the duodenal epithelium of various mammals and the epithelium of pregnant uteri of cat and snow, the observation of prostatic epithelium in mouse by BRANDES and BOURNE (1955) and the research by ALLEN and SLATER (1958) on the epithelium of the duct of the epididymis of mouse, all showed deposits of acid phosphatase in GOLGI’s region of cytoplasm. In my study of regenerative process of lens it is interesting to note that except in the early regenerate
in which the location of the reaction within the cytoplasm could not be determined due to shrinkage of the tissue in fixation, the site of reaction generally corresponded to the location of the GOLGI apparatus as previously reported by SETOGUTI (1954).

As to the biological significance of acid phosphatase, it is not so evident as for alkaline phosphatase. WACHSTEIN (1944) presumed that there was a relation with nucleic acid metabolism because of the frequent marked reaction in the nucleus. However, the histological findings of the nucleus are not consistent with the biochemical quantitative results of PALADE (1951), and also GOETSCH, REYNOLD and BUNTING (1952) have noted that the reaction is not demonstrable in the nucleus when the section is incubated without deparaffinization. Therefore, the question whether the reaction of the nucleus is non-specific or not still remains to be solved. Further, DEANE and DEMPSEY (1945) claim that the phosphatase in the GOLGI region is not related to the metabolism of the cell but is rather destined to be secreted. BODIAN and MELLORS (1945) who studied the motor nerve cells of rhesus monkey in which axon interruption had been done, recognized a close relation between regeneration of the NISSL bodies (RNA) and acid phosphatase activity, and postulated that there was a correlation between the increase of acid phosphatase and the synthesis of nucleoprotein. CHIBA (1953) observed the differentiation of polymorphonuclear leukocytes of rabbit and reported that there was close relation between acid phosphatase and DNA-turnover in the nucleus and RNA-turnover in the cytoplasm. LA VELLE, LIU and LA VELLE (1954) who studied the nerve cells of guinea pig, felt that since acid phosphatase activity changed depending upon the number of NISSL bodies, it was a part of the enzyme system which releases phosphate by acting upon ribonucleoprotein in the metabolic processes of maintenance and function of the cell. ALLEN and SLATER (1958) have reported that in the epididymis of mouse the acid phosphatase activity is controlled by androgenic hormone.

In the case of my lens regenerate phosphatase activity was the greatest in the first half of regeneration when cell multiplication, differentiation and growth are the most active, with gradual decrease in the reaction in the later half when the differentiation has progressed to some degree and there is only a trace of reaction in the terminal stage. My findings for acid phosphatase are in complete agreement with that for nucleic acid, particularly the changes in RNA noted in the course of regeneration of lens by SETOGUTI (unpublished). Consequently, the changes in these two material are similar to the findings of BODIAN and MELLORS, CHIBA, LA VELLE, LIU and LA VELLE described above. In the regenerative process of lens, as in the case of nerve cells and polymorphonuclear leukocytes, acid phosphatase is suspected to play an important role together with RNA in the metabolism of cells in the synthetic process of nucleoprotein accompanying cell multiplication, differentiation and growth.

IV. Summary.

1. The material consisted of adult newts kept for 9 to 35 days following lensectomy and the condition of acid phosphatase in the lens during the regenerative pro-
cess was investigated by GOMORI’s improved technique.

2. Moderate acid phosphatase activity was demonstrated in the first half of regeneration with high reaction near the potential posterior pole of the lens in the course of differentiation to fiber cell. On the other hand, in the later half of regeneration, the activity decreased gradually with progress in differentiation and in the terminal stage of regeneration only a trace of reaction is noted in the lens epithelium.

3. In fiber cells, acid phosphatase appeared to be deposited in the GOLGI region.

4. Acid phosphatase activity paralleled the changes in nucleic acid, particularly RNA, and it is felt that it may play an important role in the metabolism of nucleoprotein in the cell accompanying cellular multiplication, differentiation and growth in the course of regeneration.

References.