# STUDIES ON AMPHIBIAN METAMORPHOSIS. V. THE ATROPHY OF ANURAN TAIL MUSCLE DURING METAMORPHOSIS

(Five figures)

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THE total atrophy of the larval tail constitutes one of the most striking changes evidenced during anuran metamorphosis. The sequence of gross degenerations is quite definite although the time relationships between tail atrophy and other metamorphic changes is subject to some variation. As regards the former point, it may be said that the dorsal and ventral finny portions of the integument first degenerate, followed shortly by the musculature beneath. Evidence is also presented in the present article to support the idea that the anterior portions of the tail undergo atrophy more rapidly than more posterior regions. In natural metamorphosis the structure as a whole is apparently unaffected during early stages of larval transformation, suggesting the idea that the secretions of the thyroid are not directly concerned in initiating the degeneration. It is also a peculiar fact that in artificial metamorphosis the atrophy of the tail occurs much earlier in relation to other signs of transformation than is normally the case.

The problems concerned in tail atrophy may be classified under three main headings, viz., cytological, involving histolytic cellular changes; biochemical, embracing early chemical changes and later progressive changes during the ensuing histolysis; and causative problems as to the immediate fundamental agents initiating the various biochemical and cytological reactions which result in atrophy. Since the cytological and biochemical findings have been adequately reviewed elsewhere (Morse, 1918; Bradley, 1922) only the causative phase will be taken up here.

In 1891 Bataillon found that the development of the urostyle brought about a redistribution of the vascular supply to the tail owing to pressure of the rapidly growing organ on blood vessels entering the tail. Mercier (1906) identified phagocytes in the atrophying musculature of the tail which contained carmine granules previously injected into the dorsal lymph sac, thereby showing that complete occlusion of the vascular supply was not involved. Both of these workers suggested the probability that the reduced blood supply to the tail was responsible for the accumulation of metabolites with resultant tissue acidosis and autolysis. Morse (1918), commenting on this explanation of anuran tail atrophy, thought that a generalized probable lowering of the blood hydrogen-ion concentration below pH 7.0 was a more logical explanation although no blood determinations were made to lend evidence to this conception.

More recently Helff (1928), in a preliminary article, has shown that the urostyle cannot be the main causative factor since normal atrophy occurs following the removal of the larval urostyle before the latter undergoes growth. Simultaneously with this finding, Lindeman (1929) was able to demonstrate the degeneration of tail integument during metamorphosis when previously transplanted to the backs of tadpoles. These two results would tend to support the idea that the tissues of the larval tail are peculiarly susceptible to influences operative through the blood supply at a certain stage of metamorphosis, although whether these influences consist of hormonic, acid, or other changes is quite problematic. The present article describes the results of tail and other muscle transplantations designed to throw light on the probable causative factors responsible for autolysis of the musculature of the tail as it occurs during larval transformation.

## MATERIALS AND METHODS

The stock used for all operations were large *Rana clamitans* larvae obtained from the vicinity of Cincinnati, Ohio, during the months of January, February, and March, 1928. These partially neotenous forms were in their second year's growth and averaged about 80 mm. in total length. Individuals were selected which were normal larvae in all respects, the only signs of transformation being the presence of small 3 to 5 mm. hind limbs. Such individuals remain unchanged in the laboratory as typical larvae during the winter and spring months and it was therefore necessary to feed with desiccated thyroid in order to induce metamorphosis essential for the purposes of the experiments.

The technique employed in making muscle transplantations was briefly as follows: A flap of integument was first laid back and a small piece of muscle removed and placed in a few drops of lymph in a watch crystal. This piece of muscle was then accurately shaped under a dissecting microscope so that a 2-mm. cube of tissue was prepared for transplantation. In transplanting, a flap of integument was likewise laid back from the region to receive the transplant, the underlying musculature lightly scraped, and the cubical muscle transplant placed on this surface. In some cases the transplant was partially inserted in a small incision made in the muscle. The integument in either case was then replaced and the animal placed in shallow water to expose the operative area to the air and so hasten the adherence of the skin. They were then finally submerged in water to recover from the effects of the chlorotonization. All operations were autoplastic and in many cases were reciprocal, i.e., from back to tail and from tail to back of the same individual.

Following recovery from the anesthetic, the operated individuals were placed in individual containers and maintained under constant laboratory conditions. At the end of three days the integument covering the transplant was laid back and the latter examined under a dissecting microscope and generally found to be firmly attached and healthy in all respects. All cases of incomplete or abnormal grafting were discarded. The flap of integument was then replaced and the individuals fed desiccated thyroid to induce metamorphosis. The transplants were likewise examined at ten-, fifteen-, and twenty-one-day intervals following the application of the desiccated thyroid, during which time the larvae were undergoing transformation. Each time an examination was made the transplant was carefully measured, thus making possible a fairly accurate determination of its volume. In this way, knowing the original volume of 8 cu. mm., it was possible to determine the reduction in volume due to atrophy quite accurately. Typical examples of transplants examined at three-, ten-, fifteen-, and twenty-one-day intervals were selected from all operative series for sectioning and study of the histological condition of the muscle grafts.

RESULTS

1. *Transplantation of tail muscle to the back.*—The purpose involved in making tail-muscle transplants to the back of larvae was primarily to see whether or not such tissue would undergo normal atrophy at the same time the musculature of the tail degenerates. Failure of such transplants to histolyze would indicate that local influences peculiar to the tail region are normally operative while the reverse result would suggest a more generalized influence applied by way of the blood stream.

The muscle tissue transplanted was obtained from the side of the tail at a level about equidistant from the tip of the organ and its

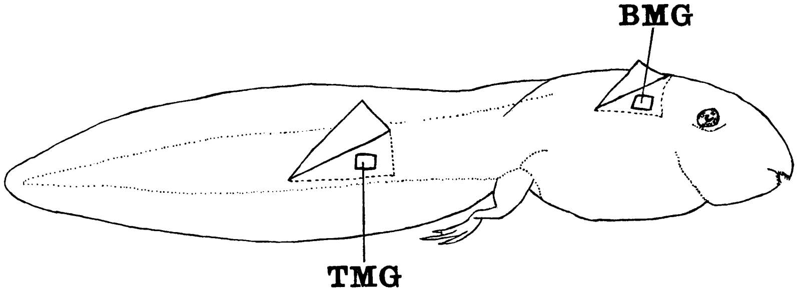



FIG. 1.—Diagrammatic outline illustrating operative procedure

junction with the body (*TMG*, Fig. 1.). The muscle, after being shaped as previously described, was then transplanted, autoplastically, to a position a little to one side of the middorsal line on the back (*BMG*, Fig. 1). This insured adequate contact of the graft with the muscle of the back; transplantation to a middorsal location being inadvisable owing to the thinness of the musculature of that region.

One hundred and thirty successful transplantations were made of this type, the percentage of abnormal grafting being very small —about 1 per cent. When examined three days following the operation and just prior to the desiccated thyroid feeding, the transplants on the average gave evidences of a slight decrease in volume. This initial shrinkage, although comparatively slight (8–11 per cent), seems to be characteristic of muscle transplantation in frog larvae and is probably the result of healing and other adjustment factors.

The reduction is not due to histological degenerative changes as can be shown by sectioning. In either case the amount of reduction was too slight to effect seriously the decided histolytic reduction in transplant volume experienced during metamorphosis.

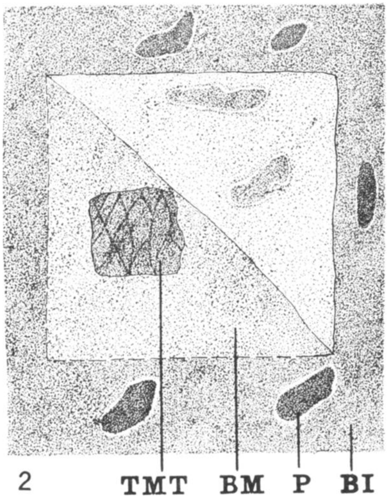
Table I represents the essential volume-reduction data, due to histolysis, for the four types of transplantations made. It will be noted that the reduction in volume of tail-muscle transplants to the back proceeds quite steadily during the period of artificially induced metamorphosis. At the end of the first ten days, the volume of tissue remaining is slightly less than half. The reduction in volume

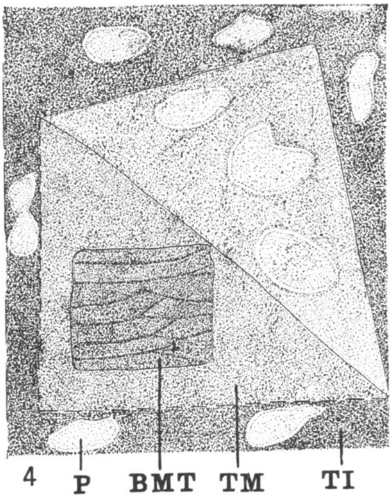
TABLE I

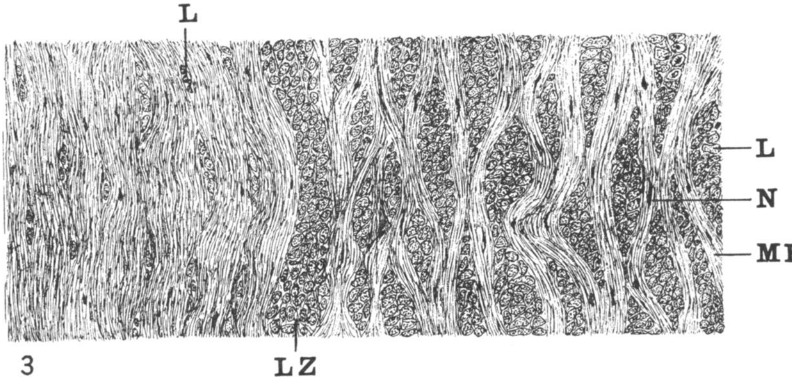
| Type of Transplantation | Number of Cases | Average Reduction in Volume Due to Atrophy | | |
|---|---|---|---|---|
| | | 10 days | 15 days | 21 days |
| Tail muscle to the back | 130 | 58% | 86% | 100 cases—100%<br>30 cases— 99.2% |
| Back muscle to the tail | 60 | 21% | 48% | 54% |
| Tail muscle to other side of tail | 15 | 38% | 40% | 49% |
| Back muscle to other side of back | 15 | 27% | 38% | 38% |

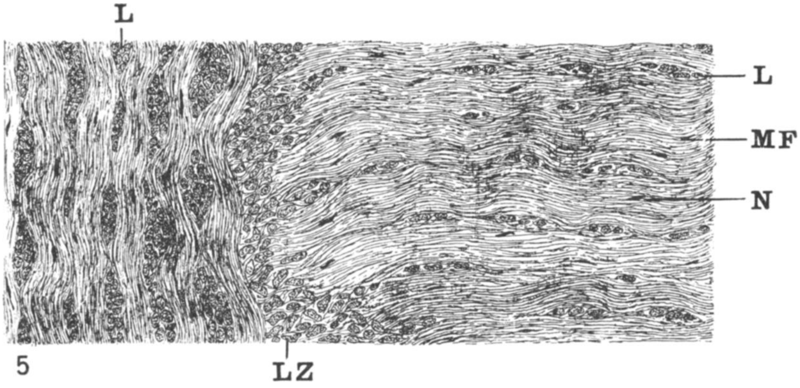
continues at a uniform rate and in the majority of cases results in the total obliteration of the graft by the end of twenty-one days. There is a small amount of variation between different individuals regarding the rate of atrophy, but in no case could more than 3 per cent of the original volume be detected at twenty-one days. The small remnants of tissue persisting in thirty cases as indicated in Table I were found to disappear within a few days.

Figure 2 represents the typical appearance of a tail-muscle graft on the back at the fifteen-day examination period. In linear dimensions the graft has been reduced from a 2-mm. cube to a 1.05-mm. cube, or a reduction in volume of 85.5 per cent. In external appearance the graft appears fairly normal, the bundles of muscle strands being still well indicated although the tissue as a whole appears slightly grayish in color as compared with the whiter shade of freshly transplanted muscle. Figure 3 represents a histological section taken through part of such a transplant to include a portion of the adjoining back muscle. It will be noted that the muscle bundles




FIGS. 2–5—*BI*, integument of the back; *BM*, muscle of the back; *BMT*, back-muscle transplant to the tail; *L*, phagocytic cells, chiefly lymphocytes; *LZ*, zone of phagocytic cells between transplant and host muscle; *MF*, dissociating muscle bundles; *N*, nuclei; *P*, pigment spots; *TI*, integument of the tail; *TM*, muscle of the tail; *TMT*, tail-muscle transplant to the back.

FIG. 2.—Semidiagrammatic sketch of a tail-muscle transplant to the back fifteen days following the onset of metamorphosis. The transplant has atrophied approximately 86 per cent in volume.

FIG. 3.—Section through tail-muscle transplant and adjacent back muscle as shown in Figure 2. Tail-muscle transplant shown to the right of *LZ;* surrounding back muscle pictured to the left of *LZ*.

FIG. 4.—Semidiagrammatic sketch of a back-muscle transplant to the tail fifteen days following the onset of metamorphosis. The transplant has atrophied approximately 48 per cent in volume.

FIG. 5.—Section through back-muscle transplant and adjacent tail muscle as shown in Figure 4. Back-muscle transplant shown to the right of *LZ;* surrounding tail muscle pictured to the left of *LZ*.

of the graft are rapidly undergoing dissociation while the tissue is heavily invaded by lymphocytes and other phagocytic cells typical of normal muscular autolysis of the tail. Contrasted to this the adjoining back muscle is much less affected, although slight signs of dissociation can be detected. Usually a heavy discontinuous zone of phagocytic cells is to be found between the transplant and host tissue. This does not necessarily indicate that the peripheral regions of the transplant always undergo more rapid histolysis than more central regions since junction points between graft and host tissue are not uncommonly found containing few, if any, phagocytes.

2. *Transplantations of back muscle to the tail.*—The reverse transplantation of back muscle to the tail was thought to be of interest in that a possible resistance of back muscle to histolysis might be shown to exist, although the fact that it would be surrounded, during metamorphosis, by rapidly autolyzing tail muscle might well serve to mask such a resistance.

The muscle transplants were obtained from regions to one side of the back (*BMG*, Fig. 1) and transplanted to the sides of the tail (*TMG*, Fig. 1). Sixty successful transplantations were obtained in all that appeared healthy and firmly attached three days after the operation. The average behavior during metamorphosis as regards volume changes is indicated in Table I. Reduction in size invariably occurred although with more variation between individuals than was true of the tail-muscle grafts as previously described. Figure 4 represents the macroscopic appearance of a typical graft examined fifteen days after the initial desiccated thyroid treatment. The grafts at this time had been reduced in linear dimensions from a 2-mm. to a 1.6-mm. cube, or a volume reduction of approximately 48 per cent. Sections made through such transplants (Fig. 5) show that atrophy is brought about through the usual methods of autolysis, the muscle fibrils being dissociated although not to as great an extent as is true of the adjacent atrophying tail muscle. Phagocytic invasion was also less pronounced in such transplants as compared with the atrophying tail musculature.

3. *Transplantation of back and tail muscle to adjacent regions.*—Histological sections through tail-muscle transplants on the back and the adjacent musculature of the back gave indications that the

latter tissue is evidently normally subjected to autolytic reactions during metamorphosis. If this were true, the rate of atrophy of foreign transplants to the back might well be increased above their normal speed. It was thought advisable, therefore, to determine the normal rate of back-muscle atrophy during larval transformation.

The operation involved consisted of the transplantation of an 8-cu. mm. piece of back muscle from one side to the other of the middorsal line on the same individual. The animals were then artificially metamorphosed and periodic examinations made of the transplants in the usual manner. It was at once evident that the back muscle of involuting larvae is subject to a decided atrophy. Reference to Table I will show that within fifteen days, following feeding of desiccated thyroid, the volume of the transplants had been reduced approximately 38 per cent. Histological examination of the transplants showed that the same type of dissociation was present as previously noted in back-muscle transplants grafted to the tail. It was furthermore noted that practically no further atrophy occurred during the next six days, at which time the transplants were again examined and measured. It would seem evident, therefore, that the musculature of the back undergoes a certain degree of atrophy, the end-stage of which is reached some time before the close of metamorphosis.

It was likewise thought profitable to determine the approximate rate of tail-muscle atrophy *in situ*. The transplantation in this case consisted of removing muscle from one side of the tail and grafting it to the opposite side of the same organ. During larval transformation the behavior of such transplants could be determined and data obtained regarding the normal rate of tail-muscle atrophy.

Table I gives the average percentage reduction of fifteen such transplantations at three stages of metamorphosis. It is evident that the rate of autolysis resulting in atrophy is quite uniform and of course progressive. The degree of atrophy also serves to illustrate the stage of metamorphosis at which the three examination periods for the other types of transplantations were made. Thus the ten-day examination was made when the volume of the tail had been reduced 27 per cent, the fifteen-day examination when the atrophy was 38 per cent, and the twenty-one-day examination when approximately 50 per cent, or one-half, of the tail had been resorbed.

It was consistently observed that muscle transplants to the tail invariably tended to move toward the junction of body and tail as metamorphosis and the resorption of the tail progressed. This did not indicate that there was an actual migration of the graft in any sense but rather that the anterior portions of the tail were undergoing a more rapid rate of atrophy than more posterior portions. If, for example, the transplant had originally been placed at a point exactly halfway distant from the anterior and posterior limits of the tail, examination twenty-one days following the onset of metamorphosis would show that it was now fairly close to the junction point of body and tail while the original distance between transplant and the tip of the tail had not been greatly diminished. The resorption of the tail would therefore appear to be more rapid in the more anterior regions, which fact probably accounts for the well-known attenuated appearance of atrophying tails.

In a histological study of the autolyzing tail musculature of involuting *Rana pipiens* larvae, Morse (1918) describes and pictures (p. 152) "dark masses running transversely over the muscle bands," which he defines as "depositions of fats bearing unsaturated fatty acids (oleic) which stain with osmium tetroxide." This condition, he further states, is precursory to phagocytosis. During the course of the present work a variety of osmium tetroxide staining methods have been used on normal tail muscle and muscle in different stages of atrophy. Our slides show only occasional depositions as described by Morse, and although their occurrence when present may always precede phagocytic invasion as he has stated, it is also true that the great bulk of tail muscle at the onset of autolysis and just prior to phagocytic invasion presents no striking reactions to osmic acid.

## DISCUSSION

Consideration of the relative degree of atrophy of the various muscle transplants during metamorphosis as compared with the normal atrophy of the same tissue *in situ* during the same time interval calls for some explanation. Thus the musculature of the tail reduces 49 per cent within twenty-one days while the same muscle transplanted to the back undergoes 100 per cent or complete resorption within the same period of time. Part of this increased rate of histolysis can be explained by the 8–10 per cent reduction in volume to which muscle tissue appears to be subject upon transplantation. The remaining further approximate increase of 41 per cent might possibly be explained by assuming that the normal 38 per cent reduction of the surrounding back muscle is additive in effect on the rate of atrophy of the tail-muscle transplants. Similarly, the 38 per cent normal reduction of back muscle, which increases to approximately 54 per cent within twenty-one days when transplanted to the tail, may be due to the usual 10 per cent transplantation reduction and to the influence of the rapidly degenerating surrounding musculature of the tail.

The suggestion might well be advanced that a comparatively highly differentiated tissue-like muscle would be likely to undergo complete resorption when transplanted for some time in non-metamorphosing larvae. In order to determine whether or not this is true of the different types of muscle transplanted in the present work, the following tests were made: ten transplantations each were made of tail muscle to the back, back muscle to the tail, back muscle to another region on the back, and tail muscle to the opposite side of the same tail. All transplantations were autoplastic and made on normal larvae showing no signs of transformation. The operated animals were maintained in cold water and gave no evidence of metamorphic changes within twenty-one days, at which time the transplants were examined. The average reduction in volume of the various muscle transplants was as follows: tail muscle to the back, 10 per cent; back muscle to the tail, 9 per cent; back muscle to another region on the back, 11 per cent; and tail muscle to the opposite side of the same tail, 8 per cent. It would appear clear, therefore, that the normal reduction in volume due to transplantation of the foregoing types of muscle could not have accounted for the much greater histolytic atrophies recorded during metamorphosis.

The results of the present article indicate, therefore, that the atrophy of anuran tail muscle during metamorphosis is not due to autolytic factors exclusively peculiar to the tail. The complete resorption of tail muscle during metamorphosis when transplanted to the back would rather suggest that the causative factors of atrophy are generalized and transported through the blood stream. The nature of this histolytic influence is, of course, quite problematic. Morse (1918) suggests that partial interruption of the blood supply everywhere results in the accumulation of carbon dioxide to the extent that the "buffer" mechanism of the blood is soon neutralized, resulting in an actual acidity of the blood. If this were true, the explanation would be sufficient to account not only for tail-muscle autolysis but for that of all other muscle undergoing similar degeneration during involution. The authors of the present article are favorably inclined toward this idea in that preliminary hydrogen-ion determinations of involuting larvae lymph indicate an increase in acidity. Work is also under way to determine the hydrogen-ion changes of anuran blood during metamorphosis. There is also a possibility that the thyroid hormone may be directly concerned. It is doubtful, however, that this is the case since the musculature of the tail remains normal for some time following the onset of metamorphosis and at a time when many other parts and organs are undergoing degeneration, development, or dedifferentiation due to the direct or indirect influence of the thyroid hormone.

## SUMMARY AND CONCLUSIONS

1. Reciprocal autoplastic transplantations of *Rana clamitans* tail and back muscle were made to determine the degree of atrophy occurring during larval transformation. The normal atrophy changes of the musculature of the back and tail were likewise investigated by transplantation to adjacent regions.

2. The musculature of the tail and back *in situ* was found to atrophy approximately 49 and 38 per cent, respectively, within twenty-one days following the onset of metamorphosis. During the same time interval, back muscle previously transplanted to the tail was 54 per cent resorbed while tail muscle previously transplanted to the back was completely obliterated.

3. Control transplantations of various muscle tissues maintained on non-metamorphosing larvae for twenty-one days gave no evidence of a sufficient amount of atrophy to affect seriously the results

of muscle-transplant atrophy as determined during metamorphosis.

4. The authors conclude that the atrophy of anuran tail muscle during involution is not due to histolytic influences exclusively peculiar to the tail but rather to more generalized factors transported by the blood stream, the probable development of an actual acidity of the blood being perhaps the main causative factor.

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