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A study of lungs in Rana catesbeiana tadpoles

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University of the Pacific

A STUDY OF LUNGS

IN *RANA CATESBEIANA* TADPOLES

A Thesis

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INTRODUCTION

The frog embryo emerges from its jelly capsule with external gills for respiration. The larva or tadpole will soon replace the external gills with the protected internal gills. The hyoid arch gives rise to a posteriorly directed flap-like membrane which covers the degenerating external gills. The flap is called the operculum. On the left side of the head the operculum remains open at its posterior margin to allow the egress of water. The opening is called the spiracle. The opercular flaps from the two sides fuse ventrally to envelop the gill or opercular chamber within (Rugh, 1951).

The tadpole embryo begins to develop lungs when it is ready to hatch. The lungs originate as a depression in the floor of the pharynx. The depression is called the laryngo-

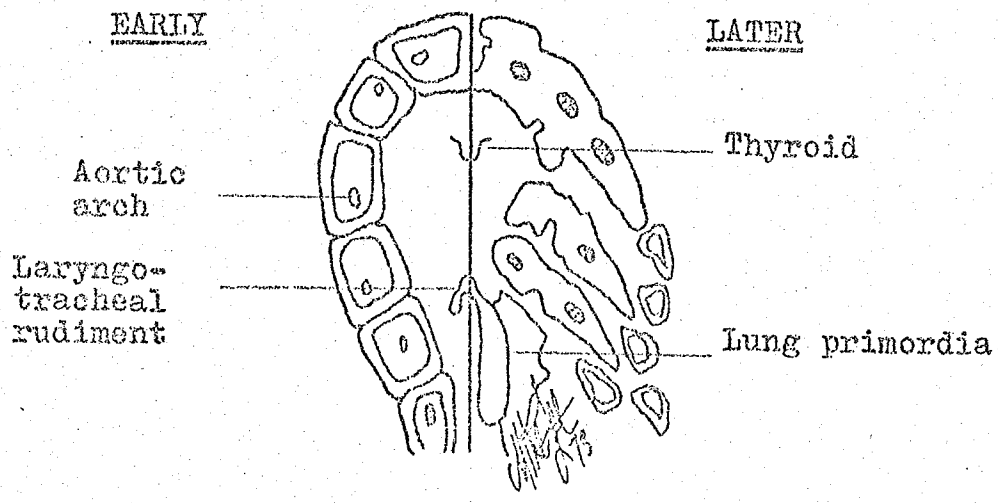


Figure 1. Frontal section of a tadpole in its early and later stages.

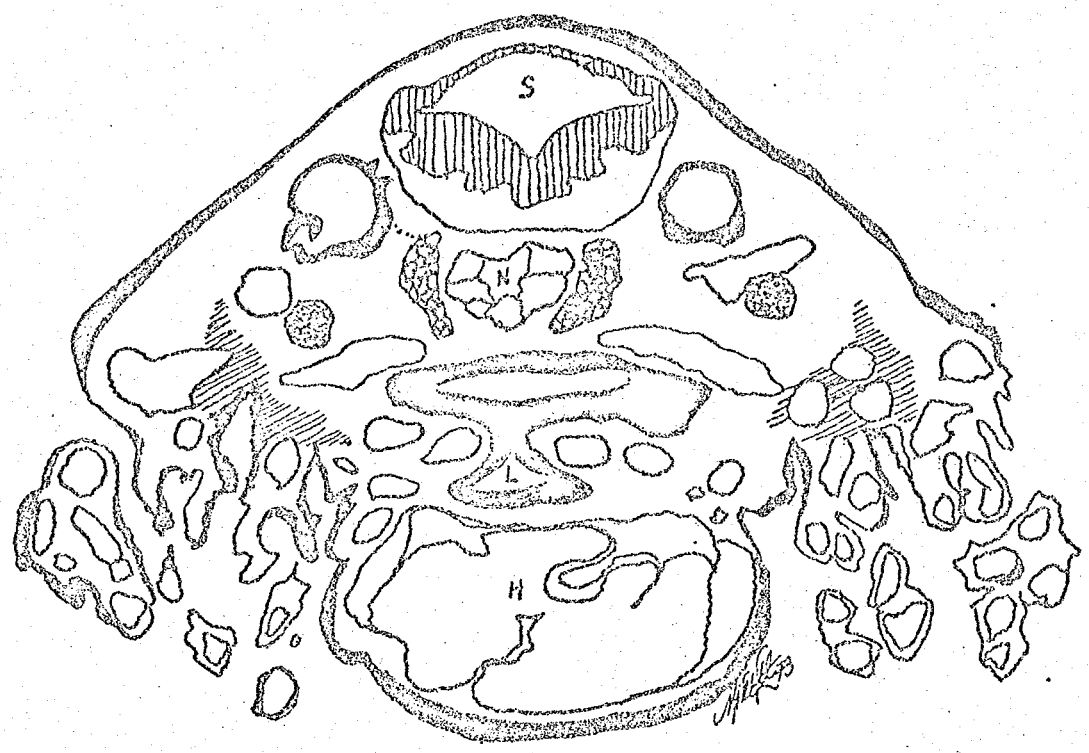


Figure 2. Medial cross section of young tadpole. S, spinal cord; N, notochord; L, laryngotracheal groove; G, gills; H, heart.

tracheal rudiment (Figure 1 and 2). From its more ventral part arise two lateral diverticula. They are the primordia of the paired bronchi and lungs. The lungs grow to form saccular structures, and by the time of metamorphosis the walls have differentiated into the highly vascularized epithelium typical of adult lungs.

The first quantitative studies of pulmonary and cutaneous respiration in the amphibians were made on Rana esculenta and Rana temporaria by Krogh (1904). He inserted a cannula connected to an air pump into the trachea and analyzed separately the air forced through the lungs by the pump and the air surrounding the frog. He was able to show that in R. tempor-

aria the lungs and skin are both important in respiratory exchange but in somewhat different ways. Oxygen enters through the lungs whereas carbon dioxide is excreted through the skin. Oxygen intake through the skin is determined solely by physical limitations while carbon dioxide excretion may vary with environmental changes. Krogh concluded that in R. temporaria the lungs dominate in oxygen consumption over the skin in a ratio of 3:1. In R. esculenta the ratio was 1:1. This probably correlated with the more aquatic habitat of R. esculenta. In the salamander, Ambystoma maculatum, Hutchison (1963) found that not only is eighty percent of the carbon dioxide produced released through the skin but the skin is also responsible for more than fifty percent of the total oxygen uptake at 15°C and below.

Strawinski (1955b) was the first to measure the number of blood capillaries in the skin of an amphibian. As the capillaries form through the skin or any other organ they develop into a net work of anastomoses (Figure 3). Strawinski

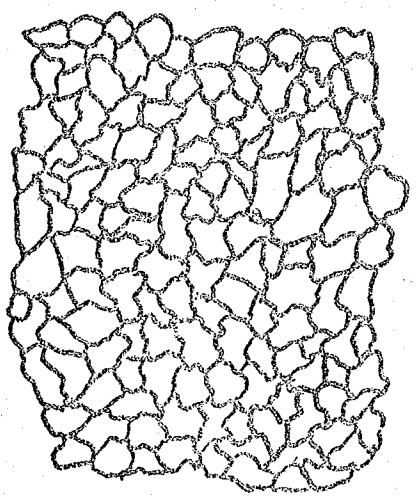


Figure 3. Capillary meshes in the lung of Rana catesbeiana. (Drawing from observations by the author)

counted the number of these crossings per unit of area. Using the skin from R. esculenta he found that on the back and thighs which are normally well exposed to the air, the meshes average 300 per mm^2, while the average count for the entire skin is 200 per mm^2. Czopek (1955a, 1955b) has used the same measuring system and calculated the percentage of the total capillaries found in the three respiratory surfaces, the skin, lungs, and buccal cavity. Most Rana species have an average of 65 percent of the total capillaries in the lungs while Leiopelma hochstetteri has 65 percent of the total capillaries in the skin. In Bombina variegata capillaries are equally distributed between the skin and lungs. In no adult anuran or urodele does the buccal cavity have a significant percentage of capillaries. These figures represent percentages in the adult animals and do not refer to embryonic or larval stages. Respiratory exchange through the body surface of the embryo is the only method during early development. Later gills and lungs develop with concommitant changes in the proportional distribution of the capillaries (Foxon, 1964). With metamorphosis changes in the degree of vascularization cease in anurans but in urodeles they may continue during growth and in response to seasonal influences.

The lungless salamanders are peculiar among most amphibians. The lungless characteristic was studied by Lapicque and Petetin (1910). By sealing the skin of the lungless salamander, Euproctus montanus, in vaseline they were able to demonstrate that cutaneous respiration is more important than

buccal respiration. The efficiency of cutaneous respiration in increased by an increase in the vascularization of the epithelium either by the thinning of the epidermis over the superficial capillaries or an increase in the penetration of capillaries into the epidermis. The efficiency of buccopharyngeal respiration is also increased in some lungless salamanders. Ritter and Miller (1899) counted buccopharyngeal vibrations of 120 to 180 per minute in Aneides lugubris.

Whether the lungs of water-dependent adult anurans and urodeles function as hydrostatic organs is questionable (Foxon, 1964). Foxon believes that in lake-living urodeles the lungs do function hydrostaticly, but Dunn (1928) questions their use as such in amphibians which live in fast moving water. The possession of organs of flotation would be a disadvantage in fast moving streams.

It has been shown by the aforementioned authors that cutaneous respiration is as important if not more so than buccopharyngeal and lung respiration in most amphibians, and cutaneous respiration replaces the lungs in the lungless urodeles. It is generally believed that the lungs are involved in respiration in adult anurans and urodeles, and in hydrostasis in adult water dependent urodeles. When comparing adult urodeles and anurans with tadpoles, little information on respiration and hydrostasis is available.

The anatomy of several species of anuran tadpoles was studied by Marshall (1970). Of interest is the author's description of the lungs of Rana catesbeiana tadpoles. At stage

XXII the lungs are inflated, becoming shorter and wider with prominent blood vessels, and blood no longer circulates through the gills. She could not pinpoint the stage at which the lungs begin to function, but perhaps they assume some respiratory function before the gills regress to the non-functional condition.

The embryology of the tadpole lungs appears to be very much related to the air bladders of the Crossopterygian fish (Noble, 1931). Both air bladders and lungs arise from endodermal pockets of the pharynx. Engel (1962) considers the respiratory system of the bullfrog, Rana catesbeiana, as a replica of developments which are likely to have occurred in the transition of fish to amphibians millions of years ago. The evolvement of choanae in the Crossopterygian fish constitute the first step in the transition from gill to lung respiration. Pulmonary rudiments appear in the tadpole at an early gill stage and the two systems exist side by side throughout the tadpole phase.

Metamorphosis has been studied comprehensively by Etkin (1932). He has observed that the mode of respiration in the bullfrog has been seen to change during metamorphosis. By placing the animal in water to a depth just covering the snout an air breather will keep the nostrils just above the surface while a water breather will not. Although there seems to be a period of indefinite reaction, this lasts less than one day and a definite change to air breathing is found to occur in the first or second day of tail resorption.

The volume of tadpole lungs has been studied by Bergerjk (1957). He placed a tadpole in a vessel completely filled with water. Through the stopper of the vessel a calibrated capillary tube protruded which was approximately half filled with water. The only free air present in the system was the air in the lungs of the tadpole. The vessel was placed in a thermo-insulated vaccum chamber and evacuated to a pre-set pressure. The rise in the water in the capillary tube represented the volume of the lungs. Using the equation: $V_1P_1 = V_2P_2$, Bergerijk was able to calculate the exact volume of the lungs. He found that the mean volume for bullfrog tadpole lungs was 0.28 cm^2 or about 2.3 percent of the total body volume. Tadpoles of Xenopus laevis had a lung volume of 0.026 cm^2, or about 3.7 percent of the total body volume.

These and many other studies on tadpoles deal with respiration as a whole and do not single out the organs that might be involved. Since lungs are present in the tadpole, a question remains as to what their function is in this aquatic stage.

METHODS AND MATERIALS

Rana catesbeiana tadpoles were used in these experiments. They were obtained from the Mokelumne River on Highway 88, two miles west of Clements, California. The tadpoles were taken from an ox bow in the river approximately 250 yards from the highway. The water flow in the river was maintained nearly constant throughout the year by a dam. The ox bow contained large quantities of elodea, small fish, and insects, and proved to be an excellent breeding ground for the bullfrog. Tadpoles for this project were taken from January to October, 1972. The animals were collected with nets and transported in plastic tubs back to the University 27 miles away. Tadpoles were collected only when needed and were kept in the laboratory no longer than a week before being used.

In the laboratory a holding tank was built. It was a galvanized metal horse trough filled with approximately 36 gallons of tap water. Water entered the tank in a continual spray and drained through an opening at the other end of the tank. Every two or three days debris from the bottom of the tank were removed and periodically the tank was drained and completely cleaned. To prevent overflows in the tank a flush valve and float were connected to the spray. If the water level were to rise too high the float would shut off the water. The tadpoles were supplied with boiled lettuce every few days.

PART A

The lungs from 71 tadpoles were removed over the 10 month period. The tadpoles were first anesthetized in 1.5 percent urethane (ethyl carbamate). The urethane left the tadpole quiescent during the time I needed to remove the lungs and yet the recovery rate from this concentration was nearly 100 percent.

Both the left and right lungs were removed during the same operation. All equipment such as iridectomy scissors, forceps, thread, etc., was sterilized before use. As I became familiar with the tadpoles I was able to see a bulge in the skin where the lungs were located. To remove the left lung an incision of 2 to 3 millimeters was made just dorsal and caudal to the spiracle. The right lung was removed on the right side in the same position. The lung was pulled from the body cavity with tissue forceps. It was measured for lenth-th and tied with thread as close to its point of origin as possible. The lung was cut off and fixed in 2 percent formalin. Very little bleeding occurred with this method. The stub was then placed back into the body cabity and the right lung was removed. The hole made by the incision was not sutured shut but allowed to heal by itself.

A number of sham operations were performed in which the body wall was cut but the lungs not removed. These tadpoles were used as controls to compare with the lungless tadpoles for health and behavior.

For the first couple of days, lungless tadpoles were placed individually in plastic tubs, 9 x 12 x 3 inches, in 2 liters of water. After 3 days they were supplied with boiled lettuce. The water was changed daily for the first week but only every 2 or 3 days after that. In the first few months of research the mortality rate was nearly 60 percent within one week after the operation. The deaths were due mainly to fungus infection. A fungicide, FungiStop by Tetra Care, was found to reduce the fatality rate. FungiStop was placed in the water of all lungless tadpoles for the duration of the experiment. At 3 week intervals lungless tadpoles of recorded stages were sacrificed and autopsied to see if lung regeneration had occurred. Several tadpoles were allowed to live through metamorphosis.

PART B

At the end of 3 weeks lungless tadpoles that were about to be sacrificed were first studied physiologically. They were placed singly in a water filled cylinder 94 centimeters high and 15 centimeters in diameter. The height of the cylinder was marked off in centimeters and the water in the cylinder was continually aerated. Along with the lungless tadpole a normal tadpole of the same stage was placed in the cylinder. The height to which they would swim was measured at one hour intervals.

PART C

Normal tadpoles were placed in one liter Erlenmeyer flasks filled to the top with tap water, one tadpole to a flask. The top was screened off so that the tadpole could not surface, that is, they could not gulp air from the atmosphere. All the flasks were aerated except one. The tadpoles were then observed for 6 hours. The experiment was run several times using different tadpoles.

PART D

The weight of each tadpole was recorded before the lungs were removed. As the lungs were removed their lengths were measured. A correlation coefficient analysis of tadpole weight and lung length was made to see if a relation did exist.

PART E

The capillary anastomoses of the operculum were counted for normal and lungless tadpoles. The operculum is a flap of skin that covers the gills, and is highly vascularized. Normal and lungless tadpoles were anesthetized in 1.5 percent urethane and injected with india ink through the truncus arteriosus and then fixed in 10 percent formalin. After fixation the operculum was cut out, dehydrated with alcohol, and sealed in Adams Histoclad. The density of the meshes (the junctions of capillaries) was measured. The ocular of the microscope was fitted with an ocular grid. The grid was then calibrated to

a length and width of one centimeter. All meshes lying along the length were counted and all the meshes lying along the width were counted. The counts for normal and lungless tadpoles were applied to a two-factor factorial analysis of variance.

RESULTS

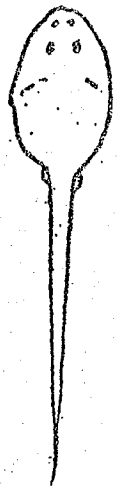
PART A

The normal development of Rana catesbeiana tadpoles is shown in Figure 4 as modified from Taylor and Kollros (1964). The stages pictured include XI through XXI.

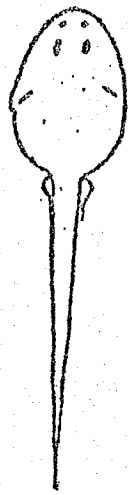
Both lungs were removed from 71 tadpoles. Three weeks after lung removal 27 tadpoles were dead from fungus disease, 26 had been sacrificed, and 11 had died of unknown reasons (Table 1). From the sham operations several tadpoles were dead from disease. The remaining tadpoles were healthy.

The 26 lungless tadpoles that were sacrificed ranged in development from stages XI to XVII. These tadpoles were autopsied to see if any lung regeneration had occurred. The epithelial stub or primary bronchus was examined. No regeneration of lung was found in any of these tadpoles. Tadpoles collected between the stages of XVIII and XXI died within 5 to 10 days after lung removal. There were no signs of disease and they were subjected to the same conditions as the other lungless tadpoles. Their death may be linked with asphyxiation. These tadpoles were autopsied for new lung tissue and none was found.

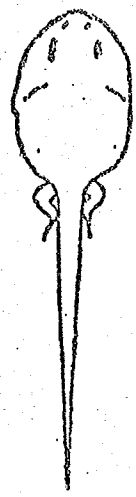
Graphed in Figure 5 is the survival time of lungless tadpoles in days. Tadpoles younger than stage XVII lived to the third week but tadpoles operated on at stages later than XVII died. As the stage in development increased, the number of days the tadpole lived after the operation decreased. Tad-



XI

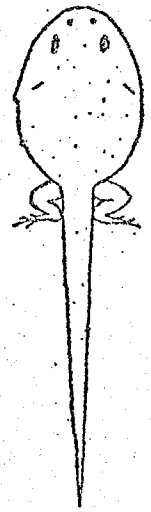


XII

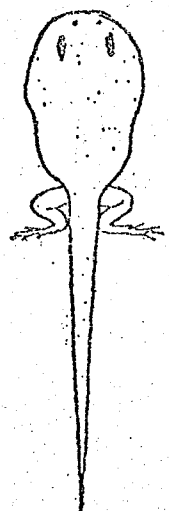


XIII

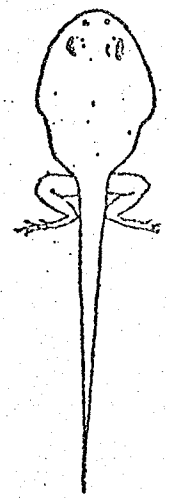
XIV



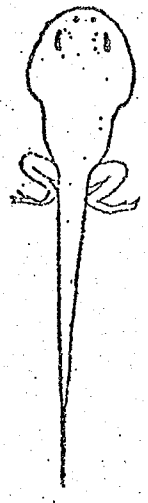
XV



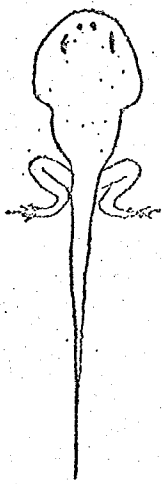
XVI



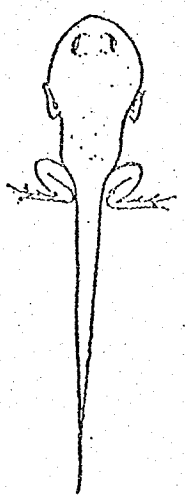
XVII



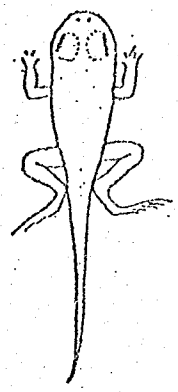
XVIII

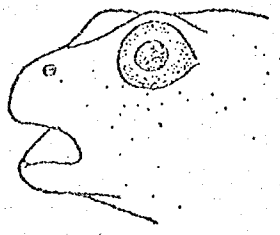


XIX



XX





XXI

Figure 4.

TABLE 1

TOTAL PNEUMONECTOMY

STAGE	NUMBER OF TADPOLES		NUMBER OF DEATHS		
	OPERATED	SURVIVING AFTER 3 WEEKS	DISEASE	SACRIFIED	UNKNOWN
XI	7	1	3	3	0
XII	5	1	2	2	0
XIII	9	2	3	4	0
XIV	10	2	4	4	0
XV	9	1	4	4	0
XVI	10	1	4	5	0
XVII	7	0	3	2	2
XVIII	5	0	1	0	4
XIX	3	0	1	0	2
XX	3	0	2	0	1
XXI	3	0	0	0	3

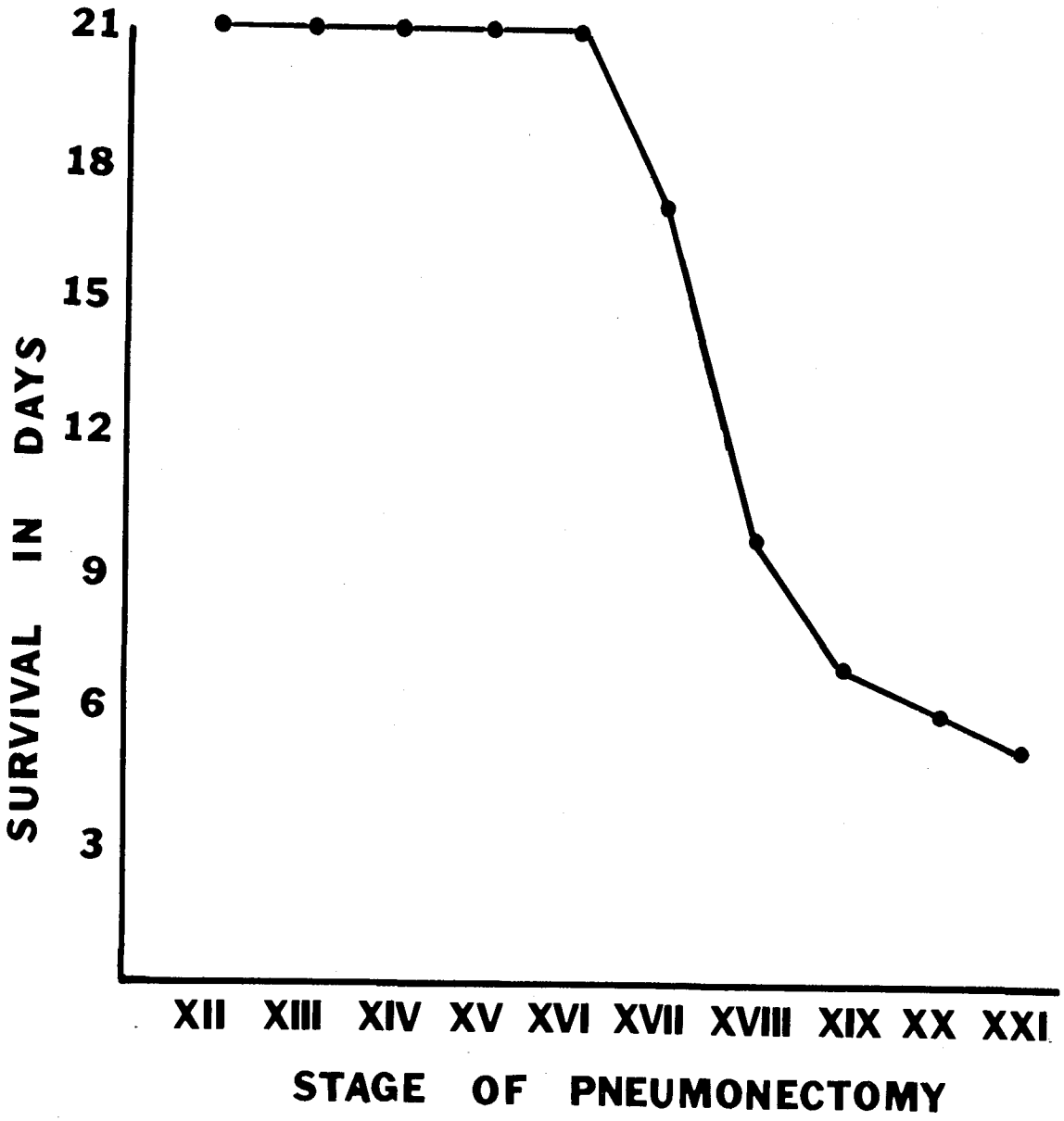

21
18
15
12
9
6
3
SURVIVAL IN DAYS
XII XIII XIV XV XVI XVII XVIII XIX XX XXI
STAGE OF PNEUMONECTOMY

FIGURE 5.

poles operated on at stage XVII lived an average of 17 days, while tadpoles at stage XVIII lived an average of 10 days, stage XIX an average of 7 days, stage XX 6 days, and stage XXI 5 days. This suggests that the lungs become more essential for life as the tadpole approaches metamorphosis.

PART B

Normal tadpoles were observed to be quiescent animals, normally lying still on the bottom or swimming slowly along the bottom scavenging for food. Only when frightened did they move swiftly for cover under debris or under other tadpoles. Occasionally a tadpole would maneuver to the surface, open its mouth to the atmosphere and gulp air. Sometimes an air bubble might appear as if air was also being released back to the atmosphere. The tadpole would spend no more than a few seconds at the surface and then descend back to the bottom. On the way down the tadpole may or may not release an air bubble. Normal tadpoles were seen to surface 6 to 11 times per hour.

When lungless tadpoles were placed in shallow tubs with 3 inches of water they exhibited the same behavior as normal tadpoles. They swam to the surface opened their mouth to the atmosphere and swam back to the bottom, usually releasing an air bubble. Occasionally the air bubble would come not from the mouth but from the incision where the lung was removed. A few of the tadpoles even became bloated and floated on top of the water. This shows that the tadpoles do draw air into

the buccal cavity and into the lungs. Air passing into the body cavity of lungless tadpoles was due to a loose ligature around the bronchus from which the lungs was removed.

Listed in Table 2 are the surfacing abilities of normal and lungless tadpoles. In each of the five sample runs, 2 tadpoles, one normal and one lungless, were allowed to swim freely in a 90 cm cylinder. It was observed that normal tadpoles ascend to a height of 85 to 90 cm. Tadpoles from the stages XVI and XVII ascended higher on the average than the younger stage XV did. The reason was not explored further but may be related to the size of the tadpole tail and/or the advanced development of the hind appendages. Lungless tadpoles ascended on the average to a height between 25 and 55 cm. The climb was one-third to one-half that of normal tadpoles. The number of ascents per hour was nearly the same for both normal and lungless tadpoles, but lungless tadpoles tended to be somewhat sluggish, making an average of 6.1 ascents per hour, while normal tadpoles ascended 7.7 times. From this test it appears that the lungs play an important role in the vertical migration of the tadpole. Removing the lungs, though, seems to have little effect on the number of ascents attempted.

A graphic representation of the surfacing abilities is presented in Figure 6. Plotting average height of climb against stage shows the definite advantage that normal tadpoles have in swimming to the surface. Normal tadpoles in every test made higher average ascents than lungless tadpoles.

TABLE 2

SURFACING ABILITY OF NORMAL AND LUNGLESS TADPOLES

SAMPLE	TADPOLES	STAGE	RUNS	NUMBER OF ASCENTS 1	2	3	AVERAGE HEIGHT OF ASCENT 1	2	3
1	normal	XVI		7	10	9	87	85	87
	lungless	XVI		5	6	8	25	37	35
2	normal	XV		9	7	6	83	85	86
	lungless	XVI		5	9	3	27	29	30
3	normal	XIII		7	7	5	87	88	86
	lungless	XIII		7	9	6	39	47	40
4	normal	XV		11	4	7	83	80	85
	lungless	XIV		9	10	6	30	55	50
5	normal	XVI		4	8	6	90	87	88
	lungless	XV		7	6	6	31	28	41

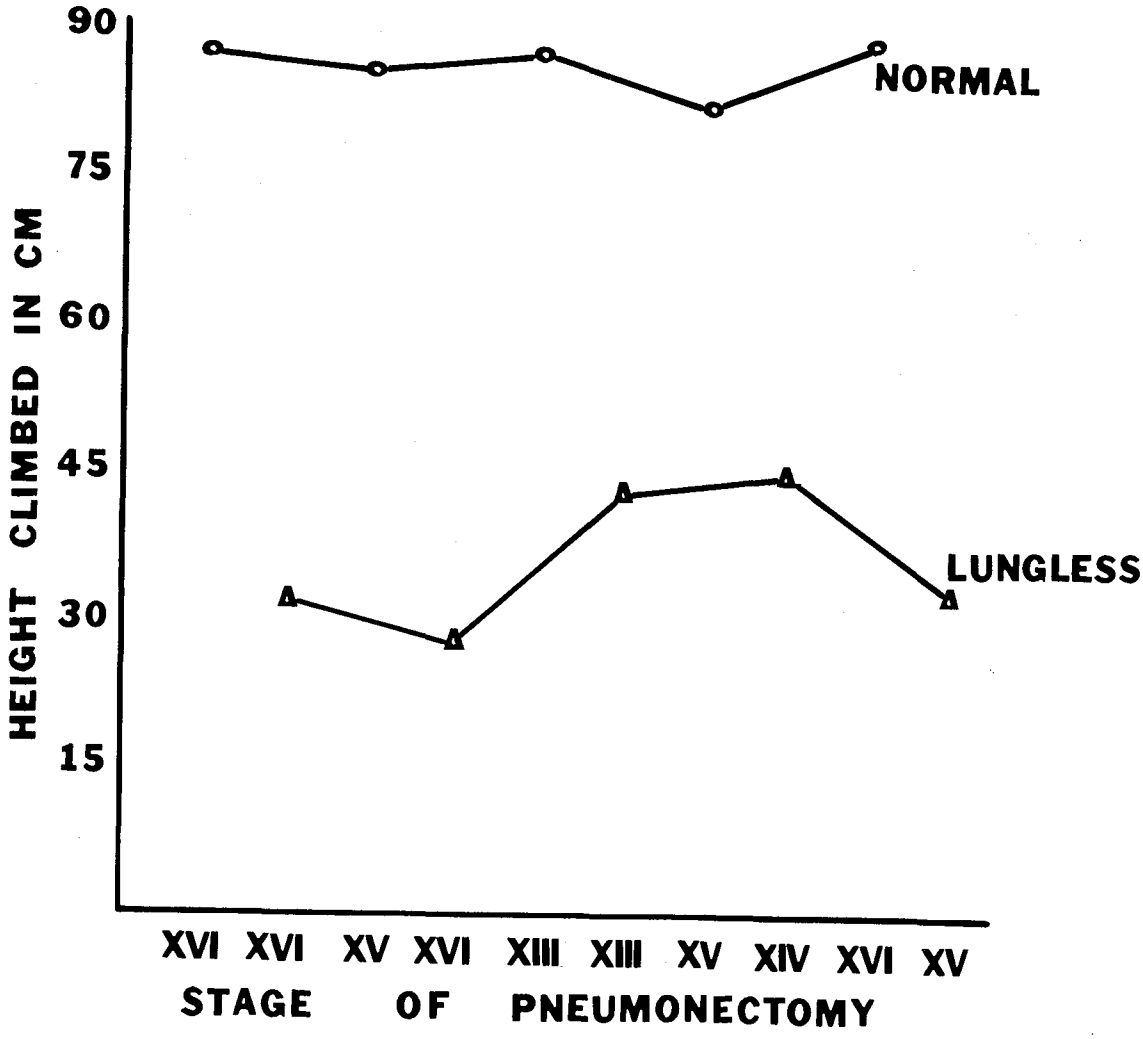

90
75
60
45
30
15
HEIGHT CLIMBED IN CM
NORMAL
LUNGLESS
XVI XVI XV XVI XIII XIII XV XIV XVI XV
STAGE OF PNEUMONECTOMY

FIGURE 6.

PART C

Twelve normal tadpoles were placed separately in Erlenmyer flasks, 8 in aerated water and 4 in unaerated water. A screen in the neck of the flasks kept the tadpoles under the surface of the water and prevented them from reaching the atmosphere. All 8 tadpoles in the aerated water lived. They showed no signs of stress and exhibited the same behavior as other normal tadpoles in the holding tank. The 4 tadpoles in unaerated water died within an hour. If normal tadpoles are kept from reaching the atmosphere to gulp air, they will remain alive if the water is continually aerated(Table 3).

PART D

The relation of tadpole lung length to tadpole weight is presented in Table 4. The average length of the lungs ranged from 7.5 mm at stage XI to 9.2 mm at stage XVIII. The lungs decreased in length after stage XVIII to a low of 8.2 mm at stage XXI. The average weight of the tadpoles ranged from a low of 9.3 g at stage XI to a high of 16.1 g at stage XVII. After stage XVII the average tadpole weight decreased to 14.6 g at stage XXI. The coefficient of correlation was calculated to be 0.11. Even though the coefficient is positive, it is too small to say that any real correlation does exist. Only 1.21 percent of the variation in the average length of the lungs, as measured by the sum of the squares was due to the linear regression of lung length to weight.

TABLE 3

NORMAL TADPOLES LIVING IN A CLOSED WATER BATH WITHOUT ACCESS TO THE ATMOSPHERE

WATER AERATED

STAGE	TESTING PERIOD	CONDITION OF TADPOLE
XI	4 hrs.	normal
XII	4	normal
XII	6	normal
XIV	4	normal
XV	4	normal
XV	8	normal
XVI	8	normal
XVI	8	normal

WATER NOT AERATED

XI	1	dead
XIV	1	dead
XV	1	dead
XV	1	dead

TABLE 4

NORMAL TADPOLE LUNG SIZE RELATED TO NORMAL TADPOLE WEIGHT

STAGE	NUMBER	AVERAGE WEIGHT OF TADPOLE	AVERAGE LENGTH OF LUNG
XI	7	9.3 gm	7.5 mm
XII	5	9.3	7.7
XIII	9	12.3	8.0
XIV	10	12.1	8.0
XV	9	15.7	8.5
XVI	10	15.8	9.0
XVII	7	16.1	9.0
XVIII	5	15.8	9.3
XIX	3	15.6	9.0
XX	3	14.8	8.7
XXI	3	14.6	8.2

Correlation Coefficient = 0.11

When this test was run the tadpoles from which the lungs were removed and measured were kept alive for other experiments. If the tadpoles had been weighed without the gut, more meaningful results might have been found. A tadpole's weight will vary with environmental conditions, due to the availability of food. Also, tadpoles cease to feed once they enter metamorphosis.

PART E

The data collected by counting the capillary meshes are presented in Tables 5 and 6, and in Figure 7. Table 5 shows the number of meshes counted per animal per stage. The three stages used were XII, XIV, and XVI, and 5 tadpoles were counted at each stage.

Beginning with normal tadpoles at stage XII the capillary density ranged from 215 to 254 meshes per cm^2 (Table 5). At stage XV the range was 220 to 251, and at stage XVI the range was 224 to 255. The number of capillary meshes for lungless tadpoles ranged at stage XII from 207 to 252 meshes per cm^2. At stage XVI the range was 239 to 262. In both normal and lungless tadpoles the average number of meshes increased with development.

Stage XII, as seen in Table 5, had the greatest variability in the number of meshes for both normal and lungless tadpoles. The distribution of capillaries was seen to vary from tadpole to tadpole. It was impossible to establish a

TABLE 5

CAPILLARY MESHES PER CM^2 OF TADPOLE OPERCULUM

		NORMAL			LUNGLESS		
STAGE		XII	XIV	XVI	XII	XIV	XVI
	1	237	242	255	253	245	262
	2	215	248	247	231	240	248
SAMPLES	3	233	220	231	248	250	242
	4	254	238	224	207	232	251
	5	243	251	252	244	233	239
AVERAGE		236	240	242	236	240	257
STANDARD ERROR		5.8	4.9	5.4	7.2	3.1	3.6

standard pattern within the capillary bed of the operculum which might be the reason for the variability in the number of meshes counted. Another reason for the variability is that many of the capillaries did not carry the dye and as a result did not appear.

The data were applied to a two-factor factorial analysis of variance. The null hypothesis was made that the number of capillaries per cm^2 does not differ between normal and lungless tadpoles. The F test was applied to the analysis with the levels of significance at 0.01 and 0.05.

In the analysis of variance the F value for normal versus lunglessness (anatomy) was 0.23 (Table 6). The standard values for F at 0.05 and 0.0a are 4.3 and 7.8, respectively. For a significant difference in the number of capillary meshes to exist the experimental number must be greater than 4.3; to be highly significant the number must be greater than 7.8. The number is much smaller and so little or no difference was found in the number of capillaries in normal and lungless tadpoles as a group. The F value for individual stages was 1.1. This compares with the standard F value for 0.05 and 0.01 of 3.4 and 5.6, respectively. The experimental is less than the standard values. There is no difference in capillary density between the stages of normal and lungless tadpoles.

The final test was for interaction between anatomy and stage. As with the first 2 comparisons, no significant difference was found. The experimental value was 0.02 while the standard values for 0.05 and 0.01 were 3.4 and 5.6, respectively.

TABLE 6

TWO-FACTOR FACTORIAL ANALYSIS OF VARIANCE OF THE NUMBER OF CAPILLARY MESHES IN THE OPERCULUM OF NORMAL AND LUNGLESS TADPOLES

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	EXPERIMENTAL F VALUE	STANDARD F VALUE 0.05	0.01
TOTAL	29	4559.5				
TREATMENTS	5	484.3				
STAGE	2	375.2	187.6	1.10	3.4	5.6
ANATOMY	1	40.5	40.5	0.23	4.3	7.8
INTERACTION	2	68.6	34.3	0.20	3.4	5.6
ERROR	24	4075.2	169.8			

The results of the count are plotted as a graph (Figure 7). Note that there is a small increase in the number of capillaries with advanced stage for both normal and lungless tadpoles, and that lungless tadpoles did not develop more capillaries in their operculum due to the loss of their lungs.

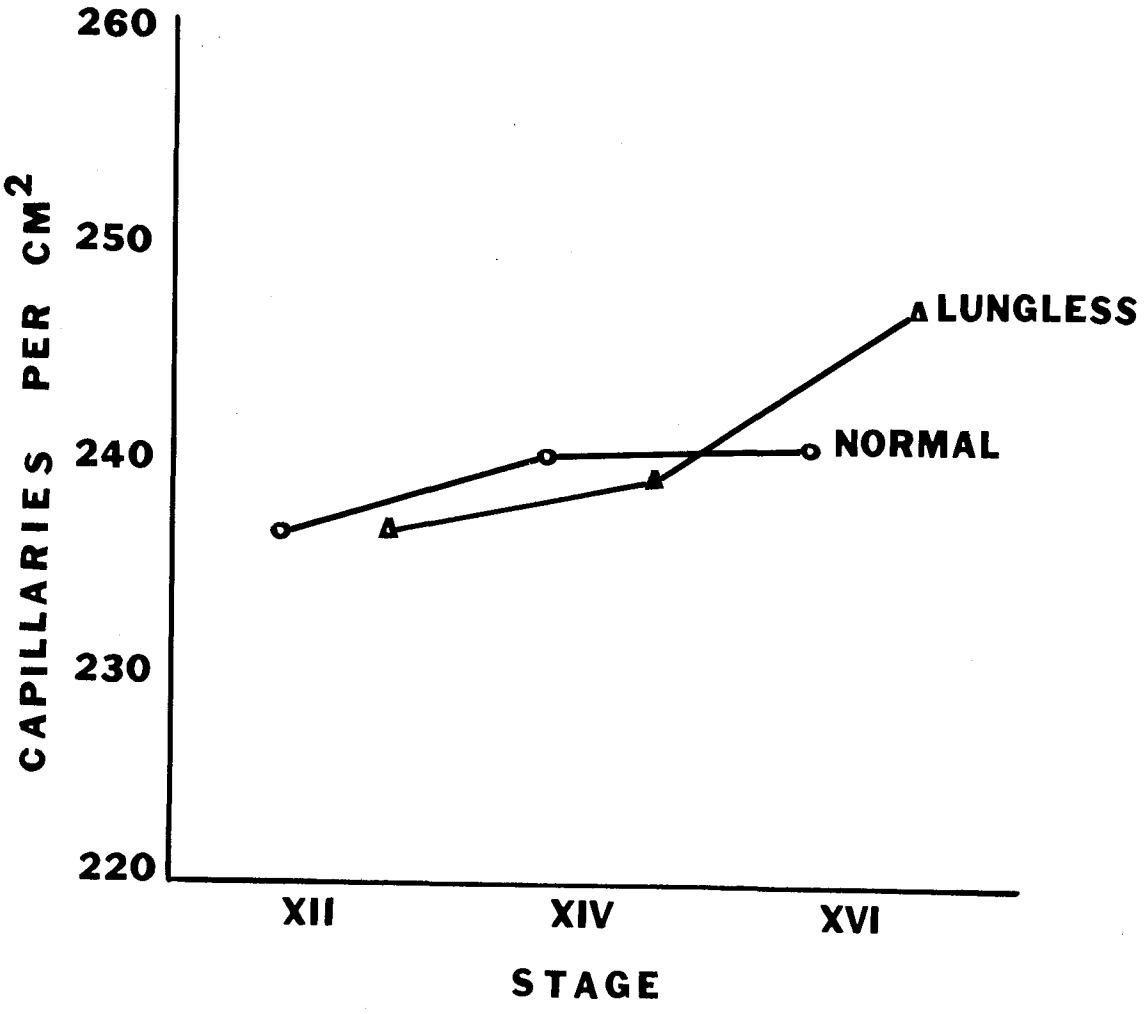

260
250
240
230
220
CAPILLARIES PER CM2
LUNGLESS
NORMAL
XII
XIV
XVI
STAGE

FIGURE 7.

DISCUSSION

This investigation attempts to establish the function of lungs in Rana catesbeiana tadpoles. Are they involved in respiration and/or hydrostasis? To see just how important the lungs are to the tadpole I removed them. This approach has not been taken by other researchers making comparisions difficult.

Savage (1952) observed that "The Rana tadpoles have lungs which become functional at an early age, because the surface of the water above colonies is often covered by the bubbles produced by the animals". Noble (1931) on the other hand observed that in Bufo this type of behavior does not occur. I found through my own observations that Savage's description of tadpole behavior is the same for R. catesbeiana, but surfacing does not appear to be a function of respiration. Lungless tadpoles in shallow tubs continued to show the surfacing behavior. When a lungless tadpole did surface to the atmosphere they inhaled air because in some lungless tadpoles the ligature had loosened and the air passed into the body cavity causing the tadpole to float.

Tadpoles at different stages reacted to lunglessness differently. Tadpoles between the stages of XII and XVI with their lungs removed lived through the 3 week testing period. Tadpoles between the stages of XVIII and XXI died within 5 to 10 days. Stage XVII was the period of transition because some

of the tadpoles could live without lungs while others died. Lungs become vital for respiration during this stage.

Normal bullfrog tadpoles could live without stress in water when they were not allowed to reach the surface. If normal tadpoles could live without surfacing (without aerating the lungs) and lungless tadpoles could also live, then the lungs must not be necessary for respiration to maintain life. They could be functional in respiration, but are not essentual. This is true only up to stage XVIII when it becomes apparent that lungs are necessary.

The only amphibians that occur naturally without lungs are the family of salamanders, Plethodontidae. The majority of American urodeles are included in this family. In the adult stage cutaneous respiration is increased by the penetration of capillaries into the epidermis or the thinning of the epidermis over the superficial capillaries (Noble, 1931). Information was found on larval respiration in the Plethodontidae family to indicate whether changes in the bull frog tadpole following lunglessness were similar.

According to Marshall the lungs of R. catesbeiana tadpole are partially inflated at stage XXII. From my own observations the lungs of the tadpoles were inflated and had extensive capillary beds as early as stage XI. The length of the lungs ranged from 7.5 mm at stage XI to 9.3 mm at stage XVIII.

Gradwell (1969) has shown that the operculum has a large capillary bed. He found that the capillary density of the op-

erculum is comparable to and even greater than that of the skin in the seven species of frogs and toads investigated by Czopek (1955a). The shape of the capillary meshes in the bullfrog tadpoles was similar also to the cutaneous samples examined by Czopek. Gradwell concluded that the capillary density and the area of the operculum of the R. catesbeiana tadpole is significantly important to respiratory gas exchange.

I found that the average number of capillary meshes in the operculum of normal tadpoles ranged from 236 to 242 for the stages XII, XIV, and XVI. For the corresponding stages in lungless tadpoles the meshes averaged 236 to 257. Statistically there is no difference. If the lungs are involved in respiration the skin does not take over their function. If time permitted it would be useful to count the capillary meshes of the gills and buccal cavity in normal and lungless tadpoles.

Lungless tadpoles in shallow tubs exhibited the same behavior as normal tadpoles. When a lungless tadpole was moved to a deep cylinder (90 cm) a striking difference appeared from normal tadpoles. Lungless tadpoles could not surface. In fact they were resticted to the lower half of the cylinder. They had lost their hydrostatic ability to climb. Both Foxon (1964), and Dunn (1928) suggested a hydrostatic function of lungs in amphibians that live in lakes. In lake dwelling species hydrostatic organs would help the tadpole in vertical movement. The only defense mechanisms that the tadpole has is its coloration and speed. Hydrostatic organs would be useful in moving the tadpole out of the range of predators.

The first stage in the evolution of respiratory organs is the transition from gills to saccular lungs. The bullfrog tadpole, which exists today in the same or similar form as it did millions of years ago, is fitted with a primitive saccular lung (Engel, 1962). Thus the respiratory system of the tadpole can be considered as a replica of developments which were likely to have occurred in the transition of fish to amphibians millions of years ago. The lungs, like the choanae of the primitive fish, Crossopterygian, probably serve not in respiration but as hydrostatic organs.

SUMMARY

The function of the lungs in Rana catesbeiana tadpoles was studied.

A. Tadpoles younger than stage XVII were able to live without their lungs. Tadpoles older than stage XVII died without their lungs.

B. Lungless tadpoles were unable to ascend to the surface from depths of 90 cm.

C. Normal tadpoles were able to live without access to the atmosphere as long as the water was aerated.

D. Removing lungs from tadpoles does not result in an increase in the number of blood capillary meshes in the operculum.

E. The author concludes that the lungs of Rana catesbeiana tadpoles function largely as hydrostatic organs.

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