THE PRODUCTION OF ACCESSORY APPENDAGES AND ANTERIOR DOUBLING IN FROG EMBRYOS BY CENTRIFUGAL FORCE

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In looking through the literature one finds that very little work has been done on the effect of centrifugal force on the blastula of the frog or salamander. We feel that this technique may be applied with advantage to problems of amphibian experimental embryology. This belief is supported by the interesting results obtained by Banta and Gortner ('15) in centrifuging the eggs of Rana sylvatica. These authors found that headless monsters and tadpoles with accessory appendages were produced when eggs in the early blastopore stage were centrifuged at speeds varying from 200 times gravity to 1200 times gravity. It was in an attempt to confirm the work of Banta and Gortner and to discover whether other types of abnormalities could be produced in the frog egg by centrifugal force, that the experiments reported here were carried out.

The eggs which were used in the present experiments were of Rana sylvatica, and were collected in the vicinity of Gambier, Ohio, during the months of March and April, 1938. Five clusters of eggs were used, each cluster containing from 150–200 eggs. Two thirds of the eggs in each cluster were centrifuged at speeds indicated below, the remaining one third of the eggs in each cluster served as controls. The first series (cluster 1), was composed of eggs in the mid-gastrula stage and these eggs were centrifuged at 1400 r.p.m. for ten minutes. The second series (cluster 2) was composed of eggs in the late blastula stage and these were centrifuged at 1400 r.p.m. for ten minutes. The third series (cluster 3) was composed of eggs in the early blastopore stage and these were centrifuged at 1800 r.p.m. for five minutes. The fourth series (cluster 4) consisted of eggs likewise in the early blastopore stage. These were centrifuged at 2900 r.p.m. for ten minutes. Eggs in the fifth series (cluster 5) were in the late blastula stage and these also were centrifuged at 2900 r.p.m. for ten minutes.

The eggs in series I, which were in the mid-gastrula stage at the time of centrifuging, developed normally. A large percentage of the eggs in series II-IV, which were either in the late blastula or early gastrula stages, developed accessory appendages or anterior doubling. It is evident from these results that the egg of Rana sylvatica has become resistant to centrifugal force by the mid-gastrula stage. Only those eggs which were in stage 10 of Pollister and Moore ('37) ever developed abnormalities. The eggs of series V gave the highest percentage of abnormals and it is interesting to note that this was the only series in which anterior doubling occurred. Very few eggs were killed by the centrifugal force. In all 60% of the eggs developed accessory appendages. In series V, 50% of the eggs gave rise to double monsters.

The accessory appendages which were produced were in all cases tail-like in appearance. These appendages are designated as tails although no true myotomes develop in them. Histologically these structures resemble the tail fin of a normal amphibian tail for they are composed largely of epidermis, dermis and connective tissue. These accessory tails may occur at any point on the body of the tadpole. The most common location is on the belly, but several have been observed on the dorsal side of the body just lateral to the normal tail fin. These accessory tails vary in size from tiny projections to the large structures shown in figure 1.

Banta and Gortner reported that several of the animals in their experiments which had accessory tails also had spina bifida. They found in these animals that each half of the unclosed neural groove in the posterior region of the embryo produced a tail. Similar cases have been found in our experiments and seem to be the result of the spina bifida condition. However, these cases are few in number.

In the animals possessing secondary tails on the dorsal side of the body, many cases are found in which the accessory tail fin makes a junction with the normal tail fin. An examination of the history of these cases shows that in the neurula stage a secondary and abortive neural fold had been formed which made junction with the posterior region of the primary neural folds. The neural groove later closed normally leaving a small section of neural fold tissue still connected with the neural tube and projecting at an angle from it. This section of neural fold material produced the accessory tail. This result may be correlated with the findings of Mangold ('32A) who induced the formation of a secondary tail in Triton by transplanting to an

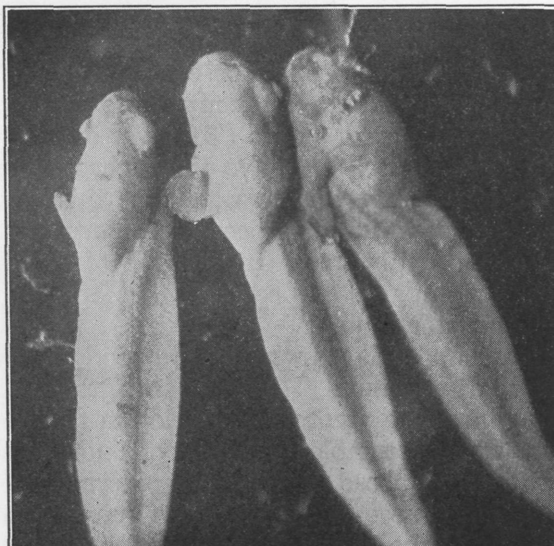

Fig. 1 (upper). Frog tadpoles showing position and appearance of accessory tail-like appendages.

Fig. 2 (lower). Photomicrograph of a frog embryo showing two neural grooves in the anterior region. This embryo died before fixation.

early gastrula the caudal quarter of the medullary plate of a Triton neurula.

The accessory tails occurring on the ventral region of the body are harder to account for, but probably arose through the transference of a portion of the tail-forming material to that region by the centrifugal force, as has been suggested by Banta and Gortner ('15). It is interesting to note that sections show neural tube-like structures at the proximal ends of these secondary tails.

In series V anterior doubling occurred in 50% of the cases. Figure 2 is a photomicrograph of an egg showing such anterior doubling. These double monsters all died in early embryonic stages, with the exception of one case which developed into a tadpole with incomplete anterior doubling. Penners and Schleip ('28A, –'28B) have obtained similar cases of anterior doubling in the frog by inverting the fertilized egg and forcing it to develop in this position. They found that in many cases the developing blastocoel was displaced, the pressure within it causing the cells which formed its walls to present an obstacle on which the blastopore lip became split. Much the same phenomenon appears to have occurred in the present experiments but due to centrifugal force. The centrifugal force acting in the blastula stage seems to have caused a displacement of the blastocoel in such a way that the dorsal roof of the blastocoel forms a wedge upon which the invaginating dorsal lip becomes split. Each portion of the dorsal lip may then give rise to as much of a neural groove as it is capable. In the experiments reported here the splitting evidently occurred in the anterior region of the embryo and anterior doubling was thus produced. Further work on this problem is in progress.

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