EXPERIMENTAL PRODUCTION OF FUNCTIONING REDUPLICATIONS—A TRIPLE AND A FUNCTIONING QUINTUPLE HINDLIMB IN THE FROG

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(With One Plate and One Text-figure)

Experimentally produced functioning reduplications, triple and quintuple hindlimbs of the frog have not yet been described.

Supernumerary limbs can be produced either by transplantation methods or by the methods described in this paper.

In order to prevent confusion between a reduplication of the primordium itself and a doubling after orthotopic transplantation of a limb bud, the author distinguished (1925) (i) reduplication of the limb primordium and (ii) pseudo-reduplication consisting of an orthotopic transplanted limb of a donor and a regenerated limb of the host. This grouping was accepted by Smook (1934).

In the following description the above-mentioned terms will be applied in the sense given.

As this paper deals with supernumerary limbs of the primordium itself the present cases are more instructive for human and animal pathology than are the transplantation experiments. Reduplication of this kind reveals the biological potency of the limb primordium as realized in abnormalities such as sometimes develop in man and animals.

Another problem dealt with in the present paper is a neurological one. The function of the triple and quintuple reduplications indicates some remarkable capacities of the central nervous system acting upon these multiple primordia since their earliest embryonic development.

There will be given in this paper a short preliminary record only of the function; further details will be published later of the movements after they have been studied by cinematography.

MATERIAL AND METHODS

A total of 288 different operations were performed in the frog embryo from the neurula stage up to the 27 mm. tadpole stage. Two methods gave the best results:

(1) Rotating method: consisting of rotation of the hindlimb primordium.

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(2) **Splitting method**: consisting of dividing the hindlimb primordium into two equal halves.

The rotating method can be applied to embryos over 7 mm. total length and to tadpoles. The best stage for splitting the hindlimb bud is at the 20 mm. tadpole stage when the hindlimb bud shows as a small round greyish swelling. More than 100 operations on neurulae at the tail bud and at later stages up to 7 mm. gave no result.

For operations the author used small dishes partly filled with beeswax. The embryo or larva was put under water into a depression in the wax cavity made of such a size and shape as to fit its body snugly. The tadpoles were anaesthetized with chloroform water. By a specially constructed curved and ground iridectomy knife the hindlimb was circumcised with the aid of a binocular microscope. After lifting, the bud was rotated 180°, re-implanted and placed under a cover-slip for an hour, and then the larva was transferred to a bowl of fresh water.

The splitting method was performed with the same iridectomy knife. The tadpole was fixed by curved pins on a wax bed. The bud was halved from its dorso-anterior to its ventro-posterior end and a small piece of tail, cut from another larva was put between the two halves to prevent fusion. The larva was then transferred to fresh water.

**DESCRIPTION OF SUPERNUMERARY LIMBS**

The following description will not include all the author's results which will be described in further papers.

**Reduplications**

*Case 841.* Splitting method.

The reduplication developed after splitting of the hindlimb primordium in the 12 mm. tadpole stage. The photograph (Pl. I, fig. 1) was taken 8 weeks after the operation. The total length of the frog from the tip of the snout to the tip of the tail is 1.3 cm. The left thigh, the two thighs on the right side and the legs are 4 mm. long. The feet of the three limbs are 6 mm. long, and both thighs of the reduplication are fused and are 3.3 mm. thick. The three legs are equal in calibre and are 1 mm. at their thickest parts.

As has been shown in the forelimbs in amphibians and in the hand and the foot in man (Brandt, 1925, 1931) the reduplications developing from a single primordium show mirror symmetry. The axis along which the arrangement of the limbs takes place is a radial or tibial axis. There are very rare exceptions to this rule. The case of the hindlimb of the frog confirms this and shows a mirror symmetry along its tibial axis. The caudal member of the reduplication is a right hindlimb, the cranial one is a left hindlimb. Therefore the animal has two left limbs, a normal left on the left side of the body and another reduplicated left hindlimb on the right side of the body. The two reduplicated limbs function normally as if they were the normal limbs of the animal. As the animal possesses a left limb on the left side of the body which functions synchronously with the left limb on the right side of the body,
the spinal cord is able to supply two left limbs, one of them on the right side of the body.

The movements of the reduplication consisted of flexion and extension, abduction and adduction at the knee and ankle during jumping. The fused thighs of the reduplication were not movable at the hip joint. The left hindlimb of the reduplication moved synchronously and identically with the normal left hindlimb; the movements of the right hindlimb were co-ordinated with those of both left limbs.

The case represents the first observation of co-ordinated movement of experimentally produced reduplicated hindlimbs in the frog.

These movements of the reduplication may be compared with those of a pseudo-reduplication in which, after transplantation of the right primordium of the forelimb of an embryo of Triton taeniatus into the normal place of development of the right forelimb of another embryo, the author (1922, case 144) first observed 3 weeks after the operation synchronous flexion, extension, abduction and adduction of the fully developed parts of the limb of the donor and of the normal regenerated right limb of the host. The details of co-ordinated movements are described in Brandt (1925, p. 224).


The reduplication is restricted to the foot showing the two members arranged in mirror symmetry along the tibial axis. There is a single thigh and a single leg only. The right thigh is 24 mm. in length, the left one 46 mm. Contrasting with the free movement in the former case the movements in this case were restricted to slight flexion and extension of the ankle and slight co-ordinated movement of the fourth and fifth toes.

Triples


The triple on the right side consists of a reduplicated foot and a round straight sprout joining the limb at its ankle. The reduplication shows mirror symmetry along its tibial axis and consists of a right and left foot, according to the arrangement of the toes. The shortest toe is the first, the longest is the fourth. The fifth and the second toes are about the same length. There is one thigh and one leg only and thus the triple is restricted to the foot.

The hip joint showed very slight movements of abduction and adduction. The third, fourth and fifth toes of the reduplication showed slight degrees of co-ordinated flexion when the animal intended to jump. After sitting, the toes were extended to their former position. The sprout remained stiff throughout. After stimulating the sole the two reduplicated feet showed some irregular and unco-ordinated movements, viz. isolated flexion or extension of single toes.
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The triple consists of a perfect reduplication of the same kind as described in case 841, and a third perfect limb developed at an angle of 90° to the reduplication (Pl. I, fig. 4). The third limb is a normal right hindlimb on the right side of the body. The reduplication lying ventral to it shows the typical arrangement of the parts in mirror symmetry. Whilst the ventral aspect of the hindlimb of the frog is unpigmented, the two reduplicated hindlimbs show a conical area of pigment on this aspect. This pigmented area must therefore be considered the dorsal aspect of the reduplication which is fixed to the pelvis in a rotated position of 180°. The pigment on the third hindlimb shows that it lies in the dorso-ventral position.

The movements of the third single hindlimb consisted of flexion, extension, abduction and adduction at the hip, knee and ankle joints and were co-ordinated with those of the opposite side but restricted to a much lesser extent. Therefore this limb must be considered as a normal regenerated limb whilst the reduplication has developed from the rotated limb bud. The reduplication itself was fixed except for sluggish and limited indications of flexion and extension in the longest toes of both feet which appeared at a later time than the movements of the normal limb of the animal.

Quintuple


The quintuple concerns the right hindlimb and shows a collection of limbs of different sizes and at different stages of development. At the time when the large limb (Text-fig. 1, no. 2) is fully developed, the foot (no. 4) is commencing to grow from the area of the knee of limb no. 3. It should be noted therefore that the quintuple does not represent a unity of limbs of equal age and that the limbs are arranged in very different positions. Seen from the ventral aspect (plate) their significance becomes clear. The ventral aspect of the thigh consists of a cranial unpigmented half and a caudal pigmented half. The upper border of the pigmented area indicates a line of demarcation between the originally rotated limb bud and a new regenerated limb. Limbs 1 and 2 turning their dorsal pigmented aspects ventrally must be considered as a unity which has developed from the rotated limb bud. The upper part of the monstrosity consisting of limbs 3, 4 and 5 is another unity and represents a regenerated stock. Limb 3 is a perfect regenerated hindlimb consisting of thigh, leg and foot in normal position, the accessory feet 4 and 5 are rudiments growing from the area of the knee of the regenerated limb.

The anatomical feature of these limbs corresponds to the physiological. Slight movements of flexion of the toes and of the ankle of limbs 2 and 3 were co-ordinated with identical movements of the normal hindlimb on the left side. The range of movement in the normal left hindlimb was more extensive than in the quintuple. The hip joint and limbs 1, 4 and 5 were fixed.
SUMMARY

1. The supernumerary limbs were produced by the rotating and splitting methods applied to the hindlimb bud in tadpoles of early stages of development.
2. The two methods gave different results. Using the rotating method, the rotated bud may develop a limb or multiples of a limb inverted at 180° and a regenerated limb of normal position. Using the splitting method, two limbs in mirror symmetry may develop besides a regenerated limb.
3. The function of supernumerary limbs is two-fold and may consist either of co-ordinated and synchronous movements of fully developed limbs or parts of limbs or the function may be restricted to some retarded and imperfect movements.
4. There is a great variety of form, structure, rate of development and arrangement of the parts of the multiples, to which a special function is related. One animal may show perfect co-ordinated movements of fully developed reduplicated limbs (case 841), another may show restricted co-ordinated movements (case 1220), while others may show co-ordinated movements of parts of other supernumerary limbs (cases 1251, 1284).
5. The co-ordinated movements in experimentally produced supernumerary hindlimbs of the frog are to be regarded as an “integration of a series of reflexes”, and they are related to the same reflex mechanism which acts in the normal ambu-
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latory cycle of the toad (Gray & Lissmann, 1940). The retarded and imperfect movements in some supernumerary deformities indicate that disturbances of the normal cycle are not the expression of a centrally determined rhythm.

REFERENCES


EXPLANATION OF PLATE I

Fig. 1. Case 841. 1937. Splitting method. Photographed 2 months after operation. Fully developed reduplication showing mirror symmetry.

Fig. 2. Case 1220. 1940. Splitting method. Photographed 53 days after operation. Reduplication of the foot. The toes are numbered to show their arrangement along the tibial axis.

Fig. 3. Case 1284. 1940. Splitting method. Photographed 47 days after operation. The reduplication of the foot is along the tibial axis. The sprout without toes represents a third limb.

Fig. 4. Case 1251. 1940. Rotation method. Photographed 47 days after operation. Triple hindlimb. The fully developed reduplication of the hindlimb shows its dorsal pigmented side at the ventral aspect of the animal. The third limb is partly covered by the reduplication.