REGENERATION OF SUPERNUMERARY LIMBS IN TADPOLES

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In tadpoles and adult specimens of the lake frog (Rana ridibunda Pall.) from certain natural reservoirs situated in the foothills of the Zailiiskii Ala-Tau we often observed supernumerary hind limbs [2, 3]. Increase in the number of limb buds occurred mainly on the right side of the body and became evident visually long before metamorphosis, at an early stage of larval development when the hind limbs appeared as oval, undifferentiated buds. Evidently the factors causing the appearance of supernumerary limbs exert their influence at a period of primary concentration of cellular material, since later may be observed fully formed buds of several limbs in a varying degree of isolation (Fig. 1).

In their external features and microscopic structure the buds of the principal and supplementary limbs are hardly distinguishable from each other, and it is often difficult to decide from which bud the principal and from which the supplementary limb will develop. In many cases the rudimentary principal right limb was identified by its symmetrical position in relation to the left, and the buds of the supernumerary limbs may be identified by their unusual situation.

In early stages of development we observed, besides a large number of cases of two or several isolated appendages, cases in which the principal and supernumerary limbs were joined together for a considerable part of their length (see Fig. 1, b, c and d). At the moment of appearance of the first outward signs of the onset of differentiation of the indentations of the forming digits, a definite spatial relationship between the combined rudiments of the limbs can already be observed. This same relationship is basically retained in the subsequent period of development, although the rate and degree of differentiation of different limbs may vary considerably. With the appearance of visible articulation in the limb at its main sections there often takes place inhibition of growth and development of the supernumerary limb both in cases of isolated supernumerary appendages and when combined with the appendage of a principal limb. Inhibition of the subsequent development of the supernumerary limb at this stage and slightly earlier is often combined with marked depigmentation of its skin covering.

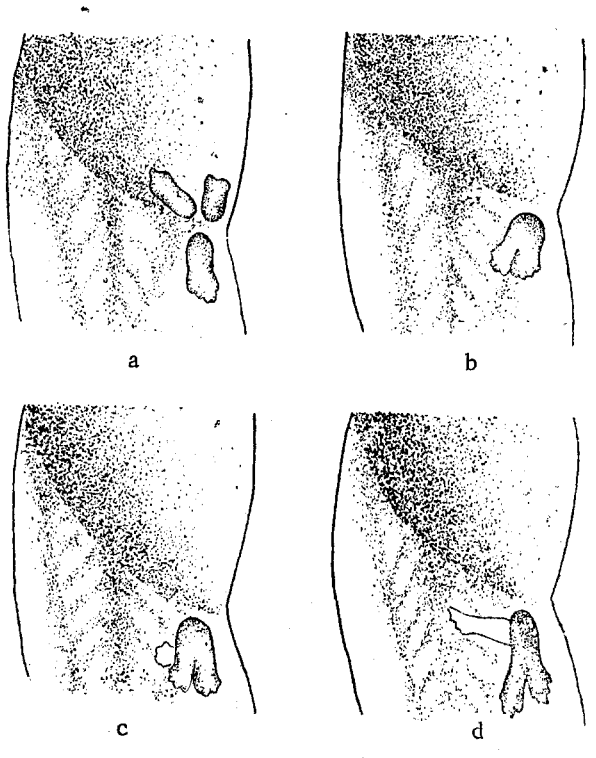

Fig. 1. Different cases (a, b, c, d) of relationship between supernumerary and principal limbs on the right side of the body at the stage of the first signs of external differentiation.

The supernumerary limb, as a rule, grows together with the principal limb, and the following relationships between them apply. Most commonly both limbs are disposed with their ventral surfaces side by side, with fan-like arrangement of the digits of the two feet; in this form the skin which is common to the combined limbs loses its polarity in pigmentation and acquires a homogeneous coloring, characteristic of the dorsal surface. In another type the supplementary limb is situated with its ventral surface on the dorsal aspect of the principal limb, while preserving the typical dorsoventral polarity in pigmentation of the skin of both limbs. The degree of combined growth of the two limbs may differ;

most commonly the limbs are joined only in the thigh region. The trophic conditions appear to be more favorable in the first type of combined growth, and the supernumerary limb often develops without any essential abnormality.

Our observations and experiments were made on anomalous tadpoles of lake frogs caught under natural conditions and continuing to develop in the laboratory. In tadpoles kept in large aquaria we studied the regeneration of the principal and supernumerary limbs. We were concerned with the problems of the extent to which the relationships formed during embryogenesis between the principal and supernumerary limb buds are stable and may be reproduced vegetatively in the process of reparative regeneration; to what extent does regeneration of supplementary limbs proceed typically, if we take into consideration our previous findings that these limbs are devoid of any motor innervation [4].

EXPERIMENTAL METHOD

Experiments lasting for a period of 3 years were performed on 62 anomalous tadpoles, of which 44 had isolated supernumerary limbs and in 18 they were joined to the principal limb bud. The number of regenerating limbs was twice this figure, since in addition to regeneration of the supernumeray limb, restoration of the principal limb was also studied. For the experiment we took tadpoles with initial signs of differentiation of the hind limbs, and not yet having lost their regenerative capacity [1, 5, 6]. The difficulty of obtaining the necessary material naturally did not permit us to perform all the experiments simultaneously. For this reason the experiments were performed at different times on single specimens as they were caught. Our annual observations were mainly made in July, just at the time when the metamorphosis of the bulk of the larvae of the lake frog is over and anomalous tadpoles can be found, developing evidently from spawn of later layings. Four large anomalous larvae, hibernating in the reservoir and found at an early stage of development were taken for an experiment at the end of April.

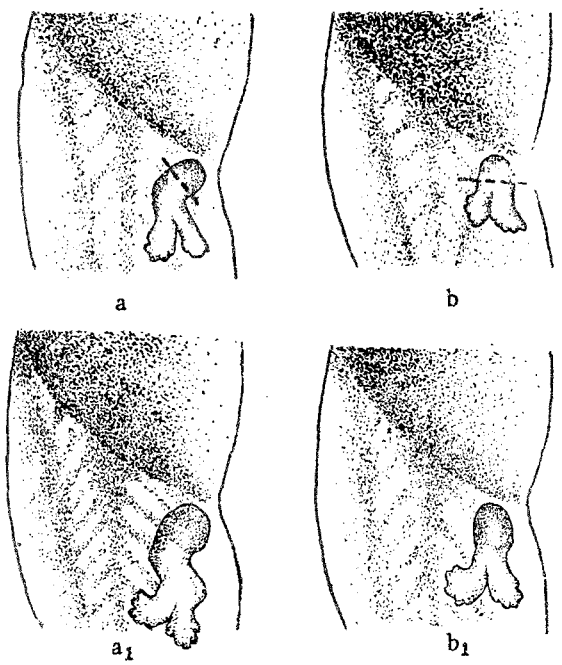

Fig. 2. Original state of the limbs before amputation of the distal portions (a, b); the level of amputation is shown by a broken line and the newly regenerating limbs in (a_1, b_1). The tadpoles were exposed to a low temperature for a short time (5 days).

The operative technic was the usual one. With a fine blade the principal and supernumerary limbs of the right side of the body were amputated perpendicularly to the main axis. The amputation was always performed at as proximal a level as possible (Fig. 2, a, b and Fig. 3, top series). The regenerating limbs were periodically drawn and a part of the material was prepared histologically.

In some experiments by Tornier [7], amputation of the limb buds which were accompanied by formation of supernumerary limbs was performed in line with the main axis, in anteroposterior or dorsoventral directions. It is relevant to remember that the power of regulated regeneration of the developing hind limb of the frog is retained for quite a long time during the larval period.

EXPERIMENTAL RESULTS

We may examine briefly the results of regeneration of isolated supernumerary limbs. Regeneration of these limbs took place synchronously with the restoration of the principal limbs amputated at the same time. They often finished up as perfectly restored limbs, with the formation of a normally formed foot; atypical regeneration consisted of a reduction in the number of digits, and diminution in size and disturbance of the relationship between the main sections of the limb. When the tadpoles were kept under optimal conditions in large aquaria with an abundance of food, the number of atypically regenerating principal limbs did not exceed 10%, while in the case of supernumerary limbs the figure was over 70%. A characteristic feature was that the atypical development of

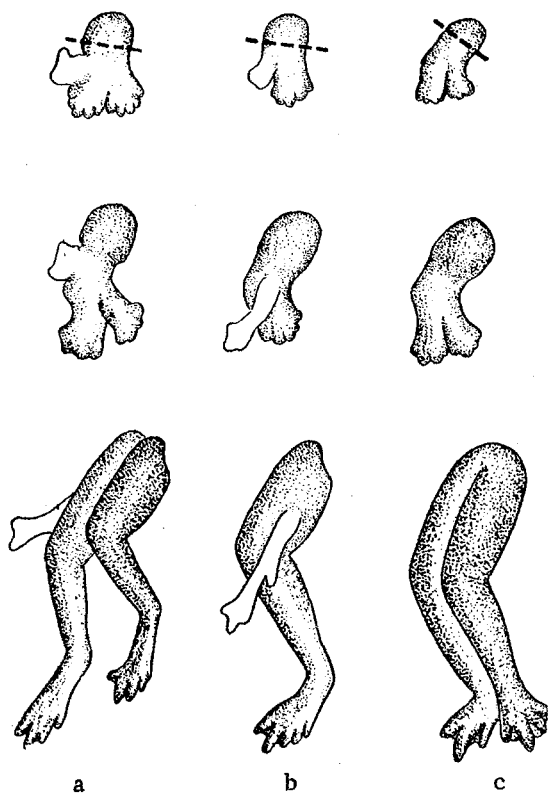

Fig. 3. Young fused limbs at the moment of amputation (top row), structure of the regenerating limbs at an early stage (middle row) and the same limbs after completion of differentiation into the typical organ at the stage of incipient functioning (bottom row).

a regenerating limb of the supernumerary type appeared and grew more pronounced at an age after the formation of a relatively normally formed foot on the young limb and the creation of the indentations of the rudimentary digits.

The most interesting findings were concerning regeneration of fused limbs, and were obtained in 18 anomalous tadpoles. During the process of regeneration the same relationships between the principal and supernumerary limbs which applied at the moment of amputation were repeated without any substantial difference. The young regenerating limbs repeated with only negligible variation the relationships which existed between the limb buds at the time of amputation. In one case we observed suppression of regeneration of a supernumerary limb with a reduction in the number of digits formed.

In another experiment performed in the following summer on different tadpoles with approximately the same anomalies, the phenomenon of repetition during regeneration of the pre-existing relationships was again confirmed (Fig. 3). Observations on the development of regenerating limbs continued for over a month, which enabled the later stages of formation of the regenerated limb to be traced. In the great majority of cases the regenerating supernumerary limbs lagged behind very considerably in their differentiation, which was combined with a partial reduction in the digits which had begun to form (see Fig. 3, a, b, c). These regenerating limbs were depigmented and, as histological examination showed, muscular tissue was absent, together with nerve fibers and vessels. In other cases the supernumerary limb regenerated without any significant abnormality, although its general growth was retarded; in these cases one of the digits in the regenerating foot of the principal limb was observed to be reduced. Where there was considerable fusion of the young buds, the regenerating combined limbs were well developed and were practically indistinguishable from each other (see Fig. 3, c).

Thus in the process of regeneration, i. e., of vegetative reproduction, there takes place a quite faithful repetition of the relationships which governed the principal and supernumerary limb buds in embryogenesis. In the process of repair we observed that same characteristic delay in differentiation which had already been noted in the development of the limbs of other anomalous specimens which had not been amputated. We consider that the absence of efferent innervation in supernumerary limbs, which we observed in conditions where there was a natural increase in the number of limb buds, could influence the nutrition and the character of the subsequent differentiation of the regenerating limbs. This did not show a negative influence in the case of the close anatomical and trophic union of two forming or regenerating limbs.

We may note in conclusion that if anomalous tadpoles are kept for two days before amputation and for 3 days after amputation in a vessel of cold water (4-6°C) they later form regenerating limbs which most clearly reproduce the previously existing relationship between the principal and supernumerary limbs. This is shown in Fig. 2; here, in contrast to the cases shown in Fig. 3, no disturbance of differentiation of the regenerating supernumerary limbs was observed, including those which develop at later stages of development. In the 4 hibernating anomalous tadpoles the regenerating limbs illustrated very clearly the stability of the morphological relationship of the principal and supernumerary limbs. Consequently, a low temperature promotes a more stable reproduction of the whole range of morphological signs of duplicate limbs and their relatively greater differentiation at a later stage of development.

SUMMARY

Amputation of supernumerary hind limbs was performed in 62 tadpoles (Rana ridibunda Pall). Regeneration of the principal and supernumerary limbs takes place with recurrence of the topographic-morphological relationships formed during the embryonic stage of development. Atypical formation of distal portions of the newly formed limbs takes place with appearance of external differentiation. Low temperature promotes a more stable recurrence of morphological signs of duplicate limbs by the regenerates.

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A TRIAL OF GROWTH HORMONE PREPARATIONS IN RATS WHOSE THYROID FUNCTION HAS BEEN BLOCKED WITH METHYLTHIOURACIL

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The discovery of Evans and Long of the growth hormone in 1921 prompted numerous attempts to isolate this substance in the pure form, which was achieved only in 1945 by the production of a highly purified preparation in the form of an individual protein [6]. However, the search for new, simpler and more convenient methods of biological testing of this hormone remains a problem of great importance; the most widely used tests, requiring preliminary hypophysectomy, are too complicated to be used as standard methods. Ia. M. Kabak and E. B. Pavlova [3] proposed the use of rats treated with methylthiouracil as test animals for growth hormone preparations, showing that blocking the thyroid gland function (as also by thyroidectomy) leads to cessation of formation of the growth hormone in the anterior lobe of the pituitary gland: the animals stop gaining weight, and in the anterior lobe of the pituitary the acidophil cells [2] which are generally held to produce the growth factor disappear; then, as a result of administration of growth hormone, growth is restored, while in the pituitary the histological changes do not take place.

* In Russian.

** See English translation.