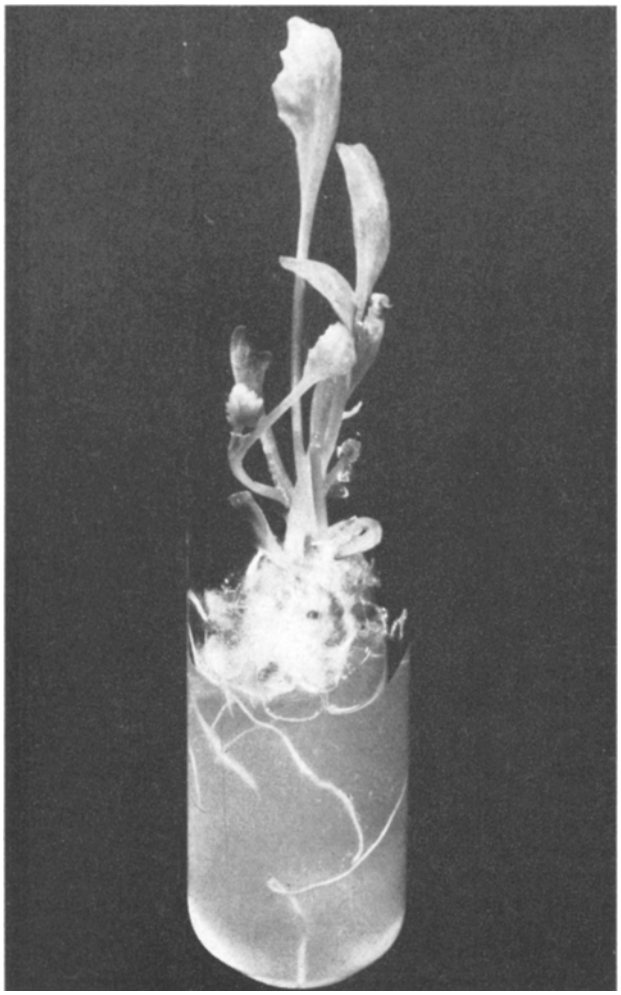

Fig. 3. Plant of marrow stem kale regenerating from the root callus in the fourth subculture. ×1.

the origin of the explants. In the case of roots and leaves they were 0.2–0.5–100 and 1.0–0.5–500 ppm respectively. In natural day-light at 20–24 °C, the explants produced in 10–30 days greyish yellow callus, which grew on the same or slightly modified media, in 1-month subcultures, for more than 1 year (Figure 1). Following 3 months of culturing, the concentration of 2,4-D was progressively lowered.

Results and discussion. Numerous roots and root trichoms were formed in 3–7 days, if the calli were transferred to the medium without 2,4-D (Figure 2). 10–30 days later, leaves and shoots appeared (Figure 3). Usually 40–70% of root calli, 10–30% of calli derived from ovaries and 5–20% of hypocotyls, cotyledons, leaves, stem pith, ovules and stamina calli could differentiate entire plants. Morphogenetic potentiality of callus tissues decreased with time. Shoot-forming ability disappeared in 6–8, and that of roots in 10 or 11 subcultures. Regenerated plants were transferred to soil in pots and then to the field where they developed normally.

Organ-forming capacities of callus tissues originating from different plant parts seem to be influenced by the degree of functional specialization of mother tissues, or by the proportion of the genome involved in its control.

Résumé. Dans les cultures de tissus repiqués de Chou moellier une néoformation de plantes entières se produit en conséquance de l'exclusion du 2,4-D du milien de culture.

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Regeneration of Limbs in Adult *Rana ridibunda ridibunda* Pallas

Studies on limb regeneration in most of the salientians among Amphibia revealed that they lose, in general, their regenerative capacity either early or later during the metamorphic period. The few salientians that partially preserve the power of regeneration at the post-metamorphic, and even till the adult stages, form heteromorphic limb outgrowths that never attain the normal pattern of limb morphogenesis.

Briefly, the salientian limb regenerative capacity falls within 3 main categories, viz. 1. early loss before the onset of the metamorphic period as in *Rana sylvatica* and *R. pipiens*[1]; 2. conspicuous decline during the metamorphic period resulting in a large cartilaginous condylar mass capping the stump skeleton as in *Alytes obstetricans*[2] and *Bufo regularis*[3,4]; and 3. better partially preserved regenerative capacity up to the adult stage in members of the families Pipidae[5–10] and Discoglossidae[10]. Their limb regenerates are heteromorphic and range from simple, spike-like to spatulate outgrowths, sometimes with rudimentary digits. In general, these outgrowths mainly consist of cartilage and connective tissue fibres with hardly any bone or muscle fibres[5,8,10].

Normally, the ranids so far studied lose completely the regenerative capacity of their limbs when transected at the adult stage[11–13]. However, this capacity could be partially enhanced by the use of various stimulating procedures[14–18]. Even these experimentally induced limb outgrowths are also mainly supported with cartilage material, with the exception of *Rana clamitans*[18] which showed ossification distally. The present investigation aimed at reporting on the power of limb regeneration in the adult ranid, *Rana ridibunda ridibunda* Pallas and on the osteogenetic pattern within the stump skeleton at, and distal to, the level of amputation.

Individuals, with average head-trunk length of 7 cm, were collected from the fields of Hamman Al-Alil (south of Mosul). One fore-limb in each was transected through the distal level of the antebrachium. Out of a total of 82 cases, 56 were operated on the left-side limb, the remaining animals on the right side one. The series operated on the left side was reared for 5 months, from April till September, while the right side series was reared for 4 months during the same season. The rate of mortality reached 12% of the total number of cases. Soon after

wound healing was accomplished during the early post-operative days, cases were reared together under more or less natural conditions, in a large ground concrete pond ($4 \times 2 \times 0.75$ m) found in the fields of the same locality. The bottom was covered with sand on which large pieces of stone were placed. The pond was kept full of water, provided with plenty of water plants. To keep the operated cases in captivity, a fence of fine wire gauze was erected, 2 m apart from the pond boundaries. Plenty of water insects as well as terrestrial ones burrowing in tunnels, served as a natural source of diet. At the end of the rearing period, cases were fixed in 10% formalin.

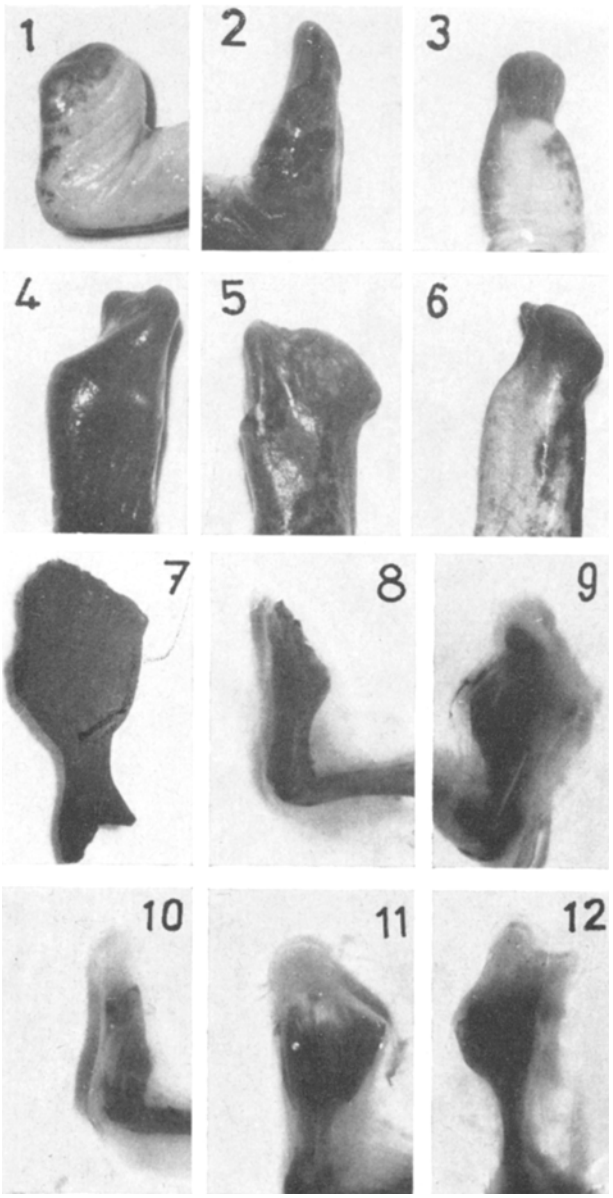

Fig. 1. Non-regenerating fore limb stump. ×2.

Figs. 2–6. 2, heteromorphic regenerates: simple and attenuating; 3, simple with a blunt apex; 4, indented with 2 digitoid lobes; 5, and paddle-shaped with digitoid protrusions that are straight; 6, or folded. Magnification ×2.

Fig. 7. Alizarin stained regenerating radio-ulna denuded of its surrounding soft tissues and skin. Magnification ×3.

Figs. 8–12. Alizarin transparencies of fore limb regenerates. Bone appears dark and opaque, while cartilage is pale and transparent. Magnification ×2.5.

Amputated limbs had failed to regenerate completely (Figure 1) in 10% of the total number of fixed cases. All the remaining cases had developed into heteromorphic limb outgrowths that were: a) simple, darkly pigmented with either an attenuating (Figure 2) or a blunt, semicircular apex (Figure 3) in 56%; b) apically indented into 2 digitoid prominences (Figure 4) in 25%; and c) paddle-shaped with digitoid protuberances (Figure 5) or with folded digitoid lobes (Figure 6) in the remaining 9% of the cases.

Alizarin transparencies[19] of various cases showed that the transected radioulna had thickened considerably at the plane of amputation. This merges distally into: a) a stout, cone-shaped bony support (Figures 7 and 8); or b) an attenuating bony, claw-like structure that is cartilaginous apically (Figure 9); or c) an ossified base which is confluent with conspicuous cartilage material that is either simple (Figures 10 and 11) or bifurcated (Figure 12). The paddle-shaped regenerates were mostly supported with cartilage material.

It may be concluded that *Rana ridibunda ridibunda* Pallas deviates from all other known adult ranids[11–13] in partially preserving the capacity of limb regeneration but at comparatively lower level than that of the members of the families Pipidae and Discoglossidae[10].

The skeletal support of the regenerates is here unique in being partially or completely ossified as compared with the dominance of cartilage material in regenerates of both normally[8–10] and majority of artificially activated[14–17] adult salientians. The only salientian, so far known, that showed ossification in the skeletal elements of the limb regenerates is *Rana clamitans*[18], after the transplantation of the adrenal gland to its jaws.

Zusammenfassung. Nachweis der Beinregeneration bei adulten Fröschen *Rana ridibunda ridibunda* Pallas. Skelett war weitgehend verknöchert.

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