(*6*) in spinal root fibers of the cat stained intravitally with methylene blue. In our material it is occasionally possible to follow the cell processes to the nodes, where there is usually an accumulation of cytoplasm studded with fine granules. Various appearances like the spiny bracelets of Nageotte are sometimes differentiated. In time, the nuclei become opaque (cf. Fig. 2) through the enlargement and fusion of the granules.

Another phenomenon appears in the fresh tissue, and as promptly as the staining of the sheath cells. The ends of the axones react intensely where they were injured by pressure or by electrical changes induced by metal instruments in the fresh tissue. Similar reactions to injury occur with methylene blue in living animals (*2*). The lead sulphide staining at sites of injury always occurs after boiling fresh tissues and has nothing to do with phosphatases. In addition, the normal myelinated fibers assume a uniform yellow tint, both in fresh and boiled material. This is presumably due to a diffuse adsorption of lead. That it is not due to staining with sulphides is indicated by the fact that a similar uniform staining occurs if the lead is visualized by means of a freshly prepared unoxidized aqueous solution of hematoxylin.[5]

If dry ice is applied to a living nerve *in situ*, all axones are shriveled. When the frozen tissue is gradually thawed in chilled glycerophosphate-lead reagent and then incubated, all axones are as intensely stained as in acetone-fixed material. In addition, all nuclei react as in fresh tissue.

In 1906 R. R. Bensley (*1*) showed that the supposed histochemical test for organically bound phosphorus was nothing more than a staining reaction. The Gomori phosphatase reactions likewise involve substitutions; the substance visualized is not the substance sought. Adsorption and diffusion may play significant roles. Until it has been shown that they do not, the specificity of the reactions must remain in doubt. It may be that both boiling and fluorides do more than merely inhibit enzymatic activity.

Certain findings indicate that the histologic acid phosphatase reactions are unreliable: (1) Both enzymes and lead phosphate can be leached out of, and hence moved about within, fixed tissues. This deprives the method of value for the localization of enzymatic activity. (2) All nuclei and the Schwann cell cytoplasm react promptly when fresh nerves are placed directly into the glycerophosphate-lead reagent. This does not occur after acetone fixation. (3) The axones of fresh myelinated fibers do not react unless they have been injured. Axones shriveled by freezing or acetone react strongly except at the nodes of Ranvier. (4) The reaction of injured tissue after the destruction of enzymatic activity shows that under certain conditions the lead of the reagent can be specifically adsorbed.

We are continuing our efforts to obtain differentiations in peripheral nerves with reagents which simulate the glycerophosphate-lead reagent but which contain no phosphate.

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[5] We owe this test to R. R. Bensley.

Polydactyly and Limb Duplication Occurring Naturally in the Tiger Salamander, *Ambystoma tigrinum*

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Few cases of polydactyly and limb duplication occurring in nature in *Ambystoma* have been reported in the literature (*8*). On the other hand, limb duplications following transplantation procedures in the laboratory are very well known from the work of Harrison and his students. Such anomalies may result from a variety of operative treatments, including the division of the limb rudiment (*11*), orthotopic and heterotopic limb transplantations (*2, 3, 5, 7, 9, 12*), and induction of supernumerary limbs by implantation of foreign tissue (*1, 4, 6*). The experimental production of limb duplications by methods other than transplantation techniques is still an unexplored field.

TABLE 1

Polydactyly and Limb Duplications in *Ambystoma tigrinum**

Animal	Left hind limb	Right hind limb	Remarks
2	Normal	8 digits	Metamorphosed
3	6 digits	6 "	
4	Normal	6 "	
5	Main limb with 6 digits; *extra limb* with 12 digits	Main limb normal; small *extra limb* with 2 digits	
6	8 digits	8 digits	
7	Normal	7 digits plus 1 very small bud	Questionable mirror-image symmetry
8	Main limb with 7 digits; *extra limb* with 7 digits	8 digits	Questionable mirror-image symmetry
9	6 digits	6 "	
10	Normal	8 "	
11	6 digits	6 "	
12	9 "	9 "	
13	Normal	8 "	
14	Main limb normal; *extra limb* with 7 digits	7 "	
15	6 digits	8 "	
16	9 "	Main limb and *extra limb* fused with a total of 14 digits	
17	9 "	6 digits plus 1 small bud	Metamorphosed
18	6 " plus 2 buds	6 digits	"
20	Normal	6 "	
21	6 digits	Normal	

* Animals 2 through 18 collected as larvae, 1946 (117–144 mm. in length); 20 and 21 collected as adults, 1947.

The anomalies reported here in the tiger salamander, *Ambystoma tigrinum*, represent the first recorded case of mass polydactyly occurring naturally. The abnormal animals were collected in Muskee Lake (altitude, 8,300 feet) approximately 15 miles west of Boulder, Cclorado. This and the surrounding lakes have been extensively studied in recent years, but the first polydactylous individuals were found in October 1946 (17 larvae) and in April and May 1947 (2 adults). The per-

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centage of abnormal individuals collected in the fall of 1946 was extremely high—of 19 animals collected, 17 showed polydactyly and limb duplication in some degree.

The abnormalities range from one extra digit to a complete extra appendage with supernumerary toes. The condition is found in both sexes, on either one or both sides of the animal,

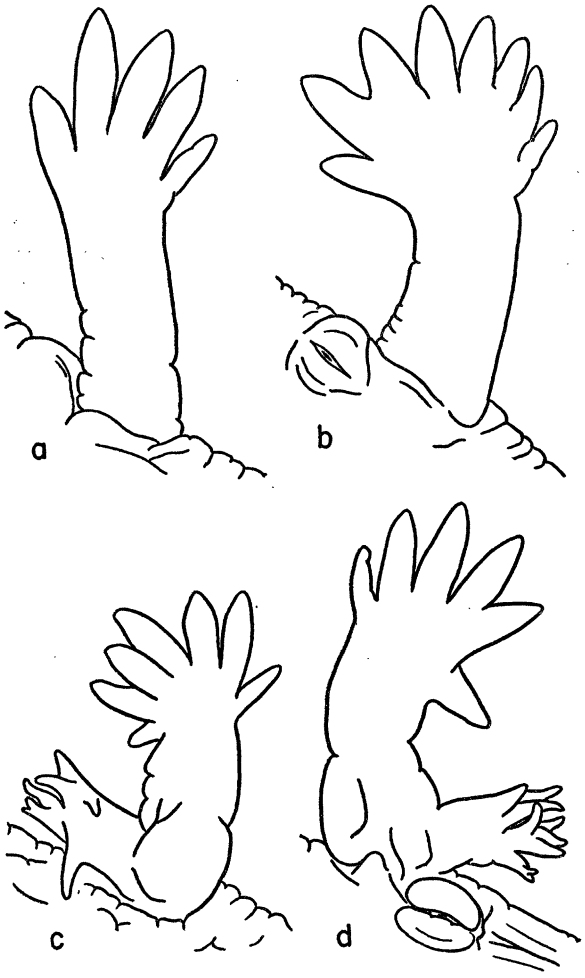

FIG. 1. Polydactylous hind limbs of *A. tigrinum* (2½×): (a) normal left limb, spec. #10, dorsal view; (b) 8-toed right limb, spec. #10, ventral view; (c) extra polydactylous left limb, spec. #8, lateral view; (d) left limb duplication, spec. #5, ventral view.

and is always limited to the hind limbs. A summary of the abnormalities occurring in larvae and adults collected both seasons is presented in Table 1. Outline drawings of polydactylous and duplicated limbs from three larvae are shown together with a normal limb for comparison (Fig. 1). The anomalous condition is not altered by metamorphosis of the animals from the larval to adult stage. Pigmentation of the extra digits is normal, and they appear in most cases to be fully functional. However, the supernumerary limbs seem to be more of an encumbrance than an advantage to the salamanders.

Polydactyly and limb duplications induced by transplantation methods usually show mirror-image symmetry between the supernumerary and the normal limb, although asymmetrical exceptions have been noted, particularly by Swett (*10*). This symmetry holds for duplications in both the dorsoventral and the anteroposterior planes and is due, according to Harrison (*6*), to the development of more than one growth center, the main center reversing the symmetry of an adjacent center. It is of significance to point out that in the naturally occurring polydactylous series noted in this paper there are only two cases—and they are doubtful—of mirror-image symmetry (Fig. 2). The right hind limb of animal #7 has 7 well-formed toes plus a small bud, and we may be dealing with a duplication involving a reversal of symmetry. In animal #8 the supernumerary left limb can be interpreted as dorsoventral mirror-image symmetry, with, however, considerable modification due to different growth rates of the several digits. These two cases of questionable mirror-image symmetry are exceptions

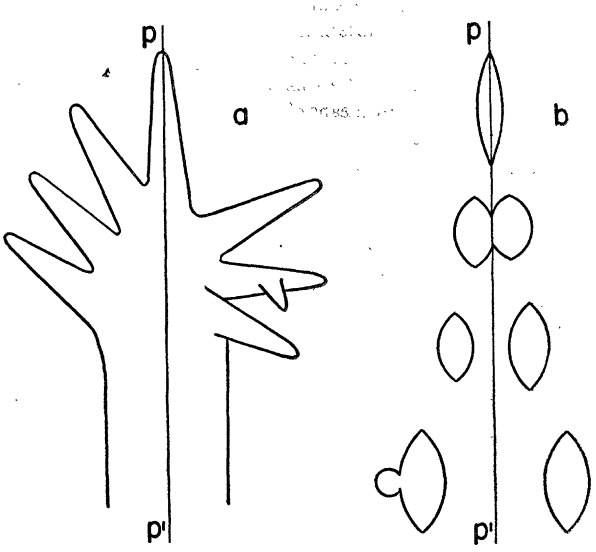

FIG. 2. Possible mirror-image symmetry in limb duplications: (a) anteroposterior symmetry in right limb of spec. #7; (b) dorsoventral symmetry in extra limb of spec. #8 (p–p' represents the plane of symmetry).

in the present series; most of the limbs show no such symmetry but rather appear to have added extra digits in series, adjacent to digit #5, as clearly shown in the right hind limb of specimen #10. (Fig. 1b).

Investigations of these polydactylous salamanders are in progress to determine the nature of the genetic background and those environmental factors, e.g. cold shock, which might have contributed to the widespread occurrence of these anomalies within a limited *Ambystoma* population.

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