agar (gel-precipitin) have been carried out between concentrates of streptococcal culture supernates and sera of various sources. Two technics of diffusion in 1 dimension (tube) were used: the single-diffusion technic of Oudin, and a double-diffusion technic. 2. Sera of rabbits previously injected with streptococcal culture supernates showed increasing numbers of bands as the course of injections progressed. Sera of such rabbits showed as many as 5 bands when tested with such concentrates, indicating that this was the minimum number of antigen-antibody systems present. 3. Sera of patients with acute rheumatic fever and of patients convalescent from an acute streptococcal infection (scarlet fever) were also tested by this technic against concentrates of streptococcal culture supernates. The group with rheumatic fever showed a greater number of bands (a range of 2-7) than did the group with scarlet fever (0-4), when tested by the double-diffusion technic. The corresponding ranges found with the single-diffusion technic were 1-5 and 0-2, respectively. In a sampling of sera from convalescents from scarlet fever the number of bands was found to be no greater 6 weeks after the onset of the infection than 3 weeks after the onset.

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Teratogenic Effects of *Lathyrus odoratus* Seeds on Development and Regeneration of Vertebrate Limbs.* (21934)

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Diets containing *Lathyrus odoratus* seeds fed to young rats produce characteristic defects of mesodermal structures(1). Chemical analysis of extracts from the seeds points to the β-aminopropionitrile (BAPN) as the active fraction of the teratogenic principle(2-5). However other aminonitriles(5) and β-mercaptoethylamine(6) when given to rats have been reported to produce similar lesions. The Lathyrus poisons gain in interest through the similarity of the induced lesions with certain skeletal and vascular diseases in man, such as slipped epiphyses, degenerative arthritis, and dissecting aneurism of the aorta. Therefore the use of aminonitriles in animal research seemed to offer an opportunity for the elucidation of these human diseases.

We reported(7) that BAPN at a concentration of 2 μl/liter of aquarium water produces characteristic deformities in Xenopus larvae. The high sensitivity of Xenopus larvae suggests their use for the detection and quantitative evaluation of lathyrism-inducing agents. The effect of BAPN appears to be highly specific, affecting particularly certain rapidly developing mesodermal tissues. In view of the similarity between the processes of embryogenesis and of regeneration, the studies were extended also on the restitution of amputated legs in the newt, *Diemictylus viridescens*.

Methods. In a *first series* of experiments

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TABLE I. Effects Produced by Lathyrus Seeds, β-aminopropionitrile, and Other Compounds in Xenopus Larvae (Witschi's Stage 28). Class 1-4 express degrees of severity, 0 indicates normal development.

Compound	Conc., μl/liter	No. of animals	Class
Powdered seeds*		20	2
Sweet pea water extract†		20	2
Acetonitrile	20	10	0
Propionitrile	20	10	0
α-aminopropionitrile	10	10	0
β-aminopropionitrile	10	20	4
	2	20	3
	1	20	2
	.5	20	1
	.2	20	0
Aminoacetonitrile (hydrosulfate)	10	10	0
γ-aminobutyronitrile	10	10	0
Acrylonitrile	10	10	0
Glutamine	20	10	0
Potassium cyanide	10	10	0
Controls		100	0

* About 1 g of powdered seeds per day was placed in each aquarium; larvae were given no other food.

† Extract of 2.5 g of dry seeds per liter of water.

larvae of the South African toad. *Xenopus laevis*, were exposed to the natural principle by feeding powdered Lathyrus seeds *ad lib.* or weak solutions of BAPN,† or solutions of other related compounds. BAPN is a liquid and mixes with water freely; therefore concentrations are expressed in μl per liter of aquarium water. Since preliminary experiments had shown that treatments of early stages are without effect on limb development, the here reported experiments were all started with nearly fullgrown larvae, 10 to 12 days before the expected breakthrough of the forelimbs. At this stage the legs are still short and not yet movable. Lots of 10 to 20 larvae were placed in aquaria containing 5 liters of dechlorinated water. In the *second series* of experiments, hen's eggs (White Rock breed) were injected with 20, 1, .2 or .1 μl of BAPN in saline. Penicillin was added at the rate of 100 units per injection. The injections were made into the yolk sac either on the fourth or the tenth day of incubation, and the eggs were candled to observe the embryos daily thereafter. In the *third series* of experiments, a hind limb of adult newts was amputated at the middle of the thigh. Immediately after the wound had healed 10 animals were placed in water containing BAPN at a concentration of 2 μl/liter; and 10 served as controls.

† Supplied through the courtesy of Dr. S. Wawzonek, who synthesized the compound from acrylonitrile and ammonia.

Results. A. *Experiments with Xenopus larvae* (Table I). BAPN in concentration of 10 μl/liter causes death of the animals during metamorphosis. The limb deformities are most severe and frequently subcutaneous hemorrhages occur in this group. The larvae exposed to lower effective concentrations complete their metamorphosis, but they are too weak to survive beyond this stage. Fig. 1 represents a metamorphosing tadpole from a lot receiving BAPN at 2 μl/liter together with a control. The legs of tadpoles treated with this concentration are so badly twisted that it is impossible to prepare longitudinal sections for histological studies. At all levels of concentration the dislocation of the knee and ankle joints represents a most characteristic deformity (Fig. 2). A loosening of leg muscles, tendons and ligaments is clearly noticeable under a binocular microscope after removal of the skin. The microscopic examination of animals treated only 5 to 6 days shows wide clefts between cartilage and the covering connective tissue in the region of the joints (Fig. 3). In control animals of corresponding stage a compact embryonic connective tissue surrounds the cartilages and connects adjoining epiphyseal ends (Fig. 4). Obviously this rent formation is the beginning of the lesions in the young toads, a process which eventually results in dislocation of the joints. The cartilage appears normal and the periosteal ossification seems unaffected.

Lesions also develop in the dorsal musculature about 10 days following the onset of the experiments. After removal of the dorsal skin of tadpoles treated with BAPN or with Lathyrus seeds one observes that the intersegmental septa are abnormally wide (Fig. 1a, b). Detailed microscopic examination shows a very loose connective tissue in the septa. The muscle segments are atrophic.

The general body growth of the treated larvae becomes increasingly retarded. Experimental animals are thinner and weaker

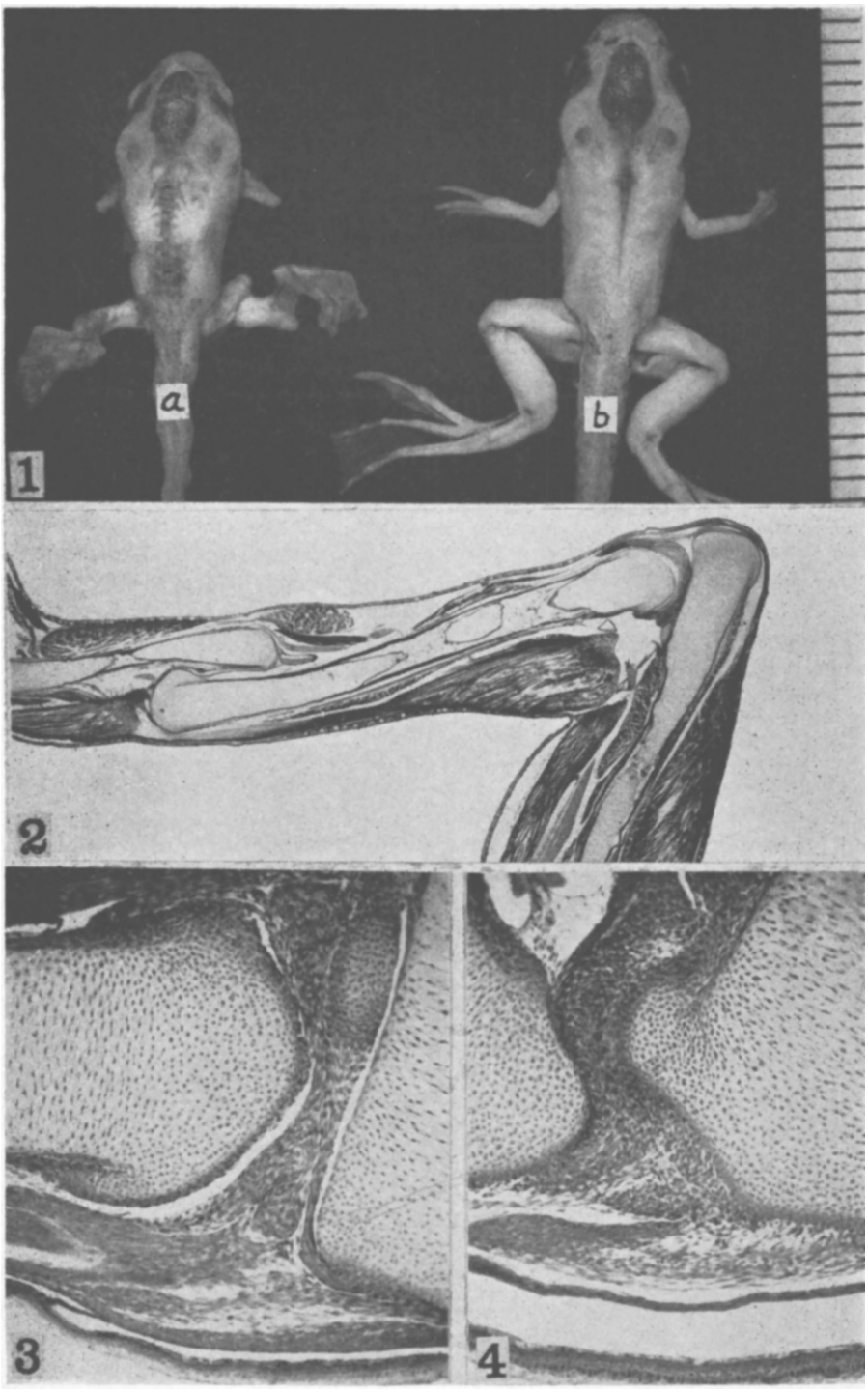

FIG. 1. a. Xenopus larva treated for 12 days with BAPN at a concentration of 2 μl/liter. Skin removed, showing dorsal musculature and widened intersegmental septa. b. Normal control of same age, skin removed.

FIG. 2. Longitudinal section through a hind leg of a Xenopus larva treated with BAPN for 10 days; concentration: 1 μl/liter; $\times$ 12.

FIG. 3. Section through the knee joint of a young tadpole, stage 29, fed with sweet pea seeds, showing the presence of clefts between the connective tissue and the epiphysis; $\times$ 120.

FIG. 4. Section through the knee joint of a control, stage 29; $\times$ 120.

TABLE II. Teratogenic Effects of BAPN on Chick Embryos.

Dosage, μl/egg	No. of eggs	No. died	Survived	Effect
20	13	13	0	Lethal
1	24	24	0	"
.2	20	13	7	Limb lesion
.1	10	6	4	" "
Control*	16	4	12	0

* Saline and penicillin.

than the controls, probably because food intake is impaired. The minimal dose producing noticeable effects is less than .5 μl/liter. It is noteworthy that within each dosage group all animals, without exception, were affected similarly.

It is of interest that treatments started after metamorphosis remain ineffective even if continued over periods of 2 months. Only slight deformity of the hind-legs developed in a few animals that had been treated over a period of 3 months with a high concentration of BAPN (4 μl/liter). However, no dislocation of joints ever occurred.

All compounds other than BAPN so far tested gave negative results. The high potency of aminoacetonitrile in producing skeletal lesions in rats as reported by Wawzonek *et al.*(5) could not be verified in tadpoles. Injections of α-tocopherol (total dose: 50 mg per tadpole) into the body cavity of tadpoles treated with BAPN did not protect the animals from developing the lesions. This result differs from that reported by Lee for the rats(8).

B. *Deformities produced in chick embryos.* In order to test the effect of BAPN on embryos of warmblooded animals, incubating hen's eggs were injected with BAPN. Since the embryonic development of the chick takes place within a closed egg shell, permitting no elimination, quantitative assays may be expected to give results of highest accuracy. The experiments are summarized in Table II. BAPN has a lethal effect at dosage levels of 1 μl and more per egg. The lesions produced in lower dosage groups are highly specific, affecting particularly the development of the legs (Fig. 5, 6). Size and weight of the animals are the same as those of the controls. Whether injected on the fourth or the tenth day, a bending of the femur end of the primary ossification center appears on the thirteenth day of incubation (Fig. 6). No dislocation of joints was observed. Cartilage and bones are histologically normal.

C. *Deformities in regenerating limbs.* Newts were used for this investigation because

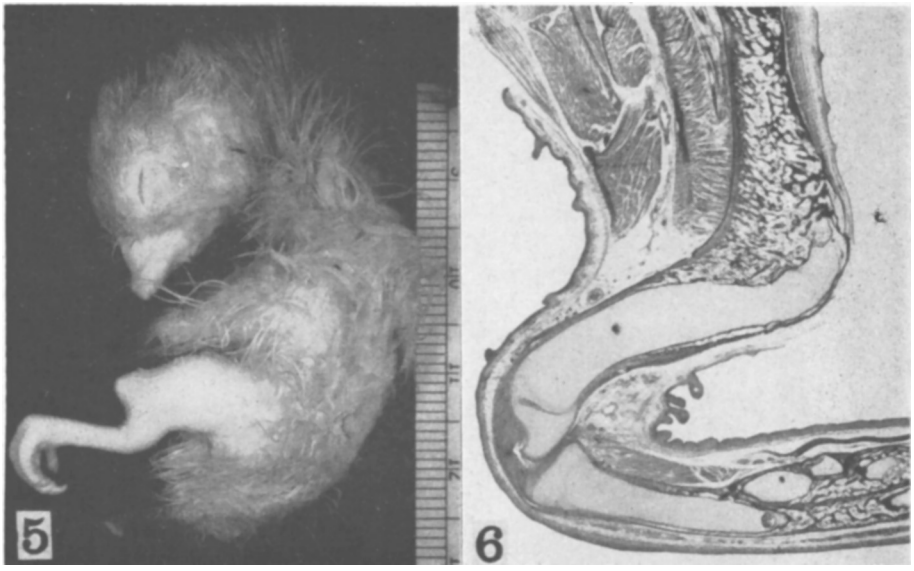

FIG. 5. Chick embryo injected with .2 μl BAPN; age 17 days of incubation.
FIG. 6. Longitudinal section through a leg of a 13-day chick embryo injected with .2 μl BAPN; × 15.

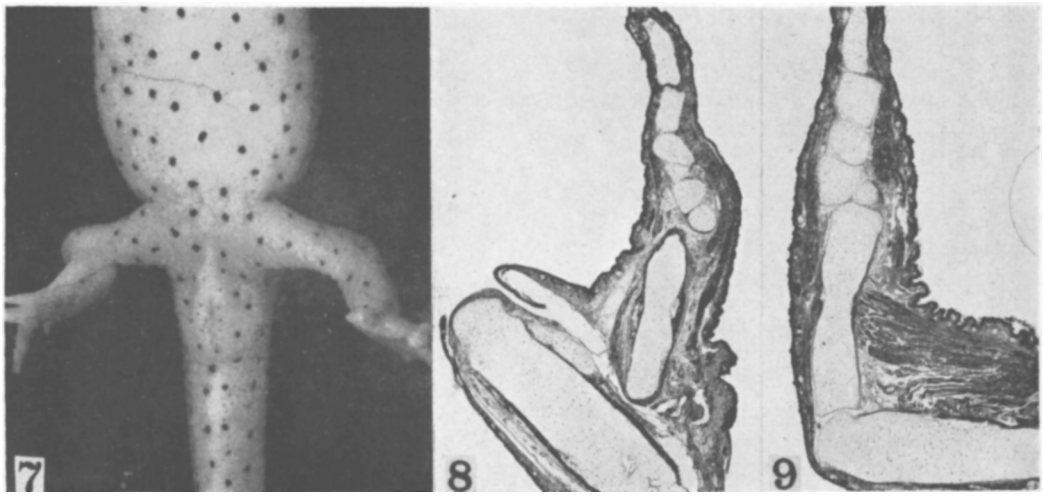

FIG. 7. Newt treated with BAPN during process of leg regeneration; concentration: 2 μl/liter. Left side intact limb, normal; right side, regenerated limb, showing dislocation of joint; × 2.

FIG. 8. Longitudinal section through regenerated limb in above figure. Note severe displacement at joint and atrophy of muscle; × 10.

FIG. 9. Longitudinal section through a normal regenerated limb; × 10.

of their well-known capacity of regeneration. After amputation of a hind limb it regenerates almost completely within about 3 months. The results of BAPN treatments are in principle as in the foregoing experimental series (Fig. 7). Microscopically the bones appear dislocated (Fig. 8, 9). Studies of serial sections also indicate atrophy of the leg muscles and loosening of the tendinous insertions.

Discussion. The results of the present series of studies indicate that BAPN is the teratogenic agent of Lathyrus seeds. In earlier experiments it was observed that 10 μl/liter of BAPN did not visibly affect cleavage and gastrulation of Xenopus eggs. However, the dorsal musculature and the skin of the hatched larvae were severely damaged. Further studies on the effect of myotome development are in progress. At the present it may be concluded that BAPN affects a definitely limited stage of the process of muscle and tendon differentiation. The action of this compound is tissue specific, affecting mainly the mesodermal tissues, probably the intercellular ground substance; but it is not species specific, producing similar lesions probably in all vertebrates. The severe teratogenic effect on embryonic and regenerating tissues suggests that the formation or normal activity of some essential morphogenic agent is inhibited by the presence of this compound.

Attempts at comparing the value of amphibians and of birds as bioassay animals are made difficult by the difference in the mode of administration of the compound. The chick as well as the amphibians are highly responsive and might be used for assay purposes in clinical work.

Summary. The evidence presented indicates that β-aminopropionitrile is the teratogenic agent of the seeds of *Lathyrus odoratus*. It produces dislocation of joints and loosening of intersegmental septa in amphibians, and bending of the femurs in chick embryos. The minimal teratogenic concentration for Xenopus larvae is .5 μl/liter, the minimal dose for chick embryos is .1 μl per egg. The effects are highly tissue specific but not species specific. The available evidence suggests that a single morphogenic process is interfered with by the presence of this compound during differentiation.

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Histochemical Studies of Effects of Cortisone on Fetal and Newborn Rats.*† (21935)

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There is a growing body of experimental evidence demonstrating the effect of cortisone on enzyme systems(1-3). These investigations, for the most part biochemical in nature, have been carried out on a variety of cellular enzymes(4-6). Much emphasis has been placed on the effect of cortisone on alkaline phosphatase. A number of workers(7), utilizing histochemical methods, have observed an increase in the alkaline phosphatase content of the brush border of the kidney tubule after injection with cortisone. Moog(8-10), obtained a precocious increase of intestinal phosphatase upon injection of fetal or newborn mice with cortisone. She interprets these results as indicating that cortisone influences the basic metabolic processes which are responsible for phosphatase synthesis.

Ribonucleic acid (RNA) has also received much attention as a substance involved in cortisone action. Decreases in hepatic RNA after cortisone injection have been observed by several investigators(11), although there is some conflict on this point(12,13). One group(7) noted an increase in renal tubule RNA after cortisone injection.

In view of the widespread interest in the mode of action of cortisone, it was decided to extend and correlate the above-cited findings. Because of the importance of the functional relationships existing between alkaline phosphatase, RNA and polysaccharide-protein complexes, histochemical methods were employed to follow the changes induced by cortisone on these materials.

Materials and Methods. Two series of animals were employed. The first series consisted of 24 female Wistar rats. Each female was placed with a male and daily vaginal smears were made until fertilization occurred (as evidenced by sperm in the smear). Beginning with the seventh day of pregnancy, the mothers were divided into 3 equal groups. Group I animals received 75 mg of cortisone per kilo of body weight daily throughout pregnancy; Group II animals received 100 mg and Group III served as controls. The cortisone was divided into 2 daily doses, given 8 hours apart.

Each of the above groups was subdivided into 2 sub-groups of equal size. In one Caesarian sections were performed on the fourteenth day and in the other on the sixteenth day of gestation. The uterine horns were exposed under moderate nembutal anesthesia, a tally of the number of fetuses present was taken and one-half of the number was removed. The incisions were then closed and

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