were approximately the same as in the isolated rabbit heart.

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Received September 20, 1956. P.S.E.B.M., 1957, v94.

Effect of STH on Experimental Lathyrism.* (22878)

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The literature on lathyrism and our earlier observations on the influence of hormones upon this condition had been reviewed elsewhere(1,2). Therefore, it will suffice here, to summarize a few facts which are particularly pertinent to the subject of this communication. It has been possible to produce skeletal deformities and aortal aneurysms, similar to those obtained by feeding sweet-pea (*Lathyrus odoratus*) seeds, by the subcutaneous administration of aminoacetonitrile (AAN) to rats. In very young AAN-treated rats aortal aneurysms are common, but in animals weighing 100 g or more, the drug tends to produce widespread skeletal deformities without any manifest lesions in the aorta. In these older rats, treatment with cortisol can suppress the skeletal lesions without sensitizing for the production of aortal aneurysms. Conversely, treatment with desoxycorticosterone exerts no marked effect upon skeletal lathyrism but sensitizes the rat to the production of aortal aneurysms. Finally, combined treatment with desoxycorticosterone and cortisol can completely reverse the normal morphologic syndrome of experimental lathyrism in that this corticoid combination causes AAN to produce aortal aneurysms in the virtual absence of bone lesions.

* These experiments were supported by Grants from the Ministry of Health of the Province of Quebec, Canada, and the National Research Council of Canada.

The object of this communication is to describe the effect of somatotrophic hormone (STH) upon intoxication with AAN. Our earlier experiments had shown that in general there is an antagonistic interaction between STH and glucocorticoids in the regulation of tissue responses to various types of injury (3).

Materials and technics. Two experiments were performed to explore the effect of STH upon acute and chronic AAN overdosage respectively. In the first experiment 20 female Sprague-Dawley rats, having a mean initial body-weight of 55 g (range 48-62 g), were subdivided into two equal groups. All animals received 5 mg of AAN (Aminoacetonitrile hydrosulfate) and 1 mg of STH (Armour), both compounds being given in 0.2 ml of water, twice daily, subcutaneously. Throughout the period of observation the rats were maintained exclusively on "Purina Fox Chow" and tap water. Towards the end of the first week all the STH-treated animals were obviously sick and developed the wobbling walk characteristic of experimental lathyrism, while the controls showed only a very slight impediment of locomotion. By the tenth day 5 of the STH-treated animals were manifestly moribund; these were killed, together with one rat of Group I, that was to serve as a control. At the same time AAN injections were stopped, while STH treatment continued at the same dose level for an ad-

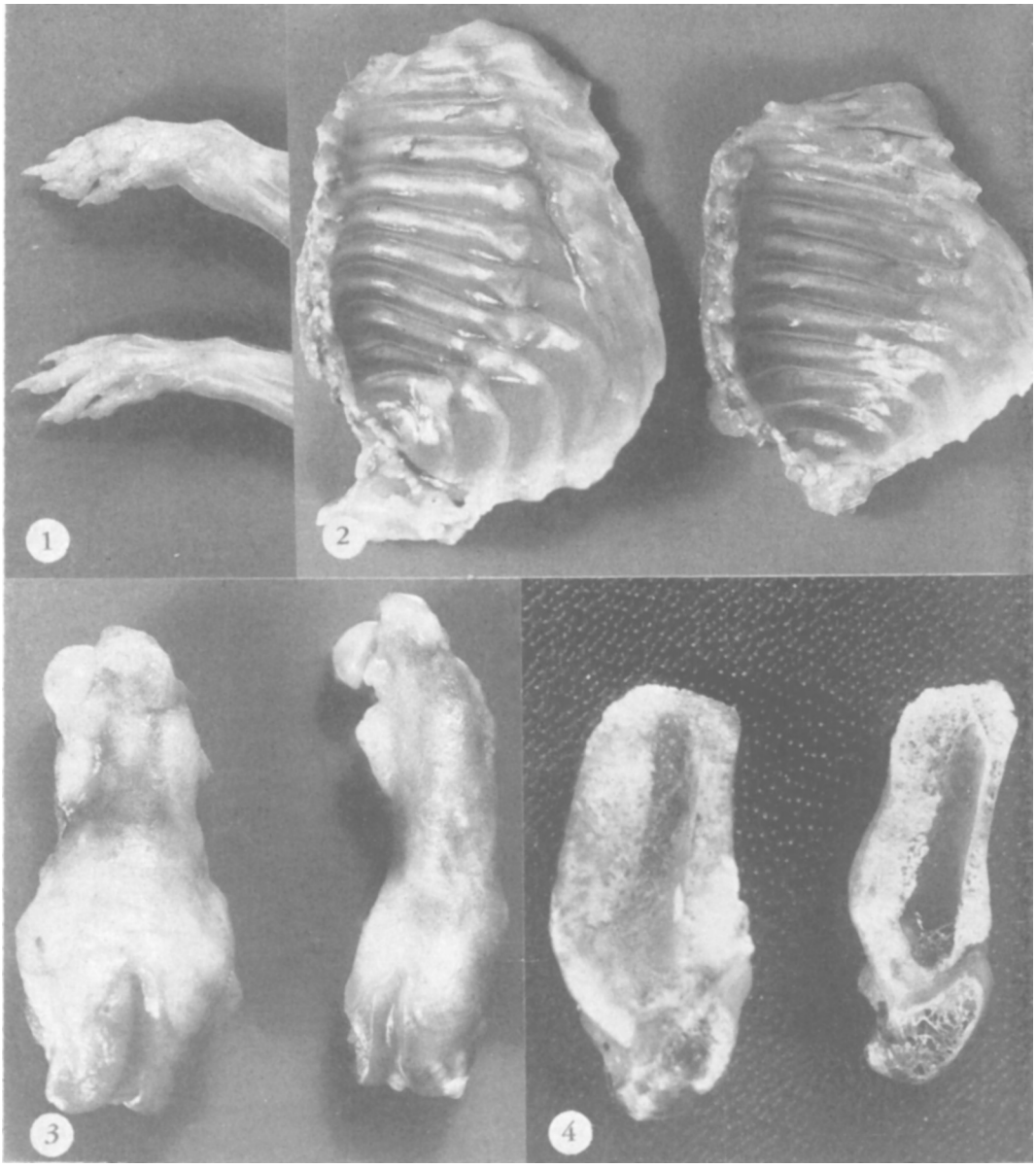

FIG. 1-4. Effect of STH upon skeletal changes characteristic of acute lathyrism (1st exp.).

FIG. 1. Excessive bone and cartilage proliferation in small wrist joints of rat treated with STH and AAN (top) in comparison with virtually normal appearance of limb in control animal treated with AAN alone.

FIG. 2. Considerable swelling of costochondral junction, with periosteal bone proliferation along entire length of ribs, in rat treated with AAN + STH (left), compared with much milder changes in control rat treated with AAN alone.

FIG. 3. Monstrous deformation of femur with excessive periosteal bone proliferation, numerous large exostoses at muscle insertion sites, and intense proliferation of cartilage in distal epiphyseal plate, after treatment with STH + AAN compared with milder but still marked deformations in femur in control rat that received AAN only.

FIG. 4. Cross-section through femurs shown in Fig. 3. Here intense proliferation of bone, complete disruption of normal epiphyseal plate structure and marked overgrowth of cartilage are even more evident.

ditional 10 days; the remaining rats of both groups were then killed.

The second experiment—designed to explore the effects of STH upon a more chronic AAN intoxication—was performed on 30 female Sprague-Dawley rats weighing 93-105 g (average 101 g). The animals were subdivided into 3 equal groups; Group I acted as untreated controls. Group II received only AAN and Group III, AAN + STH. STH (Armour) was administered, as in the previous experiment, at the dose of 1 mg in 0.2 ml of water subcutaneously twice daily throughout the experiment. AAN was given, first at the comparatively low dose level of 3 mg once a day and then raised, on the fourteenth day, to 5 mg twice a day. At both dose levels the drug was dissolved in 0.2 ml of water and injected subcutaneously. By the thirty-first day 2 of the animals in the group receiving AAN + STH had died, and showed extraordinarily marked skeletal deformities, while the others were obviously in poor condition. Therefore, AAN treatment was stopped for a period of 5 days to permit recovery of the surviving animals, but STH administration was not discontinued. After this interval AAN treatment was resumed at a dose of 5 mg twice a day and continued until the termination of the experiment on the fortieth day.

Results. In the *first experiment* the rats killed on the tenth day showed pronounced signs of acute AAN overdosage. There were marked bone deformities and hemorrhages at tendon insertion sites and along the ribs in every rat, but the changes were much more pronounced in animals receiving STH + AAN than in the controls treated with AAN alone. The blood coagulation time was not determined but it was obvious at autopsy that clot formation was greatly delayed.

Immediately after discontinuation of the AAN injections, the surviving animals showed obvious signs of recovery, but on the tenth day, when these rats were killed, the skeletal deformities were still very pronounced though much less so in Group I, which received AAN alone, than in Group II which was simultaneously treated with STH. Proliferation of bone tissue at muscle insertion sites, and excessive growth of both bone and cartilage at epiphyseal junctions, were obvious in the wrists (Fig. 1), the costochondral junctions of ribs (Fig. 2), the femur (Fig. 3) and in virtually all other parts of the skeleton. The histologic structure of these lesions was quite typical of AAN intoxication, as characterized in our earlier publications(1,2), and hence need not detain us here. It is noteworthy, however, that under these conditions STH did not stimulate anabolism; by the end of the experiment the mean bodyweight gain of the STH-treated animals was 20 g, that of the controls 22 g. Further, the dissected femurs were actually shorter in the former than in the latter group (Fig. 8).

Although STH greatly aggravated the morphologic changes and increased the mortality due to this acute overdosage with excessive amounts of AAN, it was thought advisable to repeat the experiment on larger, and hence more resistant, rats, starting with smaller doses of AAN, so as to bring out the effect of STH more clearly. This was accomplished in the *second experiment*. Here, AAN alone produced barely detectable skeletal lesions (without hemorrhages or mortality) while in combination with STH it caused considerable systemic damage and two deaths; the controls were still in excellent condition on the fortieth day when the experiment was terminated. The skeletal changes in this experiment are illustrated in our photographs (Fig. 5-8) which are self-explanatory.

Discussion. It is well known that disproportionate growth of certain parts of the skeleton and localized bony overgrowths are characteristic features of clinical acromegaly. Similar changes have also been produced in rats by partially purified STH preparations (4). It had been demonstrated furthermore, that STH sensitizes the rat for the development of "topical irritation arthritis"(5), and that arthritic changes in the knee and ankle joints can be produced in gonadectomized-adrenalectomized rats, by prolonged treatment with such hypophyseal extracts(6). The observations described in this paper

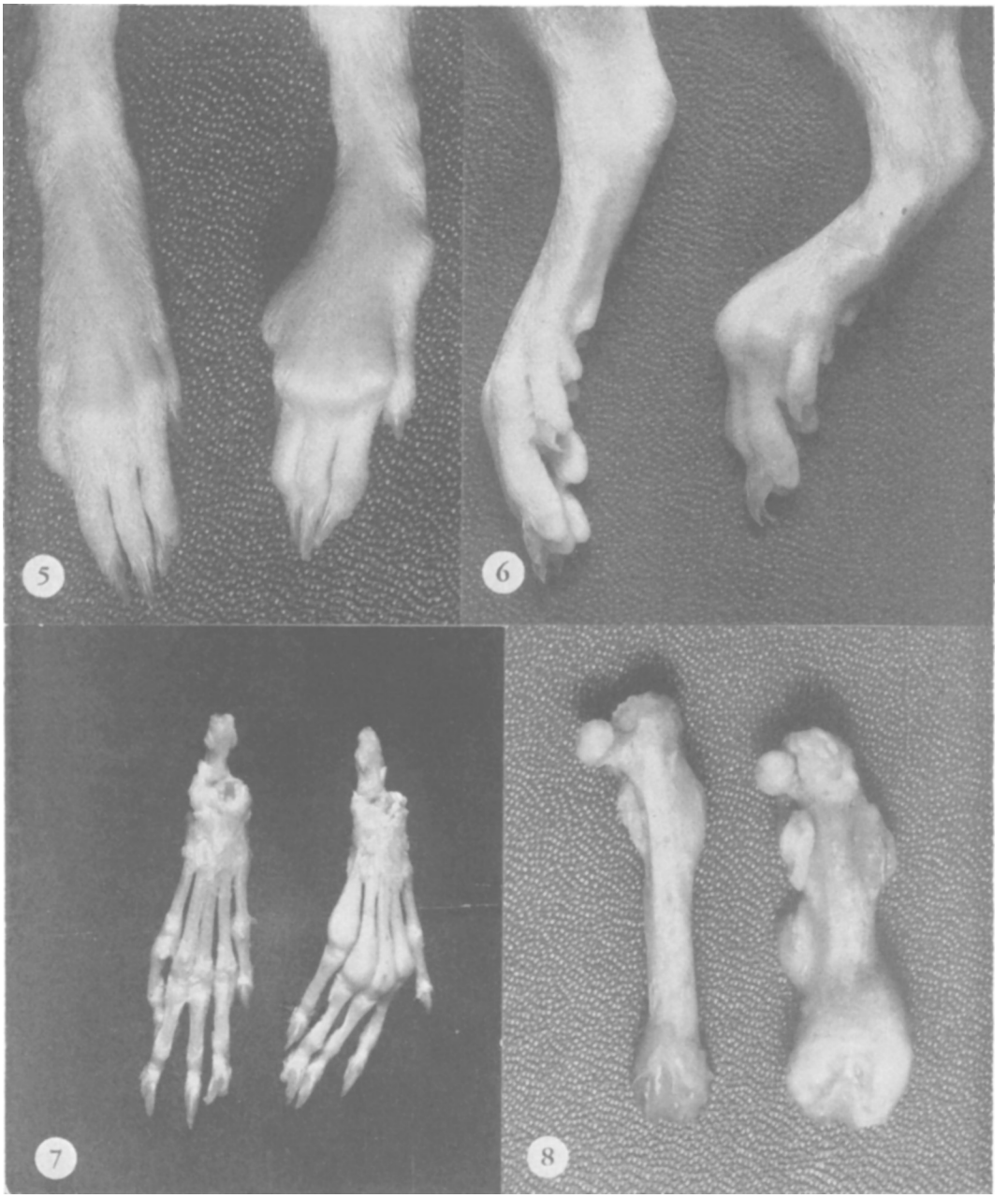

FIG. 5-8. Effect of STH upon skeletal deformities produced by chronic mild overdosage with AAN (2nd exp.).

FIG. 5. Hind paw of rat treated with AAN alone exhibits virtually normal appearance (left) while that of animal treated simultaneously with STH and AAN is greatly deformed especially in distal parts of tarsal bones.

FIG. 6. Lateral view of paws shown in Fig. 5.

FIG. 7. Skeletal parts of paws shown in Fig. 5. Here the virtual integrity of bone of rat treated with AAN alone, and characteristic clubbing of distal ends of metatarsal bones is even more evident. Metatarsophalangeal joints are deformed and digits deviated towards the outside, as result of this abnormal growth.

FIG. 8. Anterior view of left femur of rat treated with AAN alone (left) showing essentially normal bone structure, compared with corresponding femur of animal that received AAN + STH and developed marked bone deformities. Here, growth in length is actually stunted by growth hormone.

showed that the production of skeletal lathyrism by AAN can be greatly augmented by simultaneous treatment with STH. Since neither the cartilaginous nor the bony proliferations induced by AAN are inflammatory in the usual sense of the word, this observation confirms our view that *corticoids can influence tissue reactivity to various agents, and not only to those which elicit typical inflammatory responses.* It is especially noteworthy that in the first experiment in which AAN intoxication was particularly acute and severe, concurrent treatment with STH not only failed to stimulate growth but actually inhibited it; despite this the hormone was most effective in augmenting the abnormal skeletal proliferation characteristic of lathyrism. In evaluating the possible hormonal participation in non-endocrine diseases (those not primarily due to hyper- or hypo-function of an endocrine gland) we must keep in mind that, under certain conditions, a hormone can influence a pathologic process without manifesting its classical endocrine effects.

In our opinion these observations—as well as our earlier experiments on the effect of cortisol upon AAN intoxication(1)—furnish additional support for the concept that the so-called "adaptive hormones" can participate in the development of the most diverse non-endocrine diseases. Special emphasis is laid upon the fact that this is true even of non-inflammatory tissue reactions to an exogenous irritant.

Summary. Experiments in rats indicate that somatotrophic hormone (STH) can greatly aggravate skeletal manifestations of experimental lathyrism that are produced with aminoacetonitrile (AAN). This is so even under conditions whereby STH fails to stimulate growth in length.

The authors wish to thank Dr. I. V. Ponseti of Iowa State University for the supply of Aminoacetonitrile hydrosulfate.

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Received October 10, 1956. P.S.E.B.M., 1957, v94.

Determination of L- and D-Glutamic Acids by Combined Microbiological Method and Racemization Procedure.* (22879)

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The microbiological assay of L-glutamic acid with *Streptococcus faecalis* R and of total (L- plus D-) glutamic acids with any one of several other organisms‡ has been reported previously as a provisional means of determining both L- and D-glutamic acids in protein hydrolysates(1). It has been found subsequently that utilization of D-glutamic acid by these assay organisms‡ (unpublished data)

* Paper 116. This work was aided by grants from Eli Lilly and Co., Nutrition Foundation, Inc., Swift and Co., U. S. Public Health Service, National Multiple Sclerosis Soc., and Univ. of California. The authors are indebted to Mrs. Evelyn Brown for technical assistance.

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‡ *Leuconostoc dextranicum* (8086), *Leuconostoc mesenteroides* (8293), *Leuconostoc citrovorum* (797), and *Leuconostoc citrovorum* (7013). Numbers in parentheses refer to American Type Culture Collection catalog.