Selye, H.: Acta anat. *33*: 146–156 (1958)

From the Institut de Médecine et de Chirurgie expérimentales,
Université de Montréal, Montreal, Canada.

INFLUENCE OF VARIOUS HORMONES AND VITAMIN-D PREPARATIONS UPON ESTABLISHED BONE LATHYRISM

By HANS SELYE

It is a well established fact that, both in animals and in man, the ingestion of large amounts of *lathyrus odoratus* seeds produces a set of peculiar morbid changes which are known as lathyrism. Certain species of lathyrus produce predominantly nervous, others, skeletal lesions, and there is some reason to suspect that humoral and genetic factors may also determine whether osseous or neurologic signs predominate.

In rats fed *lathyrus odoratus* seeds, the disease is characterized by highly selective skeletal lesions which lead to an intense reorganization of bone structure with the formation of multiple exostoses, especially at muscle and tendon insertions. Recently, it has been shown that certain nitriles contained in the lathyrus seeds are responsible for this disease and that the skeletal lesions can be reproduced by the administration of aminoacetonitrile (AAN) while other nitriles appear to act more selectively upon the nervous system.

Bone lathyrism has been of special interest to us, because it proved to be a singularly suitable test object for the demonstration of the participation of hormones in the development of a non-endocrine disease: the skeletal changes normally produced by AAN were found to be readily prevented by cortisol, ACTH, estradiol or thyroxin (*Selye* [1957a], *Selye* and *Bois* [1956a, 1956b, 1957a]) and aggravated by STH (*Selye* and *Bois* [1957b]). The importance of humoral factors in the pathogenesis of these lesions has been further substantiated by the demonstration that thyroparathyroidectomy and partial hepatectomy (*Selye* [1957a]) greatly sensitize, while hypophysectomy (*Selye* and *Ventura* [1957]) protect the skeleton against aminoacetonitrile. Thus, bone lathyrism clearly exemplified a morbid condition which, though not due to a primary lesion of an endocrine gland, is nevertheless "conditioned" by hormones to such an extent that the latter may become the decisive factors that determine whether, under a given condition of AAN intoxication, morbid lesions will, or will not, develop.

It is the object of this communication to report on experiments designed to

determine how hormones can influence the healing of an already fully established bone lathyrism.

Materials and Techniques

Ninety female Sprague-Dawley rats, weighing 93–111 g at the beginning of the experiment, were subdivided into nine groups, each having a mean initial body weight of 100 g. All animals were treated with AAN, in the form of aminoacetonitrile hydrosulphate. The drug was administered subcutaneously, 10 mg in 0.2 ml of water, twice daily for 10 days. Then, the administration of AAN was discontinued, and during the following 12 days, the rats were treated as follows:

Group I: *Controls* without further treatment.

Group II: *Cortisol acetate* (*COL-Ac*), 1 mg of microcrystals in 0.2 ml of water once daily subcutaneously.

Group III: *Desoxycorticosterone acetate* (*DOC-AC*), 1 mg of microcrystals in 0.2 ml of water once daily subcutaneously.

Group IV: *Somatotrophic hormone* (*STH*), 2 mg in 0.2 ml of water twice daily subcutaneously.

Group V: *Sodium thyroxinate*, 500 μg in 0.2 ml of water once daily subcutaneously.

Group VI: *Estradiol*, 500 mg of microcrystals daily, in 0.2 ml of water subcutaneously.

Group VII: *Methyltestosterone*, 2 mg of microcrystals in 0.2 ml of water daily, subcutaneously.

Group VIII: *Ergocalciferol* (*Vitamin-D_2*) at the daily dose of 250 μg in 0.2 ml of sesame oil, subcutaneously.

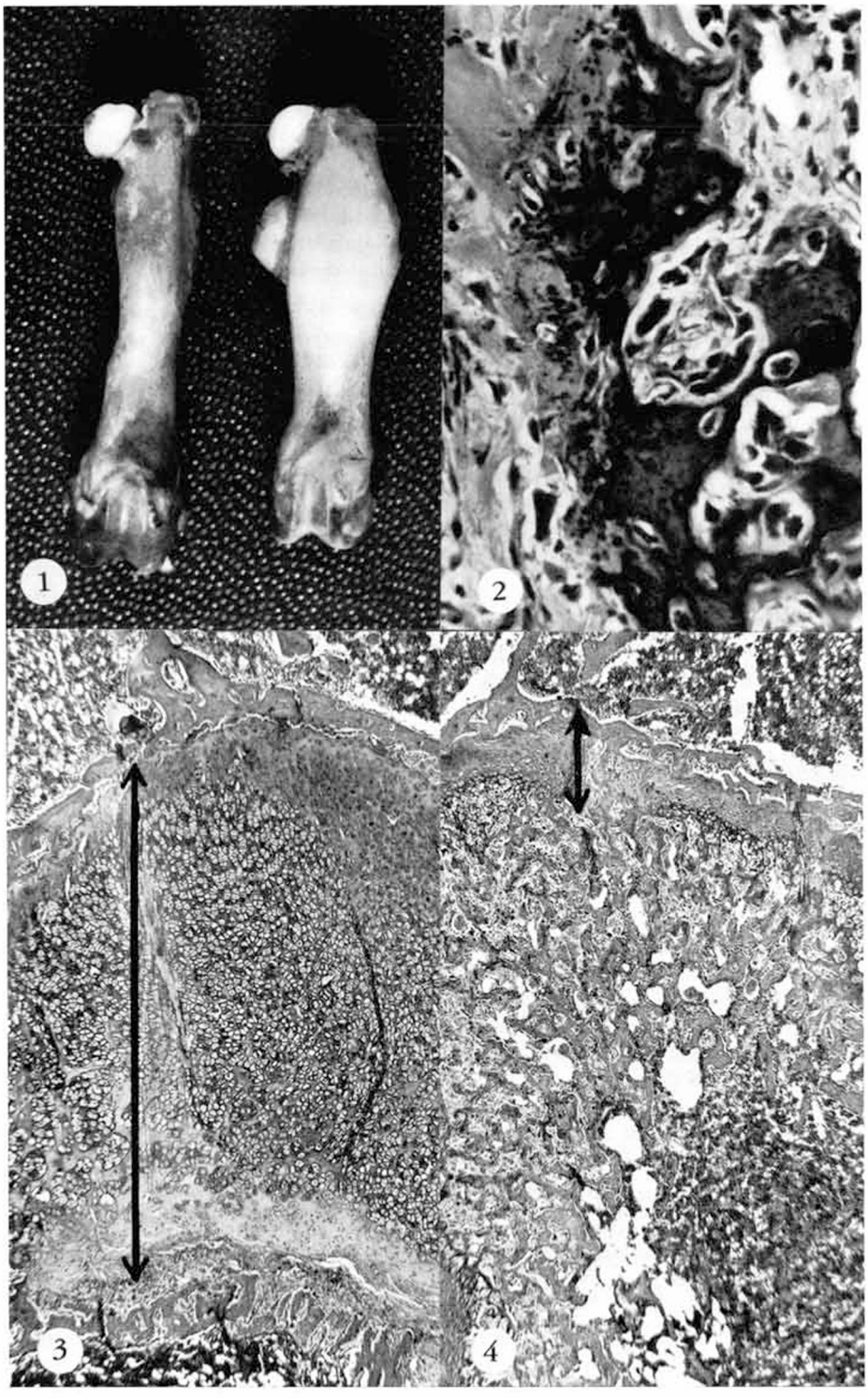
Group IX: *Dihydrotachysterol* (*AT-10*), 250 μg in 0.2 ml of sesame oil, once daily per os through a rubber catheter, because this substance is especially active when given orally.

Throughout the experiment, all animals received "Purina Fox Chow" as their sole source of nourishment.

On the 22nd day of the experiment—that is after 10 days of AAN treatment, followed by 12 days of recovery during which time the rats received the various hormone or vitamin preparations just mentioned—all the animals were killed with chloroform. Then, their skeleton were carefully inspected, and specimens of the distal two-thirds of the femur, ribs (and, in some instances, other bones) were fixed and simultaneously decalcified in Susa solution for subsequent histologic study. After complete decalcification, the bones were embedded in paraffin, and sections 6 μ in thickness were stained with hematoxylin-eosin or with *Gomori*'s aldehyde fuchsin technique.

Results

After one week of AAN treatment, signs of bone lathyrism became evident by mere clinical examination of the rats. There were palpable exostoses, particularly at the mental tubercles on each side of the mandibular symphysis and on muscle insertion sites on the long-bones, especially the femur. The growth-cartilage-



containing regions of the metaphyses were greatly swollen and deformed, a fact which could be easily ascertained by palpation, especially in the femoral and tibial metaphyses near the knee-joint. During the 12 days after cessation of AAN administration, changes induced by the various treatments did not result in marked clinical improvement, except perhaps in the rats treated with thyroxin in which the enlargement of the epiphyses tended to regress more rapidly than in any of the other groups. At autopsy, the macroscopically visible skeletal changes were not particularly striking, although the intensity of bone lathyrism was manifestly less severe in the rats treated with COL-Ac, estradiol and thyroxin, than in the other groups. The tubular bones of the animals treated with AT-10 became spindle-shaped, owing to a particular thickening of the mid-shaft region. This was especially evident in the femur (fig. 1). Another striking characteristic of the bones in the AT-10-treated rats was their chalky white color. As we shall see later, both the thickening of the shaft and the whitish discoloration were due to an extraordinarily intense apposition of periosteal new-bone.

Before discussing the histologic observations made in the present series on the regression of bone lathyrism, a few words should be said about the changes seen under similar circumstances

---

Fig. 1. Gross appearance of the femur of a control animal (left) and one treated with AT-10. In the control, the shaft is still considerably deformed, owing to irregular periosteal bone apposition but, in the rat which received AT-10, the deformity is much more pronounced. Note the chalky white fusiform thickening of the shaft and a considerable increase in the size of the muscle insertion site just beneath the head of the femur.

Fig. 2. Bone spicule from the metaphysis of the AT-10 treated rat shown in fig. 1. The dark lathyritic spicule in the middle of the field is replete with "basophilic bone globules", while the newly apposited bone along the left edge of the picture is entirely free of them. On the right side, connective tissue and osteoclast invade the basophilic old spicule (× 320, hematoxylin-eosin).

Fig. 3. Greatly enlarged and irregular growth cartilage (arrow) of a control rat. Note the transverse, almost solid, bone plate which "seals off" the growth cartilage from the bone marrow below (× 30, hematoxylin-eosin).

Fig. 4. Corresponding region of a thyroxin-treated rat. Here the cartilage plate become narrow (arrow), because endochondral ossification progresses actively in it (× 30, hematoxylin-eosin).

prior to the discontinuation of AAN treatment (*Selye* [1957a]). Among these, the following are the most outstanding: the growth-cartilage plates become extremely wide, the chondrocyte columns are irregular and often, large areas within the cartilage plate become liquefied, so that epiphyseal slipping is common. Despite this, endochondral ossification at the diaphyseal surface of the growth cartilage continues for many weeks.

On the bone surface—especially at muscle insertion sites—periosteal proliferation leads to the formation of excessive connective tissue, in which calcium deposition and membranous ossification proceed at a greatly accelerated rate. Consequently, there develop thick layers of periosteal bone made up of trabeculae, between which there is much connective tissue and blood vessels, but no marrow. Both in the periosteal connective tissue and in the newly formed trabeculae, numerous "basophilic bone globules" appear (*Selye* and *Bois* [1957b]). These globules stain intensely with hematoxylin, as well as with aldehyde fuchsin. In addition to the basophilic, fuchsinophilic material, they contain calcium, as demonstrated by the *von Kossa*'s stain (fig. 2). Similar globules do not develop in pre-existent trabeculae or in solid bone until several weeks after AAN administration has begun, nor do they occur in bone formed after the discontinuation of AAN administration. Occasionally—especially in very acute and severe AAN intoxication—there are hemorrhages in and around the periosteum throughout the skeleton. Endosteal bone proliferation is much less intensely stimulated by AAN, but when it does occur, the newly formed spicules usually contain numerous basophilic globules.

It is also characteristic of the bone formed during AAN intoxication that the osteocytes are oriented at random and not regularly arranged with their longitudinal axes parallel to each other. The osteocyte-lacunae and the blood-vessel canals are greatly dilated, so that the bone assumes an osteoporotic aspect. Both the irregular arrangement of the osteocytes and the dilatation of the blood-vessel channels are particularly evident in the shafts of tubular bones because, in the rat, these consist almost exclusively of very regular circumferential lamellae which encircle the marrow cavity, while haversian canal systems are almost completely absent. As a result of this structure, both the irregularity of the osteocytes and the dilatation of blood-vessel canals induced by AAN are especially striking in this species.

The histologic changes observed in the present series (in which the bones were examined 12 days after discontinuation of the AAN treatment) must be viewed against this background, in order to appraise the significance of the reparative processes that occurred. It should also be kept in mind that, although we shall only describe the histology of the distal half of the femur, sections through other bones showed essentially similar alterations.

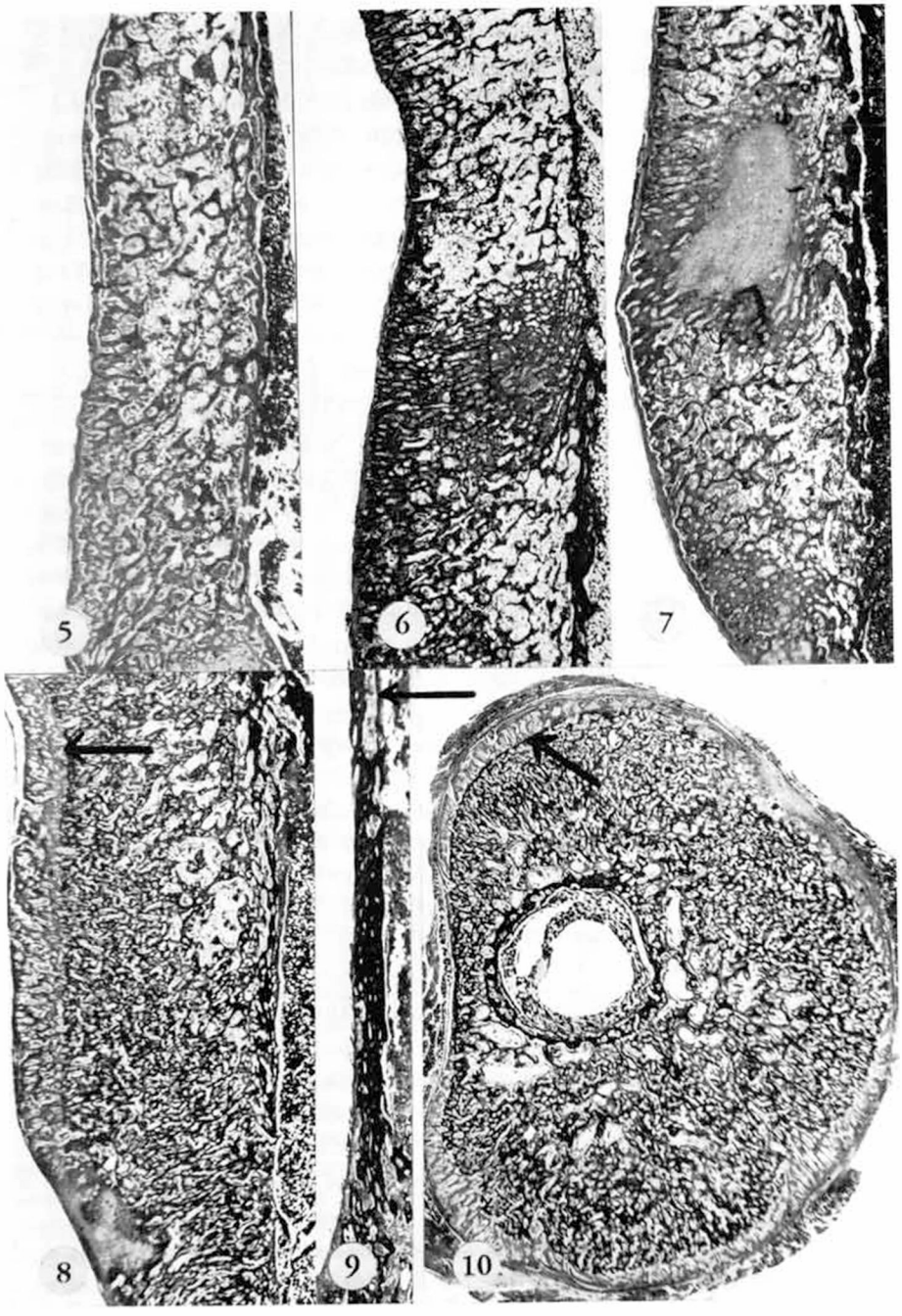
In the *normal controls* of the present series, which received no treatment after discontinuation of AAN, the growth-cartilage plates were still extremely wide and quite irregular at the time of autopsy. In most instances, the diaphyseal aspect of the growth cartilages was, at least partially, "sealed off" from the marrow cavity by a more or less solid transverse bone plate, so that endochondral new bone formation could not proceed normally.

Throughout the femur, the lathyritic bone spicules could easily be distinguished from newly formed ones, because the former were still replete with "basophilic bone globules", and their osteocytes were quite irregularly arranged, while the latter exhibited an essentially normal structure.

In rats treated with *COL-Ac*, the growth cartilages were somewhat less irregular and narrower than in the untreated controls, but the outstanding histologic characteristic in this group was the virtually complete inhibition of new bone formation. Consequently, the bone spicules of the metaphyses (as well as the trabecular bone which replaced the solid bone of the shaft during AAN treatment) remained essentially as they usually are while lathyrism is in full progress. However, at this stage, the connective tissue which fills out the spaces between the bone trabeculae during acute lathyrism has been replaced by partially fatty marrow. The bone trabeculae themselves were more or less bluish in hematoxylin-eosin sections owing to the abundance of "basophilic bone globules" in the ground substance. The osteocytes were quite irregularly arranged and abnormally numerous in proportion to the fully developed ground substance. Great dilatation of the vascular channels and of the osteocyte-lacunae throughout the bone was also quite characteristic in this group (fig. 6).

The animals treated with *DOC-Ac* did not differ significantly from the untreated controls.

STH tended to increase the thickness and irregularity of the growth-cartilage plates but, at the same time, it appeared to prevent



the "sealing off" of their diaphyseal surface; hence, endochondral ossification progressed more regularly than in the controls. In the STH-treated rats, there was also a more or less diffuse proliferation of bone, as judged by the comparatively intense deposition of regular new bone upon the old lathyritic spicules throughout the femur.

The most obvious curative effect was noted in the rats treated with *thyroxin*. In many of these, the growth-cartilage plates became essentially normal in width, and endochondral ossification appeared to progress actively, although the shape of the cartilage plates was still deformed at the end of the experiment. New bone formation on the surface of lathyritic spicules also progressed actively, and

---

Fig. 5. Longitudinal section through the compact shaft of a control rat. The dark staining tissue along the right edge of the field is bone marrow. The compact shaft tissue has been transformed into a broad layer of trabecular bone in whose broad interstices myelopoiesis progresses actively (× 30, hematoxylin-eosin).

Fig. 6. Corresponding region from the femur of a cortisol-treated rat. Since here healing has been delayed, most of the bone is replete with "basophilic bone globules" and hence assumes a dark color. The interstices are filled with fatty bone marrow. Near the center of the field, there is a nodular area of fibrosis surrounded by a network of comparatively dense trabeculae (× 10, hematoxylin-eosin).

Fig. 7. Corresponding region of the femur from an estradiol-treated rat. The picture is essentially the same as in fig. 6, except that the bone marrow is not fatty (× 10, hematoxylin-eosin).

Fig. 8. Corresponding region of the femur of a rat treated with AT-10. Here the trabecular pattern is much more dense and interstices are filled with connective tissue. To the left of the arrow, there is newly formed eosinophilic bone, which is clearly distinguishable from the dark lathyritic trabeculae towards the right (× 10, hematoxylin-eosin).

Fig. 9. Corresponding compact bone of the femur of a rat treated with AT-10, without previous AAN administration. The tip of the arrow is at the border between the shaft bone (right) and the marrow. Note the loosening of the compact bone which results in the formation of light grey cystic cavities within the shaft. At high magnification, these proved to be filled by connective tissue and osteoclasts (× 10, hematoxylin-eosin).

Fig. 10. Cross-section through the femur shown in fig. 8. Here the extreme thickening and dense trabecular pattern of the bone shaft is especially obvious. As in (fig. 8, the arrow indicates the border line between the dark lathyritic and the light newly formed bone (× 10, hematoxylin-eosin).

there was a general tendency for the "basophilic bone globules" to disappear even from old spicules (figs. 3 and 4).

Estradiol acted essentially like COL-Ac, but it produced an even more complete arrest of new bone formation than the latter hormone, so that the bone spicules (both in the metaphysis and in the now trabecular, shaft bone) retained all the characteristics of lathyrism (large number of "basophilic bone granules", comparative paucity of intercellular substance, irregular arrangement of osteocytes, widening of osteocyte-lacunae and blood-vessel canals). Here, as in the COL-Ac group, the junction cartilages were only slightly narrower than in the controls (fig. 7).

In the group treated with *methyltestosterone*, the changes were essentially the same as in the rats given STH.

Vitamin-D, caused no obvious change at the dose level at which it was administered here.

By far the most pronounced bone proliferation was noted in the rats given *AT-10*. Here, everywhere throughout the metaphysis and shaft, the old basophilic, lathyritic spicules were surrounded by eosinophilic new-bone. This resulted in a particularly compact trabecular pattern of spongy bone in which the interstices were filled out, almost exclusively, by connective tissue and vessels, with virtually no admixture of bone-marrow. In addition, underneath the periosteum and endosteum, there developed thick, circular layers consisting of trabeculae in which there were no "basophilic bone globules" This led to considerable thickening of the shaft, a deformity which had been quite obvious even upon macroscopic inspection. The deformed lathyritic growth cartilages did not appear to be significantly influenced by AT-10 treatment (figs. 2, 8 and 10).

In order to appraise the significance of the bone changes produced by AT-10 in rats previously treated with AAN, we then performed a control experiment in which 10 rats were given the same dose of AT-10, under exactly the same experimental circumstances but without previous treatment with AAN. Here, in agreement with expectations, the excess AT-10 produced a pronounced rarefication of the shaft with invasion of the vascular channels by much connective tissue and numerous osteoclasts, that is, the picture of osteitis fibrosa (fig. 9). Periosteal new-bone formation was not observed in the corresponding shaft region, and only traces of

membranous ossification were detectable in the metaphyses near the growth-cartilage plates. It is clear therefore, that the effect of AT-10 was greatly modified by the pre-treatment with AAN.

Acknowledgements

This work was supported by grants from the Gustavus and Louise Pfeiffer Research Foundation and the Abbott Laboratories.

The hormone and vitamin preparations used in these investigations were kindly supplied by the following firms: The Armour Laboratories: STH ("Somatotropin"), Abbott Laboratories: AAN (Aminoacetonitrile Hydrogen Sulphate), Winthrop Laboratories: AT-10 ("Hytakerol"), Pfizer Laboratories: cortisol acetate ("Cortril"), Schering Corporation Ltd., Montreal: desoxycorticosterone acetate ("Cortate") and estradiol, the British Drug Houses Limited: L-Thyroxine sodium salt, and CIBA Company Limited: methyltestosterone.

Summary

Severe bone lathyrism was produced in rats by treatment with high doses of aminoacetonitrile (AAN) during 10 days. Then, AAN injections were discontinued, and one group of rats remained without further treatment, while the others were given various hormone or vitamin preparations during an additional period of 12 days, to determine the effect of these substances upon a fully established bone lathyrism.

Among the substances tested, Thyroxin was the most effective in restoring the bone structure to normal. This hormone enhanced the absorption of excessively proliferating growth cartilage and the re-establishment of normal endochondral osteogenesis. At the same time, the characteristic "basophilic bone globules" of lathyrism tended to disappear throughout the skeleton.

Résumé

De fortes doses d'aminoacétonitrile (AAN) furent administrées pendant dix jours à des rats, de façon à produire un sévère lathyrisme osseux. Ce traitement fut alors arrêté et tous les groupes d'animaux, sauf un, furent traités par diverses hormones et vitamines, afin de déterminer l'effet de ces préparations sur le lathyrisme déjà établi.

Parmi les substances examinées, la thyroxine a le plus efficacement restauré la structure osseuse; elle a favorisé la résorption du cartilage de croissance, qui avait proliferé de manière excessive, et elle a rétabli l'ostéogénèse endochondrale normale. Sous l'effet de cette hormone, les «globules basophiles osseux» typiques du lathyrisme, tendent à disparaître du squelette.

Zusammenfassung

Bei Ratten wurde ein schwerer Knochenlathyrismus durch 10tägige Behandlung mit Aminoazetonitril (AAN) hervorgerufen. Dann wurden die AAN-Injek-

tionen unterbrochen. Eine Gruppe dieser Ratten wurde keiner weiteren Behandlung unterzogen und als Kontrolltiere verwendet, während die anderen Ratten noch 12 Tage lang mit verschiedenen Hormon- und Vitaminpräparaten behandelt wurden, um den Einfluß dieser Stoffe auf einen voll ausgebildeten Knochenlathyrismus zu bestimmen.

Von den untersuchten Substanzen hatte das Thyroxin bezüglich Wiederherstellung der Knochenstruktur die stärkste Wirkung. Dieses Hormon beschleunigte die Absorption des übermäßig proliferierenden Epiphysenknorpels und die Wiederherstellung der endochondralen Osteogenese. Gleichzeitig begannen die charakteristischen «basophilen Knochenkügelchen» des Lathyrismus überall aus dem Skelet zu verschwinden.

REFERENCES

Selye, H.: Über die humorale Beeinflussung des experimentellen Lathyrismus. Arch. exp. Path. Pharmak. *230:* 155 (1957a).

Selye, H. and *P. Bois:* Production of anaphylactoid inflammation and gastric ulcers with 5HT. Canad. physiol. Soc., Montreal, October 1956a. (Rev. canad. Biol. *15:* 238 (1956a).

— Effet des hormones adaptives sur le lathyrisme expérimental du rat. ACFAS, 24e Congr., Montreal, November 2–4, 1956b.

— Effect of corticoids upon experimental lathyrism. Endocrinology *60:* 507 (1957a).

— Effect of STH on experimental lathyrism. Proc. Soc. exp. Biol., N. Y. *94:* 133 (1957b).

Selye, H. and *Ventura, J.:* Effect of hypophysectomy and substitution therapy with STH upon experimental bone lathyrism. Amer. J. Path. *33:* 919 (1957).

Received February 9th, 1957