tion; the crystalline preparations consisted of two components as revealed by electrophoretic analysis. In this communication we wish to report the crystallization of growth hormone from our pure amorphous material by a technique similar to that of Fishman, *et al.*

Approximately 0.1% of the pure growth-hormone solution was adjusted to pH 10 with calcium hydroxide solution and brought to an alcohol concentration of 10% by a slow addition of 1:1 alcohol-water at 2° C. A small amount of the hormone was precipitated out and removed by centrifugation. The supernatant usually has a pH 8.5; if not, it was adjusted to this pH with 0.1 N HCl. Alcohol-water (1:1) was again added very slowly until the alcohol concentration was 15%. On standing at 2° C, crystals appeared as thin plates (Fig. 1). The crystals were highly soluble at room temperature and disappeared quickly during microscopic examination. To obtain a satisfactory crop of crystals, the protein concentration must be low, and the temperature should be below or at 2° C.

When crystalline preparations were assayed by the body growth or tibia test on hypophysectomized rats, there appeared no difference in their activity as compared with that of the starting material, indicating that further concentration or "fractionation" had not been achieved by crystallization. Electrophoretic analysis of the crystals gave results identical with those obtained with the amorphous pure preparation.

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Six-Segment Head Regenerates in an Earthworm, *Eisenia foetida* (Savigny) 1826[1]

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In heads regenerated by *E. foetida* after excision of 6 or more segments there are at most, according to Morgan (*4*), only 4 or 5 segments. A similar limitation of segment number in head regenerates (*1*) has been assumed to be characteristic of *E. foetida* and other earthworms of the same family. There are in the literature, however, at least two records of greater numbers of segments in head regenerates of this species. Morgan (*3*) included in tables two 6-segment head regenerates observed 4 and 6 months after operation. In one, the 12 anterior segments had been excised. The number of segments removed from the other was not counted at the time of operation but was estimated later to be 10(½). In 1898 Michel (*2*) reported one head regenerate of 6 segments after the removal of 8 and one of 7 after the removal of 7. Both were obtained in less than 4 weeks.

In the present study 30 specimens of *E. foetida* from Virginia were operated on in January and February. All were clitellate animals. The presence of male pores on segment xv and the clitellum in normal position were ascertained (*5*). Worms were anesthetized in dilute chloretone and the 10 anterior segments amputated with a razor blade exactly at intersegmental furrow 10/11. Animals were examined daily at first and then twice a week. Specimens were fixed after 4–8 weeks.

Twenty-one animals survived the operation, and each regenerated a head. The number of segments in the 10 normal head regenerates was as follows: 4 in 2 specimens; 5 in 5 specimens; and 6 in 3 specimens. The 11 remaining worms had head regenerates of 3–5 normal segments plus one or more partial segments.

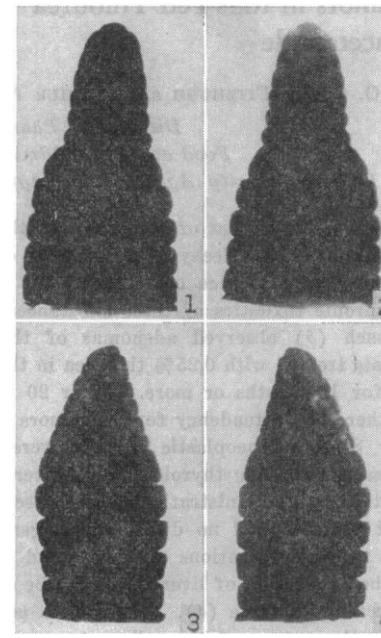

Fig. 1. (1 and 2) Dorsal and ventral views of the same 6-segment head regenerate. (3) Lateral surface view of another 6-segment head regenerate. An additional furrow shallower than the others can be seen in the proximal part of segment ii. (4) Same specimen as (3) but with the focus at the median plane. All specimens were in Cellosolve and photographed × 17.

Among the 10 normal head regenerates, three exceptions were found to the generally accepted statement concerning head regeneration in *E. foetida*. These are the first 6-segment head regenerates to be recorded for this species after amputation exactly at intersegmental furrow 10/11. Dorsal and ventral views of one of these are shown in Fig. 1 (1 and 2). Another specimen, cut through the median plane and photographed in Cellosolve, is shown in Fig. 1 (3 and 4). Segment ii (3) has

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an additional lightly marked furrow toward its proximal boundary.

The 11 head regenerates considered, for the present, to be abnormal fall into two classes: those which are asymmetrical, perhaps because of some environmental factor such as temperature and those which have additional furrows symmetrically demarcated ventrally, or ventrally and laterally. Those in the second group and the condition indicated in segment ii (3) could be interpreted as indicating the possibility that additional segments may be added to the head regenerate later.

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Liver Tumors in Rats Fed Thiourea or Thioacetamide

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The striking effectiveness of thiourea and thioacetamide in preventing orange decay (*1–3*) and the occurrence of the thiourea in the juice of treated fruit led us to study the chronic toxicities of these substances. Purves and Griesbach (*5*) observed adenomas of the thyroid glands in rats treated with 0.25% thiourea in their drinking water for 12 months or more. After 20 months of treatment there was a tendency for the tumors to become malignant. Since no neoplastic changes were observed in other tissues than the thyroid glands after this long period of thiourea administration, these observers concluded that thiourea had no direct carcinogenic action. In contrast to the observations of Purves and Griesbach, we found the production of liver tumors to be one of the chronic effects of thiourea (*4*). The present communication reports the nature of these liver tumors and their high incidence in thiourea-fed rats.

In a two-year chronic toxicity study albino rats, 21 days old, 18 to a group, were fed thiourea at levels of 1, 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01% in a diet of ground commercial rat biscuits. Control animals received the basic diet. All animals were permitted unrestricted access to both food and water.

At dosage levels of 0.25% or more thiourea, the outstanding gross lesion was enlargement of the thyroid gland. The thyroid enlargement was marked at the 1.0% level and decreased with decreasing dosage, but was distinct at 0.25% thiourea. The thyroid weights of the animals on dosage levels of 0.05% or lower were not significantly different from those of the controls. Thiourea at dosage levels of 0.25% or more stunted the growth of the rats. This effect was marked during the fast-growing period of the first three months on the experimental diet. When the rats on these higher dosage levels became adult, they were short, chubby, and very fat. They appeared listless and, when disturbed, made no effort to move around in their cages. All animals at the dosage levels of 0.25% or more thiourea died within the first 17 months of the experiment. Lower dosages had no effect on mortality.

The liver showed marked gross changes, especially in the surviving animals, at dosage levels of 0.10% and below. At levels of 1.0 and 0.5% the liver and, to a lesser extent, the viscera in general were in the majority of instances moderately pale. The pallor was not so pronounced at 0.25% and was essentially absent below this level. In no animal was there an hepatic cirrhosis or even a roughness of the liver surface, except as caused by the presence of tumors.

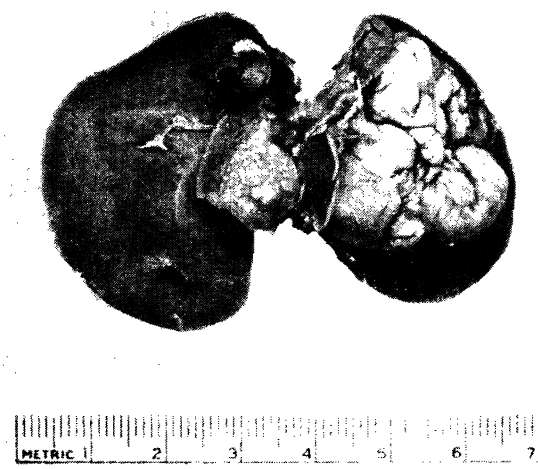

FIG. 1. Large liver tumor in rat fed 0.1% thiourea for two years.

Of the 29 experimental rats surviving the two-year feeding period, 14 showed liver tumors. There was a general correspondence between dosage level and tumor size and incidence. Only one of the nonsurviving treated rats, and none of the 18 controls, showed such a tumor. For comparison, the general run of our rats, whether controls or those fed a variety of added substances in their diets and surviving a two-year experimental period, show approximately a 1% incidence of spontaneous hepatic tumors of the type described below. In the livers of the animals with these spontaneous tumors, also, cirrhosis is absent. The significance, therefore, of an almost 50% tumor incidence in the surviving thiourea-fed animals becomes apparent.