The 6 mice in the last group (9-hour interval) were in very bad condition before treatment was started. After the second or third injection they appeared almost normal and after 24 hours they appeared to be free from any symptoms of pneumonia. All of the mice were observed for an additional 14 days without any treatment, then discarded. The antibiotic did not produce any observable toxic reaction in the mice.

Conclusions. Subtilin has been shown to exert a powerful *in vivo* action on the course of experimental pneumococcus Type III infections in mice. Animals treated with subtilin 9 hours after being injected with the organism were quickly cured of the infection. The antibiotic exhibited no apparent toxic reaction in the animals.

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Effect of Anterior Pituitary Growth Hormone on Urinary Nitrogen Loss Following Fracture.*

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Introduction. It was noted by Cuthbertson[1] that bone fracture in man produced a rise in urinary nitrogen excretion, which, at its height, was not affected by a high protein diet. It was shown that this was due to more than disuse atrophy, as splinting of the limbs in healthy individuals produced only a slight rise. Howard and co-workers[2] have confirmed Cuthbertson's work in man, and have demonstrated that there was a long period (average 35.6 days) during which patients were in actual negative nitrogen balance. In a subsequent publication[3] these same workers found no beneficial effect from increased protein in the diet, the added protein being excreted quantitatively in the urine.

In rats, Cuthbertson[4] noticed a similar rise following fracture. The maximum nitrogen excretion occurred on the third day, and the animals had returned to normal after 5 days. Cuthbertson also found that the amount of nitrogen excreted was not accounted for by the loss of weight in the limb, but rather that tissue breakdown seemed to be occurring generally throughout the body. Cuthbertson, Webster and Young[5] found that a crude alkaline extract of the anterior pituitary prevented the rise in nitrogen excretion. In view of these findings it seemed of interest to determine whether any effect could be obtained with growth hormone, inasmuch as it would be expected to cause nitrogen retention.

Methods. Rats of the Long-Evans strain approximately 50 days old were used. In order to ensure a constant food intake during the whole experimental period, all animals were restricted to 10 g of stock diet per day. Twenty-four males were studied and were divided into 4 groups of 6 each. The first group was retained as a normal control, the second unoperated but given growth hormone, the third operated, and the fourth operated and given growth hormone. The growth hormone was a homogeneous preparation and was isolated by the procedure previously de-

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1 Cuthbertson, D. P., *Lancet*, 1942, **1**, 433.

2 Howard, J. E., Parson, W., Stein, K. E., Eisenberg, H., and Reidt, V., *Bull. Johns Hopkins Hosp.*, 1944, **75**, 156.

3 Howard, J. E., Winternitz, J., Parson, W., Bigham, R. S., Jr., and Eisenberg, H., *Bull. Johns Hopkins Hosp.*, 1944, **75**, 209.

4 Cuthbertson, D. P., McGirr, J. L., and Robertson, J. S. M., *Quart. J. Exp. Physiol.*, 1939, **29**, 13.

5 Cuthbertson, D. P., Webster, T. A., and Young, F. G., *J. Endocrin.*, 1941, **2**, 468.

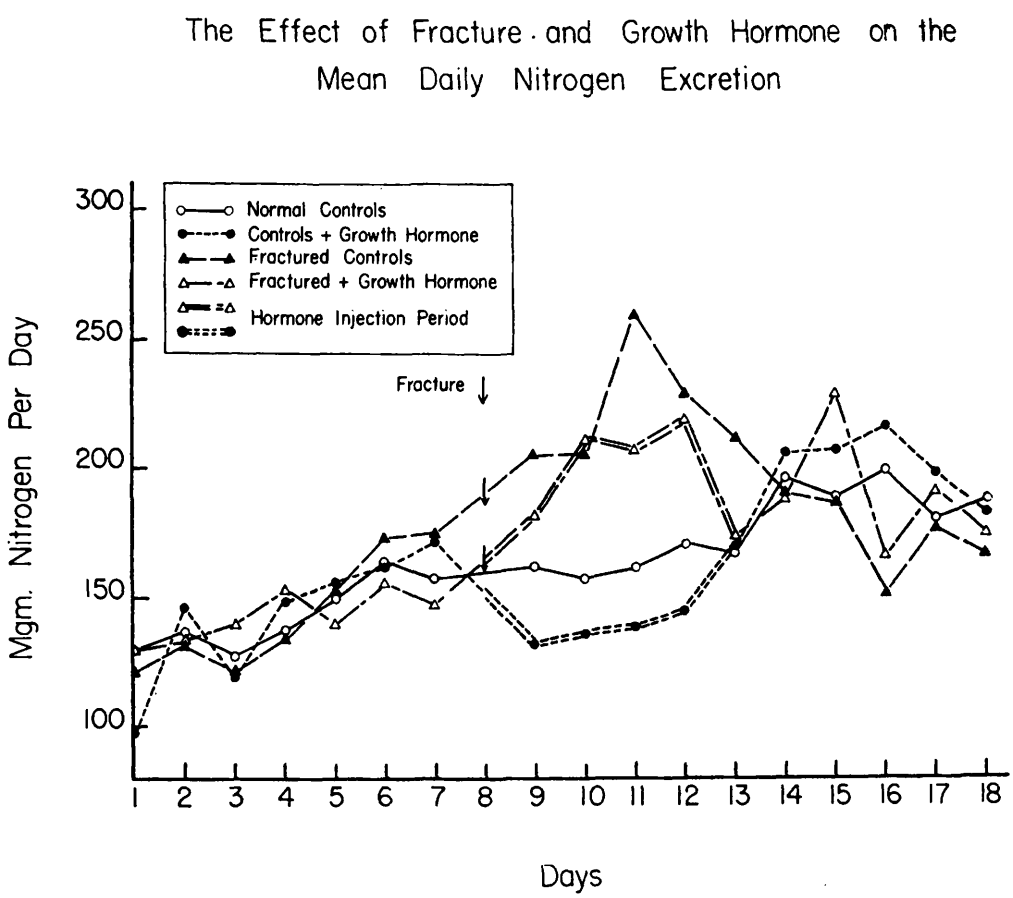

Fig. 1.

scribed.[6]

Following a preliminary period of adaptation to diet and cages, the nitrogen excretion was followed daily for a 7-day control period. At this time, both femurs were fractured under ether anesthesia. An incision was made on the lateral aspect of the thigh; the muscles were freed from the bone; and a comminuted fracture of approximately ½ cm in length was produced in the middle third of each femur. The incision was then sutured. There was little bleeding, and, although rigid asepsis was not observed, there was no evidence of infection. Daily intraperitoneal injections of 1 mg of growth hormone were begun on the day of fracture and were continued for 5 days. The daily nitrogen excretion was followed for 5 more days after growth hormone injections were stopped. Nitrogen was determined by the micro-Kjeldahl method.

Results. In Fig. 1 are shown the curves of mean daily nitrogen excretion for the 4 groups. The normal control group showed a slight but steady rise in nitrogen excretion over the entire period. In the control period before fracture, the lines representing the nitrogen excretion of the 4 groups intermingled, but at the 8th day they separated characteristically. That of the unoperated group given growth hormone dropped abruptly after the first injection and remained low during the injection period, but rose above that of the normal controls after growth hormone was stopped. In the fractured group, the excretion rose on the first day after fracture, reached a maximum on the third, and fell off in the following few days. In the last

[6] Li, Choh Hao, Evans, H. M., and Simpson, M. E., *J. Biol. Chem.*, 1945, **159**, 353.

group, fractured and growth hormone treated, the rise occurred but was less than that in the fractured untreated group.

The mean level of nitrogen excretion for each of the 4 groups during the control period was essentially the same, running from 142 mg to 144 mg of nitrogen per day with a standard deviation of from ± 3.5 to ± 5.1. In the 5-day postoperative period the average daily excretion of the control group rose to 165 ± 4.8† while that of the control group given hormone rose only to 145 ± 4.3. This represents a daily retention of 20 mg of nitrogen for the treated group as compared with the controls for the same period. The average daily excretion of the fractured group rose from 142 ± 5.1 to 223 ± 9.0, while that of the group fractured and given growth hormone rose only from 143 ± 3.5 to 199 ± 8.0, representing a retention over the untreated fractured group of 24 mg of nitrogen per day. The differences between the groups noted in this first 5-day postoperative period were all significant having p values of .05 and less.[7]

In the 5-day period after growth hormone was stopped, the nitrogen excretion of the control group averaged 30 mg per day higher than in the previous period, while that of the growth hormone treated group rose 58 mg. Also, the nitrogen excretion of the group fractured and given growth hormone was higher on the average than that of the fractured untreated group.

Discussion. In these experiments the retention of nitrogen following growth hormone was approximately the same in the fractured and unfractured rats when they were compared with their respective untreated controls. It would appear that the effect of growth hormone was to lower the base line of nitrogen excretion upon which the stress of fracture was superimposed. Cuthbertson, Webster and Young[5] reported that a crude extract of the anterior pituitary completely prevented the rise of nitrogen excretion after fracture, although it did not promote nitrogen storage beyond that occurring in the uninjected control period. There are several important differences between our experiments and theirs which could explain the different results obtained. In the present experiment 2 legs were fractured, creating a greater tendency to breakdown of protein than in the experiment of Cuthbertson, Webster and Young, where only one leg was broken. In addition younger animals were used in our experiments, which may have reduced the nitrogen retaining effect of the growth hormone.

Summary. The daily urinary nitrogen excretion was followed in growth hormone treated rats with bilateral femur fractures. The normal and fractured rats given growth hormone showed approximately the same decrease in nitrogen excretion when compared with their respective controls.

† Standard deviation of the mean.

[7] Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1936.

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Measured Dose of Gamma Hexachlorocyclohexane (γ 666) Required to Kill Flies and Cockroaches, and a Comparison with DDT.

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Hexachlorocyclohexane† or "666" has been said to be more toxic than DDT‡ for certain insects[2,5,7,8,9,11] and mammals.[3] As was also true of DDT until recently,[13] however, most of the information is in terms of percent kill after exposure for a given time to a given environmental concentration of the

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