appeared to have no significant effect on the urinary 17-ketosteroids.

The administration of 1 grain of amytal every 12 hours during the first 48 hours of the sleepless period resulted in a slight but noticeable depression in the excretion of these substances. This was most noticeable on the 2nd day. Upon stopping the amytal administration there was a gradual rise to the pre-amytal or control values.

Total urine volumes varied greatly between individuals as well as from day to day. The amount of ketosteroid excreted appeared to be practically independent of the total urine volume.

The observations that fatigue from sleeplessness did not influence the urinary excretion of 17-ketosteroids are in contradiction to the findings of Pincus and Hoagland,[2] and Pincus, Hadidian and Yeaton.[8] It must be pointed out, however, that in our experiments the output of an entire 24-hour period was measured, while the results of Pincus and Hoagland were based on samples collected during relatively short periods of stress. Furthermore, the type of stress was different and, therefore, the results are not strictly comparable.

Summary. 1. The urinary excretion of 17-ketosteroids of normal young men was not influenced by prolonged wakefulness (112 hours). 2. Amytal given during such an experiment slightly depressed the output of these substances.

8 Pincus, G., Hadidian, Z., and Yeaton, M., *Federation Proc.*, 1946, **5**, 81.

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Subtilin—Antibiotic Produced by *Bacillus subtilis*.* III. Effect on Type III Pneumococcus in Mice.†

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In a previous communication[1] subtilin was found to exert a pronounced antibiotic action against a number of types of the pneumococcus by the agar cup-plate procedure. This ac-

TABLE I.
Treatment of Pneumococcus Type III Pneumonia in Mice.

		3 mice used in each group		
Date	Time	Controls	Treated immediately	Treated after 1 hr
2/14/46	12:30 p.m.	Infected	Infected	Infected
"	12:30 "	—	0.5 cc subtilin	—
"	1:30 "	—	"	0.5 cc subtilin
"	5:30 "	—	"	"
"	9:30 "	—	"	"
2/15	8:00 a.m.	First dead	—	—
"	9:30 "	—	0.5 cc "	0.5 cc "
"	12:30 pm	Second "	"	"
"	4:00 "	Third "	—	—
"	5:30 "	—	0.5 cc "	0.5 cc "
2/16	9:30 a.m.		0.5 cc "	0.5 cc "
3/2	Results	All mice dead	All mice living, apparently normal	All mice living, apparently normal

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† The subtilin preparation used in these experiments was kindly supplied by the Western Regional Research Laboratory, Albany, Calif.

1 Salle, A. J., and Jann, Gregory J., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 60.

TABLE II.
Treatment of Pneumococcus Type III Pneumonia in Mice.

Date	Time	Six mice used in each group					
		Controls	Treated immediately	Treated after 1 hr	Treated after 3 hr	Treated after 6 hr	Treated after 9 hr
2/19/46	9 a.m.	Infected	Infected	Infected	Infected	Infected	Infected
"	9 "	—	0.5 cc subtilin	—	—	—	—
"	10 "	—	—	0.5 cc subtilin	—	—	—
"	12 m.	—	0.5 cc "	"	0.5 cc subtilin	—	—
"	3 p.m.	—	"	"	"	0.5 cc subtilin	—
"	6 "	—	"	"	"	"	0.5 cc subtilin
"	9 "	—	"	"	"	"	"
2/20	9 a.m.	1st dead	"	"	"	"	"
"	12 m.	2nd and 3rd dead	"	"	"	"	"
"	3 p.m.	—	"	"	"	"	"
"	6 "	4th dead	"	"	"	"	"
"	9 "	5th and 6th dead	"	"	"	"	"
2/21	5 a.m.	—	"	"	"	"	"
3/7	Results	All dead	All living	All living	All living	All living	All living

tion was shown to be bacteriostatic in high dilution and bactericidal in more concentrated solution.

In the present communication subtilin was tested for its effect upon Type III pneumococcus *in vivo*.

Experimental. Nine white mice, weighing between 20 and 25 g, were injected intraperitoneally with 0.1 cc of a 24-hour serum broth culture of *Diplococcus pneumoniae*, Type III. Three of the mice were not treated but served as controls; 3 were treated immediately; the remaining 3 were treated one hour later. Each mouse was injected intraperitoneally with 0.5 cc of a solution containing 0.1 mg subtilin per cc (½ unit)[2] and every 4 hours thereafter throughout the first day. Three injections were given on the second day and one on the third. The schedule of treatments and results obtained are recorded in Table I. It may be seen that all of the control mice died in about 24 hours. On the other hand, all of the treated animals were living 2 weeks after treatment was discontinued. The animals were discarded after this period of time.

In a second series a larger number of animals (36) was used to check the results obtained in the first experiment. Each mouse was injected intraperitoneally with 0.1 cc of a 24-hour serum broth culture of Type III pneumococcus. The treated animals were each given 0.5 cc of a solution containing 0.1 mg subtilin per cc. Six mice were not treated but used as controls; 6 were treated immediately; 6 were treated after an interval of 1 hour; 6 were treated after 3 hours; 6 after 6 hours; and 6 after 9 hours. The animals were given another treatment 3 hours after the first injection, and every 3 hours thereafter until 9 p.m., then continued the following morning. The treatments were continued for approximately 48 hours. The results are recorded in Table II. It may be seen that all of the control mice died in from 24 to 36 hours after being given an injection of Type III pneumococcus. On the other hand all of the treated mice survived after being given subtilin for only 48 hours.

[2] Salle, A. J., and Jann, Gregory J., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 23.

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.