extend previous studies of this enzyme system in these tissues.[8] In these studies it was also found that the dehydrogenase activity varies with the stage of the reproductive cycle, there being a steady increase in the QO_2 of the corpora lutea of pregnancy until midterm after which this level is maintained until parturition. During lactation the value declines rapidly to the early pregnancy level and remains there throughout 20 days of suckling. The QO_2 of the corpora lutea of the post-partum ovulation increases until day 20 of lactation when the highest value obtained from any ovarian tissue is reached. The QO_2 of the ovarian residue does not vary markedly during pregnancy or lactation.

Summary. The ATP-ase activity and weight of lutein and ovarian tissue were determined during estrus, diestrus, pseudopregnancy, pregnancy and lactation. The results indicate that in general the enzyme activity per unit weight is lower in functional corpora lutea than in apparently non-functional corpora. The weight and enzyme activity per corpus luteum increased and decreased at approximately the same rate during pregnancy and lactation. The enzyme activity of ovarian tissue remaining after the removal of the corpora lutea was greatest on the 4th day of pseudopregnancy, 7th day of pregnancy and 11th day of lactation.

[8] Meyer, Roland K., McShan, W. H., and Erway, Wilma F., *Endocrinology*, 1945, **37**, 431.

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Effect of Prolonged Wakefulness on the Urinary Excretion of 17-Ketosteroids.*

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Selye[1] has shown that a number of different types of stress cause a stimulation of the adrenal cortex. As a result of this stimulation, the adrenal secretes more substances of steroid nature, and the level of 17-ketosteroids in the urine is increased. Pincus and Hoagland[2] demonstrated such an increase in the output of 17-ketosteroids in pilots under conditions of stress produced by actual flight.†

Prolonged wakefulness may be considered a condition which causes considerable stress on the organism. If the response of the adrenal cortex to stress were a general phenomenon, it would be expected that prolonged wakefulness would induce a similar stimulation of the cortex with a resulting increase in the excretion of 17-ketosteroids.

Methods. Twelve subjects,‡ ranging in age from 18 to 33 years, were used. They were divided into 2 groups of 6 each. One group (B) received 1 grain of amytal§ every 12 hours for the first 48 hours of the sleepless period and then placebos every 12 hours there-

* The work described in this report was done under contract recommended by the CMR, between the Office of Scientific Research and Development and the California Institute of Technology.

[1] Selye, H., *Endocrinol.*, 1937, **21**, 169.

[2] Pincus, G., and Hoagland, H., *J. Aviat. Med.*, 1943, **14**, 173.

† After this MS. was completed, Pincus *et al.*[8] reported that the 17-ketosteroid excretion is reduced during sleep.

‡ Twelve conscientious objectors of the Glendora Civilian Public Service Camp volunteered for this and other experiments carried out under this project, and their cooperation was excellent.

§ Amytal was given in this experiment to determine its effect on the performance of men who might be forced to remain awake for prolonged periods of time. It was a constituent of a motion sickness preventive being considered for general use by the armed services.

TABLE I.
Total Daily Urinary 17-Ketosteroids.

	Control period, normal sleep days			Experimental period, no sleep days			
	1st	2nd	3rd	1st	2nd	3rd	4th
Group A							
Med.	—	—	—	Plac.	Plac.	Plac.	Plac.
KA	20.8	23.6	24.9	22.6	20.9	21.5	18.5
EB	17.0	18.1	15.7	14.0	16.1	14.8	17.0
BB	11.8	13.1	11.9	12.7	11.5	15.2	11.5
LC	21.1	22.7	23.4	18.6	18.4	19.0	21.2
DM	14.2	17.1	14.5	17.2	19.1	17.6	14.6
RT	19.1	19.3	19.3	20.6	16.7	10.7	13.7
Mean	(17.4)	(19.0)	(18.2)	(17.6)	(17.1)	(16.5)	(16.1)
Group B							
Med.	—	—	—	Amytal	Amytal	Plac.	Plac.
BA	17.6	21.8	19.8	18.8	14.4	17.3	17.9
MD	15.4	14.7	13.5	13.0	10.1	11.4	13.3
CE	20.7	20.7	23.7	22.9	17.8	20.6	17.9
EF	16.0	15.4	19.7	17.1	14.3	16.4	16.5
DL	10.5	12.9	12.3	14.6	12.0	9.2	12.3
TL	17.1	21.0	18.7	16.3	17.5	19.3	18.0
Mean	(16.1)	(17.8)	(18.0)	(17.1)	(14.4)	(15.7)	(16.0)

Values expressed as mg dehydro-iso-androsterone.

after. The other group (A) received placebos throughout. All received the same food and participated in the same activities.‖

Urine was collected, first as a control under normal conditions of sleep, for 3 days prior to the experiment, and during the sleepless period of 112 hours, as follows: Each man saved his entire output for each 24-hour period in a bottle containing 10 cc of concentrated HCl. Each sample was acidified immediately after the termination of each 24-hour period with a volume of concentrated HCl equivalent to 15% of the total urine volume, and stored at 2-4°C.

Pincus' procedures for hydrolysis and extraction,[3] and the colorimetric method of Callow[4] were employed. The concentration of total 17-ketosteroids was calculated by means of a calibration curve which had been established with graded amounts of dehydro-iso-androsterone acetate. Suggestions by Talbot *et al.*[5,6] and Engstrom and Mason[7] were followed for correction of the results for the influence of non-ketonic chromogens. In 13 representative samples, the non-ketonic fraction was separated from the ketones by the use of Girard's reagent T; from the colorimeter readings obtained with these samples, a calibration curve was drawn for the correction of all other samples.

Results and Discussion. The results are given in the table, and indicate that:

Prolonged wakefulness did not change the level of the urinary 17-ketosteroids. The values for each individual of the placebo group, as well as for this group as a whole, were not significantly different for the control and the experimental periods. This is in agreement with a previous, but incomplete, study made by one of us. (D.B.T.) on 20 subjects in which prolonged wakefulness

‖ In order to keep the men awake for prolonged periods, it is necessary that they be on a program of continuous physical activity. This is particularly important after the first day. This experiment is one of many dealing with prolonged wakefulness involving almost 700 subjects. Because of the interest at the moment in 17-ketosteroid output during various conditions of stress, it seems to be desirable to report the results of this phase of the study at the present time.

3 Pincus, G., and Pearlman, W. H., *Endocrinol.*, 1941, **29**, 413.

4 Callow, N. H., Callow, R. K., and Emmens, C. W., *Biochem. J.*, 1938, **32**, 1312.

5 Talbot, N. B., Butler, A. M., MacLachlan, E. A., and Jones, R. N., *J. Biol. Chem.*, 1940, **136**, 365.

6 Talbot, N. B., Berman, R. A., and MacLachlan, E. A., *J. Biol. Chem.*, 1942, **143**, 211.

7 Engstrom, W. W., and Mason, H. L., *Endocrinol.*, 1943, **33**, 229.

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

* This investigation was aided by a grant from Eli Lilly and Company, Indianapolis, Indiana.

† 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.