

Such doses appear to be "massive" only when expressed as "units." The advantages of therapeutic administration of penicillin in a single oral dose, or in oral doses given once or twice a day, are obvious.

Further work is in progress to determine the effectiveness of single oral doses of dif-

ferent lots of penicillin in the streptococcal and other infections in mice.

Summary. Survival of 97% was obtained in a streptococcal infection in mice with crude penicillin powder containing 650 units per mg, administered in a single oral dose of 25 mg per kilo.

15363 P

Adenosine Triphosphatase Activity and Weight of Corpora Lutea During Reproductive Cycle of the Rat.*

CLYDE BIDDULPH, ROLAND K. MEYER, AND W. H. MCSHAN.

From the Department of Zoology, University of Wisconsin, Madison.

Adenosine triphosphate (ATP) and the ATP-splitting enzyme, adenosine triphosphatase (ATP-ase), have been found in tissues other than muscle,¹ and evidence has been presented that the ATP system links the energy-requiring reactions of tissues with the energy-yielding reactions.² These results suggested an investigation of the ATP-ase activity of ovarian structures since these tissues undergo rapid changes in growth and function during which large amounts of energy must necessarily be involved.

Materials and Methods. The method of DuBois and Potter¹ was used in determining the ATP-ase activity of corpora lutea and remaining ovarian tissue of female rats during estrus, diestrus, pseudopregnancy and lactation at the intervals indicated in Table I. This method measures the amount of inorganic phosphorus released by the enzyme of homogenized tissue when ATP is used as substrate in a buffered solution (pH 7.4) containing calcium. The corpora lutea were dissected from the ovaries using sharp-pointed scalpel blades and spectacle loupes. The identification of the corpora of pregnancy and corpora of the post-partum ovulation through-

out lactation was based on the differences in size, shape and color. That these criteria were adequate was confirmed by marking the corpora of pregnancy with fine silk thread. The corpora and ovarian residue were weighed and homogenized prior to adding to the incubation mixture. Each ATP-ase value in the table is the average of 9 determinations (3 for each of 3 rats), and each weight is the average of the corpora lutea obtained from 3 rats.

Results and Discussion. In diestrus there was a slight increase in the ATP-ase of the corpora lutea and ovarian residue above the estrus level. The average weight of the diestrous corpora was essentially the same as that found in estrus. During pseudopregnancy the weight of the corpora increased somewhat from the estrus level, but the ATP-ase activity did not change significantly. As pseudopregnancy progressed a definite decrease in the value of the ovarian residue was noted. At 11 days estrus was imminent, and the results suggest an inverse relationship between estrogen level and ATP-ase of the extra-luteal tissues.

The ATP-ase activity of the corpora lutea during pregnancy, when calculated on a per mg of tissue basis, did not show marked variation despite the fact that the average weight of the corpora lutea progressively increased. Late in pregnancy an increase in activity was found which continued until the

* This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

¹ DuBois, K. P., and Potter, V. R., *J. Biol. Chem.*, 1943, **150**, 185.

² Potter, V. R., *J. Cell. and Comp. Physiol.*, 1945, **26**, 87.

TABLE I.

Tissue	Stage											
	Pseudopreg.				Pregnancy				Lactation			
	4	7	11		4	7	11	15	20	22	23	
Estrus												
1.2												
Corpora lutea												
1.1	1.5	1.6	—		1.5	1.4	2.1	4.0	4.5			
Avg wt. (mg)												
2.4†												
1.5												
15.9†												
±1.3												
5.5												
±2.4												
16.6												
±7.6												
24.9												
±9.5												
20.6												
±4.3												
6.7												
±2.8												
9.1												
±1.3												
12.3												
±.57												
16.0												
±.83												
14.7												
±.87												
16.4												
±1.3												
9.8												
±.40												
8.1												
±.54												
7.9												
±.32												
7.2												
±.25												
18.0												
±.27												
15.7												
±.54												
13.6												
±.27												
17.6												
±.84												
11.2												
±.57												
13.5												
±.74												
19.7												
±.66												
13.8												
±.22												
14.3												
±.82												
12.6												
±.20												
16.3												
±.64												
17.7												
±.43												
19.8												
±1.3												
16.8												
±.59												
15.0												
±.23												
16.0												
±.78												
14.7												
±.83												

* Days. † First line, corpora of pregnancy which persist during lactation; second line, corpora lutea of the post-partum ovulation.

‡ A unit is the quantity of ATP-ase which liberates 1 μ of inorganic phosphorus from ATP in 15 minutes at 37°C. The values are expressed as units per mg of fresh tissue, each value being the average of 9 determinations (3 for each of 3 rats). § Standard error of the mean.

11th day of lactation, during which time the average weight of the corpora lutea decreased abruptly.

During the first 20 days of lactation the ATP-ase activity of the corpora lutea of the post-partum ovulation paralleled closely the activity of the corpora of pregnancy during gestation. The increased ATP-ase after day 20 was correlated with decreasing lactation and reappearance of estrous cycles.

When the above results are calculated on the basis of change per corpus luteum rather than change per mg of tissue, the weight and the ATP-ase activity increase at practically identical rates during the periods of gestation in which there is active progesterone secretion.^{3,4} The same relationship holds for the corpora of the post-partum ovulation during the first 20 days of lactation. Immediately before parturition and late in lactation the relationship changes so that the rate of change in ATP-ase activity exceeds the weight change rate. Following parturition both the weight and ATP-ase activity decrease, but the latter lags considerably behind the former. These results indicate a parallel increase in size and ATP-ase activity of the functional lutein cell, which suggests, together with other evidence linking the ATP system with lipid metabolism,^{5,6} that ATP-ase may be involved in the production of progesterone by the functional corpus luteum.

The enzyme activity of the ovarian residue during pregnancy increased to a high value on day 7, then decreased significantly on day 11, and remained approximately unchanged until after parturition. Following littering a maximum was reached on day 11 of lactation.

We have also studied the succinic dehydrogenase activity of lutein and ovarian tissues throughout pregnancy and lactation⁷ to

³ Atkinson, W. B., and Hooker, C. W., *Anat. Rec.*, 1945, **93**, 75.

⁴ Laqueur, G. L., and Koets, P., *Proc. Soc. Exp. Biol. and Med.*, 1945, **60**, 239.

⁵ Lardy, H. A., Hansen, R. G., and Phillips, P. H., *Arch. Biochem.*, 1945, **6**, 41.

⁶ Lehninger, A. L., *J. Biol. Chem.*, 1945, **157**, 363.

⁷ Unpublished data.

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 mid-term 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.

15364

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

DAVID B. TYLER, WALTER MARX, AND JOSEPH GOODMAN.

From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, Calif.

Selye¹ 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 Hoagland² 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.