been found to be directly proportional to the amount of carrier, as is predicted.

If membranes based on this mechanism are used with more than two solutes, they may exhibit selective countertransport, separating and concentrating one of the ions. We have made experiments using monensin-containing membranes which show the selective countertransport of sodium ion in the presence of potassium ion. The data are very similar to those of Fig. 1, with the maximum sodium concentration difference about three times larger than the maximum concentration difference observed for potassium.

For the simultaneous transport of several solutes against this concentration gradient, the chemical requirements on the carrier are not stringent. If a *p*H gradient is the energy source, cations may be moved against this gradient with any weak acid as a carrier, and the anions with any weak base as a carrier. In addition, the energy source for this effect is not limited to acid-base reactions, but can, in principle, be applied to any diffusional process that can be coupled via the carrier to the solute being transported. We have observed such nonselective effects with membranes containing octanol or trichloroethane solutions of silicones. Others have reported similar nonspecific membranes which employ pentanol solutions of stearic acid (*7*) or aqueous acetate solutions (*8*). However, because of an inadequate theoretical basis, the interpretation of previous experiments has often been uncertain.

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Ornithine Decarboxylase Stimulation in Rat Ovary by Luteinizing Hormone

Abstract. *In normal albino rats with a 4-day estrous cycle, the activity of ovarian ornithine decarboxylase undergoes a transitory rise on the evening of proestrus and only at that time. The response could be elicited by the administration of either luteinizing hormone or human chorionic gonadotrophin. When antiserum to luteinizing hormone was injected at 2 p.m. on the day of proestrus, the induction of ornithine decarboxylase was blocked, an indication that the enzyme is under luteinizing hormone control. The strategic positioning of the induction of ornithine decarboxylase between the normal release of luteinizing hormone and ovulation implies that putrescine is associated with the ovulatory process, and opens a new avenue of research on the control of ovulation.*

Mammalian ornithine decarboxylase is a key enzyme in the biosynthesis of the polyamines, spermine and spermidine (*1*, *2*). It responds rapidly to various stimuli, and high levels of the enzyme occur in the rat liver after partial hepatectomy (*1–3*) or after the administration of growth hormone (*4*). In the ventral prostate of rat, testosterone regulates ornithine decarboxylase activity (*5*); and we now report on the possible role of polyamines in female reproductive physiology.

Female Sprague-Dawley rats (150 to 180 g) were followed through three consecutive estrous cycles. Each was killed at 10 a.m., 2 p.m., or 8 p.m., at the stage of the estrous cycle indicated. Ornithine decarboxylase activity of the ovaries was assayed as described by Russell and Snyder (*2*), except that the reaction was carried out in Warburg vessels and the evolved carbon dioxide was trapped on Hyamine-impregnated filter paper (*6*). The specific activity of the DL-[1-^{14}C]ornithine used was 1 mc/mmole. The assay medium contained 100 mg of homogenized tissue, 0.2 μmole of DL-[1-^{14}C]ornithine, 0.2 μmole of pyridoxal phosphate in 0.1*M* phosphate buffer, *p*H 7.2, in a final volume of 2 ml. The incubation time was 30 minutes at 37°C in air. The reaction was stopped with 0.2 ml of 1*M* citric acid injected through a rubber septum and shaken for an additional 30 minutes. The filter paper was transferred to a glass vial containing 3 ml of ethanol and 7 ml of toluene [0.4 percent of 2,4-diphenyloxazole and 0.01 percent of *p*-bis-(*O*-methylstyryl)benzene] and assayed for radioactivity in a liquid scintillation spectrometer. The amount (90 percent) of $^{14}CO_2$ from [1-^{14}C]ornithine was nearly equivalent to that obtained as [^{14}C]putrescine from [5-^{14}C]ornithine.

The characteristic feature of ornithine decarboxylase of the ovary was the pronounced transitory rise in enzyme activity which occurred only on the evening of proestrus (Fig. 1). A more detailed study of the development of ornithine decarboxylase activity during late proestrus verified this pattern of enzyme activity and showed that the major increase in enzyme activity occurred between 5 and 8 p.m. and was nearly normal by 10 p.m. (Fig. 2). A similar study of the other periods of the estrous cycle did not reveal any significant change in ornithine decarboxylase activity. The unique timing of this rise in ornithine decarboxylase in the ovary leads us to suspect that this enzyme may be under hormonal control.

In cycling female albino rats, lutein-

Table 1. Effect of hormonal treatment on ornithine decarboxylase activity in rat ovary. Animals in proestrus were injected subcutaneously with the designated hormone at 9 a.m. on the morning of proestrus, and the ornithine decarboxylase in the ovary was determined 4 hours later. The number of animals is shown in parentheses. Activity is expressed as the number (± S.E.) of nanomoles of CO_2 formed per gram of tissue per hour.

Hormone	Dose (per kg)	Ornithine decarboxylase activity (nmole g^{-1} hr^{-1})
None	None	4.15 ± 0.63 (6)
LH	100 μg	37.78 ± 3.55 (6)
HCG	50 units	46.65 ± 5.78 (9)
Estradiol	1 mg	4.38 ± 0.98 (6)
Progesterone	5 mg	4.98 ± 0.98 (6)
Thyroxine	2 mg	2.43 ± 0.75 (3)
Testosterone	50 mg	7.40 ± 4.98 (3)
FSH	25 units	7.65 ± 3.93 (6)

Table 2. Inhibition of the induction of ornithine decarboxylase in rat ovary by bovine antiserum to luteinizing hormone. The enzyme activity is expressed as nanomoles (± S.E.) of CO_2 formed per gram of tissue per hour.

Treatment	Ornithine decarboxylase activity (nmole g^{-1} hr^{-1})
Proestrus 2 p.m.	5.43 ± 1.35 (3)
Proestrus 7 p.m.	29.70 ± 13.43 (3)
Proestrus 7 p.m.*	2.03 ± 0.73 (3)

* Injected with antiserum (0.3 ml) intraperitoneally at 2 p.m.

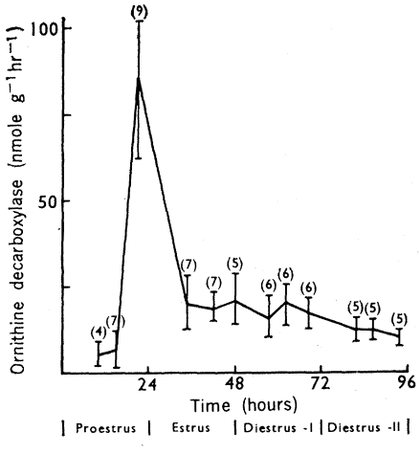

Fig. 1. Ornithine decarboxylase in the rat ovary during the estrous cycle. Rats with a 4-day cycle were used, and animals were killed at 10 a.m., 2 p.m., and 8 p.m. The number of animals in each group is indicated in parentheses and enzyme activity is expressed as nanomoles (±S.E.) of CO_2 per gram of tissue per hour.

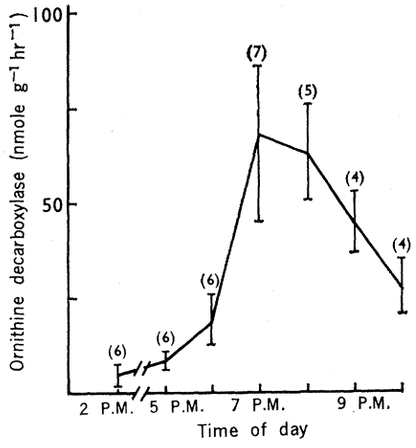

Fig. 2. Ornithine decarboxylase in the rat ovary during late proestrus. Rats with a 4-day cycle were killed, as indicated, during late proestrus between 2 p.m. and 10 p.m. The number of animals in each group is indicated in parentheses, and enzyme activity is expressed as nanomoles (± S.E.) of CO_2 per gram of tissue per hour.

izing hormone (LH) release occurs on the afternoon of proestrus, usually between 3 and 7 p.m., and ovulation occurs about 12 hours later (*7*). Therefore, LH and a variety of other hormones were tested as follows: rats in proestrus were injected subcutaneously at 9 a.m. with the designated hormone, and the ovarian ornithine decarboxylase activity was determined 4 hours later (Table 1). Both LH and human chorionic gonadotrophin (HCG) produced a substantial increase in enzyme activity, whereas the other hormones had little or no stimulatory effect upon this enzyme. In fact, thyroxine appeared to be inhibitory. The results with HCG and follicle-stimulating hormone (FSH) may have been due to the presence of LH, which is known to contaminate these two hormones. In order to test this hypothesis further, rats at proestrus were injected at 2 p.m. with bovine antiserum to LH, which can neutralize rat LH, and the ovarian ornithine decarboxylase activity was measured at 7 p.m. and compared to that of untreated rats (Table 2). The antibody to LH prevented the rise in enzyme activity which normally occurs on the evening of proestrus; these results suggest that ovarian ornithine decarboxylase activity is under LH control.

In the rat, there are now four hormones that induce this enzyme in the appropriate target tissue: testosterone in the prostate (*5*), estradiol in the uterus (*8*), growth hormone in the normal and regenerating liver (*1–3*), and LH in the ovary. The short half-life (10 to 15 minutes) of induced ornithine decarboxylase in regenerating liver (*9*), the hormone specificity of tissue response, and the rapid inducibility of the enzyme suggest to us that polyamine biosynthesis is a finely modulated process closely associated with the metabolic activity of growing tissues that are under hormonal regulation. In the rat ovary, the strategic positioning of the rise in ornithine decarboxylase activity between the release of LH and ovulation implies that polyamines may be intimately associated with the ovulatory process and may be related to the synthesis of new protein and RNA believed to be involved in the early phase of LH action (*10*). However, whether specific inhibition of ornithine decarboxylase would influence ovulation is not known. Nonetheless, the possibility that the control of ovulation might be achieved at a site more specific than that afforded by the pituitary-ovarian axis should stimulate the search for an inhibitor of ornithine decarboxylase.

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Acetoacetyl–Coenzyme A Thiolase in Brain, Liver, and Kidney during Maturation of the Rat

Abstract. *The development of thiolase in various rat tissues from 7 days before birth to adulthood was studied. Enzyme activities in brain, liver, and kidney during the perinatal period reflect the nutritional environment, whereas those in the adult tissues do not.*

Itoh and Quastel (*1*) have shown that in slices of immature rat brain acetoacetate was a more potent source of acetyl-coenzyme A (CoA) than glucose. The sequential pathway of acetoacetate degradation involves acetoacetyl-CoA transferase (E.C. 2.8.3.5) and acetoacetyl-CoA thiolase (E.C. 2.3.1.16) neither of which was measured by Itoh and Quastel. Thiolase furthermore is implicated in the beta oxidation of fatty acids. Inasmuch as the young rats during the first 3 weeks of life are nursing and thus are getting a diet high in fat, it became important to ascertain whether thiolase activity of brain and possibly of kidney reflects the increased utilization of ketone bodies and furthermore whether thiolase activity of liver would be an index of increased fatty acid degradation and ketone body formation. It was for these reasons that the development of thiolase activity was followed in rat brain, liver, and kidney during maturation of these tissues.

All experiments were conducted with albino Wistar rats. No sexual differentiation was made of rats less than 4