apply to these springs as to the rising thermals. Shrimp appear less concentrated over the springs in cloudy weather or at night.

Populations of migrating phalarope stop at the lake, and a bird frequently swims in a tight circle, occasionally pecking at shrimp drawn into the vortex created by it. The concentration mechanisms suggested above may also aid this process.

It appears, then, that littoral solar warming of the near-bottom water causes it to rise in localized convective density currents, often along natural barriers that provide easy upward paths —for example, ripple marks and rocks. The water is warmer, less dense, and, coming from intimate sedimentary contact, probably richer in dissolved organic compounds than the main water mass. In these rising plumes brine shrimp become trapped in a revolving toroid through exercise of a negative photokinesis, which may be combined with one or more hypothetical mechanisms causing an apparent positive rheokinesis. The presence and activity of the shrimp may decrease the density and augment the upward flow of the current. It should be recognized that according to this model the shrimp will circulate within the plume, while the water is continually drawn from below and released above.

Application of portions of this model to aerial swarms of insects in thermally convective air requires only ethological treatment of the kinetic behavior of the organisms involved in plumes.

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References and Notes

1. E. B. Peterson and A. W. H. Damman, *Science* **148**, 392 (1965).
2. D. B. Ward, J. Beckner, R. Hackman, *ibid.* **149**, 764 (1965); P. S. Corbet, J. A. Downes, G. Steyskal, *ibid.* **150**, 1629 (1965).
3. S. W. Garman, *Amer. Naturalist* **6**, 771 (1872).
4. W. H. Bradley, *Science* **150**, 1423 (1965).
5. Average of 61 and 63 mg/liter found by J. D. H. Strickland and P. M. Williams for pelagic Mono Lake water taken 23 October 1965 (personal communication).
6. J. W. Hoyt and G. Soli, *Science* **149**, 1509 (1965).
7. D. T. Mason, "The limnology of Mono Lake," dissertation, in preparation.
8. T. H. Waterman, Ed., *The Physiology of Crustacea* (Academic Press, New York, 1961), vol. 2.
9. Investigation assisted by NIH fellowship 1-F1-GM-21,467-01,02, and by NSF grant GB-2436 to C. R. Goldman whose criticism of the manuscript I gratefully acknowledge.

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Progesterone Retards Postpartum Involution of the Rabbit Myometrium

Abstract. *Postpartum involution of the uterus was significantly reduced in rabbits by immediate and repeated injections of progesterone. Progesterone had no effect if the injections commenced 24 hours after delivery. The decrease in concentration of progesterone that accompanies birth results in release of proteolytic and other degradative enzymes from subcellular particles; these enzymes cause the involution, and, once the enzymes are released into the cellular milieu, progesterone has no effect.*

The mechanism by which involution, or decrease in size and weight of the mammalian uterine myometrium, occurs after termination of pregnancy is poorly understood. Histological study (*1*) has shown little cell autolysis, and that loss of weight by the myometrium reflects decrease in size of individual cells in the tissue. It has been proposed (*2*) that proteolytic enzymes may participate in this process, but the "trigger mechanism" that begins postpartum involution is not known. Csapo *et al.* (*3*) recently suggested that the loss of chronic stretch, which occurs after expulsion of the fetal mass during delivery, may initiate postpartum involution in the rabbit, since maintenance of this stretch appears to prevent complete involution of the tissue.

A recent report (*4*) is that estradiol-17β stimulates increased synthesis of several degradative enzymes in the uterine myometrium of ovariectomized rabbits; the same enzymes are also much more abundant in the myometrium of pregnant than of nonpregnant rabbits. Of particular interest is the increase in proteolytic enzyme activity in the myometrium during these periods; it was proposed that this increase is important for the process of postpartum involution.

It was also reported (*4*) that these degradative enzymes, including the proteolytic enzyme activity, can be isolated in a subcellular particulate fraction from homogenates of pregnant-rabbit myometrium. It was proposed that progesterone may participate in growth and involution of the rabbit myometrium by interaction with the subcellular particles in the following manner. Although exogenous estradiol-17β stimulates increased synthesis of the proteolytic enzymes, concurrently with the stimulation of growth of the uterine myometrium of ovariectomized rabbits, a source of exogenous progesterone is needed for continued growth of the tissue. During normal pregnancy, a source of progesterone, either ovary or placenta, is needed to ensure continued growth of the uterus and maintenance of the pregnancy until term. In either case, loss of the source of progesterone results in cessation of tissue growth and the beginning of tissue involution.

Progesterone could prevent involution by stabilizing the subcellular particles so that their content of proteolytic enzyme is not released into the cellular milieu, or by inhibiting the proteolytic enzymic activity after the enzymes had been released from the particles. These two possibilities could combine. Removal of the source of progesterone, which occurs with the loss of the placenta at birth, would then allow the particulate-bound degradative enzymes to become active. Involution of the tissue would result from activity of these degradative enzymes.

These propsals can be tested very simply. The concentration of progesterone in the circulating blood can be maintained in animals postpartum by administration of exogenous hormone. If progesterone either stabilizes the particles or inhibits the enzymic activity, the weight of the uterus should not decrease rapidly as it does during normal postpartum involution. A test of these proposals entailed consideration of the following points: The daily production rate of progesterone during pregnancy is very high and the turnover rate is very rapid (*5*). The concentration of progesterone in the circulating blood of the rabbit falls very rapidly following the birth process (*6*). These facts mean that large amounts of progesterone must be administered daily, with the total amount divided into frequent injections throughout the experimental period. Secondly, it must be assumed that enough of the injected hormone reaches the cells of the uterus to maintain the intracellular content of progesterone after the termination of pregnancy. Thirdly, the decrease in chronic tension in the uterus, which results from loss of the fetal mass at birth, should not by itself result in some uterine change that would mask an effect of the hormone.

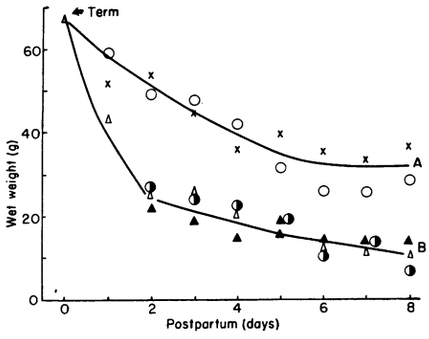

Fig. 1. Uterine wet weight at daily intervals postpartum. Curve *A* is for animals injected immediately after delivery: crosses, progesterone, two animals per data point; hollow circles, estradiol and progesterone combined, one animal per data point. Curve *B*: hollow deltas, normal involution, four animals per data point; solid deltas, progesterone injections beginning 24 hours postpartum, one animal per data point; half-solid circles, estradiol and progesterone combined beginning 24 hours postpartum, one animal per data point. *Term* represents the average weight of uteri of three animals.

The following experimental design was used to test these proposals. Pregnant New Zealand white rabbits (*7*) were allowed to reach term and deliver normally. Only 70 mothers of more than six live young were used; their average live litter numbered 7.7.

At the time of delivery the rabbits were grouped as follows (all injections of progesterone were of 25 mg administered every 4 hours; of estradiol-17β, 2.5 μg every 4 hours): group 1, given progesterone; group 2, given estradiol-17β and progesterone; group 3, given progesterone, with injections beginning 24 hours postpartum; group 4, given estradiol-17β and progesterone, with injections beginning 24 hours postpartum; and group 5 (the controls), injected with similar amounts of vehicle containing no hormones. The hormones injected were contained in total volumes of 1 ml of corn oil containing 10 percent ethanol. The intramuscular injections (hind thigh) continued until death. Animals from each group were killed daily for 8 days postpartum; the uteri were removed, dissected free of fat and connective tissue, and weighed wet to the nearest 0.5 g.

The results are summarized in Figs. 1 and 2. In the animals of groups 1 and 2, the wet weight of the uterus decreased very slowly (relative to the controls) after an early drop (Fig. 1*A*); after 8 days the difference was significant, the experimental uteri averaging more than 20 g heavier. The early loss of weight in the experimental animals probably reflected the loss of tension in the uterus at delivery. Uterine weight is normally determined not only by hormonal condition but also by fetal mass during pregnancy (*8*). Tension results in net synthesis of protein in the uterus of the rabbit, and uterine weight can be maintained postpartum to some extent by the maintenance of chronic tension (*3*). Chronic tension and hormonal status act synergistically. It should be possible to maintain the maximum weight of the uterus, attained during pregnancy, by a combination of hormone administration and maintenance of the chronic tension. Significantly these data show that progesterone alone prevented involution of the tissue, but they do not show whether the hormone acted on the subcellular particles or inhibited the enzyme. Estradiol-17β added nothing to the effect of progesterone.

In the animals of groups 3 and 4 the injections had no effect on the course of involution (Figs. 1*B* and 2). The data indicate that these hormones have little if any inhibitory effect on proteolytic activity, and that progesterone acts on the subcellular particle. Once the process of involution is under way, it is irreversible.

My data strongly support the following proposals. First, progesterone allows uterine growth to continue and enables maintenance of pregnancy by stabilizing, in a manner unknown, subcellular particles that contain degradative enzymes such as the proteolytic enzymes referred to above. Second, postpartum involution occurs because the concentration of progesterone in the blood and myometrial cells is suddenly decreased at birth by the loss of the placental source of this hormone; the loss results in decreased stability of the subcellular particles that contain the proteolytic enzymes, and these enzymes are subsequently released into the cellular milieu. These enzymes are responsible for the decrease in cellular mass that is manifest as decrease in uterine weight. This process may be prevented, or at least delayed, by maintenance of a high concentration of progesterone in the animal. But the synergistic effects of chronic tension (*3*) should not be discounted. Third, involution, which accompanies the decrease (by the loss of the placenta at the time of delivery) in progesterone, is irreversible; this fact indicates that progesterone acts on the subcellular particles and not by inhibiting the enzymes.

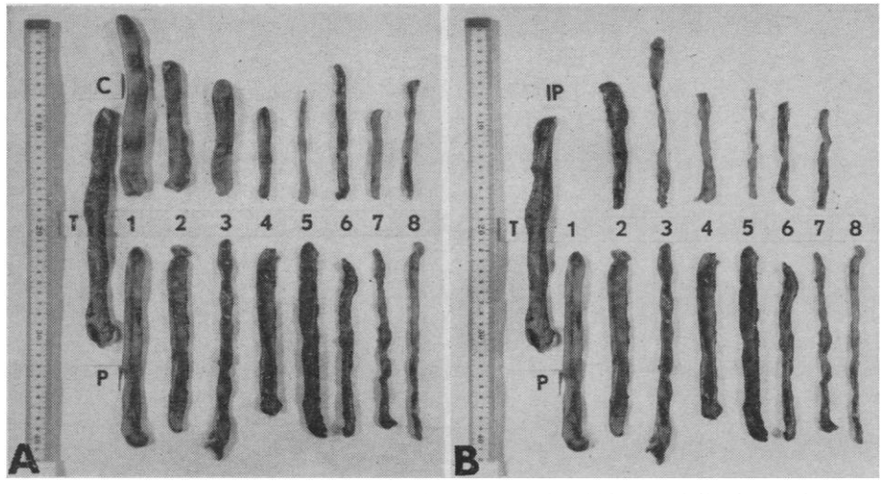

Fig. 2. Typical gross appearance of uterine horns of rabbits at daily intervals postpartum; scale, centimeters. In *A*, individual horns from animals injected with progesterone immediately after delivery (*P*) are compared with horns from animals in which involution preceeded normally (*C*) and with the term horn (*T*). In *B*, individual horns from progesterone-treated animals (*P*) are compared with horns from animals in which progesterone injections began 24 hours postpartum (*IP*). The numbers are of days postpartum.

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References and Notes

1. C. Kurimitsu and L. Loeb, *Amer. J. Physiol.* **55**, 422 (1921); A. B. Dawson, *Amer. J. Anat.* **79**, 241 (1946); H. G. Maibenco, *Anat. Rec.* **136**, 59 (1960).
2. J. F. Woessner, Jr., *Biochem. J.* **83**, 304 (1962); ——— and T. H. Brewer, *ibid.* **89**, 75 (1963).
3. A. Csapo, T. Erdos, C. R. de Mattos, E. Gramss, C. Moscowitz, *Nature* **207**, 1378 (1965).
4. F. R. Goodall, *Arch. Biochem. Biophys.* **112**, 403 (1965).

5. W. H. Pearlman, in *Hormonal Steroids*, L. Martini and A. Pecile, Eds. (Academic Press, New York, 1964), vol. 1, pp. 115–24; *Ciba Found. Colloq. Endocrinol.* **11**, 233 (1957).
6. M. X. Zarrow and G. M. Neher, *Endocrinology* **56**, 1 (1955).
7. From a colony inbred for 8 years; from G. M. Rabbitry, Brooks St., Upton, Mass.
8. S. R. M. Reynolds, *Cold Spring Harbor Symp. Quant. Biol.* **5**, 84 (1937).
9. Assisted by grants from NSF (G-22004) and NIH (GM-09808) to A. G. Szent-Györgyi, and by grants to F.R.G. from the Lalor Foundation and the American Heart Association (65 G 133). I thank A. G. Szent-Györgyi for assistance and discussion.

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Ornithine-Urea Cycle Enzymes in the African Lungfish, Protopterus aethiopicus

Abstract. *The presence of all five enzymes of the ornithine-urea cycle has been demonstrated in the liver of the African lungfish* Protopterus aethiopicus. *Levels of activity of the rate-limiting enzymes, carbamoyl phosphate synthetase and argininosuccinate synthetase, are similar to those in the premetamorphic tadpole of* Rana catesbeiana *and considerably lower than the levels reported for other ureotelic animals. They are thus consistent with the predominantly ammonotelic metabolism of the lungfish in an aquatic environment.*

In a recent report, Brown (*1*) demonstrated the presence of ornithine carbamoyltransferase in the liver of African lungfish, but he was unable to detect carbamoyl phosphate synthetase activity. Evidence is given in this report for the occurrence of all five enzymes of the ornithine-urea cycle of Krebs and Henseleit (*2*) in the liver of the free-living lungfish.

Lungfish were collected by one author (P.A.J.) during a visit to Makerere College, Uganda, from the University of Sheffield, England, in the spring of 1964, and were identified as *Protopterus aethiopicus*. Identification was based on the following criteria: (i) The fish were collected from Lake Victoria in which *P. aethiopicus* is the sole representative of the genus (*3*). (ii) After the livers were removed for enzyme analysis the fish were examined and found to have between 38 and 40 pairs of ribs, which is characteristic of the species (*4*). The fish were flown to Sheffield in 1964 and then to Madison in October 1965, where they were kept in individual plastic tanks in still tap-water at a temperature of 23°C. They were fed twice weekly on minced lamb heart or chopped calf liver, and the containers were cleaned out on the day after each feeding.

Activities of carbamoyl phosphate synthetase (*5*), ornithine carbamoyltransferase, argininosuccinate synthetase, and argininosuccinate lyase (*6*) were measured by the method of Brown and Cohen (*7*); arginase (*6*) activity was assayed by the method of Balinsky and Baldwin (*8*). The tissue was prepared by the following procedure. Lungfish were weighed and then killed by decapitation. The liver was removed, weighed, and immediately homogenized in four volumes of 0.1 percent CTB (cetyltrimethylammonium bromide) in a glass homogenizer with a Teflon pestle. Homogenization was effected with a top-drive motor while the homogenizer was immersed in a mixture of ice and water. The homogenate was centrifuged at 10,000*g* for 10 minutes at 3°C, and the supernatant fraction (S_1) was decanted. The pellet was rehomogenized in three volumes of 0.1 percent CTB, and the homogenate was centrifuged at 10,000*g* for 10 minutes at 3°C. Supernatant fraction (S_2) was decanted. The following enzyme assays were carried out: argininosuccinate synthetase activity on fraction S_1; carbamoyl phosphate synthetase on fraction S_3 (four volumes of fraction S_1+ three volumes of fraction S_2); ornithine carbamoyltransferase and argininosuccinate lyase on fraction S_3 diluted with four volumes of water; and arginase on fraction S_3 diluted with 24 volumes of water and then mixed with an equal volume of 28 m*M* manganous sulfate.

Assays indicated presence of all five enzymes of the ornithine-urea cycle in the liver of *Protopterus* (Table 1). In each case, enzyme activity was linear with respect to both enzyme concentration and time of incubation under the experimental conditions employed. The presence of carbamoyl phosphate synthetase, which Brown (*1*) was unable to detect (for reasons which we cannot comment on since experimental conditions were not revealed), was established by the demonstration that enzyme activity was dependent on the presence of acetylglutamate in the incubation medium (Table 1).

Values for enzyme activities reported here are similar to those previously given for ornithine carbamoyltransferase (*1*) and arginase (*9*) in the lungfish. Argininosuccinate synthetase appears to be the rate-limiting enzyme in the ornithine-urea cycle (*10*); the level of activity of this enzyme in lungfish is similar to that in the premetamorphic tadpole of *Rana catesbeiana* but considerably lower than in adult *R. catesbeiana* (*10*) and the great majority of ureotelic animals that were examined (*11*). The same pattern is evident for levels of activity of both carbamoyl phosphate synthetase and ornithine carbamoyltransferase. The level of arginase activity is extremely high in lungfish but this is also the case in *Necturus maculosus maculosus* Rafinesque (*11*), an amphibian which is almost exclusively ammonotelic (*12*). Fed lungfish of a group from which the experimental animals were taken excreted both ammonia and urea in the ratio 8.5 : 1.5 (*13*), which is in agreement with previous work by Smith (*14*). The only data to the contrary (*15*) was obtained with a large fish (450 g) in a small volume of water (500 ml) in which, on the first day of the experiment, the ammonia concen-

Table 1. Levels of enzymes of the ornithine-urea cycle in the liver of *Protopterus*.

Animal	Body wt. (g)	Liver wt. (g)	Carbamoyl phosphate synthetase	Ornithine carbamoyl-transferase	Arginino-succinate synthetase	Arginino-succinate lyase	Arginase
			Micromoles of product per milligram of protein per hour				
1	92	1.52	0.90	36.8	0.20	2.12	790
2	60	0.95	.50	38.6	.12	0.55	800
3	37	.65	.50				
3	37	.65	.08*				
			Micromoles of product per gram of wet tissue per hour				
1	92	1.52	37.2	1530	7.7	88.3	32,000
2	60	0.95	23.8	1820	5.5	25.4	37,600
3	37	.65	32.7				
3	37	.65	4.8*				

* Acetylglutamate omitted from incubation medium.