that this stimulation would have been observed within 4 hours of perfusion. It has been shown that rats which received 20,000 units of vitamin D_3 intrajugularly have increased intestinal calcium transport within 4 hours (*16*), and rats dosed with only 2000 units of D_3 intraperitoneally have increased intestinal transport by 6 hours after the dose (*17*). Furthermore, experiments with ^{3}H-labeled vitamin D_3 show that the intestine receives about 2 percent of an intrajugular dose of vitamin D_3 (*18*). In this light the 10,000 units given to the isolated intestines is equivalent to a considerably larger dose given to the whole animal (approximately 500,000 international units).

This is the first report of an in vitro effect of a vitamin D on intestinal calcium transport. This effect complements the in vitro effect of the metabolite in mobilizing calcium from bone tissue culture (*12*). These in vitro data, together with the results of earlier in vivo experiments showing the effectiveness of 25-HCC in bone mobilization and intestinal calcium transport (*8*), provide an important insight into the basic mechanism of vitamin D action. It seems clear that at the tissue level 25-HCC is the active form of vitamin D_3. To exert its calcium-mobilizing and intestinal-transport effects it is apparent that vitamin D must be converted to this active metabolite. Neither the intestine nor the bone tissue seems capable of catalyzing this reaction, and most likely the liver is the only organ capable of significant conversion of the vitamin D_3 to 25-HCC (*9–11*). However, it must be noted that it is possible that in the target tissues 25-HCC may be converted further to another metabolite before it is active. This possibility requires further study.

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References and Notes

1. A. Windaus, O. Linsert, A. Luttringhaus, G. Weidlich, *Ann. Chem.* **492**, 226 (1932).
2. A. Windaus, F. Schenck, F. von Weider, *Z. Physiol. Chem.* **241**, 100 (1936).
3. F. A. Askew, R. B. Bourdillon, H. M. Bruce, R. G. C. Jenkins, T. A. Webster, *Proc. Roy. Soc. Ser. B* **107**, 76 (1930).
4. J. Lund and H. F. DeLuca, *J. Lipid Res.* **7**, 739 (1966).
5. J. W. Blunt, H. F. DeLuca, H. K. Schnoes, *Chem. Commun.* **1968**, 801 (1968).
6. ———, *Biochemistry* **7**, 3317 (1968).
7. J. W. Blunt and H. F. DeLuca, *ibid.* **8**, 871 (1969).
8. J. W. Blunt, Y. Tanaka, H. F. DeLuca, *Proc. Natl. Acad. Sci. U.S.* **61**, 717 (1968).
9. G. Ponchon and H. F. DeLuca, *J. Clin. Invest.*, in press.
10. G. Ponchon, A. L. Kennan, H. F. DeLuca, *J. Clin. Invest.*, in press.
11. M. Horsting and H. F. DeLuca, *Biochem. Biophys. Res. Comm.*, in press.
12. G. Trummel, L. G. Raisz, J. W. Blunt, H. F. DeLuca, *Science* **163**, 1450 (1969).
13. H. Steenbock and D. C. Herting, *J. Nutrition* **57**, 449 (1955).
14. E. B. Olson and H. F. DeLuca, in preparation.
15. Dextran 70, Travenol Laboratories, Morton Grove, Illinois.
16. Y. Tanaka and H. F. DeLuca, unpublished results.
17. J. E. Zull, E. Czarnowska-Misztal, H. F. DeLuca, *Science* **149**, 182 (1965).
18. P. F. Neville and H. F. DeLuca, *Biochemistry* **5**, 2201 (1966).
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Female Specific Protein: Biosynthesis Controlled by Corpus Allatum in Leucophaea maderae

Abstract. *A specific protein is present in serums of all females of* Leucophaea maderae *that mature eggs but not in serums of males, nymphs of either sex, or females that are reproductively inactive. Ablations, microsurgery, and reimplantations showed that this protein is produced* de novo *under the influence of the corpus allatum hormone. This protein, essential for egg maturation, is synthesized in isolated abdomens of allatectomized females treated with 0.08 to 0.12 microgram of the* t,t,t-*isomer of the authentic juvenile hormone. The corpus allatum hormone also stimulates to a similar degree the synthesis of other proteins which appear to be essential for egg maturation as well; these are not specific to females.*

Protein metabolism associated with egg maturation is reportedly controlled by hormones liberated by the neurosecretory system in *Schistocerca gregaria* (*1*) and *Tenebrio molitor* (*2*). In *Rhodnius prolixus* (*3*), *Leucophaea maderae* (*4*), and *Periplaneta americana* (*5*), however, the corpus allatum hormone appears to control specific protein metabolism during yolk deposition. Circumstantial evidence in additional species shows that several hormones may control different phases of protein metabolism. It has been suggested, furthermore, that the neurosecretory hormones and corpus allatum hormone are functionally interdependent in their control of egg maturation. Results of experiments involving surgery in the brain may be misleading (*4*); since it is difficult to confine surgery in the insect brain to the neurosecretory system alone, observed effects may be the result of nonspecific brain damage unrelated to neurosecretory phenomena. The purpose of this study on *Leucophaea* is to help clarify the endocrine control of protein metabolism and egg maturation in a species whose endocrinology is relatively well worked out.

As in other insect species, the concentrations of blood proteins in this ovoviviparous cockroach fluctuate with egg maturation cycles (*4*). Allatectomy and particularly, cautery of the pars intercerebralis or brain surgery are followed by a very low concentration of protein in the hemolymph. Since any kind of operation, particularly brain surgery, interferes with normal activities essential for reproduction (such as mating and feeding), data on total pro-

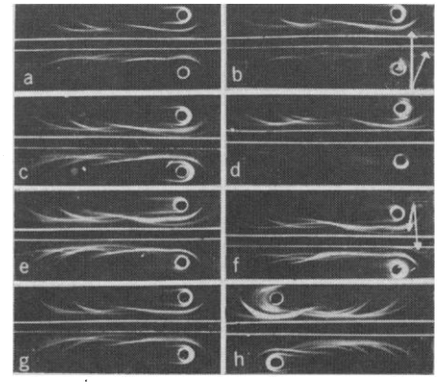

Fig. 1. Immunoelectrophoresis of serums from normal and operated females, of serums from males, and of extracts from nearly mature oocytes. (a) Serum from female adult at 6 days. (b) Saline extract from oocytes. (c) Serum from allatectomized female. (d) Distilled-water extract from oocytes. (e) Serum from female subjected to cautery of the pars intercerebralis, no eggs matured. (f) Serum from female subjected to cautery of the pars intercerebralis, eggs matured. (g) Serum from adult male at 25 days. (h) Serum from pregnant female at 40 days. The upper trace in each case contains serum from egg-maturing females which served as control. After electrophoresis the trough was filled with antiserum to blood from egg-maturing females. Antiserums were obtained from rabbits repeatedly injected with the serum of cockroaches (*4*). The arrows indicate the female specific protein.

tein concentrations after surgery are difficult to interpret in the context of egg maturation.

An estimation of qualitative changes in serum proteins seemed therefore more meaningful and promising. In serums of egg-maturing, unoperated females, one specific protein stays at the origin or moves slightly toward the cathode during electrophoresis. This protein is reliably detected immunologically. It was never found in serums of those females that are not maturing eggs. It was never found in males (Fig. 1) or nymphs of either sex. It was detected in serums of all 14 females with cauterized pars intercerebralis that matured eggs 4 to 5 weeks after this operation but not in the remaining 48 operated females that did not mature eggs (Fig. 1). The complete removal of the median and lateral neurosecretory cells of the pars intercerebralis was verified histologically in all of these operated animals. This result suggests that the presence of the female specific protein is correlated with active corpora allata and is unrelated to the availability of neurosecretory hormones from the pars intercerebralis. This observation, supporting earlier conclusions (*4*), shows that the corpus allatum hormone is perhaps involved in the biosynthesis of a specific protein in females and that this protein is essential for egg maturation. Saline extracts of fat bodies, midguts, and inactive ovaries of allatectomized females did not contain the specific protein. It was, however, extracted from fat bodies of egg-maturing females. This latter observation shows that presumably the fat bodies synthesize the specific protein; whether the corpus allatum hormone also stimulates its release from the fat bodies is not known. The female specific protein, together with four of the other serum proteins, was present in saline extracts from mature oocytes, but not in distilled-water extracts (Fig. 1). As has been shown (*6*), yolk proteins are practically insoluble in distilled water.

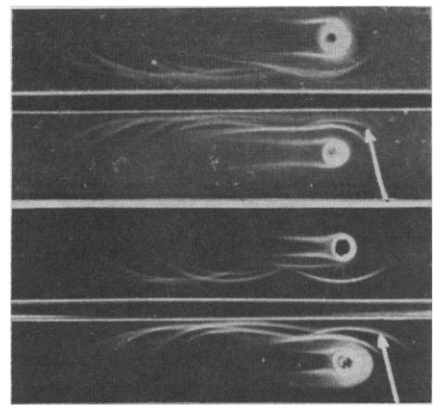

Fig. 2. Immunoelectrophoresis of serums from allatectomized females to whose isolated abdomens the *t,t,t*-isomer of the authentic juvenile hormone had been applied (upper) 400 TU; (lower) 600 TU. These females had never produced the female specific protein before application of the hormone and therefore its appearance denotes *de novo* synthesis and not release of stored protein. The arrows indicate the female specific protein.

In a further series, females of *Leucophaea* were allatectomized 1 to 3 days after emergence before they had received food as adults and before the initiation of synthesis of the specific protein. They then received food for 4 to 5 weeks (in order to allow the accumulation of reserves) and the *t,t,t*-isomer (*7*) of the juvenile hormone (JH) was topically applied to the dorsum of the ligated abdomens (*8*). It was found that 0.08 to 0.12 μg (400 to 600 *Tenebrio* units) of the JH caused the appearance of the female specific protein within 3 to 4 days (Fig. 2); traces of yolk were observable in the oocytes on the 7th or 8th day. Thus this isomer of the authentic JH has the same biological activity in adult female cockroaches as the corpora allata. Since the isolated abdomens do not contain known endocrine glands involved in egg maturation and since ovariectomized females produce the specific protein, it is unlikely that the induced synthesis of the female protein after application of the hormone is caused by other mechanisms than the one suggested here.

It was essential to determine whether the corpus allatum hormone stimulates the synthesis of the female specific protein alone or whether it also causes an increased rate of synthesis of the nonsex specific proteins. All females used in the following experiments were adults, 4 to 5 weeks old. Females which were at their peak rate of yolk deposition and those allatectomized shortly after emergence were injected with 1.4 μc of C^{14}-leucine. Allatectomized females with ligated necks received four active corpora allata or were treated with 1 μg of the synthetic JH 5 days before injection of the labeled leucine. Four hours after injection of labeled leucine the animals were bled by puncturing the neck membrane; the animal was then centrifuged head down in a tapered centrifuge tube at low speed. The proteins of a sample of serum were precipitated with 10 percent trichloroacetic acid. Additional samples were used for precipitation of all of the nonspecific proteins by use of antiserum to male hemolymph; the female specific protein was precipitated with the female specific antibody. After the antigen-antibody precipitates had been washed in saline, they were again pre-

Table 1. Incorporation rate of C^{14}-leucine into serum proteins of normal and treated *Leucophaea maderae* females. Proteins from equal samples of serums were either precipitated by 10 percent trichloroacetic acid or exhaustively treated with antiserums to male hemolymph (which do not contain the female specific antibody) or with female specific antibody. The female specific antibody was obtained by precipitation of the nonsex specific antibodies from female antiserums with male serum. The amounts of the total radioactivity in nonspecific and female specific proteins were calculated from the percentage of radioactive material precipitated by the antiserums. Data in these cases are not expressed as specific activity because the amounts of precipitated antibody proteins from the antiserums were several times those in the blood serum proteins and because the cockroach serum always contains relatively more nonspecific than specific proteins; the obtained values would thus lead to misinterpretations. The radioactivity of the proteins was determined in a Nuclear-Chicago Scintillation Counter and is expressed as disintegrations per minute (dpm). Numbers after ± are standard errors. Numbers in parentheses are the number of animals used per series.

Test animals	Specific activity of the total serum proteins (dpm/mg protein)	Fractions of label in proteins precipitable with antiserums	
		Nonsex specific serum proteins (dpm)	Female specific serum protein (dpm)
Egg-maturing females	12,300 ± 1,600 (7)	4,600 ± 240 (5)	4,600 ± 870 (5)
Allatectomized females	4,300 ± 450 (5)	1,700 ± 370 (4)	0 (4)
Allatectomized and neck-ligated females with corpus allatum implants for 5 days	6,900 ± 1,900 (6)	4,300 ± 1,100 (6)	2,300 ± 1,400 (6)
Allatectomized and neck-ligated females treated with 1 μg of JH for 5 days	5,700 ± 890 (7)	2,400 ± 550 (7)	4,100 ± 750 (7)

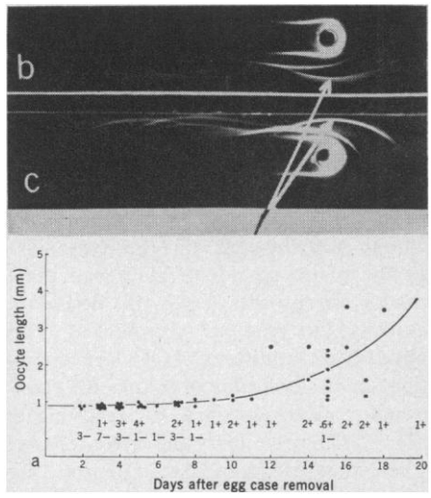

Fig. 3. (a) Initiation of egg maturation on removal of the egg cases in pregnant females (4 to 5 weeks) whose serums were subjected to electrophoresis. The number of animals which had the female specific protein in the hemolymph is indicated by a +. (b) Egg-maturing female. (c) Egg-maturing female 4 days after removal of the egg case. The arrows indicate the female specific protein.

cipitated in 10 percent trichloroacetic acid and then used for protein and radioassay. The specific activity of the total serum proteins of egg-maturing females was nearly three times that of allatectomized females (Table 1). The rate of the synthesis of the female specific protein in normal egg-maturing females was about as high as that of the other serum proteins together. The rate of synthesis of the nonspecific proteins was three times higher in the egg-maturing females than in allatectomized ones. Treatment of serums from allatectomized females with the antibody to female specific protein yielded no precipitate. Implantation of active corpora allata into allatectomized females with ligated necks caused both a high rate of synthesis of the female specific protein and an increase in the synthesis of the nonspecific protein (Table 1). Application of 1 μg of JH caused the *de novo* synthesis of the female specific protein at a somewhat higher rate than the implantation of four active corpora allata (*9*).

One can conclude that the corpus allatum hormone causes not only the *de novo* synthesis of a female specific protein but also increased general protein synthesis. Since the head was ligated in the allatectomized females, neurosecretion from the pars intercerebralis or hormones from the corpora cardiaca probably can be excluded as controlling agents of the observed protein synthesis. These results do not, however, negate any additional roles that those latter endocrine glands may play.

My data are in agreement with the hypothesis that the presence of the female specific protein is a prerequisite for egg maturation and not merely a concomitant of egg maturation. Additional observations strengthen this conclusion. The egg cases of females that had been pregnant for 4 to 5 weeks were removed, an interference that leads to a renewed egg maturation visible after about 8 to 12 days. Samples of serum were taken from these animals at daily intervals. Immunoelectrophoresis showed that some females had begun to produce the specific protein 3 to 4 days after removal of the egg cases (Fig. 3). This is 3 to 5 days before any traces of yolk deposition are detectable in the oocytes and before the accessory sex glands exhibit signs of activity. This finding corroborates analogous observations after treatment with JH; several days before yolk deposition begins, the female protein is present in the hemolymph. The importance of this protein for egg maturation is also suggested by the fact that more than 80 percent of the protein extractable from mature eggs is identical with the female specific protein (*6*). Although other nonspecific proteins contribute relatively little to the yolk, the fact that their synthesis is stimulated by the corpus allatum hormones suggests that they are essential in egg maturation.

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References and Notes

1. L. Hill, *J. Insect Physiol.* **8**, 609 (1962); K. C. Highnam, O. Lusis, L. Hill, *ibid.* **9**, 587 (1963).
2. W. Mordue, *ibid.* **11**, 617 (1965).
3. G. C. Coles, *Nature* **203**, 323 (1964); *J. Insect Physiol.* **11**, 1325 (1965).
4. F. Engelmann, *Arch. Anat. Microsc. Morphol. Exp.* **54**, 387 (1965); *Amer. Zool.* **5**, 673 (1965); F. Engelmann and D. Penney, *Gen. Comp. Endocrinol.* **7**, 314 (1966).
5. W. J. Bell, *Amer. Zool.* **8**, 755 (1968).
6. V. J. Brookes and R. K. Dejmal, *Science* **160**, 999 (1968).
7. The juvenile hormone is methyl-10-11-epoxy-7-ethyl-3, 11-dimethyl-10, 11-*cis*-trideca-2-*trans*-6-*trans*-dienoate.
8. I thank Dr. H. Röller, Texas A & M University, for supply of the juvenile hormone.
9. The amount of radioactivity precipitated by the specific and nonspecific antibodies does not always add up to the total radioactive precipitable by trichloroacetic acid, an indication that the hemolymph contains antigens for which no antibodies are available.
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Field Potentials Generated by Dendritic Spikes and Synaptic Potentials

Abstract. *Predictions from the cable model and equations for field potentials generated by single neurons are computed and compared with extracellular recordings from synaptically activated cerebellar Purkinje cell dendrites. Neither theory predicts the results, nor does the experimental situation satisfy the assumptions of either theory. Theoretical calculations from a recent formulation developed by Rall compare favorably with potentials recorded by other authors. Applications of these formulations are discussed.*

Some controversy has arisen over the interpretation of potentials recorded extracellularly from alligator cerebellar cortex (*1*). At issue is the applicability of the cable model of neurons as opposed to the volume conductor theory (*2, 3*). The problem is of interest as an example of the general difficulty of interpreting extracellularly recorded potentials from complex neural tissue. I will analyze the predictions of each theory and apply a new formulation developed by Rall and Shepherd (*4*) which overcomes many of the failures of the classical approximations.

Llinás *et al.* (*1*) have adduced evidence in favor of propagating dendritic action potentials. They stimulate electrically a surface sheet or beam of parallel fibers which form the main excitatory input to Purkinje cells and record extracellularly at various depths in the cortex. A negative wave appears and its latency is increased with deeper electrode placements. They contend that this progressive delay with increasing depth implies active propagation of spikes through the dendritic tree. A conditioning surface stimulus abolishes this transient response to a subsequent test stimulus, presumably via interneuronal inhibitory pathways. A slow surface-negative, depth-positive potential remains which is interpreted as an excitatory postsynaptic potential (EPSP). Inhibition may not only abolish spikes, but also can reduce the amplitude of some EPSP's and enhance others, depending on the postsynaptic geometry (*5*); hence this inhibitory effect cannot be used to determine whether the first wave is a spike or an EPSP. Other evidence contributes to the interpretation of the fast transient as a dendritic spike, but these supporting results are inconclusive, and Llinás