from the intravenous injection of atropine (0.5 mg/kg) (A to C, part 4). This procedure was used in the last neuron examined in a given cat and only after completion of electrophoretic drug tests. Reticular stimulation before drug application raised the mean neuronal firing rate during the 2 seconds following each stimulus (D to F, part 1) to one and a half times the spontaneous firing rate (A to C, part 1). Acetylcholine increased the poststimulus firing, especially in the first 600 msec after application of the stimulus (D to F, part 2). Atropine electrophoresis reduced poststimulus firing (D to F, part 3) and especially abolished the early increase in firing after reticular stimulation. Atropine applied intravenously reduced the firing after reticular stimulation (D to F, part 4) to the level that was obtained during spontaneous firing after intravenous injection of atropine (A to C, part 4). In other words, systemic administration of atropine completely abolished the activating effect of reticular stimulation. In contrast, local applications of ACh and atropine had no effect upon transmission of the light response in this neuron (G to I, parts 1 to 3). However, injection of atropine intravenously diminished firing during the bright phase of the light stimulus (G to I, part 4).

Acetylcholine facilitation and atropine depression of reticular excitatory action were observed in several neurons, many of them not showing a similar drug effect while firing in response to intermittent retinal illumination, to electrical shocks to the optic radiation, to transcallosal afferents, or while firing spontaneously. Some neurons that did show drug effects under these circumstances also showed, when tested, facilitation of the drug-susceptible activities by a conditioning stimulus to the MRF. In a few neurons, the response to electrical stimulation of the visual path was altered both by reticular stimulation and by drug application, but neither manipulation had an effect upon the flash response. In these instances, showing drug effects upon firing not induced by reticular stimulation, it can be assumed that drugs acted upon excitatory reticular input which then modified neuronal firing under the other conditions. The same mechanism could apply in cases in which reticular stimulation and drugs altered the spontaneous firing rate. Since the contribution of reticular input to the spontaneous firing rate differs from neuron to neuron and varies in a given neuron with time, level of alertness, and so forth, this mechanism would explain why testing of the spontaneous firing rate, especially in animals under general anesthesia, is not likely to reveal the nature of ACh action.

Electrophoretic application of atropine occasionally has a local anesthetic effect on single neurons (*6*). This action was seen in some instances and lasted rarely longer than a few minutes after the end of electrophoresis. It could be distinguished by the fact that it reduced all types of neuronal activity indiscriminately and was not limited to neurons reacting to ACh.

The differential effect of ACh and atropine upon transmission of reticular excitatory impulses, although not excluding the possibility of drug action upon presynaptic terminals of fiber connections from the MRF, is in agreement with the hypothesis that ACh mediates synaptic transmission of reticular excitation to cortical neurons. This hypothesis is supported by evidence accumulated with different methods (*7*). The difference in the effects of electrophoretic and intravenous application of atropine suggests that the latter reaches receptors which are presumably located at a distant link in a chain of afferent neurons and not available to the former.

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25-Hydroxycholecalciferol: Direct Effect on Calcium Transport

Abstract. *The perfused small intestine from a vitamin D deficient rat exhibits about one-half the calcium transport of the intestine from a rat given vitamin D. These levels of calcium transport can be maintained for at least 4 hours. Addition of 2.5 micrograms of 25-hydroxycholecalciferol added to the vitamin D deficient intestine via the arterial blood perfusate induces a rise in calcium transport to +D levels within 2 hours. In contrast, 250 micrograms of vitamin D_3 given in the same manner has no effect on the calcium transport level over a 4-hour period. These data provide strong evidence that 25-hydroxycholecalciferol represents the metabolically active form of vitamin D_3.*

Since the identifications of vitamins D_2 and D_3 by Windaus *et al.* (*1*, *2*) and Askew *et al.* (*3*), it has been assumed that the unaltered vitamin D itself carries out the vitamin's physiologic functions. In 1966 Lund and DeLuca established the existence of a highly biologically active vitamin D metabolite in rats (*4*). This metabolite has recently been isolated and identified as 25-hydroxycholecalciferol (25-HCC) (*5*, *6*) and has been chemically synthesized (*7*). When injected into the jugular vein of a vitamin D deficient rat, 25-HCC is approximately 1.4 times more active than vitamin D in curing rickets (*6*). More importantly, 25-HCC exhibits its in vivo activity in stimulating bone mobilization and intestinal calcium transport more rapidly than an equivalent dose of vitamin D_3 (*8*). This time lag suggests that vitamin D_3 must be converted to 25-HCC before its biological activity is manifested. Studies of early vitamin D metabolism implicated the liver in this conversion (*9*) and recently, by the use of hepatectomized rats, it has been demonstrated that the liver is the major if not the only site of 25-HCC production (*10*). In addition, a liver enzyme system has been obtained which catalyzes the conversion of vitamin D_3 to 25-HCC (*11*).

Direct evidence is now available in

support of the hypothesis that 25-HCC is responsible for vitamin D action. Trummel *et al.* have demonstrated that 25-HCC at a concentration of 0.9 unit/ml is effective in stimulating in vitro release of Ca^{45} from bone tissue cultures, while 400 units of vitamin D_3 per milliliter has little or no effect in this system (*12*). The results in the present report demonstrate that 25-HCC is highly effective in stimulating calcium transport in an isolated perfused intestine, while large amounts of vitamin D_3 have no effect.

Male, 20-day-old albino rats were maintained on a vitamin D deficient diet with normal mineral content (0.47 percent Ca, and 0.3 percent P) as described earlier (*13*). After 4 weeks the animals were vitamin D deficient, as shown by reduced growth and low serum calcium. All experimental animals were starved in a tubular restraining case (to minimize coprophagy) for 24 hours prior to use. At 12 hours prior to use, vitamin D treated rats received a single dose of 2000 units of vitamin D_3 in 0.1 ml of cottonseed oil via a blunt needle inserted well into the esophagus. The experimental rats were anesthetized with sodium pentobarbital and the small intestine was isolated for perfusion as described (*14*). A solution of 5 percent blood serum from vitamin D deficient rats, 4.7 percent dextran of high molecular weight (*15*), and 0.1 percent glucose in Krebs-Ringer bicarbonate buffer was pumped at 120 pulses/min under a pressure of about 100 mm-Hg into the superior mesenteric artery. After passing through the capillary bed of the small intestine this blood perfusate drained from the portal vein at 2 to 3 ml/min. A reservoir of the same solution containing Ca^{45} was pumped (Harvard pump) at 0.08 ml/min through the intestinal lumen from the duodenum to the ileum.

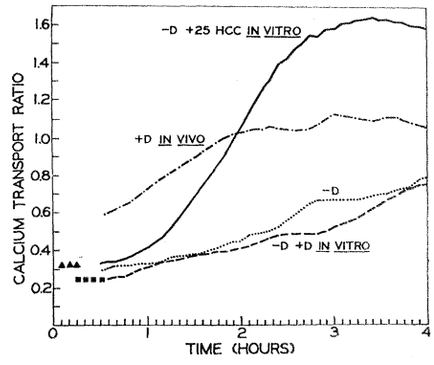

Fig. 1. Time course of calcium transport in perfused rat intestines in response to vitamin D_3 and 25-HCC. Intestines were treated as described in the text. The solid triangles and squares represent infusion of 25-HCC and vitamin D_3, respectively.

It took 10 to 20 minutes for this solution to fill the intestine and begin to drain from the ileal cannula. After this time, parallel 5-minute fractions of the blood and lumen perfusates were collected. The ratio of the amount of Ca^{45} in the blood perfusate to the concentration of Ca^{45} in the lumen effluent was taken as an index of calcium transport. (The use of the word "transport" is not intended to imply that the observed flux of Ca^{45} is necessarily the result of active transport. This experimental design will not differentiate between any of the processes involved in intestinal absorption.)

Intestines from deficient rats were treated with 25-HCC or vitamin D_3 as follows. One-fourth microgram of 25-HCC or 250 μg of vitamin D_3 dissolved in 10 μl of 95 percent ethanol was suspended in 15 ml or 20 ml, respectively, of the blood perfusate. This solution was infused into the arterial perfusate at approximately 1 ml/min.

The time course of calcium transport as measured by the amount of Ca^{45} in the blood perfusate divided by the concentration of Ca^{45} in the lumen (calcium transport ratio) is shown in Fig. 1. Small intestines from vitamin D deficient rats have a low rate of calcium transport as characterized by a calcium transport ratio of about 0.3 at ½ hour, gradually rising to a calcium transport ratio of about 0.7 at 4 hours. The classical effect of vitamin D on calcium transport is shown by intestines from vitamin D deficient rats which have been dosed with 2000 units of vitamin D_3 12 hours prior to the intestinal isolation. The calcium transport ratios of these intestines were about 0.6 at one-half hour and rose to about 1.1 after 4 hours. Thus a high but physiological dose of vitamin D doubled the calcium transport as observed in this system.

Vitamin D deficient intestines which have been exposed to 100 units of 25-HCC during the first 15 minutes of perfusion exhibit a pronounced change in calcium transport over the 4 hours of the perfusion. Initially these intestines have a low calcium transport ratio, as is expected for vitamin D deficient intestines. However, 45 to 50 minutes after the introduction of 2.5 μg of 25-HCC into the arterial blood perfusate, the level of calcium transport begins to rise, and within 2 hours after 25-HCC administration the calcium transport of these intestines is at the level exhibited by intestines from vitamin D treated animals. This elevated calcium transport is maintained for the remainder of the 4-hour perfusion. In direct contrast, intestines from vitamin D deficient rats exposed to 10,000 units of vitamin D_3 during the first 30 minutes of the perfusion showed the same time course of calcium transport as untreated intestines from vitamin D deficient animals. The confidence limits of the results presented in Fig. 1 are given in Table 1. An "overshoot" in calcium transport at 3 hours in 25-HCC treated intestine is frequently noted, although not highly significant statistically (Fig. 1). Several possible reasons for this can be visualized. Since it is unknown if such an overshoot is noted in vivo in response to 25-HCC, additional comment would seem premature.

These results clearly demonstrate that within the time of the perfusion the isolated small intestine responds readily to 25-HCC while massive amounts of vitamin D_3 produce no response. If vitamin D_3 were capable of stimulating calcium transport in an isolated intestine, there is good evidence

Table 1. The influence of vitamin D_3 and 25-HCC on calcium transport in perfused intestine. Intestines were treated as described in the text. The numbers in parentheses represent the number of rats used to determine the mean and the standard error of the mean.

Time (hours)	Calcium transport ratios			
	− D (vitamin D deficient)			
	No treatment ($N = 5$)	+10,000 units of vitamin D_3 ($N = 5$)	+ 100 units of 25-HCC ($N = 4$)	+D (not deficient) ($N = 5$)
1	0.33 ± 0.01	0.31 ± 0.02	0.41 ± 0.02	0.73 ± 0.04*
1½	.38 ± .01	.37 ± .02	.67 ± .05†	.89 ± .02*
2	.45 ± .03	.41 ± .02	1.05 ± .09*	1.03 ± .05*
3	.67 ± .02	.52 ± .03	1.58 ± .12*	1.12 ± .07*

* Different from the −D (no treatment) transport ratio at better than the 99 percent confidence level. † Different from the −D (no treatment) transport ratio at better than the 97.5 percent confidence level.

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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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-

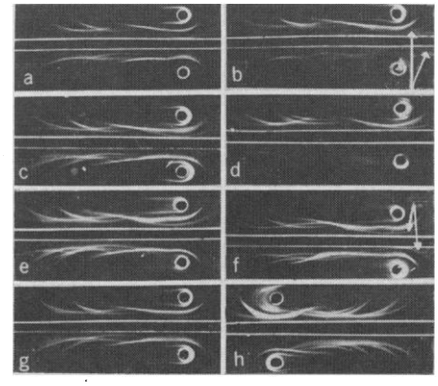

Fig. 1. Immunoelectrophoresis of serums from normal and operated females, of serums from males, and of extracts from nearly mature oocytes. (a) Serum from female adult at 6 days. (b) Saline extract from oocytes. (c) Serum from allatectomized female. (d) Distilled-water extract from oocytes. (e) Serum 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.