The brains of diapausing borers implanted in brainless diapausing larvae did not prevent the photoperiodic response (Table 1, *A*).

Search for an endocrine organ in the 7th and 8th abdominal segments resulted in the discovery of secretory activity in the epithelial cells of the portion of the hindgut lying in the 7th and 8th abdominal segments. The secretory activity was detected in borer larvae undergoing diapause development, at which time the lumen of the intestinal tract was empty. No evidence of secretion into the gut lumen was detected; it was considered likely that the secretory products were being released into the hemolymph. This hypothesis was circumstantially supported by the observation that the intestinal muscularis was found to be very scant in this part of the digestive tract, and blood and hemocytes could be seen to be separated from the epithelial cells by only a very thin membrane. Cytological signs of secretory activity observed were: (i) multilobate nuclei, (ii) numerous cytoplasmic granular inclusions that stained dark purple with paraldehyde-fuchsin, and (iii) numerous small cytoplasmic vacuoles. This apparent secretory activity increased in intensity in response to biochemical treatments that have been shown to accelerate the termination of diapause (*5*). Evidence of a photoperiodically regulated daily secretory cycle was also found. Aqueous extracts of the proctodeal epithelium in question caused the termination of the diapause state when injected into diapause larvae that had undergone partial diapause development (Table 1, *E* and *F*). From this experimental evidence, it was concluded that the proctodeal epithelium constitutes a site of hormone production. Because of its source, the name PROCTODONE is proposed for this hormone.

A series of surgical experiments demonstrated the role of proctodone in the activation of the larval brain. Inactive (from diapause) or fully active (from nondiapause) larval brains were implanted into diapausing larvae, some of which had been ligated between the 6th and 7th abdominal segments (Table 1, *A*, *B*, *C*, *D*). The operated larvae were held under an appropriate photoperiod and observed for pupation occurring within 20 days after treatment. The results showed that a brain of diapausing borer implanted into a diapause larva can be activated, unless the recipient larva had been subjected to an abdominal ligation. A brain from a nondiapausing insect, however, induced pupation whether or not the recipient larva had been ligated.

Like the ligation experiments already discussed, the surgical results indicated that the neuroendocrine processes of the fully activated brain were independent of the hormonal output of the proctodeal system. We were, therefore, led to the conclusion that the physiological function of proctodone is activation of the endocrine centers of the brain. The details of these experiments and of the probable relationships between endocrine functions and photoperiodism are being published elsewhere (*5*).

Using larvae of *Galleria mellonella*, which is a nonphotoperiodic, nondiapause species, we have found that a proctodone function also occurs. Larvae that had completed feeding and had left the rearing medium preparatory to pupation were subjected to abdominal ligation. Those ligated before cocoon spinning had begun were unable to complete their development, although they survived for several weeks. Ligatures applied after cocoon spinning had begun did not prevent pupation. As with the corn borer, *Galleria* larvae showed a proctodone requirement over a period of time that was somewhat shorter than the period of dependence on the brain hormone system. In both of these species, proctodone is apparently required for the activation of the endocrine functions associated with the brain. No other species have been investigated (*6*).

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Inhibition of Antibody Synthesis by L-Phenylalanine

Abstract. *Antibody synthesis in response to the injection of diphtheria toxoid into rabbits and rats was used to study the effects of an excess of the amino acid* L*-phenylalanine on protein synthesis. Injections of phenylalanine produced a marked inhibition of antibody synthesis in both the rat and the rabbit. The dosage of phenylalanine used caused an increase over the normal concentration of phenylalanine in the plasma and spleen of rats, but did not cause a loss of weight or serum protein changes in the treated animals.*

Sufficient evidence is available to indicate that antibodies are formed from the pool of free amino acids in the cell and that such synthesis requires a full complement of amino acids (*1*). It would appear that interference with this pool may have serious consequences on the antibody-forming system.

The administration of an excess of an amino acid to an animal on a nutritionally adequate diet might be expected to produce an alteration in some of the pools of free amino acids of the body. Such alterations could occur because the excess amino acid interfered with the transport of other amino acids into the cell. For example, Neame (*2*) has reported that phenylalanine interferes with the uptake in vitro of histidine, arginine, ornithine, and tyrosine by the brain. Other studies (*3*) have indicated that this type of inhibition may result from many amino acids entering the cell by way of a common carrier. An excess of one amino acid could saturate the carrier, thereby interfering with normal cellular transport.

Table 1. Hemagglutination titers of rats immunized with diphtheria toxoid. Group A received 0.85 percent saline. All groups also received an intraperitoneal injection of 0.2 ml of diphtheria toxoid adsorbed on aluminum phosphate which was followed by a second injection 3 weeks later. Serums were obtained 10 days after the second injection of toxoid. Initial injections of L-phenylalanine and diphtheria toxoid were given on the same day. The L-phenylalanine was injected intraperitoneally.

Group	Animals (No.)	Phenylalanine dosage (mg/kg)	Average titer*
A	7	0	3600
B	6	125	1200
C	6	180	700
D	6	240	180
E	5	300	140

* Reciprocal of dilution.

Table 2. Hemagglutination titers of rabbit antiserums to diphtheria toxoid. Intraperitoneal injections of L-phenylalanine (48 mg/kg per day; five times per week for 5 weeks) were started 1 week before immunization. All animals received an intramuscular injection of 0.5 ml of diphtheria toxoid and a similar injection 3 weeks later. Serums were obtained 7 days after the second injection. The figures below represent the secondary response. Control animals received 0.85 percent NaCl.

Control rabbit	Titer*	Exptl. rabbit	Titer*
1	625	1	5
2	3,125	2	
3	3,125	3	5
4	15,625	4	25
5	625	5	25
6	3,125	6	25
7	3,125	7	5
8	5		

* Reciprocal of dilution.

The inhibition of amino acid transport by phenylalanine or other amino acids may reduce the capacity of cells to synthesize proteins by limiting the availability of essential amino acids. Because of the similarities between antibody synthesis and the synthesis of other proteins (*4*), the inhibition of amino acid transport was employed in this study as a model to determine the effect of excess L-phenylalanine on protein synthesis.

Antibody was measured by the hemagglutination technique of Boyden (*5*) as modified by Stavitsky (*6*). Fluid diphtheria toxoid (*7*) was used to coat the red blood cells treated with tannic acid. Diphtheria toxoid (0.15 ml, Lederle) adsorbed on aluminum phosphate was injected intramuscularly into 2.0- to 3.0-kg male rabbits and intraperitoneally into 80- to 100-g male Sprague-Dawley rats. Blood was obtained from the rats by decapitation and from the rabbits by cardiac puncture.

The concentration of phenylalanine (0.56 g of DL-phenylalanine/kg), approximated that previously used (*7*), where it was found that this concentration would increase the plasma concentration of phenylalanine from 16 μg/ml to 24 μg/ml at 5 hours. However, Hier (*7*) used dogs and the amino acid was administered orally. To determine if the concentrations used here would increase the concentration of plasma amino acid, ten rats were injected intraperitoneally with 300 mg of L-phenylalanine per kilogram of body weight. Three hours after injection, the plasma was analyzed for phenylalanine (*8*). The plasma phenylalanine increased from 0.05 μmole/ml to 0.21 μmole/ml (average of ten control and ten experimental animals). In addition, the phenylalanine concentration in the spleen was increased from 0.17 to 0.86 μmole per gram of spleen (wet weight). Since the animals were injected 5 days each week, presumably the phenylalanine in the plasma and spleen was elevated during a part of each 24-hour period. Measurements of the phenylalanine in the serum of rats 24 hours after the injection of 300 mg of phenylalanine per kilogram of body weight indicated no significant difference from the normal. The addition of 0.21 μmole of phenylalanine per milliliter to the serum of the control animals did not inhibit the hemagglutination titer.

Table 3. Average titers of rat serums 1, 2, 3, and 4 weeks after a second injection of diphtheria toxoid. Phenylalanine-treated rats received daily 300 mg per kilogram of body weight; methionine-treated rats received daily 300 mg per kilogram of body weight; control rats received 0.85 percent sodium chloride. Each result is the average of duplicate antibody determinations on 15 rats. Titers are reciprocals of dilutions.

Week	Control	Methionine	Phenylalanine
1	1664	1480	84
2	570	336	90
3	64	64	28
4	27	136	100

The effects of L-phenylalanine at various dosages on the antibody response to diphtheria toxoid are summarized in Table 1. A graded response to the amino acid is apparent with the most significant inhibition occurring at the two highest dosages.

Similar experiments with rabbits were conducted to determine if this phenomenon could be reproduced in another laboratory animal. The results of this experiment are presented in Table 2. These data indicate that the immune response of the rabbit is more sensitive to the presence of excess phenylalanine than that of the rat since a more pronounced inhibition of antibody response occurred at lower concentrations of phenylalanine.

Since the possibility exists that the results could be explained as a minor delay in antibody synthesis rather than as a real suppression, antibody titer was measured 1, 2, 3, and 4 weeks after the booster injection of diphtheria toxoid. The results in Table 3 suggest that although the titer for the control animals does decrease with time, the values for the L-phenylalanine animals remain low during the 4-week period. Furthermore, methionine at comparable dosages, has no effect on antibody synthesis and thus it would appear that the phenylalanine effect is real and not the result of stress from repeated injections nor the result of a delay of synthesis.

The rats and the rabbits were weighed weekly and there was no significant difference in weight gain between the experimental and control groups. In addition, quantitative paper electrophoresis of the serums showed no significant difference in the concentration of the total or of the individual serum proteins.

From the foregoing, it may be tentatively concluded that selective inhibition of protein synthesis follows the administration of phenylalanine. However, a decrease in antibody response must be interpreted with caution since the observations noted could also be explained by an inhibition of antibody release or an accelerated degradation (*9*).

However, if the effects reported are the result of impairment of antibody synthesis, then the capacity of excess phenylalanine to depress this synthesis suggests a possible mechanism for the brain damage in phenylketonuria (*10*). The high concentration of phenylalanine in the plasma, by interfering with the transport of other amino acids, could alter the pattern of cellular amino acids resulting in the inhibition of the synthesis of certain types of proteins.

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