likely that the inhibition by reserpine of the olfactory blockage of pregnancy results from the release of prolactin from the anterior pituitary, which counteracts the accelerating influence of the pheromones produced by male mice on estrus in females (*10*). My results are direct evidence of hypothalamic mediation in the male-induced olfactory blockage of ovo-implantation in mice.

C. J. DOMINIC*
Physiological Laboratory, University of Cambridge, Cambridge, England

References and Notes

1. C. J. Dominic, *J. Reprod. Fertility* **8**, 266 (1964); *ibid.*, in press.
2. H. M. Bruce, *Nature* **184**, 105 (1959).
3. ———, *J. Reprod. Fertility* 1, 96 (1960); A. S. Parkes and H. M. Bruce, *Science* **134**, 1049 (1961).
4. C. J. Dominic, *J. Reprod. Fertility* **10**, 469 (1965).
5. H. M. Bruce and A. S. Parkes, *J. Endocrinol.* **20**, 29 (1960); A. S. Parkes, in *Proc. Intern. Congr. Anim. Reprod. 4th, The Hague* (1961), p. 163.
6. C. J. Dominic, thesis, Univ. of Cambridge, 1965.
7. No difference could be detected in the reactions of females to inbred and outbred CBA males, which therefore have not been differentiated.
8. R. Gaunt, J. J. Chart, A. A. Renzi, *Ann. Rev. Pharmacol.* 3, 109 (1963); J. Meites, C. S. Nicol, P. K. Talwalker, in *Advances in Neuroendocrinology*, A. V. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963), p. 238.
9. J. L. Pasteels, *Ann. Endocrinol.* **22**, 257 (1961).
10. Estrus in unmated females also is accelerated by males [W. K. Whitten, *J. Endocrinol.* **17**, 307 (1958); H. M. Marsden and F. H. Bronson, *Science* **144**, 1469 (1964); C. J. Dominic, unpublished data].
11. Supported by the Population Council and the Ford Foundation. I thank A. S. Parkes and H. M. Bruce, University of Cambridge, for suggestions.
* Present address: Department of Zoology, Banaras Hindu University, Varanasi 5, India.

16 March 1966

Gibberellic Acid: Effects of Feeding in an Artificial Diet for Honeybees

Abstract. *Complete larval and pupal development occur in colonies of honeybees when adult bees are allowed to feed upon an artificial diet containing gibberellic acid. In the absence of gibberellic acid larvae die in the 3rd or 4th day of development.*

Pollen is the natural food of adult honeybees, *Apis mellifera* L., whereas larvae are fed on royal jelly or worker jelly secreted by glands in the head of nurse bees. Efforts to find substitutes for pollen have failed or have had only limited success (*1*). The failure to maintain larval rearing in a caged laboratory colony when adults are fed simple, inexpensive artificial foods has hindered study of the physiology of caste differentiation, nutrition, and behavior in honeybees.

We have developed a semi-defined diet which permits excellent egg-laying by the queen and allows larval development to an age of 3 to 4 days. However, for larvae to develop beyond the age of 4 days and pupate normally, we have had to add a minimum of 7.5 percent by weight of natural pollen or certain appropriate fractions of pollen to the artificial diet (in preparation). We now report that we have been able to rear bees through more than one generation by replacing all pollen in an artificial diet (Table 1) with gibberellic acid (*2*).

Small colonies of bees were confined outside in screened cages (1.8 by 1.8 by 1.8 m) and given 60 percent sugar syrup as desired. A fresh cake of food (20 g) was supplied to each colony daily (Table 1). Data showing the area of brood (eggs, larvae, and pupae) being reared by three colonies receiving 0.85 mg of gibberellic acid per gram of dry food compared with the control colony (colony 4) are given in Fig. 1. The control colony received the same artificial base diet fortified by addition of 7.5 percent natural pollen. Brood-rearing activities decreased in all colonies during late August 1965, which may possibly be the result of environmental factors. During the latter part of September and early October brood rearing in all colonies with the exception of colony 2 was stable, with 60 to 80 square inches (1 in^2 = 6.45 cm^2) of brood being maintained. In all colonies larvae developed beyond the 4th day of age and, after 2 weeks' feeding on the gibberellic acid diet, all stages of brood development were present in each colony. Al-

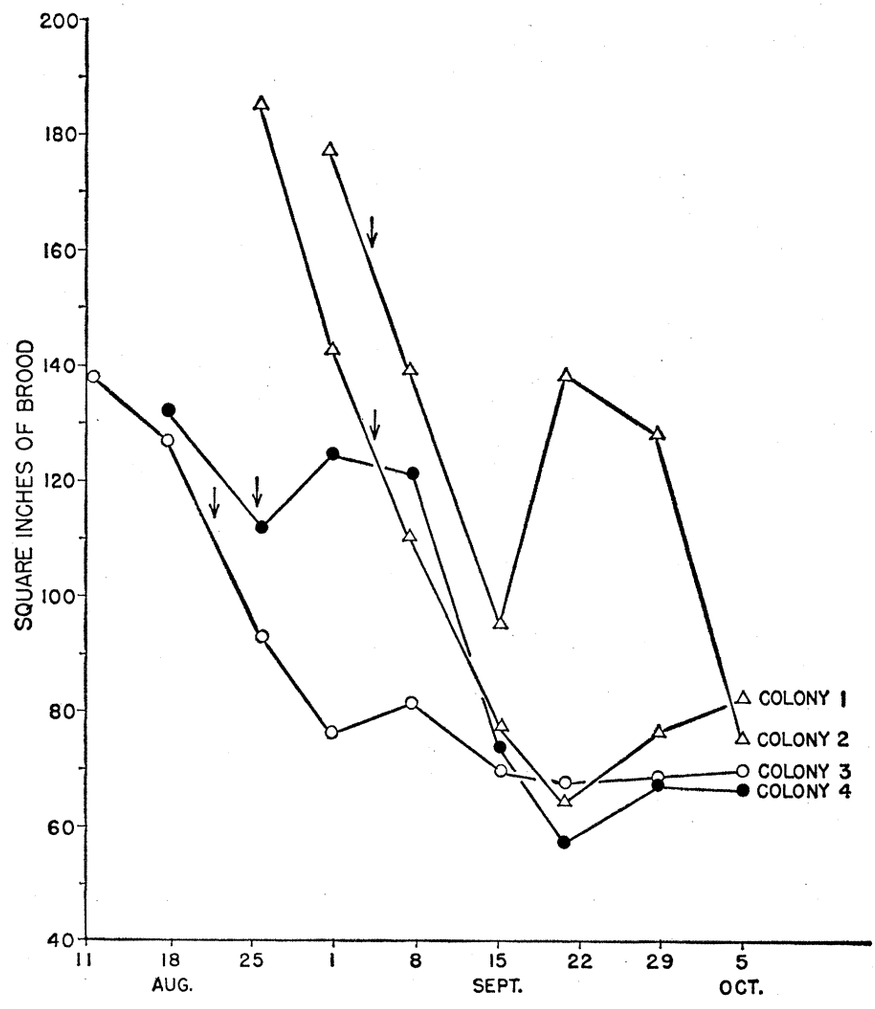

Fig. 1. The total square inches (1 in^2 = 6.45 cm^2) of brood in all developmental stages present in colonies feeding on an artificial diet containing gibberellic acid. The arrows indicate the dates on which the experimental diet was first fed. All stages of brood development were present in all colonies when the experiment was terminated on 11 October 1965. ○—○, Gibberellic acid (0.85 mg) plus traumatic acid (1 mg) per gram of dry diet; ● — ●, Artificial diet plus 7.5 percent mixed whole pollen; △—△, 0.85 mg of gibberellic acid per gram of dry diet.

though bees were not marked in any of these experiments, colony 3 bees received the gibberellic acid diet for nearly 8 weeks, during which time all stages in development were present.

The diet of colony 3 throughout this experiment also contained plant wound hormone, traumatic acid (1 mg of traumatic acid per gram of dry diet) (*2*). In further experiments, however, we found that traumatic acid alone in the artificial diet failed to allow larval development beyond 3 days of age. Gibberellic acid alone added to the artificial diet in colonies 1 and 2 did permit all stages of brood to develop. The value of gibberellic acid in promoting larval development seemed to depend upon a high concentration in the basic diet. Two colonies receiving 0.43 mg of gibberellic acid and 0.5 mg of traumatic acid per gram of dry diet for 1 to 3 weeks did not rear young larvae beyond the 4th day of development.

Gibberellic acid may partly replace or substitute for some essential nutrient present in limited quantity or entirely absent from our basic diet. Gibberellic acid influences development in the desert locust, *Schistocerca gregaria* Forsk., and ecdysone purified from the locust had gibberellin-like activity on plants (*3*). It may be significant that *Apis mellifera* larval prothoracic glands, which produce ecdysone, develop rapidly about the 3rd day of larval life (*4*), the time approximating that prevailing in our experiments when the larvae died unless gibberellic acid or natural pollen was added to the artificial diet.

Whether a specific stage of development is influenced by gibberellic acid, or whether a more general effect occurs, cannot be determined from our experiments. Possibly gibberellic acid appears in the glandular secretions which nurse bees feed to larvae and thus exerts its effect directly on larval development, or gibberellic acid may somehow function in the adults to permit secretion of an adequate larval food while not appearing in the secretions.

Regardless of its function, incorporation of gibberellic acid into artificial diets for adult bees promises to become useful in the study of bee nutrition.

Table 1. Composition of artificial diet.

Compound*	Weight (%)
Casein, vitamin-free	5.0
Gelatin	5.0
Zein	5.0
Egg albumin	5.0
Wesson's salt mixture	5.0
Mazola corn oil	5.0
Cholesterol	0.25
RNA	1.0
Sucrose	45.0
Cellulose	23.75

* A thin cake made from 20 g of the dry diet mixed with filtered honey contained the following vitamins (in milligrams): riboflavin, 1.2; pyridoxine, 1.0; niacinamide, 8.0; thiamine, 1.0; ascorbic acid, 60.0; choline, 12.0; panthenol, 3.0; inositol, 12.0; carnitine, 0.46; *p*-amino benzoic acid, 0.48; glutathione, 1.18; biotin, 0.0298; vitamin B_{12}, 0.00064.

J. L. NATION

Zoology Department,
University of Florida, Gainesville 32601

F. A. ROBINSON

Department of Entomology, Institute of Food and Agricultural Sciences

References and Notes

1. M. H. Haydak, *J. Econ. Entomol.* **30**, 637 (1937); M. H. Haydak, *Amer. Bee J.* **97**, 90 (1957); N. Weaver, *Gleanings in Bee Culture* **92**, 550 (1964).
2. Gibberellic acid was purchased from Eastman Organic Chemicals Co. as an 85 percent pure preparation. It was not purified further. Dr. J. Joiner, Institute of Food and Agricultural Sciences, provided a sample of 85 percent gibberellic acid. Traumatic acid was obtained from Eastman Organic Chemicals Co., other diet chemicals from Nutritional Biochemicals. Corn oil was purchased locally.
3. D. B. Carlisle, D. J. Osborne, P. E. Ellis, J. E. Moorhouse, *Nature* **200**, 1230 (1963); P. E. Ellis, D. B. Carlisle, D. J. Osborne, *Science* **149**, 546 (1965).
4. F. Lukoschus, *Naturwissenschaften* **39**, 116 (1952).
5. This report is Florida Agricultural Experiment Station Journal Series No. 2373.

28 March 1966

Neurological Defect: Manganese in Phenocopy and Prevention of a Genetic Abnormality of Inner Ear

Abstract. *A specific congenital ataxia may be caused by presence of mutant genes and by manganese deficiency during prenatal development in normal mice. Supplementation of the diet of mutant mice with manganese during prenatal development rectifies the aberrant development, resulting in normal behavior. The congenital ataxia results from defective development of the otoliths.*

Previous work of Hurley *et al.* (*1*) has shown that a maternal dietary deficiency of manganese in rats and guinea pigs results in the birth of offspring affected with an ataxic condition characterized by incoordination, lack of equilibrium, and retraction of the head. This congenital ataxia was irreversible and was associated with defective morphogenesis of the vestibular portion of the inner ear. In studies on genetic-nutritional interactions with respect to manganese, we have first established that manganese deficiency in the mouse produces an ataxic condition similar to that seen in rats and guinea pigs. This was accomplished by using a purified diet in which the level of manganese was specified. Table 1 shows the results of experiments in mice derived from a cross of four inbred strains. The mice were continuously maintained on the respective diets through three litters, and some are being continued into succeeding generations. The defect, as scored by the inability of animals to orient themselves when submerged in water, increased in incidence with the length of time on the deficient diet. It is apparent that as the body reserves were depleted the incidence of the ear defect increased greatly. The same congenital defect has also been induced in each of three inbred lines (BALB/c, C57BL, and DBA).

Lyon (*2*, *3*) has shown that three nonallelic genes in the mouse affect the differentiation of the otoliths within the sacculus and utriculus of the inner ear. The resulting loss of postural reflexes is characterized by behavior of which the ataxic condition of manganese-deficient mice appears to be a phenocopy. One of the mutants studied by Lyon, the pallid gene, therefore has been tested for its response to manganese supplementation. Female

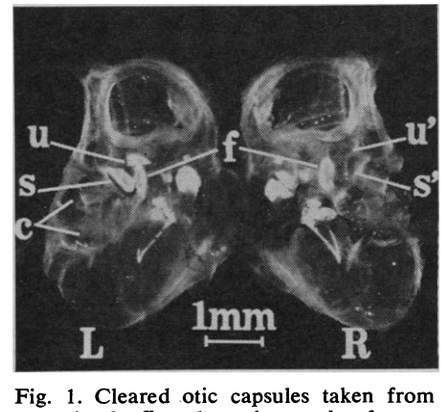

Fig. 1. Cleared otic capsules taken from an animal affected as the result of manganese deficiency. This animal had normal otoliths in the left utriculus and sacculus but was lacking them in the right ear. The unlabeled areas of density are portions of the ear ossicles. Symbols: *u*, utricular otolith present; *s*, saccular otolith present; *u'* and *s'*, otoliths absent; *f*, fenestra ovalis; *c*, cochlea; *L*, left ear; *R*, right ear.