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.

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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.
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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

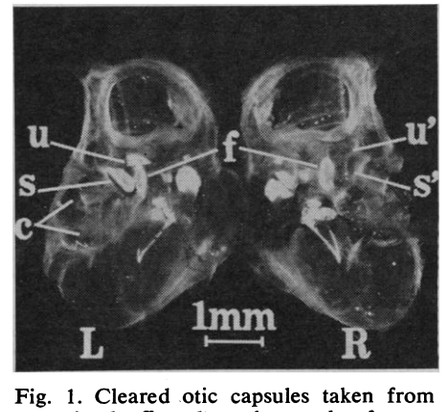


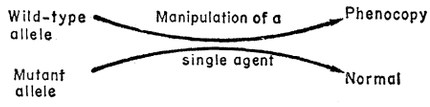
Fig. 1. Cleared otic capsules taken from an animal affected as the result of manganese deficiency. This animal had normal otoliths in the left utriculus and sacculus but was lacking them in the right ear. The unlabeled areas of density are portions of the ear ossicles. Symbols: *u*, utricular otolith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

mice homozygous for the pallid gene were given, during pregnancy, a purified diet possessing a high manganese content. Under these conditions none of the progeny of these females showed the postural defect characteristic of the pallid gene. When pregnant pallid females were given the normal diet used in the laboratory for maintenance of stock mice, a high proportion of their offspring showed the defect (see Table 2). This incidence is comparable to that reported by Lyon in strains in which she found variation due to genetic background and to maternal effects such as litter size and maternal age. It is plausible that such effects may, in fact, be related to manganese utilization. It should be pointed out that neither deficiency nor supplementation of manganese has any apparent effect on pigmentation itself.

It is with regard to manganese utilization that the report of Cotzias *et al.* (*4*) became especially relevant to our findings. They have shown by neutron activation that manganese is several times more abundant in pigmented than in nonpigmented tissues and have suggested that this fact explains some neurophysiological defects. For developmental phenomena the simplest hypothesis would require that manganese be available in or near the sensitive tissues and that the presence of pigmentation might insure an adequate supply of manganese. Since the membranous labyrinth appears to be the organ most sensitive to manganese deficiency, the recent reports of Mayer (*5*) are especially interesting. He found that normally pigmented strains of mice do have moderate pigmentation in the membranous labyrinth, and, furthermore, that certain white-spotted mutants have tissue-specific variation in the pattern of pigmentation.

We have now examined manganese-deficient and mutant mice by means of cleared, whole-mount specimens of the otic capsule. Following the method of Mayer we fixed the tissues in neutral formalin, dehydrated them in ethyl alcohol, and cleared them in wintergreen (methyl salicylate). More recently, Lyon's technique (*3*), by which she studied the penetrance of the pallid defect, has come to our attention. However, her fixation was carried out in 70 percent alcohol and clearing in benzyl alcohol. This very simple technique not only permits a comparison of pigmentation but, because of the refractive properties of the granular otoliths, the otoliths can be seen as white plates (Fig. 1). It is possible to score readily for their presence under an ordinary dissecting microscope. Moreover, morphological and quantitative differences can be observed in both manganese-deficient and mutant animals, and when all four otoliths are lacking the animal is completely incapable of orienting itself in the water. No other abnormalities in the labyrinth were detected. In addition, cases of asymmetry confirm those reported by Lyon—that is, the affected side is usually cocked upward, and the absence of utricular otoliths caused no obvious behavioral defects. It is interesting that the two coat-color mutants, pallid and muted, are lacking in pigmentation of the membranous labyrinth, while the "unbalanced" gene does not alter the coat color or pigmentation within the membranous labyrinth. Moreover, observation of large crystals in place of the normal otolith which was associated with some ability of the unbalanced mutants to orient themselves suggests a different mode of action for this gene. Supplementation of this mutant with manganese, although doubling the mean number of crystals, did not result in a normal otolith.

There are numerous examples wherein environmental manipulation, including temperature shock and various teratogenic agents, will mimic, or phenocopy, developmental effects of known genetic mutants. However, we know of no other demonstration in which the phenocopy-inducing agent has a reciprocal effect on the mutant itself:



The Himalayan gene present in many species, including rabbits and mice, may produce pigment when the skin is naturally or artificially reduced in temperature, but this is a repetitive, physiological phenomenon associated with hair growth.

Perhaps the nearest analogy to our results is the genetic defect in humans known as phenylketonuria. It is possible to circumvent the deleterious effects of this disease by a persistent restriction of the dietary uptake of phenylalanine during childhood. By contrast, a single, specifically timed supplement of manganese produces a complete and permanent remedy for a congenital defect associated with the inner ear. Conversely, once that specific time of development is past the defect is irreparable in both the mutant and the phenocopy.

Table 1. Birth, survival, and incidence of ear defect in control and manganese-deficient mice (*6*).

Group and No. of litters	Born (No.)	Survival to 21 days (%)	Unable to swim (%)
Control			
1st litters, 7	46	76	0
2nd litters, 8	54	91	0
3rd litters,5	41	95	0
Mn-deficient (3 ppm)			
1st litters, 8	71	46	9
3rd litters, 6	49	77	24
2nd generation, 8	63	84	83
Mn-deficient (1 ppm)			
1st litters, 7	42	64	4
2nd litters, 8	48	65	61
3rd litters, 6	40	70	100

Table 2. Effect of manganese supplementation on incidence of ear defect in pallid mice.

Group	Litters (No.)	Offspring (No.)	Unable to swim (%)
Stock diet control	29	162	68
Mn-supplemented (1000 ppm)	7	45	0

The role of manganese in the phenocopy and prevention of this genetic defect appears to be more directly involved in the differentiation of otoliths than other agents are in their respective processes. It was, in fact, the induction of a phenocopy by manganese deficiency that gave the first clue as to the nature of the "competition for food substances, either general or particular" postulated by Lyon (*3*). These results suggest the existence of an important interaction between these genes and manganese metabolism.

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greatly reduced penetrance. The gene, unbalanced, and a fourth one, tilted head, do not affect pigmentation but do show complete penetrance of imbalance. Our observations of these two mutants reveal abnormal crystals in place of the otoliths.
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6. These experimental animals were F_2 progeny of a four-way stock derived from the AKR, C57BL, C3H, and DBA inbred strains of mice. The synthetic diet is prepared in our laboratories with casein and cerelose as the basic ingredients and is fortified with oil, vitamins, and salts. The manganese content is in parts per million (ppm) of dry weight of complete diet; control diets contain approximately 50 ppm Mn^{++}.
7. Supported by NIH grants GM 701-05 and HD 01743-01.

24 March 1966

Visual Receptive Fields in the Cat's Retina: Complications

Abstract. *Visual receptive fields have been mapped with moving patterns in the cat's retinal ganglion cells. A small, general-purpose computer was used to collect a matrix of 2500 data points covering a 25°-by-25° region of space. The analysis of 40 units reveals the existence of many nonconcentric receptive fields and also the presence of line and edge detectors.*

Various attempts have been made to classify unit responses recorded from retinal ganglion cells. Perhaps the most generally accepted classification deals with the "on-center" and "off-center" aspects of the recordings, the surround showing a response "opposite" to that of the center of the field.

The suggestion has been that the fields are more or less concentric and that the more complex responses obtained from higher stations in the visual system are composed or integrated from these elementary concentric units. There have been, however, a few indications that such a view of the structure of the receptive field recorded from ganglion cells may be oversimplified. For example, Rodieck and Stone (*1*) point out that all of the receptive fields they mapped were to some extent radially asymmetric, and that in some cases the surround region could be detected over only part of the receptive field or not at all. Kuffler (*2*) had also noted the "asymmetry" of some fields.

The present investigation was undertaken as part of a larger program delineating efferent control of input in the visual system. In these studies the control mechanisms of an *XY* plotter were used to move a white disc on a black background on any *X* or *Y* dimension. A Computer for Average Transients (CAT 400A) was used to compute averaged response histograms in a fashion similar to that described by Rodieck and Stone (*1*). While this method affords a great deal of precision, it is not very flexible, so that the shape of the receptive field, which is two-dimensional, has either to be inferred or reconstructed laboriously from a number of such scans, or to be attained by mapping point-by-point by hand.

To gain a better understanding of receptive-field organization we decided to take full advantage of the flexibility of the *X-Y* stimulus control system used and to use a small, general-purpose computer (PDP-8), to collect and display the data. A program was designed that could achieve the following:

1) Generate appropriate electrical functions for the *X* and *Y* servo ampli-

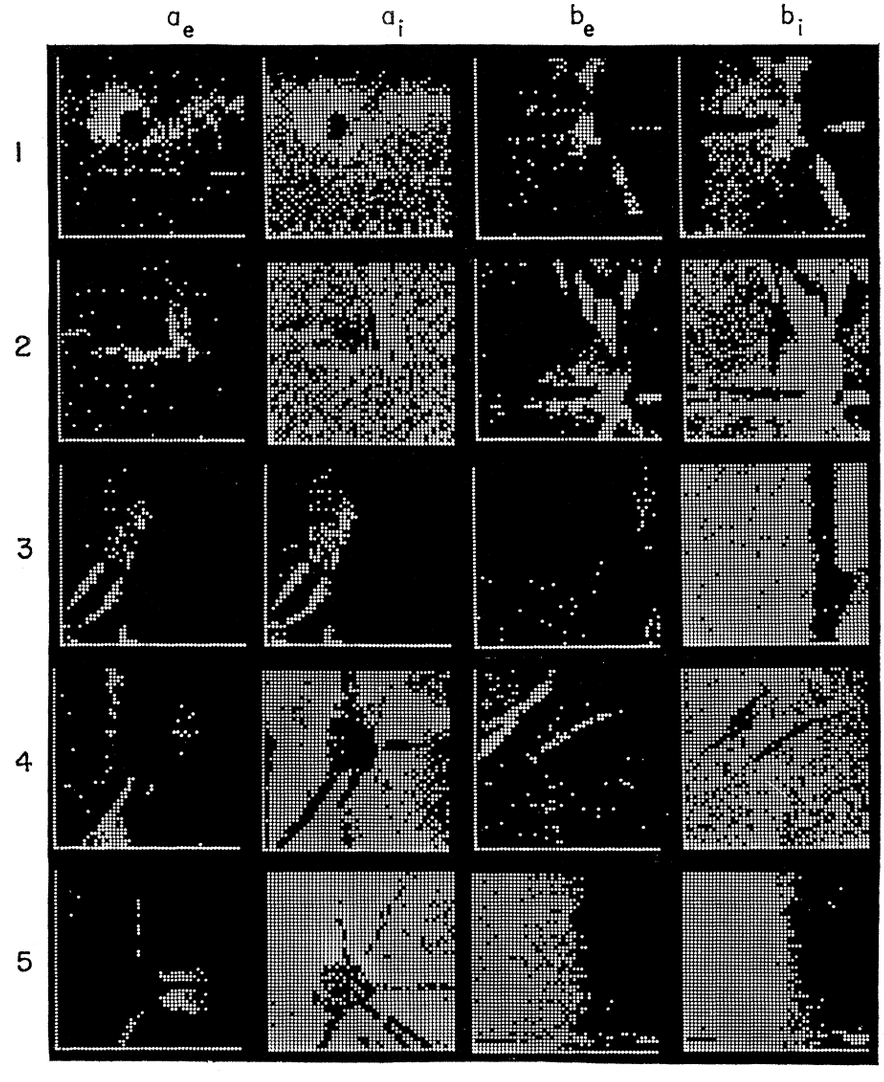

Fig. 1 (right). Columns a_e1 through b_e5 show, in order of "increasing complexity," ten of the receptive fields studied with this method. In column a_e, 1 through 5, the excitatory regions of five receptive fields are displayed by showing points where activity was greater than the mean background plus three standard deviations. In column a_i, 1 through 5, the inhibitory regions of the same five receptive fields are displayed as dark areas by showing all points with one or more counts. The same considerations apply to columns b_e and b_i, 1 through 5.