The presence of alcian-blue staining intracytoplasmic vesicles at the contact surfaces of the papillary growths may be interpreted as a stage in the secretion of "lubricants."

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Starch Formation Induced by a Plant Parasitic Nematode

Abstract. *This is the first report that a nematode causes plant tissues to produce starch. The formation of starch granules in the syncytial cells of several plant species is induced by* Nacobbus batatiformis. *The starch, which is associated with the feeding of the nematode, appears within a few days after the animal has become situated in the plant roots and diminishes in amount during nematode reproduction. The starch granules show the usual optical properties.*

Nacobbus batatiformis Thorne and Schuster (*1*), the Nebraska root-galling nematode, induces the formation of starch granules in plants. Such granules are not found in normal roots of certain plant species and varieties. Stimulation of starch formation in plant tissues infected by parasitic nematodes has not been reported previously. Owens and Novotny (*2*), who studied *Meloidogyne incognita* in tomato and cucumber roots, stated that "comparative chemical analyses show that . . . in the galls . . . starch disappeared." We found a decrease of starch in the vicinity of *Pratylenchus scribneri* in potato tubers and in the vicinity of *Heterodera trifolii* in roots of *Trifolium repens*. Starch formation was induced during the early stages of development of *Heterodera trifolii*. Starch was not detected in the walls of giant cells of soybean (*3*) and tomato (*4*) infected with *Meloidogyne* species. We have not found starch in giant cells of *Beta vulgaris* infected with *Meloidogyne incognita, Meloidogyne javanica, Meloidogyne hapla,* or *Heterodera schachtii*. Nor does *Pratylenchus penetrans* invoke obvious starch production in roots of wheat and *Chrysanthemum* species. DuCharme (*5*) noted a disappearance of starch around the lesions in grapefruit roots induced by *Radopholus similis*. Kostoff and Kendall (*6*) noted a starch accumulation in Nicotiana roots infected with root-knot nematodes but photographs showed no starch in giant cells or in their immediate vicinity.

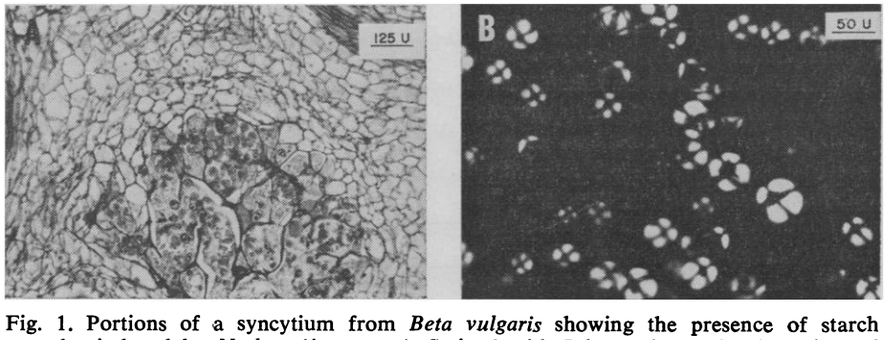

Fig. 1. Portions of a syncytium from *Beta vulgaris* showing the presence of starch granules induced by *N. batatiformus*. *A*, Stained with Johansen's quadruple stain and photographed through bright light (part of female nematode is in lower left-hand corner); *B*, photographed through crossed polaroids.

The syncytium that arises within the gall adjacent to the anterior portion of *N. batatiformis* is more translucent in appearance than the other cells (*7*). It may reach 3 mm in length and contain hundreds of cells. Starch can be demonstrated within syncytial cells in thin sections (10 μ) of galls by staining (Fig. 1*A*) with Johansen's quadruple stain (*8*) and can be identified by the use of crossed polaroids (Fig. 1*B*). In general, starch grains are more numerous and larger near the anterior of the nematode and decrease in size and number away from the feeding area. Some syncytial cells are completely filled with starch granules, whereas others contain a few small or large granules, or some of various sizes, even when such cells are equidistant from the nematode's stylet. Because the starch granules are located only near the anterior, their formation is probably associated with feeding.

Starch granules are formed soon after the nematode infects a root and before the syncytium is formed. The granules first appear at the time that the nuclei and nucleoli enlarge and before hypertrophy of the cortical and epidermal cells. Initially, the granules are located around the nucleus and later are scattered throughout the cell. The high concentration of starch in the syncytium indicates that a considerable amount of nutrients are drawn to this area. *Nacobbus batatiformis* apparently utilizes the starch after transforming it into a soluble substance, since the amount of starch decreases during nematode reproduction.

The starch granules induced by *N. batatiformis* do not appear to differ in optical properties from those that occur naturally in other plant tissues. The granules have conspicuous concentric layering around the hilum when observed under oil immersion with bright light or polarized light. Birefringent crosses typical of starch are evident when the granules are viewed through crossed polaroids (Fig. 1*B*). In the root galls of sugar beets, spinach, and common purslane, the granules, which are usually spherical, range from 1 to 30 μ in diameter; most of them measure from 5 to 10 μ, and the average diameter is 11 μ.

In roots grown in organ culture, males as well as females of *N. batatiformis* are capable of inducing the formation of galls and their associated

starch granules. However, under such conditions the starch granules are not so localized because the nematodes move around more actively than in soil-grown roots (*9*).

In an attempt to determine whether *N. batatiformis* induces starch production in other susceptible hosts, we found starch granules in infected roots of *Beta vulgaris, Spinacia oleracea, Lycopersicon esculentum, Chenopodium album, Portulaca oleracea, Opunitia tortispina, Kochia scoparia,* and *Euphorbia maculata.* These plants represent five different families. Syncytia in all freshly infected galls in all host species examined contained starch. Since starch granules cannot be detected as readily in preserved specimens, fresh material was examined.

Our findings suggest that the ability to induce starch formation in plants may be a general property of the nematode. Thus, tests for starch may prove valuable in the diagnosis of *N. batatiformis* infections.

The syncytium caused by *N. batatiformis* represents a peculiar type of pathological change in host tissues and is necessary for the nutrition of the nematode. The nutrients do not necessarily have to traverse vascular elements as has been postulated for species of *Meloidogyne* and *Heterodera.* The syncytium does not intercept translocated nutrients from the vascular system in order to supply *N. batatiformis* with adequate nutrition. This nematode has been found to develop and reproduce when the syncytium arises in the cortex completely divorced from the stele. In roots grown in organ culture, and in soil-grown sugar beets and other crops, *N. batatiformis* is found in cortical parenchyma in contrast to *Meloidogyne* and *Heterodera* which feed in the stelar area.

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Thalidomide: Effects on Enzymes of Glutamic Acid Metabolism in Mice

Abstract. *Thalidomide caused no significant alteration in the activity of glutamic decarboxylase in extracts of brain or of glutamic dehydrogenase or glutamic oxalacetic transaminase in extracts of brain or liver when added in vitro or injected into normal mice. Administered to pregnant mice, thalidomide had no effect on the activity of the latter enzymes in extracts of embryos with adhering uterus tissue.*

In the aftermath of the recent discovery of the pronounced teratogenic effect of thalidomide on the human embryo at a specific stage of development (*1*), numerous investigations are being devoted to determining the mechanism of action of this compound. From these studies and from experiments on the metabolic fate of thalidomide, several hypotheses have emerged which await experimental test. One of these (*2*) takes note of the fact that thalidomide gives rise in the body to a number of derivatives of glutamic acid and postulates that these derivatives, or thalidomide itself, may interfere in some way with the biochemical and physiological functions of this amino acid. This hypothesis is not supported by our experiments.

Glutamic decarboxylase (GDC) was determined manometrically in brain homogenates (*3*). The other assays were carried out separately in extracts of brain, liver, or embryos containing the loosely bound—that is, water-soluble—and the tightly bound—that is, digitonin-extractable—forms of the enzymes (*4*). To prepare these extracts, freshly excised tissue was homogenized in a Potter-Elvehjem homogenizer in distilled water at a concentration of 100 mg of tissue per milliliter. The homogenate was centrifuged at 4°C for 30 minutes at 15,000*g*. The supernatant constituted extract A. The residue was resuspended in the original volume of 1 percent digitonin in water; the suspension was allowed to stand for 10 minutes with occasional stirring and was centrifuged at 4°C for 30 minutes at 15,000*g*. This supernatant constituted extract B.

Spectrophotometric determinations were made of glutamic dehydrogenase (GDH) in the direction from α-ketoglutarate to glutamate (*5*), glutamic oxalacetic transaminase (GOT) (*6*), and lactic dehydrogenase (LDH) (*7*). All results were expressed on the basis of the total nitrogen content of the extracts or homogenates.

For tests in vitro, thalidomide, DL-*N*-(2,6-dioxo-3-piperidyl)phthalimide, at a final concentration of $1 \times 10^{-3}M$ was added to the reaction mixtures for the assay of glutamic decarboxylase of mouse brain, glutamic dehydrogenase of mouse liver, and glutamic oxalacetic transaminase in mouse brain or liver. No significant alterations in enzyme activity were observed.

For experiments in vivo, normal C57 black mice were given a series of three subcutaneous injections, on succeeding days, of thalidomide in 30 percent propylene glycol at a dose of 250 mg/kg of body weight per day. Control mice received three injections of the

Table 1. Effects of thalidomide administration in vivo on enzyme activities of mouse liver and brain. Values are expressed as change in optical density per minute per milligram of total nitrogen for all determinations except for glutamic decarboxylase for which the units are microliters of CO_2 per 30 minutes per milligram of total nitrogen. Results are the means ± the average arithmetic deviation.

Enzyme	Extract*	10^2 Units of enzyme activity	
		Control	Treated
		Liver	
GDH	A	7.7 ± 1.2	9.8 ± 2.4
	B	52 ± 16	66 ± 24
GOT	A	45 ± 8	59 ± 14
	B	66 ± 17	76 ± 18
LDH	A	185 ± 61	195 ± 12
	B	30 ± 16	43 ± 6
		Brain	
GDH	A	2.8 ± 1.3	1.9 ± 0.4
	B	6.6 ± 2	4.7 ± 0.5
GOT	A	79 ± 9	85 ± 14
	B	60 ± 4	57 ± 9
LDH	A	138 ± 35	133 ± 3
	B	65 ± 2	69 ± 3
GDC	H†	0.2 ± 0.02	0.26 ± 0.07

* Extract A contains the water-soluble portion and extract B the digitonin-extractable portion of the enzymes. † Total homogenate.