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.

M. L. SCHUSTER
ROBERT SANDSTEDT
LARRY W. ESTES
Plant Pathology Department,
University of Nebraska, Lincoln

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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.

suspending medium. Tissues were removed 1 hour after the last injection of the drug in order to determine enzyme activity. The mean results of three consecutive experiments are summarized in Table 1. It is evident that there is no significant difference in the activities of three enzymes taking part in glutamic acid metabolism in brain nor is there any significant difference in the activities of glutamic dehydrogenase and glutamic oxalacetic transaminase in liver. Lactic dehydrogenase, an enzyme requiring diphosphopyridine nucleotide but without any direct relation to glutamic acid metabolism, also was unaffected by thalidomide treatment in vivo. The injection schedule in these experiments may be considered adequate for producing the various derivatives of the drug in the body, and it may therefore be concluded that neither thalidomide nor its catabolic products cause a significant alteration in the activity of these enzymes in these two mouse tissues.

These results are not surprising since thalidomide has proved quite nontoxic in a variety of tests even at very high doses in various animal species, with the exception of its now well-known, highly specific, and selective teratogenic effect in man. Since it was shown recently (*8*) that congenital malformations can be produced by thalidomide in mice, an experiment was carried out to assess the effect of this drug on the enzyme content of the embryos of pregnant mice. A group of Paris mice were given three successive daily doses of thalidomide (250 mg/kg per day subcutaneously) on days 6, 7, and 8 after the presumed date of conception. Controls were given three injections of 30 percent propylene glycol, the solvent for the drug, on the same days. All mice were sacrificed about 20 hours after the last treatment; the mice which were found not to be pregnant were discarded. From the remaining mice (that is, 32 out of 95 in the thalidomide group and 10 out of 31 in the control group), the embryos, together with the contiguous portions of the uterus, were removed and extracted. The extracts were promptly assayed for glutamic dehydrogenase, glutamic oxalacetic transaminase, and lactic dehydrogenase by the procedures already outlined. No appreciable differences in enzyme activity, expressed on the basis of the total nitrogen of extracts A and B, were observed between the control and thalidomide groups.

These experiments were undertaken to test the working hypothesis—advanced by several groups of investigators—that this teratogen or its catabolic products may interfere with the biochemical or physiological functions of glutamic acid. Our results lend no support to this postulate.

Note added in proof: In independent studies on rat brain and rabbit fetuses, Fabro *et al.* reached the same conclusions (*9*).

ERICH HIRSCHBERG, MARTHA OSNOS
SYLVIA BRYANT, JOHN E. ULTMANN
Departments of Biochemistry and *Medicine* and *Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York*

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Experimental Reversal of Germ Cells in Ovaries of Fetal Mice

Abstract. *When heterosexual pairs of gonads of fetal mice were homotransplanted in close contact with each other below the kidney capsule of castrated adult hosts, the testis developed normally for this environment, but the ovary became an ovotestis. The medulla of the ovotestis contained dilated seminiferous tubules in which spermatogenesis progressed to the point at which secondary spermatocytes were produced. Under these conditions, germ cells, genetically determined as ova, underwent differentiation toward spermatozoa. This is the second clear case of germ cell reversal by experimental means in a mammalian species.*

Postgenetic sex reversals, under natural and experimental conditions, have been studied in a variety of vertebrates including teleosts (*1*), elasmobranchs (*2*), amphibians (*3*), reptiles (*4*), and birds (*5*). The conclusion emerging from investigations of this type is that the germ cells differentiate in response to internal environment rather than to their own genetic constitution. There are good reasons for believing that the region of the gonad in which the germ cells reside is a more potent factor in determining the direction of differentiation than are genic and chromosomal elements in the germ cells. The breeding of sex-reversed fishes, amphibians, and birds has provided unequivocal evidence that the genome is not impaired or otherwise changed by the procedures employed in producing the reversal.

While there are no reasons for believing that these principles do not apply to mammals, the mammalian gonads possess a remarkably stable organization and are difficult to modify or reverse. With few exceptions, attempts to alter the gonads have either yielded negative results or produced changes so minor in character as to be unconvincing. The masculinized ovaries of cattle freemartins are well known (*6*), and the condition has probably been duplicated experimentally by homotransplanting embryonic ovaries of the rat and mouse to the testes of adult hosts (*7*). The clearest and most complete instance of experimental gonad reversal in mammals, and the only one to be produced by the administration of exogenous steroids, has been obtained by Burns (*8*) in studies on young opossums (*Didelphis virginiana*). Burns found that when proper amounts of estradiol dipropionate were administered to pouch young in the ambisexual stage of development the testis became an ovotestis containing ovocytes in the cortical component. Jost (*9*), working with rabbits, attempted to transplant testes from 20-day fetuses contiguous to the intact ovaries of fetal recipients and, in a single instance, a testis and an ovary grew together for 8 days, causing the latter to become modified into a kind of ovotestis.

Macintyre (*10*) homotransplanted heterosexual pairs of fetal rat gonads beneath the kidney capsule of castrated adult hosts and, when the gonads were in close contact and of the same age, the testis differentiated normally, whereas the ovary generally formed tubular structures resembling testis tubules. The tublar elements of the modified ovaries contained ovocytes. The conclusion was reached that the capacity of contiguous heterosexual grafts to modify one another was due to the release of diffusible substances considered to be of the nature of corticomedullary in-