pad; intravenous injection of 0.5 to 1.0 ml into the right saphenous vein; or abrasion of the ear lobes followed by treatment with inoculum.

During approximately 3 years after inoculation, eight of these animals developed severe lesions containing myriads of *M. leprae* (Table 2). All infected animals died or were killed, and the disease was diagnosed as leprosy by histopathologic examination (*3, 5*). Seven animals are still alive and negative as determined by biopsy. Five animals died from causes unrelated to leprosy and at the time of death were negative. These animals died 21 to 31 months after inoculation, so at least some of them were probably resistant to the infection and would not have developed leprosy had they survived longer.

The slowness with which the disease developed in adult armadillos captured from the wild and inoculated with leprosy bacilli is illustrated by the fact that severely infected animals died from leprosy or its complications 15, 26, 30, 31, 33, 34, 37, and 41 months after inoculation. If it is assumed that all animals that are now negative will remain negative and that none of the animals that died from other causes would have developed leprosy, 40 percent of the animals inoculated developed lepromatous leprosy.

These estimates are provisional. Factors other than immunologic competence which could influence incidence of infection include routes of inoculation, number of bacilli injected, strain of bacilli, and nutritional status of the animals.

The salient features of leprosy in the armadillo are the high incidence of susceptibility (estimated at 40 percent), the high degree of pathologic involvement, and the enormous numbers of bacilli produced. In some cases, the central nervous system and lungs became seriously infected, a finding not reported in man. Although some involvement of the bone marrow is usually observed in human lepromatous leprosy, the degree and distribution of involvement in the armadillo far exceeds that found in human disease with the exception of leprotic osteitis occasionally seen in digits. It seems likely that armadillos in the late stages of disease become depressed immunologically because of massive invasion of the bone marrow and related reticuloendothelial tissues by leprosy bacilli.

The most important finding, from the standpoint of the biochemist, is that armadillos that died of leprosy 26 to 41 months after inoculation yielded 988 g of lepromas at autopsy (Table 2). Since removal, this tissue has been stored at −70°C and is estimated to contain 15 to 20 g of *M. leprae* as judged from bacterial counts averaging about 10^{10} per gram compared to 10^7 to 10^8 per gram in advanced human cases. This material contains very little stroma, so isolation of 95 percent "pure" bacilli is easily achieved. This material and the live bacilli harvested from living animals should make possible studies on the biochemistry and the metabolism of the leprosy bacillus which were hitherto impossible.

Table 2. Data on armadillos necropsied with advanced leprosy; I.V., intravenous.

Animal	Route of inoculation	Inoculation to death (months)	Lepromatous tissue harvested (g)
		Group 1	
5	Dermal	30	116
8	Dermal	15	*
		Group 2	
9	Dermal	41	13
		Group 3	
14	Dermal	34	172
16	Dermal	33	354
17	I.V.	31	85
18	I.V.	26	127
24	Dermal	37	121

* Not determined.

We conclude that it is now possible to produce disseminated leprosy in an intact animal system and that studies related to the epidemiology, immunology, and chemotherapy of the disease can now be performed. It is felt that information obtained from these pursuits will be of value in other biomedical research areas as well as in leprosy research.

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Site of Biosynthesis of Galactolipids in Spinach Chloroplasts

Abstract. *The envelope of the spinach chloroplast is the site of galactolipid synthesis.*

The chloroplast envelope is a continuous boundary of two osmiophilic membranes and has an important role in thylakoid synthesis (*1*). This conclusion is based on electron micrographs showing that membranes are continuously produced by the invagination of the inner membrane of the plastid envelope (*1*). With biochemical evidence lacking it was interesting to determine whether the envelope is the site of the synthesis of some structural component of the thylakoid. I chose to investigate the site of synthesis of galactolipids because they represent more than 70 percent of the total polar lipid of the thylakoid (*2*). Their active synthesis in isolated spinach chloro-

Table 1. Specific activities (micromoles of P_i formed per hour per milligram of protein) of marker enzymes, chlorophyll content (micrograms per milligram of protein), and galactolipid synthesis (picomoles of galactose incorporated per minute per milligram of protein) in fractions obtained after disruption of intact chloroplasts by hypotonic treatment.

Fraction	Specific activity (μmole hr^{-1} mg^{-1})			Chlorophyll (μg/mg protein)	Galactolipid synthesis (pmole min^{-1} mg^{-1})
	Trypsin-activated Ca^{2+}-dependent ATPase	Fructose-1-6-diphosphatase	Mg^{2+}-dependent ATPase		
Intact chloroplast	28	7.5	0.5	65	62
Thylakoid	60	0.7	0.3	147	39
Stroma	0.1	14	0.3	0	2
Envelope	0.1	0	12.9	0.8	1200

plasts was demonstrated recently by Ongun and Mudd (*3*).

Chloroplasts from whole green spinach leaves were prepared according to the aqueous method of Walker (*4*). Purification of intact chloroplasts (class 1) was carried out according to a modification of the method of Leech (*5*). The envelope, thylakoid, and stroma of the chloroplasts were prepared and characterized as described previously (*6*). The method makes use of the fact that gentle swelling of intact chloroplasts caused rupture and total detachment of the envelope with the liberation of the stroma material.

The following enzyme assays were carried out on the various fractions: trypsin-activated Ca^{2+}-dependent adenosine triphosphatase (ATPase) activity of coupling factor 1 (*7*), Mg^{2+}-dependent ATPase insensitive to *N,N'*-dicyclohexylcarbodiimide (*6*), fructose-1,6-diphosphatase (*6*), and reduced nicotinamide adenine dinucleotide (NADH) : cytochrome c oxidoreductase (*6*). Galactolipid synthesis was measured at 37°C in a 0.4-ml incubation mixture containing tricine-NaOH buffer (*p*H 7.5), 4 μmole; $MgCl_2$, 0.8 μmole; diglyceride, 0.4 μmole; and appropriate amounts of enzyme. Reactions were initiated upon addition of uridine diphosphate—[U-^{14}C]galactose (18 nmole, 500,000 count/min), and incubated for 20 minutes. The reaction mixtures were extracted according to the method of Bligh and Dyer (*8*). Labeled galactolipids were isolated from the chloroform-methanol phase and identified by thin-layer chromatography (*6*). The ^{14}C in the isolated lipids was measured in a liquid scintillation counter, and the amount was converted to picomoles of galactose for each lipid. Protein, chlorophyll, and polar lipids were determined according to the methods of Douce *et al.* (*6*).

Trypsin-activated Ca^{2+}-dependent ATPase of coupling factor 1 and chlorophyll content were markers for the thylakoid membrane fraction; fructose-1,6-diphosphatase was the marker for the stroma; Mg^{2+}-dependent ATPase insensitive to *N,N'*-dicyclohexylcarbodiimide was the marker for the chloroplast envelope (*6*). The marker activities were distributed among the fractions as anticipated, thus validating the fractionation procedure used (Table 1). The galactolipid synthesis occurred primarily in the envelope. The galactolipid synthesis activity found in the envelope is not attributable to microsomal contamination; the envelope is strikingly different from the microsomal subfractions in that it exhibits no NADH : cytochrome c oxidoreductase activity, no b-type cytochrome (*6*), and no phosphatidylethanolamine (Table 2). During the chloroplast preparation some envelope material released from the broken chloroplasts contaminated the other cell fractions. This may explain why other investigators observed galactolipid synthesis activity in the microsomal fraction obtained from spinach leaves (*3*). It should be pointed out that the demonstration of the preferential association of galactolipid synthetic activity with the envelope does not preclude association of a small fraction of this activity with the thylakoid system.

Table 2. Polar lipid composition of chloroplast envelopes and microsomes of spinach. The identification of the polygalactolipids was determined by the method of Webster and Chang (*12*). Microsomes from whole green leaves were prepared by the method of Marshall and Kates (*9*); there was some contamination by chloroplast material.

Polar lipid	Lipid (percent by weight)	
	Envelopes	Microsomes
Monogalactosyldiglyceride	24	12
Digalactosyldiglyceride	32	8
Trigalactosyldiglyceride	5	0
Tetragalactosyldiglyceride	1	0
Sulfolipid	7	2
Phosphatidylcholine	21	35
Phosphatidylethanolamine	0	30
Phosphatidylglycerol	8	3
Phosphatidylinositol	2	7
Phosphatidylserine	0	3

It is well established that the main location of the polar lipid synthesizing enzymes in plant cells (*9*) and animal cells (*10*) is the endoplasmic reticulum. However, the data presented here show that the outer membrane of the chloroplast, like that of the mitochondrion (*11*), can be a site of synthesis of an organelle's major structural lipids.

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Amorphous Semiconductor Switching in Melanins

Abstract. *Melanins produced synthetically and isolated from biological systems act as an amorphous semiconductor threshold switch. Switching occurs reversibly at potential gradients two to three orders of magnitude lower than reported for inorganic thin films, and comparable to gradients existing in some biological systems. Of a number of other biological materials tested, only cytochrome c acted similarly, but at the high potential gradients reported for thin film amorphous semiconductors.*

There has been a suggestion that the biological pigment melanin may qualify as an amorphous semiconductor (*1*). Recent experimental evidence has demonstrated that this material exhibits properties consistent with a more exotic form of an amorphous semiconductor, a threshold switch. Threshold switching has become a central issue in the development of amorphous electronic devices, but until now only inorganic materials have been demonstrated to possess these properties.

Synthetic melanins were produced