Table 1. Estimated number of sympathetic neurons in the thoracolumbar intermediolateral nucleus of cats of both sexes. The mean (and standard error of the mean) weight and number of neurons for female cats were 2.75 ± 0.19 kg and 35,543 ± 1,411, respectively. For male cats the mean weight was 2.63 ± 0.13 kg; the mean number of neurons was 45,765 ± 2,556.

Cat number	Weight (kg)	Neurons (No.)
	Female cats	
9	3.0	32,790
12	2.2	37,570
13	3.0	38,350
15	2.8	33,460
	Male cats	
11	2.6	53,340
14	3.0	43,300
16	2.5	42,170
17	2.4	44,350

mol in saline for at least 2 weeks. Each spinal cord, trimmed to include segments C_6 to L_5, was divided into four parts which were embedded in paraffin. Serial transverse sections 15-μm thick were cut, and every tenth section was mounted and stained with thionine (*8*). A total of 9400 sections was studied at a magnification of × 320. We identified ILN neurons by well-defined criteria (*9*), and estimated their total number by multiplying by 10 the number of nucleoli counted in the ILN of both sides. We assessed the accuracy of this method of counting by comparing the estimated count with the actual count of nucleoli in a series of 200 consecutive serial sections in one specimen; the numbers of counted and estimated nucleoli were 2022 and 1980, respectively.

The ILN extends from the junction of segments C_8 and T_1 to L_4 and is located within the lateral horn of the gray matter. Total neuron counts, body weights, and sexes of the eight animals studied are shown in Table 1. The mean counts for males and females are significantly different ($t = 3.5$, $P < .02$), although no significant difference is apparent between the mean weights of the two groups ($t = 0.54$, $P > .9$).

The data presented demonstrate a sex difference in the number of preganglionic sympathetic neurons in the cat. We are not aware of a sex difference in the number of central neurons having been reported before. However, this finding does not necessarily imply that the total number of neurons in the central nervous system is greater in the male. This sex difference may be limited to the sympathetic nervous system as it has been demonstrated that the number and size of neurons in sympathetic ganglia may be increased by the administration to newborn mice of a nerve growth factor isolated from the submaxillary gland of male mice; the amount of this factor present in the gland is decreased by ablation of the testes and increased by administration of testosterone to female rats (*10*). In addition, as it has been demonstrated that the development of chick dorsal root ganglia is influenced by the size of the tissue innervated (*11*), a possibility to be considered is that the sex difference in the number of ILN neurons may be secondary to a sex difference in the size of the viscera supplied by the sympathetic nervous system. Because no information is available on the size in the two sexes of organs supplied by the sympathetic nervous system, this possibility cannot be excluded. Finally, because no information was available on the age of these animals, it is possible that a difference in the body weights of cats of the two sexes was not demonstrated because some of the animals in our sample were not fully developed. This possibility is given substance by the demonstrated sex difference in weights of adult cats (*12*). The interpretation of our results would then be that the greater number of ILN neurons in the male is related to the statistical expectation of a greater body mass in the fully developed animal. However, whether or not this difference in the number of ILN neurons is related to body or visceral mass as well as to sex, a confirmation of our findings in other areas of the central nervous system would raise interesting questions regarding the influence of genetic and environmental factors on the developmental dynamics of neurogenesis in the two sexes.

FRANCO R. CALARESU
JAMES L. HENRY
Department of Physiology, University of Western Ontario, London 72, Ontario, Canada

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9. The neurons of the ILN are either bipolar or multipolar and are usually pyriform in shape with the long axis two to three times the length of the short axis. Their nuclei are located in a central position and contain darkly stained nucleoli. The scanty cytoplasm contains Nissl substance arranged in packets of irregular size and shape. The distinction between neurons and glial cells is always unequivocal because neurons contain nucleoli clearly surrounded by nucleoplasm. Neurons of the ILN are easily differentiated from those in neighboring structures at the lateral and dorsal borders because of intervening white matter. The ventral border is usually distinct because ILN and ventral horn neurons differ in size and are clearly separated. The medial border, however, is occasionally difficult to establish because of the presence of scattered neurons between the ILN and the intermediomedial nucleus; in these cases only those neurons which clearly appear as part of a group of other ILN cells are included.
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26 April 1971; revised 4 June 1971

Glycogen Plastids in Müllerian Body Cells of Cecropia peltata—A Higher Green Plant

Abstract. *A glycogen-containing plastid has been found in the cells of Müllerian bodies on* Cecropia peltata *(Moraceae) trees. Plastids in cells of the leaf blade, petiole, and stem are of the usual chloroplast type and contain starch grains only. Müllerian bodies exist for the purpose of providing a food supply for a protective, mutualistically inhabiting ant population. A series of chemical tests and observations indicates that this glycogen is identical to animal glycogen.*

Traditionally there has been a sharp distinction in the terminology used to describe the method of glucose accumulation and storage between plants and animals. Higher plants have been found to store their excess glucose in the form of starch, whereas animals produce a polysaccharide termed glycogen. Starch usually takes the form of a grain, a few microns in diameter, and is located in

a plastid, whereas glycogen usually appears as small granules 200 to 400 Å in diameter found free in the cytoplasm.

Most plant starch is composed of amylose and amylopectin. Amylose is a straight-chain polymer of α-D(1-4)-glucose molecules, while amylopectin contains the straight chains of α-D(1→4) linkages, but also possesses a number of branch points caused by α-D-(1→6) bonds. The α-D-(1→4) chains in starch can be from 200 to 2100 molecules in length. Glycogen is a very highly branched molecule containing a large number of α-D-(1→6) bonds, and has α-D-(1→4) chains of only about 12 glucose molecules in length. The designation between starch and glycogen became cloudy as various types of starch were found in which amylopectin began to predominate. Thus, the storage product, by virtue of the higher number of α-D-(1→6) linkages, becomes more highly branched, and thus more analogous to glycogen. Certain starches, called waxy, found in various strains of corn consist almost entirely of amylopectin (*1*), and another amylopectin type of starch, termed floridean starch, occurs in the red algae (*2*).

The designation between plant and animal glucose storage products was further confused by the findings of Morris and Morris (*3*), in certain strains of sweet corn, of a water-soluble polysaccharide that precipitated similarly to glycogen in acetic acid solutions. Amylose and amylopectin are not water-soluble, whereas glycogen is highly soluble in water. Thus, it was rather easy to distinguish even a highly branched amylopectin from glycogen on the simple basis of solubility in water. Morris and Morris also compared the water-soluble corn polysaccharide with glycogen on the basis of their cupric chloride crystallization patterns, the classic color reaction with iodine, and the rate of digestion when malt amylase was used. The water-soluble polysaccharide was essentially indistinguishable from animal glycogen.

Workers after Morris and Morris have continued to study the properties of this water-soluble polysaccharide, and the term *phytoglycogen* has been used to refer to it. Phytoglycogen has been biochemically compared to glycogen from a wide variety of animal sources, amylopectin, and selected beta-dextrin starches by Archibald *et al.* (*4*). Their work contrasted such characteristics as chain length, degree of polymerization, absorption wavelength, and extinction of absorption value at given wavelengths, and, although they discovered a wide spectrum of values for glycogen, their conclusion was that the water-soluble polysaccharide of *Zea*, or phytoglycogen, was indistinguishable from animal glycogen. It seems that the main difference between phytoglycogen and animal glycogen is now one of location in either plant or animal tissue respectively and, with the invention of the electron microscope, morphology of the polysaccharide, rather than its molecular structure.

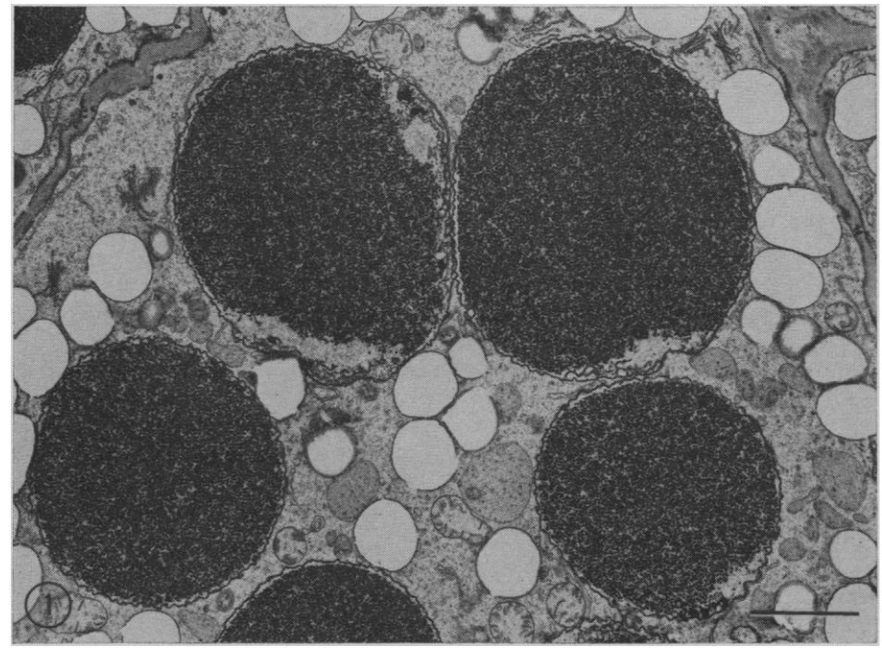

Fig. 1. Portion of a $KMnO_4$ fixed cell showing a group of glycogen plastids. The glycogen stains intensely with lead and uranium. The electron transparent areas in the cytoplasm represent sites of lipid that was leached out of the tissue during dehydration. (× 7000). Scale equals 2 μm.

In 1966 Lavintman (*5*) described the enzymological events surrounding glucose conversion to various polysaccharides. As a part of this study she presented an electron micrograph of centrifuged material that was reported to be pure phytoglycogen. The picture obtained shows a series of rather evenly distributed rosettes. Each large rosette measures 550 to 750 Å in diameter, and the subparticles making up a rosette have a diameter of 250 to 400 Å. This picture appears to be the only published evidence of the morphology of phytoglycogen and is similar to published pictures of the α-particles of animal glycogen (*6*, *7*).

Badenhuizen (*8*) presents several micrographs of *Zea* var. Golden Bantam endosperm at various stages of development with no mention made of any possible ultrastructural localization of the phytoglycogen. In his new book on starch granules, Badenhuizen (*9*) did not discuss the cytological location of this polysaccharide even though he has a detailed discussion of possible biochemical pathways of glycogen synthesis.

As part of a study on plant tissue that has been evolutionarily modified under selection pressure in the direction of producing an ant food, in mid 1970 I received some trichilia from a Costa Rican *Cecropia* plant. A trichilium is a pad of tissue, located at the abaxial junction of the petiole and the stem, that produces a feltlike surface of unicellular hairs. From this pad of tissue, small (0.75 by 0.25 mm), egg-shaped, cellular structures are produced which ants collect and utilize as a food source. These structures, called Müllerian bodies, are formed by the plant alone, and are not, in any way, dependent on an ant stimulus for their production.

The material from Costa Rica had been fixed in formalin–acetic acid–alcohol. After embedding the material in paraffin and staining the tissue with safranin-fast green-crystal violet, large, spherical, clear zones were apparent in the cytoplasm of the Müllerian body

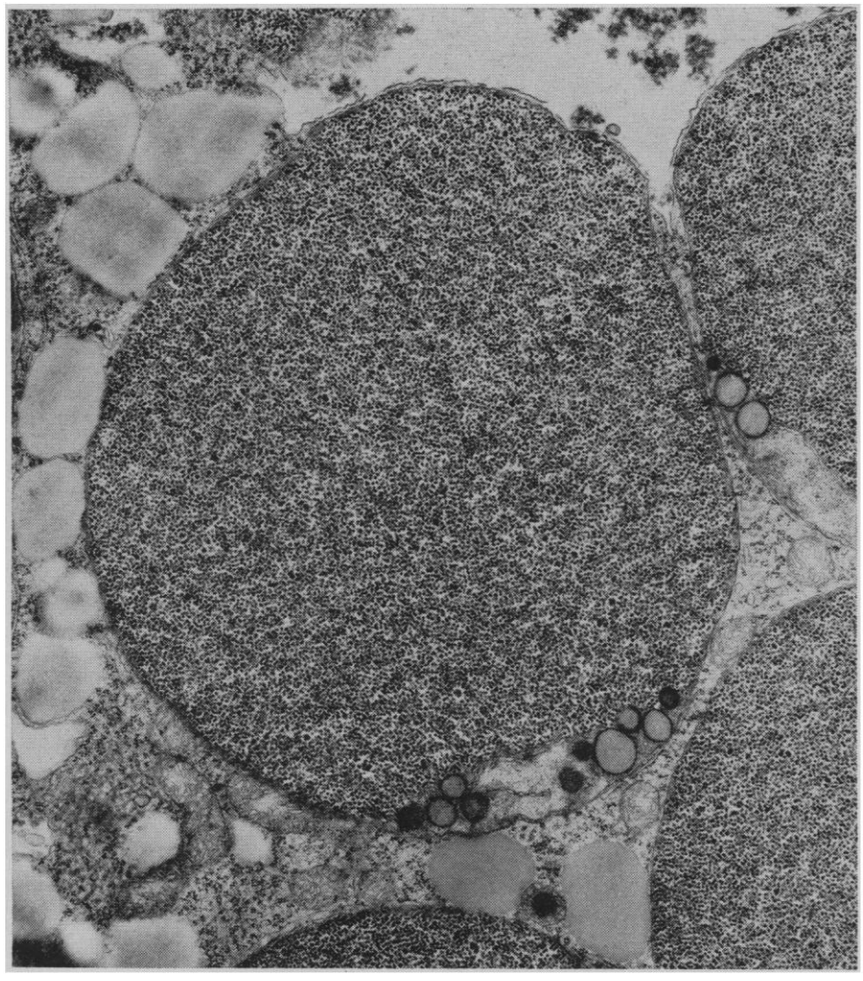

Fig. 2. A glycogen plastid from a Müllerian cell of *Cecropia peltata* (Moraceae). Cells of Müllerian bodies constitute the food supply for ants that defend the parent plant. Diameter of the plastids averages 9 μm.

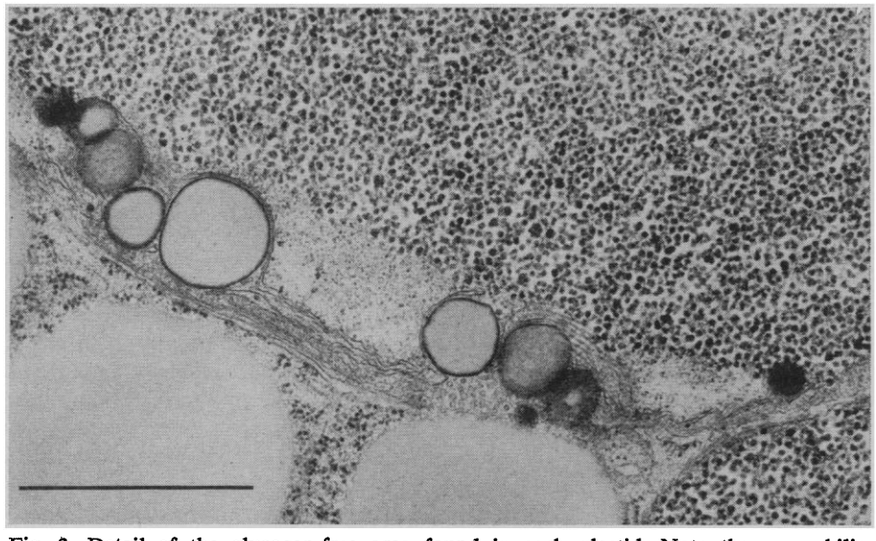

Fig. 3. Detail of the glycogen-free area found in each plastid. Note the osmophilic droplets and membrane system. Glutaraldehyde-osmium fixation. ($\times$ 31,400). Scale equals 1 μm.

cells. After some standard histochemical tests it was found that the clear areas were negative for nucleic acids or proteins but were extremely periodic acid–Schiff's reagent positive. This reaction indicates some type of polysaccharide. The spherical bodies were too large for starch grains, but since the material was already fixed in the alcohol solution there was no way that the techniques of electron microscopy could be applied to it.

In the latter part of 1970, three root stalks of *Cecropia peltata* from Costa Rica were obtained from Dr. Daniel Janzen of the Department of Biology, University of Chicago, and were grown in greenhouses at Madison, Wisconsin, under continuous illumination. Müllerian bodies were produced on trichilia and were collected for study. This greenhouse material was collected at such a time as to approximate that developmental stage at which the ants harvest the bodies in the wild. By use of the electron microscope with either $KMnO_4$ or glutaraldehyde-osmium fixation and uranyl acetate–lead poststaining it was quickly apparent that the spherical structures noted in the light microscope were enlarged plastids containing a particulate material that closely resembled animal glycogen (Fig. 1). The fact that they were plastids was readily confirmed by observing an ontogenetic series in very young Müllerian bodies. At maturity each plastid is approximately 8 to 10 μm in diameter and essentially spherical (Fig. 2). It is surrounded by an envelope consisting of two normal-appearing unit membranes. Along one edge of a plastid there is a clear space containing a number of spherical inclusions (Fig. 3), which, by their electrondense limiting margin and their general staining qualities, are similar to those osmophylic globules found in many other plastids. Also in this clear space there is a slight elaboration of membrane material, some small electrondense particles of the same size and morphology as plastid ribosomes, and elements of the rigid tubular membrane system reported from other plastids (Fig. 3). Finally, they are ontogenetically derived from normal plastids found in cells of the trichilium tissue before differentiation of a Müllerian body. Thus, this particular organelle is undoubtedly a plastid in the truest sense of the word.

The individual particles that occupy most of the organelle measure 350 to

400 Å in diameter, are irregular in their outlines, and show various degrees of clumping into groups of two, three, four, or five individuals. The particles are extractable at the ultrastructural level with alpha amylase after long periods of time (12 to 18 hours), or in a short period of time (1 hour) following a pretreatment for 1 hour with pullulanase (*10*). Pullulanase is known to be selective for α-D(1→6) bonds (*11*), and although these data indicate a high degree of branching in the polysaccharide, they do not allow for an in vivo distinction between amylopectin and glycogen (*10*). The particles stain intensely with the periodic acid–thiocarbohydrazide–silver protein method of carbohydrate localization developed by Seligman *et al.* (*12*) and modified for ultrastructural use by Thiéry (*13*).

A complete biochemical analysis of this material is difficult because of the small size of each Müllerian body. However, 200 to 300 individual Müllerian bodies were collected in a microhomogenizer and ground in cold water. After centrifugation the supernatant had a very opalescent appearance. The material was initially precipitated with ethyl alcohol, and reprecipitated three times with ethyl alcohol, after which the final alcohol precipitate was evaporated at 40°C over silica gel. A small amount of the resulting white fluffy material was resuspended to 0.5 percent in water and tested with the Sumner and Somers (*14*) procedure for phytoglycogen and glycogen. As a control, glycogen from oyster was used. Both with and without the addition of a saturated ammonium sulfate solution, the oyster glycogen and the material from the Müllerian body remained colorless or turned slightly yellow to yellow-brown depending on the amount of I_2KI added. A pink color, indicative of phytoglycogen, was never detected in either the glycogen or Müllerian body material solutions.

Therefore, it seems reasonable to conclude that the material in the glycogen plastids of the Müllerian body is similar in every respect to oyster glycogen if not all animal glycogens. The location in a plastid is particularly significant if one considers the cytological machinery available for carbohydrate metabolism in a plant cell. Further, there are many interesting evolutionary ramifications to this system in that a survey of the ultrastructure of cells from trichilium tissue, leaf tissue, and green photosynthetic stem tissue of *Cecropia* reveals the presence of typical higher plant chloroplasts complete with a grana-lamella system and starch grains. Further study, on the ultrastructure of the young, maturing, mature, and germinating endosperm tissue of Golden Bantam sweet corn, is needed: the location of the glycogen in those cells should shed light on the question of glycogen metabolism in relation to ultrastructure, in higher green plants.

FRED R. RICKSON*
Department of Botany,
University of Wisconsin,
Madison 53706

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16. Supported in part by NSF grant GB7934.
* Present address: Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

6 May 1971

Cerumen Genetics and Human Breast Cancer

Abstract. *International mortality and frequency rates for breast cancer seem to be associated with the frequency of the allele for wet-type cerumen. A preliminary retrospective case-control study in California indicates that phenotypically wet-type cerumen is found in Japanese women with breast cancer more often than in healthy Japanese women. Findings support the hypothesis that the apocrine system's genetically determined variation may influence susceptibility to breast cancer.*

Mortality rates from breast cancer are exceptionally low in Oriental populations and intermediate in those of Eastern Europe and the Middle East, as compared with women in Western Europe and the United States (*1*)—an epidemiological finding that so far is unexplained. The recently established genetic dimorphism in human cerumen (earwax) may be a significant trait for the studies of these international variations in rates of breast cancer.

Matsunaga (*2*) showed that cerumen occurs in two phenotypic forms, wet (sticky) and dry, and that the quality of cerumen is controlled by a single pair of genes in which the allele for the sticky trait is dominant to the allele for the dry. The homozygous wet type is phenotypically indistinguishable from the heterozygous form. Races vary widely in their frequencies of alleles for wet and dry cerumen: in Mongoloid populations of Asia and the Americas (Japanese, Koreans, Mongols, Chinese, and American Indians) frequencies of the dry allele are high; in Caucasians and U.S. Negroes they are low; and in populations in some countries of the Middle East and Southeast Asia (Turkey, Iran, Afghanistan, India, and Malaysia) they are intermediate (*2–5*).

This report presents data supporting an association between wet cerumen and rates of breast cancer in diverse population groups. Such an association is plausible since the ceruminous, mammary, and certain axillary sweat glands are histologically of the apocrine type, and their secretions are biochemically similar (*6–8*).

These histological and functional relations suggested the hypothesis that the disparate rates for breast cancer between Mongoloid and Caucasian populations might in part reflect genetic differences between persons with alleles for dry and wet cerumen expressed through the apocrine gland system. Such differences might differentially influence the susceptibility