These effects on isolated TMS (but not N) mitochondria include the stimulation of the rate of succinate or exogenous NADH oxidation, the stimulation of swelling, the inhibition of combined malate and pyruvate oxidation, and the inhibition of adenosine diphosphate (ADP)–induced state 3 oxidation.

Figure 3 shows the percentage of transmittance and O_2 curves of mitochondrial suspensions treated with methomyl or with *B. maydis* (race T) toxin before they were fixed for the electron micrographs presented in Fig. 4. The addition of 3 m*M* methomyl (Fig. 3C) or *B. maydis* (race T) toxin (Fig. 3D) resulted in an immediate and dramatic increase in the transmittance of light through the mitochondrial suspensions. This extensive swelling was not observed in the control (Fig. 3A) nor the 1 m*M* methomyl treatment (Fig. 3B). The electron micrographs in Fig. 4 confirm our interpretation of swelling, based on the percentage of transmittance curves in that mitochondria treated with 3 m*M* methomyl (Fig. 4C) or *B. maydis* (race T) toxin (Fig. 4D) exhibited a very dilute matrix and few cristae, yet had intact membranes. At 1 m*M* methomyl stimulated a moderate increase in the rate of NADH oxidation, but caused only a slight increase in the percentage of transmittance curve possibly due to a rounded cristae conformation (Fig. 4B).

While the observation of selective effects due to a potent insecticide on isolated maize mitochondria is significant in itself, the potential for the use of methomyl in assessing the difference in TMS and N maize mitochondria is likely of more interest to plant physiologists and geneticists. Studies aimed at delineating the precise site of *B. maydis* (race T) toxin binding to or its effect on isolated mitochondria have not been as exact as possible, largely because of the still poorly defined nature of the toxin or toxins. Methomyl is a relatively simple compound to which a radioactive label can be incorporated (*9*). The fact that membrane components are made labile with compounds such as digitonin (*10*), combined with methomyl binding studies, should distinguish the functional or structural difference between N and TMS mitochondria, provided that the methomyl and *B. maydis* (race T) toxin effects are as similar as we hypothesize them to be.

In view of the previous whole plant studies of Humaydan and Scott (*8*), our work defines at the organelle level the nature of the selective damage of methomyl to whole plants. That methomyl and certain other carbamate insecticides have been reported to damage other plant species in specific circumstances (*11*) opens the question of whether the genetic vulnerability of these plants to certain insecticides is conferred cytoplasmically through the mitochondrial genome.

The magnitude of the *B. maydis* (race T)–induced epiphytotic through much of the corn belt in 1970 (*12*) called renewed attention to the developing problem of a lack of genetic diversity in many of our crop species. It now seems clear that, while plant breeders and geneticists have developed heritable nuclear genomes of limited diversity, the much smaller and largely overlooked cytoplasmic genome probably contains even less diversity, not only among varieties of one crop but between many crops as well. Further studies with methomyl and closely related compounds known to effect plant damage should be of value in assessing the homogeneity, or lack of it, of a portion of the cytoplasmic genome associated with mitochondria.

In summary, the methomyl effect on TMS mitochondria of maize seems (i) to provide the potential for understanding a component of the extranuclear mitochondrial genome and (ii) to discriminate between detrimental and positive effects often present with the use of man-introduced environmental contaminants such as pesticides.

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α_1-Antitrypsin: The Presence of Excess Mannose in the Z Variant Isolated from Liver

Abstract. *The Z variant of α_1-antitrypsin was isolated by a new technique from the liver of a patient homozygous for the Z allele of the protease inhibitor locus. The material was homogeneous and antigenically competent but had no protease inhibiting capacity. An interesting correlation was found between the subcellular localization and the carbohydrate composition of the Z variant from liver. Carbohydrate analysis of this glycoprotein showed an absence of galactose and sialic acid, an appreciable decrease in* N*-acetylglucosamine, and an almost twofold increase in mannose residues. These data indicate a considerable slowdown in the processing of the oligosaccharides of liver Z variant. In spite of the absence of sialyl residues, the liver Z variant was microheterogeneous by analytical isoelectric focusing. The isoproteins of liver Z variant coincided with those of asialo M variant in the focusing field.*

In persons homozygous for the Z allele of the Pi (protease inhibitor) locus, the concentration of α_1-antitrypsin in the plasma is decreased to 10 to 20 percent of the normal value and a substantial amount of antitrypsin is retained in the liver (*1*). Heterozygous carriers of this allele have a correspondingly higher concentration of the protein in the serum, but they too show accumulation of this material in the liver. These findings strongly suggest an impairment either in the biosynthesis or the secretion of the Z variant. The fact that glycoproteins other than antitrypsin do not accumulate in the liver in this condition and the reported substitution of a glutamyl residue with lysine in the Z variant isolated from serum (*2*) suggest that an alteration of the Z variant, rather than alteration of the glycoprotein processing apparatus, is the primary cause of retention. We isolated and analyzed Z variant from the liver to find out how and at what particular stage the biosynthesis of this glycoprotein was affected.

Liver tissue was obtained at autopsy from a 50-year-old female, Pi ZZ patient.

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Antitrypsin-rich inclusion bodies were prepared by two consecutive steps of centrifugation in sucrose gradients, essentially by the method of Jeppson *et al.* (*3*). The preparation was extracted with 0.5 percent methylamine, resulting in the removal of substantial amounts of pigment, some lipid, and protein. The methylamine extracts were discarded. After the last extraction, the residue was twice washed with water and then antitrypsin was repeatedly extracted with 12 percent 2-chloroethanol. During the last step the suspension was briefly subjected to sonication. All purification steps were carried out at 0°C. The antitrypsin-containing extract was dialyzed first against 12 percent 2-chloroethanol, then against two changes of distilled water. Some precipitate that formed during the latter step was removed by centrifugation at 6000 rev/min for 30 minutes and the slightly opaque supernatant was collected and analyzed.

After 24, 48, and 72 hours of acid hydrolysis, the amino acid composition of the protein was the same as that of the normal M variant within a margin of 10 percent. By double immunodiffusion the Z variant from the liver formed lines of precipitation with monospecific antiserum, and these lines fused with precipitation lines of authentic M antitrypsin to give reactions of serological identity. Electrophoresis in polyacrylamide gel in the presence of 0.1 percent dodecyl sulfate showed the presence of a single protein band in the preparation (Fig. 1) which, in agreement with an earlier observation (*3*), migrated slightly faster than the normal M variant.

Analysis of the carbohydrate compositions of the Z and M variants (Table 1) shows a virtual absence of galactose and sialic acid, an appreciable decrease in the hexosamine, and a nearly twofold increase in the mannose content of the Z variant isolated from the liver. Similar results were obtained by analyzing the carbohydrates of pronase glycopeptides of the Z variant from the liver of a dif-

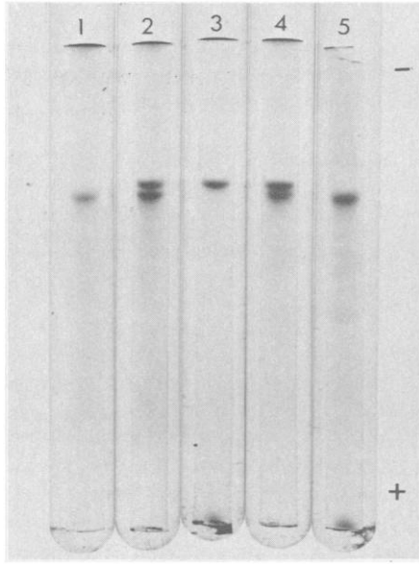
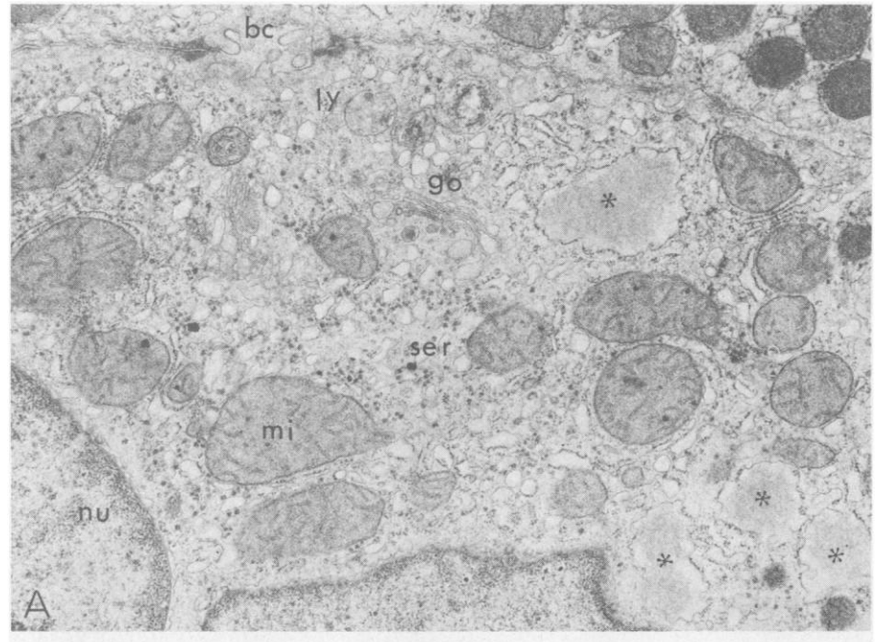
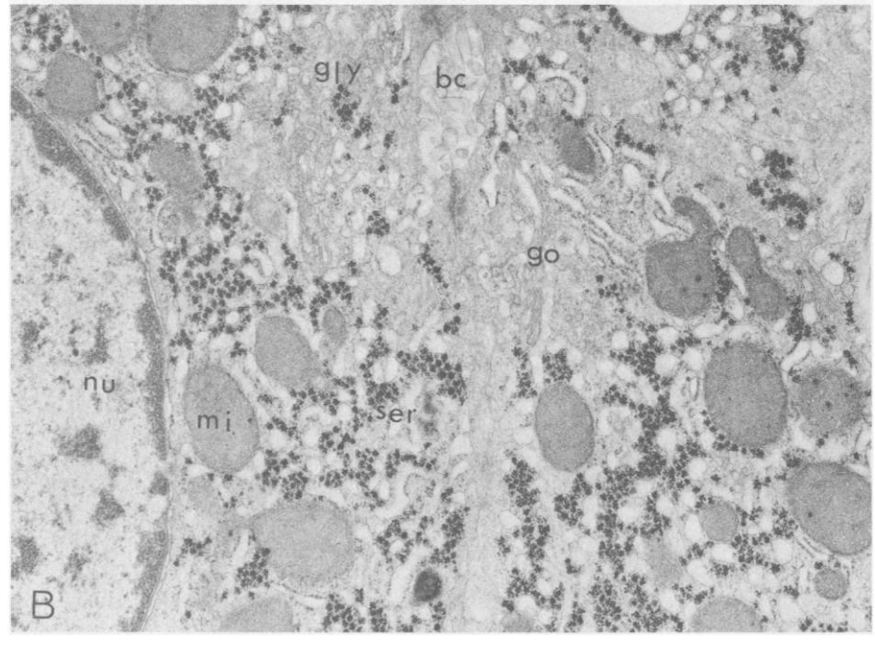


Fig. 1 (left). Comparison of two different preparations of purified liver antitrypsin with serum antitrypsin by electrophoresis in dodecyl sulfate–containing polyacrylamide gel. The two preparations were obtained separately, but essentially by the same method. Gel 1, preparation A (liver); gel 2, preparation A mixed with M variant of serum antitrypsin; gel 3, M variant of antitrypsin; gel 4, preparation B mixed with M variant of serum antitrypsin; and gel 5, preparation B (liver). Anode is at the bottom. Fig. 2 (right). (A) Electron micrograph of a portion of a hepatocyte from a liver specimen obtained by biopsy showing the early stage of inclusion formation. The specimen was obtained from a patient with the ZZ phenotype. The moderately electron opaque material, corresponding to antitrypsin-containing inclusions (asterisks) appear within the cisternae of the rough endoplasmic reticulum. The other cell organelles, including Golgi complexes (*go*), lysosomes (*ly*), mitochondria (*mi*), and smooth endoplasmic reticulum (*ser*) appear normal. Parts of the cell nucleus (*nu*) and a bile canaliculus (*bc*) are also shown. (B) Electron micrograph of a portion of a normal hepatocyte. The small electron-opaque particles correspond to glycogen (*gly*) (×18,000).





ferent patient (*4*). For comparison, the corresponding figures reported from another laboratory (*3*) are also shown in Table 1. Although there are wide differences between the two sets of data, the mannose to hexosamine ratios are increased significantly from the M variant to the liver Z variant by approximately the same factor in both cases [3.9 in this work and 3.6 in (*3*)].

The subcellular localization of the Z variant is relevant for the interpretation of the carbohydrate data. Electron microscopic examination of numerous liver specimens (for example, see Fig. 2) invariably showed that the Z variant from the liver was confined exclusively to the cisternae of the rough endoplasmic reticulum (RER). It has been known for several years that while the polypeptide portion and core carbohydrates of glycoproteins are assembled in the RER, the peripheral carbohydrates (sialic acid, galactose, and some of the *N*-acetylglucosamine residues) are attached to the core subsequently in the smooth endoplasmic reticulum (SER) and Golgi apparatus (*5*). Without specifying glycosidic linkages, one can express the core structure of numerous asparagine-linked complex type of oligosaccharides as follows:

$$\text{Asn}-\text{GlcNAc}-\text{GlcNAc}-\text{Man}\begin{matrix}\nearrow \text{Man} - \\ \searrow \text{Man} -\end{matrix}$$

where Asn is asparagine, GlcNAc is *N*-acetylglucosamine, and Man is mannose. In a recent report, the M variant of antitrypsin was found to contain four oligosaccharides per mole (*6*).

On these grounds, the Z variant from liver would not be expected to contain galactose and sialic acid (see Table 1), but it should contain eight *N*-acetylglucosamine and 12 mannose residues per mole. The presence of approximately six residues of *N*-acetylglucosamine in this preparation indicates that this protein contains on the average only three oligosaccharides with two *N*-acetylglucosamine and seven mannose residues per chain.

Because of the extra mannose residues, these oligosaccharides are substantially larger than expected from the composition of the core of the finished product. In various tissues at the initial stages of glycoprotein biosynthesis, oligosaccharides destined for incorporation into protein contain one to two glucose, two *N*-acetylglucosamine, and 11 mannose residues (*7*). There is evidence to suggest that all the glucose residues and some of the mannose residues are removed at subsequent stages of the biosynthetic process (*8*). Presumably, under normal conditions, excess mannose is removed from α_1-antitrypsin by oligosaccharide processing. If one assumes that the oligosaccharides originally present on the Z variant from the liver also contained 14 to 15 carbohydrate units, the data in Table 1 would indicate that this protein did undergo the early steps of processing but that there was a block in the removal of the last few mannose residues.

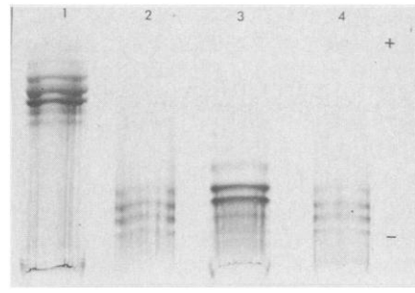


Fig. 3. Comparison of liver antitrypsin with variant M of serum antitrypsin by isoelectrofocusing (*p*H 4.0 to 6.0) in 6.0*M* urea-containing polyacrylamide gel. Sample 1, variant M (24 μg); sample 2, liver antitrypsin (18 μg); sample 3, variant M (24 μg) desialylated with *Vibrio cholera* neuraminidase; and sample 4, liver antitrypsin (12 μg). The anode is at the top.

Although our findings are novel with respect to mammalian glycoproteins, a very similar situation occurs in the biosynthesis of the G glycoprotein of vesicular stomatitis virus. In its completed form this glycoprotein contains oligosaccharides with only three mannose residues per oligosaccharide; peripheral sialic acid, galactose, and *N*-acetylglucosamine residues are attached to the core structure. When the virus was grown in a mutant line of Chinese hamster ovary cells lacking a glycoprotein *N*-acetylglucosaminyltransferase, the oligosaccharides of G glycoprotein did not contain peripheral carbohydrates, but did contain an excess of mannose (*9*).

The two major aspects of our carbohydrate analysis are (i) the apparent absence of one oligosaccharide of antitrypsin and (ii) the composition of the remaining three, which defines the stage at which oligosaccharide processing came to a near halt. Because many details of normal glycoprotein processing are still unknown, we can only tentatively describe from these observations the pathological processing of liver Z variant. The presence of only three oligosaccharides in this protein indicates an interference with the glycosylation of a particular asparagine residue. This situation could be the consequence of the reported amino acid substitution in the Z variant (*2*), especially if the substitution occurred in the proximity of a particular asparagine residue. Since transfer of core carbohydrates from lipid-bound to protein oligosaccharide can take place only in the RER, glycoproteins may not be free to leave the RER until the process is completed. Defective glycosylation may interfere with the passage of glycoproteins from the RER to the SER, and lead to stagnation of liver Z variant in the former. The simplest way to explain the presence of excess mannose in the oligosaccharides of liver Z variant is to suggest that the enzyme responsible for their removal resides in the SER or Golgi apparatus. As long as liver Z variant was confined to the RER, extra mannose residues in the oligosaccharides would remain. The fact that the carbohydrate composition of serum Z variant is essentially the same as that of the M variant (*10*) indicates that some liver Z variant molecules may be fully glycosylated and subsequently leave the RER to undergo the normal stages of glycoprotein processing and addition of peripheral sugars.

Observations by analytical isoelectric focusing showed liver antitrypsin to be microheterogeneous (Fig. 3). Each of the

Table 1. Carbohydrate compositions of α_1-antitrypsin preparations. Figures are expressed as residues per mole. With the exception of hexosamines the carbohydrates were measured by gas-liquid chromatography (*13*). Samples were methanolyzed in dry methanolic HCl (0.035 ml of acetyl chloride per milliliter of dry methanol) at 80°C for 20 hours. Because of the resistance of *N*-glycosidic linkages to these conditions, the *N*-acetylglucosamine figures in this work are lower than the actual figures. Hexosamines were measured with the Ehrlich reagent (*14*).

Carbohydrate	M variant (serum)		Liver Z variant	
	This work	Ref. (*3*)	This work	Ref. (*3*)
Galactose	8.1	8.0	<0.1	1.7
Mannose	12.0	8.0	21.2	3.6
N-Acetylglucosamine	8.9	12.0	3.9	1.5
Sialic acid	6.3	6.5	<0.1	0.0
Glucose	0.0		0.0	
Fucose	0.0		0.0	
Hexosamine	12.6		5.6	
Mannose/hexosamine ratio	0.95	0.67	3.70	2.40

isoproteins of liver antitrypsin nearly or completely coincided in the focusing field with a corresponding isoprotein in an authentic sample of asialo M variant, that is, not containing sialic acid (compare sample 2 with sample 3, Fig. 3). The microheterogeneity of some glycoproteins is largely due to uneven sialylation of their individual components (*11*). From observations on the M variant, we concluded earlier that uneven sialylation was not the cause of the microheterogeneity of antitrypsin (*12*). The demonstration of microheterogeneity of sialic acid–free liver antitrypsin confirms the earlier conclusion. Our results indicate that the microheterogeneity of antitrypsin becomes established before it leaves the RER. Hence, microheterogeneity is probably caused by variations in the content of charged amino acids of the isoproteins.

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Decrease of Human Serum Fucosyltransferase as an Indicator of Successful Tumor Therapy

Abstract. *Surgical removal of colon carcinomas leads to a decrease in the rate of incorporation of [^{14}C]fucose into its endogenous acceptor in human serum; normal incorporation rates are attained within 14 days. A similar time course has been determined for α_2- and α_3-fucosyltransferase when either desialo- or desialodegalactofetuin are employed as exogenous acceptors. A correlation has also been seen between transferase activity and the therapeutic response of patients with breast cancer. These results indicate that the determination of fucosyltransferase activity can facilitate the diagnosis of neoplasia, and the success of surgery, chemotherapy, or radiation.*

The glycosyltransferases that add the terminal sugars L-fucose and *N*-acetylneuraminic acid to nascent glycoconjugates have been studied in different tissues (*1*), especially for their role in oncogenic processes (*2*). Elevations in the serum glycosyltransferases sialyltransferase (*3*), fucosyltransferase (*4*), and galactosyltransferase (*5*) have been found in patients with neoplastic disease. At least two fucosyltransferases have been detected in human serum: α_2-fucosyltransferase transfers L-fucose from guanosine diphosphate (GDP)-L-fucose to the terminal galactose residues of oligosaccharides, desialylated glycoproteins (*6*), or glycolipids (*7*) by forming (1 → 2) linkages; α_3-fucosyltransferase adds L-fucose at the C-3 atom of free or protein-bound *N*-acetyl-D-glucosamine (*6*). Fucosyltransferase activity is especially high in the serums of patients suffering from highly malignant or metastatic tumors (*4*). We now show a correlation between these enzyme levels and the patient's response to tumor therapy.

Randomly selected blood samples were collected in anticoagulant-free tubes, and the serum was separated for the assay. A characteristic decrease in the incorporation of [^{14}C]fucose into its endogenous acceptor was found 4 to 6 days after surgery for colon carcinoma (Fig. 1); this rate approached normal values within 14 days. However, a transient minor elevation of the incorporation rate may also be seen (Fig. 1). When desialofetuin, or fetuin from which both *N*-acetylneuraminic acid and galactose have been removed, were employed as exoge-

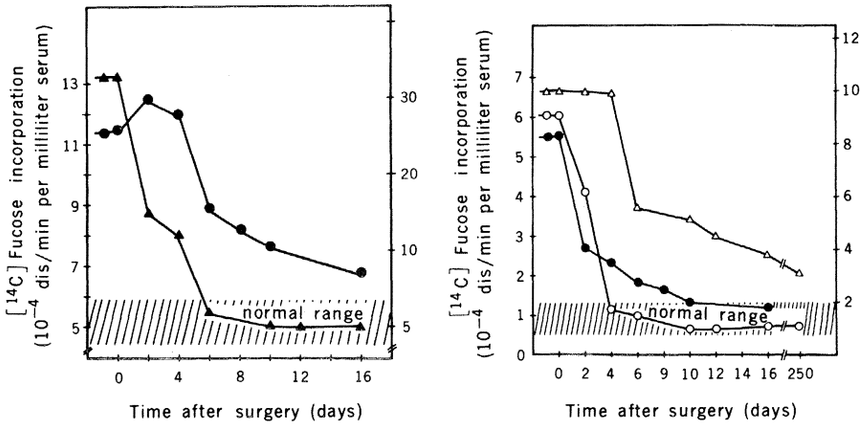

Fig. 1 (left). Incorporation of [^{14}C]fucose into endogenous acceptors of human serum before and after removal of colon carcinoma by T. W. (●) (72-year-old male, values indicated on left ordinate) and G.K. (▲) (59-year-old female, values indicated on right ordinate). The total volume of the incubation mixture (*p*H 5.5) was 120 μl, comprised of morpholinoethane sulfonic acid (4 μmole), $MgCl_2$ (2 μmole), sodium azide (0.4 μmole), GDP-L-[U-^{14}C]fucose (100,000 dis/min), and 30 μl of serum. The mixture was incubated for 21 hours at 37°C, and protein-bound radioactivity was determined (*4*). Each point represents the average value of duplicate determinations. The normal range is 40,000 to 60,000 dis/min per milliliter of serum. Fig. 2 (right). Pattern of fucosyltransferase activities in human serum at different times after surgery for colon carcinoma. T. W. (●) (values on left ordinate) and G. K. (○) (values on right ordinate) were assayed for α_2-fucosyltransferase. G.K. (△) (values on right ordinate) was assayed for α_3-fucosyltransferase. Incubation conditions were the same as described in the legend of Fig. 1, except that either 0.5 mg of desialofetuin or desialodegalactofetuin were added as exogenous acceptors. The normal range for α_2-fucosyltransferase was 4,000 to 20,000 dis/min per milliliter of serum; and for α_3-fucosyltransferase it was 10,000 to 30,000 dis/min per milliliter of serum.

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