Effects of high-glucose and high-fat diets on concanavalin A binding to rat liver plasma membranes and on the amount and pattern of their glycoprotein carbohydrates

Diane S. Henriquez, Helen M. Tepperman, and Jay Tepperman[1]

Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, NY 13210

Abstract Purified liver plasma membranes were prepared from rats fed a high-fat, carbohydrate-free diet or a high-glucose, fat-free diet. Membranes from rats fed the high-fat diet bound significantly less ^{125}I-labeled concanavalin A (Con A) than did those from rats fed the fat-free diets. The magnitude of the binding difference increased with increasing concentrations of Con A. Neither association nor dissociation rates of the lectin–receptor complex was affected by diet. The extent of degradation of Con A by liver plasma membrane preparations from rats fed either diet was the same. Chemical analysis of delipidated liver plasma membrane showed that membranes prepared from high-fat diet-adapted rats had significantly lower values for all carbohydrate components measured with the exception of galactose. The results indicate that, in liver cells, a change in plasma membrane glycoproteins is part of the complex adaptation to altered diet composition.—**Henriquez, D. S., H. M. Tepperman, and J. Tepperman.** Effects of high-glucose and high-fat diets on concanavalin A binding to rat liver plasma membranes and on the amount and pattern of their glycoprotein carbohydrates. *J. Lipid Res.* 1979. **20:** 624–630.

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The plasma membrane is a dynamic structure whose constituents are in a constant state of turnover. This continuous degradation and renewal of membrane components suggests the possibility that the chemical morphology as well as the functions of the plasma membrane may be susceptible to adaptive modifications.

Consistent with this view, members of this laboratory have described in previous reports the adaptation of a series of plasma membrane-associated functions in response to changes in diet composition. Hormone-stimulated adenylate cyclase activity (1), insulin binding capacity (2), and glucose transport (3) were all observed to be significantly increased in adipose tissue preparations from rats fed a high-carbohydrate diet compared to those from animals fed a high-fat, low-carbohydrate diet. Similar differences in insulin binding capacity were described recently in purified liver membrane preparations isolated from carbohydrate diet-adapted and fat diet-adapted rats (4).

Concanavalin A (Con A), a plant lectin, binds selectively to α-D-mannopyranoside and α-D-glucopyranoside residues present in glycoprotein molecules. In recent years Con A has proven to be a useful probe for the study of membrane-associated functions involving complex carbohydrates (5). This lectin has been reported to have an insulin-like effect (6) and to decrease insulin binding to target cells (7). Previous experiments reported from this laboratory (2, 4) indicated that Con A decreased insulin binding to fat cells and liver plasma membranes of rats fed a glucose diet to a greater extent than was observed for membranes of rats fed a high-fat diet.

These observations suggested the possibility that diet adaptations may involve changes in the chemical morphology of plasma membranes and, specifically, in the glycoproteins of the cell surface.

It seemed of interest, therefore, to study, first, the binding of ^{125}I-Con A to liver plasma membranes isolated from fat diet-adapted and carbohydrate diet-adapted rats and, second, to determine the sugar composition of glycoproteins of membranes from rats fed a high-glucose or high-fat diet. This report provides evidence that the liver plasma membranes from fat diet-adapted rats have a decreased capacity to bind Con A and contain in their glycoproteins smaller amounts of all sugar components measured with the exception of galactose.

Abbreviations: Con A, concanavalin A; ^{125}I-Con A, ^{125}I-labeled concanavalin A; BSA, bovine serum albumin.

[1] To whom all correspondence should be addressed.

MATERIALS AND METHODS

Diet and treatment of animals

Male Sprague-Dawley rats weighing 90–100 g were obtained from Taconic Farms, Germantown, NY. The animals were fed Purina rat chow for one day before they were fed either a high-glucose (G) or a high-fat (L) diet (isocaloric) for 5–6 days. During this period food and water were available ad libitum. As described in a previous report (2), both diets included 32% of calories as casein with 68% of calories in the form of lard (L diet) or glucose (G diet). Adequate amounts of vitamins, minerals, and cellulose were added. The average weight gain throughout the feeding period was 6.1 ± 0.7 g/day for the G diet-fed rats and 6.9 ± 1.0 g/day for the L diet-fed animals.

Membrane preparation

Purified plasma membrane fractions were prepared from rat liver according to the procedure of Neville (8), and aliquots were frozen at −70°C until use. Routine studies with marker enzymes, 5′-nucleotidase (9), NADH oxidase (9), and succinic dehydrogenase (10), were performed. The extent of purification was the same for both diet groups (**Table 1**). The purification of 16–17-fold for the enzyme marker 5′-nucleotidase and the yield of membrane protein (G = 3.8 mg/10 g of liver tissue; L = 3.5 mg/10 g of liver tissue) were similar for both types of membranes. Electron micrographs of these preparations (not shown) were indistinguishable and revealed plasma membranes composed mainly of paired sheets with no identifiable organelle contamination.

[125]I-Concanavalin A preparation

Carrier-free [125]iodine was purchased from New England Nuclear, Boston, Massachusetts. Con A (3×

recrystallized, obtained from Miles-Yeda) was iodinated by the chloramine T method as described by Cuatrecasas (11), and purified by affinity chromatography on Sephadex G-100 (Pharmacia). The labeled lectin had a specific activity of 13.74 ± 0.92 μCi/μg. Over 74.2 ± 4.73% of the radioactivity of the ^{125}I-Con A preparation used for binding studies was precipitable by 7% trichloroacetic acid. All storage of ^{125}I-Con A was in plastic disposable tubes at −20°C for no longer than a month.

Binding conditions. Specific binding of ^{125}I-Con A to isolated liver plasma membranes was measured according to Gurd and Evans (12). All binding assays were performed in triplicate in disposable polystyrene tubes (12 × 75 mm). Incubations were carried out at 24°C for 90 min.

The incubation mixture contained 200 μg/ml of membrane protein, 11.6 ng/ml of ^{125}I-labeled Con A (100,000 cpm), and increasing amounts of unlabeled Con A (0.2 μg/ml to 500 μg/ml). Krebs-Ringer phosphate buffer, pH 7.6, containing 0.1% bovine serum albumin (BSA) was added to a total volume of 0.15 ml.

At the end of the incubation period, the mixture was rapidly filtered through Whatman GFR glass fiber filters previously soaked in 0.1% BSA. This was followed by washing under vacuum with 10 ml of 0.1% BSA. The radioactivity remaining on the filter was measured with 10 ml of Instabray (Yorktown Research) in a Nuclear Chicago Isocap 300 liquid scintillation counter.

All binding data were corrected for nonspecific binding, measured in the presence of 2.5% α-methyl-mannopyranoside (αMDMP). The nonspecific binding in all experiments was less than 10% of the total binding.

Rate of association. Liver membranes (200 μg/ml) were incubated at 24°C for increasing intervals of time (0–120 min) with ^{125}I-Con A (11.6 ng/ml) and unla-

TABLE 1. Enzyme activities in liver homogenates and plasma membranes of glucose (G)- and lard (L)-fed rats

Purification Fraction	5′-Nucleotidase		Succinic Dehydrogenase		NADH Oxidase	
	Specific Activity[a]	Relative Specific Activity[b]	Specific Activity	Relative Specific Activity	Specific Activity	Relative Specific Activity
Homogenate						
G	3.0 ± 0.51[c]	1.0	13.0 ± 4.01	1.0	140.0 ± 30.1	1.0
L	3.2 ± 0.59	1.0	11.0 ± 3.15	1.0	160.0 ± 25.08	1.0
Plasma membrane						
G	52.0 ± 2.80	17.3	2.0 ± 0.22	0.15	30.0 ± 1.88	0.21
L	50.0 ± 3.23	16.2	1.8 ± 0.30	0.16	30.0 ± 1.56	0.18

[a] Specific activities are expressed as nmol of substrate metabolized per milligram of protein per min.
[b] Relative specific activity is specific activity of plasma membrane/specific activity of whole homogenate.
[c] Values are given as the means ± SE of three separate determinations; each determination represents the pooled livers of eight animals.
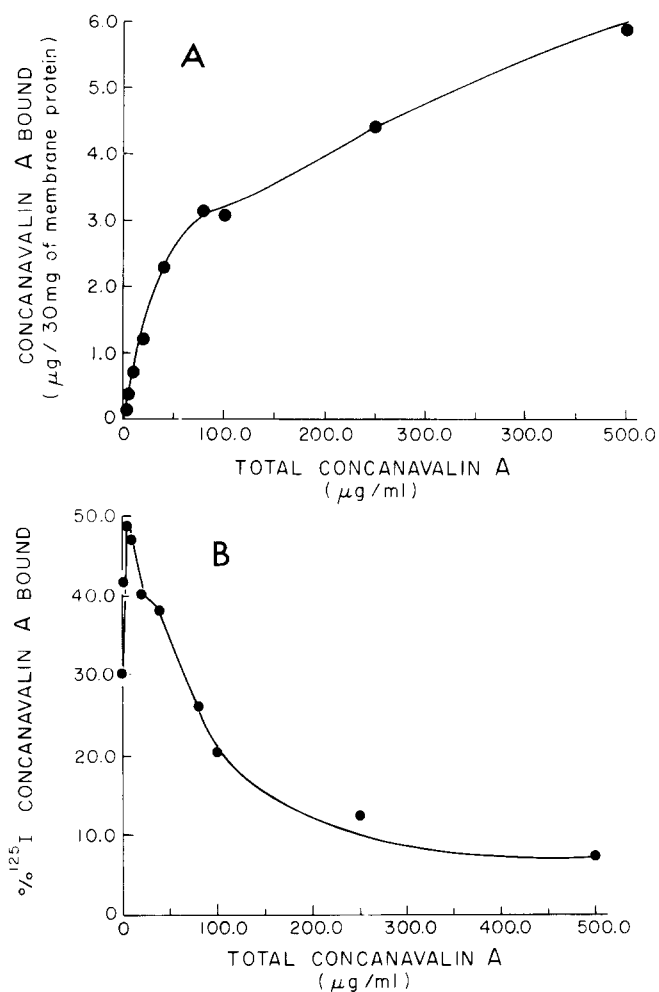
Fig. 1. Specific binding of ^{125}I-Con A to liver plasma membranes from chow-fed rats. Liver membranes, 200 μg protein/ml, were incubated with 11.6 ng/ml of ^{125}I-Con A and with unlabeled Con A over the range of 0.2–500 mg/ml (total). The binding conditions were described in detail in Methods. *A*. The specific Con A bound is plotted as a function of the total Con A concentration. *B*. Percent of Con A bound is plotted as a function of total Con A concentration.

beled Con A (100 μg/ml). At the end of the incubation period reaction mixtures were filtered, and radioactivity was determined as previously described. Nonspecific binding was determined in the presence of 2.5% αMDMP.

Rate of dissociation. Dissociation of ^{125}I-Con A was initiated after 90 min of incubation. Duplicate 50-μl aliquots of the incubation mixture (200 μg/ml of membrane protein, 11.6 ng/ml of ^{125}I-Con A and 100 μg/ml of unlabeled Con A) were then diluted in 5 ml of Krebs-Ringer phosphate buffer, pH 7.6, or 5 ml of the same buffer containing 500 μg/ml of unlabeled Con A. Incubations were carried out for increasing intervals of time, after which membrane-bound ^{125}I-Con A was determined as previously described (12).

Concanavalin A degradation. Determination of the

amount of ^{125}I-Con A degraded during the binding assay was performed at the end of each experiment. For this, 150 μl of the incubation mixture (200 μg/ml membrane, 11.6 ng/ml ^{125}I-Con A, and increasing concentrations of cold Con A) were centrifuged for 10 min in plastic microfuge tubes. The supernatant was removed and a trichloroacetic acid (TCA) precipitability test was done to calculate the amount of free ^{125}I-Con A still precipitable by the acid.

Carbohydrate analysis of delipidated membranes

Liver plasma membranes were extensively dialyzed (96 hr) against distilled water at 2°C to remove any sucrose that was still present in the membrane suspension after the fractionation procedures. Dialyzed membranes were delipidated according to the method of Folch, Lees, and Sloane Stanley (13).

Bound hexosamines in the lipid-free membrane fraction were estimated after hydrolysis of the fraction in 2 N HCl for 16 hr at 100°C in a sealed tube, under an atmosphere of N_2. Hydrolysates were passed through a Dowex 50-X8 (200–400 mesh, H^+ form) resin column (14) and the hexosamine was eluted with 2 N HCl. The eluate was dried by lyophylization. Hexosamine content was determined by the method of Gaff and Berman (15) with galactosamine as reference standard. Tracer amounts of N-acetyl-[1-^{14}C]-glucosamine (New England Nuclear) were added to each fraction prior to hydrolysis in order to correct for incomplete recovery (G membranes, 81.7%; L membranes, 84.2%).

Galactose and mannose were estimated after hydrolysis of the membrane fraction in 2 N H_2SO_4 for 5 hr at 100°C in a sealed tube. Diluted hydrolysate was passed sequentially through columns of Dowex 50-4X and 1-8X (16). The eluate was concentrated by lyophilization, applied on Whatman No. 1 paper, and the chromatogram was developed in n-butanol–ethanol–water 10:1:2 (v/v) (16) for 96 hr. The mobilities of the different sugars were compared to standards run simultaneously and located by the silver nitrate reagent (17). Quantitative determinations of the individual sugars were made after elution of the spots. Mannose was determined by the Park and Johnson ferricyanide method (18). As the procedure described above did not allow separation of galactose and glucose, the entire glucose–galactose area was eluted. One aliquot of this eluate was analyzed for glucose (Statzyme, Reagent, Worthington Biochemical Corp.), and another aliquot was analyzed by the ferricyanide method to measure the sum of the galactose and glucose. The value of the galactose component was obtained by subtracting from the total the value obtained for glucose. To correct for incomplete recovery, tracer

amounts of [1-^{14}C]galactose and [1-^{14}C]mannose (New England Nuclear) were added to each fraction prior to hydrolysis (galactose, 76 ± 8%; mannose, 73 ± 6%).

Fucose was determined separately by the cysteine–sulfuric acid reaction of Dische and Shettles (19). Sialic acid was determined by the thiobarbituric acid method of Warren (20) after its release from the protein by hydrolysis in 0.1 N H$_2$SO$_4$ at 80°C for 60 min.

Other chemical analyses. Protein was determined by the method of Lowry et al. (21) with albumin as standard.

Statistical analysis. Values in figures and tables are presented as means ± standard errors. Student's *t* test (22) was used to compare differences between means. A *P* value < 0.05 was considered significant.

RESULTS

General features of the binding of Con A to liver plasma membranes

The binding of ^{125}I-Con A to liver plasma membranes isolated from control chow-fed rats is shown in **Fig. 1.** When the binding of ^{125}I-Con A was plotted as a function of total lectin concentration (Fig. 1*A*) a complex binding curve was obtained. No saturation of the binding process was observed even at the highest concentration of lectin tested (500 μg/ml).

When the percent of ^{125}I-Con A bound to liver plasma membranes was plotted as a function of total Con A concentration (Fig. 1*B*), a biphasic curve was observed. At low concentrations the unlabeled lectin clearly enhanced the binding of ^{125}I-Con A, whereas at high concentrations the binding of ^{125}I was readily decreased by the unlabeled lectin.

Effect of fat feeding upon the binding of concanavalin A to liver plasma membranes

Diet-induced differences were found in the binding of concanavalin A to liver plasma membranes (**Fig. 2**). While the overall shapes of the binding curves for the two groups were similar, a significantly greater binding of Con A to G membranes was observed over the range of Con A concentration from 2.0 to 500 μg per ml. This binding difference increased as the concentration of the lectin was increased; at concentrations below 4 μg/ml, G membranes showed approximately 16% more binding of Con A, while at higher concentrations (>40 μg/ml) a 26% difference was observed.

An attempt was made to see whether differences in the rates of association or dissociation could account for the differences in lectin binding to the different groups of liver plasma membranes. **Fig. 3** shows the time courses of association expressed as
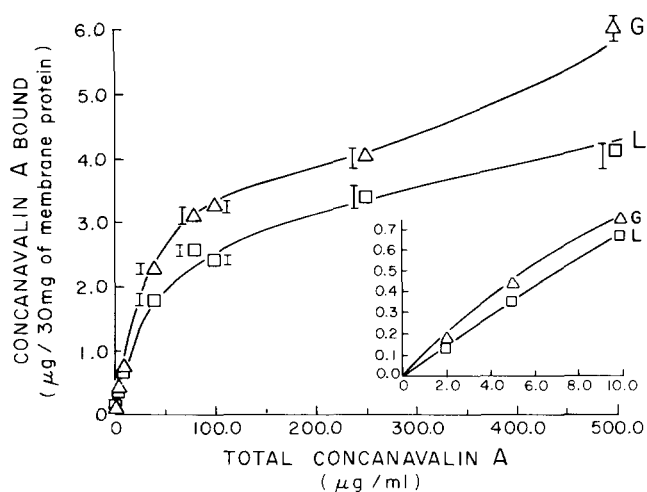

Fig. 2. Specific binding of Con A to liver plasma membranes from glucose- (△) or lard-fed (□) rats. Liver membrane, 200 μg protein/ml, was incubated with 11.6 ng/ml of ^{125}I-Con A and unlabeled Con A over a range of 0.2–500 μg/ml (total). The binding conditions were described in detail in Methods. The specific Con A bound is plotted as a function of total Con A concentration. The binding observed with low concentrations of Con A is depicted in the insert. Data for G and L membranes are the mean ± SEM of duplicate determinations in five experiments for concentrations higher than 40 μg/ml and of eight experiments for concentrations less than 40 μg/ml.

percent of maximal ^{125}I-Con A binding. No detectable difference was observed in the rate of association between G and L membranes. In both cases, Con A binds rapidly to liver plasma membranes and binding is completed within 30–40 min. After 90 min of incubation with ^{125}I-Con A, dissociation was initiated by incubation in the presence and absence of an excess of unlabeled Con A after a 100-fold dilution of lectin–receptor complex. Under both conditions dissociation of
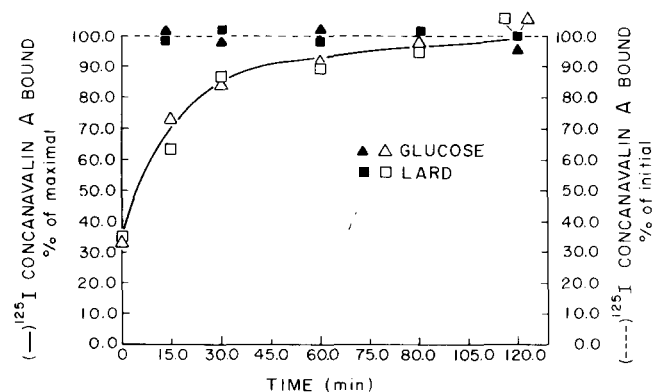

Fig. 3. Rates of association and dissociation of liver plasma membrane–concanavalin A complex. For association (—) ^{125}I-Con A was incubated with liver membranes from glucose- or lard-fed animals. The specific ^{125}I-Con A bound was determined and plotted as the percentage of maximal ^{125}I-Con A binding. For dissociation (– – –), total ^{125}I-Con A bound was determined and plotted as percentage of initial ^{125}I-Con A binding. See Materials and Methods for experimental details.

TABLE 2. Carbohydrate composition of isolated liver plasma membranes
of glucose-fed rats (G) and lard-fed rats (L)

	Glucose	Lard	L/G	
			%	
Sialic acid	4.77 ± 0.35 (8)[a]	3.31 ± 0.50 (8)	60.7	$P < 0.05$
Hexosamine	13.16 ± 0.70 (5)	8.49 ± 0.60 (5)	62.4	$P < 0.01$
Fucose	7.54 ± 0.60 (5)	5.20 ± 0.53 (5)	68.9	$P < 0.05$
Mannose	17.03 ± 1.30 (5)	11.84 ± 1.17 (5)	69.5	$P < 0.05$
Galactose	25.30 ± 2.22 (5)	27.29 ± 3.21 (5)	107.8	N.S.

Determinations were performed as given in Methods and Materials. Values
are means ± SE expressed as μg/mg of membrane protein.
[a] Number of determination; each one performed in triplicate.

the Con A receptor complex was extremely slow and at 24°C negligible dissociation was observed during a 2-hr period (Fig. 3). Similar results were obtained for G and L membranes.

The decreased Con A binding observed in L membranes could be due to an increase in Con A degradation during the incubation period, and therefore to a decrease in functionally active Con A. To test this hypothesis the extent of Con A degradation was estimated at the end of each experiment by trichloroacetic acid precipitability. Over a range of Con A concentrations from 0.2 to 500 μg/ml, no significant difference could be observed between the two groups after 90 min. The average precipitable Con A was 84.78 ± 3.36% for G membranes and 85.90 ± 3.65% for L membranes. The failure to observe differences in lectin degradation eliminated this factor as a cause for the observed differences in Con A binding.

Carbohydrate composition of isolated liver plasma membranes

Analyses of the carbohydrate compositions of delipidated G and L membranes are shown in **Table 2.** The major sugar components for both types of membranes were galactose, mannose, and hexosamines while smaller amounts of sialic acid and fucose were also present.

The total sugar content for G membranes was significantly higher than for L membranes (G, 69.77 μg/mg of membrane protein; L, 55.84 μg/mg of membrane protein). Significantly lower concentrations of all carbohydrate constituents except galactose were found in the L membranes. Although glucose was detected in both types of membranes, little significance was attributed to its presence, since this sugar can easily represent residual contamination from the fractionation procedures (23).

DISCUSSION

General features of the binding of concanavalin A to liver plasma membranes

The initial studies of binding of concanavalin A to control liver of plasma membranes (Fig. 1A,B) showed results similar to those previously reported by others in liver plasma membranes (24), thymocyte membranes (25), and fat cells (11). Binding curves suggested the presence of multiple binding sites of differing affinities. This would be in agreement with recent reports that show the involvement of multiple glycoproteins in the binding of Con A to liver plasma membranes (12). Furthermore, a detailed examination of the binding curves at low lectin concentrations (Fig. 1A, insert; Fig. 1B) suggests the existence of interactions among binding sites. At these low lectin concentrations (<10 μg/ml), unlabeled lectin clearly enhanced the binding of ^{125}I-Con A. It has been suggested by others (26, 27) that positive interactions of this kind may be the result of either an increase in the affinity of the receptor for the lectin or an increase in the number of available receptors caused by the unmasking of cryptic sites. Either one of these phenomena may be caused by conformational changes in membrane components or by their redistribution across a fluid lipid matrix.

The possibility that interactions among receptor sites may be due to cross-linking of such receptors by a multivalent ligand such as Con A has been supported by Schmidt, Ulrich, Wallach, and Hendricks (25). These authors reported that binding of Con A to rabbit thymocyte membranes induces the dimerization of its receptor (55 K dalton glycoprotein) through a cooperative process that leads to an increased affinity for the lectin. The enhanced binding affinity at low lectin concentrations could be artificially obtained by cross-linking of membrane glycoproteins by glutaral-

dehyde. In experiments not reported here, we could observe similar results when liver plasma membranes were pretreated with glutaraldehyde.

Effect of diet composition on the binding of ^{125}I-Con A to liver plasma membranes

Con A has been reported to have an insulin-like effect and to decrease insulin binding in target cells (6, 7). Previous experiments performed by members of this laboratory are consistent with these observations, and furthermore indicate an overall decrease in the effect of Con A on the interaction of insulin and its receptor in liver plasma membranes isolated from L-adapted rats.

As suggested by these observations, our present results indicate a significantly decreased binding of Con A to L membranes (Fig. 2). Data from several experiments indicate that L membranes bound 16% less Con A as compared to the G membranes at the lowest Con A concentrations (<40 μg/ml) and 26% less at higher concentrations. Since we found (as did others) that Scatchard analysis of Con A binding gives uninterpretable curves (11, 24), we are unable to say on this basis whether this reflects a change in affinity or number of one or more types of binding sites; however, rates of association and dissociation were not different and therefore could not account for differences in lectin binding to the different groups of liver plasma membranes. The decreased binding of Con A to L membranes could not be explained either by differences in contamination with other cellular components (Table 1) or increased Con A degradation during the incubation period.

These experiments suggested, therefore, a generalized diet-induced alteration in cell surface glycoproteins.

Evidence exists that membrane glycoproteins may be altered in other adaptive states. Chang, Huang, and Cuatrecasas (28) have reported that the decreased insulin binding capacity of liver plasma membranes from obese mice compared to that of their lean littermates is associated with a decreased capacity to bind Con A and wheat germ agglutinin. More recently Chandramouli et al. (24) have observed a decreased binding of Con A and ricin to plasma membranes prepared from livers of streptozotocin-diabetic rats compared to normal controls.

Carbohydrate analysis of G and L membranes

The values obtained for sialic acid and hexosamines in G membranes fell within the range of values reported by others who used rat liver plasma membranes prepared by Neville's method or variations of it (29, 30). No values are available in the literature for individual neutral sugars in purified rat liver mem-

branes; however, the total hexose content of membranes is similar to that reported by Glossman and Neville (29).

As suggested by our previous results, when carbohydrate analysis was performed on G and L delipidated membranes significant differences were observed for all sugar components measured with the exception of galactose (Table 2). Compared to G membranes there was a 39.3% decrease in sialic acid, 31.1% in fucose, 37.6% in total hexosamine, and 30% in mannose in the L membranes. No significant difference could be observed for galactose levels. The percentage decrease in mannose levels was similar to the average decrease in binding of Con A to L membranes. The explanation for the similar galactose content in both membranes as compared to the differences observed for the other sugar components is not clear. One possibility is that additional galactose residues are added to incomplete oligosaccharide units present in the L membranes as a consequence of failure to add outer residues (sialic acid, fucose).

Further work will be required to explain the mechanisms involved in these changes in chemical morphology. It is conceivable that the observed differences are a manifestation of substrate availability. In recent reports Pouyssegur and collaborators (31, 32) observed that the glycosylation of a major glycoprotein present in the cell surface of Balb/c 3T3 fibroblasts is inhibited by glucose deprivation as well as by high concentrations of glucosamine and 2-deoxyglucose. Under these conditions a nonglycosylated form of the protein appears in the cell surface. Alternatively, the morphological differences observed may reflect adaptations in membrane oligosaccharide biosynthesis. A logical point of adaptation would be in the enzymatic machinery of the cell. It is known that long-term regulation occurs when an enzyme or a sequence of enzymes in a metabolic pathway is challenged with chronically altered levels of substrate. Several dietary procedures such as those used in this work have previously resulted in alterations in the activity of several enzymes (33). It would seem possible, therefore, that the equilibrium concentrations of enzymes involved in glycoprotein synthesis may be maintained at different levels depending on whether the cell had been exposed to a metabolic mixture high or low in carbohydrate.

We conclude that adaptive responses of cells to changes in their substrate environment include alterations in the chemical morphology of their plasma membranes. Our results suggest an overall decrease in the sugar components of liver plasma membranes from L-adapted rats when compared to G-adapted animals. How these changes in membrane glycoprotein composition correlate with previous observations of

functional differences in diet adapted rats is not clear.■■

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