Tishler, M. G., Stokes, J. L., Trenner, N. R., and Conn, J. B. (1941), *J. Biol. Chem. 141,* 197.
Urry, D. W. (1971), *Proc. Nat. Acad. Sci. U.S. 68,* 672.
Urry, D. W. (1972), *Proc. Nat. Acad. Sci. U.S. 69,* 1610.
Urry, D. W., Glickson, J. D., Mayers, D. F., and Haider, J. (1972), *Biochemistry 11,* 487.
Urry, D. W., Goodall, M. C., Glickson, J. D., and Mayers, D. F (1971), *Proc. Nat. Acad. Sci. U.S. 68,* 1907.

Fractionation of Synaptic Plasma Membrane Glycoproteins by Lectin Affinity Chromatography†

James W. Gurd* and Henry R. Mahler‡

ABSTRACT: Synaptic plasma membranes were prepared from rat brain and extracted with 1% DOC–5 mM Tris-HCl buffer (pH 8.4), and the extract was chromatographed on an affinity column consisting of *Lens culinaris* phytohaemagglutinin attached to Sepharose 4B (LcH Sepharose). Most of the 7–8 glycoproteins present in the membrane extract were retained by LcH Sepharose and eluted with 2.5% α-methyl D-mannopyranoside. When a DOC extract of synaptic membranes labeled with [^{3}H]fucose was chromatographed on LcH Sepharose 45% of the radioactivity was retained; 25% of the radioactivity was retained when a similar extract was applied to an affinity column made with wheat germ agglutinin (WGA Sepharose). A membrane extract labeled with [^{3}H]fucose was chromatographed on LcH Sepharose and WGA Sepharose columns which had been connected in sequence. Under these conditions four fractions containing [^{3}H]fucose labeled glycoproteins were obtained. Sodium dodecyl sulfate gel electrophoresis indicated that each of the four fractions possessed a characteristic glycoprotein composition. The results are discussed in terms of the composition and possible functional significance of the membrane glycoproteins. The general applicability of the procedure for the purification of both membrane and soluble glycoproteins is suggested.

Glycoproteins are present as constituents of cell surface (plasma) membranes and have been implicated in a variety of surface related phenomena (see review by Hughes, 1973). The number, nature, and distribution of surface glycoproteins have been investigated by studying the reaction of lectins, proteins which bind in a highly specific manner to sugar residues (Sharon and Lis, 1972), with the surface membrane (Cuatrecasas, 1973; Henning and Uhlenbruck, 1973; Nicolson and Singer, 1971; Sela *et al.,* 1971; Fox *et al.,* 1971). Carbohydrates are present on the surface of neuronal cells (Rambourg and Leblond, 1967; Pfenninger, 1973) and receptors for the plant lectin concanavalin A have been demonstrated on the surface of isolated synaptosomes as well as in the synaptic cleft (Matus *et al.,* 1973; Bosmann, 1972). Isolated synaptic plasma membranes are enriched in protein bound glucosamine and sialic acid (Gombos *et al.,* 1971) and have been shown by gel electrophoresis to contain six to eight molecular weight classes of glycoproteins (Gurd *et al.,* 1974; Banker *et al.,* 1972).

Lectins have been used for the isolation of both soluble and membrane associated glycoproteins (Allen *et al.,* 1972; Lis and Sharon, 1973). As an initial step in a study of the functional and biosynthetic properties of synaptic plasma membrane glycoproteins we now describe their isolation and fractionation by affinity chromatography using the lectins isolated from *Lens culinaris* and wheat germ as the affinity ligands. A preliminary report of this work has been given (Gurd and Mahler, 1974).

Materials and Methods

Preparation of Synaptic Plasma Membranes and Administration of Isotope. Synaptic plasma membranes were prepared from the cortices of 30–35-day old Sprague Dawley rats as previously described (Gurd *et al.,* 1974). For experiments in which the distribution of [^{3}H]fucose was determined the isotope (250–500 μCi) was taken to dryness under N_2 at 4°, dissolved in 50 μl of 10 mM PO_4/0.9% NaCl (pH 7.6), and administered by intracerebral injection of 10 μl of this solution to each of five rats. After 16 hr the animals were killed, labeled cortices were mixed with an equal number of unlabeled cortices, and plasma membranes were prepared as above.

Isolation of Lectins and Preparation of Affinity Columns. Affinity columns were prepared using lectins isolated from the common lentil, *Lens culinaris* (LcH),[1] and from wheat germ (WGA). LcH was isolated essentially as described by Hayman and Crumpton (1972) using adsorption of the lectin on G-100 as the primary purification step. A Mn^{2+} requirement for LcH has been described (Paulová *et al.,* 1971) and it was necessary to include 5 mM $MnCl_2$ in all solutions used during the isolation procedure in order to minimize the loss of carbohydrate binding activity. WGA

† From the Brain Research Group, Department of Chemistry, Indiana University, Bloomington, Indiana 47401. *Received July 1, 1974.* Supported by Research Grant NS 08309 from the Institute of General Medical Sciences. Publication No. 2527.

* Author to whom correspondence should addressed. Present address: Department of Zoology, Scarborough College, University of Toronto, West Hill, Ontario, Canada.

‡ Recipient of a Research Career Award K06 GM 05060 from the Institute of General Medical Sciences.

[1] Abbreviations used are: LcH, *Lens culinaris;* WGA, wheat germ; SDS, sodium dodecyl sulfate; DOC, sodium deoxycholate; GlcNAc, *N'*-acetylglucosamine; αMDMP, methyl α-D-mannopyranoside; PAS, periodate-Schiff procedure.

was isolated from wheat germ acid phosphatase (Miles-Seravac) using affinity chromatography on ovomucoid-Sepharose, prepared using CNBr activated Sepharose (Pharmacia) exactly as described by Marchesi (1972). The isolated lectins were homogeneous as indicated by SDS gel electrophoresis.

Affinity columns were prepared using CNBr activated Sepharose obtained from Pharmacia. Prior to reaction with CNBr Sepharose the purified lectins were dialyzed against 100 mM NaH_2CO_3 (pH 8.4) containing either 0.2 M methyl α-D-glucopyranoside or 0.2 M N'-acetylglucosamine (GlcNAc) in the case of LcH and WGA, respectively. Lectins (20–25 mg) were incubated with CNBr Sepharose (3 g) in a total volume of 10 ml of the dialysis buffer overnight at 4° and the reaction was stopped by the addition of 4 mM ethanolamine (pH 8.4) and incubation for an additional hour. The Sepharose–lectin complexes were washed successively with 250 ml of 100 mM NaH_2CO_3–1 M NaCl (pH 8.4), 100 mM sodium acetate–1 M NaCl (pH 4.0), glass-distilled water, and then exhaustively with 1% DOC–5 mM Tris-HCl buffer (pH 8.4) (DOC–Tris).

Extraction and Fractionation of Membrane Proteins. The isolated membrane fraction was washed once with glass-distilled water in order to remove sucrose and the washed membranes were suspended in 1% DOC–5 mM Tris-HCl buffer (pH 8.4) at a final protein concentration of 1–2 mg/ml. Following incubation at 37° for 15 min the membrane–DOC suspension was centrifuged at 100,000*g* for 30 min. The supernatant was removed and used for fractionation studies. Fresh extracts were prepared for each experiment.

For affinity chromatography the membrane extract was applied to either LcH Sepharose or WGA Sepharose and the column washed with DOC–Tris buffer until the E_{280} or radioactivity of the eluent had returned to background. The buffer was then changed to include, in addition to DOC–Tris buffer, 2.5% methyl α-D-mannopyranoside (αMDMP) or 2.5% GlcNac for LcH Sepharose and WGA Sepharose, respectively. When LcH Sepharose and WGA Sepharose columns were connected in sequence the sample was applied to LcH Sepharose and the eluent from this column was directed to the top of the WGA Sepharose column from which fractions were collected. The sequence of buffers used for elution was (1) DOC–Tris, (2) DOC–Tris–2.5% GlcNAc, (3) DOC–Tris, (4) DOC–Tris–2.5% αMDMP, and (5) DOC–Tris–2.5% GlcNAc. Steps 2, 3, and 5 were performed by disconnecting the columns and eluting WGA Sepharose directly. All columns were run at 4° and were washed with 50 ml of DOC–Tris buffer immediately before use. When appropriate, portions of the eluted fractions were removed for the determination of radioactivity as described below.

SDS Gel Electrophoresis. Pooled fractions from the affinity columns were concentrated by ultrafiltration against a PM10 filter (Amicon) and the concentrated fractions were dialyzed overnight at 4° against 10 mM Tris-HCl buffer (pH 8.4). Dialyzed samples were precipitated at 4° by the addition of one-tenth volume of 4% acetic acid. The precipitate was washed two times with 3 ml of ethanol to remove DOC and lipids and was dissolved in 4 M urea, 1% sodium dodecyl sulfate, and 1% 2-mercaptoethanol solution by heating at 100° for 3–5 min. In some instances the SDS solution did not clarify and smeared gel patterns were obtained. This could be avoided by passing the cloudy SDS suspension through a Millipore filter (0.22-μ clearance) prior to electrophoresis and this procedure was routinely followed in the present experiments. Although 25–30% of the radioactivity was lost by the inclusion of this step the distribution of radioactivity on the gel was not altered. Plasma membranes were dissolved by heating in 4 M urea, 1% sodium dodecyl sulfate, and 1% 2-mercaptoethanol at 100° for 3–5 min. Polyacrylamide gels [7.5% (w/v) polyacrylamide, 10 cm × 6 mm] were prepared as described by Weber and Osborn (1969) and run at 6–8 mA/tube for 10 hr. After electrophoresis gels were stained either for protein with Coomassie Blue or for carbohydrate by the periodate–Schiff procedure (PAS) (Zacharius *et al.*, 1969). For the determination of radioactivity, gels stained with Coomassie Blue were sliced into 2-mm sections and counted as described below.

Determination of Radioactivity. Portions of the column fractions and of membrane extracts were counted in 10 ml of scintillant consisting of 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis[2-(5-phenyloxazoly)]benzene/l. of toluene–Triton X100 (2:1 v/v). Gel slices were dissolved in 0.5 ml of 30% H_2O_2 by heating at 50° overnight prior to the addition of 10 ml of the above scintillant. Radioactivity was determined in a Nuclear Chicago Mark 1 liquid scintillation counter with a counting efficiency for ^3H of 35%.

General Methods. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Dansylated bovine serum albumin and ovalbumin were prepared as described by Inouye (1971). The molecular weights of the dansylated products were determined by comparison with standards on SDS gels to be 77,000 and 45,000 respectively.

Materials. All materials and reagents were of analytical grade and were obtained from Sigma Chemical Co., Ltd. [^3H]Fucose (13.4 Ci/mmol) was obtained from New England Nuclear. Glass-distilled water was used throughout.

Results

Synaptic plasma membranes were prepared as previously described (Gurd *et al.*, 1974). On the basis of enzymic and morphological criteria 75–80% of the isolated fraction was estimated to consist of presynaptic membranes. This was the fraction used for the following studies.

Fractionation of Proteins on LcH Sepharose. Extraction of the purified membranes with DOC–Tris buffer solubilized 75–80% of the protein. When this extract was chromatographed on LcH Sepharose, which selects for mannoside and glucoside residues (Stein *et al.*, 1971), 80–85% of the protein was washed directly through the column and 15–20% was retained and subsequently eluted with 2.5% αMDMP (Figure 1). Analysis of the unretained and retained fractions by SDS gel electrophoresis indicated that the former consisted of a large number of proteins ranging in molecular weight from less than 30,000 to greater than 180,000, only two of which gave a positive PAS stain indicative of glycoproteins. In contrast the retained fraction consisted predominately of proteins with a molecular weight in excess of 60,000, the majority of which were associated with carbohydrates (Figure 1). These results indicated that chromatography of the membrane extract on LcH Sepharose had effected a separation of most of the glycoproteins from the remainder of the membrane constituents.

Fractionation of [^3H]Fucose Labeled Proteins on LcH and WGA Sepharose. In order to label the carbohydrate moiety of the glycoproteins, and thereby facilitate their de-

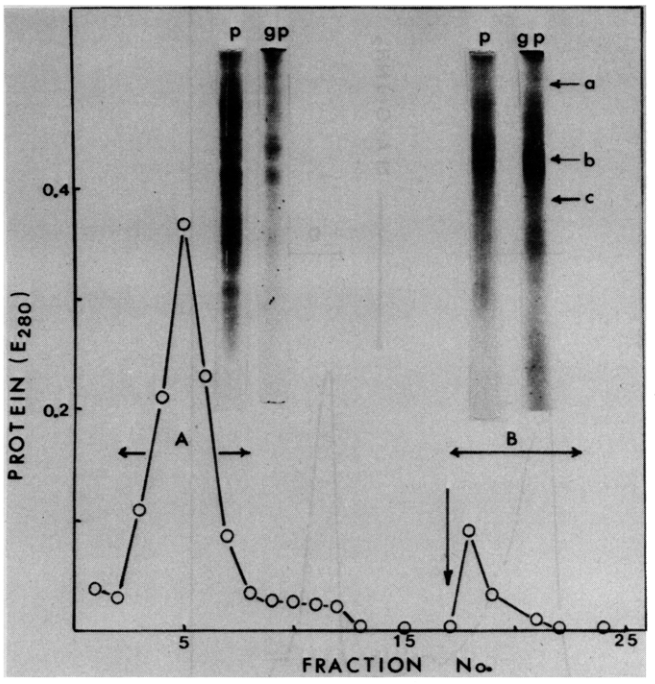

FIGURE 1: Fractionation of synaptic plasma membrane proteins on LcH Sepharose. Synaptic plasma membranes were extracted with 1% DOC–5 mM Tris-HCl buffer (pH 8.4) and 2.2 mg of protein was applied to the column and eluted with DOC–Tris buffer. At the arrow 2.5% αMDMP was added to the elution buffer; 130-drop fractions were collected. Fractions A and B were pooled as indicated and contained 1.4 and 0.4 mg of protein, respectively. Samples were prepared for SDS gel electrophoresis as described in the Materials and Methods section. Gels were sliced longitudinally and stained either for protein with Coomassie Blue (p) or for carbohydrates by the PAS procedure (gp). Molecular weight standards were: (a) I_gG 160,000, (b) bovine serum albumin 68,000, and (c) ovalbumin 45,000.

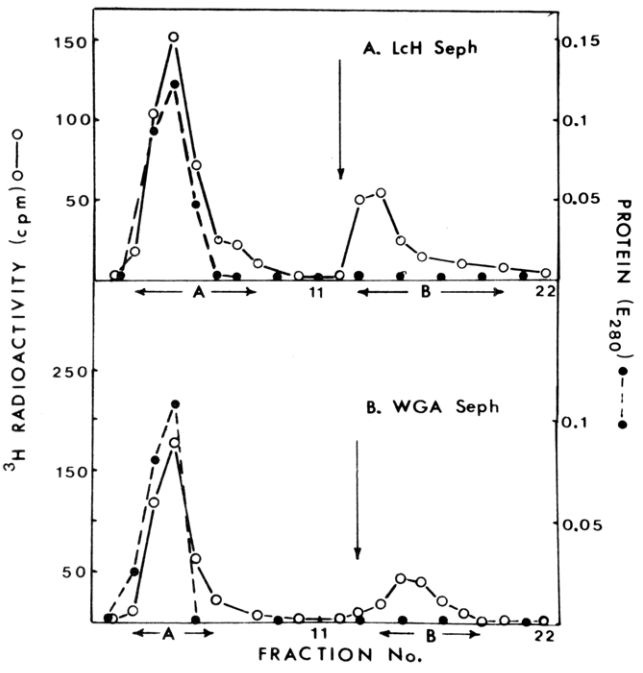

FIGURE 2: Fractionation of [^{3}H]fucose labeled synaptic plasma membrane proteins on LcH and WGA Sepharose affinity columns. [^{3}H]Fucose was administered 16 hr prior to preparation of synaptic plasma membranes. At the arrows the DOC–Tris elution buffer was changed to contain either 2.5% αMDMP (LcH Sepharose) or 2.5% GlcNAc (WGA Sepharose). Fractions were pooled as indicated. Additional experimental details are as in Table I.

tection, [^{3}H]fucose was administered 16 hr prior to preparation of the synaptic membranes. Fucose has been shown to be incorporated uniquely into brain glycoproteins (Zatz and Barondes, 1969) and to appear at the nerve terminal within 3–4 hr after injection (Zatz and Barondes, 1971). Extraction of the labeled membranes with DOC–Tris buffer solubilized 80–90% of the radioactivity and 80% of the protein. When this extract was applied to LcH Sepharose 40–45% of the radioactivity was recovered in the retained fraction which represented a three- to fourfold increase in specific radioactivity (Figure 2, Table I). Application of a similarly labeled extract to WGA Sepharose, which selects for GlcNAc residues (Burger and Goldberg, 1967), resulted in only 25–30% of the radioactivity being retained (Figure 2, Table I).

Sequential Fractionation of [^{3}H]Fucose Labeled Proteins by LcH and WGA Sepharose. The above results indicated a differential retention of glycoproteins from the synaptic membrane by LcH and WGA Sepharose. In addition, the specific nature of the lectin binding reaction suggested that fractionation of the glycoproteins might be achieved by running the two affinity columns in sequence. LcH and WGA Sepharose columns were therefore arranged in tandem as described in the Materials and Methods Section and a membrane extract labeled with [^{3}H]fucose was applied. Under these conditions an initial peak of radioactivity representing proteins which were not retained by either LcH or WGA Sepharose was obtained (Frac A, Figure 3). Successive buffer changes to 2.5% GlcNAc, 2.5% αMDMP, and 2.5% GlcNAc eluted three additional fractions of the following lectin specificities: LcH negative, WGA positive (Frac B), LcH positive, WGA negative (Frac C), and LcH positive, WGA positive (Frac D). Of the radioactivity which was LcH positive 24–28% was WGA negative and 17–20% was WGA positive. Similarly the WGA positive proteins, which accounted for 30% of the radioactivity, were subdivided into LcH positive and LcH negative fractions. Only 20% of the labeled glycoproteins were retained by both affinity columns (Table II).

Analysis of each of the four affinity column fractions by SDS gel electrophoresis indicated that we had achieved a fractionation of the glycoproteins (Figure 4). Although there was some overlap of molecular weight classes each of

TABLE I: Fractionation of [^{3}H]Fucose Labeled Synaptic Plasma Membrane Proteins on LcH Sepharose and WGA Sepharose Affinity Columns.[a]

Lectin	Fraction	[^{3}H]-Fucose (cpm)	Protein (μg)	Specific Activity (cpm/μg)
LcH	Unretained (A)	5300	850	6
	Retained (B)	5100	250	21
WGA	Unretained (A)	4640	n.d.	n.d.
	Retained (B)	1180	n.d.	n.d.

[a] [^{3}H]Fucose (250 μCi) was administered 16 hr prior to the preparation of synaptic plasma membranes; 1.2 mg (10,400 cpm) and 0.7 mg (6300 cpm) of protein were applied to LcH and WGA Sepharose, respectively. Results are from the experiments described in Figure 2. n.d. = not determined.

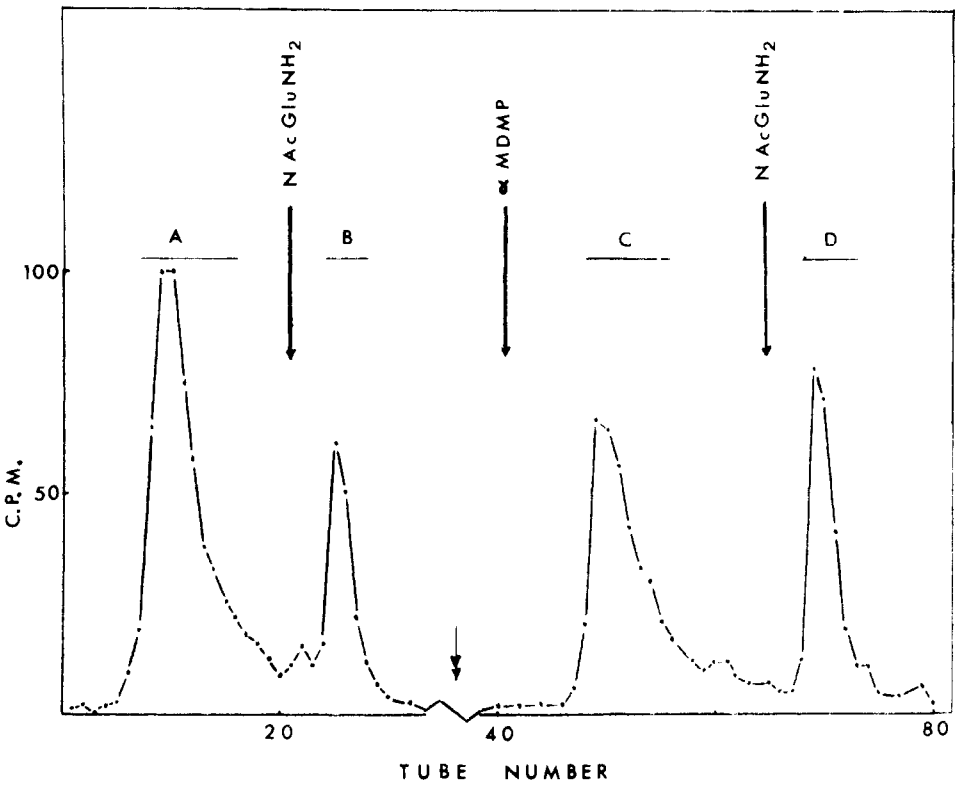

FIGURE 3: Fractionation of [^{3}H]fucose labeled synaptic plasma membrane proteins on LcH Sepharose and WGA Sepharose affinity columns connected in sequence. At the arrows the DOC–Tris buffer was changed to contain either 2.5% αMDMP or 2.5% GlcNAc as indicated. WGA Sepharose was washed with 20 ml of DOC–Tris buffer prior to the addition of αMDMP (indicated by double-headed arrow); 160-drop fractions were collected and 0.1 ml portions taken for the determination of radioactivity. Fractions were pooled as indicated. Additional details are as given in Table II, experiment 1.

the fractions exhibited a characteristic profile of radioactivity. Of the seven [^{3}H]fucose labeled proteins present in the intact membrane two, corresponding to peaks 5 and 6, were concentrated in fraction A, the latter being recovered exclusively in this fraction. The radioactivity present in fraction D was recovered predominantly as a single protein, of apparent molecular weight 83,000, corresponding to peak 4 of the intact membrane.

TABLE II: Fractionation of [^{3}H]Fucose Labeled Synaptic Plasma Membrane Proteins on LcH and WGA Sepharose Columns Connected in Sequence.

	Lectin Specificity		% of Recovered Radioactivity	
Fraction	LcH	WGA	Expt 1	Expt 2
A	Neg	Neg	42	44
B	Neg	Pos	13	11
C	Pos	Neg	28	24
D	Pos	Pos	17	20
A[1]	Neg	Neg	n.d.	86

[a] [^{3}H]Fucose (500 μCi) was administered 16 hr prior to preparation of synaptic plasma membranes. In experiment 1 41,000 cpm (1.8 mg of protein) was applied and 30,300 cpm was recovered from the columns. In experiment 2 74,500 cpm (1.7 mg of protein) was applied and 61,200 cpm was recovered. Fraction A[1] was the unretained material obtained upon a second passage of fraction A through the columns. n.d. = not determined.

Discussion

In the present paper four classes of glycoproteins present in the synaptic plasma membrane have been identified on the basis of their ability to bind to LcH and WGA lectin affinity columns. Because the reaction between a lectin and glycoprotein depends on the recognition by the lectin of a particular sugar residue and/or sequence on the protein it may be assumed that the fractionation of membrane glycoproteins now described has occurred on the basis of their carbohydrate composition. The utilization of [^{3}H]fucose as a marker for the distribution of glycoproteins facilitated the analysis of small amounts of material. However, it should be emphasized that the particular distribution of radioactivity obtained may reflect only the fucose content or the biosynthetic state of the proteins in question rather than their actual distribution. In this respect it is relevant that the distribution of [^{3}H]fucose labeled glycoproteins obtained when membranes which had been labeled for 6 days were fractionated on LcH/WGA columns was similar to that obtained following a 16-hr labeling period (J. W. Gurd, unpublished results), indicating that differential incorporation and/or breakdown of synaptic membrane fucosyl proteins did not occur after the earlier time.

The use of multilectin specificities in conjunction with each other should find general application for the purification and fractionation of glycoproteins. Although only two lectins were used for the present experiments there is no *a priori* limitation on the number of different lectins which might be used in combination with each other. Indeed with the appropriate choice of specificities purification of individual glycoproteins should be possible.

Although the involvement of surface carbohydrate

moieties in a variety of membrane activities has been described (Hughes, 1973) the specific function(s) of the various synaptic membrane glycoproteins is not known. A number of surface membrane enzymes are glycoproteins (Evans and Gurd, 1973; Evans *et al.*, 1973) and both acetylcholine esterase (Wenthold *et al.*, 1974) and acetylcholine receptor (Hucho and Changeux, 1973) have been shown to bind to lectin affinity columns. It has been suggested that glycoproteins present at the nerve terminal may play a role in the formation and function of synaptic contacts (Brunngraber, 1969) and that surface carbohydrates may be involved in the regulation of neuronal cell growth and differentiation (Kaplowitz and Moscona, 1973; Glick *et al.*, 1973; Treska-Ciesielski *et al.*, 1971) as well as in the formation of specific histogenetic associations during development of the central nervous system (Moscona, 1971). Neither LcH nor concanavalin A inhibited the energy dependent, high affinity uptake of a number of putative CNS transmitters (Y.-J. Wang and J. W. Gurd, unpublished results) indicating that the receptors for these lectins are not directly associated with this process.

Previous reports have shown that synaptic plasma membranes contain 6–8 glycoproteins (Gurd *et al.*, 1974; Banker *et al.*, 1972). These yielded two types of glycopeptides upon extensive digestion with Pronase, one consisting almost exclusively of mannose and glucosamine and the other containing most of the sialic acid, one-third of the mannose and glucosamine as well as fucose, galactose, and galactosamine residues (Gombos *et al.*, 1971). Although the distribution of these glycopeptides among the individual glycoproteins is not known some inferences may now be drawn from the specificities of the two lectins used in the present study. In addition to the requirement for mannose residues, glucosamine also appears to be involved in the binding of glycoproteins to LcH (Kornfeld *et al.*, 1971). WGA is specific for GlcNAc residues and binds more strongly to oligosaccharides than to the simple sugar (Allen *et al.*, 1973). These specificities suggest that the former class of glycopeptides may be concentrated in the LcH and WGA positive fractions whereas the latter may be present primarily in the unretained fraction. It is significant in this context that only 15% of the sialic acid present in an extract of total rat brain particulate proteins bound to concanavalin A (Susz *et al.*, 1973) which has a specificity similar to that of LcH (Young *et al.*, 1971) and that rat brain microsomal glycopeptides which bound to polymerized concanavalin A were deficient in both fucose and sialic acid (Gombos *et al.*, 1972).

Each of the four glycoprotein fractions now described possessed fucose containing glycoproteins of apparently similar molecular weight. Although the possibility that such overlap between fractions represents cross-contamination cannot be ruled out, the reproducibility of the elution profile and the fact that the fractions eluted at the same point on a second passage argue against it. Electrophoresis in SDS separates proteins on the basis of molecular weight and a single molecular weight class may consist of a mixture of proteins (Knufermann *et al.*, 1973). The occurrence of proteins of similar molecular weight but apparently different lectin specificities may therefore represent membrane constituents which differ both in their protein and carbohydrate portions or similar proteins which differ only with respect to the composition of their carbohydrate side chains, alternatives which cannot be distinguished on the basis of the present results. The synaptic plasma membrane

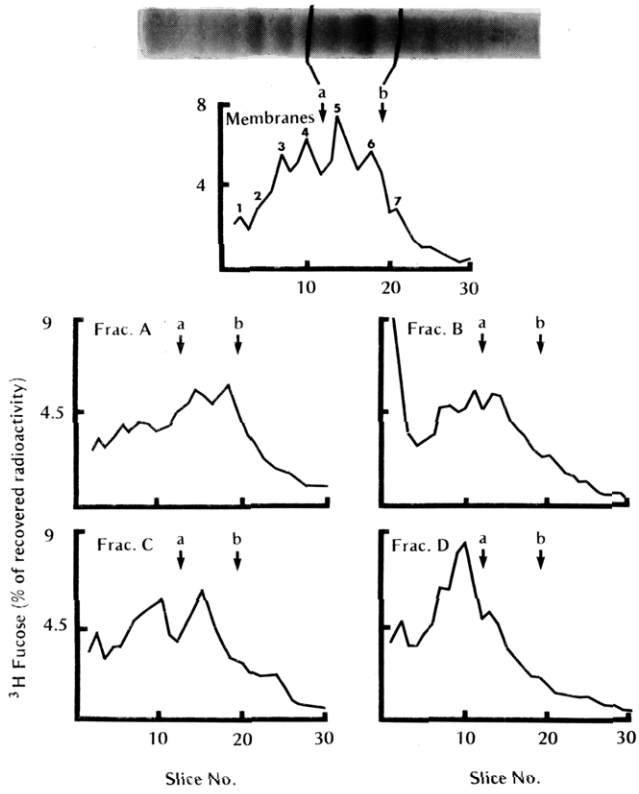

FIGURE 4: Distribution of [^{3}H]fucose labeled synaptic plasma membrane proteins between LcH Sepharose and WGA Sepharose fractions. Samples were obtained from experiment 2 described in Table II and were prepared for SDS gel electrophoresis as described in the Materials and Methods section. Following electrophoresis the positions of the internal molecular weight standards, dansylated bovine serum albumin and dansylated ovalbumin were marked with a piece of wire prior to staining and slicing; 2-mm slices were made. The direction of electrophoresis was from left to right. The stained gel represents the intact membrane stained for protein with Coomassie Blue. (a) dansylated bovine serum albumin mol wt 77,000; (b) dansylated ovalbumin, mol wt 45,000. The total counts applied to each gel were: intact membranes, 11,000; Frac A, 11,700; Frac B, 3,320; Frac C, 8,800; Frac D, 6,500.

fraction used for the present experiments was derived from a functionally heterogeneous population of synaptosomes (*i.e.*, from the cerebral cortex) and the diversity of its protein and glycoprotein constituents may reflect this heterogeneity. It is hoped that experiments using more homogeneous synaptosome fractions as starting material (McGovern *et al.*, 1973) will help to distinguish between these possibilities.

Acknowledgments

We thank Dr. W. J. Moore for helpful discussions during the early part of this work and Jeanene Ondrik for excellent technical assistance.

References

Allen, A. K., Neuberger, A., and Sharon, N. (1973), *Biochem. J. 131,* 155–162.

Allen, D., Auger, J., and Crumpton, M. J. (1972), *Nature (London), New Biol. 236,* 23–25.

Banker, G., Crain, B., and Cotman, C. W. (1972), *Brain Res. 42,* 503–513.

Bosmann, H. B. (1972), *FEBS (Fed. Eur. Biochem Soc.) Lett. 22,* 97–100.

Brunngraber, E. G. (1969), *Perspectives Biol. Med. 12,* 467–470.

Burger, M. M., and Goldberg, A. R. (1967), *Proc. Nat. Acad. Sci. U. S. 57,* 512–515.
Cuatrecasas, P. (1973), *Biochemistry 12,* 1312–1323.
Evans, W. H., and Gurd, J. W. (1973), *Biochem. J. 133,* 189–199.
Evans, W. H., Hood, D. O., and Gurd, J. W. (1973), *Biochem. J. 135,* 819–826.
Fox, T. O., Sheppard, J. R., and Burger, M. M. (1971), *Proc. Nat. Acad. Sci. U. S. 68,* 244–247.
Glick, M. C., Kimhi, Y., and Littauer, U. Z. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 1682–1687.
Gombos, G., Hermetot, J. C., Reeber, A., Zanetta, J. P., and Treska-Ciesielski, J. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 24,* 247–250.
Gombos, G., Morgan, I. G., Waehneldt, T. V., Vincendon, G., and Breckenridge, W. G. (1971), *Advan. Exp. Med. Biol. 25,* 101–113.
Gurd, J. W., Jones, L. R., Mahler, H. R., and Moore, W. J. (1974), *J. Neurochem. 22,* 281–290.
Gurd, J. W., and Mahler, H. R. (1974), *Trans. Amer. Soc. Neurochem. 5,* 110.
Hayman, M., and Crumpton, M. J. (1972), *Biochem. Biophys. Res. Commun. 47,* 923–930.
Henning, R., and Uhlenbruck, G. (1973), *Nature (London), New Biol. 242,* 120–122.
Hucho, F., and Changeux, J. P. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 38,* 11–15.
Hughes, R. C. (1973), *Progr. Biophys. Mol. Biol. 26,* 189–268.
Inouye, M. (1971), *J. Biol. Chem. 246,* 4834–4838.
Lis, H., and Sharon, N. (1973), *Annu. Rev. Biochem. 42,* 541–574.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265–275.
Kaplowitz, P. B., and Moscona, A. A. (1973), *Biochem. Biophys. Res. Commun. 55,* 1326–1333.
Kornfeld, S., Rogers, J., and Gregory, W. (1971), *J. Biol. Chem. 246,* 6581–6586.
Knufermann, H., Bhakdi, S., Schmidt-Ullrich, R., and Wallach, D. F. (1973), *Biochim. Biophys. Acta 330,* 356–361.
Marchesi, V. T. (1972), *Advan. Enzymol. 288,* 354.
Matus, A., de Petris, S., and Raff, M. C. (1973), *Nature (London), New Biol. 244,* 278–280.
McGovern, S., Maguire, M. E., Gurd, R. S., Mahler, H. R., and Moore, W. J. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 31,* 193–198.
Moscona, A. A. (1971), *Science 171,* 905–907.
Nicolson, G. L., and Singer, S. J. (1971), *Proc. Nat. Acad. Sci. U. S. 68,* 942–945.
Paulová, M., Tichá, M., Entlicher, G., Koštíř, J. V., and Kocourek, J. (1971), *Biochim. Biophys. Acta 252,* 388–395.
Pfenninger, K. H. (1973), *Progr. Histochem. Cytochem. 5,* 1–86.
Rambourg, A., and Leblond, C. P. (1967), *J. Cell Biol. 32,* 27–53.
Sela, B. A., Lis, H., Sharon, N., and Sach, L. (1971), *Biochim. Biophys. Acta 249,* 564–568.
Sharon, N., and Lis, H. (1972), *Science 177,* 949–959.
Stein, M. D., Howard, I. K., and Sage, H. T. (1971), *Arch. Biochem. Biophys. 146,* 353–355.
Susz, J. P., Hof, H. I., and Brunngraber, E. G. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 32,* 289–292.
Treska-Ciesielski, J., Gombos, G., and Morgan, I. G. (1971), *C. R. Acad. Sci., Ser. D 273,* 1041.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406–4412.
Wenthold, R. J., Mahler, H. R., and Moore, W. J. (1974), *J. Neurochem. 22,* 945–949.
Young, N. M., Leon, M. A., Takahashi, T., Howard, I. K., and Sage, H. J. (1971), *J. Biol. Chem. 246,* 1596–1601.
Zacharius, R. M., Zell, T. F., Morrison, J. H., and Woodlock, J. J. (1969), *Anal. Biochem. 30,* 148–152.
Zatz, M., and Barondes, S. H. (1969), *J. Neurochem. 17,* 157–163.
Zatz, M., and Barondes, S. H. (1971), *J. Neurochem. 18,* 1125–1133.