trate each region is enhanced sequentially (*17*). Further evidence for sperm selection in the oviduct comes from the observation that the motility of sperm recovered from the isthmus is poorer than that of sperm that have reached the ampulla (*4*). Both the cervix and the fallopian tube in humans contain large amounts of β-amylase (*18*), an enzyme that in the rabbit both aids capacitation of spermatozoa in vitro and causes antigenic changes similar to those found in sperm recovered from the ampulla (*19*). No explanation has been advanced for the sperm filtering and possible capacitating functions of the tubal isthmus, although the same functions in the cervix are attributed to its mucus.

These observations indicate that the ability of the oviductal isthmus to transport, bidirectionally, sperm and ova may depend, in part, on the presence or absence of a column of viscous mucus. Before ovulation the transport of sperm distally appears to be independent of cilial activity, and the mucus may serve a role in sperm selection similar to that proposed for the cervix. Within several days after ovulation the mucus disappears, allowing cilia to become erect and, presumably, able then to function efficiently in transporting the egg to the uterine cavity.

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Red Cell Membrane Glycophorin Labeling from Within the Lipid Bilayer

Abstract. *Human red blood cell membranes were labeled from within the lipid bilayer by the apolar photosensitive reagent, 5- [^{125}I]iodonaphthyl-1-azide. Glycophorin, the major sialoglycoprotein of the red cell membrane, was purified by two different methods; it contained approximately half of the total label incorporated into membrane proteins. The label was confined to the trypsin-insoluble peptide of glycophorin that includes a sequence of 20, mainly apolar, amino acids. These findings provide direct evidence that the labeled segment resides within the membrane in direct contact with the lipid bilayer, and support the suggestion that glycophorin spans the bilayer through its hydrophobic domain.*

Proteins in biological membranes are thought to have segments of their polypeptide chains in contact with or spanning the phospholipid bilayer (*1, 2*). It is likely that these domains contain specific sequences of amino acids that structurally and functionally distinguish membrane proteins from other soluble cellular proteins. The isolation and characterization of lipid-embedded polypeptides have been curtailed by the lack of direct methods to identify them. Only in the case of glycophorin, the major glycoprotein of the human red blood cell (RBC), does evidence exist for its configuration within the lipid bilayer. Glycophorin has been labeled from both surfaces of the membrane, isolated with water-soluble reagents, and digested with trypsin; the water-soluble, labeled peptides that were released could be identified as those residing in contact with the aqueous environment, while the unlabeled, indigestible peptide was suggested to be that region of the polypeptide chain buried within the bilayer (*3, 4*). Although this experimental approach was valid for glycophorin, a relatively short (131 amino acids) linear polypeptide chain, it could lead to erroneous results if significant regions of the polypeptide chain (or chains) in the aqueous environments were not labeled.

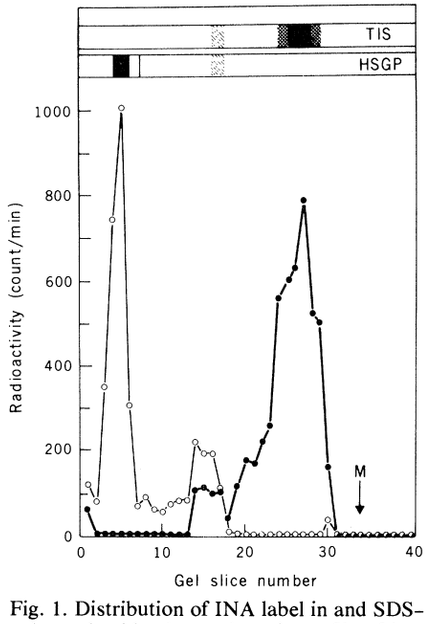

Fig. 1. Distribution of INA label in and SDS-polyacrylamide electrophoretic profile of isolated glycophorin (human sialoglycoprotein, *HSGP*) and its insoluble peptide *(TIS)* after trypsin digestion. Membranes were labeled with INA as described in the text and in (*6*), and glycophorin was isolated by affinity chromatography (Table 1) (*7*). A portion of labeled glycophorin was added to 200 μg of unlabeled glycophorin and trypsinized, and the insoluble peptide *(TIS)* was isolated (*3*). Intact labeled glycophorin (○) and *TIS* (●) were subjected to electrophoresis on 12 percent acrylamide gels in the presence of 1 percent SDS (*3*). The gels were stained for protein with Coomassie brilliant blue and cut into 2-mm slices, and the radioactivity was counted; *M* denotes the transfer dye.

A direct procedure for labeling those portions of membrane proteins in contact with the bilayer lipids had been recently developed (*5, 6*). The very hydrophobic, light-sensitive compound 5-[^{125}I]iodonaphthyl-1-azide (INA), is nonreactive in the dark, and more than 98 percent of it partitions into the lipid phase of the membrane (*6*). Subsequent short irradiation at 314 nm converts INA into a reactive nitrene that attaches covalently to integral proteins and lipids of the membrane from within the bilayer (*5, 6*).

We have labeled RBC membranes with INA and, after isolating the labeled glycophorin, have determined the distribution of the label within the polypeptide chain. Our results indicate that the trypsin-insoluble segment (TIS) contains nearly all the incorporated label. The results further provide direct evidence that TIS is located within the bilayer, and confirm the usefulness of INA in identifying polypeptides embedded in the lipid phase.

Ghosts from RBC were prepared from freshly drawn blood and were labeled with INA (*6*). To 2.0 ml of the ghost suspension (1 mg of protein per milliliter) in phosphate-buffered saline were added 20 μl of an ethanol solution of [^{125}I]INA (1.3 Ci/mmole), producing a final concentra-

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Table 1. Distribution of INA label during the isolation of glycophorin.

Fraction	Percentage of radioactivity when isolated by	
	Lithium diiodosalicylate–phenol	Wheat germ agglutinin–affinity chromatography
Membranes*	100	100
Membranes after dialysis†	60	61
Phenol phase	27	
Material not adsorbed to affinity matrix		53
Glycophorin	10	8

*The membrane preparation contained INA (8.2×10^6 and 5.6×10^6 count/min) for the lithium diiodosalicylate–phenol extraction and the wheat germ agglutinin–affinity chromatography procedure, respectively. †Irradiated membranes were dialyzed for 24 hours at 4°C against 500 ml of 0.05*M* tris-HCl, *p*H 7.4, with bovine serum albumin (2 mg/ml).

tion of about $10^{-6}M$ INA. The suspension was incubated at 37°C for 5 minutes in the dark and then irradiated at 314 nm for 6 minutes (*6*). The suspension was cooled to 4°C, and a sample was withdrawn for sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (*6*); the rest of the membrane preparation was used to isolate glycophorin. Electrophoretic analysis of the labeled membranes confirmed the observation (*6*) that approximately 20 percent of the INA was covalently attached to the membrane proteins; it was chiefly confined to the regions of band 3, other periodic acid–Schiff–positive bands, and band 7, while very small amounts of label were found in the region of bands 1 and 2 [the numbering system of polypeptide bands is in accord with (*2*)].

Glycophorin that was isolated both by affinity chromatography on wheat germ agglutinin conjugated to Sepharose 4B (*7*) and by lithium diiodosalicylate–phenol (*8*) was highly labeled. The fraction of the radioactivity incorporated into glycophorin was about 10 percent of the total initial radioactivity associated with the membrane (Table 1). Thus, approximately one-half of the [^{125}I]INA attached to the membrane proteins (*6*) resides in glycophorin.

The purified glycophorin was digested with trypsin and the TIS was isolated (*3*). In four separate experiments TIS contained from 80 to 90 percent of the total radioactivity present in glycophorin, almost all of which migrated in SDS–polyacrylamide gel electrophoresis as a broad band typical of TIS (Fig. 1). The other small peak in the TIS fraction was also present in intact glycophorin (Fig. 1); its nature is not known. The proportion of the label incorporated into TIS is probably greater than observed since small amounts of this peptide escape precipitation by the isolation procedure we employed. The radioactivity (less than 10 to 20 percent) not associated with TIS could not be identified as a defined species. It is possible that either some nonspecific labeling by INA takes place or, alternatively, other regions of the protein may have some contact with the lipid bilayer.

The TIS (33 amino acids) represents approximately 25 percent of the glycophorin polypeptide chain and 10 percent of the total molecule (60 percent carbohydrate and 40 percent protein). The finding that it contains between 80 and 90 percent of the radioactivity derived from [^{125}I]INA directly supports the concept that glycophorin is anchored in the lipid backbone of the membrane through its hydrophobic domain (most of TIS) inserted into the lipid bilayer (*3, 4, 9*). These results encourage the use of INA to identify directly segments of other membrane proteins in contact with the lipid bilayer (*5, 6, 10*).

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Kainic Acid Lesions of the Striatum Dissociate Amphetamine and Apomorphine Stereotypy: Similarities to Huntington's Chorea

Abstract. *Kainic acid lesion of cell bodies in the dorsal striatum enhanced the stereotypy-producing effects of* d-*amphetamine without affecting the stereotypy produced by the direct receptor agonist apomorphine. This pattern of results parallels that found in patients suffering from Huntington's chorea, thus strengthening the parallels between the kainic acid animal model and the human disease state initially suggested on biochemical grounds. The present results further suggest a dissociation of the mechanisms involved in the production of stereotypy by these two drugs, perhaps in terms of differential involvement of the striato-nigral negative feedback loop.*

Huntington's chorea is a genetically transmitted autosomal dominant degenerative disease (*1*) characterized by involuntary choreic movements (*2*). Evidence indicates an involvement of the basal ganglia in this disease (*3*), and it has been proposed that rats in which kainic acid has been injected into the striatum provide a model (*4*) that shows considerable biochemical similarities (*5*) to the human disease state. One of the best demonstrated functions of the striatum in animal experiments has been a role in the mediation of the stereotyped behavior patterns (*6*) seen in animals given high doses of the psychomotor stimulant drug amphetamine (*7, 8*). Electrolytic lesion of the entire stratum convincingly blocks amphetamine stereotypy (*9*), as does total destruction of afferent dopamine terminals by means of the selective neurotoxin 6-hydroxydopamine (6-OHDA) (*10*). On the other hand, amphetamine and other dopamine releasing agents, when administered to patients with Huntington's chorea, seem to exacerbate the choreic movements (*11*), that is, they have an enhanced effect compared to an identical dose in normal humans. L-Dopa has, in fact, been suggested to be of use in revealing "presymptomatic" chorea in otherwise apparently normal humans (*12*). Apomorphine, a direct dopamine receptor agonist (*13*), is devoid of this effect (*14*) despite its being highly effective in elicit-

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