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# Incorporation of Glucosamine-$^{14}$C into Membrane Proteins of Reticulocytes*

Edward D. Harris† and Clarence A. Johnson

ABSTRACT: Reticulocytes from phenylhydrazine-treated rabbits were found to incorporate radioactive glucosamine into glycoproteins of the cell membrane. Hexosamines accounted for 90% of the bound activity, while less than 5% was found in sialic acid. Radioactive uridine diphosphate *N*-acetylglucosamine was identified in the acid-soluble fraction. The incorporation was inhibited by puromycin. Added glucose was also inhibitory. Labeled components were cleaved from the intact cell surface by trypsin. These components showed a number of radioactive peaks on DEAE-cellulose and were found to be associated with glycopeptides of high molecular weight. About 10% of the radioactivity was extractable from stroma by organic solvents.

The biosynthesis of proteins by reticulocytes has been studied by many investigators. It has been established that hemoglobin is the major protein synthesized (Dintzis *et al.*, 1958). However, reticulocytes may also have the capacity to make other proteins, among these glycoproteins, as suggested by the incorporation of glucosamine-$^{14}$C into these cells (Eylar and Matioli, 1965) and into related bone marrow cells (Dukes *et al.*, 1964; Dukes and Goldwasser, 1965). In connection with our studies of glycoproteins in erythrocyte membranes, it seemed plausible that the reticulocyte system may be used to investigate the biosynthesis of these complex substances. Toward this end we have studied the incorporation of radioactive glucosamine into reticulocytes hoping to gain some insight into a mechanism. A partial characterization of radioactive products has also been made.

## Materials and Methods

*Chemicals.* Surfactant DN-65 was a gift from Rohm and Haas Corp., Chicago, Ill. Crystalline heparin (136 units/mg) was purchased from General Biochemicals Corp. UDP-*N*-acetylglucosamine was obtained from

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† Present address: Department of Physiology, University of Chicago.

Sigma Chemical Co. Puromycin dihydrochloride and trypsin (twice crystallized-salt free) were purchased from Nutritional Biochemicals Corp. Sodium pyruvate was purchased from Calbiochem Corp. Penicillin G and streptomycin sulfate were products of E. R. Squibb and Sons, Inc. Radioactive D-glucosamine-1-$^{14}$C (10–40 mCi/mmole) and lysine-1-$^{14}$C (3.3 mCi/mmole) were purchased from New England Nuclear Corp.

*Preparation of Reticulocytes.* Male, New Zealand albino rabbits (6 and 8 lb) were fed *ad libitum* throughout experimental periods. Abnormally high levels of reticulocytes were induced with phenylhydrazine (recrystallized from 95% ethanol) as described by Borsook *et al.* (1952). Animals received five subcutaneous injections, 1 ml each; vitamin supplements were omitted. Blood was collected by cardiac puncture 24–72 hr after the final injection. The heparinized whole blood amounting to 90–100 ml (35–50% reticulocytes) from each animal was chilled to ice-bath temperatures and centrifuged at 1200*g*. The cells were washed twice with centrifugation in six volumes of pH 7.4 Krebs–Ringer phosphate buffer discarding the buffy coat after each washing. The final washing was followed by a 25–30-min centrifugation at 1200*g* yielding a tightly packed cell preparation. Reticulocyte counts were performed on cells stained first with brilliant cresyl blue and then counterstained with Wright's solution (Cruz, 1941). Total cell counts were determined in a Coulter Counter, Model F.

*Reaction with Glucosamine-$^{14}$C.* One volume of packed washed cells was suspended in three volumes of Krebs–Ringer phosphate buffer containing 20 mM pyruvate, glucosamine-1-$^{14}$C (0.1–1.0 $\mu$Ci/ml of cells), and 100 units of penicillin G and 100 $\mu$g of streptomycin sulfate per ml. Buffer solutions were rendered aseptic by filtration through 0.45-$\mu$ filters. Incubations were carried out in the air at 37° in a metabolic shaker. Flasks were fitted with air condensers to minimize water loss. Unless otherwise indicated, cell suspensions were temperature equilibrated for 30 min before adding the isotope and pyruvate.

*Preparation of Acid-Soluble Extracts.* At selected times during the incubation, 1 ml of suspension was removed, centrifuged, and the cells were washed free of unincorporated radioactivity with three 10-ml portions of Krebs–Ringer buffer. Ice-cold 5% trichloroacetic acid (1 ml) was added to the sedimented cells and after thorough mixing and centrifugation, the supernatant was tested for radioactivity. Radioactive compounds in the extracts were examined after first treating the supernatant with two volumes of ether to remove trichloroacetic acid. The UDP-*N*-acetylhexosamine in the extracts was isolated separately and prepared for chromatography by the method of Cabib *et al.* (1953).

*Preparation of Stroma for Radioactive Counting.* Stroma were prepared and washed free of hemoglobin by the procedure of Dodge *et al.* (1963). Washing buffers used for this purpose contained 0.1% cold glucosamine hydrochloride to remove adsorbed radioactive compounds. Bound radioactivity was determined on preparations of stroma which had been washed at least five times with buffer and then suspended in 1 ml of 0.4 N NaOH containing 0.1% (v/v) DN-65 detergent. Heating this suspension in a water bath at 80° for 1 or 2 min dissolved the stroma. Aliquots were then tested for radioactivity and protein contents.

A more rapid estimate of bound radioactivity could be made by using pellets obtained from cold acid treatment of washed cells. In this procedure the pellets were first washed with four 10-ml portions of cold 5% trichloroacetic acid to remove soluble radioactive compounds, and then heated in 1 ml of the acid solution at 80° for 1 hr. Such treatment solubilized about 40% of the bound radioactivity in the pellet.

*Determination of Radioactivity in Sialic Acid and Hexosamines.* Mild acid treatment (5% trichloroacetic acid, 1 hr, 80°) released sialic acid and some acetylated hexosamine. The radioactivity present as sialic acid was determined by the method of Molnar *et al.* (1965). Strong acid treatment (2.0 N HCl, 15 hr, 100° in sealed tubes) released glucosamine and all of the bound radioactivity in the stroma. Hydrolysates were dried *in vacuo*, resuspended in small portions of distilled water, and after filtering out undissolved residue, chromatographed on Dowex-50 hydrogen columns. Hexosamines were eluted with 2.0 N HCl collecting the eluates in 50-ml round-bottom flasks. The contents were dried *in vacuo*, redissolved in 1 ml of distilled water, and aliquots of this were tested for hexosamines and radioactivity. The radioactivity in glucosamine and galactosamine was determined on the long column of the amino acid analyzer equipped with a scintillation flow counter (Packard Model 314 AX 500).

*Paper Chromatography.* Acid-soluble radioactive products and sugars were identified on Whatman No. 1 paper using the following solvents: (A) 95% ethanol–1 M ammonium acetate (7.5:3) (pH 3.8), (B) 95% ethanol–1 M ammonium acetate (7.5:3) (pH 7.5), and (C) ethyl acetate–pyridine–water (10:4:3). Hexosamines were detected with ninhydrin (0.4% in acetone), *N*-acetylhexosamines by the method of Partridge (1948), and sialic acid by the thiobarbituric acid reagent (Warren, 1960). *cis*-Hydroxy compounds in general were also visualized by using a modified periodic acid–Schiff base stain adapted for paper by Bonner (1960). Nucleotides were detected with a short-wave ultraviolet mineral lamp.

*Radioactivity Measurements.* Samples of aqueous solutions (not more than 0.5 ml) were transferred to a scintillation vial containing 10 ml of scintillation fluid (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(phenyloxazolyl)]benzene to 500 ml each of toluene and methyl Cellosolve) and counting was carried out in a Packard liquid scintillation counter. Radioactivity appearing on paper was determined quantitatively by cutting out 1.8-cm$^2$ strips and placing them in scintillation vials containing 5 ml of scintillation medium consisting of 0.5 g of 2,5-diphenyloxazole in 1 l. of toluene.

*Chemical Determinations.* Hexoses were determined by the method of Winzler (1955) omitting those steps needed to precipitate proteins. Proteins were measured by the biuret reaction (Gornall *et al.*, 1949) and sialic acid by the Warren (1959) method. Total hexosamines were determined following hydrolysis with 2.0 N HCl in sealed tubes for 15 hr using the method described by Gatt and Berman (1966). The analysis of neutral sugars

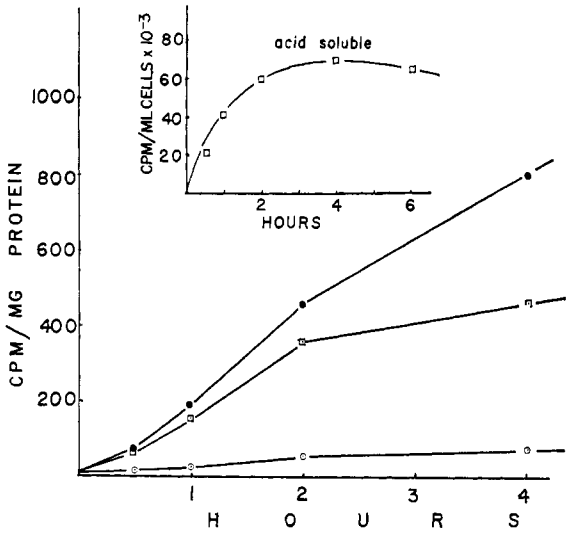


FIGURE 1: Time course of incorporation of glucosamine-$^{14}$C into cells. Incubation of cells (48% reticulocytes) and determination of bound and acid-soluble radioactivity were carried out as described in Methods. (-●—●-) Without glucose and pyruvate; (-□—□-) with 20 mM pyruvate; (-○—○-) with 20 mM glucose.

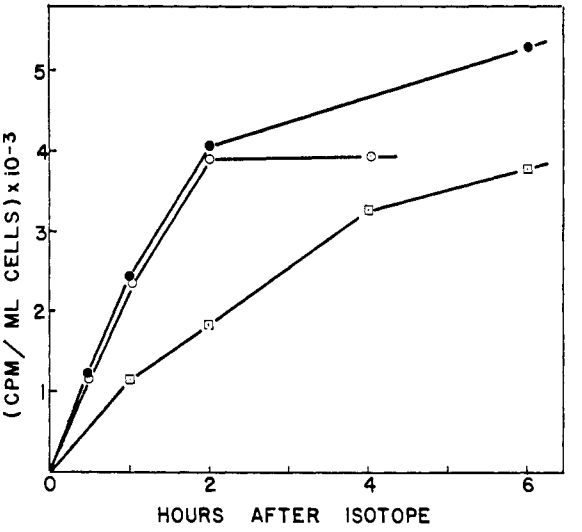


FIGURE 2: The effect of preincubation time in salt media on the rate of radioactivity uptake into stroma. (-□—□-) Not preincubated; (-●—●-) 2-hr preinubation; (-○—○-) 4-hr preincubation. Washed sedimented cells were treated with 5% trichloroacetic acid and the resulting precipitate was washed four times with 10-ml portions of the acid to remove the last traces of soluble radioactivity. An estimation of the bound radioactivity was then obtained by determining the counts released upon heating the pellet in the acid solution at 80° for 1 hr.

in the DEAE-cellulose pools by gas–liquid partition chromatography were performed at SUNY, Buffalo.[1]

## Results

*Incorporation of Glucosamine-$^{14}$C into Cells.* The incorporation of glucosamine-$^{14}$C into membrane proteins was slow in the first 30 min of incubation, but then proceeded at a faster, nearly linear rate for at least 4 hr (Figure 1). A cellular pool of acid-soluble radioactive compounds accumulated during these times. When glucose was added, the uptake of glucosamine-$^{14}$C was almost completely inhibited. Added pyruvate at the same concentration (20 mM) was also inhibitory but to a lesser extent. Faster initial rates of incorporation were achieved when the cells were incubated for 2 or 4 hr in the salt media before adding the isotope and pyruvate (Figure 2). The more rapid uptake, however, was not sustained for more than 2 hr. This suggests that inhibitory factors initially present in the cells were overcome by the preincubation. The extent of the incorporation was proportional to the concentration of reticulocytes in the blood samples (Table I). These data, therefore, are in accord with the conclusion that reticulocytes took up most of the radioactive glucosamine.

*Effects of Puromycin.* Puromycin has been shown to inhibit protein synthesis in intact rabbit reticulocytes (Allen and Schweet, 1962) and to block the incorporation of glucosamine-$^{14}$C into membrane proteins of liver and ascites carcinoma cells (Molnar *et al.*, 1964; Cook *et al.*, 1965). As shown in Table II puromycin added simultaneously with isotopes inhibited the incorporation of lysine-$^{14}$C into stromal proteins by 80% while at the same time reduced glucosamine-$^{14}$C incorporation by 55%. When the antibiotic was added 2 hr before glucosamine-$^{14}$C, inhibition was increased to nearly 70%. These findings suggest that peptide synthesis is required for the optimum binding of glucosamine-$^{14}$C.

*Radioactive Constituents in Acid-Soluble and Bound Products.* Table III summarizes the distribution of radioactivity in the cells after 4-hr incubation with glucosamine-$^{14}$C. At this time the total amount of radioactivity in 1 ml of cells was 15–20% of that added to the media; 0.4–0.5% was bound to the stroma. It can be seen that

TABLE I: The Effect of Reticulocyte Concentration on the Incorporation of Glucosamine-$^{14}$C.[a]

| Treatment Days | % Reticulocytes | cpm/mg of Protein |
|---|---|---|
| 1 (normal) | 2.8 | 66 |
| 2 | 3.5 | 87 |
| 3 | 6.0 | |
| 4 | 42.0 | 285 |
| 5 | 66.0 | 924 |

[a] The above data is from a single rabbit. On each treatment day the animal was bled and given 2.5 mg of phenylhydrazine. The cells were washed and incubated with glucosamine-$^{14}$C (0.5 μCi/ml of cells) for 20 hr. Bound activity in the stroma was determined as described in Methods.

[1] We wish to thank Dr. Richard J. Winzler for his assistance in performing these analyses.

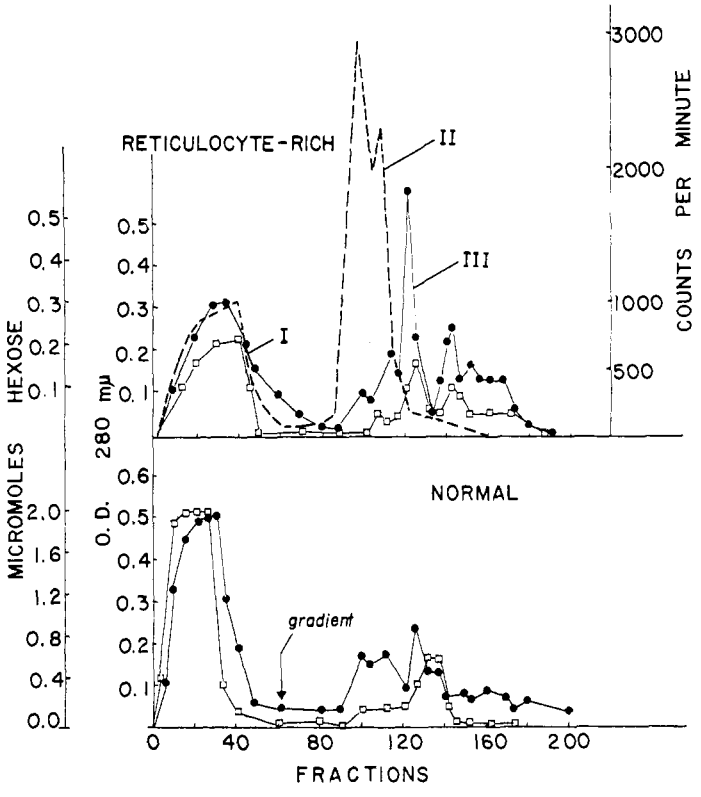


FIGURE 3: Resolution of trypsin fragments from normal and reticulocyte-rich cells on DEAE-cellulose. Tryptic products solubilized from 300 ml of normal and 60 ml of reticulocyte-rich cells (40% reticulocyte) were collected. The equivalent of 66 mg of protein from normal and 47 mg from reticulocyte rich were dissolved in 80 ml of 0.05 M sodium acetate buffer (pH 4.5) and applied to a 1.9 × 17.5 cm column equilibrated against this same buffer. The products were eluted with 200 ml of starting buffer followed by 1 l. of a linear NaCl gradient ending with 1.0 M NaCl. Fractions (5–6 ml) were collected and analyzed for (-●—●-) optical density at 280 mμ, (-□—□-) hexose, and (- - - - - -) counts per minute).

nearly half of the counts in the acid-soluble fraction was in the UDP-*N*-acetylhexosamine, while one-fourth was in glucosamine.[2] The remaining 25% was in the material which remained at the origin following chromatography in solvents A and B. Almost 90% of the counts bound to the stroma was in the hexosamine residues. Of this amount glucosamine accounted for 82% and the remainder was galactosamine. Furthermore, chromatographic evidence based on mild acid hydrolysis suggested that these amino sugars were in the acetylated form when bound to stroma. Sialic acid made up less than 5% of the bound radioactivity and the remaining was associated with constituents not yet identified.

*Extraction of Stroma with Organic Solvents.* The preceding experiments showed that radioactivity from glucosamine-$^{14}$C was bound to components in the cell membrane. For a further characterization, washed stroma were extracted with organic solvents in the Soxhlet apparatus. Table IV shows that chloroform–methanol (1:3) solubilized 12% of the total bound radioactivity while at the same time decreased the dry weight by 28%. Using ethanol–ether (3:1), 3% of the activity was extracted while the dry weight was decreased by 40%. In both of these experiments, radioactivity not extracted by the solvent was recovered in the residue. These findings suggest that glycolipid complexes in the membrane are not extensively labeled.

*Sonication of Stroma.* To get additional insight into the chemical nature of the labeled products, dried stroma were suspended at a concentration of 1 mg/ml in 20 mM phosphate buffer (pH 7.4) and irradiated at 4° for 5–7 min using a 20-kcycle Branson sonifier at 1.5–1.8-A output. This treatment resulted in an opalescent suspension which was not clarified completely by centrifugation at 30,000*g* for 35 min. More than half the total radioactivity was found in the supernatant. Further centrifugation of this supernatant (100,000*g* for 75 min) produced a pellet fraction and a water-clear supernatant which contained 40% of the radioactivity. When the 100,000*g* supernatant was examined on Sephadex G-25, 60% of the radioactivity appeared in the excluded

TABLE II: Puromycin Inhibition of Glucosamine-$^{14}$C and Lysine-$^{14}$C Incorporation into Membrane Components.[a]

| Isotope | Puromycin (μmoles) | Protein (cpm/mg) | % Inhibn |
|---|---|---|---|
| Lysine | 0.0 | 566 | 0.0 |
| | 2.0 | 109 | 81.7 |
| Glucosamine | 0.0 | 393 | 0.0 |
| | 2.0 | 172 | 56.2 |
| | 2.0[b] | 126 | 68.0 |

[a] Cells (35% reticulocytes) were incubated in salt media for 2 hr, then 4 hr with pyruvate and isotope (2.5 μCi of lysine-$^{14}$C or 1 μCi of glucosamine-$^{14}$C per ml of cells). Except where indicated puromycin was added with the isotope. Bound radioactivity was determined as described in Methods. [b] Added 2 hr before isotope.

[2] The pool of free glucosamine may represent radioactivity which has been taken up but not metabolized subsequently by the erythrocytes present.

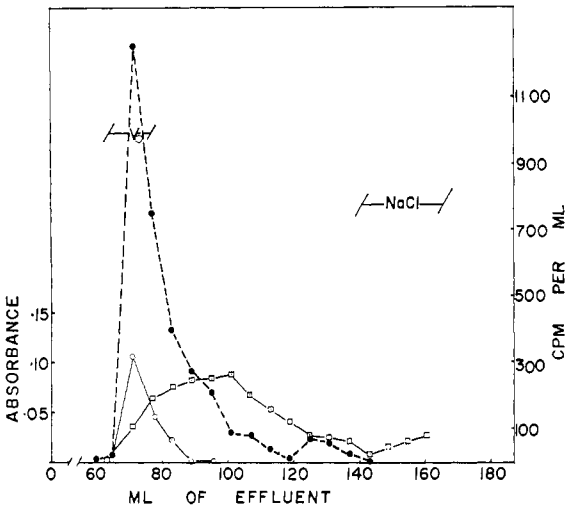


FIGURE 4: Sephadex G-25 chromatogram of peak I fraction from reticulocyte-rich cells. Components of this peak (see Figure 3) were dialyzed against distilled water and dried. The residue was taken up in 2.0 ml of 0.05 M sodium acetate (pH 4.5) and applied to a 2.5 × 38 cm column equilibrated against the same buffer. (-□—□-) Optical density at 280 mμ; (-○—○-) hexose; (—●- - -●-) counts per minute.

TABLE III: Distribution of Radioactivity in Cells after 4-hr Incubation with Glucosamine-$^{14}$C.

| Fraction | cpm/ml of Cells[a] | % Total Act. |
|---|---|---|
| Cold acid extract | 123,800 | 100 |
| UDP-*N*-acetylhexosamine | 62,920 | 50.8 |
| Glucosamine | 31,450 | 25.4 |
| Unidentified | 29,430 | 23.7 |
| Insoluble residue | 5,600 | 100 |
| Glucosamine | 3,990 | 71.2 |
| Galactosamine | 880 | 15.7 |
| Sialic acid | 245 | 4.4 |
| Unidentified | 485 | 8.7 |

[a] One milliliter contained approximately 9.5 × $10^9$ cells.

volume of the column. Radioactive compounds of lower molecular weights (as judged by their retention volumes on the Sephadex) were also present. These findings suggested that part of the bound radioactivity can be released by sonication and is likely to be associated with macromolecules in the stroma. A further characterization of the soluble components was not undertaken.

*Treatment of Intact Labeled Cells with Trypsin.* In previous studies reported from this laboratory, trypsin was used to release protein fragments from the erythrocyte surface (Winzler *et al.*, 1967). Among the split products, glycopeptides rich in hexosamine and sialic acid were isolated and characterized. This approach was used in the present study. Reticulocyte-rich cells were incubated with glucosamine-$^{14}$C for 20 hr. After washing the cells were suspended in Krebs–Ringer buffer (adjusted to pH 7.8) containing 0.25 mg of trypsin/ml of packed cells. Incubation was continued for 2 hr at 37°. For comparative purposes trypsin fragments were also prepared from normal rabbit erythrocytes which not been exposed to glucosamine-$^{14}$C. The isolation of the split products in the digests was carried out as described for human erythrocytes by Winzler *et al.* (1967).

On DEAE-cellulose at pH 4.5 fragments from both reticulocyte-rich and normal cells yielded a peak I fraction rich in hexose and ultraviolet-absorbing components which eluted with the starting buffer (Figure 3). The reticulocyte fragments in this peak showed appreciable radioactivity. Additional peaks containing radioactivity and carbohydrate coincidental with ultraviolet absorbancy were eluted with a linear NaCl gradient. Of the various peaks shown in Figure 3, only three have been investigated in detail.

When the fractions in peak I from reticulocyte-rich cells were pooled and examined on Sephadex G-25, all of the radioactivity and hexose emerged with components in the void volume of the gel (Figure 4). Clearly the carbohydrate and radioactivity in this peak were bound to components of higher molecular weight. A similar profile without radioactivity was obtained with fragments from normal cells. An analysis of the carbohydrate in these components from peak I is shown in Table V. It is of interest that glucosamine was the only amino sugar present and that glucose appeared to be a more important structural moiety of reticulocytes.

TABLE IV: Extraction of Radioactive Products from Stroma with Organic Solvents.[a]

| Solvent | Fraction | cpm Total | % Total (cpm) | Wt (mg) |
|---|---|---|---|---|
| None | Whole stroma | 8200 | 100 | 12.6 |
| $CHCl_3$–MeOH | Extract | 984 | 12 | |
| $CHCl_3$–MeOH | Residue | 7133 | 87 | 9.1 |
| 95% EtOH–ether | Extract | 212 | 2.6 | |
| 95% EtOH–ether | Residue | 6770 | 82.7 | 7.5 |

[a] Stroma were prepared from cells after 4-hr incubation with glucosamine-$^{14}$C. The dried stroma were extracted for 3 hr in a Soxhlet apparatus.

TABLE V: Carbohydrates in Higher Molecular Weight Components of Peak I.[a]

| Component | Erythrocytes | | Reticulocytes | |
|---|---|---|---|---|
| | % Dry Wt | μmoles/mg of Protein | % Dry Wt | μmoles/mg of Protein |
| Hexose (orcinol) | 22.4 | 2.2 | 10.6 | 1.6 |
| Hexose (gas chromatography) | 18.7 | 1.8 | 11.7 | 1.7 |
| Glucose | 1.6 | 0.2 | 3.3 | 0.5 |
| Galactose | 10.9 | 1.1 | 5.7 | 0.8 |
| Mannose | 6.3 | 0.6 | 2.7 | 0.4 |
| Fucose | 1.8 | 0.2 | 0.8 | 0.1 |
| *N*-Acetylhexosamines[b] | 13.8 | 1.1 | 5.6 | 0.7 |
| Glucosamine[c] | | 1.0 | | 0.67 |
| Galactosamine[c] | | 0.0 | | 0.0 |
| Sialic acid | 2.3 | 0.1 | 0.7 | 0.06 |

[a] Peak I fraction shown in Figure 3 from reticulocyte-rich and normal cells was chromatographed on Sephadex G-25, the respective void volumes pooled, and analyzed for carbohydrates. [b] Determined by colorimetric analyses. [c] Determined on the long column of the amino acid analyzer.

An examination of peak II applying the same procedures yielded radioactive compounds which emerged in a symmetrical peak near the salt volume of the Sephadex column. These radioactive compounds migrated identically with the standard UDP-*N*-acetylglucosamine in solvent B. Radioactive constituents in peak III appeared mainly in the excluded volume of the Sephadex, indicating that these too were of higher molecular weights. This peak contained about two-thirds of the recovered sialic acid.

A more extensive characterization of the materials from DEAE-cellulose is currently under way. Taken as a whole the trypsin experiments provided additional evidence that bound glycoproteins in the cell membrane were labeled by the glucosamine-$^{14}$C.

## Discussion

The *in vitro* incubation of reticulocyte-rich cells with radioactive glucosamine has led to the labeling of macromolecules in the cell stroma. Tests have shown that the radioactive sugar was not simply adsorbed to the surface or entrapped in the membrane, but that it was covalently attached to glycoprotein structures comprising the cell surface. Binding of glucosamine-$^{14}$C was inhibited by glucose and occurred at a faster rate when cells were preincubated. Incorporation required *de novo* synthesis of polypeptides and very likely occurred through the utilization of UDP-*N*-acetylhexosamine. Based on these findings it is concluded that reticulocytes have a capacity to synthesize structural glycoproteins of the cell membrane.

Since we have used cells which were composed of a mixture of different types of red cells, it was important to determine whether reticulocytes were responsible for the incorporated radioactivity. Inasmuch as white cells were removed before incubation and bacterial growth was controlled by antibiotics it was possible to test this hypothesis directly. Thus, the finding that mature erythrocytes did not appreciably bind the isotope and, more important, that incorporation levels rose with the reticulocyte count clearly showed that reticulocytes played a major role in the labeling process.

In spite of using mixed cell populations, some insight into the mechanism of glycoprotein biosynthesis in erythroid cells has been gained through these studies. Of importance in this connection was the finding of radioactivity in UDP-*N*-acetylhexosamine. This compound is known to be both an immediate precursor of protein-bound glucosamine and intermediate in the biosynthesis of sialic acid from glucosamine (Johnston *et al.*, 1966; Roseman, 1962; Warren, 1966). Evidently the pathway of glucosamine incorporation into reticulocyte proteins is not unlike that described for other tissues.

The inhibition of incorporation by puromycin showed further that peptide synthesis must take place before the binding of the glucosamine label to the stroma. No doubt these peptides contain the binding sites for the labeled sugars. The failure of puromycin to inhibit glucosamine-$^{14}$C incorporation to the extent of isotopic lysine implies that some of these binding sites were available initially or that groups other than protein can accept the radioactive sugars. It should be noted, however, that stromal lipids showed little affinity to bind glucosamine-$^{14}$C in this system. More work on this aspect of the problem is warranted.

The nature of the intracellular site of glycoprotein formation in reticulocytes has not been clarified by these studies. The fact that radioactivity was bound mainly in membrane proteins is in accord with the observation of Laico and Eylar (1966) that transferase enzymes which assemble oligosaccharide units to peptide chains are present in the cell membrane. In liver tissue it has been suggested that the rough microsomal membranes are the site of attachment of hexose and hexosamine to protein (Sarcione, 1964; Molnar *et al.*, 1965). In contrast,

we have observed that reticulocyte microsomes accumulate or retain very little radioactivity during the exposure of intact cells to glucosamine-$^{14}$C (Harris and Johnson, 1968). Such a finding was perhaps to be expected since reticulocyte microsomes show little evidence of membranous structures (Warner *et al.*, 1963) and hence lack the acceptor sites for the labeled sugars.

Studies carried out with trypsin provided further evidence of the protein character of the radioactive products in the membrane. It was of interest that the trypsin fragments from reticulocytes showed properties similar to unlabeled fragments from normal cells when chromatographed on DEAE-cellulose and on Sephadex columns. This suggests that they may have arisen from common trypsin-accessible sites in the cell membrane. Confirmation of this interpretation must await a more thorough analysis of the structure of fragments from the two cell sources.

In conclusion it has been shown that phenylhydrazine-induced reticulocytes obtained from rabbits can incorporate radioactive glucosamine into membrane glycoproteins. These studies, therefore, extend the scope of known metabolic activities of these cells. The approach adopted in this report should prove useful in revealing, in greater detail, the steps in the synthesis of these proteins and, hopefully, to shed further light on their function in the membrane.

Acknowledgment

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