Biosynthesis of a Fucose-containing Glycopeptide from Rat Small Intestine in Normal and Vitamin A-deficient Conditions*

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SUMMARY

The number of goblet cells in the small intestine decreased in the vitamin A-deficient rat. Incorporation of \(^\text{14}C\)-D-glucosamine into a fucose-containing glycopeptide declined markedly. Ultracentrifugation of this glycopeptide in H\(_2\)O gave only a single component, with a sedimentation value of 3.6 S, whereas, in 0.1 M phosphate buffer (pH 7.5) two components appeared, with much higher sedimentation constants (6.2 S and 7.2 S).

The glycopeptide was found to contain glucosamine, galactose, fucose, and sialic acid in the molar ratios of 3:3:1:0.25, respectively.

Synthesis of the fucose-containing glycopeptide in vitro, using uridine-diphosphate-N-acetyl-D-glucosamine-1-\(^\text{14}C\) and \(^3\)H-serine, showed a 2- to 3-fold decrease in the incorporation of the labels by rough endoplasmic reticulum prepared from vitamin A-deficient animals as compared with normal animals.

Vitamin A deficiency in rats has been shown to decrease by 50 to 60% the incorporation of \(^\text{14}C\)-leucine into proteins in vitro when rough endoplasmic reticulum is used, but does not appear to affect protein synthesis with free polyribosomes (1). Since vitamin A deficiency causes a reduction in the number of mucus-secreting goblet cells in the small intestine (1), one would expect that glycoproteins, the principal constituents of goblet cells, would also be decreased. Furthermore, since secreted proteins, such as glycoproteins, are thought to be synthesized on bound rather than free polyribosomes (2, 3), such a decrease would be consistent with our findings in vitro. (1).

Much work has been done on the role of vitamin A in the secretion of mucus and biosynthesis of glycosaminoglycans. Some workers found a pronounced effect of the deficiency on this biosynthesis; others could not observe any effect. Most of the research dealing with the effect of the vitamin on glycosaminoglycan biosynthesis concerned itself only with incorporation of radioactive precursors into total glycosaminoglycans. Very few attempts were made to study a single species of macromolecule (4, 5).

EXPERIMENTAL PROCEDURE

Animals and Diets—These were identical to those described in a previous report (1). For the experiment on recovery from vitamin A deficiency, 500 \(\mu\)g of vitamin A acetate in Tween 80 were injected intraperitoneally according to the method of Levi and Wolf (6); concurrently, the same amount was given in oil by mouth, and the animals were killed at the times indicated in Table I. In all experiments, normal control and vitamin A-deficient rats were pair-fed. The latter were used after the weight plateau stage, 3 to 5 days after weight loss had begun.

Isolation of \(^\text{14}C\)-Glucosamine-labeled Material from Cell Fractions—\(^\text{14}C\)-Glucosamine was injected intraperitoneally (25 \(\mu\)Ci/200 g, body weight; specific activity, 49 \(\mu\)Ci per \(\mu\)mol) into vitamin A-normal and -deficient rats. The animals were killed at the time intervals indicated in Fig. 2, and the mucosa was scraped as previously described (1). The scrapings were homogenized in 6 volumes of ice-cold Medium A of Littlefield and Keller (7) in a Dounce tissue homogenizer with 8 strokes of the loose pestle and 6 strokes of the tight pestle. An aliquot of the homogenate was diluted twice with a 170 solution of cold glucosamine, and then added to 5 oh trichloracetic acid. The precipitate was collected on a Millipore filter (0.45 \(\mu\)m pore size), dissolved in NaOH (10X), and added to 15 ml of Bray’s solution (8), was counted in a liquid scintillation counter (Nuclear Chicago). Free and membrane-bound polysomes were prepared by the following procedure. The whole homogenate was centrifuged at 7,600 \(\times\) g for 10 min to remove intact cells, cell debris, nuclei, and mitochondria. The supernatant fraction was then placed on a discontinuous sucrose gradient (3.1 ml of 0.5 M sucrose on top of 2.3 ml of 2.0 M sucrose) and centrifuged at 155,000 \(\times\) g for 5 hours in an R40 rotor of a Beckman

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Yosizawa (10). The tissue (about 2.5 g/200 g, body weight) was incubated for 42 hours at 37°. At the end of the incubation, were added together with 0.05 ml of toluene; the suspension was added, and was incubated at 30° for 5 hours. Calcium chloride was adjusted to 8 with sodium hydroxide, and 4 mg of pronase of water to which 2 mg of trypsin and 0.1 ml of toluene were the residue (about 200 mg per animal) was resuspended in 10 ml being immersed in a boiling water bath for 15 min, the homogeo- prepared from the intestinal scrapings as described by Inoue and polysomes. Their radioactivity was determined as described aliquots were precipitated in cold 5% trichloracetic acid, plated plasma membranes). After the pellet was redissolved in water, membrane-bound polysomes were pelleted with all the smooth vesicles (smooth endoplasmic reticulum, Golgi apparatus, and 2.0 and 0.5 for radioactivity in Bray's medium. Hours after vitamin A administration 14C incorporated into Peak III 14C incorporated into Peak V Ratio of Peak III to Peak V

| hrs | dpm | dpm | Ratio |
|-----|-----|-----|-------|
| 0   | 425 | 4,500 | 0.10  |
| 4   | 630 | 3,975 | 0.16  |
| 18  | 1,207 | 3,235 | 0.56  |
| 26  | 12,750 | 3,300 | 3.86  |

The vitamin A-deficient rats were fed 500 μg of retinyl acetate dissolved in oil. An identical aliquot was injected intraperitoneally in Tween 80 (6). The animals were killed at the following time intervals: 0, 4, 18, and 26 hours after vitamin A injection; they received 2.5 μCi of 14C-glucosamine per 200 g, body weight, 2 hours prior to death. After washing with 0.9% NaCl solution, the mucosa was scraped in the usual manner. The glycopeptides were prepared as previously described, dissolved in 2 ml of water, and placed on a DEAE-Sephadex A-50 column. Fractions of 15 ml were collected with an automatic fraction collector and assayed for radioactivity in Bray's medium.

Preparation of Glycopeptides—The glycopeptides were prepared from the intestinal scrapings as described by Inoue and Yosizawa (10). The tissue (about 2.5 g/200 g, body weight) was dilute with buffer containing of Tris (0.05 M), MgCl₂ (5 x 10⁻⁴ M), and KCl (0.025 M) at pH 7.6, and centrifuged at 105,000 x g for 1 hour. The membrane-bound polysomes were pelleted with all the smooth vesicles (smooth endoplasmic reticulum, Golgi apparatus, and plasma membranes). After the pellet was redissolved in water, aliquots were precipitated in cold 5% trichloracetic acid, plated on a Millipore filter, dissolved in Hymamine Hydroxide 10X, and counted in Bray's solution (8). The translucent pellet at the bottom of the discontinuous sucrose gradient represented free polysomes. Their radioactivity was determined as described above.

Preparation of Glycopeptides—The glycopeptides were prepared from the intestinal scrapings as described by Inoue and Yosizawa (10). The tissue (about 2.5 g/200 g, body weight) was dilutted approximately 1:10 with 80% ethanol and homogenized for 15 min at medium speed in a Virtis homogenizer. After being immersed in a boiling water bath for 15 min, the homoge- nate was cooled, centrifuged, decanted, and extracted with 80% ethanol, with 100% ethanol, and with ether. After air drying, the residue (about 200 mg per animal) was resuspended in 10 ml of water to which 2 mg of trypsin and 0.1 ml of toluene were added, and was incubated at 30° for 5 hours. Calcium chloride was then added to give a final concentration of 0.1 m, the pH was adjusted to 8 with sodium hydroxide, and 4 mg of protamine were added together with 0.05 ml of toluene; the suspension was incubated for 42 hours at 37°. At the end of the incubation, cold trichloracetic acid was added to give a final concentration of 7%. The suspension was centrifuged and the precipitate discarded. Two volumes of cold ethanol were added to the super- natant solution and the precipitated glycopeptides were collected by centrifugation, washed with alcohol and ether, dried, and weighed (about 20 mg/ 900 g, body weight).

**DEAE-Sephadex A-50 Chromatography**—The components of the glycopeptide preparation were separated by chromatography on DEAE-Sephadex A-50 columns. The resin was suspended in water and the fine particles were removed by decantation. The resin was then washed several times in 0.5 M HCl and rinsed with water until all chloride ions had disappeared from the washings. Columns (2 x 100 cm) were packed and the glycopeptides were applied to the columns after being dissolved in 2 ml of water. Elution was done in steps with different concentrations of lithium chloride: 0.2, 0.4, 0.5, and 1 M. Aliquots were counted in Bray's solution at an efficiency of 65%.

**Sepharose 4B Chromatography**—The glycopeptide portion eluted from DEAE-Sephadex with 0.4 M LiCl (Peak III) was further fractionated on Sepharose 4B columns (2 x 50 cm), which had been equilibrated with a 0.01 M phosphate buffer, pH 7.4, that was 0.1 M in EDTA and 1 M in mercaptoethanol. Two identical columns were used for the normal and vitamin A-deficient glycopeptides. Aliquots were counted in Bray's solution at a counting efficiency of 65%. Fucose and uronic acid were assayed on the eluate.

**Large Scale Preparation of Fucose-containing Glycopeptide and Ultracentrifugal Analysis**—Forty rats were killed to prepare F-glycopeptide in amounts suitable for chemical analysis. The glycopeptide mixture (847 mg) was placed on three columns of DEAE-Sephadex A-50 (2 x 50 cm). The fractions eluted with 0.4 M LiCl were pooled, flash evaporated to approximately 30 ml, and precipitated with 2 volumes of cold ethanol. This material (110 mg) was then placed on a large column of Sepharose 4B (5 x 60 cm) equilibrated with phosphate buffer as described. Three distinct peaks of radioactivity were found in the 20-mL fractions. Only tubes 18 to 30 were pooled, and the F-glycopep- tide was further purified by chromatography on Sepharose 2B (2.5 x 50 cm) equilibrated with the same phosphate buffer as Sepharose 4B. The eluate showed only one component. This material was used for ultracentrifugal analysis in H₂O and 0.1 M phosphate buffer at pH 7.5 in the model E Beckman ultracen- trifuge at 22° and 175,000 x g average.

**Synthesis in vitro of 14C, 3H-Labeled Fucose-containing Glyco- peptide**—Rough endoplasmic reticulum and pH 5 enzyme frac- tions were prepared as previously described (1) from two vitamin A-normal and two vitamin A-deficient rats 18 hours after liga- tion of the common bile duct. The cell fractions were incubated for 45 min at 37° in the presence of 1.25 μCi of uridine dipophosphate-N-acetylglycosamine-14C (40 mCi per mmole) and 2.5 μCi of L-serine-3H (5 Ci per mmole); 8.2 mg of rough endoplasmic reticulum protein and 5 mg of pH 5 enzyme protein were used in each incubation. All other components of the incubation were identical with those used previously (1).

At the end of the incubation, 2 mg of cold F-glycopeptide were added as carrier to both preparations. The preparations were digested as described for F-glycopeptide, and the glycopeptides were placed successively on DEAE-Sephadex (1 x 12 cm) and Sepharose 2B (1 x 12 cm) columns. The activity of 3H and 14C was measured in Bray's medium with a Beckman LS-150 liquid scintillation counter.

**Analytical Techniques**—Uronic acid was determined by the method of Bitter and Muir (11). Hexosamines were measured by the method of Rondle and Morgan (12) and by chromatog- raphy on the short column of the Beckman amino acid analyzer at pH 5.2 after hydrolysis in 4 N HCl for 5 hours in a sealed ampoule under nitrogen at 100° (15). Total hexoses were de-
terminated by the anthrone method of Trevelyan and Harrison (14) and galactose by the galactostat reagent method (after hydrolysis in 1 N HCl for 1 hour at 80°). Paper chromatography of the sugars was done according to Trevelyan, Procter, and Harrison (15) after hydrolysis in 0.1 N H2SO4 for 1 hour at 80°. N-Acetylneuraminic acid was determined by the method of Warren (16) after hydrolysis of the glycopeptide by 0.1 N HCl for 1 hour at 90° in a closed ampoule under nitrogen, fucose according to Aminoff and Morgan (17), aldopentoses by Disher and Bornfreund's method (18), SO3 by the method of Antonopoulos (19), and protein by the method of Lowry et al. (20).

The amino acid composition of F-glycopeptide was determined on the Beckman amino acid analyzer after hydrolysis of a sample at 105° in 6 N HCl for 12 hours.

Materials—$\beta$-glucosamine hydrochloride (14C; 30 to 50 mCi/mole), uridine diphosphate-$N$-acetylglucosamine-1-14C (40 mCi per mmole), and L-serine-3H (5 Ci per mmole) were purchased from New England Nuclear. N-acetyl neuraminic acid and fucose were obtained from Sigma. Male albino rats were obtained from Holtzman. The vitamin A-deficient diet was procured from General Biochemicals and prepared according to the method of Wolf, Lane, and Johnson (21), and vitamin A was obtained from Eastman Kodak. DEAE-Sephadex A-50 and Sepharose were products of Pharmacia, Uppsala, Sweden.

RESULTS

As shown in Fig. 1, vitamin A deficiency did not affect incorporation of 14C-glucosamine into a mixture of trichloroacetic acid-precipitable macromolecules at different time intervals after injection. Similarly (see Fig. 2), 14C-glucosamine incorporation into free and membrane-bound polyribosomes was determined at different time intervals; although the level of incorporation into membrane-bound polyribosomes was about 10 times that into the free ones, no effect of the vitamin deficiency was detectable. It is noteworthy that maximum incorporation for both deficient and normal mucosa is attained 2 hours after intraperitoneal injection of the label (Fig. 1). All subsequent experiments were performed at this time interval after injection of 14C-glucosamine.

Glycopeptides from eight normal and eight vitamin A-deficient rats were prepared by the method described in "Experimental Procedure." The total amount of material obtained for the two groups was almost identical (122 mg of glycopeptide material from the normal and 120 mg from the deficient), as was the total radioactivity (about 1,200,000 dpm for both). Therefore, no difference could be detected when we considered the mixture of all glycopeptides. Since our aim was to ascertain whether or not the vitamin was necessary for synthesizing a specific glycopeptide, the normal and deficient preparations were placed on two identical DEAE-Sephadex A-50 columns.

Fig. 1. Incorporation in vivo of 14C-glucosamine into total trichloroacetic acid-precipitable proteins from whole homogenates of vitamin A-normal (●—●) and vitamin A-deficient (○—○) rat intestinal mucosa. Glucosamine-1-14C was injected (25 μCi in 0.9% NaCl solution per 200 g, body weight) intraperitoneally into vitamin A-normal and vitamin A-deficient rats. The deficient rats lost about 5% of their maximum weight and the normal animals were pair-fed. The animals were killed at the indicated time intervals after injection of the label, and the small intestines were processed as described under "Experimental Procedure." Wet weight of the mucosa from each animal was about 2.5 g.

Fig. 2. Incorporation study in vivo of 14C-glucosamine into free and membrane-bound polyribosomes from vitamin A-normal (●—●) and vitamin A-deficient (○—○) rat intestinal mucosa. Free (A) and membrane-bound (B) polysomes were prepared as described under "Experimental Procedure" after injection of 14C-glucosamine. The protein assay was performed on resuspended pellets prior to precipitation by trichloroacetic acid.
stepwise elution with increasing concentrations of lithium chloride was performed. Two peaks of radioactivity were eluted by 0.2 M LiCl; in both preparations these two peaks contained the same amount of radioactivity (about 210,000 dpm). The 0.4 M LiCl eluted a peak that we henceforth term "Peak III" and that was greatly affected by vitamin A deficiency. The peak from deficient rats contained only about 40% the radioactivity of the normal (Fig. 3). By increasing the concentration of LiCl to 0.5

![Fig. 3. DEAE-Sephadex A-50 chromatography of the glycopeptides prepared from vitamin A-normal (O—O) and vitamin A-deficient (O—O) rat intestinal mucosa. The rats were injected with 14C-glucosamine 2 hours prior to killing, and the glycopeptides were prepared according to the method of Inoue and Yosizawa (10). This experiment used eight normal and eight vitamin A-deficient rats. In all, 122 mg of the normal glycopeptide mixture and 120 mg of the deficient were obtained. The glycopeptides were dissolved separately in 27 ml of water, and 22 ml were applied to two identical columns (2 X 100 cm) of DEAE-Sephadex; 12.5-ml fractions were collected with an automatic fraction collector, and the radioactivity was assayed on 0.5 ml.

Table II

| Time after injection | Number of goblet cells per crypt of Lieberkühn |
|----------------------|-----------------------------------------------|
| 0                    | 13.88 ± 2.04a                                 |
| 4                    | 14.35 ± 2.08                                 |
| 10                   | 15.11 ± 2.19                                 |
| 18                   | 19.58 ± 2.41                                 |
| 23                   | 19.81 ± 2.34                                 |

* Mean ± standard deviation of the mean.

Counts of goblet cells were performed as previously described (1). Ten sections were made for each intestine, representing one time point, and were then stained; 220 counts of goblet cells per crypt were made on each section. Statistical significance between zero time and 4 hours is at the level of p < 0.01; between 4 and 10 hours and between 10 and 18 hours, p < 0.01; between 18 and 23 hours, p < 0.2.

Table III

Comparison of total amounts and 14C-glucosamine-specific radioactivity between vitamin A-normal and -deficient fucose-containing glycopeptide after Sepharose 4B chromatography

| F-glycopeptide | Total 14C-glucosamine in F-glycopeptide | 14C-glucosamine |
|----------------|----------------------------------------|-----------------|
| F-glycopeptide, normal | 14.1 mg | 250,600 dpm | 16,904 dpm/mg F-glycopeptide |
| F-glycopeptide, deficient | 24.7 mg | 147,420 dpm | 5,988 dpm/mg F-glycopeptide |

* Per group of six rats. Normal F-glycopeptide was prepared from tubes 6 to 12 (Fig. 4A); deficient F-glycopeptide was prepared from tubes 10 to 25 (Fig. 4B). Normal and vitamin A deficient F-glycopeptide were prepared as described under "Sepharose 4B Chromatography" in "Experimental Procedure."
FIG. 5. Synthesis of FG in vitro by rough endoplasmic reticulum and pH 5 enzyme fractions from vitamin A-normal and vitamin A-deficient animals. DEAE-Sephadex A-50 chromatography of $^{14}$C, $^3$H-labeled glycopeptides in vitro from normal and deficient rats. The conditions of incubation and preparation of the glycopeptide mixture in vitro are described under "Experimental Procedure."

Fig. 6. Synthesis of F-glycopeptide in vitro by rough endoplasmic reticulum and pH 5 enzyme fractions from vitamin A-normal and vitamin A-deficient animals. Sepharose 2B chromatography of the $^{14}$C, $^3$H-labeled Peak III from normal and deficient rats in vitro.

Fig. 7. Large scale preparation of F-glycopeptide. Details of the experiment are described under "Experimental Procedure."

A, elution pattern of the Peak III material upon Sepharose 4B chromatography (column, 5 x 60 cm; void volume, 300 ml) in 0.01 M phosphate buffer at pH 7.4. Fractions of 20 ml were collected. B, elution pattern of F-glycopeptide prepared as follows. Tubes 18 to 30 from Sepharose 4B chromatography were combined, and, after extensive dialysis against twice-distilled water and concentration to 80 ml, F-glycopeptide was precipitated by adding 80 ml of cold ethanol. The precipitate was placed on a column (2.5 x 50 cm; void volume, 50 ml) of Sepharose 2B equilibrated in the same way as for the Sepharose 4B. Fractions of 25 ml were collected and radioactivity assayed on 0.1 ml. F-glycopeptide was then precipitated after dialysis, washed with ethanol and ethyl ether, and used for composition analysis.

m, another broad peak of radioactivity could be detected; the peak from deficient animals had less radioactivity than the normal. The effect of the vitamin A deficiency, however, was not as pronounced as in Peak III. The 1 M LiCl eluted a final peak of radioactivity that showed an increased incorporation of $^{14}$C-glucosamine in the deficient preparation. From these results it is clear that if total glycopeptides were analyzed, no difference between the vitamin A-normal and -deficient preparations could be detected.

The ratio of Peak III to Peak V (Fig. 3) for the vitamin A-normal preparation is 0.5; the same ratio in vitamin A deficiency is 0.1 due to decreased incorporation of $^{14}$C-glucosamine into Peak III (from 145,000 dpm for the normal to 60,000 for the deficient) and increased incorporation into glycopeptide V (from 240,000 for the vitamin A normal to 350,000 for the vitamin A...
deficient). This experiment was repeated three times with identical results.

We next wanted to determine whether vitamin A administration to deficient rats initiated Peak III biosynthesis and restored to normal the ratio of Peak III to Peak V. Table I provides the answer. Four hours after vitamin A injection the ratio was unchanged. After 18 hours the ratio increased to 0.56, and after 26 hours the value was higher than normal (3.86).

**Table IV**

| Chemical composition of fucose-containing glycopeptide from vitamin A-normal rat intestinal mucosa |
|------------------------------------------------------------------------------------------------|
| Methods of analysis described under “Experimental Procedure.” |

| Component | % of total weight |
|-----------|------------------|
| Fucose    | 9.5              |
| Galactose | 27.0             |
| Glucosamine | 20.0          |
| N-Acetyl neuraminic acid | 6.0          |
| Uronic acid | 4.0             |
| Total sugar | 75.5            |

| Amino acid | % mg/100 ìg F-glycopeptide |
|------------|---------------------------|
| Aspartic   | 1.15                      |
| Threonine  | 3.45                      |
| Serine     | 1.28                      |
| Glutamic   | 1.97                      |
| Proline    | 1.39                      |
| Glycine    | 0.33                      |
| Alanine    | 0.27                      |
| Isoleucine | 1.60                      |
| Leucine    | 1.50                      |
| Lysine     | 1.15                      |
| Methionine | 0.638                     |
| Total amino acid (%) | 14.72                   |

Further attempts to fractionate Peak III on Sephadex G-200 were fruitless because of the large size of the glycopeptides. On Sepharose 4B we were able to obtain two major radioactive peaks and a shoulder of radioactivity after the second peak. The first eluted peak is the only one to contain fucose, as determined both by the fucose assay (17) and by paper chromatography (15). This latter technique also showed galactose to be the only hexose present in the first eluted peak. From Fig. 4 it is evident that the only peak to be affected by vitamin A deficiency is the fucose-containing glycopeptide of larger molecular weight. From Table III we can see that, although the total amount of F-glycopeptide seems to be higher in vitamin A deficiency, the specific radioactivity of 14C-glucosamine is decreased 4-fold. This indicates that there is an accumulation of F-glycopeptide (perhaps due to lack of secretion) in vitamin A deficiency and at the same time a decrease in synthesis. This is also found in the F-glycopeptide synthesized in vitro. Figs. 5 and 6 show a 2- to 3-fold increase in the incorporation of UDP-N-acetylglucosamine-14C and serine-3H in F-glycopeptide synthesized on vitamin A-deficient rough endoplasmic reticulum.

To characterize F-glycopeptide better, we prepared it in large quantities from 40 rats 2 hours after the injection of 14C-glucosamine. After DEAE-Sephadex chromatography, Peak III, eluted with 0.4 M LiCl, was placed on Sepharose 4B. Fig 7A clearly shows the presence of three radioactive components. To
avoid any contamination from the second peak, F-glycopeptide was prepared from tubes 18 to 30 by the usual ethanol precipitation method. It was then rechromatographed on Sepharose 2B (Fig. 7B), which gave a single peak just after the void volume. As can be seen in Table IV, F-glycopeptide contains glucosamine, galactose, fucose, and sialic acid in the molar ratios 3:3:1:0.25. No sulfate and aldopentoses were found. The uronic acid present is probably due to contamination from the adjacent peak. Typically for glycopeptides, threonine is the most abundant amino acid and the amino acid content represents 15% of the total F-glycopeptide. When ultracentrifugal patterns at three different concentrations of F-glycopeptide in H₂O were run, only one sharp peak could be detected, with a theoretical sedimentation value of 3.6 S. However, when the same analysis was run in 0.1 M phosphate buffer at pH 7.5, two distinct peaks appeared with sedimentation values of 6.2 and 7.2 (Fig. 8). The possibility exists that the two components are two subunits of the same glycoprotein and that they radically change their configuration from linear to spherical in an alkaline environment. Further studies are in progress to clarify this point.

**DISCUSSION**

In an attempt to find a metabolic function for vitamin A at the molecular level, we showed in previous experiments (1) that in intestinal mucosa, a target organ of the vitamin, protein synthesis on membrane-bound but not on free polyribosomes is partially dependent on the vitamin. Having observed that the number of mucous-secreting goblet cells in the intestine of vitamin A-deficient rats is decreased (1), and since secreted proteins are thought to be synthesized on membrane-bound polyribosomes, we considered it pertinent to determine whether the synthesis of any intestinal glycopeptide in vivo and in vitro would be affected by the vitamin. If we could relate the biosynthesis of a particular and defined macromolecule in vitro to the presence of the vitamin, the mechanism whereby the latter influences the former might become apparent.

The effect of vitamin A on the synthesis of mucopolysaccharides and glycoproteins in a variety of tissues has been investigated by many workers with varying results. Colon mucopolysaccharide was reported to be depressed (22), whereas there was no change in mast cell heparin (5). Bone mucopolysaccharide was actually found to increase upon deficiency (23). The difficulty in interpreting many of these reports has been the lack of separation of the isolated material into definite chemical entities.

We have focused our attention on the biosynthesis of total glycopeptide as well as that of specific components. We found no difference in the 1⁴C-glucosamine incorporation into total glycoprotein in intestinal mucosa from vitamin A-deficient and pair-fed normal control rats (Fig. 1). Similarly, vitamin A deficiency did not affect the synthesis of bulk glycoproteins in vivo on membrane-bound polyribosomes or on free polyribosomes at various times after a pulse of labeled glucosamine (Fig. 2). Kinetic data of incorporation were also very similar for deficient and normal mucosa. It should be noted that the incorporated radioactivity was about 10 times greater in rough endoplasmic reticulum than in free polyribosomes, consistent with recent reports (24) that glycopeptides are synthesized mainly on rough endoplasmic reticulum.

When total intestinal mucosal glycopeptides were prepared 2 hours after the intraperitoneal injection of labeled glucosamine, no difference was noticed in total amounts and total radioactivity between normal and deficient preparations, a result that would indicate that the vitamin has no effect on glycopeptide biosynthesis. However, upon separation on DEAE-Sephadex of bulk glycopeptide into its constituents (Fig. 3), it was obvious that ¹⁴C-glycosamine incorporation into the glycopeptide fraction eluted with 0.4 M LiCl was strongly depressed in vitamin A deficiency (Peak III, Fig. 3). Simultaneously, it became apparent that another glycopeptide (Peak V) increased in vitamin A deficiency. These results suggested a precursor-product relationship between glycopeptides III and V, with accumulation of the precursor when the product synthesis is interrupted. They also show that the effect of the vitamin could not be detected by measuring bulk glycopeptide synthesis because the effect was obscured by a variety of unaffected glycopeptides, in addition to a glycopeptide that increased during deficiency. This explains the extreme variability of the results obtained by other researchers.

Recovery studies (Table 1) demonstrated that the vitamin was able to restore glucosamine incorporation into glycopeptide III and glycopeptide V to normal levels 18 hours after injection. At the same time an increase in the number of goblet cells per crypt of Lieberkühn was found. This result implies that the vitamin stimulates primarily the differentiation of basal epithelium to produce new goblet cells, and the synthesis of new glycoprotein may result from the growth of new cells.

When Peak III was further fractionated on Sepharose, three components emerged, of which only one, a fucose-containing glycopeptide, was affected by vitamin A deficiency. The specific radioactivity of glucosamine in F-glycopeptide was 4-fold lower than in normal F-glycopeptide (Table III and Fig. 4). Table III shows that the actual amount of F-glycopeptide present in vitamin A-deficient mucosa is increased, although the synthesis is impaired, possibly because of lack of secretion.

Because the decreased biosynthesis of F-glycopeptide parallels its accumulation in vitamin A deficiency, one can hypothesize that the rate of degradation of F-glycopeptide is decreased to a higher extent than the rate of synthesis, resulting in accumulation.

The chemical composition of F-glycopeptide is very similar to that of the blood group substances of which fucose, glucosamine, galactose, and sialic acid are common constituents (25). Moreover, the amino acid composition of the different human blood group-specific substances isolated from ovarian cyst fluids (25) shows threonine as the most abundant amino acid; this is also the most abundant amino acid in F-glycopeptide.

The ultracentrifugal behavior of F-glycopeptide in H₂O and phosphate buffer indicates that, below pH 6, F-glycopeptide has an elongated, rod-shaped configuration, whereas, at pH 7.5, it has a globular or hydrated structure that allows faster sedimentation and the separation into two components (Fig. 8).

The study of the biosynthesis in vitro of the fucose-containing glycopeptide in the presence of rough endoplasmic reticulum and pH 5 enzyme fractions clearly indicates that the biosynthesis of both the polysaccharide and the peptide moieties is affected by vitamin A deficiency. Moreover, this experiment excludes any effect of the vitamin prior to the formation of UDP-N-acetylgalactosamine.

We have recently reported (26) that 15⁴C-retinol, or a metabolite thereof, and mannose from GDP-1⁴C-mannose are incorporated into a mannose-containing lipid compound in the
presence of a microsomal preparation from rat liver. The compound has the same properties as the mannosyl-phosphoryl undecaprenol isolated from Micrococcus lysodeikticus (27), which has been shown to participate in the biosynthesis of a membrane-associated mannan. Since vitamin A is a Tetrametric derivative of isoprene, it might be performing in mammals the same function as the undecaprenol performs in microorganisms: that of carrying mono- or oligosaccharides for the biosynthesis of glycoproteins.

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