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Research Article

Elucidation of mechanisms involved in the control of colonic production of mucus requires direct examination of glycoprotein synthesis and secretion by colonic mucosa. In the past, the limited viability of intestinal mucosa in vitro has hampered such investigations. When maintained in an organ culture system, mucosal biopsies of rabbit colon and human rectum remained viable for 24 h as documented by morphologic appearance and a steady rate of protein synthesis and secretion. These biopsies also incorporated ^{14}C-labeled glucosamine into tissue glycoproteins and secreted labeled glycoproteins at a steady rate for 24 h. Glucosamine was predominantly incorporated into macromolecules that were ultimately secreted, in contrast to leucine, which was predominantly incorporated into tissue macromolecules. When studied by autoradiography, cultured rabbit colonic biopsies synthesized and secreted glycoproteins in vitro at cellular sites and over a time-course similar to those previously described for the intestine of intact animals. Acetylcholine consistently stimulated secretion of labeled glycoproteins but did not alter glycoprotein synthesis. In contrast, cycloheximide inhibited glycoprotein synthesis but had no effect on the secretion of newly synthesized glycoproteins. Rectal biopsies from patients with active ulcerative colitis incorporated increased amounts of [^{14}C]glucosamine into glycoproteins during organ culture and secreted labeled glycoproteins more rapidly into the incubation medium when compared to biopsies obtained from healthy volunteers These findings indicate that organ culture provides a useful […]

Glycoprotein Synthesis and Secretion by Mucosal Biopsies of Rabbit Colon and Human Rectum

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ABSTRACT Elucidation of mechanisms involved in the control of colonic production of mucus requires direct examination of glycoprotein synthesis and secretion by colonic mucosa. In the past, the limited viability of intestinal mucosa in vitro has hampered such investigations. When maintained in an organ culture system, mucosal biopsies of rabbit colon and human rectum remained viable for 24 h as documented by morphologic appearance and a steady rate of protein synthesis and secretion. These biopsies also incorporated ^{14}C-labeled glucosamine into tissue glycoproteins and secreted labeled glycoproteins at a steady rate for 24 h. Glucosamine was predominantly incorporated into macromolecules that were ultimately secreted, in contrast to leucine, which was predominantly incorporated into tissue macromolecules. When studied by autoradiography, cultured rabbit colonic biopsies synthesized and secreted glycoproteins in vitro at cellular sites and over a time-course similar to those previously described for the intestine of intact animals. Acetylcholine consistently stimulated secretion of labeled glycoproteins but did not alter glycoprotein synthesis. In contrast, cycloheximide inhibited glycoprotein synthesis but had no effect on the secretion of newly synthesized glycoproteins. Rectal biopsies from patients with active ulcerative colitis incorporated increased amounts of [^{14}C]glucosamine into glycoproteins during organ culture and secreted labeled glycoproteins more rapidly into the incubation medium when compared to biopsies obtained from healthy volunteers These findings indicate that organ culture provides a useful means of directly examining the synthesis and secretion of glycoproteins by healthy and diseased colonic mucosa.

INTRODUCTION

The regulation of mucus production by colonic mucosa remains poorly understood although previous work (1–4) has clarified the sequence of events involved in intestinal synthesis of glycoproteins. Neutra and Leblond (2) injected radioactive precursors of glycoproteins into intact rats and at intervals thereafter examined colonic mucosa by autoradiography. These workers showed that packaging of glycoproteins into mucus granules occurs in the Golgi region of goblet cells. These mucus granules then coalesced to form globules which migrated to the apex of the cell where mucus is discharged into the lumen. Using cell fractionation techniques, Forstner (3) demonstrated the progression of radioactively labeled glycoproteins from a microsomal fraction to a brush border fraction of small intestinal cells and then into the intestinal lumen. Similarly, Kim, Perdomo, and Nordberg (4) showed that after synthesis of the peptide portion of the molecule in the rough endoplasmic reticulum, the sugar moieties are added in the smooth endoplasmic reticulum by stepwise glycosylation. Thus, within the channels of endoplasmic reticulum membrane-bound glycosyl transferases attach one sugar at a time to the growing glycoprotein.

In spite of this work, however, little is known about the rates of glycoprotein synthesis and secretion by the colon or about factors which influence these rates. In order to examine these processes directly, it is necessary to maintain isolated colonic mucosa under steady-state conditions for reasonably prolonged periods of time and

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to assess secretion independent of synthesis. The limited viability of intestinal mucosa in vitro has previously prevented such an approach. Recently, however, an organ culture system has been developed which allows human and rabbit small intestinal biopsies to be maintained in vitro for 24 h (5, 6). Cultured biopsies of rabbit small bowel mucosa synthesized and secreted proteins at a steady rate for 24 h with good histological preservation (6). In addition, human rectal biopsies remained viable for 24 h when maintained in organ culture (7). We have therefore used this technique to examine the biosynthesis and secretion of glycoproteins by biopsies of rabbit and human colonic mucosa.

METHODS

Culture technique. A 10–15-cm segment of distal colon was excised from anesthetized 2–5-kg white male New Zealand rabbits. After opening the segment lengthwise, multiple mucosal biopsies were taken with a multipurpose biopsy tube (8) (Quinton Instruments, Seattle, Wash.). For human studies, biopsies were obtained from rectums of normal volunteers or from patients with active ulcerative colitis. Up to four biopsies were taken from each volunteer, of which one was fixed in Bouin's solution (7) to assess morphology. Normal colon was obtained at the time of surgery from two subjects undergoing colostomy revision after previous colectomy for diverticulitis. The blood supply of the colonic segment to be resected was maintained up to the moment of removal. The 4–5-cm segment was then opened lengthwise, and multiple mucosal biopsies were taken with a multipurpose biopsy tube. Biopsies were cultured as described in detail previously (5). In brief, biopsies were oriented mucosal side up on a stainless steel wire screen in plastic organ culture plates (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.). The screens were floated on 1 ml of organ culture medium composed of 90% Trowells T8 medium (Grand Island Biological Co., Grand Island, N. Y.) and 10% fetal calf serum to which 100 U per ml penicillin and 100 μg per ml streptomycin had been added. The biopsies were cultured for periods of up to 24 h at 37°C in a sealed McIntosh jar (Torsion Balance Co., Clifton, N. J.) in 95% O_2–5% CO_2.

Glycoprotein and protein synthesis and secretion. To assess radioactive glucosamine incorporation into glycoproteins, 1.25 μCi D-[1-^{14}C]glucosamine (sp act 56.5 mCi per mmol, New England Nuclear, Boston, Mass.) was added to each milliliter of medium. To measure incorporation into tissue glycoprotein, cultured biopsies were homogenized in ground glass homogenizers in 1.5 ml of chilled 0.04% $CaCl_2$. Total biopsy protein was determined from an aliquot of this homogenate by the method of Lowry, Rosebrough, Farr, and Randall (9). Another aliquot was precipitated with 10% trichloroacetic acid (TCA) [1] and 1% phosphotungstic acid (PTA) and centrifuged at 15,000 rpm for 10 min. The pellet was washed twice with 10% TCA-1% PTA and then washed twice with chloroform-methanol (1:1 by volume). After drying in room air, the pellet was dissolved in 1 cm^3 of NCS (Amersham-Searle, Arlington Heights, Ill.) and counted in Liquiflour-Toluene (New England Nuclear) in a Beckman LS-250 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Counting efficiency was determined by the external standard-channels ratio method. To study protein synthesis, 1.25 μCi of L-[U-^{14}C]leucine (sp act 305 mCi per mmol, New England Nuclear) or 2 μCi of L-[4,5-^{3}H]leucine (sp act 55.2 mCi per mmol, New England Nuclear) were added to each milliliter of medium. After culture, biopsies were homogenized and treated as above.

To measure secretion of radiolabeled glycoproteins or proteins, the incubation medium was collected from each biopsy. Before collection of the medium, however, the biopsy was gently rinsed with a few drops of the medium to obtain secreted macromolecules adherent to the top of the biopsy. The medium was then centrifuged at 15,000 rpm for 10 min to remove cellular debris. The supernate was dialyzed in Visking tubing for 72 h against three exchanges of 0.15 M NaCl (200:vol:vol). After dialysis, an aliquot of the medium was counted in Bray's solution (10), to determine the amount of radioactive precursor incorporated into nondialyzable macromolecules. Control dialysis bags contained radioactive incubation medium which had not been incubated with a biopsy. To correct for variations in biopsy size, synthesis and secretion of radiolabel were expressed as disintegrations per minute per milligram of total biopsy protein as determined by the Lowry method (9). The amounts of RNA and DNA per biopsy were determined by previously described methods (11, 12) so that synthesis and secretion could also be expressed in terms of micrograms of RNA and DNA.

Pulse-chase studies. To measure macromolecular secretion independent of synthesis, a "pulse-chase" system was utilized. During the pulse period, biopsies were cultured for 1 h with 5.0 μCi [^{14}C]glucosamine in the medium. Biopsies were then cultured during the chase period over new medium containing 20 mM unlabeled glucosamine and no radioactive glucosamine. By dilution, the nonradioactive glucosamine minimizes further labeling of tissue glycoproteins. Therefore, during the chase period labeled glycoproteins are secreted into the medium, and when the biopsies and medium were processed as described above, secretion could be distinguished from synthesis.

Autoradiography. To localize the site of glucosamine incorporation into macromolecules and to determine its subsequent pattern of movement within epithelial cells, biopsies were cultured over medium to which 10 μCi of D-[6-^{3}H]glucosamine (sp act 3.6 Ci per mmol, New England Nuclear) per ml had been added. The biopsies were cultured over isotope-containing medium for 40 min, after which the radioactive medium was removed and replaced by medium containing 20 mM unlabeled glucosamine. Biopsies were then harvested after culture over this medium for ½, 1, 2, 3, 4, 6, 8, or 24 h. After fixation in buffered osmium tetroxide and embedment in epoxy resin, autoradiographs were prepared as described previously (5, 7). To determine the distribution of radioactivity within goblet cells, the location of radioactivity was determined in 100 well oriented goblet cells for each time interval in coded autoradiographs. An ocular micrometer was used to divide each goblet cell into basal, middle, and upper thirds. If the pulse of radioactivity could not be localized to a specific third of the cell, the cells were scored as diffusely labeled. Cells without appreciable radioactivity were tabulated as unlabeled cells.

Drug studies. The effect of cycloheximide was determined by adding 1 mg of cycloheximide (Sigma Chemical Co., St. Louis, Mo.) to each milliliter of medium. Acetyl-

[1] *Abbreviations used in this paper:* PTA, phosphotungstic acid; TCA, trichloroacetic acid.

choline chloride (Sigma Chemical Co.) in concentrations varying from 10^{-3} to 10^{-12} M was added to the medium along with 3×10^{-4} M eserine (Calbiochem, La Jolla, Calif.). Atropine sulfate 10^{-6} M (Sigma Chemical Co.) was used to block the effects of acetylcholine.

Column chromatography. Dialyzed medium was subjected to gel filtration on Sepharose 2B (Pharmacia Fine Chemicals, Piscataway, N. J.) with pH 6.8, 0.1 M phosphate buffer.

RESULTS

To investigate the stability of the biopsies over the 24-h incubation period, we cultured rabbit biopsies over ^{14}C-labeled leucine for 6 h ("0–6 hour" biopsies) and compared the incorporation of [^{14}C]glucosamine into tissue and secreted glycoproteins with the incorporation which occurred when biopsies were cultured over nonradioactive medium for 18 h and then over medium containing labeled leucine for 6 h ("18–24 hour" biopsies). As shown in Fig. 1, biopsies incorporated similar amounts of radioactivity into glycoproteins and secreted similar amounts of labeled glycoprotein into the medium whether studied immediately or after 18 h of culture. In addition, the biopsies showed maintenance of near normal histological architecture at the end of the 24-h incubation period.

When rabbit biopsies were continuously incubated for 24 h, the amount of radioactive [^{3}H]leucine incorporated into tissue proteins plus secreted proteins was linear (Fig. 2B). In addition, glucosamine incorporation into both tissue and secreted glycoproteins (total glucosamine incorporation) was also essentially linear over the 24 h (Fig. 2A). During the first 12 h most of the ^{14}C-labeled glucosamine was incorporated into glycoproteins extracted from the tissue. Subsequently, however, increasing amounts of incorporated radioactivity were found in the medium, so that at 24 h the amount of macromolecular radioactivity recovered from the medium, which constituted radioactivity incorporated into secreted glycoproteins, was greater than that present in the tissue. In contrast to the results obtained with glucosamine, the rate of incorporation of radioactive leucine into tissue proteins and also into proteins secreted into the medium remained reasonably constant throughout the 24-h period. Furthermore, the amount of radioactivity present in tissue protein greatly exceeded that found in secreted proteins throughout the period of culture.

Cycloheximide, a known inhibitor of protein synthesis (13), affected leucine and glucosamine incorporation into tissue macromolecules differently. When biopsies were not preincubated with cycloheximide but were cultured directly for 18 h over medium containing cycloheximide with either [^{14}C]glucosamine or [^{14}C]leucine, the drug almost completely abolished leucine incorporation into tissue proteins but only moderately reduced glucosamine incorporation into glycoproteins (Fig. 3). To show that

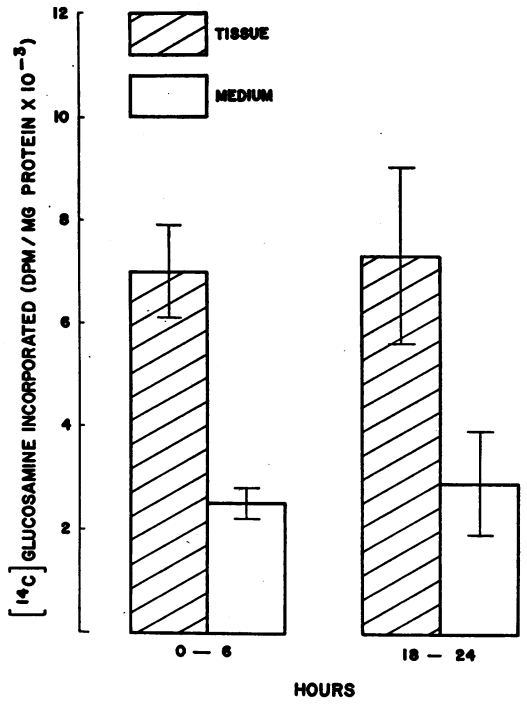

FIGURE 1 Incorporation of [^{14}C]glucosamine into tissue and secreted glycoproteins by biopsies at the beginning (0–6 hours) and the end (18–24 hours) of a 24-h culture period. The 0–6 hour biopsies were cultured over medium containing ^{14}C-labeled glucosamine for 6 h and then processed, while the 18–24 hour biopsies were cultured over unlabeled medium without added glucosamine for 18 h and then transferred to medium containing radioactivity for the remaining 6 h. The results are expressed as mean dpm/mg biopsy protein ±1 SEM. Each bar represents the mean result for six biopsies.

radiolabeled glucosamine was not simply binding nonspecifically to the tissue but was incorporated into macromolecules, biopsies were first preincubated with cycloheximide for 1, 6, or 12 h and then cultured for an additional 18 h with either [^{14}C]glucosamine or [^{14}C]leucine. With progressively lengthening periods of preincubation (Fig. 3), glucosamine incorporation was increasingly inhibited.

The pattern of movement of labeled glycoproteins through colonic cells was examined by autoradiography. Fig. 4 shows autoradiographs of tissue fixed 1 and 6 h after a 40-min exposure to radioactive glucosamine. In the top panel, at 1 h, label was seen primarily in the Golgi regions of goblet cells, in mucus granules just above the Golgi region, and throughout the cytoplasm of absorptive cells. In the bottom panel, by 6 h, labeled glycoprotein was concentrated in secreted mucus in the crypt lumen, in the apical mucus granules of goblet cells, and in the glycocalyx of the absorptive cells. Determination of the distribution of radioactivity in goblet cells in coded slides showed the sequential progression of glu-

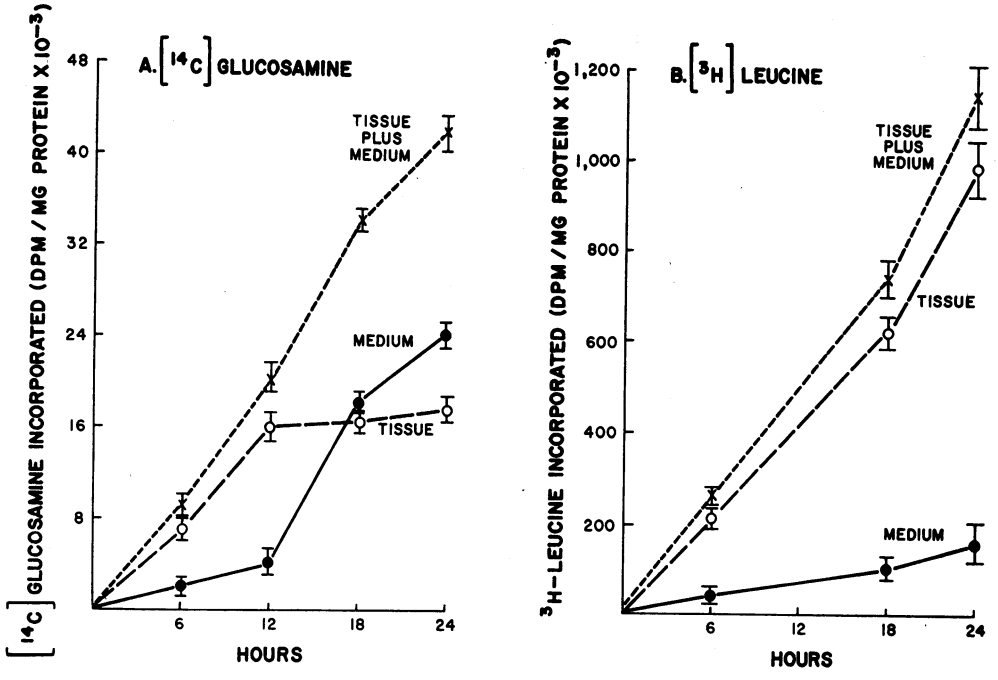

FIGURE 2 Incorporation of radioactivity by rabbit colonic biopsies continuously exposed to 1.25 μCi of [^{14}C]glucosamine or [^{14}C]leucine over a 24-h period. The amount of radioactivity incorporated into macromolecules in the medium and tissue was determined as described in Methods. The results are expressed as mean dpm/mg protein ±1 SEM. Each point represents the mean value for six to fourteen biopsies.

cosamine-labeled macromolecules from the basal cytoplasm at ½–2 h to the apical cytoplasm by 6–8 h and out of the cells by 24 h (Table I).

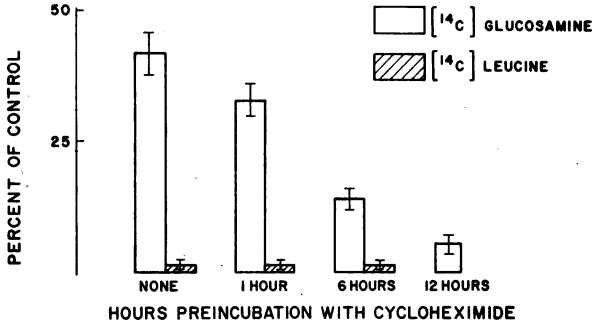

FIGURE 3 Effect of cycloheximide on [^{14}C]glucosamine and [^{14}C]leucine incorporation into tissue macromolecules by rabbit colonic biopsies. The biopsies were preincubated for 1, 6, or 12 h with cycloheximide. Cycloheximide was kept in the incubation medium for an 18-h further incubation with either [^{14}C]glucosamine, as shown by the open bars, or [^{14}C]leucine, as shown by the hatched bars. One group of biopsies was not preincubated with cycloheximide. The vertical axis shows the radioactivity incorporated as percent of control values. The control biopsies, not shown, were incubated for the same period of time, but were not all exposed to cycloheximide. Each bar represents the mean ±1 SEM for six to eight biopsies.

This pulse-chase system was also used to quantitate the uptake, incorporation, and secretion of radioactive glucosamine and leucine. The rabbit biopsies were first pulsed with ^{14}C-labeled glucosamine or leucine for 1 h and then transferred to chase medium containing large amounts of unlabeled precursor. We could then observe the distribution of radioactivity and correlate the findings with the results of autoradiography. Since the excess of unlabeled precursor prevents further incorporation of labeled precursor molecules into the biopsy, once labeling of the tissue macromolecules had occurred, secretion could be followed independent of tissue synthesis.

Fig. 5 shows the changing distribution of radioactive leucine and glucosamine for 24 h after the initial 1-h pulse period. We determined: (*a*) the percent of recovered radioactivity which was found in the TCA supernate of homogenized biopsies (TCA-soluble glycoproteins, proteins, and precursors), (*b*) the percent of recovered radioactivity in the TCA precipitate of homogenized biopsies (TCA-precipitable glycoproteins and proteins), and (*c*) the percent of recovered radioactivity in nondialyzable macromolecules recovered from the medium (secreted glycoproteins and proteins). For both [^{14}C]glucosamine and [^{14}C]leucine, most of the radioactivity was initially present in the TCA supernate (acid-

soluble form). Radioactivity subsequently disappeared gradually from the supernate and was incorporated into tissue macromolecules. The radioactivity in the TCA supernate was completely removed by dialysis, indicating that the TCA-soluble radioactivity represented small molecules. After the pulse period there was progressive formation of ^{14}C-labeled TCA-precipitable glycoproteins and proteins. Peak incorporation of [^{14}C]glucosamine into tissue glycoproteins occurred at 3–4 h after the pulse period, while peak incorporation for [^{14}C]leucine into tissue proteins occurred at 2–3 h after the pulse period. Secreted radiolabeled proteins and glycoproteins appeared in the medium approximately 2–3 h after the pulse period and continued in more or less linear fashion over the remaining 24 h. After 24 h approximately 40% of the incorporated [^{14}C]leucine and [^{14}C]glucosamine was recovered as macromolecules which had been secreted into the medium. These findings allowed selection of a period of time suitable for measuring secretion of labeled glycoproteins independent of tissue synthesis. Thus measurement of secretion into the medium was begun 3 h after completion of pulse labeling. At this point in time peak incorporation of radioactivity into tissue glycoproteins had already occurred and labeled macromolecules were beginning to be secreted into the incubation medium. Medium was then collected for an additional 3 h.

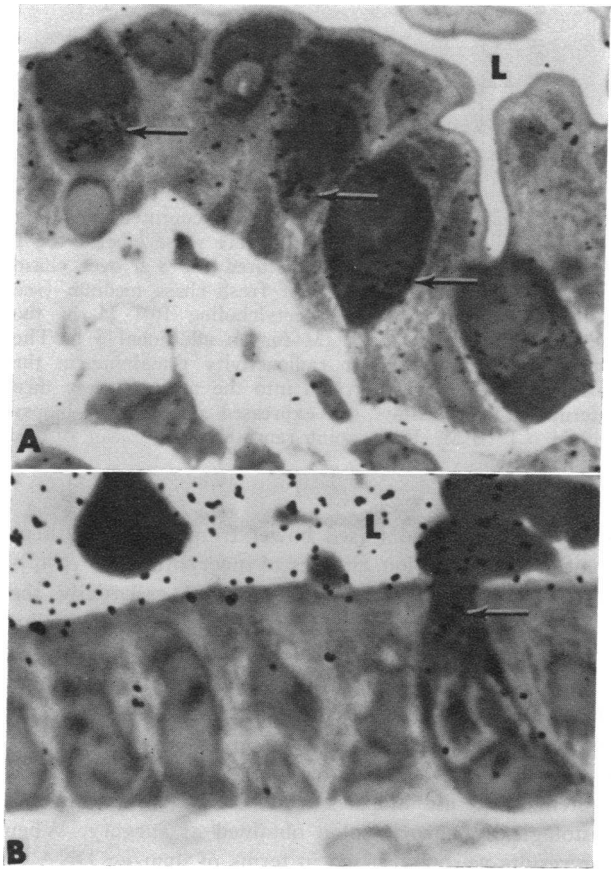

FIGURE 4 Autoradiographs of epithelium from biopsies cultured for 40 min over D-[6-^{3}H]glucosamine containing pulse medium and then transferred to unlabeled glucosamine containing chase medium for 1 h (A) and for 6 h (B). × 1,400. (A) After 1 h over chase medium the radioactivity in goblet cells is concentrated in the Golgi zone (arrows) in the lower third of the cells. Absorptive cell cytoplasm is lightly but diffusely labeled and the lumen (*L*) contains no radioactive material (B) After 6 h over chase medium radioactivity in many goblet cells is found near their luminal surface (arrow) and the lumen (*L*) contains much radioactive material. Most of the radioactivity in absorptive cells is at their apical surface in the brush border region.

TABLE I

*Localization of Pulse of Radioactivity in Goblet Cells of Cultured Rabbit Colon**

Time	Number of goblet cells				
	Basal third	Middle third	Apical third	Diffuse	Unlabeled
h					
½	88	4	0	8	0
1	72	9	0	19	0
2	55	22	0	23	0
4	42	28	5	25	0
6	21	35	20	23	1
8	10	14	47	20	9
24	4	6	11	27	52

* Biopsies were cultured over medium containing D-[6-^{3}H]-glucosamine for 40 min and then transferred to isotope-free medium containing an excess of unlabeled glucosamine for an additional ½–24 h of culture. 100 goblet cells were scored for each time period.

To determine whether acetylcholine stimulated the secretion of labeled glycoproteins, we incubated rabbit colonic biopsies in chase medium for 3 h after pulse labeling with [^{14}C]glucosamine and then exposed the biopsies to fresh chase medium containing 10^{-6} M acetylcholine (Fig. 6). As seen in the top panel, the amount of macromolecular radioactivity remaining in control biopsies decreased over 3 h (a period extending from 3 to 6 h after the pulse labeling). However, exposure of biopsies to acetylcholine resulted in a much greater fall in tissue radioactivity. Concomitantly, as seen in the bottom panel of Fig. 6, the amount of radioactive macromolecular material recovered from the medium was markedly increased when biopsies were exposed to acetylcholine. As shown in Fig. 7, maximum stimulation of glycoprotein secretion by acetylcholine occurred with concentrations of 10^{-8}–10^{-6} M and acetylcholine-stimulated secretion was completely blocked by atropine. Although acetylcholine stimulated secretion of labeled glycoproteins by rabbit colonic biopsies, acetylcholine over the same dose

range had no effect on overall incorporation of glucosamine into glycoproteins when examined under continuous culture conditions for 24 h (Table II). In contrast to the effects of acetylcholine, cycloheximide failed to inhibit secretion of previously synthesized glycoproteins as demonstrated by pulse-chase experiments (Table III) even though this agent clearly inhibited tissue synthesis of glycoproteins (Fig. 3).

To observe the influence of inflammation on glycoprotein synthesis and secretion, we obtained rectal biopsies from patients with active ulcerative colitis and compared them to rectal biopsies obtained from healthy subjects

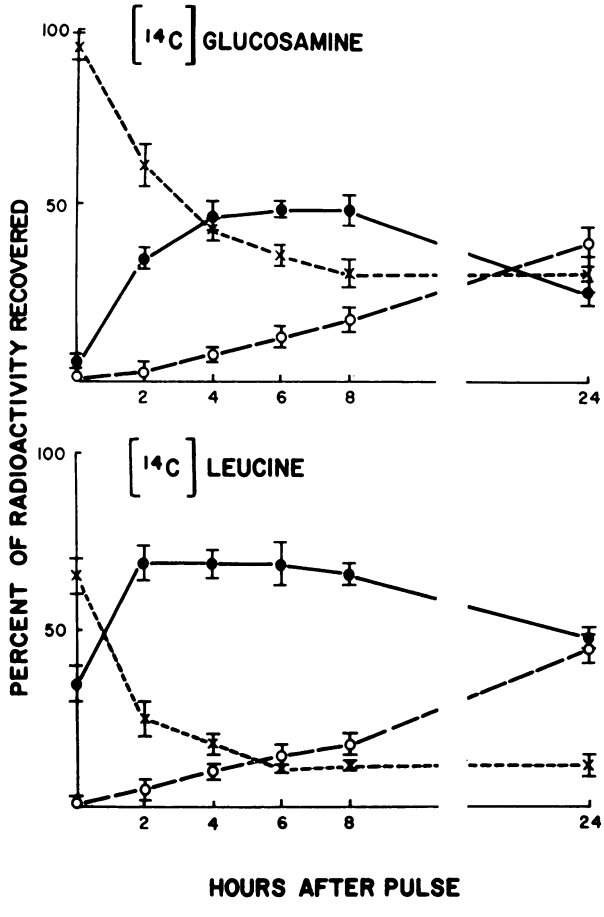

FIGURE 5 Recovery of [^{14}C]glucosamine and [^{14}C]leucine from rabbit colonic biopsies cultured by the pulse-chase technique. Biopsies were pulse labeled with radioactivity for 1 h and then cultured over chase medium for varying periods of time. The amount of radioactivity found in secreted macromolecules (○--○), tissue macromolecules (TCA-precipitable radioactivity, ●—●), or in the TCA supernate (×---×) was then determined. The results are expressed as the percent of recovered radioactivity present in the TCA precipitate, the TCA supernate, or in macromolecules secreted into the medium. The bars represent ±1 SEM. Each point represents the mean of 10 to 14 biopsies.

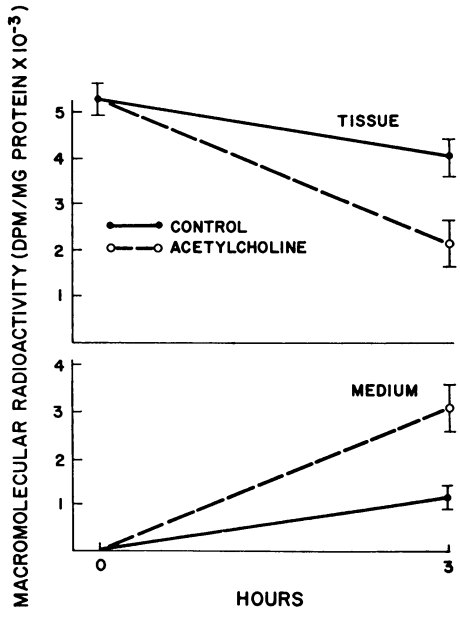

FIGURE 6 Effect of acetylcholine on glycoprotein secretion by rabbit colonic biopsies. Biopsies were pulse labeled for 1 h with [^{14}C]glucosamine, then cultured for 3 h over chase medium, and finally cultured over fresh chase medium (see Methods) with or without acetylcholine 10^{-6} M in the presence of eserine 3×10^{-4} M for an additional 3 h. The amount of macromolecular radioactivity remaining in the tissue and the amount secreted into the medium were then determined. The results are expressed as dpm/mg biopsy protein ±1 SEM. Each point represents the mean of six to nine biopsies.

and biopsies of undiseased bowel from a resected segment of sigmoid colon. Studies of incorporation of [^{14}C]-glucosamine and [^{14}C]leucine revealed essentially the same patterns of incorporation and secretion of radioactivity for normal human colonic and rectal biopsies as described in Figs. 1 and 2 for rabbit colonic biopsies. As shown in Fig. 8, biopsies from patients with active ulcerative colitis incorporated a significantly ($P < 0.01$) greater amount of [^{14}C]glucosamine into tissue glycoproteins than did biopsies from normal volunteers and histologically normal colon obtained at surgery. When the results were expressed in terms of dpm/μg DNA or dpm/μg RNA, the findings were not significantly altered (data not shown). The amount of incorporated [^{14}C]glucosamine recovered from the medium was also significantly ($P < 0.01$) greater from the cultured ulcerative colitis biopsies (Fig. 8), whether expressed in terms of protein, DNA, or RNA.

Since the increase in secreted radiolabeled glycoproteins observed in biopsies from patients with ulcerative colitis might be secondary to increased incorporation of [^{14}C]glucosamine into tissue glycoproteins, secretion of glycoproteins by these biopsies was measured inde-

pendently of tissue synthesis. Biopsies were pulse labeled for 1 h in medium containing [^{14}C]glucosamine, and after 2 h in chase medium the biopsies were transferred to fresh chase medium for a 4-h collection period. After the chase period, there was no significant difference ($0.2 < P < 0.5$) in the macromolecular radioactivity remaining in the tissue of eight rectal biopsies from four active ulcerative colitis patients in comparison to 12 rectal biopsies from four normal volunteers (Fig. 9). However, as also seen in Fig. 9, there was a fourfold increase in the amount of radiolabeled glycoproteins found in the medium of the biopsies from ulcerative colitis patients compared to that of control biopsies ($P < 0.01$). Furthermore, when the results were expressed as the percent of total macromolecular radioactivity which was recovered from the medium (Fig. 9), this percentage was significantly ($P < 0.01$) increased in the case of ulcerative colitis biopsies. The observed variation in results occurred not only from patient to patient but also from biopsy to biopsy in those cases where two biopsies were obtained from a single patient. There was no apparent correlation between glycoprotein production and severity of the lesion as observed histologically.

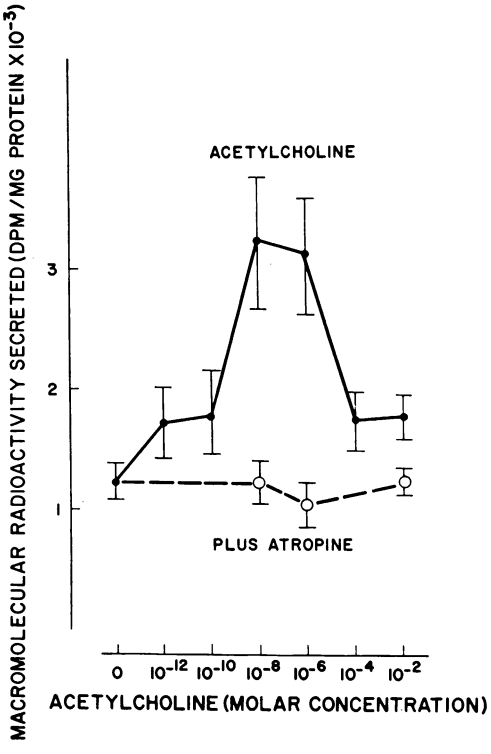

FIGURE 7 Effect of increasing concentrations of acetylcholine on glycoprotein secretion by rabbit colonic biopsies. Biopsies were cultured utilizing the pulse-chase system as described in Methods. Conditions of culture were as described in the previous figure. The solid line shows the effect of acetylcholine in doses from 10^{-2} to 10^{-12} M. The broken line shows the effect of acetylcholine, when atropine 10^{-6} M was also added. Eserine, 3×10^{4} M, was included at all times. The macromolecular radioactivity secreted is expressed as dpm/mg biopsy protein ±1 SEM. Each point represents the mean of six to nine biopsies.

TABLE II

*Lack of Effect of Acetylcholine on Glycoprotein Synthesis by Colonic Biopsies**

	Total incorporation of [^{14}C]glucosamine
	dpm/mg biopsy protein $\times 10^{-3}$
Control	166±19
10^{-2} M Acetylcholine	147±23
10^{-4} M Acetylcholine	156±9
10^{-6} M Acetylcholine	133±14
10^{-8} M Acetylcholine	150±16
10^{-10} M Acetylcholine	166±21

* The medium contained eserine 3×10^{-4} M alone or together with varying concentrations of acetylcholine. Biopsies were cultured for 24 h with 5.0 μCi of [^{14}C]glucosamine per ml after which the amount of radioactivity incorporated into tissue glycoproteins and into glycoproteins recovered from the medium was measured. The results show total incorporation into tissue and secreted glycoproteins and are expressed as the mean±SE for 6–12 biopsies.

Dialyzed media from two normal and two ulcerative colitis rectal biopsies were subjected to gel filtration on Sepharose 2B. As seen in Fig. 10, two peaks of radioactivity were observed. The first peak, within the void volume, most likely represents aggregates of large molecular weight glycoproteins, presumably mucus. Media from two control biopsies had 37% of the secreted radioactivity recovered from the column present in this first peak, while media from ulcerative colitis biopsies had 64% of the recovered counts in the first peak.

TABLE III

*Effect of Cycloheximide on Glycoprotein Secretion by Rabbit Colonic Biopsies**

Biopsies	Percent of incorporated glucosamine secreted
Control	37%±8.2%
1,000 μg/ml Cycloheximide	34%±5.6%

* The biopsies were pulsed for 1 h with [^{14}C]glucosamine. They were then placed in chase medium (see Methods) with or without cycloheximide for 18 h. The percent of incorporated radioactivity secreted into the medium was then determined by dividing the amount of radioactivity found in the medium by the amount found in the tissue plus the medium (total incorporation). The results are expressed as the mean±SE for six biopsies.

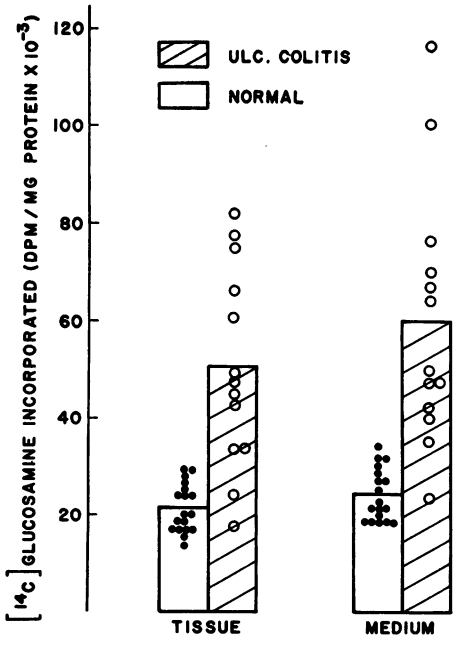

FIGURE 8 Incorporation of [^{14}C]glucosamine into tissue and secreted glycoproteins by 13 biopsies from eight active ulcerative colitis patients (hatched bars), in comparison to 18 biopsies from five normal volunteers and two surgically obtained normal colon specimens (open bars). Bars show mean values in dpm/mg biopsy protein and each circle represents one biopsy. All biopsies were cultured over [^{14}C]-glucosamine continuously for 24 h.

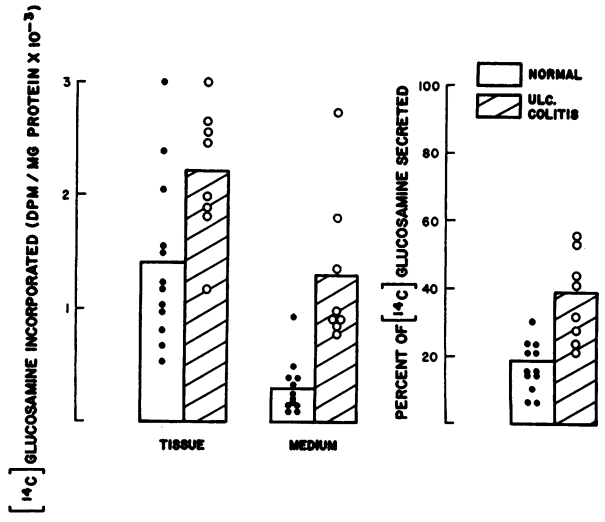

FIGURE 9 Secretion of prelabeled glycoproteins by nine biopsies from four active ulcerative colitis patients (hatched bars) in comparison to 12 biopsies from four normal volunteers (open bars). Each point represents one biopsy. Biopsies were pulse labeled for 1 h with [^{14}C]glucosamine. After a 2-h chase period (see Methods), biopsies were transferred to fresh chase medium and the medium collected for 4 h. The results in the first two sets of bars are expressed as the mean dpm/mg protein of [^{14}C]glucosamine incorporated into glycoproteins remaining in the tissue or secreted into the medium. The third set of bars represents the percent of incorporated [^{14}C]glucosamine recovered from the medium: 100 × (dpm/ml protein in medium)/(dpm/mg protein in medium + tissue).

DISCUSSION

Biopsies of rabbit colon cultured for 24 h maintained an intact and morphologically near normal mucosa as determined by light microscopy. Comparable results were previously obtained with biopsies of human rectal mucosa (7). Sustained viability was confirmed by the fact that cultured mucosal biopsies from rabbit colon and human rectum incorporated leucine into proteins and glucosamine into glycoproteins at a steady rate for 24 h. This incorporation probably represents macromolecular synthesis rather than nonspecific attachment of radioactive precursors since incorporation was almost completely prevented by the presence of cycloheximide, which prevents the transfer of amino acids from aminoacyl tRNA to a growing protein chain (13). However, cycloheximide had only a modest inhibitory effect on glycoprotein synthesis except when biopsies were preincubated with cycloheximide for at least 6 h before culturing with [^{14}C]glucosamine. During glycoprotein synthesis, the peptide portion of the molecule is synthesized first, after which the sugar moieties are incorporated (1). Thus, it is only after prolonged exposure to cycloheximide that the incorporation of glucosamine into glycoproteins is halted (14, 15), presumably because of

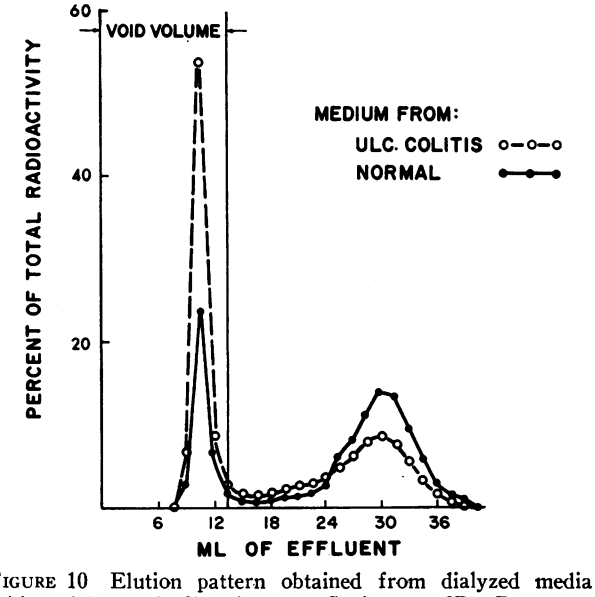

FIGURE 10 Elution pattern obtained from dialyzed media subjected to gel filtration on Sepharose 2B. Data are plotted as percent of total number of counts recovered from the column. The broken line indicates mean results for media of two biopsies from two ulcerative colitis patients and the solid line indicates mean results for media of two biopsies from two control subjects.

the depletion of either peptide precursors or the required glycosyl transferases.

Total incorporation of radioactivity into macromolecules by cultured colonic biopsies was linear with time when biopsies were continuously incubated in the presence of either ^{14}C-labeled leucine or glucosamine. Nevertheless, the pattern of distribution of incorporated radioactivity was quite different depending upon which labeled precursor was used. When biopsies were incubated with ^{14}C-labeled leucine, both the amount of incorporated radioactivity extracted from biopsy tissue and the amount of macromolecular radioactivity recovered from the medium increased steadily over the 24-h period. Throughout the incubation, the amount of incorporated radioactivity found in the tissue greatly exceeded that present in the medium. Thus, most of the newly synthesized protein containing labeled leucine remained within the biopsy, while only a small but relatively constant proportion of this newly synthesized protein was released into the incubation medium. In contrast, colonic biopsies incubated with ^{14}C-labeled glucosamine incorporated this radioactivity into glycoproteins which for the most part were ultimately secreted into incubation medium. Indeed, by the end of 24 h more macromolecular radioactivity was recovered from the incubation medium than was extracted from the tissue. This preferential secretion of newly labeled glycoproteins is consistent with previous observations by Forstner (3) who found that intestinal mucosa incorporated ^{14}C-labeled glucosamine into three glycoprotein pools: a large, poorly labeled pool of structural glycoproteins with a relatively slow turnover rate and two smaller, more intensely labeled pools which turned over more rapidly, one present in the glycocalyx and the other secreted into the intestinal lumen.

When examined by autoradiography after pulse labeling, cultured biopsies were found to synthesize and secrete glycoproteins at cellular sites similar to those previously described in the intestine of intact animals (2, 16). In addition, both the biochemical data and the autoradiographic studies were consistent with the time-course observed in previous in vivo studies (2, 3). The interval between exposure of intestinal mucosa to labeled glucosamine and initial secretion of labeled glycoproteins was 3–4 h (2, 3).

During pulse-chase experiments the amount of labeled glucosamine incorporated into acid-precipitable glycoproteins continued to increase during the chase period and reached a maximum 3–4 h after the initial pulse. This gradual increase in macromolecular radioactivity was accompanied by a decrease in dialyzable, acid-soluble radioactivity extracted from biopsy tissue. Presumably this labeled material was in the process of being incorporated into glycoproteins. Other investigators have shown this acid-soluble form of radioactivity to be predominantly UDP-*N*-acetylhexosamine (15). However, the [^{14}C]glucosamine might also be converted into galactose, fucose, galactosamine, sialic acid (17), and to a slight extent into leucine (14) before it is incorporated into glycoprotein molecules.

Acetylcholine markedly increased the rate of secretion of newly synthesized glycoproteins by biopsies of rabbit mucosa, an effect which was readily blocked by atropine. Thus, isolated colonic biopsies respond to cholinergic stimulation in a manner analogous to that of intact colon which is known to secrete goblet cell mucus in response to vagal stimulation or parasympathomimetic drugs (18).

The responses of colonic biopsies to acetylcholine and cycloheximide suggest that in organ culture glycoprotein synthesis and secretion can be clearly differentiated. Acetylcholine markedly stimulated secretion of newly synthesized glycoproteins, but had no effect on glycoprotein synthesis. In contrast, cycloheximide impaired glycoprotein synthesis, but had no effect on secretion.

Increased colonic production of mucus has been frequently observed in patients with ulcerative colitis and other inflammatory disorders of the colon (18). We therefore cultured rectal biopsies from patients with active ulcerative colitis to determine whether the inflammatory state influenced the biosynthesis and secretion of glycoproteins. The results indicated that both biosynthesis and secretion were substantially increased in biopsies from patients with colitis when compared to those from healthy volunteers. The increased incorporation of [^{14}C]glucosamine into tissue glycoproteins is consistent with the finding (19) that cultured biopsies from patients with ulcerative colitis have increased rates of epithelial cell turnover and migration, which would require increased rates of glycoprotein synthesis for incorporation into new cells. In addition, the increased synthesis and secretion of glycoproteins observed in vitro in cultured biopsies reflects the increased mucus production observed in vivo in patients with ulcerative colitis. Not only was there an increased secretion of [^{14}C]glucosamine-labeled glycoproteins, but the elution pattern of the dialyzed media fractionated by Sepharose 2B gel filtration suggested an increase in the proportion of large molecular weight glycoproteins. The elution patterns obtained showed only a change in the relative amounts present in the two peaks. No new peaks were observed to suggest an obvious change in glycoprotein type, a finding consistent with previous studies indicating that the composition of rectal mucus is similar in normal subjects and patients with ulcerative colitis (20).

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