Biosynthesis of proteins by human gastric mucosa *in vitro*

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Incorporation of L-[U-¹⁴C]leucine and of D-[U-¹⁴C]glucose into proteins of fresh human gastric mucosa *in vitro* was studied after incubation of homogenized tissue and of intact mucosal pieces. CsCl centrifugation was used to separate high-density mucus glycoproteins from other mucosal proteins, and the macromolecular nature of radioactive mucosal glycoprotein fractions was confirmed by SDS/polyacrylamide-gel electrophoresis and autoradiography of the polyacrylamide gels. In all experiments a substantial proportion of total incorporated radioactivity was associated with gastric-mucosal glycoprotein fractions (CsCl fraction L3), indicating their biosynthesis. Radioactivity of these fractions was shown to co-chromatograph with carbohydrates when fractionated either directly or after reduction and alkylation (1) by Sephadex G-200 chromatography in the excluded fractions and (2) by DEAE-cellulose ion-exchange chromatography. On incubation of intact mucosa, the major portion of radioactivity associated with the glycoprotein fractions of both leucine- and glucose-labelled specimens was secreted into the mucosal media during the course of the experiment. It is suggested that biosynthesis of mucus *in vivo* by gastric mucosa may be associated with rapid secretion of the synthesized macromolecules into the lumen of the stomach and that investigations of the metabolic processes within the mucosa should consider the products of secretion of the tissue. Incorporation of L-[U-¹⁴C]leucine implies biosynthesis of the polypeptide components of the macromolecules.

INTRODUCTION

Practical problems of studies of the structure, function and metabolism of mucous secretions have been reviewed, and lack of knowledge about the biosynthesis of the core protein of mucus, and also controversy in the understanding of the mechanism of mucus secretion, were emphasized (Carlstedt *et al.*, 1985). Since the early work of Kent & Allen (1968), biosynthesis of gastric mucus has been investigated by a number of laboratories (Dekanski *et al.*, 1975; Jentjens *et al.*, 1984; Jentjens & Strous, 1985; Kent, 1972; Lukie & Forstner, 1972; Parke & Symons, 1977; Snary & Allen, 1971, 1972; Spohn & McColl, 1980, 1986; Starkey *et al.*, 1974; Trotman & Greenwell, 1979; Yeomans & Millar, 1980), but so far the mechanisms involved and the factors controlling them remain obscure. Moreover, information about the biosynthesis and secretion of mucus by human gastric mucosa is scarce.

Jentjens & Strous (1985) have formulated a hypothesis which states that the backbone polypeptide of mucus only accounts for a small part of protein synthesis of mucus-producing cells. Our evidence, based on investigations of synthesis of proteins *in vitro* by intact gastric mucosa and by isolated gastric epithelial-cell preparations from the guinea pig (Spohn & McColl, 1980, 1986), as also by fresh human gastric mucosa, conflicts with this hypothesis, but suggests an alternative explanation for the findings. It is suggested that, although the polypeptide component of tissue glycoproteins is poorly labelled by radioactive precursors both *in vitro* and *in vivo*, synthesis of glycoprotein-associated polypeptides does take place within the mucosa. *In vivo*, such synthesis may be followed by rapid secretion of newly synthesized macromolecules into the lumen of the stomach. The observations by Jentjens & Strous (1985), whose experimental design was based on analysis of pulse-labelled tissue proteins after vascular perfusion, exclude

any consideration of the products of secretion of the mucosa.

In the work presented here, we have investigated the synthetic capability of human gastric mucosa *in vitro* by looking at the incorporation of radioactive precursors into proteins. L-[U-¹⁴C]Leucine was chosen to radiolabel the polypeptide chain of proteins, whereas D-[U-¹⁴C]glucose was expected to radiolabel all actively synthesized components of the mucosa.

MATERIALS AND METHODS

Reagents

Tissue samples were incubated in unsupplemented Dulbecco 'A' phosphate-buffered saline (pH 7.3–7.5; from Oxoid, Basingstoke, Hants., U.K.: 2.68 mM-KCl, 1.47 mM-KH₂PO₄, 137 mM-NaCl, 8.1 mM-Na₂HPO₄), in phosphate-buffered saline containing penicillin G (100 units/ml), and in Dulbecco's modified minimum essential medium (pH 7.2; GIBCO Europe) containing glucose (1000 mg/l), sodium pyruvate (110 mg/l), a range of amino acids, including 802 nmol of leucine/ml, and vitamins.

L-[U-¹⁴C]Leucine [sp. radioactivity 330 mCi (12.21 GBq)/mmol and 348 mCi (12.88 GBq)/mmol] and D-[U-¹⁴C]glucose [sp. radioactivity 270 mCi (9.99 GBq)/mmol] were from The Radiochemical Centre, Amersham, Bucks., U.K. Unless otherwise specified, all other chemicals, which were of analytical grade, were from BDH Chemicals (Poole, Dorset, U.K.).

Experimental procedure

Pieces of fresh human antral mucosa removed at operation for duodenal ulcers and stripped of the submucosal layers were incubated with radioactive precursors within 20 min of resection as tissue homogenates (Series A), as intact mucosa in Ussing-type

Perspex chambers (Series B), or as intact mucosal pieces (Series C) in a manner similar to the homogenized samples. The experiments were designed to compare extents of incorporation of the precursors into different proteins within a single experimental tissue sample, and no attempt was made to study the absolute rates of the synthesis of proteins under different experimental conditions, nor to correlate the incorporation of [^{14}C]glucose with that of [^{14}C]leucine.

The period of incubation and the concentrations of the precursor in the original media were altered arbitrarily during the course of several years of investigation, in an attempt to augment the radioactivity of the resulting proteins. All incubations were at 37 °C for specified lengths of time.

After incubation, unused radioactive precursors were generally removed from experimental samples by exhaustive dialysis, and the dialysed fractions were freeze-dried. In some experiments proteins were precipitated directly from the reaction mixtures by addition of trichloroacetic acid (final concn. 10%).

Series A. Gastric-mucosal samples, homogenized in a Teflon/glass Potter-Elvehjem homogenizer (clearance 0.15–0.23 mm) with five up-and-down strokes over a period of 1 min, were incubated with L-[U- ^{14}C]leucine (0.1–1.0 $\mu\text{Ci/ml}$) or D-[U- ^{14}C]glucose (1.0–5.0 $\mu\text{Ci/ml}$) in a shaking water bath.

Series B: Ussing-chamber incubations. Intact mucosal tissue discs (254 mm²) were bathed independently on the mucosal (luminal) and the serosal sides by 15 ml of circulating media gassed with O₂/CO₂ (97:3) (Spohn & McColl, 1980). Radioactive precursors L-[U- ^{14}C]leucine (0.06–0.50 $\mu\text{Ci/ml}$) or D-[U- ^{14}C]glucose (0.30–2.50 $\mu\text{Ci/ml}$) were added to the mucosal media, and proteins which were secreted into these media during the course of incubation were isolated separately from tissue-bound proteins. The period of incubation was 1–24 h in the experiments of Series A and B.

Series C. Intact mucosal pieces (1–10 mg of protein/sample) were incubated for 2 h directly in phosphate-buffered saline containing L-[U- ^{14}C]leucine (0.1 $\mu\text{Ci/ml}$) with and without penicillin G (100 units/ml).

Isolation and separation of proteins

Series A: incubation of mucosal homogenates. After incubation, the homogenates were centrifuged (15 min at 1500 g), and the residues resuspended in phosphate-buffered saline and again centrifuged as above. The combined supernatants and washings (SUPT. A) and the corresponding insoluble residues (IR) were collected.

Series B. All serosal media which contained no significant amounts of ^{14}C radioactivity were discarded. Secreted proteins, together with any proteins of cell debris which may have been sloughed off by the mucosa into the medium during incubation, were either precipitated directly by mixing with an equal volume of 20% trichloroacetic acid or dialysed and freeze-dried. For tissue proteins, experimental tissue specimens were washed by passage through five changes of phosphate-buffered saline and homogenized. The homogenates either were dialysed and freeze-dried as whole tissue fractions, or were first centrifuged (15 min at 1500 g)

and the resulting supernatants and residues collected separately.

Series C. Proteins were precipitated by addition of trichloroacetic acid (final concn. 10%). The samples were centrifuged (15 min, 1500 g), the sediments homogenized in water, and proteins isolated by repeated precipitation with 10% trichloroacetic acid and centrifugation.

Extraction of lipids

Lipids were extracted from freeze-dried fractions with chloroform/methanol (2:1, v/v) and removed by filtration through Whatman no. 1 paper. Lipid extracts were counted for radioactivity, but not analysed further.

Reduction and alkylation of proteins

Delipidated insoluble residues, and also the delipidated material of supernatant glycoprotein CsCl fractions L3, were incubated for 2 h at 37 °C in 0.1 M-dithiothreitol/5 M-guanidinium chloride/0.5 M-Tris/HCl/2 mM-EDTA (pH 7.4). Reduced proteins were alkylated by addition of iodoacetamide (final concn. 0.13 M). The reaction mixtures were centrifuged (15 min, 1500 g) and the separated supernatants and residues dialysed and freeze-dried.

Isopycnic CsCl-gradient fraction

The original freeze-dried supernatants, and some selected delipidated and reduced insoluble residues, were suspended in 0.1 M-Tris/HCl/5 mM-EDTA, pH 8.3, at concentrations not exceeding 50 mg of solid/ml. The suspensions were mixed with solid CsCl (0.425 g/ml) and the mixtures centrifuged at 4 °C for 72 h at 100000 g in a Beckman model L5-65 ultracentrifuge. Then 1 ml layers were collected from the top of the gradients and the specific radioactivities of proteins in the separated layers determined.

CsCl-gradient fractionation is used routinely in most laboratories to separate high- M_r glycoproteins, which sediment at the bottom of the gradient as clear viscous gels (fraction L3, sp.gr. 1.29–1.33), from the remaining proteins of the mucosa (Bhaskar & Creeth, 1974; Jentjens & Strous, 1985; Spohn & McColl, 1977; Starkey *et al.*, 1974).

SDS/polyacrylamide-gel electrophoresis

Dry proteins suspended in reducing sample buffer (0.01 M-Tris/borate buffer, 5 mM-EDTA, 2% SDS, 0.5 M-urea, 2% 2-mercaptoethanol and 10% glycerol) were heated for 3 min in a boiling-water bath. Electrophoresis was carried out in 0.1 M-Tris/borate/5 mM-EDTA, pH 8.2, at constant current (25 mA) in 80 mm-long 2 mm-thick gel slabs. Gel slabs were made up of 3.5% acrylamide and 0.5% agarose in 0.1 M-Tris/borate/5 mM-EDTA containing 2% SDS and 0.5 M-urea. After electrophoresis, protein bands were fixed in 10% trichloroacetic acid and stained with Coomassie Brilliant Blue. The gels were destained with 25% ethanol/8% acetic acid, shrunk with 55% methanol, immersed for 1 h in En³Hance (New England Nuclear), washed for 1 h in running water and dried on a Pharmacia gel-slab drier GSD 4.

Some selected gels were stained for glycoproteins by the periodic acid-Schiff method of Zacharius *et al.* (1969).

For autoradiography, dry SDS/polyacrylamide elec-

Table 1. Series A: incubation of homogenized human gastric mucosa with L-[U-¹⁴C]leucine and D-[U-¹⁴C]glucose

Distribution of ¹⁴C radioactivity between fractions is shown. Results are expressed as total incorporated radioactivity/mg of protein and as percentage distribution of total radioactivity between different fractions. Total radioactivity = 100% = radioactivity of (insoluble residue + SUPT. A). Abbreviations: PBS, phosphate-buffered saline; MEM, minimum essential medium containing non-radioactive leucine.

Incorporation	Incubation medium and time	Precursor (μ Ci/ml of medium)	Incorporated radioactivity (d.p.m./mg of protein)	Distribution of ¹⁴ C radioactivity (% of total)		
				Insol. residue	SUPT. A	Fraction L3
Leucine	PBS, 4 h	1.0	—	—	—	16.4
	PBS, 8 h	0.1	1170	65.0	35.0	25.0
	PBS, 8 h	0.1	—	60.0	39.0	30.1
	PBS, 18 h	1.0	4274	68.5	31.5	—
	MEM, 24 h	0.1	1817	56.0	44.0	18.7
Glucose	PBS, 3 h	1.0	8798	73.5	26.5	18.8
	PBS, 4 h	5.0	—	—	—	17.8
	PBS, 5 h	5.0	10093	—	—	25.8
	PBS, 18 h	1.7	14913	50.2	49.8	10.5

trophoresis gels were exposed to D7P X-ray film for periods of 1–4 weeks.

Sephadex G-200 chromatography

This was carried out on unreduced or reduced material of radioactive CsCl fractions L3 in Tris-buffered saline (0.1 M-Tris/HCl/0.15 M-NaCl/5 mM-EDTA, pH 7.0).

DEAE-cellulose ion-exchange chromatography

This was done by stepwise elution of the column as described by Porter (1973). Buffers: 1, 0.01 M-NaH₂PO₄, pH 7.6; 2, 0.02 M-NaH₂PO₄, pH 6.3; 3, 0.05 M-NaH₂PO₄, pH 5.0; 4, 0.3 M-NaH₂PO₄, pH 4.6; 5, 2 M-NaCl/0.4 M-NaH₂PO₄, pH 3.9.

Hydrolysis of proteins

Freeze-dried proteins were hydrolysed in air-tight Teflon-capped glass tubes in 6 M-HCl for 18 h. HCl was removed by evaporation under a stream of N₂.

T.l.c. of amino acids

Dry residues of hydrolysed proteins were suspended in 0.1 M-HCl and quantitatively applied to Merck pre-coated cellulose plates together with non-radioactive leucine marker. Chromatograms were developed in ethanol/conc. NH₃ (sp.gr. 0.880)/water (20:1:4, by vol.), and then sprayed with 0.5% ninhydrin in butan-1-ol saturated with water. Spots were scraped as soon as they became visible (5–10 min), and the powder was suspended in 1 ml of 0.1 M-HCl, mixed with 5 ml of Unisolve-100 and counted for radioactivity. The recovery of total radioactivity from the plates was $91.8 \pm 9.2\%$ ($n = 57$).

Specific radioactivity of proteins

Proteins precipitated from aqueous fractions by addition of trichloroacetic acid (final concn. 10%) were measured on the Technicon Autoanalyser by the method of Lowry *et al.* (1951) after 18 h hydrolysis at 37 °C in 0.5 M-NaOH. Samples of the hydrolysed solutions were also counted for radioactivity.

Carbohydrates

Hexose content of aqueous samples was determined by the method of Dubois *et al.* (1956) with glucose as standard. Carbohydrate contents indicate only the relative amounts of hexose found in different samples. They cannot be treated as absolute, since the sugars which are associated with gastric-mucosal glycoproteins comprise mixtures of different carbohydrates, all of which contribute in different yields to the intensity of colour produced by the colorimetric reaction used. Carbohydrate composition of the different CsCl fractions of human gastric-mucosal proteins has been reported (Spohn & McColl, 1977).

Radioactivity determination

This was done on aqueous samples by counting in liquid scintillator Unisolve-100 (Koch-Light) in an LKB-Wallac 81000 liquid-scintillation counter set to 1% error. Counts below twice the background were rejected. The efficiency of counting was 76%.

RESULTS AND DISCUSSION

Series A: incubation of gastric-mucosal homogenates

Insoluble residue. In all experiments, over 50% of the protein content and of the radioactivity of proteins was associated with the original insoluble residue (Table 1); these fractions also contained $45.3 \pm 16.6\%$ (8) of total hexose of the samples. Although after reduction and alkylation of delipidated residues some 20–30% of ¹⁴C radioactivity of leucine-labelled samples was found at the bottom of CsCl gradients in fractions L3, the nature of the material in these fractions was not investigated further, and the contribution that glycoproteins of the residue might make to the radioactivity of mucus glycoproteins as presented in Table 1 has not been taken into account.

About 70% of the ¹⁴C radioactivity of glucose-labelled original insoluble residues could be extracted with chloroform/methanol (2:1, v/v), signifying active incor-

Fig. 1. Distribution of hexose and of protein of human gastric-mucosal supernatants on isopycnic CsCl gradients

A typical tracing is shown of results of fractionation of material of SUPT. A (Series-A experiments). The stippled area represents layers that were combined to give fractions L3. Results are expressed as percentages of total contents in the mucosa (supernatant + insoluble residue = 100%): ●, hexose; ■, protein.

poration of the precursor into the lipids of the mucosa and thus lipid synthesis.

Supernatant proteins (SUPT. A). When fractionated on isopycnic CsCl gradients, most proteins layered in the middle part of the gradient (layers 1–9), well separated from the bulk of hexose-rich clear gastric mucus at the bottom of the tubes in fractions L3 (Fig. 1). However, a substantial proportion of the radioactivity of both leucine- and glucose-labelled specimens was associated with fractions L3 (Table 1). The specific radioactivities of proteins of these fractions exceeded those of other fractions from a given experimental sample by several orders of magnitude.

Extraction of the glucose-labelled glycoprotein fractions L3 with chloroform/methanol (2:1, v/v) resulted in the loss of 30–40% of the radioactivity, suggesting association of lipids with gastric-mucosal glycoproteins, in agreement with the findings of Slomiany *et al.* (1983) and Witas *et al.* (1983).

No significant loss of radioactivity was detected after lipid extraction of leucine-labelled fractions L3, and $80.6 \pm 10.9\%$ (8) of ^{14}C radioactivity of these fractions was recovered as free leucine after hydrolysis and t.l.c.

After reduction and alkylation of both the leucine- and the glucose-labelled delipidated fractions L3, most (over 85%) of the radioactivity was again recovered as a clear

viscous layer at the bottom of isopycnic CsCl gradients after centrifugation.

On Sephadex G-200 chromatography, over 90% of the radioactivity of unreduced fractions L3, and after their reduction and alkylation, was found together with the carbohydrates in the excluded fractions. DEAE-cellulose ion-exchange chromatography of the excluded fractions again showed co-chromatography of over 80% of radioactivity of both leucine- and glucose-labelled material with the bulk of the carbohydrates (Fig. 2).

Although incorporation of glucose into hexose-rich glycoproteins of the mucosa cannot be meaningfully elucidated at this stage, owing to the complicated metabolic pattern of this precursor, incorporation of [^{14}C]leucine does imply active synthesis of the polypeptide components of high- M_r mucosal glycoproteins.

Series B: incubation of intact mucosa in Ussing-type chambers

In these experiments, proteins which were released into the mucosal media during the course of incubation (secreted proteins) were isolated separately from tissue-bound proteins.

Fig. 2. Recovery of hexose and of ^{14}C radioactivity from DEAE-cellulose ion-exchange columns

Chromatography of excluded Sephadex G-200 fractions of the products of reduction and alkylation of original glycoprotein CsCl fractions L3 from Series-A experiments. A_{254} indicates relative protein contents of the eluates. ▼, [^{14}C]Leucine; ●, [^{14}C]glucose.

Fig. 3. Series B experiments: distribution of leucine-labelled tissue and secreted proteins on isopycnic CsCl gradients

Intact mucosal tissue was incubated in phosphate-buffered saline for 18 h with L-[U- 14 C]leucine (0.5 μ Ci/ml). Results are expressed as percentages of total radioactivity (tissue + mucosal medium = 100%) and as specific radioactivity (d.p.m./mg of protein). The tissue insoluble-residue fraction (IR) was not delipidated. The lightly stippled area represents layers which were combined to give the corresponding CsCl fractions L3.

Secreted (mucosal-medium) proteins. The amount of protein found in the mucosal media after incubation of intact tissue samples was quantitatively low in all experiments; even after 18 h of incubation, less than 10% of the protein content of tissue specimens was detected in the media. However, in all experiments the major portion (over 50%) of total incorporated radioactivity was associated with the secreted proteins, of which the glycoprotein fraction L3 was the most prominent (Figs. 3 and 4); this fraction also contained 50–70% of the hexose content of the mucosal media. The specific radioactivities of the secreted glycoprotein fractions L3 were several orders of magnitude greater than those of other proteins of the sample (layers 1–9 of CsCl gradients), and tens of orders of magnitude greater than for any fraction of the corresponding tissue samples (Figs. 3 and 4).

Tissue proteins. CsCl-gradient fractionation of tissue proteins revealed that the glycoprotein fractions L3 accounted for only a small proportion of total incorporated radioactivity (Table 2 and Figs. 3 and 4), in agreement with the findings of Jentjens & Strous (1985). The specific radioactivities of leucine-labelled tissue glycoproteins (fractions L3) did not differ significantly from those of other tissue proteins (Fig. 3), including the corresponding insoluble tissue residues (IR).

Glucose-labelled tissue fractions L3 (not corrected for contribution by radioactive lipids), although of higher

specific radioactivity than proteins of other CsCl layers, were less radioactive than the corresponding insoluble tissue residues, and considerably less radioactive than the glycoprotein fractions L3 of the secreted material (Fig. 4).

The distribution of 14 C radioactivity between various fractions of the secreted and of the corresponding tissue proteins in different experimental specimens (Series B) is given in Table 2. The results suggest that, although most proteins of the mucosa are radiolabelled by both precursors, the major and the most notably labelled single products of incorporation *in vitro* of [14 C]leucine and of [14 C]glucose by human gastric mucosa are the secreted glycoproteins of the mucosal-medium fractions L3.

Series C

Direct incubation of gastric-mucosal pieces for 2 h in phosphate-buffered saline showed no effect of penicillin G (100 units/ml) on the incorporation of L-[U- 14 C]leucine into proteins (Table 3).

Autoradiography of SDS/polyacrylamide gels

After electrophoresis of reduced fractions L3, autoradiographic results showed that the most clearly detectable radioactivity of soluble proteins of Series A was associated with the high- M_r periodic acid-Schiff-positive material found at the origins, barely if at all penetrating the acrylamide/agarose gels (Fig. 5). This

Fig. 4. Series B experiments: distribution of glucose-labelled tissue and secreted proteins on isopycnic CsCl gradients

Intact mucosal tissue was incubated in phosphate-buffered saline for 3 h with D-[U- 14 C]glucose (2.5 μ Ci/ml). Results are expressed as percentages of total radioactivity (tissue + mucosal medium = 100%) and as specific radioactivity (d.p.m./mg of protein). The tissue insoluble-residue fraction (IR) was not delipidated. The lightly stippled area represents layers which were combined to give the corresponding CsCl fractions L3.

implies that the M_r of these highly radiolabelled proteins is in excess of 200000, consistent with that of glycoproteins of gastric mucus.

Similarly, SDS/polyacrylamide-gel electrophoretograms of reduced mucosal-medium fractions L3 showed that most of the radioactivity remained at the origin of the gels, suggesting radiolabelling of the secreted high- M_r gastric-mucosal glycoproteins.

General conclusions

Practical difficulties associated with biochemical investigations of secretory mechanisms are well recognized. In the human, ethical considerations make studies *in vivo* of gastric-mucosal metabolism almost impossible; moreover, scarcity of normal fresh human mucosa, combined with dependence on the operating surgeon and on theatre personnel for supply of the tissue, considerably limits the range of metabolic investigations *in vitro* that can be meaningfully controlled and interpreted.

In our work each tissue specimen was investigated individually. Assuming that the mucosa had lost an incalculable degree of viability in the period between resection and incubation, in addition to wide biological variations, no attempt was made to assess absolute rates of biosynthesis of the proteins in the different specimens;

the experiments were designed to establish the capacity of human gastric mucosa to synthesize mucus glycoproteins, by comparing the proportion of radioactivity incorporated into different fractions within a single experimental tissue specimen.

The results demonstrate that the major radioactive products of incubation of gastric mucosa *in vitro* are associated with the high- M_r mucosal glycoprotein fractions, which are traditionally isolated from other proteins of the mucosa as clear, homogeneous, viscous gels at the bottom of isopycnic CsCl gradients; incorporation of radioactive leucine into these fractions and recovery of around 80% of the incorporated activity as leucine after hydrolysis and t.l.c. implies biosynthesis of the polypeptide components of glycoproteins.

Incorporation of radioactive glucose presents a more complex problem of interpretation of the results, because this precursor can be incorporated into the polypeptide, carbohydrate and lipid components of macromolecular complexes. It is noteworthy that in glucose-labelled samples some 30–40% of the radioactivity of CsCl fractions L3 is lost by extraction with chloroform/methanol, suggesting association of lipids with mucus glycoproteins.

The incorporation of radioactive precursors into

Table 2. Series B: incubation of intact human gastric mucosa in phosphate-buffered saline with L-[U-¹⁴C]leucine and D-[U-¹⁴C]glucose

Distribution of ¹⁴C radioactivity between secreted and tissue-bound proteins and the corresponding glycoprotein fractions L3 is shown. Results are expressed as total incorporated radioactivity/mg of protein of the sample, and as percentage distribution of total radioactivity between different fractions. Total radioactivity = 100% = radioactivity of (secreted proteins + tissue proteins).

Incorporation	Time of incubation	Precursor (μ Ci/ml of medium)	Incorporated radioactivity (d.p.m./mg of protein)	Distribution of ¹⁴ C radioactivity (% of total)			
				Secreted proteins		Tissue proteins	
				Whole	L3	Whole	L3
Leucine	1 h	0.37	35963	54.5	25.3	45.5	7.9
	2 h	0.17	—	74.6	26.6	25.4	2.4
	4 h	0.13	—	88.0	34.0	12.0	2.4
	4 h	0.40	—	60.8	15.5	39.2	6.7
	5 h	0.06	8518	64.2	15.0	35.8	2.8
	6.5 h	0.50	16198	55.2	15.2	44.8	2.4
	8 h	0.50	8819	73.6	26.9	26.3	7.6
	18 h	0.50	44932	79.0	57.6	21.0	7.8
Glucose	3 h	2.50	83296	89.4	71.8	10.6	2.8
	4 h	1.00	—	59.0	15.1	40.9	13.3
	18 h	1.70	346330	82.2	21.8	17.8	3.1

Table 3. Series C: incubation of intact mucosal pieces

Tissue samples were incubated for 2 h with and without penicillin G (100 units/ml) in 1 ml of unsupplemented phosphate-buffered saline (PBS) with L-[U-¹⁴C]leucine (0.1 μ Ci/ml). Results are expressed as means $\pm$ S.D. for five samples.

Treatment	Protein (mg/sample)	Sp. radio-activity (d.p.m./mg of protein)
Control (PBS)	5.24 $\pm$ 1.47	533 $\pm$ 138
Penicillin G	4.95 $\pm$ 2.34	699 $\pm$ 286

glycoprotein fractions by human gastric mucosa *in vitro* is associated with the release, presumed to be active secretion, of highly radioactive newly synthesized material into the incubation media.

On prolonged incubation *in vitro*, tissue viability decreases, as assessed by precursor uptake reaching a plateau (M. Spohn & I. McColl, unpublished work), and debris of cells sloughed off during the course of incubation adds to the composition of the mucosal media. Consequently our results cannot be used to establish the relative rates of synthesis or of secretion of different proteins. Moreover, experiments *in vitro* cannot be compared with conditions prevailing *in vivo*. However, as long as the factors that regulate and control the synthesis and secretion of proteins by gastric mucosa *in vivo* remain obscure, it is of interest to know that mucosal tissue under certain conditions can incorporate radioactive precursors into fractions which represent gastric-mucosal glycoproteins. The finding that the incorporated radioactivity cannot be dissociated from the glycoproteins by classical methods of purification and separation even after reduction suggests chemical binding.

Fig. 5. Autoradiographic tracings after SDS/polyacrylamide-gel electrophoresis

(a) Series B experiments: glucose-labelled proteins of the mucosal medium. (b) Series A experiments: glucose-labelled glycoprotein CsCl fraction L3. (c) Series A experiments: leucine-labelled glycoprotein CsCl fraction L3.

In vivo, mucosal glycoproteins are known to be secreted into the lumen of the stomach, and therefore investigations, both qualitative and quantitative, of the metabolic processes of gastric mucosa must take these products of secretion of the tissue into account.

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