Age-related changes in chemical composition and physical properties of mucus glycoproteins from rat small intestine

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Mucus glycoproteins from newborn and adult rat small intestine were radiolabelled *in vivo* with Na₂³⁵SO₄ and isolated from mucosal homogenates by using Sepharose 4B column chromatography followed by CsCl-density-gradient centrifugation. Non-covalently bound proteins, lipids and nucleic acids were not detected in the purified glycoproteins. Amino acid, carbohydrate and sulphate compositions were similar to chemical compositions reported for other intestinal mucus glycoproteins, as were sedimentation properties. There were, however, important differences in the chemical and physical characteristics of the mucus glycoproteins from newborn and adult animals. The buoyant density in CsCl was higher for the glycoproteins from newborn rats (1.55 g/ml versus 1.47 g/ml). On sodium dodecyl sulphate/polyacrylamide/agarose-gel electrophoresis, the glycoprotein from newborn rats had a greater mobility than the adult-rat sample. Although both preparations had similar general amino acid compositions, variations were observed for individual amino acids. The total protein content was greater in the glycoprotein from newborn animals (27%, w/w, versus 18%, w/w). The molar ratio of carbohydrate to protein was less in the newborn, primarily owing to a decreased fucose and *N*-acetylgalactosamine content. Comparison of the molar ratio of fucose and sialic acid to galactose for both glycoproteins demonstrated a reciprocal relationship similar to that described by Dische [(1963) *Ann. N.Y. Acad. Sci.* **106**, 259–270]. The sulphate content was greater in the glycoprotein from newborn rats (5.5%, w/w, versus 0.9%, w/w). Both had similar sedimentation coefficients in a dissociative solvent. These results suggest an age-related difference in the types of mucus glycoproteins synthesized by small intestine.

The small intestinal tract of the rat, which does not reach mature biochemical function until the fourth postnatal week, has been studied extensively as a model for postnatal development (Henning, 1979). Most investigations, however, have centred on studying the biochemical maturation of absorptive epithelial cells, with particular emphasis on development of brush-border components. Histological studies have demonstrated cell-surface changes during postnatal development of the small intestine (Etzler & Branstrator, 1979). Differences in electrophoretic mobility (Galand & Forstner, 1974) and glycosylation (Bresson *et al.*, 1982) of

microvillus-membrane glycoproteins have also been noted. It seems reasonable, therefore, to expect that there may be age-related changes in the chemical structure of mucus glycoproteins.

Purified small-intestinal mucus glycoproteins from adult animals of various species have been isolated and characterized (Bella & Kim, 1972; Forstner *et al.*, 1973; Jabbal *et al.*, 1976; Forstner *et al.*, 1979; Mantle & Allen, 1981). These mucus glycoproteins are complex polydisperse large-molecular-mass molecules that possess oligosaccharide side chains covalently attached to a protein core. It has also been shown (Mantle & Allen, 1981) that highly purified preparations of mucus glycoproteins, which were essentially free from non-covalently bound contaminants, retained the viscous and gel-forming properties of the native mucus.

By using similar techniques to those described by

Abbreviation used: SDS, sodium dodecyl sulphate.

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Mantle & Allen (1981), we have isolated and partially characterized large-molecular-mass water-soluble mucus glycoproteins from newborn and adult rat small intestine. In the present paper we report the chemical composition and physical properties of these mucus glycoprotein preparations and conclude that there are age-related differences in the composition and structure. A preliminary communication has been reported previously (Shub *et al.*, 1982).

Materials and methods

Materials

The $\text{Na}_2^{35}\text{SO}_4$ (1 mCi/ml) was obtained from New England Nuclear (Boston, MA, U.S.A.). Sepharose 4B was obtained from Pharmacia (Piscataway, NJ, U.S.A.). CsCl (optical grade) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All reagents used for polyacrylamide/agarose-gel electrophoresis were purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.), except agarose, which was purchased from Sea-Kem (Rockland, ME, U.S.A.). All other chemicals were of analytical-grade quality and obtained through commercial sources.

Animals

Sprague-Dawley rats were used in all experiments and were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.).

Newborn animals weighing 5–7 g were allowed to suckle and were always killed at less than 24 h of

age. Adult animals were female, over 2 months of age and weighed 200–300 g.

Isolation and purification of small-intestinal mucus glycoproteins from newborn and adult rats

Adult and newborn rats were anaesthetized and given intraperitoneal injections of $\text{Na}_2^{35}\text{SO}_4$ (1.0–4.0 $\mu\text{Ci/g}$ body weight). At 2 h after injection animals were re-anaesthetized, and the small intestines were dissected out from the distal portion of the duodenum to just proximal to the ileocaecal valve. The intestines were flushed with iced 0.15 M-NaCl and either kept at 4°C for immediate processing or stored at –20°C. No differences were noted in the purified mucus glycoproteins obtained from frozen intestines. All subsequent steps were performed at 4°C.

The mucosal surface was exposed by longitudinal dissection, and a microscope slide was used to obtain the mucosal scrapings. On histological examination of the intestine it was noted that this technique removed mainly the mucosa, leaving most of the submucosa and all of the deeper structures intact. Batch preparations from 50–90 newborn-rat small intestines and two or three adult-rat small intestines were used. This procedure yielded 6–10 g (wet tissue weight) of mucosal scrapings.

The mucus glycoproteins were purified by a modification of the method of Mantle & Allen (1981) as outlined in the flow diagram in Scheme 1. Scrapings were collected in 100 ml of 5.0 mM-EDTA (pH 7.4)/g of wet tissue weight, and solubilized by homogenization in a Waring blender for 20 s. The

Scheme 1. Flow diagram of the purification of mucus glycoproteins from the small intestines of newborn and adult rats. For experimental details (modified from Mantle & Allen, 1981) see the text.

soluble fraction was separated by centrifugation at 25000g for 30 min. The supernatant was dialysed against deionized distilled water for 48 h, and non-diffusible material was freeze-dried. The crude soluble mucus glycoprotein fraction was then resuspended in 10 ml of 10 mM-potassium phosphate buffer, pH 7.0, containing 0.02% NaN₃ and centrifuged at 36000g for 30 min. The supernatant was then applied to a column (85 cm × 2.5 cm) of Sepharose 4B and eluted with the 10 mM-potassium phosphate buffer, pH 7.0, at a flow rate of 0.45 ml/min. The column fractions were analysed to determine the absorbance at 280 nm and also the radioactivity. The fractions eluted at the column void volume were pooled, and the mucus glycoprotein was further purified by equilibrium density-gradient centrifugation in aqueous CsCl (initial density 1.42 g/ml; 150000g for 48 h at 4°C) according to methods previously described (Creeth & Denborough, 1970; Starkey *et al.*, 1974; Mantle & Allen, 1981). The gradient fractions were analysed to determine absorbance at 280, 260 and 230 nm, and also radioactivity. The fractions containing glycoprotein were pooled and dialysed, initially against 1 M-NaCl for 24 h, then against deionized distilled water for 48 h. The dialysed samples were divided into several portions and stored at -20°C for further analysis. Between 2 and 5 mg of mucus glycoprotein was obtained in each preparation.

SDS / polyacrylamide / agarose-slab-gel electrophoresis

SDS/polyacrylamide/agarose-gel electrophoresis was conducted by the method of Holden *et al.* (1971), modified for use with slab gels. The mucus glycoprotein samples (25–50 µg) were electrophoresed under the conditions outlined in Fig. 3. The gels were stained with either periodic acid/Schiff reagent for glycoproteins or Coomassie Brilliant Blue for proteins.

Analytical procedures

The presence of protein constituents in column effluents was determined by measuring absorbance at 280 nm. The presence of protein constituents or nucleic acid constituents in CsCl-density-gradient fractions was determined by measuring the absorbance at 280 and 230 nm or at 260 nm respectively. Radioactivity was measured in a liquid-scintillation counter. The amino acid composition and glucosamine and galactosamine contents of samples were determined by acid hydrolysis followed by analysis with an amino acid analyser (Swann & Mintz, 1979). Neutral sugars and *N*-acetylneuraminic acid were measured by g.l.c. (Reinhold, 1972). [These analyses were kindly performed by Mr. K. Lindsley

in the Carbohydrate Research Laboratory, Massachusetts General Hospital (Director Dr. R. W. Jeanloz).] The sulphate content was determined by the method of Antonopoulos (1962). Lipid content was measured by g.l.c. after methanolysis and hexane extraction of the fatty acid methyl esters (Heckers *et al.*, 1977) (kindly performed by Mr. K. Lindsley). Quantification was accomplished by comparison with standard free fatty acid markers. Nucleotides were determined by high-pressure-liquid-chromatographic analysis by the method of Nelson *et al.* (1976). (These analyses were kindly performed by Dr. J. A. McGowen in the Department of Surgery, Shriners Burn Hospital, Boston, MA, U.S.A.) Quantification was accomplished by comparison with standard nucleotide markers. Analysis for ribose was done by g.l.c.

Ultracentrifugation analysis

Ultracentrifugation studies were performed in a Beckman model E analytical ultracentrifuge in 4 M-guanidinium chloride/50 mM-Tris/HCl buffer, pH 7.0. Sedimentation-velocity measurement was made with the use of an An-D rotor operated at 20°C and 42000 rev./min. Glycoprotein concentrations used for newborn-rat samples were 3.5 mg/ml and for adult-rat samples were 1.5 mg/ml and 5.5 mg/ml. Sedimentation coefficients were calculated by using values for partial specific volume ($\bar{v}$) of 0.63 ml/g and 0.65 ml/g for adult-rat and newborn-rat mucus glycoproteins respectively (Snary *et al.*, 1971).

Results and discussion

The elution profile of the mucus glycoproteins after column chromatography was similar for samples from both the newborn (Fig. 1) and the adult (not shown) animals. Most of the radiolabelled crude soluble mucus glycoprotein was present in the exclusion volume of the Sepharose 4B gel-filtration column. This excluded fraction was pooled and further purified by centrifugation in a CsCl density gradient. The density-gradient profile of both mucus glycoprotein preparations was similar to that observed previously for other mucus glycoproteins (Creeth & Denborough, 1970; Starkey *et al.*, 1974; Meyer, 1977; Spee-Brand *et al.*, 1980). The major constituent was a radiolabelled glycoprotein, which was present between 1.45 g/ml and 1.55 g/ml. Small quantities of nucleic acids and proteins were present at 1.60 g/ml and 1.30 g/ml respectively. Analyses were performed to determine if ³⁵S incorporation into the newborn-rat and adult-rat glycoprotein samples was present as ester [³⁵S]sulphate by acid hydrolysis (6 M-HCl at 100°C for 24 h in sealed tubes under vacuum). Over 90% of

Fig. 1. *Sepharose 4B* chromatography of crude soluble ^{35}S -labelled mucus glycoprotein from newborn-rat small intestines. The column (85 cm $\times$ 2.5 cm) was eluted with 10 mM-potassium phosphate buffer, pH 7.0, containing 0.02% NaN_3 at a flow rate of 0.45 ml/min at 4°C. The column fractions were analysed for protein at A_{280} (----) and for ^{35}S radioactivity (●). The fractions were pooled as indicated to obtain partially purified mucus glycoprotein. The arrow marked V_0 indicates the elution position of Blue Dextran 2000. A representative chromatogram is shown.

the recoverable radioactivity was not retained on a Dowex 50W-X2 (H^+ form) column and was precipitable by benzidine and pentanol in 95% (v/v) ethanol (Antonopoulos, 1962). This suggests that most of the ^{35}S radioactivity was incorporated as ester sulphate in both the newborn-rat and the adult-rat samples. Analysis of fractions from the density gradient revealed that the mucus glycoprotein from newborn-rat preparations had maximum ^{35}S radioactivity occurring at a buoyant density of 1.55 g/ml, whereas maximum absorbance at 230 nm occurred at a density of 1.52 g/ml (Fig. 2a). This difference in buoyant density appeared to be significant and reproducible. The mucus glycoprotein from adult animals had a lower buoyant density (1.47 g/ml), and the ^{35}S -radioactivity peak coincided with the absorbance peak at 230 nm (Fig. 2b). Differences in buoyant density between mucus glycoproteins from newborn and adult rats persisted when samples were dialysed against 4 M-guanidinium chloride and run in a CsCl density gradient containing 4 M-guanidinium chloride (1.50 g/ml and 1.41 g/ml for samples from newborn and adult rats respectively).

Gel electrophoresis

The isolated small-intestinal mucus glycoproteins from both animals gave a single diffuse band on

SDS/polyacrylamide/agarose-gel electrophoresis. The mucus glycoproteins stained intensely with periodic acid/Schiff stain (Fig. 3) but only poorly with Coomassie Blue (results not shown). Other protein constituents were not detected. The newborn-rat mucus glycoprotein was consistently found to migrate a greater distance through the gel when run in parallel with samples from adult rats (Fig. 3).

Chemical analysis

The chemical composition of the newborn-rat and adult-rat glycoproteins are summarized in Table 1. As others have reported for purified glycoproteins (Scawen & Allen, 1977; Mantle & Allen, 1981), only small quantities of mannose were present in the purified glycoproteins. This indicates the presence of either minimal contamination with serum glycoproteins or a few *N*-glycosidically linked oligosaccharides in these mucus glycoproteins. Xylose and uronic acid were not detected by g.l.c. analysis, indicating the absence of detectable glycosaminoglycans. Lipids were not detected by g.l.c.

The amino acid, carbohydrate and sulphate contents of the mucus glycoproteins from newborn rats (Table 1) were similar to those of other mucus-type glycoproteins (Gottschalk, 1972). Several variations in composition were observed, however, when the glycoproteins from newborn-rat

Fig. 2. Fractionation of partially purified mucus glycoproteins on CsCl density gradients (a) Newborn-rat and (b) adult-rat fractions were analysed for ^{35}S radioactivity ($\bullet$), density ($\circ$), A_{280} (Δ) and A_{230} ($\blacktriangle$). The starting density of the gradients was 1.42 g/ml. Purified mucus glycoproteins from newborn and adult rats were obtained by pooling and dialysing fractions 3–7 (a) and 5–9 (b) respectively. A representative analysis is shown. For full experimental details see the text.

Fig. 3. SDS/polyacrylamide/agarose-slab-gel electrophoresis of purified mucus glycoprotein

Gel electrophoresis was performed on a 0.1% (w/v) SDS/2% (w/v) polyacrylamide/0.5% (w/v) agarose gel. No stacking gel was used. Lanes were loaded with 25–50 μg of purified mucus glycoproteins from small intestines of (1) adult and (2) newborn rats. Glycoprotein samples were solubilized in 17.8 mM-Tris / 6.8 mM-boric acid / 0.5 mM-EDTA buffer, pH 8.2, containing 1% (w/v) SDS and 0.73 M-2-mercaptoethanol. The mixture was heated at 60°C for 5 min, then layered on to the gel. Electrophoresis (75 V/slab gel) was performed in the presence of 89 mM-Tris/34 mM-boric acid/2.5 mM-EDTA buffer, pH 8.2, containing 0.1% (w/v) SDS. Gels were stained for carbohydrate with periodic acid/Schiff reagent. Migration was towards the anode (bottom).

and adult-rat small intestines were compared. The protein contents, determined by summation of the amino acids after drying the samples to a constant weight, were 27% and 18% by weight for the glycoproteins from newborn and adult rats respectively. Differences were also observed in the amino acid compositions. The glycoprotein from newborn-rat intestine had a higher content of threonine, alanine and valine and a lower content of serine, proline, tyrosine and lysine. The sums of the threonine and serine residues for both glycoproteins, however, were similar. The carbohydrate constituents also differed for the two glycoproteins. Expressed as mol/1000 mol of total amino acid residues, the mucus glycoprotein from newborn rats had a smaller total carbohydrate content compared with the sample from adults (1726.3 versus 2164.7). This difference was due to the lower fucose and *N*-acetyl-galactosamine content of the glycoprotein from newborn rats. The molar ratio of galactosamine to serine plus threonine was 0.78 in the glycoprotein from newborn rats and 1.30 in the glycoprotein from adults. If the oligosaccharide side chains in these glycoproteins have a similar structure, then a greater proportion of the serine/threonine residues would be *O*-glycosidically substituted with carbohydrate side chains in the glycoprotein from adults. Both glycoproteins contained a large number of negatively charged groups as sialic acid and ester sulphate, which is similar to that described for other small-intestinal mucus glycoproteins (Mantle & Allen, 1981). The sialic acid content was similar for both glycoproteins, but the glycoprotein from newborn rats had a higher sulphate content (5.5%, w/w, versus 0.9%, w/w).

Table 1. *Chemical composition of purified mucus glycoproteins from rat small intestines*

The amino acid composition and hexosamines were determined with an amino acid analyser (Swann & Mintz, 1979), and the neutral sugars and *N*-acetylneuraminic acid were measured by g.l.c. The results were the averages of multiple determinations on three independent glycoprotein preparations and are expressed as means $\pm$ s.e.m. The sulphate analyses were the result of multiple determinations on two independent preparations.

	Amino acid content (mol/1000 mol of total protein)	
	Newborn	Adult
Aspartic acid	87.3 $\pm$ 7.5	60.0 $\pm$ 2.1
Threonine	354.7 $\pm$ 5.2	300.0 $\pm$ 5.0
Serine	118.7 $\pm$ 8.0	178.7 $\pm$ 4.5
Glutamic acid	52.0 $\pm$ 2.5	65.0 $\pm$ 4.9
Proline	110.7 $\pm$ 7.7	153.0 $\pm$ 3.8
Glycine	63.7 $\pm$ 6.8	59.7 $\pm$ 4.3
Alanine	55.3 $\pm$ 3.8	33.7 $\pm$ 4.1
Half-cystine	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
Valine	65.7 $\pm$ 4.7	26.0 $\pm$ 3.5
Methionine	1.6 $\pm$ 0.6	3.6 $\pm$ 1.3
Isoleucine	21.7 $\pm$ 1.2	19.3 $\pm$ 1.9
Leucine	15.3 $\pm$ 2.6	23.3 $\pm$ 3.7
Tyrosine	3.6 $\pm$ 0.5	8.0 $\pm$ 0.4
Phenylalanine	7.8 $\pm$ 0.9	10.4 $\pm$ 0.6
Lysine	18.3 $\pm$ 2.2	29.3 $\pm$ 2.8
Histidine	10.6 $\pm$ 2.5	10.0 $\pm$ 1.6
Arginine	13.9 $\pm$ 2.5	19.7 $\pm$ 0.7
	Carbohydrate content (mol/1000 mol of total protein)	
	Newborn	Adult
Fucose	94.2 $\pm$ 16.3	265.8 $\pm$ 64.0
Mannose	6.4 $\pm$ 1.3	22.0 $\pm$ 2.0
Galactose	575.6 $\pm$ 78.9	617.9 $\pm$ 86.7
<i>N</i> -Acetylglucosamine	427.8 $\pm$ 13.3	435.4 $\pm$ 10.4
<i>N</i> -Acetylgalactosamine	376.8 $\pm$ 6.1	601.2 $\pm$ 3.5
<i>N</i> -Acetylneuraminic acid	245.5 $\pm$ 1.9	222.4 $\pm$ 26.2
Sulphate	262.4 $\pm$ 34.3	54.2 $\pm$ 2.0

Sedimentation-velocity analysis of the purified glycoproteins produced single sedimenting boundaries. The $s_{20,w}$ values for the mucus glycoproteins at 3.5 mg/ml in 4.0M-guanidinium chloride buffer were 9.3S and 10.4S for newborn-rat and adult-rat samples, respectively. The $s_{20,w}$ values for the adult-rat sample was calculated by interpolation between the 1/S values for the two concentrations analysed.

General discussion

Newborn-rat small-intestinal mucus glycoprotein was obtained from mucosal homogenates without the use of proteolytic enzymes, reducing agents or other harsh techniques so that the intact mucus glycoprotein could be studied. This purified glyco-

protein was essentially devoid of non-covalently bound nucleic acids or lipids, and there was no evidence for contamination by glycosaminoglycans and other proteins. The contamination by serum glycoproteins appears to be minimal. The glycoprotein from newborn rats is quite similar to other purified intestinal mucus glycoproteins with regard to major compositional features (Bella & Kim, 1972; Forstner *et al.*, 1973; Jabbal *et al.*, 1976; Mantle & Allen, 1981), buoyant density (Spee-Brand *et al.*, 1980), electrophoretic mobility in polyacrylamide/agarose gels (Spee-Brand *et al.*, 1980) and $s_{20,w}$ value (Forstner *et al.*, 1973; Spee-Brand *et al.*, 1980; Mantle & Allen, 1981).

There were, however, significant differences in chemical and physical characteristics of the glycoprotein from newborn-rat and adult-rat small intestines. The glycoprotein from newborn rats had a higher buoyant density in CsCl than did the glycoprotein from adults. Although we have no explanation for the age-related difference in buoyant density observed, the difference is not the result of non-covalently bound 'contaminants', since no lipids or nucleic acids were detected in the purified fraction and the higher density persisted in the presence of 4M-guanidinium chloride. Newborn-rat mucus glycoprotein migrated faster in SDS/polyacrylamide/agarose-gel electrophoresis than did adult-rat mucus glycoprotein. Since glycoproteins demonstrate unusual behaviour in gel electrophoresis in the presence of SDS, it is not reliable to correlate the mobility in SDS/polyacrylamide/agarose-gel electrophoresis to molecular mass or chemical composition (Leach *et al.*, 1980). When the chemical compositions were compared (Table 1), both glycoproteins had similar amino acid compositions, although variations were observed. Additional variations were observed in the carbohydrate composition. The newborn-rat glycoprotein had a significantly lower fucose content, but the sialic acid content was similar for both glycoproteins. Comparison of the molar ratio of fucose and sialic acid to galactose for both glycoproteins demonstrated a reciprocal relationship, as was originally described by Dische (1963). This might suggest alterations in the synthesis of the terminal sequence of the carbohydrate chain (Beyer *et al.*, 1979). *N*-Acetylgalactosamine is located at the reducing terminal of the mucus glycoprotein oligosaccharide (Carlson, 1977) and the non-reducing terminal of blood-group-A substance (Watkins, 1966). Since *N*-acetylgalactosamine residues were substantially decreased and were less than the sum of serine and threonine residues in the newborn-rat mucus glycoprotein, it is likely that the newborn-rat mucus glycoprotein has fewer carbohydrate side chains or a lower percentage of side chains with *N*-acetylgalactosamine at the non-reducing termini.

In summary, we have isolated and partially characterized purified mucus glycoproteins from newborn-rat and adult-rat intestine. These glycoproteins have a number of physical and compositional differences. We speculate that these differences are due to age-related differences in the types of glycoproteins synthesized by rat intestine during development. Further investigation will be necessary to understand the molecular basis for these developmental changes.

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References

- Antonopoulos, C. (1962) *Acta Chem. Scand.* **16**, 1521–1522
- Bella, A. & Kim, Y. S. (1972) *Arch. Biochem. Biophys.* **150**, 679–689
- Beyer, T. A., Rearick, J. I., Paulson, J. C., Prieels, J. P., Sadler, J. E. & Hill, R. L. (1979) *J. Biol. Chem.* **254**, 12531–12541
- Bresson, J. L., Hercovics, A. & Walker, W. A. (1982) *Gastroenterology* **82**, 1025
- Carlson, D. M. (1977) in *Mucus in Health and Disease* (Elstein, M. & Parke, D. V., eds.), pp. 251–273, Plenum Press, New York and London
- Creeth, J. M. & Denborough, M. A. (1970) *Biochem. J.* **117**, 879–891
- Dische, Z. (1963) *Ann. N.Y. Acad. Sci.* **106**, 259–270
- Etzler, M. E. & Branstrator, M. L. (1979) *Ciba Found. Symp.* **70** (New Ser.), 51–68
- Forstner, J. F., Jabbal, I. & Forstner, G. G. (1973) *Can. J. Biochem.* **51**, 1154–1166
- Forstner, J. F., Jabbal, I., Qureshi, R., Kells, D. I. C. & Forstner, G. G. (1979) *Biochem. J.* **181**, 725–732
- Galand, G. & Forstner, G. G. (1974) *Biochem. J.* **144**, 293–302
- Gottschalk, A. (ed.) (1972) *Glycoproteins*, Elsevier, Amsterdam and New York
- Heckers, H., Dittmar, K., Melcher, F. W. & Kalinowski, H. O. (1977) *J. Chromatogr.* **135**, 93–107
- Henning, S. J. (1979) *Environ. Health Perspect.* **33**, 9–16
- Holden, K. G., Yim, N. C. F., Griggs, L. J. & Weisbach, J. A. (1971) *Biochemistry* **10**, 3110–3113
- Jabbal, I., Kells, D. I. C., Forstner, G. G. & Forstner, J. F. (1976) *Can. J. Biochem.* **54**, 707–716
- Leach, B. S., Collawn, J. F., Jr. & Fish, W. W. (1980) *Biochemistry* **19**, 5734–5741
- Mantle, M. & Allen, A. (1981) *Biochem. J.* **195**, 267–275
- Meyer, F. A. (1977) *Biochim. Biophys. Acta* **493**, 272–282
- Nelson, J. A., Rose, L. M. & Bennett, L. L. (1976) *Cancer Res.* **36**, 1375–1378
- Reinhold, V. N. (1972) *Methods Enzymol.* **25**, 244–249
- Scawen, M. & Allen, A. (1977) *Biochem. J.* **163**, 363–368
- Shub, M. D., Pang, K. Y. & Walker, W. A. (1982) *Gastroenterology* **82**, 1179
- Snary, D., Allen, A. & Pain, R. H. (1971) *Eur. J. Biochem.* **24**, 183–189
- Spee-Brand, R., Strous, G. J. A. M. & Kramer, M. F. (1980) *Biochim. Biophys. Acta* **621**, 104–116
- Starkey, B. J., Snary, D. & Allen, A. (1974) *Biochem. J.* **141**, 633–639
- Swann, D. A. & Mintz, G. (1979) *Biochem. J.* **179**, 465–471
- Watkins, W. M. (1966) *Science* **152**, 172–181