Biochem. J. (1968) **106**, 293
Printed in Great Britain

Biosynthesis of Intestinal Mucins

SIALIC ACIDS OF SHEEP COLONIC EPITHELIAL MUCIN

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(*Received* 30 *June* 1967)

1. Sheep colonic mucin contains three types of sialic acids, separable from the macrostructure by mild acidic hydrolysis. These are composed chiefly of *N*-acetyl- and *N*-glycollyl-neuraminic acid in ratios between 1:1·2 and 1:3·5 for different preparations of the mucin. The third sialic acid appears to be a diacetylated neuraminic acid. 2. A particle-free enzyme preparation, obtained from sheep colonic mucosa by gentle homogenization and high-speed centrifugation, catalyses a series of reactions involving *N*-acylamino sugars and leading to the formation of sialic acids *in vitro*: (i) phosphorylation by ATP of D-glucosamine, *N*-acetyl- and *N*-glycollyl-D-glucosamine; (ii) conversion of *N*-acetylglucosamine 6-phosphate into *N*-acetyl-D-glucosamine 1-phosphate; (iii) formation of sialic acids from phosphoenolpyruvate and *N*-acetyl- or *N*-glycollyl-D-glucosamine; (iv) formation of *N*-acetylneuraminic acid from uridine diphospho-*N*-acetylglucosamine or from *N*-acetylmannosamine; (v) incorporation of L-[U-^{14}C]serine into the mucin by whole mucosal preparations.

In a previous paper (Draper & Kent, 1963) the presence of sialic acids (3·5%) was reported in mucin fractions obtained from sheep colonic mucosal scrapings by digestion with papain. The [^{14}C]sialic acids, obtained from surviving mucosa incubated *in vitro* with [1-^{14}C]glucose, have now been examined chromatographically and some features of their synthesis have been investigated.

Warren & Felsenfeld (1961*a*,*b*) and Roseman, Jourdain, Watson & Rood (1961) obtained a particle-free enzyme system from rat liver and pig submaxillary glands that promoted condensation of *N*-acylmannosamines with phosphoenolpyruvate to form sialic acids. Three enzymes catalysing steps in the process are known in partly purified forms, namely *N*-acetylmannosamine kinase, sialic acid synthetase and sialic acid 9-phosphatase. A soluble enzyme system has now been prepared from mucosal scrapings able to perform these and other reactions that may participate in the biosynthesis of sialic acids *in vivo*.

We have also investigated the incorporation of L-[^{14}C]serine, which is a possible source of *N*-glycollyl groups, into mucin by sheep colonic mucosal scrapings. A preliminary report of this work has already appeared (Kent & Draper, 1963).

METHODS AND MATERIALS

Preparation of mucin. Mucin fractions were obtained from fresh or incubated scrapings of sheep colonic mucosa, by papain digestion in 0·04M-citrate buffer (pH 5·5) containing EDTA and cysteine as described by Draper & Kent (1963).

Incubations. Surviving scrapings (approx. 1g. wet wt.) were incubated at 37° in 20ml. of Krebs medium III (Krebs, 1950) with [1-^{14}C]glucose (Draper & Kent, 1963) or L-[U-^{14}C]serine (sample CFB18; The Radiochemical Centre, Amersham, Bucks.; 0·5–2·5μc). Control experiments were performed with ethanol-treated scrapings. Manometric methods were as described by Draper & Kent (1963).

Hydrolysis and paper chromatography. Sialic acids were cleaved from isolated mucin fractions by hydrolysis with 0·1N-H_2SO_4 at 80° for 20min. The hydrolysate, neutralized with $BaCO_3$, was treated with 3vol. of ethanol to precipitate residual mucin and was centrifuged briefly at 700*g*.

The supernatant solutions were evaporated to dryness at 30° *in vacuo* and the resulting sialic acids were separated on Whatman no. 1 filter paper (previously washed with 2N-acetic acid and water) with the following solvents: butan-1-ol–propan-1-ol–0·1N-HCl (1:2:1, by vol.); ethanol–aq. NH_3 (sp.gr. 0·88)–water (40:1:10, by vol.); butan-2-ol–acetic acid–water (4:1:5, by vol.) (Gottschalk, 1960). The acids were detected with orcinol–trichloroacetic acid (Gottschalk, 1960), thiobarbituric acid reagent (Warren, 1960) or alkaline $AgNO_3$ (Trevelyan, Procter & Harrison, 1950).

Amino acids were obtained from mucin by hydrolysis with 2N-HCl at 102° for 16hr. The hydrolysates, freed from humin by centrifuging, were evaporated to dryness at room temperature *in vacuo*, and amino acids and amino sugars so obtained were separated on Whatman no. 1 paper with the following solvents: butan-1-ol–butanone–water (2:2:1, by vol.) (Mizell & Simpson, 1961); butan-1-ol–acetic acid–water (25:6:25, by vol.) (Fischer & Nebel, 1955); phenol

saturated with water. They were detected with ninhydrin (0·1% in butan-1-ol containing 1% of acetic acid).

Particle-free enzyme preparation. Scrapings of sheep colonic mucosa were collected in water at 0°, and fragmented by two strokes in a Teflon–glass Potter–Elvehjem homogenizer. The material was centrifuged at 105000*g* for 60min. at 4°. The clear supernatant (designated 'mucosal preparation') used in enzymic experiments was kept at 0°.

Enzymic acylation of amino sugars. The system contained: 0·08ml. of mucosal preparation; 0·34μmole of acetyl-CoA; 0·5μmole of glucosamine hydrochloride, or 0·6μmole of glucosamine 6-phosphate; 20μmoles of phosphate buffer, pH7·27, in a total volume of 0·2ml. To measure the activation and transfer of acetate and glycollate, acetyl-CoA was omitted in certain experiments and replaced by the following: 5μmoles of sodium acetate or glycollate; 4units of CoA; 20μmoles of tris–HCl buffer, pH7·2; 6·7μmoles of KCl; 4μmoles of $MgCl_2$; 1μmole of ATP; 1μmole of cysteine. After incubation of the mixture for 1hr. at 37°, the protein was precipitated with trichloroacetic acid (final concn. 5%). *N*-Acylamino sugars were estimated in the supernatant solution.

Phosphorylation of acylamino sugars. The incubation mixtures contained, in 0·2ml.: 0·1ml. of mucosal preparation; 30μmoles of tris-HCl buffer, pH7·2; 6μmoles of $MgCl_2$; 10μmoles of KCl; 1μmole of ATP; 0·19μmole of *N*-acetylglucosamine or 0·5μmole of *N*-glycollylglucosamine. Protein was precipitated, after incubation of the mixtures for 1hr. at 37°, either with trichloroacetic acid (final concn. 5%) or by adding 0·3ml. each of 5% (w/v) $ZnSO_4$ and 0·3N-$Ba(OH)_2$ (Somogyi, 1945). *N*-Acylamino sugar was estimated in the supernatant solutions. In some experiments the solutions were treated with 0·1 or 0·67N-HCl at 100° for 5min. before estimation.

Formation of sialic acids. The system contained, in 0·4ml.: 0·3ml. of mucosal preparation; 30μmoles of tris–HCl (pH7·2); 10μmoles of KCl; 0·5μmole of phosphoenolpyruvate; 20mμmoles each of NAD and NADP; 0·3μmole of *N*-acetylmannosamine or 0·19μmole of *N*-acetylglucosamine or 0·5μmole of *N*-glycollylglucosamine or 0·1 or 0·3μmole of UDP-*N*-acetylglucosamine. UMP (0·1μmole) was added in some experiments. After incubation for 1hr. at 37°, sialic acids in the mixtures were detected by the thiobarbituric acid method of Aminoff (1961). To identify the reaction product from *N*-acetylmannosamine more positively, an incubation was carried out with ten times the above quantities. Protein was precipitated with trichloroacetic acid (final concn. 1%), which was then extracted with diethyl ether. Sialic acid in the aqueous solution was absorbed on a column of charcoal (Ultrasorb SC 120/240; British Carbo-Norit Ltd., West Thurrock, Essex) (cf. Hughes & Whelan, 1958), eluted with 20% ethanol and identified by paper chromatography. Phosphorylation of *N*-acetylmannosamine was observed with a system identical with that used for *N*-acylglucosamines.

Radioactive assays. Radioactive mucin and chromatograms of radioactive materials were counted as described by Draper & Kent (1963). In addition, radioactive materials on paper chromatograms were counted at higher levels of efficiency in a liquid-scintillation counter (Isotope Developments Ltd., Beenham, Berks.), with 3cm.×2·2cm. paper rectangles, presented normal to the photomultiplier tubes, immersed in 10ml. of liquid phosphor in a vessel of 2·2cm. diam. Radioactive sialic acids were counted absorbed on lens-tissue disks on aluminium planchets (2·1cm. diam.) by using a mica and end-window counter EHM 2 operating at 1600v.

Preparations and estimations. Sialic acids were generally estimated by the thiobarbituric acid method of Aminoff (1961), by the same author's 'alkaline Ehrlich's' method, by the thiobarbituric acid method of Warren (1959) and by the resorcinol method (Svennerholm, 1957). *N*-Acylamino sugars were estimated by the modified Morgan–Elson method of Reissig, Strominger & Leloir (1955). Acidic solutions were made just alkaline to phenolphthalein (with NaOH) before the estimation. Dry weights of mucosal preparations were determined by heating known volumes at 102° overnight.

N-Acetylglucosamine was prepared from glucosamine (Inouye, Onodera, Kitaoka & Hirano, 1956). *N*-Glycollylglucosamine was made from glucosamine HCl by the method of Jourdain & Roseman (1962). Acetyl-CoA was prepared from CoA and acetic anhydride (Ochoa, 1957).

RESULTS AND DISCUSSION

Identification of sialic acids. Paper chromatography of liberated sialic acids showed that there were at least three compounds present giving orcinol and thiobarbituric acid reactions. Of these the slowest-moving was identified as *N*-glycollylneuraminic acid, since it ran on chromatograms in the same position (in three solvents) as authentic ^{14}C-labelled *N*-glycollylneuraminic acid. A second, faster-running, component migrated with synthetic *N*-acetylneuraminic acid (L. Light and Co. Ltd., Colnbrook, Bucks.) in three solvents. The third and fastest component was not identified. When eluted it gave a strong reaction for sialic acid with the resorcinol reaction and a relatively much weaker one with thiobarbituric acid. These colour reactions and its R_F suggest that it may be an *NO*-diacylneuraminic acid. These hydrolysates also contained fucose, identifiable on paper by its R_F and staining properties, but no galactose or ninhydrin-reacting material was present.

Amounts of sialic acids. Sialic acids (*N*-acetyl- and *N*-glycollyl-neuraminic acid), eluted from chromatograms, were obtained in yields of 1·3–4·4% of the weight of mucin hydrolysed (seven experiments: expected yield 5·5%). The higher figure probably represents an almost quantitative recovery if the presence of a third sialic acid is considered. The ratios of *N*-glycollyl- to *N*-acetylneuraminic acid in mucins from different samples of scrapings varied between 1·2:1 and 3·5:1. Variations have been noted by Gibbons (1959) and Dische (1963) in fucose:sialic acid ratios among different samples of mucins from various tissues. In a single experiment the amount of the third sialic acid obtained was about 10% of the *N*-glycollylneuraminic acid and 20% of the *N*-acetylneuraminic acid content measured by the thiobarbituric acid method.

Table 1. *N-Acylation of glucosamine and its 6-phosphate by a particle-free preparation of sheep colonic mucosa*

Incubation mixtures contained, in 0·2 ml. final vol.: 0·08 ml. of mucosal preparation, 0·34 μmole of acetyl-CoA, 0·5 μmole of glucosamine or 0·6 μmole of glucosamine 6-phosphate, 20 μmoles of phosphate buffer, pH 7·27. Incubation was for 1 hr. at 37°. Protein was precipitated with trichloroacetic acid (final concn. 5%, w/v), and *N*-acetylhexosamine was estimated in the supernatant solutions.

Additions						
Glucosamine 6-phosphate	+	−	−	+	+	−
Glucosamine	−	+	+	−	−	−
ATP	−	+	−	−	−	−
Acetyl-CoA	+	+	+	−	+	−
Mucosal preparation	+	+	+	+	−	+
Product*	0·038 ± 0·007 (S.D.)	0·037	0·004	0·002	0·002	0·001
No. of expts.	5	2	2	2	2	2

* *N*-Acetylhexosamine formed (μmole/hr./mg. dry wt. of mucosal preparation).

Table 2. *Activation of acetate and transfer to amino sugars by particle-free preparation of sheep colonic mucosal scrapings*

Incubation mixtures contained, in 0·2 ml. final vol.: 0·08 ml. of mucosal preparation, 4 units of CoA, 0·5 μmole of glucosamine or 0·6 μmole of glucosamine 6-phosphate, 5 μmoles of sodium acetate or glycollate, 20 μmoles of phosphate buffer, pH 7·27, 20 μmoles of tris–HCl buffer, pH 7·2, 6·7 μmoles of KCl, 4 μmoles of $MgCl_2$, 1 μmole of cysteine. Incubation was for 1 hr. at 37°.

Additions							
Glucosamine 6-phosphate	+	+	+	−	+	+	−
Glucosamine	−	−	−	+	−	−	+
Acetate	+	+	+	+	−	−	−
Glycollate	−	−	−	−	−	+	+
ATP	+	+	−	+	+	+	+
Mucosal preparation	+	−	+	+	+	+	+
Product*	0·024 ± 0·004 (S.D.)	0	0	0·037	0·014	0·013	0·013
No. of expts.	3	2	2	2	2	4	2

* *N*-Acetylhexosamine formed (μmole/hr./mg. dry wt. of mucosal preparations).

Enzymic acylation of amino sugars. Since isolated scrapings of colonic mucosa can synthesize sialic acids (Draper & Kent, 1963) we investigated some enzymic reactions which may form part of the biosynthetic pathway, with a particle-free preparation from the scrapings. Attempted *N*-acetylation of amino sugars gave results shown in Table 1 (with acetyl-CoA) and Table 2 (with acetate and glycollate). The mucosal preparation was able to transfer acetyl groups from acetyl-CoA to glucosamine 6-phosphate, and to glucosamine itself in the presence of ATP. Also, as seen in Table 2, acetate, but not glycollate, could be significantly activated and transferred in the presence of CoA and ATP. Pasternak (1961) has shown a similar preparation from rat colon to phosphorylate glucosamine and to acetylate its 6-phosphate, and the acetylating enzyme has been isolated from a variety of other tissues (Davidson, Blumenthal & Roseman, 1957).

Phosphorylation of N-acylamino sugar. Both *N*-acetyl- and *N*-glycollylglucosamine were phosphorylated by the mucosal preparation with ATP. Results obtained appear in Table 3. The Somogyi reagents were used to precipitate protein and

Table 3. *Phosphorylation of N-acylhexosamines by a particle-free preparation of sheep colonic mucosa*

Incubation mixtures contained, in 0·2 ml. final vol.: 0·1 ml. of mucosal preparation, 30 μmoles of tris–HCl buffer, pH 7·2, 10 μmoles of KCl, 6 μmoles of $MgCl_2$, 1 μmole of ATP and *N*-acylamino sugar as shown. Incubation was for 1 hr. at 37°. Protein was precipitated with $ZnSO_4$ and $Ba(OH)_2$. Disappearance, corresponding to the amount phosphorylated, is measured in μmole/hr./mg. dry wt. of mucosal preparation. Numbers of measurements are shown in parentheses.

	N-Acylamino sugar added (μmole)	*N*-Acylamino sugar esterified (μmole ± S.E.M.)
N-Acetylglucosamine	0·19	0·07 ± 0·03 (6)
N-Acetylglucosamine	0·38	0·11 ± 0·02 (2)
N-Glycollylglucosamine	0·50	0·13 ± 0·02 (14)

phosphates after the incubation, and the residual unchanged *N*-acylamino sugar was measured.

Leloir, Cardini & Olavarria (1958) found an *N*-acetylglucosamine kinase in a variety of rat tissues, and showed that *N*-acetylglucosamine 6-phosphate was the product. Leloir & Cardini (1956) were able to show, by using properties of the phosphates discovered by Reissig *et al.* (1955), that a phospho-acylglucosamine mutase in rat liver and kidney produced an equilibrium mixture of 1- and 6-phosphates. *N*-Acetylglucosamine 6-phosphate gave 100% colour yield as *N*-acetylglucosamine in the modified Morgan–Elson reaction, whereas the 1-phosphate gave no colour, but could be hydrolysed to *N*-acylamino sugar with full colour yield in 5min. at 100° by 0·1N-hydrochloric acid. Further, Somogyi's method precipitated all the phosphates from the solution along with the protein, whereas trichloroacetic acid removed only the protein, so that a Morgan–Elson estimation after use of the latter precipitant measured both *N*-acylhexosamine and its 6-phosphate. By appropriate hydrolysis to convert the 1-phosphate into free *N*-acylamino sugar, a further increment of colour was obtained.

With a similar technique we showed that both 1- and 6-phosphate were formed by the mucosal preparation from *N*-acylglucosamines and ATP. The results (Table 4) were insufficiently consistent to obtain a ratio of 1-phosphate to 6-phosphate, but the latter predominated. Neither hydrolysis in 0·1N- nor in 0·67N-hydrochloric acid decreased the colour obtained from added *N*-acetylglucosamine, i.e. no deacetylation occurred under the conditions of the experiments.

Formation of sialic acids. The mucosal preparation catalysed the condensation of phosphoenolpyruvate with *N*-acetylmannosamine, and UDP-*N*-acetylglucosamine, as well as with the two *N*-acylglucosamines (in which case the reaction was stimulated by UMP). These results are shown in Table 5.

The formation of sialic acid from *N*-acetylmannosamine and phosphoenolpyruvate was linear with time over 1hr., and the product was identified chromatographically as *N*-acetylneuraminic acid.

Table 4. *Phosphorylation of* N-*acylglucosamine by a particle-free enzyme preparation from sheep colonic mucosa*

Incubation mixtures contained, in 0·2ml. final vol.: 0·1ml. of mucosal preparation, 30μmoles of tris–HCl buffer, pH 7·2, 10μmoles of KCl, 6μmoles of ATP, 6μmoles of $MgCl_2$ and *N*-acylglucosamine as indicated. Details are given in the text. Values are given as phosphate formed (μmole/hr./mg. dry wt. of mucosal preparation). Numbers of experiments are given in parentheses.

	N-Acylglucosamine added (μmole)	Product formed	
		6-*O*-phosphate	1-phosphate
N-Acetyl	0·19 (4)	0·04	0·01
	0·38 (2)	0·08	*
N-Glycollyl	0·50 (5)	0·07	0·03

* Not measured.

Table 5. *Formation of sialic acids from precursors by a particle-free enzyme preparation from sheep colonic mucosa*

Incubation mixtures contained, in 0·4ml. final vol.: 0·3ml. of mucosal preparation, 30μmoles of tris–HCl buffer, 1μmole of ATP, 0·5μmole of phosphoenolpyruvate, 20mμmoles each of NAD and NADP, 10μmoles of KCl and 6μmoles of $MgCl_2$. Incubations were for 1hr. at 37°. Values, as μmole of sialic acid formed/hr./mg. dry wt. of mucosal preparation, are means of duplicate experiments, which were accurate to within ±10%.

Added precursor	UMP (0·1μmole)	Sialic acid synthesized (μmole)
N-Acetylmannosamine (0·3μmole)	Absent	0·0084* ± 0·0002 (S.D.)
N-Acetylglucosamine (0·19μmole)	Absent	0·0033
N-Acetylglucosamine (0·19μmole)	Present	0·0041
N-Glycollylglucosamine (0·5μmole)	Absent	0·0006
N-Glycollylglucosamine (0·5μmole)	Present	0·0013
UDP-*N*-Acetylglucosamine (0·3μmole)	Absent	0·0054
UDP-*N*-Acetylglucosamine (0·1μmole)	Absent	0·0034

* Mean of 47 experiments.

Both ATP and NAD (or NADP) were necessary for the conversion, whose rate was limited by the amount of mucosal preparation present. Fluoride (1·8 μmoles) fully inhibited the conversion.

The requirement for small amounts of NAD and NADP has been found also (Warren & Felsenfeld, 1961*b*) in crude extracts of rat liver. The ATP was needed for the preliminary synthesis of *N*-acetylmannosamine phosphate, shown by Warren & Felsenfeld (1961*a*,*b*; 1962) and Ghosh & Roseman (1961) to be a step in the reaction sequence. This first step was demonstrated in the present system by observing the esterification of Morgan–Elson-reacting material when *N*-acetylmannosamine was incubated with mucosal preparation and ATP. In the described conditions 0·054 μmole of *N*-acetylmannosamine/hr./mg. dry wt. of enzyme was phosphorylated. The reaction was inhibited (20%) by 1·8 μmoles of fluoride.

Formation of sialic acid from *N*-acetylmannosamine was undiminished in mucosal preparation stored frozen for several weeks, but synthesis from other substrates declined in enzymes stored at 0°. Mucosal preparation kept for 4hr. at 0° had only about 20% of its original activity, and became quite inactive after freezing and thawing or storage overnight at 2°. Activity was not restored by cysteine, cyanide or ascorbate. Similar lability has been noted by Comb & Roseman (1958) and Glaser (1960) in the reaction converting UDP-*N*-acetylglucosamine and *N*-acetylglucosamine into *N*-acetylmannosamine in rat liver, first observed (but incorrectly interpreted as yielding *N*-acetylgalactosamine) by Cardini & Leloir (1957). It seems likely that the same reaction step is involved in the present system. Partial destruction of activity during incubations with fresh mucosal preparation probably accounts for the observed lower rates of formation of sialic acids from these earlier precursors compared with rates from *N*-acetylmannosamine (cf. Table 5).

Formation of UDP-*N*-acetylglucosamine from *N*-acetylglucosamine 1-phosphate by a calf-liver enzyme has been described by Strominger & Smith (1959); McGarrahan & Maley (1962) have shown that the nucleotide is among the radioactive compounds formed in rat liver within 5min. of an intraperitoneal injection of [1-^{14}C]glucosamine.

Ghosh & Roseman (1962) showed that in pig kidney a direct interconversion of *N*-acylglucosamine and *N*-acylmannosamine, not involving nucleotides and requiring only traces of ATP, could occur. Such a direct conversion by the colonic mucosal enzyme might account for the incomplete recovery of *N*-acylamino sugar noted in Table 4. (*N*-Acetylmannosamine gives a lower colour yield than *N*-acetylglucosamine in the Morgan–Elson reaction.) On the other hand the stimulatory effect of UMP on the formation of sialic acids from *N*-acylhexosamines (cf. Table 5) suggests that the route through uridine nucleotides also occurs.

An aldolase catalysing the reversible synthesis of sialic acids from *N*-acylmannosamine and pyruvate has been observed in several tissues (Comb & Roseman, 1960; Brunetti, Jourdain & Roseman, 1962), though not in those tissues in which sialic acids are synthesized in large quantities. The colonic preparation described in the present paper was distinguished from the aldolase in that it did not destroy *N*-acetylneuraminic acid and that the formation of sialic acid from *N*-acetylmannosamine and phosphoenolpyruvate required ATP.

The observed activities of the mucosal preparation account for the formation of sialic acids from glucosamine or its 6-phosphate, and hence from glucose, and provide a route to radioactive sialic acids from [^{14}C]glucose in mucosal scrapings. The mechanism of incorporation of sialic acids into the polymer was not investigated, but may involve CMP-sialic acids, which were originally found in bacteria but which also occur in pig submaxillary gland (Roseman, 1962). On the basis of the observed rate of formation of sialic acid from *N*-acetylglucosamine, a colon segment could form about 3·3mg. of mucin (0·17mg. of sialic acid)/hr. Since after 3hr. incubation of scrapings with [^{14}C]-glucose about 4mg. of radioactive mucin could be obtained, the observed rate was more than sufficient to account for the synthesis of radioactive mucin.

Incorporation of radioactive serine. The experiments on the conversion of *N*-acylamino sugars into sialic acids agree with the results of investigations on other tissues (Jourdain & Roseman, 1963), in that *N*-glycollylglucosamine was utilized by the mucosal preparation. The preparation was able to activate acetate for the *N*-acetylation of D-glucosamine 6-phosphate, but could not correspondingly activate glycollate. A possible precursor of *N*-glycollyl groups in mammalian systems is 2-(1,2-dihydroxyethyl)thiamine pyrophosphate ['active glycollaldehyde' (da Fonseca-Wollheim, Bock & Holzer, 1962), analogous to 'active acetaldehyde'], which may be formed from hydroxypyruvate or from fructose. Serine, which would yield hydroxypyruvate by deamination, was therefore investigated as a possible precursor of the *N*-glycollyl groups in sialic acid.

Table 6 shows the observed incorporation into mucin isolated from scrapings incubated with L-[^{14}C]serine. Serine was incorporated in amounts dependent on, and ranging from 0·5 to 1·0% of, the quantity added. D-Glucose in the incubation medium did not affect the incorporation. Chromatograms of amino acids from the [^{14}C]mucin were

Table 6. *Incorporation of* L-[U-^{14}C]*serine into mucin by scrapings of sheep colonic mucosa*

Basal medium was Krebs medium III without glucose (Krebs, 1950). Details of incubation and extraction are given in the text and by Draper & Kent (1963). Percentage recovery of radioactivity was calculated on the basis of an efficiency of 7% for the Geiger–Müller counter, measured with a sample of [^{14}C]glucose of known absolute activity. No radioactivity was incorporated into mucin by ethanol-treated scrapings.

Expt.	Q_{O_2} of scrapings	Radioactivity added (μc)	Radioactivity incorporated (counts/min./mg. ± S.E.M.)	Approx. radioactivity recovered (counts/min.) [Yield (%) in parentheses]	Additions to basal medium
1	−4·0	0·5	9·9 ± 0·9	6 × 10^2 (0·7)	4 mM-Glutamine, 40 μmoles of glucose
			8·0 ± 0·7	6 × 10^2 (0·7)	4 mM-Glutamine
2	−3·0	1·0	23·5 ± 1·3	1·7 × 10^3 (1·1)	No organic salts, 40 μmoles of glucose
			18·9 ± 0·9	1·3 × 10^3 (0·8)	No organic salts
3	Not measured	2·5	27·9 ± 0·9	1·5 × 10^3 (0·4)	No organic salts, 40 μmoles of glucose
			27·8 ± 0·8	1·9 × 10^3 (0·5)	No organic salts

radioactive in the positions corresponding to serine and to glycine. Eluted specimens of the separated ^{14}C-labelled amino acids were examined in a scintillation counter. Serine so recovered (second experiment in Table 6) gave 59 ± 1·4 (S.E.M.) counts/min./mg. of mucin, and glycine 6 ± 1 counts/min./mg. of mucin. In these experiments, chromatograms of sialic acid had no measurable radioactivity. A larger quantity (100 μg.) of sialic acid, obtained by acidic hydrolysis of the pooled mucins from three experiments and purified on a column of Dowex 1 (formate form) (Gottschalk, 1960), was counted on lens-tissue disks and was slightly but significantly radioactive (10 ± 3 counts/min./mg. of sialic acid, or 0·6 count/min./mg. of mucin). This value was, however, too small to allow further experiments, and it is not known whether the radioactivity resided in the *N*-glycollyl group or elsewhere in the sialic acid molecule.

The experiments with radioactive serine show that the peptide part of the mucin was synthesized in mucosal scrapings, and suggest that a total synthesis of mucin occurs in the system, but it is not clear whether serine is a precursor of *N*-glycollyl groups.

We are grateful to Dr S. Roseman for a gift of ^{14}C-labelled *N*-glycollylneuraminic acid and to Dr J. R. Quayle for a gift of trisodium phosphoenolpyruvate. This work was carried out with the help of Departmental grants from the Rockefeller Foundation and from the U.S. Public Health Service and with the award (to P.D.) of a Medical Research Council Scholarship.

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