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The Biosynthesis of Intestinal Mucins

THE EFFECT OF SALICYLATE ON GLYCOPROTEIN BIOSYNTHESIS BY SHEEP COLONIC AND HUMAN GASTRIC MUCOSAL TISSUES *IN VITRO*

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1. Incubation of sheep colonic mucosal scrapings in Krebs–Ringer buffer for 2½ hr. in the presence of salicylate (15mM) resulted in decreased incorporation of radioactivity into the epithelial glycoprotein from the following labelled precursors: 16·6 μM-D-[2-^{14}C]glucose (83·9% inhibition), 20 μM-L-[U-^{14}C]threonine (82%) and $^{35}SO_4^{2-}$ (79%). Oxygen uptake measured simultaneously was diminished to 41% of the control value. 2. At lower concentrations of salicylate (e.g. 3·75mM), incorporation of 20 μM-L-[U-^{14}C]threonine was little affected (3–6% inhibition), whereas utilization of 4 μM-D-[U-^{14}C]glucose and $^{35}SO_4^{2-}$ was inhibited (41–48% and 40–59% of the control values respectively). 3. Analysis of the papain-digested glycoprotein from tissue incubations with 16·6 μM-D-[2-^{14}C]glucose in the presence of salicylate (3·75mM) showed large decreases in labelling of *N*-acetylneuraminic acid and *N*-glycollylneuraminic acid residues (57% and 34% of the control values respectively) and of hexosamine constituents (glucosamine, 55% inhibition; galactosamine, 33% inhibition). Labelling of neutral sugars (galactose and fucose) was relatively little affected (9 and 11% inhibition respectively). 4. Glucose 6-phosphate transaminase and glucosamine 6-phosphate acetylase in particle-free enzyme preparations of the sheep tissue were unaffected by salicylate at the above concentrations. Acetyl-CoA synthetase was markedly inhibited. 5. Human gastric mucosa (from operation), on incubation as above, had in one experiment an oxygen consumption of 9·9 μl./hr./mg. dry wt. of tissue and incorporated 5 μM-D-[U-^{14}C]glucose (15·8% of the total radioactivity added) into bound hexosamine (20·6% of the total radioactivity incorporated), hexoses (glucose and galactose, 5·7%) and fucose (14·2%). The presence of salicylate (15mM) decreased the incorporation of 5 μM-D-[U-^{14}C]glucose into the glycoprotein by 74%, all sugar constituents being affected, without influence on the rate of oxygen consumption. 6. The results suggest an inhibitory effect of salicylate on glycoprotein biosynthesis at the level of the amino sugar intermediates.

Studies on the action of salicylates in alleviating rheumatic diseases (Whitehouse & Boström, 1961, 1962) have shown inhibitory effects on the incorporation *in vitro* of $^{35}SO_4^{2-}$, D-[^{14}C]glucose and [^{14}C]acetate into the sulphated mucopolysaccharides of bovine heart valves, cartilage and cornea. Corresponding effects were found both when salicylate was administered intravenously to rats and *in vitro* with rat cartilage slices incubated in Krebs III medium (Whitehouse, 1963). Inhibition by salicylate of $^{35}SO_4^{2-}$ incorporation into granulation tissue in guinea pigs (Kodicek & Loewi, 1955) and into sulphated mucopolysaccharides in developing cartilage of chick-embryo somites in tissue culture (Whitehouse & Lash, 1961) has also been demonstrated.

There is much clinical evidence indicating that salicylates (e.g. aspirin) can cause considerable bleeding and damage to gastric membranes (Wood, 1963; Muir, 1963). The disruptive effect of salicylates on the human gastric mucosa is supported by gastroscopic observations after aspirin ingestion (Pitman, Weiss & Graham, 1963). Croft (1966) has made extensive studies of the exfoliation of gastric cells, as measured by the increased content of DNA in stomach wash-outs after aspirin ingestion. Extensive studies *in vivo* on the production of gastric lesions, with concentrations of aspirin comparable with those taken by man, have also been achieved in experimental animals, e.g. guinea pigs (Anderson, 1964), dogs (Hurley & Crandall, 1963) and cats (Roth & Valdes-Dapena, 1963).

In view of these effects, the possible inhibitory action of the drug on glycoprotein biosynthesis in the sheep colonic system has now been investigated. This is a well-characterized system in which it is possible to investigate more precisely the action of salicylate on some of the individual enzymic steps in the pathway of biosynthesis of the protective epithelial mucin, with a view to identifying pacemaker reactions. Previous studies on the sheep colonic system (Draper & Kent, 1963; Kent & Allen, 1966; Allen & Kent, 1968) have shown that surviving scrapings possess extensive biosynthetic activity, radioactivity from a number of labelled substrates being incorporated into a defined glycoprotein fraction (Kent, Ackers & Marsden, 1967). The enzymes leading to the biosynthesis of the *N*-acetylhexosamines and sialic acids have been demonstrated (Kent & Draper, 1968). Puromycin also acts as an inhibitor of glycoprotein biosynthesis in this system (Allen & Kent, 1966).

MATERIALS AND METHODS

The preparation of sheep colonic scrapings, measurement of the oxygen uptake and the isolation of glycoprotein fractions were as described by Draper & Kent (1963). Glycoprotein fraction H, obtained by papain digestion and preparative ultracentrifugal fractionation, has a molecular weight of 212000 ($c \rightarrow 0$) and is composed of peptide (25%, including 5·7% threonyl residues) and carbohydrate [75%; *N*-acetylneuraminic acid (5·1%), *N*-glycollylneuraminic acid (8·6%), fucose (4·7%), galactose (13·8%), *N*-acetylglucosamine (27·4%) and *N*-acetylgalactosamine (13·7%)]. On average, the glycoprotein has a content of 4·6% hydrolysable sulphate, corresponding to 42 ester sulphate groups per $2{\cdot}12 \times 10^5$ mol.wt. Incubation of colonic scrapings with radioactive substrates, and the analysis of the labelled glycoprotein, were as described by Allen & Kent (1968). Incubations were carried out in the presence of polybactrin to suppress bacterial growth. This reagent has no significant influence on the oxygen consumption of sheep colonic epithelial cells (Allen & Kent, 1968), as is further shown in Table 1. The absence of effects of addition of potential metabolites on the oxygen consumption suggests a high rate of metabolism of endogenous substrates for energy production in the tissue, probably derived from lipids.

Chemicals. D-[2-^{14}C]Glucose (sp. radioactivity 28·4–31·3 μc/μmole), D-[U-^{14}C]glucose (sp. radioactivity 123–131 μc/μmole), L-[U-^{14}C]threonine (sp. radioactivity 25·6 μc/μmole) and carrier-free $Na_2{}^{35}SO_4$ (sp. radioactivity approx. 15 mc/μg. of S) were obtained from The Radiochemical Centre (Amersham, Bucks.). Salicylic acid was obtained from British Drug Houses Ltd. Poole, (Dorset) and recrystallized from water.

Cell-free preparations. These were prepared by the method of Allen & Kent (1968). Scrapings (3 vol.) were homogenized in 2 vol. of medium containing tris–HCl buffer, pH 7·4 (20 mM), KCl (0·125 M) and EDTA (2 mM). The tissue was homogenized by two strokes of a loose-fitting Teflon–glass Potter–Elvehjem homogenizer. The 'particle-free' preparation was the supernatant obtained by centrifuging the homogenate for 1 hr. at 105000*g*.

Human gastric mucosal incubations. Tissue removed surgically during partial gastrectomy was transferred to frozen 0·9% NaCl immediately upon removal and washed with cold 0·9% NaCl, and the mucosal surface scraped off into ice-cold modified Krebs medium III (D-glucose omitted, but L-glutamine, 3·2 mM final concn., added). Each Warburg flask contained (in a total volume of 4·05 ml.): tissue (1·0 ml.), D-[U-^{14}C]glucose (0·05 ml. containing 2·5 μc) and Krebs medium III (3 ml.). Carbon dioxide was taken up by 0·2 ml. of 2 N-KOH in the centre well. Incubation was for 3½ hr. at 37°, after which trichloroacetic acid (0·5 ml. of a 12%, w/v, solution) was added from the side arm. The precipitate was centrifuged, resuspended in water and dialysed for 5 days against water (three changes daily) and finally freeze-dried. For measurement of the specific radioactivity of the resulting material, a solution (5 mg./ml.) in 0·2 N-NaOH was prepared, known volumes of which were then transferred to planchets for drying and counting.

RESULTS

Incubation media containing salicylic acid were adjusted to pH 7·4 with 0·2 N-sodium hydroxide before addition of tissue. The pH of the incubation mixture remained constant (pH 7·4) over a 3 hr. incubation period. Because of the variations in the activities (oxygen consumption and uptake of

Table 1. *Effect of added metabolites on the respiratory activity of sheep colonic mucosal scrapings*

Incubations were carried out in Krebs III medium (glucose omitted) at 37° with 0·1 ml. (4 mg. dry wt.) of mucosal scrapings. The total volume was 4 ml.

Addition (final concn.)	Oxygen consumption (μl./mg./hr.) difference from control ± S.E.M.	Student's *t* value	No. of expts.	Significance
D-Glucose (10 μM)	−1·04 ± 0·33	3·15	6	$P < 0{\cdot}05$
L-Fucose (10 μM)	−0·08 ± 0·89	0·1	2	None
Sodium pyruvate* (5 μM)	−0·4 ± 0·35	1·1	2	None
Insuline (0·05 unit/ml.)	−1·55 ± 0·90	1·7	3	None
Polybactrin† (0·1%)	0·25 ± 0·5	0·5	2	None

* Sodium pyruvate omitted from the medium in this experiment.
† In the presence of 10 μM-glucose.

Table 2. *Incorporation of radioactivity from* D-[2-^{14}C]*glucose into a glycoprotein fraction from sheep colonic tissue incubated with salicylate*

The incubation medium contained modified Krebs III medium (7·5 or 15 ml.) (D-glucose omitted, L-glutamine, 3·2 mM, added), tissue (2·5 or 5 ml., 74·3 mg. dry wt./ml.) and D-[2-^{14}C]glucose (0·1 ml., 5–12 μc). Incubation was for 2½ hr. at 37°. Oxygen uptake in the controls was: 7·3, 7·4, 5·4 μl./2½ hr./mg. dry wt. of tissue for Expts. 1, 2 and 3 respectively.

	Additions to incubation		Glycoprotein isolated			
Expt. no.	Concn. of salicylate (mM)	10^{-5} × Radioactivity added (counts/min./ml.)	Sp. radioactivity (counts/min./mg.) (mean ± S.D.)	Incorporation of radioactivity (% of added radioactivity)	Phosphorus content (% dry wt.)	Sialic acid content* (% dry wt.)
1	Control	20·27/20·1	11760 ± 170	21·6	1·2	2·5
	3·75	20·27/20·1	6100 ± 86	5·3	0·9	2·6
	Zero-time control	20·27/20·1	8 ± 15	—	—	—
2	Control	20·27/20·1	11950 ± 144	33·0	1·6	4·4
	3·75	20·27/20·1	7050 ± 120	19·1	1·8	5·4
	Zero-time control	20·27/20·1	Nil	—	—	—
3	Control	8·11/10·1	4282 ± 52	7·7	0·9	4·0
	15·00	8·11/10·1	687 ± 12	1·25	0·8	2·9
	30·00	8·11/10·1	13 ± 6	0·27	1·1	2·9

* Determined as *N*-acetylneuraminic acid by the thiobarbituric acid method (Aminoff, 1961).

Table 3. *Incorporation of* [2-^{14}C]*glucose into the monosaccharide constituents of a glycoprotein fraction from sheep colonic scrapings incubated with* 3·75 mM-*salicylic acid*

	Expt. 1			Expt. 2		
	Control (counts/min./mg.)	3·75 mM-Salicylate	Inhibition (%)	Control (counts/min./mg.)	3·75 mM-Salicylate	Inhibition (%)
Glycoprotein sample	11760	6100	48	11950	7050	41
	(10^{-3} × counts/min./μmole)			(10^{-3} × counts/min./μmole)		
Sialic acids						
N-Acetyl	4·16	2·07	51	8·26	2·27	63
N-Glycollyl	0·43	0·32	26	0·62	0·36	41
Hexosamines						
Glucosamine	3·02	1·32	56	3·54	1·65	53
Galactosamine	1·83	1·36	26	4·11	2·50	39
Neutral sugars						
Fucose	4·90	5·23	−5	4·06	3·52	13
	(counts/min.)*					
Galactose	501	424	15	2·65	2·48	6

* The galactose values from Expt. 1 are estimates from the areas under peaks on chromatograms, after chromatography of the total hydrolysate of equal weights of glycoprotein. Amino sugars on the same chromatograms gave a value of 52% inhibition compared with that calculated from the above specific radioactivities of 48%.

labelled substrates) of different preparations of the tissue, results are expressed as % of control, samples made from the one mucosal preparation being used for each experiment. The sialic acid contents (measured by the thiobarbituric acid method of Aminoff, 1961) and the phosphorus contents (Chen, Toribara & Warner, 1956) of glycoprotein samples are in the ranges 2·5–6·3% and 0·8–2·2% respectively, but values are reproducible for each experiment (Tables 2 and 3). The sialic acids are found as constituents of the major glycoprotein component (fraction H; Kent & Marsden, 1963) of the tissue. Similarly, phosphorus is confined almost entirely to the nucleic acid components present in the unfractionated glycoprotein fraction (Kent *et al.* 1967). Of the three components of the glycoprotein fraction, fraction H, a minor glycoprotein component (fraction L) and nucleic acid, only fraction H

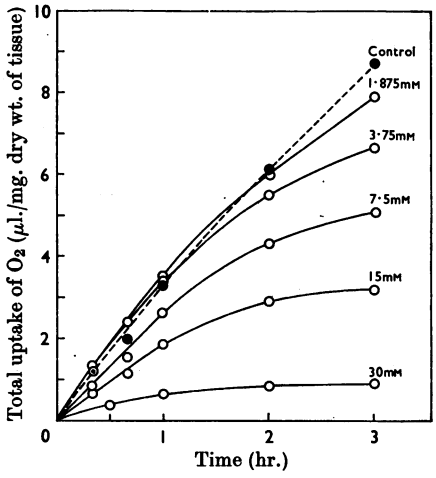


Fig. 1. Oxygen uptake of sheep colonic scrapings in the presence of salicylic acid at the concentrations shown. The incubation medium (total volume 4·0 ml.) contained: 1·0 ml. of tissue preparation (70 mg. dry wt.) and 3·0 ml. of modified Krebs medium III (D-glucose omitted, L-glutamine, 3·2 mM, added). Incubation was at 37° in Warburg flasks.

becomes radioactively labelled on incubation of tissue with labelled substrates (Draper & Kent, 1963; Allen & Kent, 1968).

Effect of salicylate on oxygen uptake by sheep colonic mucosal tissue. The effect of salicylate (1·875–30 mM final concn.) on the oxygen uptake of sheep colonic mucosal tissue incubated in modified Krebs medium III (D-glucose omitted, L-glutamine, 3·2 mM final concn., added) is shown in Fig. 1. Salicylate in concentrations up to 30 mM produces a progressive decrease in the oxygen consumption. The influence of the inhibitor is observable within 20 min. and becomes progressively more profound over a period of 3 hr. With low concentrations of salicylate (1·875 mM and 3·75 mM) an initial stimulation of oxygen uptake was noted, suggesting that some uncoupling of oxidative phosphorylation occurred. Incubations were performed for up to 2½ hr. to give the greatest differences between control and inhibited conditions at low inhibitor concentrations.

Incorporation of radioactivity from D-[^{14}C]*glucose into the glycoprotein fraction from sheep colonic mucosa in the presence and absence of salicylate.* The

Table 4. *Incorporation of* L-[U-^{14}C]*threonine,* D-[U-^{14}C]*glucose and* $^{35}SO_4^{2-}$ *into a glycoprotein fraction by sheep colonic mucosal tissue incubated with salicylate*

The incubation medium contained, in a total volume of 10·1 ml.: 7·5 ml. of modified Krebs III medium (D-glucose omitted, L-glutamine, 3·2 mM, added), tissue (2·5 ml.; 74 mg. dry wt.), radioactive substrate (0·1 ml.) (D-[U-^{14}C]-glucose, 5 μc, L-[U-^{14}C]threonine, 5 μc, or $^{35}SO_4^{2-}$, 25 μc). Incubation was for 2½ hr. at 37°. Oxygen uptake for control was 3·64 and 8·8 μl./2½ hr./mg. dry wt. of tissue for Expts. 1 and 2 respectively.

	Incubation mixture		Glycoprotein isolated			
Tracer added	Concn. of salicylic acid (mM)	10^{-5} × Radioactivity added [counts/min./total volume (10·1 ml.)]	Sp. radioactivity (counts/min./mg.) (mean ± S.D.)	Incorporation of radioactivity (% of added radioactivity)	Phosphorus content (% dry wt.)	Sialic acid content* (% dry wt.)
Expt. 1						
L-[U-^{14}C]Threonine	Control	8·11	305 ± 10·0	0·76	2·2	5·1
	3·75	8·11	294 ± 11·2	0·76	2·1	5·1
	15·00	8·11	20 ± 5·3	0·06	1·6	4·7
D-[U-^{14}C]Glucose	Control	8·11	5245 ± 56·6	15·60	1·9	5·6
	3·75	8·11	3900 ± 47·0	10·65	2·1	5·8
	15·00	8·11	420 ± 14·7	1·11	1·6	4·9
$^{35}SO_4^{2-}$	Control	40·54	327 ± 10·4	0·64	2·0	4·7
	3·75	40·54	135 ± 7·5	0·33	2·0	5·4
	15·00	40·54	61 ± 6·9	0·11	2·0	5·5
Expt. 2						
L-[U-^{14}C]Threonine	Control	8·11	250 ± 10·2	0·98	1·1	4·6
	3·75	8·11	265 ± 10·0	0·94	1·4	4·6
	15·00	8·11	73 ± 6·0	0·18	1·3	4·7
$^{35}SO_4^{2-}$	Control	40·54	339 ± 14·8	1·98	1·2	5·7
	3·75	40·54	202 ± 9·4	0·43	1·4	6·3
	15·00	40·54	73 ± 6·1	0·21	1·2	4·7

* Determined as *N*-acetylneuraminic acid by the thiobarbituric acid method (Aminoff, 1961).

amounts of labelling of the glycoprotein fractions from colonic tissues incubated with trace amounts of 16·6 μM-D-[2-^{14}C]glucose (5 or 12·5 μC) in modified Krebs III medium (10 or 20ml.) in the presence and absence of salicylate (3·75mM, 15mM, and 30mM final concn., are given in Table 2. In the presence of 3·75mM-salicylate the incorporation of radioactivity from D-[2-^{14}C]glucose is decreased by 52% and 59% in duplicate experiments. With 30mM-salicylate the inhibition of incorporation of radioactivity from the same substrate is almost complete (sp. radioactivity of the glycoprotein 0·31% of the control value without salicylate). Similar results were obtained with D-[U-^{14}C]glucose (Table 4).

^{14}C-labelled glycoprotein from incubations with D-[2-^{14}C]glucose in the presence of 3·75mM-salicylate was further analysed for the distribution of radioactivity in the monosaccharide constituents (Table 3). The radioactivity of fucose and galactose residues is relatively undisturbed, compared with the salicylate-free control. In contrast, the labelling of the hexosamines (glucosamine and galactosamine) and the sialic acids (*N*-acetylneuraminic acid and *N*-glycollylneuraminic acid) is considerably diminished.

Electrophoresis (in borate buffer, pH 9·3; Draper & Kent, 1963) of radioactive glycoprotein from incubations with D-[2-^{14}C]glucose in the presence and absence of salicylate (final concn. 3·75mM and 15mM) showed there is no difference in the mobilities of these constituents. In both cases there are only three bands staining with Alcian Blue (fractions H and L and nucleic acid fraction), and as before only one band (fraction H) is radioactively labelled.

Effect of salicylate on the incorporation of radioactivity from D-[U-^{14}C]*glucose,* L-[U-^{14}C]*threonine and* $^{35}SO_4^{2-}$ *into a glycoprotein fraction from sheep colonic mucosa.* The results of simultaneous incubations of samples of the one preparation of sheep colonic tissue with 4 μM-D-[U-^{14}C]glucose (5 μC) or 20 μM-L-[U-^{14}C]threonine (5 μC) or $^{35}SO_4^{2-}$ (25 μC) in the presence and absence of salicylate (3·75mM and 15mM) are shown in Table 4. At a final concentration of 3·75mM-salicylate the radioactivities of the glycoprotein isolated from incubations with 4 μM-D-[U-^{14}C]glucose (74·5% of control) and $^{35}SO_4^{2-}$ (41·4% and 59·6% of control) are markedly affected, whereas the radioactivity of glycoprotein isolated from the 20 μM-L-[U-^{14}C]-threonine incubations is relatively unaltered (97% and 106% of the control specific radioactivities). At a salicylate concentration of 15mM the specific radioactivity of the glycoprotein from incubations with radioactive precursors is considerably diminished, and in all cases is below 30% of the radioactivity in the control.

Though no values are as yet available for the intracellular concentrations of glucose or of SO_4^{2-} ions in the sheep tissue, threonine is present in concentrations of the order of 2·33 μmoles/g. dry wt. of scrapings (measured by ion-exchange analysis of supernatants of homogenized scrapings after precipitation of proteins and glycoproteins).

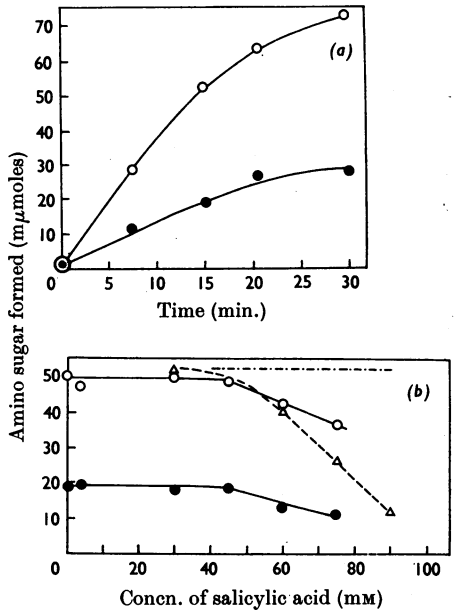


Fig. 2. Effect of salicylate on glucose 6-phosphate transamination by glutamine in a particle-free preparation of sheep colonic mucosal tissue. The incubation medium (total volume 0·5ml.) contained: particle-free supernatant (0·25ml.; 14·6mg. of protein/ml.), glucose 6-phosphate (5 or 1 μmoles), L-glutamine (7·5 or 1·5 μmoles), 2-mercaptoethanol (3·75 μmoles) and KH_2PO_4–KOH buffer, pH 7·4 (25 μmoles). Incubation was at 37°; the reaction was stopped by the addition of 0·5ml. of trichloroacetic acid (12%, w/w) and samples (0·5ml.) were assayed for hexosamines by the method of Levvy & McAllan (1959). (*a*) Time versus activity; (*b*) incubation for 15min. with varying concentrations of salicylic acid. ○, 5 μmoles of glucose 6-phosphate and 7·5 μmoles of glutamine; ●, 1 μmole of glucose 6-phosphate and 1·5 μmoles of glutamine; △, 5 μmoles of glucose 6-phosphate and 7·5 μmoles of glutamine, but with preincubation at the given salicylate concentration for 15min. and assay at half the given salicylate concentration. —•—•—, Reversible inhibition, theoretical.

Effect of salicylate on enzyme activities in a 'particle-free' supernatant from sheep colonic mucosa. (1) D-Glucose 6-phosphate transaminase activity. The quantitative formation of hexosamine 6-phosphate in incubations of particle-free supernatant with substrate amounts of glucose 6-phosphate and glutamine was assayed in the presence of salicylate in the range 3·75–90mM final concn. (Fig. 2*a*). During incubation (15min.) the rate of

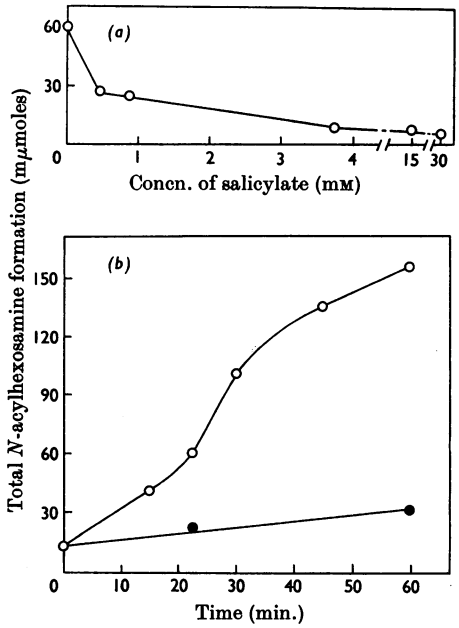


Fig. 3. Effect of salicylate on the *N*-acetylation of glucosamine with acetate in a particle-free preparation of sheep colonic mucosal tissue. Incubation medium (total volume 0·5ml.) contained: KH_2PO_4–KOH buffer, pH 7·4 (25μmoles), KCl (16·75μmoles), $MgCl_2$ (10μmoles), $MnCl_2$ (0·613μmole), 2-mercaptoethanol (3·25μmoles), ATP (2·5μmoles), CoA (0·1μmole), glucosamine hydrochloride (1·25μmoles), sodium acetate (12·5μmoles) and particle-free preparation (0·125ml.; 13·7mg. of protein/ml.). Incubation was at 37°; the reaction was stopped by 0·5ml. of trichloroacetic acid (12%, w/w) and *N*-acetylhexosamine (0·5ml. of neutralized solution, 2N-NaOH) was assayed by the method of Reissig, Strominger & Leloir (1955). (*a*) Effect of different concentrations of salicylate with acetate (12·5μmoles), incubation time 22·5min.; (*b*) time versus activity: ○, with acetate (12·5μmoles); ●, without acetate.

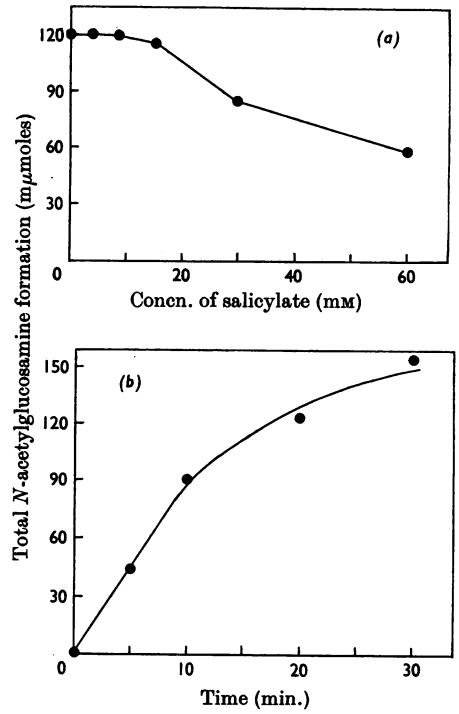


Fig. 4. Effect of salicylate on the *N*-acetylation of glucosamine with acetyl-CoA by a particle-free preparation of sheep colonic mucosal tissue. Incubation medium (total volume 0·5ml.) contained: KH_2PO_4–KOH buffer, pH 7·4 (25μmoles), KCl (16·75μmoles), $MgCl_2$ (10μmoles), 2-mercaptoethanol (3·25μmoles), ATP (2·5μmoles), glucosamine hydrochloride (1·25μmoles), acetyl-CoA (0·16μmole), and particle-free preparation (0·125ml.; 13·7mg. of protein/ml.). Incubation was at 37°; the reaction was stopped with trichloroacetic acid (12%, w/w) and *N*-acetylhexosamine (0·5ml. of sample neutralized with 2N-NaOH) was assayed by the method of Reissig *et al.* (1955). (*a*) Effect of different concentrations of salicylate, incubation time 22·5min.; (*b*) time versus activity.

amino sugar formation was linear. At different concentrations of substrates, 5μmoles and 1μmole of glucose 6-phosphate with 7·5μmoles and 1·5μmoles of glutamine respectively, salicylate below 60mM had no effect on the formation of hexosamine (Fig. 2*b*). With 60mM-salicylate there was decreased synthesis of hexosamine (15 and 25% of control values respectively). Preincubations of enzyme for 15min. with salicylate, final concn. 30–90mM, followed by a twofold dilution and assay for transamination activity, showed that irreversible inhibition of the enzyme occurred at concentrations of 45mM-salicylate or more. At lower concentrations, salicylate appeared to be non-inhibitory to this enzyme.

(2) *N*-Acetylation of glucosamine. The effect of different concentrations of salicylate (0·235–30mM, final concn.) on the quantitative *N*-acetylation of glucosamine by acetate ions in the particle-free supernatant was investigated (Fig. 3). The kinetics of this system proved to be complicated and a progress plot of *N*-acetylglucosamine formation gave a non-linear relationship. The effect of salicylate was assayed with a 22·5min. incubation time. With 0·235mM and 0·94mM final concn. of salicylate the acylation activity was decreased to 46% and 41% of the salicylate-free control respectively. There was also a progressive decrease in activity up to 30mM-salicylate; with this salicylate concentration only 10% of this activity in the control remained. Replacement of the sodium acetate by acetyl-CoA and incubation under the same conditions relieved

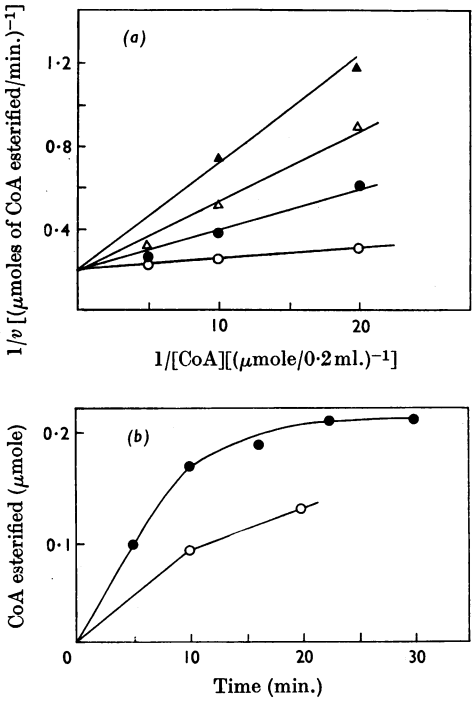


Fig. 5. Effect of salicylate on acetyl-CoA synthetase activity in a particle-free preparation of sheep colonic mucosal tissue. Incubation medium (total volume 0·2ml.) contained: particle-free preparation (0·1ml.; 10·7mg. of protein/ml.), CoA (0·2–0·05μmole) ATP, (1·1μmoles), tris–HCl buffer, pH 7·4 (10μmoles), $MgCl_2$ (0·5μmole) and sodium acetate (2·5μmoles). Incubation was at 37°; samples (0·08ml.) were taken for estimation of free thiol groups by the method of Stadtman (1957). (*a*) Inhibition at different concentrations of salicylate and CoA: ▲, 2·5μmoles of acetate and 3·75mM-salicylate; △, 2·5μmoles of acetate and 0·94mM-salicylate; ●, 2·5μmoles of acetate and 0·235mM-salicylate; ○, inhibitor-free control; (*b*) time versus activity: ●, 2·5μmoles of acetate; ○, acetate-free.

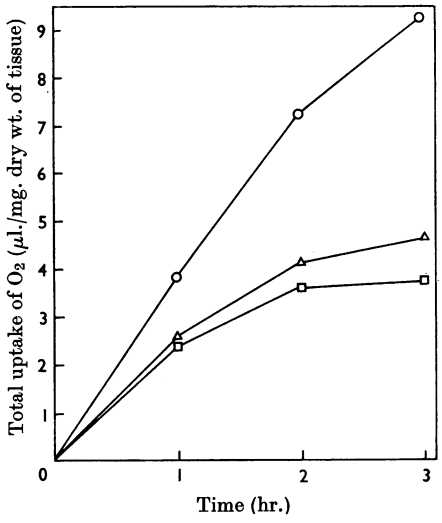


Fig. 6. Effect of aspirin on the oxygen uptake of sheep colonic mucosal tissue. The incubation medium (total volume 4·0ml.) contained: tissue preparation (1ml.; 70mg. dry wt.) and 3ml. of modified Krebs III medium (D-glucose omitted, L-glutamine, 3·2mM final concn., added). Incubations were at 37° in Warburg flasks. ○, Control; △, 15mM-aspirin; □, 30mM-aspirin.

the inhibition at low salicylate concentrations (below 15mM) of the acetylation of glucosamine (Fig. 4). No effect on the acetylation of glucosamine by acetyl-CoA was observed until a final concentration of 15mM-salicylate was reached, and thereafter there was a progressive increase in inhibition to 60mM final concentration of salicylate, when 48% of the activity of the control remained.

(3) Acetyl-CoA synthetase activity (EC 6.2.1.1). The above effect of added acetyl-CoA, relieving the inhibition by salicylate (below 15mM) of the acetylation of glucosamine with free acetate, suggested acetyl-CoA synthetase as a sensitive site of salicylate action in the system. The activity of this enzyme was assayed in the particle-free supernatant (105000*g*, 60min.). With concentrations of CoA between 1·0mM and 0·25mM there was a linear removal (Fig. 5) of free thiol groups from the solution over the first 10min., as measured by the nitroprusside reaction (Webster, 1965). Sodium acetate (2·5μmoles) increased the uptake of free thiol groups from solution by 40–50% of the control value without acetate.

In the presence of salicylate at concentrations as low as 0·235mM (final concn.) there was an inhibition of the uptake of free thiol groups (49% of the activity in the control at a concentration of CoA of 0·25mM). The results of an experiment (in duplicate) where the concentrations of salicylate and CoA were varied are shown in Fig. 5. Increase in the salicylate concentration (or decrease in the CoA concentration), caused increased inhibition until, at 3·75mM-salicylate (final concn.), with a CoA concentration of 0·25mM, only 2·5% of the activity in the control remained. The endogenous thiol-reacting material in the enzyme preparation was equivalent to 28mμmoles/0·4ml. of enzyme (10·7mg. of protein/ml.). Incubation with ATP and acetate, omitting CoA, produced no change in the thiol-reacting content. The acetyl-CoA synthetase activity was unstable; after 2hr. at 4°, 10% of the original activity remained. Freezing at − 70° immediately after preparation and storing below 0° preserved the enzyme activity.

Effect of aspirin on the metabolism of sheep colonic

Table 5. *Incorporation of radioactivity from* D-[U-^{14}C]*glucose into a glycoprotein fraction by sheep colonic scrapings incubated with aspirin*

The incubation mixture contained in a total volume of 10·1 ml.: 7·5 ml. of modified Krebs III medium (D-glucose omitted; L-glutamine, 3·2 mM, added), tissue (2·5 ml.; 74 mg. dry wt.) and D-[U-^{14}C]glucose ($8{\cdot}11 \times 10^5$ counts/min., 5 μc, in 0·1 ml.). Incubation was for 2½ hr. at 37°. Total oxygen uptake was 8·1 and 9·1 μl./2½ hr./mg. dry wt. of tissue, for Expt. 1 and Expt. 2 respectively.

	Glycoprotein isolated			
	Sp. radioactivity		Incorporation of radioactivity	
Concn. of aspirin (mM)	(counts/min./mg.) (mean ± S.D.)	(% of control)	(% of added radioactivity)	(% of control)
Expt. 1				
Control	2392 ± 36	—	13·10	—
3·75	2060 ± 38	86·5	12·25	93·5
15·0	1752 ± 24	73·5	9·67	73·0
Expt. 2				
Control	4593 ± 44·6	—	10·50	—
15	3130 ± 20·9	67·4	5·67	54·0
30	1783 ± 32·2	39·0	4·66	44·4

mucosal scrapings. Since aspirin (in contrast with salicylate) may be the active principle in causing gastric mucosal disturbances, its effect on the mucosal systems was investigated.

(1) Oxygen uptake. The effect of a commercial preparation of aspirin (15 mM and 30 mM) on the oxygen consumption of the tissue is shown in Fig. 6. Aspirin inhibited the oxygen uptake (47% and 56% for 15 mM- and 30 mM-aspirin respectively), but not so markedly as salicylate (58% and 87·4% inhibition for 15 mM- and 30 mM-salicylate respectively).

(2) Incorporation of radioactivity from D-[U-^{14}C]-glucose into the glycoprotein fraction. The results of this incorporation of radioactivity by sheep colonic mucosal scrapings incubated in the presence and absence of aspirin (15 mM and 30 mM) are shown in Table 5. In duplicate experiments aspirin decreased the specific radioactivity of the glycoprotein by 26·5% and 32·6% for 15 mM-aspirin; again the decrease was not so marked as with salicylate (Tables 2 and 4).

Oxygen uptake and utilization of D-[U-^{14}C]-*glucose by human gastric mucosal tissue in the presence of salicylate* (15 *mM*). The preparations were from the pyloric region of specimens of human stomach obtained at surgical partial gastrectomy of patients with a duodenal ulcer (Table 6, Expt. 1) and stomach cancer (Expt. 2). Both preparations had high rates of oxygen consumption; average oxygen consumption for the first hour was 9·9 (Expt. 1) and 5·8 μl. (Expt. 2)/mg./hr. (Table 6). Radioactivity from added D-[U-^{14}C]glucose was incorporated into the non-diffusible trichloroacetic acid-precipitated fractions giving specific radioactivities of 1236 and 3375 counts/min./mg. dry wt. of tissue (average of two incubations in each case). Although zero-time controls were not performed (owing to the small amount of tissue available), it was thought reasonable in the light of previous experience to assume that dialysis for 5 days was adequate to remove all unbound radioactivity.

The dried tissue obtained from Expt. 1 was hydrolysed in 2N-hydrochloric acid for 16 hr. at 105° and analysed chromatographically for amino sugars and neutral sugars in ethyl acetate–pyridine–water (2:1:2, by vol.) and ethyl acetate–pyridine–acetic acid–water (5:5:1:3, by vol.). Besides a wide spectrum of amino acids, the following sugars were present: glucosamine, galactosamine, glucose, galactose, mannose and fucose. The distribution of radioactivity in the hydrolysed samples (specific radioactivities 1196 and 1276 counts/min./mg. respectively) in the different monosaccharides was determined chromatographically and expressed as percentage of total counts in the glycoprotein (Allen & Kent, 1968): hexosamines (glucosamine) 21·4% and 19·7%, glucose and galactose 5·2% and 8·2%, fucose 13·6% and 14·7%. There was also detected a large unidentified radioactive peak (25·2% and 21·5% of total counts incorporated) [$R_{glucosamine}$ 0·42 and 0·93 in ethyl acetate–pyridine–water (2:1:2, by vol.) and ethyl acetate–pyridine–acetic acid–water (5:5:1:3, by vol.) respectively].

The presence of 15 mM-salicylate did not have a marked effect (Table 6) on the oxygen uptake of the tissue. However, the incorporation of radioactivity into the trichloroacetic acid-insoluble fraction was considerably decreased; the specific radioactivities of 379 and 607 counts/min./mg. dry wt. of tissue represented 31% and 18% of the control values without salicylate (Expts. 1 and 2 respectively;

Table 6. *Oxygen uptake and utilization of* D-[U-^{14}C]*glucose by human gastric mucosal cells in the presence of* 15 mM-*salicylate*

The incubation mixture contained in a total volume of 4·05 ml.: 3 ml. of modified Krebs medium III (D-glucose omitted, L-glutamine, 3·2 mM, added), 1 ml. of tissue (40 mg. dry wt.) and 0·05 ml. of D-[U-^{14}C]glucose (2·5 μC, 4·05 × 10^5 counts/min.)

	Expt. 1				Expt. 2			
Concn. of salicylate (mM)	0	0	15	15	0	0	15	15
Consumption of O_2 (μl./hr./mg. dry wt. of tissue)								
First hr.	8·2	11·5	11·7	8·8	4·5	7·1	4·9	4·7
Second hr.	8·0	10·0	7·6	7·5	2·8	3·9	2·3	2·5
Third hr.	6·6	6·7	5·7	3·6	2·1	2·7	1·6	1·7
Total uptake of O_2 (μl./3½ hr./mg. dry wt. of tissue)	26·1	31·1	27·1	22·3	10·0	14·7	9·4	9·3
Sp. radioactivity (counts/min./mg. dry wt. of tissue) (mean ± S.D.)	1196 ± 31·4	1276 ± 19·6	319 ± 19·6	438 ± 13·1	3270 ± 56	3480 ± 44	641 ± 6·0	572 ± 9
Incorporation of radioactivity (% of added radioactivity)	16·2	15·4	3·7	4·5	28·0	19·5	6·0	7·6

Table 6). Hydrolysis and chromatography of the samples from Expt. 1 showed that the decreased incorporation of radioactivity from D-[U-^{14}C]-glucose in the presence of salicylate (15 mM) was reflected in the diminished labelling of all the monosaccharide constituents. The percentages of the total radioactivity distributed in the individual sugars of the non-diffusible fractions from 15 mM-salicylate incubations (specific radioactivity of tissue 319 and 438 counts/min./mg. respectively) were: hexosamines (glucosamine and galactosamine) 13·4% and 9·7%, hexoses (galactose and glucose) 10·8% and 7·5%, and fucose 14·1% and 12·4% (determined after chromatographic separation).

DISCUSSION

The initial stimulation of oxygen uptake by sheep colonic mucosa (oxygen consumption 3·64–8·8 μl./hr./mg. dry wt. of tissue) in the presence of low concentrations of salicylate (1·875 and 3·75 mM) is in keeping with its known uncoupling effects on isolated mitochondria. Brody (1956) showed complete uncoupling of rat liver mitochondria by 2 mM-salicylate. At higher concentrations (7·5 mM and above) of salicylate the inhibitory effect of the drug on other respiratory enzymes becomes marked, contributing to a lowering of the oxygen consumption by the colonic tissue. This effect overtakes the increased consumption of oxygen due to uncoupling, until with 30 mM-salicylate the oxygen uptake after 2½ hr. incubation is only 11·4% of that of the control (Fig. 1). These findings are compatible with those of Smith and co-workers (e.g. Hines, Bryant & Smith, 1963; Smith, 1964), who have shown that at higher concentrations of salicylate (5 mM and above) there was inhibition of several enzymes of the respiratory chain. Progressive decreases in incorporation of radioactivity from [2-^{14}C]glucose into glycoprotein and in the oxygen consumption by the tissue are brought about by the presence of salicylates (Fig. 7).

In the presence of 15 mM-salicylate (oxygen consumption 58% of control value) the incorporation of radioactivity from 20 μM-D-[U-^{14}C]threonine, 16·6 μM-D-[2-^{14}C]glucose and $^{35}SO_4^{2-}$ into the glycoprotein was substantially decreased to less than 30% of the control value. At 30 mM-salicylate (oxygen consumption 11·4% of control value) the incorporation of radioactivity from D-[2-^{14}C]glucose into mucin was virtually abolished (0·27% of control value). Assuming that the incorporation of radioactive precursors is truly an indication of glycoprotein biosynthesis (Allen & Kent, 1968) and provided that the pool sizes of intermediates are small, then the most reasonable explanation is that salicylate under these conditions is causing the

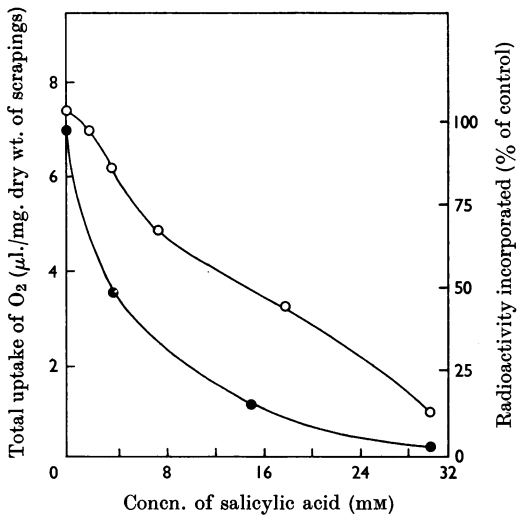


Fig. 7. Oxygen consumption and isotopic labelling of glycoprotein from [2-^{14}C]glucose by sheep colonic scrapings in the presence of salicylate. ○, Uptake of O_2; ●, ^{14}C incorporated into glycoprotein. Average specific radioactivity (control value) was 13500 counts/min./mg.

inhibition of glycoprotein biosynthesis in this system.

In the results of similar incorporation experiments at a concentration of 3·75mM-salicylate, a differential effect on the incorporation of radioactivity into the glycoprotein was detectable. The incorporation of L-[U-^{14}C]threonine into the protein moiety was relatively unaffected, whereas the incorporation of radioactivity from D-[2-^{14}C]glucose and $^{35}SO_4^{2-}$ was noticeably decreased (52–59% of control for the former and 42–59% for the latter). Analysis of the labelling in the constituent sugar residues of the glycoprotein (from incubation of the tissue with D-[2-^{14}C]glucose in the presence of 3·75mM-salicylate, final concentration) shows that the hexosamine and sialic acid residues are prominently affected whereas the neutral sugars (galactose and fucose) are relatively unaffected. [U-^{14}C]-Threonine is incorporated solely as threonyl residues and $^{35}SO_4^{2-}$ solely as ester sulphate (Allen & Kent, 1968).

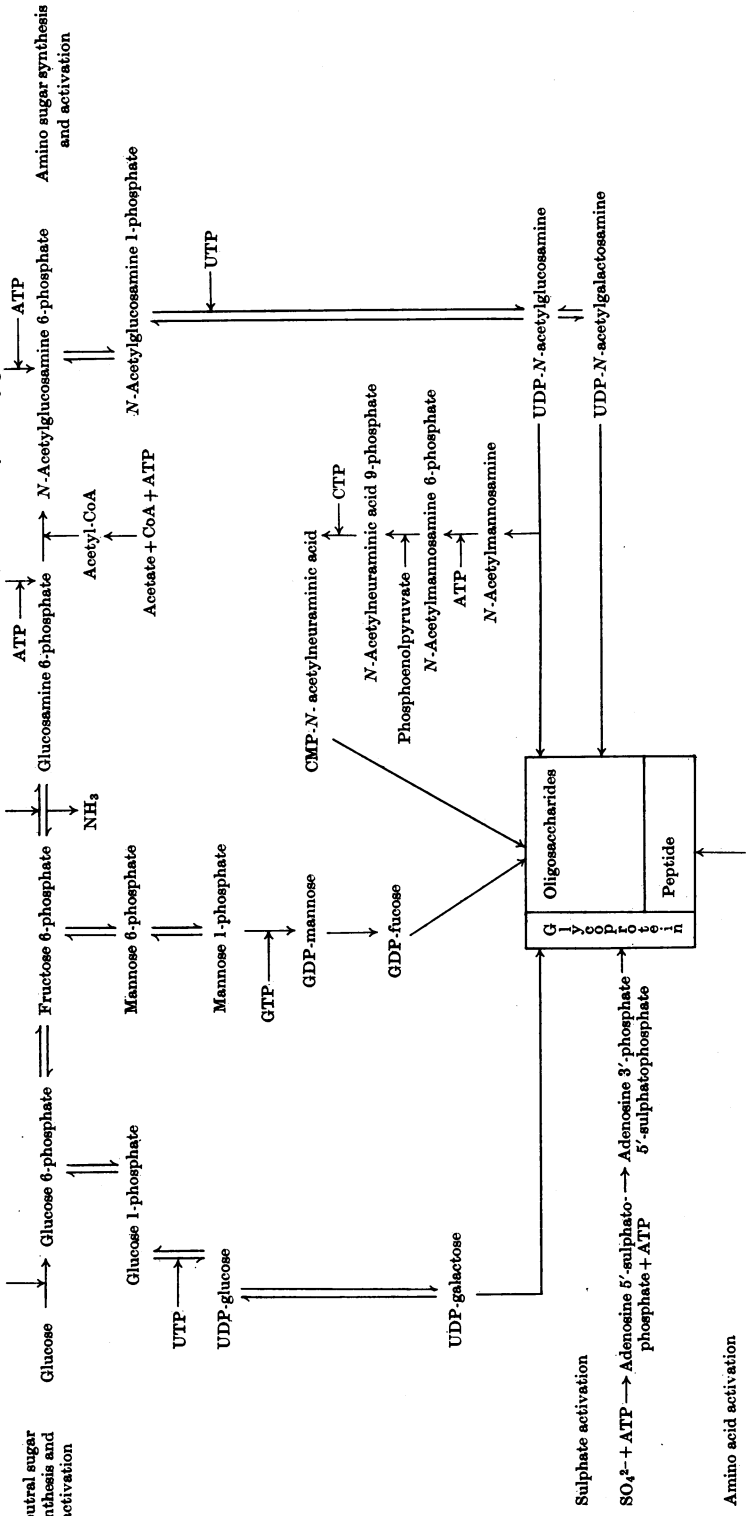
These results were obtained with concentrations of salicylate known to cause inhibition of the incorporation of radioactively labelled substrates into sulphated mucopolysaccharides in bovine cartilage, cornea and heart valves (Whitehouse & Boström, 1962). For example, those workers reported the inhibition of incorporation of D-[^{14}C]-glucose (30% with 4mM-salicylate) and $^{35}SO_4^{2-}$ (41% with 2·5mM-salicylate) into sulphated mucopolysaccharides of cartilage.

The inhibitory action of salicylate on the incorporation of radioactivity from ^{14}C-labelled precursors into the glycoprotein can be considered at two levels: first, the depletion by salicylate of the supply of energy and hence effects on such essential substrates as glutamine; secondly, direct effects on the enzymes involved in biosynthesis of the monomer precursors and the incorporation of these precursors at the nucleotide polymerase level into the macromolecule.

The biosynthesis of the glycoprotein (Scheme 1) from glucose, threonine and SO_4^{2-} depends on a supply of energy for the biosynthesis of the nucleotide intermediates. The depletion of available energy sources by salicylate, particularly at high concentrations (e.g. 15mM and above), may be sufficient to account for most of the observed decrease in the incorporation of substrates into the glycoprotein by the sheep colonic tissue. The selective inhibition of the incorporation of D-[2-^{14}C]-glucose into the hexosamines and the sialic acids by 3·75mM-salicylate is not, however, readily explicable on this basis alone. The known enzymic pathways for the conversion of glucose into the nucleotide hexosamines and nucleotide neutral sugars (e.g. Roseman, 1959; Ginsburg, 1964) have similar nucleotide triphosphate requirements. The only extra energy requirement in the biosynthesis of UDP-acetylhexosamines is that required for the formation of acetyl-CoA from acetate (if free acetate is the main source of *N*-acetyl groups) and for the biosynthesis of glutamine. Since glutamine (3·2mM) is included in the incubation medium it is unlikely that this selective effect of salicylate is due to its decreasing the supply of intracellular glutamine.

Besides ATP deprivation, salicylate may influence the incorporation of radioactivity from D-[2-^{14}C]glucose into the *N*-acetylhexosamines by directly inhibiting the enzymes concerned in their metabolism. Enzymes involved in the conversion of glucosamine into UDP-*N*-acetylglucosamine and *N*-acetylneuraminic acid have been demonstrated in sheep colonic tissue (Draper & Kent, 1963; Kent & Draper, 1968). In the work reported in the present paper a further enzyme system is demonstrated in particle-free preparations involving the transamination of glucose 6-phosphate to glucosamine 6-phosphate, probably via fructose 6-phosphate, although the rate of transamination was identical with either hexose phosphate as substrate. Since UDP-*N*-acetylglucosamine is on the accepted pathway for *N*-acetylneuraminic acid biosynthesis, sites of salicylate action affecting the biosynthesis of the former will affect the biosynthesis of the latter.

Bollet (1961) and Jacobsen & Boström (1964) have shown that salicylate inhibits glutamine–



Scheme 1. Pathway of biosynthesis of epithelial glycoprotein. t-RNA, transfer RNA.

fructose 6-phosphate transaminase in rat granulation tissue (2·1 mM-salicylate, 39% inhibition) and bovine heart valves (3·5 mM-salicylate, 80% inhibition). In 'particle-free' preparations of sheep colonic mucosa the amination of glucose 6-phosphate is unaffected by salicylate up to a final concentration of 60 mM. At higher concentrations of salicylate irreversible denaturation of the enzyme is found (Fig. 2). A similar species difference in the sensitivity of a particular enzyme to salicylate has been demonstrated with histidine decarboxylases (Whitehouse & Skidmore, 1965).

Another step common to the biosynthetic pathway of all other amino sugars is the enzymic acetylation of glucosamine 6-phosphate (Davidson, Blumenthal & Roseman, 1957). The overall enzymic activity of acetylation of glucosamine with acetate in the presence of ATP and CoA in a cell-free supernatant of sheep colonic mucosa is inhibited by salicylate at very low concentrations. For example, the 54% inhibition of this activity by 0·235 mM-salicylate (Fig. 3) is at a concentration of salicylate well within that (i.e. 3·75 mM) of the selective inhibition of amino sugar labelling in the above experiments *in vitro*. In these experiments, sodium acetate was used throughout; further study would be needed to establish whether Na^+ is inhibitory to the enzyme and whether other cations, e.g. K^+ and Mg^{2+}, had any stimulatory role. The system for the acetylation of glucosamine with acetate comprises at least three enzymes: acetyl-CoA synthetase, hexokinase and glucosamine 6-phosphate acyltransferase. Further steps were taken to identify the salicylate-sensitive enzyme step. Replacement of acetate by acetyl-CoA in the above system relieved inhibition by salicylate at concentrations below 15 mM (Fig. 4). These results suggest that the most sensitive site of salicylate inhibition in the acetylation of glucosamine with acetate may be the formation of acetyl-CoA by acetyl-CoA synthetase and that *N*-acetylglucosamine 6-phosphate may play a central role in the regulation of glycoprotein biosynthesis in the epithelial tissue.

Addition of salicylate inhibits acetyl-CoA synthetase activity (measured by the disappearance of CoA thiol groups) in concentration ranges (49% inhibition at 0·235 mM-salicylate, Fig. 5) comparable with that leading to inhibition of the overall glucosamine-acetylation reaction. Moreover, plots of $1/s$ against $1/v$ at different concentrations of inhibitor and CoA are consistent with competitive inhibition (Dixon & Webb, 1964) at low CoA concentrations (0·1 and 0·05 μmole). In the acetyl-CoA synthetase assay the high substrate blank without added acetate (comprising about two-thirds of the activity with added acetate) may involve a CoA-requiring reaction other than acetyl-CoA synthetase. The enzyme was not sufficiently stable to permit removal of endogenous materials by dialysis, nor to allow further purification for more detailed studies on the mechanism of salicylate inhibition.

Nothing is known of the mechanism of action of salicylate on acetyl-CoA synthetase in this tissue. It remains to be discovered whether this enzyme resembles the counterpart in bovine heart mitochondria in requiring activating metal ions (Webster, 1967). If so, salicylate may act by sequestering such ions.

From these results the selective decreased labelling in the presence of salicylate (3·75 mM) of the hexosamines and sialic acids of the glycoprotein can be accounted for, in part at least, by the inhibition of acetyl-CoA synthetase activity. Free acetate is important in ruminant metabolism (Annison, Leng, Lindsay & White, 1963) and can be readily utilized by surviving sheep colonic mucosal tissue for the *N*-acetyl groups of the amino sugars (Allen & Kent, 1968).

Besides amino sugar (including sialic acid) biosynthesis being selectively inhibited at partially inhibitory concentrations of salicylate (3·75 mM), incorporation of $^{35}SO_4^{2-}$ is also affected. Two main possibilities can be contemplated to explain the latter observation: (*a*) decrease of *N*-acetylhexosamine biosynthesis or of its incorporation into the glycoprotein or both; (*b*) a specific effect of sulphate metabolism.

In connexion with (*a*), the $^{35}SO_4^{2-}$ is thought to be incorporated as ester sulphate attached to hexosamine residues (Marsden, 1964) by the adenosine 3′-phosphate 5′-sulphatophosphate sulphotransferase mechanism (Kent & Pasternak, 1958). If the transfer of sulphate ester from adenosine 3′-phosphate 5′-sulphatophosphate to the hexosamine residues accompanies polymerization, then changes in the number of hexosamine residues may be reflected correspondingly in the number of ester sulphate groups. From the structure of the purified fraction H (the sole radioactively labelled component of the glycoprotein fraction) the neutral sugars are located nearer the terminal ends of the carbohydrate side branches than the hexosamines, which are considered to be proximal to the peptide chain (Kent *et al.* 1967). Since the labelling of the neutral sugars is relatively unaffected in the presence of salicylate (3·75 mM) it is suggested that incorporation of galactose and fucose residues is not closely dependent, under these conditions, on the prior incorporation of hexosamines. Therefore if the reduced incorporation of $^{35}SO_4^{2-}$ observed under these conditions is accounted for by lack of hexosamine precursor (free or bound) it is necessary to postulate the formation of an atypical glycoprotein preparation with an increased neutral

sugar:hexosamine or sulphate ratio. Preliminary attempts to find electrophoretic evidence for the formation of an incomplete or atypical glycoprotein at partially inhibitory concentrations of salicylate (3·75mM) were unsuccessful. No difference between glycoprotein isolated from salicylate-inhibited and control experiments has been detected by electrophoresis in the number or the mobility of the Alcian Blue-staining components (fractions H and L and nucleic acid) or of the single radioactive peak moving with fraction H, from D-[2-^{14}C]glucose, L-[U-^{14}C]threonine or $^{35}SO_4^{2-}$ incubations.

The alternative interpretation (*b*) of the labelling pattern in the presence of salicylate (3·75mM) envisages that the differences reflect changes in the specific radioactivity of the precursor pools, especially of hexosamine precursors, and their partition between cytoplasmic organelles, although without significant effect on the total concentration of the pool intermediates. Consequently the specific radioactivity of the pool of glycoprotein precursors and hence of glycoprotein would be decreased, but the rate of glycoprotein biosynthesis would be unaffected. Although this inference is consistent with the accompanying small change in the labelling of the neutral sugar constituents of the glycoprotein, it suggests that other specific effects of salicylate (i.e. apart from that on hexosamine metabolism) on sulphate metabolism may be operative.

Before aspirin (or salicylate) administered therapeutically can be considered to be implicated in damage to the gastric mucosal surface by inhibiting the biosynthesis of the gastric mucins (as inferred from the present experiments on sheep colonic tissue) three important features must be considered: (*a*) whether the action of salicylate *in vitro* occurs at concentrations and in the time prevailing in the stomach during therapeutic administration; (*b*) whether aspirin or salicylate itself is the active inhibitor in the biosynthesis; (*c*) whether the effect of salicylate on sheep colonic mucosa is identical in human gastric mucosa.

In the stomach much higher topographical concentrations of drug may occur in the vicinity of the mucosa after aspirin ingestion than in the blood (with large doses of aspirin its concentration in blood may rise to 2mM; Whitehouse, 1965). For example, a therapeutic dose (600mg.) in the average human stomach volume (100ml.; *Documenta Geigy*, 1962) gives concentrations within the range resulting in almost complete inhibition of incorporation of radioactivity into the glycoprotein. Within 20min. of incubation with 20mM-salicylate there is a noticeable decrease in the uptake of oxygen (Fig. 1). Evidence that ingested aspirin exerts a local effect on the gastric mucosa, in contrast with its more general action, comes from experiments (e.g. Hurley & Crandall, 1963) showing that experimentally produced lesions in animals are caused only by ingestion of aspirin or salicylate, and not by intravenous injection. At the pH prevailing in the stomach, local high concentrations of the drug as well as pH disturbances may occur in the microenvironment of the mucous epithelium (Kent, 1967), owing to actual precipitation of salicylic acid.

In man, aspirin taken by mouth appears in the blood almost wholly in the deacetylated form or as conjugates (cf. Milne, 1963). However, since aspirin is the ingested form of the drug, its effect on the gastric mucosa is relevant. Aspirin when incubated with sheep colonic tissue inhibited the oxygen uptake and incorporation of radioactivity from D-[U-^{14}C]glucose into the glycoprotein fraction by the tissue, but to a lesser degree than free salicylate (Fig. 6 and Table 5). With 15mM-aspirin, 44% inhibition of oxygen uptake and 30% inhibition of incorporation of radioactivity from D-[U-^{14}C]glucose occurred, compared with 54% inhibition of oxygen uptake and 92% inhibition of incorporation of radioactivity from D-[U-^{14}C]-glucose with 15mM-salicylate.

In two experiments with human mucosa, the preparations (each with duplicate incubations) showed considerable metabolic activity when incubated in Krebs III medium (Table 5); the oxygen consumption in the first hour's incubation was 9·9 and 5·8 for Expts. 1 and 2 respectively. Radioactivity was incorporated from D-[U-^{14}C]-glucose into bound hexosamines (glucosamine and possibly galactosamine, 20·6% of radioactivity incorporated), hexoses (glucose and galactose, 5·7% of radioactivity incorporated) and fucose (14·2% of radioactivity incorporated). These residues, apart from glucose, are the main constituents of the blood-group substances secreted into human gastric juice (Morgan & Watkins, 1959). Salicylic acid (15mM) decreased the incorporation of D-[U-^{14}C]-glucose into bound monosaccharides by 70% and 82% of the control values without salicylate. At the same time, there was little observable inhibition of the oxygen uptake (Table 6), an effect that requires further investigation. These results suggest that, as in the sheep colonic mucosa, salicylate inhibition of glycoprotein biosynthesis may also occur in human gastric mucosa. Further, the inhibition by salicylate or aspirin of glycoprotein metabolism in the gastric mucosa may in part be responsible for the known disruptive effects of these drugs on this tissue.

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