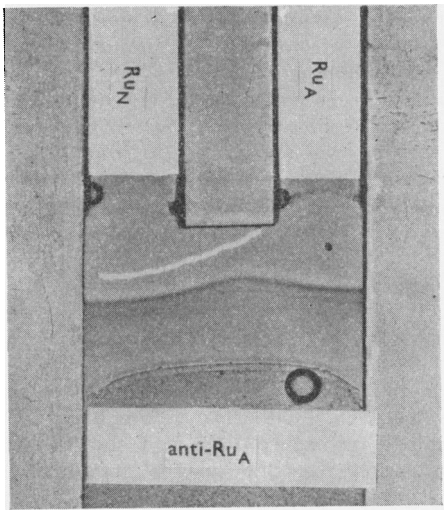

Fig. 3. Gel-diffusion precipitin pattern obtained by the method of Ouchterlony (1948). Solutions (about 1% in 0·05M-veronal buffer, pH 8·6) of thyroglobulin from normal (Ru_N, 0·1 ml.) and abnormal (Ru_A, 0·1 ml.) thyroid tissue of a patient with a follicular carcinoma of the thyroid gland and an anti-serum prepared against Ru_A in a rabbit, were placed in the positions marked Ru_N, Ru_A and anti-Ru_A respectively and allowed to diffuse together. At least three precipitin bands formed; the fourth and lowest band, visible also under Ru_A to the naked eye, was too diffuse to show in the photograph. The white streak is an artifact.

of Ru_N and Ru_A at the same concentration appears to be due to a structural difference which is not reflected in the antigenic properties of the proteins.

SUMMARY

1. Thyroglobulins prepared from normal and carcinomatous thyroid tissue of one patient migrated as single components during electrophoresis in 0·05M-veronal buffer (pH 8·6, I 0·05) at concentrations of 1 and 0·4% (w/v). They differed significantly in mobility.

2. Analysis by the gel-diffusion method of Ouchterlony revealed the presence of three, possibly four, antigenic components in each thyroglobulin but no qualitative difference between the two thyroglobulins.

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Biosynthesis of Intestinal Mucins

1. SURVEY OF INCORPORATION OF [^{35}S]SULPHATE BY ISOLATED GASTROINTESTINAL TISSUES

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When [^{35}S]sulphate is injected into mammals most of it is eliminated in the urine and faeces (Dziewiatkowski, 1949). The comparatively small amount of isotope which is retained is incorporated by a number of tissues, notably the epithelium of the gastrointestinal tract and cartilaginous tissue (Lewison, Levi, Jones, Jones & Silberstein, 1951; Dziewiatkowski, 1953; Bélanger, 1954). By the use of radioautographic techniques it has been shown (Bélanger, 1953; Boström, 1953; Davies & Young, 1954; Jennings & Florey, 1956) that in the gastrointestinal tract the various mucin-secreting cells are the principal sites of localization and that here inorganic sulphate is incorporated into sulphated amino polysaccharides (Crevier & Bélanger, 1954; Kent, Whitehouse, Jennings & Florey, 1956).

In order to study in greater detail the biosynthesis of such polymeric substances, which are major organic components of epithelial mucin, a survey has now been made of the utilization of [^{35}S]sulphate *in vitro* by surviving slices and segments of various types of gastrointestinal

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tissue. [The term 'mucin' has the meaning defined by Kent & Whitehouse (1955*a*), throughout this paper.] Previous workers have shown the uptake *in vitro* of this ion by mammalian cartilage (Boström, 1953; Clark & Umbreit, 1954), chick embryo (Layton, 1950*a*; Jones & Gerarde, 1953; Boyd & Neumann, 1954) and granulation tissue (Layton, 1950*b*; Kodicek & Loewi, 1955) and have demonstrated that it becomes incorporated into sulphated polymers.

Part of the present results have already been reported in brief (Pasternak, Whitehouse & Kent, 1955).

METHODS

Preparation of tissues

Sheep and calves. The whole stomach was removed immediately after slaughter, rinsed with cold water and placed in ice-cold saline. The gastric mucosa from the pyloric region of the abomasum (fourth stomach) was separated from the muscular layer, and pieces (20–30 mg. dry wt.) were blotted, weighed and placed in chilled manometer flasks containing ice-cold incubation medium.

Frogs. Frogs were killed by pithing. The stomach was removed and pieces of gastric mucosa prepared as described above. The dry wt. of tissue was 10–20 mg./flask.

Guinea pigs. Adult guinea pigs were killed by cervical fracture. The stomach was removed and pieces of gastric mucosa (45–65 mg. dry wt. per flask) were prepared as described above. Portions of duodenum, ileum and colon were separated from adhering fat and rinsed with ice-cold incubation medium; whole segments (approx. 2 cm. in length) were blotted, weighed, everted and placed in chilled manometer flasks (45–75 mg. dry wt./flask). The liver was used in some experiments, 35–45 mg. dry wt. of thin tissue slices being added to flasks containing gastric or intestinal tissue.

Incubation

The incubation medium used was the 'medium III' described by Krebs (1950) from which the organic acids had been omitted, i.e. a phosphate buffer containing 0·02M-glucose (total vol. 4 ml./flask). For the experiments with frog gastric mucosa 22 ml. of water was added/100 ml. of this medium. A solution (0·1 ml.) of $Na_2{}^{35}SO_4$ (Radiochemical Centre, Amersham), diluted to contain 1–10 μC, was added. In certain experiments the following additions were made to this medium: sodium acetate, sodium α-oxoglutarate, L-glutamine, (final concn. 0·002M); a mixture of 20 common amino acids (final concns. 0·004M for DL-acids and 0·002M for L-acids and glycine) and aqueous yeast extract A of Whitehouse, Kent, Peters & Foulkes (1954) (0·1 ml. added to 4 ml. of incubation medium). Incubation was at 37° in oxygen for 3 hr. With frog gastric mucosa the temperature was 25°.

Oxygen uptake was measured throughout incubation, carbon dioxide being absorbed by 0·2 ml. of 2M-sodium hydroxide; values of oxygen uptake (Q_{O_2}) are expressed as μl. of O_2/mg. dry wt. of tissue/hr.

At the end of incubation, the contents of each flask were transferred to centrifuge tubes and heated at 100° for 10 min. Tissue was then separated by centrifuging from the supernatant medium, which contained mucin liberated during incubation.

Extraction of tissues

Tissues after incubation and centrifuging were twice extracted with 10 ml. of 5% (w/v) sodium acetate for 12 hr. at room temp., and the resulting extracts were centrifuged before dialysis.

Dialysis

Incubation medium (4 ml.) and tissue extract (20 ml.) were dialysed in cellophan at 2° against distilled water (2·5 l.) which was changed every 12 hr. Sodium sulphate [0·2 ml. of 5% (w/v)] and a mixture containing 1% of penicillin G and 1% of streptomycin phosphate (0·2 ml.) were added to the dialysing solutions at every change of water. Duplicate samples of the incubation medium (0·05 ml.) and tissue extract (0·2 ml.) were removed before dialysis for radioactive assay; further samples (0·2 ml.) were removed at intervals during dialysis. Dialysis was continued until constant radioactivity of the internal solution was attained (90–130 hr.). At the end of dialysis the volumes of the dialysed medium and tissue extract were measured; for certain experiments both fractions were then freeze-dried. The products isolated after dialysis of the medium and tissue extract are termed 'extruded' and 'extracted' mucin respectively. (The word 'extruded' is not used in any physiological sense, but merely to denote the non-dialysable material liberated from the tissue during incubation.)

Analytical procedures

Dry-weight determination. The dry wt. of tissues was determined after heating duplicate samples for 12 hr. at 105°.

Radioactive assay. Samples (0·05 or 0·2 ml.) of the solutions being dialysed were plated directly on to Perspex disks (1·8 cm.^{2}) and allowed to dry at room temp. before the radioactivity was measured (GM mica end-window tube EHM 2s operating at 1640v). All counts were corrected for background (approx. 10 counts/min.), self-absorption (by extrapolation to infinite thinness) and dead-time. Allowance for decay of ^{35}S was made by expressing the final radioactivity of extruded and extracted mucin fractions as total counts/min./100 000 counts/min. initially added/mg. of tissue dry wt., abbreviated subsequently to counts/mg. of tissue.

Amino sugar determination. Amino sugar content of freeze-dried material was determined after hydrolysis with 4N-HCl (4 hr.) or 3N-HCl (13 hr.) at 100° by the Elson & Morgan (1933) reaction. The modification of Johnston, Ogston & Stanier (1951) was employed, with concentrated reagents as suggested by Exley (1957).

Ester sulphate determination. Ester sulphate was determined by the method of Kent & Whitehouse (1955*b*).

Nitrogen determination. Nitrogen was determined by the micro-Kjeldahl method.

Blood-group specificity. Blood-group specificity was estimated by an iso-agglutination-inhibition technique suggested by Dr R. G. Macfarlane, F.R.S. Two series of doubling dilutions of a 0·5% (w/v) solution of mucin in 0·85% saline were prepared; to one of the series of tubes an equal volume (0·2 ml.) of human anti-A serum was added, and to the other an equal volume of anti-B serum. The contents of each tube were mixed and allowed to stand for ½ hr. at room temp. (20–25°). Human A_1 erythrocytes in

citrate [0·2 ml. of a 5% (v/v) suspension containing 0·5% (w/v) trisodium citrate and 0·85% (w/v) sodium chloride] were then added to the tubes containing anti-A serum, and a similar volume of B erythrocytes to the tubes containing anti-B serum. Both series of tubes were mixed and allowed to stand for 1 hr. at room temperature. At the end of this time the degree of agglutination in each tube was compared with a saline control.

Penetration of tissue by [^{35}S]*sulphate.* The method of Deyrup & Ussing (1955) was used. Tissues were prepared and incubated as described above. The radioactivity of medium and of aqueous tissue extract (5 ml.) was also determined as described above.

RESULTS

Oxygen uptake and yield of mucin. The rate of consumption of oxygen by tissue slices showed a slow regular decline during incubation. Table 1 gives Q_{O_2} values and the corresponding yield of mucin fractions. Owing to the finely divided state of freeze-dried mucins, slight losses were incurred during isolation. The values are therefore to be regarded as minimal figures.

Composition of mucin fractions. Table 2 shows values for the total ester sulphate and amino sugar content of mucin fractions derived after incubation from sheep gastric tissue and guinea-pig colonic tissue. In the latter case 30% of the mucin occurs in the incubation medium, the major part being obtained only by chemical extraction of the tissue. In both the extruded and extracted fractions of guinea-pig colon, ester sulphate and amino sugar are present in the molar ratio of 1:1. This suggests that the mucin fractions contain amino polysaccharides which are sulphated to a considerable degree.

[^{35}S]*Sulphate uptake.* A preliminary investigation with sheep gastric mucosa indicated that this tissue incorporates relatively small amounts of [^{35}S]sulphate under the described conditions of incubation (Table 3). These values were not increased by addition of the following substances to the medium: sodium acetate, L-glutamine, sodium α-oxoglutarate, amino acid mixture or yeast extract. Substitution of the medium by sheep serum was also without effect on uptake of [^{35}S]-sulphate.

Under the same conditions of incubation, calf gastric mucosa showed somewhat greater uptake of [^{35}S]sulphate, particularly in the extruded mucin fraction (1·4 counts/mg. of tissue). Incubation of gastric mucosa of frog and guinea pig, with or without the additional substrates mentioned above, gave no appreciable uptake of [^{35}S]sulphate, even when these tissues were incubated in the presence of liver slices. Control liver slices incorporated [^{35}S]sulphate to a value of 0·7 count/mg. of tissue.

The behaviour of other gastrointestinal tissues was studied. Guinea-pig duodenum, ileum and colon were found to incorporate considerable amounts of [^{35}S]sulphate. Additional substrates or liver slices were without effect on the level of incorporation.

With gastric tissue the mucosa alone was studied, whereas with colon segments of whole intestine were used. Since the musculature appears to be almost inactive in fixing [^{35}S]sulphate (Jennings & Florey, 1956), the results (Table 3) indicate that the mucosa of colon is very much more active than that of stomach in this respect.

Table 1. *Yield of mucin and* Q_{O_2} *of gastrointestinal tissues*

Values are averages derived from several experiments, the numbers being given in parentheses. For experimental details see text. The Q_{O_2} values were calculated from the slope of the Q_{O_2}/time curve at 150 min. The S.E.M. of the results for the sheep gastric mucosae experiments are given.

Tissue	Q_{O_2}	Extruded mucin (μg./mg. of dry tissue)	Extracted mucin (μg./mg. of dry tissue)
Sheep gastric mucosa	−5·5±1·0 (14)	80±40 (10)	220±90 (9)
Calf gastric mucosa	−3·8 (2)	60 (1)	—
Frog gastric mucosa	−1·9 (3)	20 (1)	—
Guinea-pig gastric mucosa	−10·8 (1)	70 (1)	—
Guinea-pig colon	−6·5 (4)	100 (2)	—

Table 2. *Composition of mucin fractions from gastrointestinal tissues*

Values (in μg./mg. of dry tissue) ± S.E.M. are averages derived from several experiments, the numbers being given in parentheses. For experimental details see text.

Tissue	Mucin fraction	Ester sulphate	Amino sugar
Sheep gastric mucosa	Extruded	2·4 (1)	3·5±1·3 (9)
	Extracted	1·7 (1)	6·2±2·4 (10)
Guinea-pig colon	Extruded	2·9±1·2 (13)	4·7±0·6 (6)
	Extracted	5·8±1·4 (5)	10·0±0·9 (6)

It was subsequently found that for sheep, as for guinea pig, the uptake of [^{35}S]sulphate by gastrointestinal tissues was in the descending order: colon, duodenum, stomach.

It was decided to examine the behaviour of guinea-pig gastric and colonic tissue in more detail, since the difference in incorporation of ^{35}S by the two tissues is so marked. The ester sulphate content of the freeze-dried extruded fractions was measured and found to be similar (24 μg. of sulphate/mg. of gastric mucin and 22 μg. of sulphate/mg. of colonic mucin); the chemical nature of these mucins in terms of total ester sulphate does not therefore provide an explanation of the differences in fixation of ^{35}S.

Penetration of tissue by [^{35}S]*sulphate.* In view of the low uptake of [^{35}S]sulphate by guinea-pig gastric mucosa compared with colon, the penetration of these tissues by [^{35}S]sulphate was determined. Values, calculated from the relation

$$[SO_4^{2-}]_{\text{tissue water}} = \frac{^{35}S_{\text{tissue water}}}{^{35}S_{\text{medium}}} \times [SO_4^{2-}]_{\text{medium}},$$

where $[SO_4^{2-}]$ is expressed in μmoles/ml. and ^{35}S in counts/min./ml., were found to be similar, namely 0·88 μmole of sulphate in tissue water of gastric mucosa and 0·89 μmole in that of colon segments, compared with 1·40 μmoles of sulphate in the incubation medium. Calculation of the tissue sulphate relative volume,

$$100 \times \frac{\text{(concn. of } ^{35}\text{S in tissue water)}}{\text{(concn. of } ^{35}\text{S in medium)}},$$

gives a value of 64 for guinea-pig colon segments, a figure similar to that (95) found for rat-colon segments by Deyrup & Ussing (1955), in so far as 'active uptake' of [^{35}S]sulphate from the medium is not indicated. Differences of concentration of K^+ ion may account for the higher value found by Deyrup & Ussing (1955).

Blood-group specificity. The results (Table 4) show that gastric mucin fractions are potent inhibitors of isoagglutination, whereas intestinal mucins are inactive.

DISCUSSION

Oxygen consumption. The rates of uptake of oxygen observed with the various tissues studied are in agreement with those reported by previous workers (Davies, 1948; Crane & Davies, 1951; Davenport & Chavré, 1952). Steady respiration by gastric mucosa was observed for longer periods than was noted by Lutwak-Mann (1947). The present results for the Q_{O_2} of guinea pig, pig and sheep tissues are similar to the findings of Dickens & Weil-Malherbe (1941), who studied rat tissues,

Table 3. *Incorporation of* [^{35}S]*sulphate by gastrointestinal tissues*

Values (in counts/100 000 added/mg. of dry tissue) are averages derived from several experiments, the numbers performed being given in parentheses. For experimental details see text.

Tissue	Extruded mucin	Extracted mucin	Total
Sheep gastric mucosa	0·03 (4)	0·02 (4)	0·05
Calf gastric mucosa	1·4 (2)	0·2 (2)	1·6
Frog gastric mucosa	1·0 (3)	0·0 (3)	1·0
Guinea-pig gastric mucosa	0·7 (3)	0·03 (3)	0·73
Guinea-pig duodenum	4·0 (1)	17·5 (1)	21·5
Guinea-pig ileum	8·7 (1)	3·1 (1)	11·8
Guinea-pig colon	37·7 (6)	20·5 (5)	58·2

Table 4. *Inhibition of isoagglutination by gastrointestinal mucins*

The numbers of animals examined are shown in parentheses. For experimental details see text. The sign – means that the sample showed no activity.

Tissue	Group of serum inhibited: Extruded mucin	Group of serum inhibited: Extracted mucin	Highest dilution exhibiting inhibition
Sheep gastric mucosa	A (3)	A (1)	1:2000–1:10 000
Calf gastric mucosa	B (1)	B (1)	1:2000
Frog gastric mucosa	*AB (3)	—	1:30 000
Guinea-pig gastric mucosa	B (2)	B (1)	1:10 000
Guinea-pig duodenum	B (1) (slight)	–(1)	–
Guinea-pig ileum	–(1)	–(1)	–
Guinea-pig colon	–(2)	–(1)	–

* Pooled sample from three frogs.

Table 5. *Composition of gastrointestinal mucins*

All values are mg./100 mg. of mucin.

Tissue	N (%)	Amino sugar (%)	Ester sulphate (%)	Authors
Sheep stomach§	7·7	3·1*	1·7	Present work
Pig stomach	11·7	7·7	0·14†	Werner (1953)
Pig stomach	—	16·7	0·34	Whitehouse (1955)
Pig colon	13·2	5·0	1·0†	Werner (1953)
Guinea-pig colon§	7·3	4·7‡	2·1	Present work

* Confirmed by pyrrole method of Exley (1957)
† Calculated from Werner's (1953) data for fractionated material.
‡ From Table 2.
§ Extruded, freeze-dried mucin.

and found a descending order for the Q_{O_2} values of duodenum, ileum and colon.

Properties of extruded and extracted mucin. Werner (1953) and Whitehouse (1955), in contrast with other authors, have studied gastrointestinal mucins by techniques designed to avoid degradation during isolation; their results are compared with those obtained during the present experiments in Table 5. The low values of amino sugar content observed by Werner (1953) and by us suggest the presence of considerable amounts of non-carbohydrate material, and Werner (1953) was indeed able to increase the amino sugar content of his preparations two- to three-fold by removal of protein by tryptic digestion. Interpretation of the high ester sulphate/amino sugar ratios found in these studies (see also Table 2) must await the results of purification and fractionation of the freeze-dried material.

Blood-group activity (Table 4) was found in the gastric mucins of sheep, calf (cf. Hartmann, 1941; Jorpes & Thaning, 1945; Beiser & Kabat, 1952), frog and guinea pig. Identification of the nature of the blood-group substance from frog is difficult, since the material was obtained after pooling the extruded mucin fractions from three stomachs; it is therefore not possible to say whether the AB activity is due to one frog or whether it represents a mixture of A and B substances from different frogs. Nevertheless, the finding of any A activity is interesting in view of the apparent lack of A specificity in the red blood cells from frogs (Ashhurst, 1956).

Among the mucins of the gastrointestinal tract, only those from stomach appear to exhibit marked blood-group activity (cf. Witebsky & Neter, 1935; Satoh, 1949). Zittle, Smith & Krejci (1948) seem to be the only authors to have isolated intestinal mucins possessing strong blood-group specificity. The possibility that guinea-pig intestinal mucins contain blood-group substances, but in an inactive state, cannot be excluded (cf. Witebsky & Neter, 1935). In view of a recent study relating the presence of blood-group specific substances in the gut of humans to the incidence of duodenal ulcer (Clarke *et al.* 1956), it seems of considerable importance to investigate such compounds further; we have made an attempt to correlate blood-group activity and the chemical composition of mucins produced by different regions of the gastrointestinal tract (Pasternak & Kent, 1957).

Incorporation of [^{35}S]sulphate during incubation. Calculation of incorporation of ^{35}S (in terms of μg. of sulphate) as a fraction of the total ester sulphate content of extruded and extracted mucin (Table 2) gives a value for the proportion of mucin sulphate groups which are newly formed during incubation. In guinea-pig colon this is 4·5% for the extruded fraction and 1·2% for the extracted mucin fraction. Since the sulphate content of other gastric mucins is similar and the uptake of ^{35}S is considerably less, the percentage incorporation in such mucins is even smaller (less than 1%). In all the tissues studied, therefore, most of the extruded or extracted mucin is not synthesized during incubation, but exists preformed at the start of the experiment. It has not been possible to decide whether the newly synthesized material represents a net increase of mucin or whether it is accompanied by an equivalent breakdown (see Pasternak & Kent, 1958).

The mechanism of incorporation of [^{35}S]sulphate will be considered in greater detail elsewhere (Pasternak & Kent, 1958). There does not appear to be a relation between oxygen uptake and sulphate incorporation by different tissues of the gastrointestinal tract (Tables 1 and 3); since sulphate fixation is only one of the many functions exhibited by these tissues during incubation *in vitro*, such a relation is hardly to be expected.

A feature of the present study is the relative inactivity of gastric tissue, as opposed to intestinal tissue, in fixing [^{35}S]sulphate (Table 3). This result is in accord with experiments on incorporation of [^{35}S]sulphate by whole animals: Jennings & Florey (1956), for example, find that several mammalian species exhibit only slight uptake of ^{35}S in the neck cells of gastric epithelium, whereas greater fixation

is apparent in Brunner's glands of duodenum and in goblet cells of the small and large intestine.

The inactivity of gastric tissue is unlikely to be a reflexion of the type of mucin which is produced, since the degree of sulphation of gastric and colonic mucins is similar; it is worth noting, however, that Satoh (1949) has found more 'hydrolysable sulphur' in human colonic mucin than in that from the small intestine.

It is considered improbable, moreover, that lack of uptake of [^{35}S]sulphate by gastric tissue is caused by conditions *in vitro*; permeability of the tissue to [^{35}S]sulphate, for instance, is similar for stomach and colon. Insufficient energy is also unlikely to be the cause, since gastric tissues have a higher Q_{O_2} than intestinal ones (Table 1). An explanation of the low level of incorporation of ^{35}S-sulphate by stomach, in contrast with colon, may lie in differences in the concentrations of certain enzymes or coenzymes within the tissue.

SUMMARY

1. The oxygen uptake and incorporation of [^{35}S]sulphate by isolated tissues of the gastrointestinal tract of several animals have been measured.

2. The incorporation of [^{35}S]sulphate by gastric mucosa of sheep, calf, frog and guinea pig was found to be negligible (less than 2 counts/mg. of dry tissue); uptake by lower regions of sheep and guinea-pig intestine, especially by colon, is considerable (10–60 counts/mg. of dry tissue).

3. The penetration of gastric and colonic tissue by ^{35}S-sulphate has been determined and found to be similar for these two tissues (approx. 60%).

4. The ester sulphate and amino sugar contents of gastric and colonic mucins have been measured.

5. Differences in incorporation of [^{35}S]sulphate by gastric and colonic tissue are not accounted for on the basis of (*a*) penetration of [^{35}S]sulphate into the tissue or (*b*) the degree of sulphation of the mucins produced by the tissue.

6. The blood-group specificity of several types of gastrointestinal mucin has been determined: activity appears to be restricted to mucins isolated from gastric tissue.

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