alkaline pH and into *p*-hydroxyphenylacetic acid [^{35}S]sulphate in the presence of urine at neutral pH.

3. The implications of these various findings are discussed.

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Studies on L-Tyrosine *O*-Sulphate

3. THE METABOLIC FATE OF THE L-TYROSINE *O*[^{35}S]-SULPHATE RESIDUE OF ^{35}S-LABELLED RABBIT FIBRINOPEPTIDE B

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L-Tyrosine *O*-sulphate is known to be a component of mammalian fibrinogens (Bettelheim, 1954; Blombäck, 1960). In the ox, pig and rabbit the ester occurs in one of the two peptides (fibrinopeptide B) that are liberated from fibrinogen by the action of bovine thrombin. In man, however, although L-tyrosine *O*-sulphate is present in fibrinogen (Bettelheim-Jevons, 1958), there is some evidence to suggest that it is not present in fibrinopeptide B (Korff & Bronfenbrenner, 1958; Blombäck, Blombäck, Edman & Hessel, 1962).

Attempts made in these and other Laboratories (e.g. Segal & Mologne, 1959; Grimes, 1959; Vestermark & Boström, 1959) to achieve the enzymic sulphation of free L-tyrosine *in vitro* by the normal adenosine 3′-phosphate 5′-sulphatophosphate–phenol-sulphokinase route have all failed, and there is some evidence to suggest that the amino acid must be present in a peptide-bound form before sulphation can occur. This lends support to the possibility that the relatively large amounts of L-tyrosine *O*-sulphate that are found in normal human urine (up to 30 mg./day; Tallan, Bella, Stein & Moore, 1955) arise from the degradation of protein, presumably fibrinogen. In contrast with humans, rats, mice and guinea pigs do not appear to excrete L-tyrosine *O*-sulphate in urine (Grimes 1959), but this is not surprising in view of the ability of these species to deaminate the ester to yield the sulphate esters of *p*-hydroxyphenylpyruvic acid and *p*-hydroxyphenylacetic acid (Dodgson, Powell, Rose & Tudball, 1961; Powell, Rose & Dodgson, 1962, 1963). In an attempt to throw further light on the metabolism of L-tyrosine *O*-sulphate residues when present in peptide-bound form, a study has been made of the metabolic fate, in rabbits, of free L-tyrosine *O*[^{35}S]-sulphate and of rabbit fibrinopeptide B in which the L-tyrosine *O*-sulphate residue is labelled with ^{35}S.

MATERIALS AND METHODS

Sulphate esters. The ^{35}S-labelled sulphate esters of L-tyrosine and *p*-hydroxyphenylacetic acid were prepared as described by Dodgson *et al.* (1961).

^{35}S-labelled rabbit fibrinopeptide B. Eight virgin female rabbits (Flemish Giants, each weighing approx. 3 kg.) were each injected intraperitoneally with a total of 6 mc of $Na_2{}^{35}SO_4$ (code SJS 1, specific activity greater than 100 mc/mg. of S; The Radiochemical Centre, Amersham, Bucks.) over a period of 3 days. Sixteen hours after the last injection the animals were anaesthetized with ether and the blood was drawn from the abdominal aorta and collected in vessels containing a 4% solution of sodium citrate. The volume of sodium citrate solution used was such that the final concentration of citrate in the blood sample was approx. 0·4%.

Red cells were separated by centrifuging at 2000*g* and 10° for 1 hr. and the ^{35}S-labelled fibrinogen was isolated from the plasma by method 6 of Cohn *et al.* (1946). Subsequently, the fibrinogen was purified by the glycine method of Blombäck & Blombäck (1956) as far as stage I-o (yield approx. 7·5 g. wet wt.). The fibrinogen was then clotted with bovine thrombin and fibrinopeptide B separated by fractionation of the clot supernatant on Dowex 50 according to the procedures outlined by Blombäck,

Boström & Vestermark (1960) (yield from eight rabbits approx. 20 mg.). The fibrinopeptide exhibited electrophoretic and spectrophotometric properties identical with the ones reported by these workers.

For the determination of the radioactivity of the preparation, portions (1 mg.) were hydrolysed with 15 ml. of N-HCl and the liberated inorganic [^{35}S]sulphate was precipitated in the presence of 3 ml. of aqueous 0·15M-K_2SO_4 by the addition of 4 ml. of aqueous 10% $BaCl_2$. Precipitated $Ba^{35}SO_4$ was plated and counted as described below. The radioactivity of the preparation corresponded to 100 counts/min./mg.

Experimental animals. For the metabolism studies, mature Dutch rabbits were used. These animals were usually injected intraperitoneally and were subsequently placed in metabolism cages designed to permit separate collection of urine and faeces. The animals were allowed water without restriction and were fed once daily with cabbage. Urine and faeces were collected after 24 and 48 hr., the cages being washed down on each occasion with approx. 100 ml. of water. Urine and washings were pooled and clarified by centrifuging. Precipitated material was washed twice (with intermediate centrifuging) with 40 ml. portions of water, the washings being added to the clear urine. No radioactivity remained in the precipitated material that had been washed in this way. At the end of the experimental period the rabbits were killed by exsanguination and fibrinogen was isolated from the blood by the method of Cohn *et al.* (1946).

Preparation of samples for determination of radioactivity. The inorganic sulphate fraction of the urine was precipitated as $Ba^{35}SO_4$ by the procedure described by Lloyd (1961).

For the determination of the combined inorganic and ester [^{35}S]sulphate fraction of urine the procedure of Lloyd (1961) was followed except that during acid hydrolysis the urine became cloudy and was therefore clarified by centrifuging before precipitating with $BaCl_2$. The material that sedimented during the centrifuging was washed twice (with intermediate centrifuging) with 5 ml. of water, the washings being added to the clear hydrolysate.

Preliminary experiments showed that there was no co-precipitation of L-tyrosine *O*[^{35}S]-sulphate or of fibrinopeptide B and no hydrolysis of either compound during the precipitation of the inorganic sulphate fraction of rabbit urine.

The inorganic and combined inorganic and ester sulphate fractions of rabbit bile were also precipitated as $Ba^{35}SO_4$. A precipitate that formed during the initial acidification of the bile samples was removed by centrifuging, the sedimented precipitate being washed as usual and the washings added to the clear bile.

For determination of the total ^{35}S content of faeces, the 24 and 48 hr. samples were pooled and dissolved in fuming HNO_3 before oxidization by the method of Denis (1910). The oxidized material was dissolved in N-HCl (15 ml.) before precipitation of the ^{35}S as $Ba^{35}SO_4$ as above.

Determination of radioactivity. The dried $Ba^{35}SO_4$ precipitates were plated at 'infinite thickness' in plastic planchets as described by Lloyd (1961). Measurements of radioactivity were made in automatic scaling equipment (Nuclear-Chicago Corp., Ill., U.S.A.) with the D. 37A thin mica end-window Geiger–Müller tube. The usual corrections for background, decay and coincidence were made. The standard error of counting was less than ±2%, except in measurements on the inorganic sulphate fractions of urines from rabbits receiving fibrinopeptide B, when the standard error was of the order of ±10%.

EXPERIMENTAL AND RESULTS

Fate of L-*tyrosine* O[^{35}S]-*sulphate*

Six rabbits (three of each sex) were injected intraperitoneally with 2 mg. of L-tyrosine *O*[^{35}S]-sulphate (specific activity approx. 6 μc/mg.) dissolved in 1 ml. of 55 mM-sodium citrate adjusted to pH 6·4 with N-hydrochloric acid. The ^{35}S content of the urines and faeces of these animals was then determined. The results (Table 1) showed that the bulk of the injected ^{35}S was eliminated as ester sulphate within 24 hr., only a small amount appearing as inorganic [^{35}S]sulphate. The ^{35}S content of the 48 hr. samples of faeces in no case exceeded 0·5% of the injected dose. These results

Table 1. *Distribution of* ^{35}S *in the urine of rabbits* (1–2 *kg.*) *injected intraperitoneally with free* L-*tyrosine* *O*[^{35}S]-*sulphate or with fibrinopeptide B containing a* L-*tyrosine* *O*[^{35}S]-*sulphate residue*

Results are average values with the range in parentheses. ^{35}S was precipitated and counted as $Ba^{35}SO_4$ as outlined in the text. Radioactivity of the dose of the respective compounds corresponded to 10^5 counts/min. (L-tyrosine *O*[^{35}S]-sulphate) and 10^3 counts/min. (fibrinopeptide B).

				Distribution of ^{35}S in urine (% of ^{35}S injected)			
				Inorganic sulphate fraction		Combined inorganic and ester sulphate fraction	
Compound	Dose (mg.)	Sex	No. of animals	Time 24 hr.	Time 48 hr.	Time 24 hr.	Time 48 hr.
L-Tyrosine *O*[^{35}S]-sulphate	2	Male	3	1·5 (1·3–2·3)	0·6 (0·0–1·2)	91·5 (79·7–103)	4·6 (1·3–7·6)
	2	Female	3	2·6 (1·6–3·2)	0·5 (0·3–0·7)	98·4 (95·8–103)	5·1 (1·1–10·0)
Fibrinopeptide B	10	Female	1	2·9	0·9	74·8	17·0
	10	Female	1*	4·0	1·4	90·4	12·4

* Injected intravenously (ear vein).

are similar to those recorded for the rat by Dodgson *et al.* (1961). No radioactivity could be detected in the fibrinogen that was subsequently isolated from the rabbits at the end of the experiment.

In further experiments, urine and bile were collected at hourly intervals, via bladder and bile-duct cannulae, from two anaesthetized (Nembutal) rabbits that had been injected intravenously with L-tyrosine *O*[^{35}S]-sulphate. The results (Table 2) showed that the bulk of the injected dose of ^{35}S was eliminated in urine within the first 3 hr. after injection, and approx. 85% of the dose was recovered in 6 hr. During this period less than 1% of the dose was excreted into the bile duct.

Table 2. *Excretion of total* [^{35}S]*sulphate (inorganic and ester) in the urine of rabbits during the 7 hr. immediately after intravenous injection of* L-*tyrosine* O[^{35}S]-*sulphate*

Results are average values of two experiments with the ranges in parentheses. Each rabbit was anaesthetized (Nembutal), injected with 2 mg. of potassium L-tyrosine *O*[^{35}S]-sulphate, and urine was collected via a bladder cannula.

Time (hr.)	Vol. of urine (ml.)	Excretion of total [^{35}S]sulphate (inorganic and ester) (% of ^{35}S injected)
1	10·1 (9·7–10·5)	28·1 (24·2–31·9)
2	7·4 (7·0–7·8)	23·7 (22·8–24·5)
3	7·7 (6·5–8·9)	14·1 (12·2–15·9)
4	9·0 (7·5–10·5)	7·9 (6·9–8·8)
5	7·4 (6·0–8·9)	5·0 (3·9–6·1)
6	6·3 (3·5–9·2)	3·3 (3·1–3·4)
7	11·8*	2·9*

* Results for a single animal only.

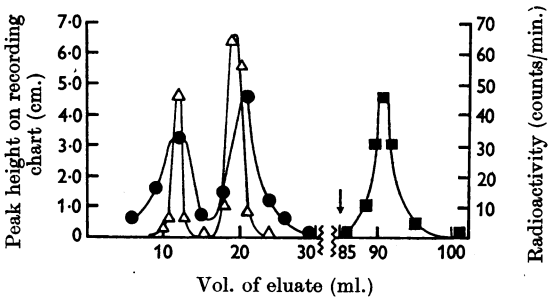

Fig. 1. Behaviour, on Dowex 50 (H^+ form) ion-exchange columns, of ^{35}S-labelled rabbit fibrinopeptide B and of the metabolites in urine resulting from the injection of potassium L-tyrosine *O*[^{35}S]-sulphate and ^{35}S-labelled fibrinopeptide B into rabbits. The columns were eluted with 50 ml. of water, followed by 50 ml. of aqueous 1 M-ammonia solution, the arrow indicating the approximate point of emergence of an alkaline eluate. See text for further details. △, Urine from rabbits receiving potassium L-tyrosine *O*[^{35}S]-sulphate (left-hand ordinate); ●, urine from rabbits receiving fibrinopeptide B (right-hand ordinate); ■, normal rabbit urine to which had been added ^{35}S-labelled fibrinopeptide B (right-hand ordinate).

Paper electrophoresis and chromatography experiments had previously indicated that the bulk of injected L-tyrosine *O*[^{35}S]-sulphate is eliminated unchanged by the rabbit, although small amounts of *p*-hydroxyphenylacetic acid [^{35}S]sulphate are formed (Mr T. Flanagan, unpublished work; cf. Powell *et al.* 1963). Confirmation of this was obtained as follows. Urine samples (12 hr.) from two rabbits that had each been injected with 2 mg. of L-tyrosine *O*[^{35}S]-sulphate were concentrated approx. 50-fold by freeze-drying. After clarifying by centrifuging, each concentrate was applied to a column (10 cm. × 2 cm.) of Dowex 50 ion-exchange resin (200–400 mesh; H^+ form), which was subsequently washed with 50 ml. of water followed by 50 ml. of aqueous 1 M-ammonia solution. The effluent was collected in fractions of 1 ml., which were then checked for radioactivity. Fraction samples (10 μl.) were placed at intervals of 4 cm. on a single strip of Whatman no. 1 filter paper, which, after drying at room temperature, was passed through the C.100 Actigraph automatic chromatogram scanner (Nuclear-Chicago Corp.) at a speed of 12 in./hr., with a slit width of 0·5 in., an integration time of 40 sec. and a scale setting of 150 counts/min. The relative radioactivity of each sample was then assessed by measuring the height of the corresponding deflexion on the scanning chart and the radioactive peaks in the eluate were deduced accordingly. Two such peaks were detected in the acidic eluate (total vol. approx. 30 ml.) that first emerged from the column (Fig. 1), the remainder of the eluates containing no detectable radioactivity.

The pooled fractions corresponding to each peak were neutralized with aqueous 1 M-ammonia solution and concentrated to about 1 ml. *in vacuo* at 35° on a rotary evaporator. Paper electrophoresis and paper chromatography, under the conditions described by Rose & Powell (1963), showed that the radioactive peak first emerging from the column contained material with electrophoretic and chromatographic mobilities identical with those of authentic *p*-hydroxyphenylacetic acid [^{35}S]sulphate, whereas the mobilities of the radioactive component of the second peak were identical with those of L-tyrosine *O*[^{35}S]-sulphate.

Fate of the L-*tyrosine* O[^{35}S]-*sulphate residue of rabbit fibrinopeptide B*

A female rabbit was injected intraperitoneally with 10 mg. of the ^{35}S-labelled fibrinopeptide dissolved in 1 ml. of 55 mM-sodium citrate ad-

justed to pH 6·4 with N-hydrochloric acid. A second animal was given the same dose intravenously (ear vein). Urine and faeces from these animals were examined quantitatively for radioactivity as before (Table 1). As with free L-tyrosine *O*[^{35}S]-sulphate, the bulk of the ^{35}S appeared in urine in an ester-bound form. No radioactivity could be detected in faeces or in the fibrinogen that was isolated from the blood of the animals at the end of the experiment.

Attempts to identify the labelled fibrinopeptide metabolites in the remainder of the urine were hindered by the low radioactivity and it proved impractical to detect metabolites by paper electrophoresis or paper chromatography. Accordingly, one-half of the remaining urine was freeze-dried and reconstituted in 5 ml. of 0·1 M-ammonium formate adjusted to pH 3·0 with formic acid. After the pH of the solution had been checked and re-adjusted to 3·0 with formic acid, the solution was applied to a column (10 cm. × 2 cm.) of Dowex 50 resin that had been equilibrated with 0·1 M-ammonium formate adjusted to pH 3·0 with formic acid. The column was treated successively with portions (100 ml.) of (*a*) 0·1 M-ammonium formate, adjusted to pH 3·0 with formic acid, (*b*) the same buffer at pH 3·7, (*c*) aqueous 0·1 M-ammonia solution. After each treatment the resultant effluent was freeze-dried; the freeze-dried residue was transferred to a polythene planchet (sufficient to fill the planchet) and compressed with a close-fitting stainless-steel plunger (see Lloyd, 1961) before counting for radioactivity. Only the pH 3·0 eluate contained any measurable radioactivity.

The type of column used in the above experiment was also employed in the initial separation of fibrinopeptide B from the clot supernatant. The peptide is retained on such a column at pH 3·0 or less, elution occurring at pH 3·7 or above (see also Blombäck *et al.* 1960). Preliminary experiments, in which ^{35}S-labelled fibrinopeptide B was added to normal rabbit urine (2 mg./30 ml. of urine) before subjecting the whole to the fractionation procedure described above, showed that urine did not significantly affect the behaviour of the peptide on such a column, 90 % of the radioactivity being recovered in the pH 3·7 eluate. These results show that the radioactivity present in the urine of rabbits injected with ^{35}S-labelled fibrinopeptide B could no longer be attributed to the intact peptide.

The remainder of the original urine was subsequently concentrated to 3 ml. and fractionated on a Dowex 50 column (H^+ form) exactly as described above for the urines of animals receiving free L-tyrosine *O*[^{35}S]-sulphate. The effluent from the column was collected in fractions of 3 ml. and sufficient 4 N-hydrochloric acid was added to each fraction to give a final concentration of 1·0 N before hydrolysing for 4 hr. at 100°. After cooling, 3 ml. of 0·15 M-potassium sulphate and 4 ml. of aqueous 10 % (w/v) barium chloride were added to each hydrolysate and the radioactivity of the precipitated $Ba^{35}SO_4$ was determined. The results showed that two peaks of radioactivity were present in the first acidic eluate (30 ml.) leaving the column. The positions of emergence of the peaks coincided approximately with those obtained with the urines of rabbits that had received free L-tyrosine *O*[^{35}S]-sulphate (Fig. 1). No radioactivity could be detected in the remainder of the water eluate or in the ammonia eluate. The behaviour of authentic rabbit fibrinopeptide B on such a column was checked as follows. The ^{35}S-labelled peptide (2 mg.) was dissolved in 30 ml. of normal rabbit urine and the whole concentrated to 3 ml. by freeze-drying. The concentrated solution was then fractionated on Dowex 50 resin exactly as described above. Fig. 1 shows that the fibrinopeptide was retained on the column during the elution with water but was eluted by aqueous 1 M-ammonia solution. These results again indicate that injected fibrinopeptide B is degraded *in vivo*, and provide strong presumptive evidence that free L-tyrosine *O*-sulphate and *p*-hydroxyphenylacetic acid sulphate are products of this degradation.

DISCUSSION

The present work and that of Dodgson *et al.* (1961) and Powell *et al.* (1963) shows that similarities and differences exist between the ways in which free L-tyrosine *O*[^{35}S]-sulphate is metabolized by the rat and rabbit respectively. Thus, with both species, no appreciable hydrolysis of the ester occurs *in vivo* and this is in accordance with the finding (Dodgson, Rose & Tudball, 1959) that mammalian arylsulphatases show little or no activity *in vitro* towards L-tyrosine *O*-sulphate. In both rat and rabbit the ester is rapidly eliminated in the urine and only traces appear to find their way into the intestine via the bile. In the rat, however, L-tyrosine *O*[^{35}S]-sulphate is apparently rapidly deaminated to yield the [^{35}S]sulphate esters of *p*-hydroxyphenylpyruvic acid and *p*-hydroxyphenylacetic acid, and unchanged starting material is not often detected in the urine (at a dose 1·5 mg./200 g. body wt.). In contrast, the greater part of the dose (2 mg./rabbit) of the ester is eliminated by the rabbit in an unchanged form and similar findings have been made (Mr T. Flanagan, unpublished work) with a dose equivalent to that given to rats. The rabbit therefore appears to be less able to deaminate L-tyrosine *O*[^{35}S]-sulphate than the rat and under these circumstances the ester might perhaps have been expected to be

present in normal rabbit urine (cf. human urine; Tallan *et al.* 1955). In this connexion Grimes (1959), during studies on rabbits that had received $Na_2{}^{35}SO_4$ by stomach tube, failed to detect L-tyrosine *O*[^{35}S]-sulphate in the 24 hr. urine samples. However, it is doubtful whether this type of experiment is valid if urinary L-tyrosine *O*-sulphate has its origin in fibrinogen, the turnover rate of which is relatively low (half-life 70 hr. in rabbits; see Blombäck *et al.* 1960).

A further difference between rat and rabbit is that *p*-hydroxyphenylpyruvic acid [^{35}S]sulphate has not been detected in the rabbit urines that have so far been examined. The reason for this difference is not yet clear. However, the compound readily undergoes spontaneous conversion into *p*-hydroxyphenylacetic acid [^{35}S]sulphate (Powell *et al.* 1963) and, although the point has not been checked experimentally, there is reason to believe that this conversion would be accelerated under the slightly alkaline conditions that normally prevail in rabbit urine and retarded in the presence of rat urine, which is usually slightly acid (cf. Bloxam, Day, Gibbs & Woolf, 1960).

The failure to detect incorporation of injected L-tyrosine *O*[^{35}S]-sulphate into fibrinogen is in agreement with present knowledge about the inability of free L-tyrosine to undergo biological sulphation. However, too much emphasis cannot be placed on the present findings in view of the relatively low specific activity (from the point of view of incorporation studies) of the L-tyrosine *O*[^{35}S]-sulphate used and the speed with which the ester is eliminated by the kidneys.

The metabolism studies on fibrinopeptide B have established that the L-tyrosine *O*[^{35}S]-sulphate residue of the peptide does not undergo hydrolysis *in vivo*. In this connexion, Tudball (1959) has failed to achieve the enzymic desulphation of bovine fibrinopeptide B *in vitro*. It has also been shown that the labelled metabolites which appear in urine are not retained on Dowex 50 resin under conditions of pH known to result in the binding of the peptide. Further, fractionation of the urine on the acid (H^+) form of Dowex 50 resin results in the separation of two radioactive fractions. The positions of emergence of these fractions from the column coincide with those obtained when the urines of rabbits receiving free L-tyrosine *O*[^{35}S]-sulphate are similarly fractionated. In the latter the fractions have been shown to contain *p*-hydroxyphenylacetic acid [^{35}S]sulphate and unchanged starting material respectively, in order of emergence from the column. Although the evidence is not unequivocal it is strongly presumptive of the fact that the injected fibrinopeptide has been degraded *in vivo* with the consequent liberation of free L-tyrosine *O*[^{35}S]-sulphate. The results therefore support the view (Segal & Mologne, 1959; Dodgson, 1958; Blombäck *et al.* 1960) that the L-tyrosine *O*-sulphate which is found in human urine originates from fibrinogen or other, as yet unknown, proteins containing L-tyrosine *O*-sulphate residues.

SUMMARY

1. The bulk of the dose of intraperitoneally injected potassium L-tyrosine *O*[^{35}S]-sulphate was rapidly eliminated by rabbits in an unchanged form in the urine. Small amounts of *p*-hydroxyphenylacetic acid [^{35}S]sulphate also appeared in the urine.

2. Fibrinopeptide B containing a L-tyrosine *O*[^{35}S]-sulphate residue was prepared from the fibrinogen of rabbits that had been injected with $Na_2{}^{35}SO_4$.

3. Intravenous or intraperitoneal injection of the labelled fibrinopeptide into rabbits was followed by the appearance of the ^{35}S in an ester-bound form in the urine. The behaviour on ion-exchange columns of this labelled urinary material showed that it was no longer associated with fibrinopeptide B.

4. Two radioactive metabolites could be separated from the urine on Dowex 50 (H^+ form) resin. The positions of emergence of these compounds from the column coincided approximately with those of free L-tyrosine *O*[^{35}S]-sulphate and *p*-hydroxyphenylacetic acid [^{35}S]sulphate.

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The Occurrence of Bis-(2-Amino-2-Carboxyethyl) Trisulphide in Hydrolysates of Wool and other Proteins

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Acid hydrolysates of wool have been found to contain sulphur compounds other than cystine, cysteine and methionine, and experiments in which wool was hydrolysed in the presence of [^{35}S]cystine showed that most of these unidentified sulphur compounds were formed by the decomposition of cystine during hydrolysis (Lewis, Robson & Tiler, 1960). The fact that these decomposition products had the same specific activity as the cystine in the hydrolysate has been made the basis of a method for determining cystine plus cysteine in proteins (Fletcher & Robson, 1962*a*). Most of these compounds reduced phosphotungstic acid, either alone or in the presence of sodium bisulphite, and their presence in wool hydrolysates provided an explanation for the discrepancy between the chromatographic and colorimetric analyses for cystine (Corfield & Robson, 1955). The most abundant of these compounds (given the trivial designation 'S 1' by Lewis *et al.* 1960) accounted for 6% of the sulphur in a 40 hr. hydrolysate of wool. The present paper describes its isolation and characterization as bis-(2-amino-2-carboxyethyl) trisulphide. A short account of this work has already appeared (Fletcher & Robson, 1962*b*).

Lewis *et al.* (1960) showed that small amounts of S 1 were formed when cystine was boiled in 5N-hydrochloric acid in the presence of all the other protein amino acids, and that its formation appeared to depend on the presence of tryptophan. However, the finding of a trace of ^{35}S-labelled S 1 in a solution of [^{35}S]cystine in 0·1N-hydrochloric acid which had stood for some time suggested that the influence of tryptophan was indirect, and possibly connected with the interaction of cystine and tryptophan to form cysteine (Olcott & Fraenkel-Conrat, 1947). The chromatographic behaviour of S 1 showed that it was very labile under neutral and alkaline conditions, and that cystine and *meso*-cystine were among the products of decomposition, indicating a close structural relationship between S 1 and cystine.

Thus far investigations had been conducted on a few milligrams of impure S 1, but much larger amounts were required for a more detailed study. However, before the isolation of S 1 on a large scale from wool hydrolysates was attempted, other possible sources were investigated. The appearance of traces of S 1 in an aged acid solution of [^{35}S]-cystine might have been due to self-irradiation. To test this possibility, an acid solution of inactive cystine was given a massive dose of γ-radiation from a ^{60}Co source. Although S 1 was present in the irradiated solution, the yield was not sufficient to make this a practicable method of preparation. Subsequently, hydrolysates of insulin, ribonuclease, bovine plasma albumin, lysozyme and β-lactoglobulin were examined for the presence of S 1. The amounts of S 1 present in hydrolysates of the first three proteins increased with increasing time of hydrolysis: after 40 hr. the percentages of protein sulphur present as S 1 were 1·9, 2·0 and 2·7 for insulin, bovine plasma albumin and ribonuclease, respectively; S 1 was not found in hydrolysates of lysozyme or β-lactoglobulin. The yields of S 1 in these experiments were much less than those obtained from wool hydrolysates, and the latter, therefore, were used exclusively for the preparation of S 1 on a large scale.

For the preparative isolation of S 1, wool hydrolysates were chromatographed initially on columns of Zeo-Karb 225 (H^+ form; X 4) resin by elution with 2·5N-hydrochloric acid. This pro-