FURTHER STUDIES OF THE METABOLISM OF CYSTINE IN SHEEP

By A. M. Downes*, L. F. Sharry*, and A. R. Till*

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Summary

Further evidence for the binding of cystine to plasma proteins *in vivo* is presented.

Ovine plasma albumin was labelled *in vitro* with L-[^{35}S]cystine (to give albumin–S–^{35}S–Cy) and with ^{131}I. The doubly labelled albumin was then administered intravenously to a sheep and the rate of disappearance of the two labels from the circulating plasma was measured. Another sheep was similarly dosed with [^{131}I]albumin–S–S–[3-^{14}C]Cy. The initial rate of disappearance of the ^{131}I was much slower (half-time *c.* 12 hr) than that of either the ^{35}S or ^{14}C (half-time 150, 105 min respectively). The rate of disappearance of the ^{35}S or ^{14}C was therefore presumed to represent the rate of the thiol–disulphide exchange reaction of albumin–S–S–Cy with free cysteine in sheep plasma *in vivo*, and not simply the rate of equilibration of the circulating and extravascular plasma proteins.

Three experiments were performed in which the fate of intravenous doses of L-[^{35}S]cysteine was studied. Relatively much less of the ^{35}S became disulphide-bound than after intravenous doses of L-[^{35}S]cystine.

In three other experiments a small amount (less than 10 mg) of L-[^{35}S]cystine was infused intravenously at a constant rate into a sheep, and the degree of labelling of some of the constituents of the plasma was measured. After 4–5 hr the concentrations of free cystine-^{35}S and of disulphide-bound cystine-^{35}S became approximately constant, more than half (56–70%) of the non-peptide cystine-^{35}S then being in the disulphide-bound form.

When an intravenous tracer dose of L-[^{35}S]cystine was followed 30–60 min later by a relatively large intravenous infusion of unlabelled cyst(e)ine, the disulphide-bound ^{35}S was rapidly displaced from the plasma proteins and was then accounted for as free cystine.

The concentration of free cyst(e)ine in the plasma from five sheep was found to range from 0·16 to 0·82 mg/100 ml. These values are lower than those reported for other species.

The significance of the results is discussed.

I. Introduction

The results of previous experiments have shown that cystine takes part in a thiol–disulphide exchange reaction with plasma proteins and that at least some half-cystine residues are bound to these proteins *in vivo* in the sheep (Downes 1961*b*). The mixed disulphide between a plasma protein and cystine will be symbolized as P–S–S–Cy throughout this paper. ^{35}S in the P–S–^{35}S–Cy form will be referred to as "disulphide-bound"; ^{35}S in other positions of the protein molecule will be called

* Division of Animal Physiology, CSIRO, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

"peptide-bound". To our knowledge there are no published values for the concentration of free cysteine or cystine (together called "cyst(e)ine") or of P–S–S–Cy in sheep plasma. Even for other species the metabolic relationships between cysteine and cystine are not fully understood (Brigham, Stein, and Moore 1960). The present experiments were carried out to fill part of this gap in our knowledge and to provide a background to studies of the mechanism of incorporation of cystine into wool keratin.

When P–S–^{35}S–Cy is injected intravenously into a sheep the ^{35}S disappears exponentially from the circulating plasma with a half-time of 1–2 hr (Downes 1961*b*). This was attributed to the thiol–disulphide exchange reaction. However, the possibility remained that the label was simply being removed by equilibration of the circulating plasma proteins with the extravascular pool. If this had been so, the rate of equilibration would have been much faster than that observed in animals of a comparable size but of a different species (Sterling 1951; Forker, Chaikoff, and Reinhardt 1952; Baker and Wycoff 1961; Takeda and Reeve 1963). This possibility has now been ruled out by measuring simultaneously the rates of disappearance of intravenously administered albumin–S–^{35}S–Cy (or albumin–S–S–[3-^{14}C]Cy) and [^{131}I]albumin. From these results it follows that, on infusing L-[^{35}S]cystine intravenously at a constant rate into a sheep, an equilibrium between the free and disulphide-bound [^{35}S]cystine should be established within a few hours, at which stage the concentrations of ^{35}S in these two forms will be proportional to the actual concentrations of cystine in the two forms. Thus the relative concentrations of free and disulphide-bound cystine should be measurable by radiochemical techniques. The results of three experiments of this type are presented here, together with the results of chemical analyses of the concentration of free cyst(e)ine in sheep plasma. Further evidence for the thiol–disulphide exchange reaction *in vivo* is presented, and the fate of intravenously administered L-[^{35}S]cysteine is compared with that of L-[^{35}S]cystine.

II. Materials and Methods

(*a*) *General*

The animals used for the tracer experiments were kept in metabolism cages of the type described by Till and Downes (1963), in a room held at 22($\pm$ 3)°C. Some of the sheep whose blood plasma was analysed for free cyst(e)ine were housed in single or grouped pens in an animal house. All sheep, except those listed in Table 3, were fed once daily, between 9 and 10 a.m., a ration comprising 700–1000 g of equal parts of lucerne and wheaten chaff.

The doses of L-[^{35}S]cystine (from Schwarz BioResearch Inc., New York, and from the Radiochemical Centre, Amersham, England) were prepared and the radiochemical purity checked as described previously (Downes and Lyne 1961). L-[^{35}S]Cysteine was prepared by reducing L-[^{35}S]cystine by the method of Emiliozzi, Pichat, and Herbert (1959) and was injected immediately into the experimental sheep. Samples of the solution were taken before the reduction and just before the injection and were chromatographed on paper with n-butanol–water–formic acid (75 : 15 : 10

by volume). Radioautography of the chromatograms revealed the presence of only a trace of cystine after the reduction. Unlabelled L-cystine was obtained from L. Light & Co. Ltd., Colnbrook, England.

The methods of blood analysis and of radioassay have been described (Downes 1961*a*, 1961*b*). Blood samples were collected in precooled bottles containing heparin, and centrifuged immediately at 4000 *g* and 5°C for 15 min. The precipitation of plasma proteins with trichloroacetic acid (TCA) was carried out within 30 min of the blood sample being taken. Under these conditions the binding of cystine by the plasma proteins *in vitro* is reduced to less than 10% (Downes 1961*b*).

(*b*) *Preparation of Labelled Albumin*

(i) *Albumin–S–^{35}S–Cy.*—Albumin (0·2 g), which had been isolated from sheep plasma as described by Schwert (1957), was dissolved in 4 ml phosphate buffer (M/15, pH 7·4). An aqueous solution of L-[^{35}S]cystine hydrochloride (0·2 ml; 0·46 mg cystine; 17 μc) was added and the solution was incubated at 39°C for 6 hr. The protein was then separated from unbound cystine by elution with 0·9% (w/v) NaCl from a column (17 by 30 cm) of Sephadex G-25. The column eluate was collected in fractions, whose ^{35}S contents were measured as follows. Portion (0·01 ml) of each fraction was applied to a disk of Whatman 3 MM paper ($\frac{15}{16}$ in. diam.) placed in the bottom of a glass vial and dried under an infrared lamp. A solution of a scintillator [2 ml of toluene containing 2,5-diphenyloxazole (0·4% w/v) and 1,4-bis-2-(5-phenyloxazolyl)-benzene (0·01% w/v)] was then added and the sample was counted in a Packard Tricarb liquid scintillation spectrometer, model 314 EX. The fractions corresponding to the first peak eluted from the column were combined and were then ready for use.

(ii) *Albumin–S–S–[3-^{14}C]Cy.*—This was prepared as described above, except that (DL + meso)-[3-^{14}C]cystine (0·32 mg cystine; 19 μc; from the Radiochemical Centre, Amersham) was substituted for L-[^{35}S]cystine.

(iii) [^{131}I]*Albumin.*—This was prepared at a level of 1 atom of iodine per molecule by the method of McFarlane (1958). The unbound ^{131}I was removed on a column of Sephadex G-25 as described for albumin–S–^{35}S–Cy.

(*c*) *Analysis of Free Cystine in Plasma*

About 50 ml of blood was withdrawn from the jugular vein of each sheep studied and the plasma was collected rapidly as described above in Section II(*a*). Picric acid [50 ml 1% (w/v) in water] was added to each of two 10-ml samples of the plasma within 1 hr of the time of bleeding and the precipitated proteins were removed by centrifugation. L-[^{35}S]Cystine (0·1 ml solution containing 0·1 μc, 1·8 μg) was then added to 50 ml of the filtered supernatant to provide a recovery factor for the estimation, since trial analyses had shown a wide variation in the recovery of known amounts of cystine as cysteic acid.

The picric acid was removed on a column (1·3 by 10 cm) of Deacidite FF (acetate form) as described by Stein and Moore (1954). The column eluate was collected in a weighed flask and a portion kept for measurement of ^{35}S and total

α-amino nitrogen. The rest was freeze-dried and the product oxidized with performic acid (5 ml) for 15 min at 0°C. Ice-cold distilled water (50 ml) was then added and the solution was again freeze-dried. The residue was dissolved in 2–3 ml of acetate buffer (0·2N, pH 2·2) and the cysteic acid separated from the other amino acids by chromatography on a column (0·9 by 150 cm) of Amberlite IR120 at 50°C as described by Stein and Moore (1954). The column effluent was collected in 2-ml

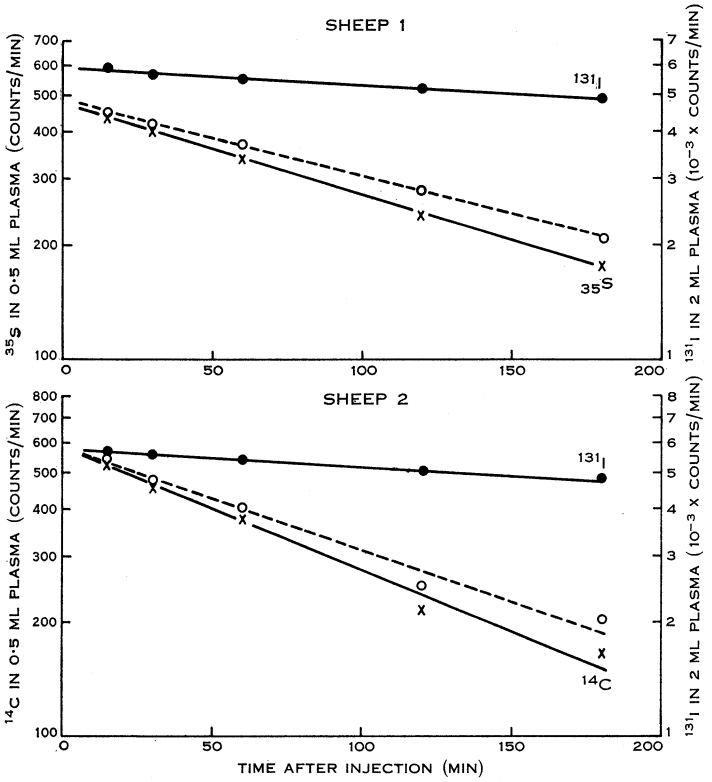

Fig. 1.—Sheep 1: Comparison of rates of disappearance from circulating plasma of ^{131}I and ^{35}S following intravenous administration of [^{131}I]albumin–S–^{35}S–Cy. Sheep 2: Similar comparison of ^{131}I and ^{14}C following intravenous dose of [^{131}I]albumin–S–S–[3-^{14}C]Cy. The rate of loss of ^{131}I is presumed to indicate the rate of passage of albumin into extravascular sites. The faster rates of loss of ^{35}S and ^{14}C are presumably due to the thiol–disulphide exchange reaction in the plasma *in vivo*. The difference between the two rates is indicated by the broken line, which presumably indicates the net rate of loss due to the exchange reaction.

fractions, the first 60 of which were analysed for α-amino nitrogen as described by Moore and Stein (1954) and for ^{35}S. The amount of cysteic acid was estimated by comparing the colour yields of the appropriate fractions with those of standard amounts of cysteic acid, and was then corrected by dividing by the proportion of the added ^{35}S recovered in the same fractions. This proportion varied from 0·3 to 0·8. The rest was eluted just before cysteic acid and was probably present as sulphate, which was shown to appear in this position on the chromatogram. Within experimental error, all of the ^{35}S applied to the column was recovered in these two peaks.

III. Experimental Results

(*a*) *Rate of Equilibration of Plasma- and Extravascular Albumin*

Sheep plasma albumin which had been labelled with ^{131}I and with [^{35}S]cystine (albumin–S–^{35}S–Cy) was injected intravenously into a sheep. Another sheep was given [^{131}I]albumin–S–S–[3-^{14}C]Cy. Blood samples were taken at intervals and the concentration of each isotope in the plasma was measured. The results (Fig. 1)

Table 1

Plasma volumes of sheep dosed with doubly labelled albumin–S–S–Cy

Sheep	Isotope	Plasma Volume* (ml)
1	^{131}I	2120
	^{35}S	1970
2	^{131}I	2140
	^{14}C	2120

* Calculated from dose of each isotope and concentration in plasma at zero time, obtained by extrapolation.

showed that the concentration of ^{35}S and ^{14}C fell exponentially ($T_{\frac{1}{2}} = 125$, 90 min respectively) at about the same rate as observed previously (Downes 1961*b*), while the concentration of ^{131}I fell much more slowly. The difference between the slopes of the two lines is shown in Figure 1 as a broken line, which presumably indicates

Table 2

Comparison of labelling of plasma proteins from sheep dosed intravenously with L-[^{35}S]cysteine and L-[^{35}S]cystine

Dose	Sheep	Disulphide-bound ^{35}S / Peptide-bound ^{35}S		Peptide-bound ^{35}S (% of total plasma ^{35}S)	
		30 Min*	60 Min*	30 Min*	60 Min*
L-[^{35}]Cysteine	1	1·8	0·6	15	41
	2	4·5	1·6	8·0	27
	3	2·5	—	6·3	—
L-[^{35}S]Cystine	4	8	3·0	4·0	15
	5	40	7·6	1·3	8·0
	6	23	8·0	2·4	7·7
	7	22	—	3·0	—

* Period of time from dosing to blood sampling.

the true rate of the exchange of half-cystine and between albumin–S–S–Cy and cyst(e)ine in the plasma of these sheep *in vivo* ($T_{\frac{1}{2}} = 150$, 105 min). Two estimates of the plasma volume were made in each sheep, from the dose of each isotope and the concentration in the plasma at zero time, obtained by extrapolation. The two sets of results (Table 1) agreed reasonably well, showing that the observed rate of fall of the ^{35}S or the ^{14}C was in fact the rate of disappearance of the disulphide-bound half-cystine residues which had been injected.

(*b*) *Fate of Intravenous Doses of* L-[^{35}S]*Cysteine*

Three sheep were given intravenous tracer doses of L-[^{35}S]cysteine. In two of these sheep the dose was followed a short time later by an intravenous infusion of a relatively large amount of unlabelled cyst(e)ine [see Section III (*d*)]. Blood samples (10–20 ml) were taken at intervals, and the concentrations of total ^{35}S, peptide-bound ^{35}S, disulphide-bound ^{35}S, and free cystine ^{35}S in each plasma sample were determined.

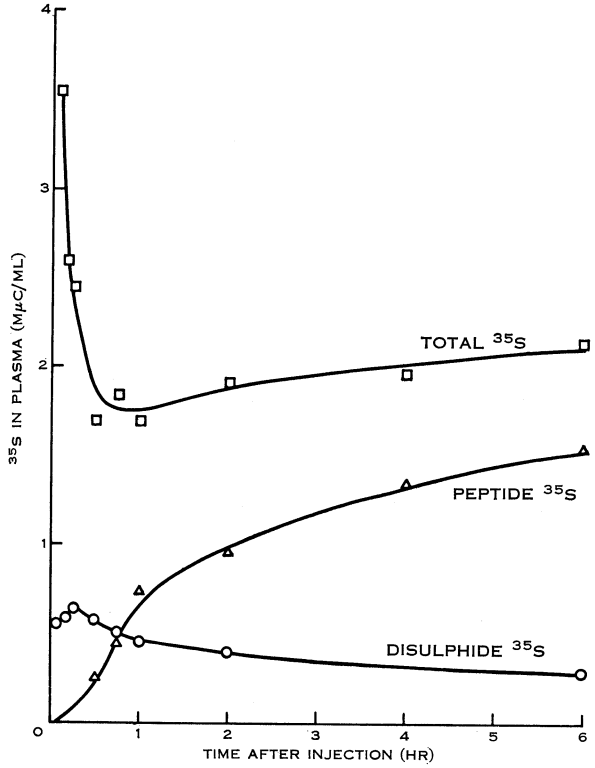

Fig. 2.—Distribution of ^{35}S in plasma at various times following the intravenous administration of L-[^{35}S]cysteine (18 mg; 86 μc) to a sheep.

In each experiment some of the label appeared in the disulphide-bound form. Figures 2 and 7(*a*) show this for two of the sheep. However, the distribution of the ^{35}S was different to that found after intravenous doses of L-[^{35}S]cystine, and the concentrations of ^{35}S as P–S–^{35}S–Cy fell more slowly from its peak value than after doses of L-[^{35}S]cystine. These differences are illustrated by the results in Table 2 which show that relatively much more of the ^{35}S became disulphide-bound following doses of L-[^{35}S]cystine than following L-[^{35}S]cysteine.

(*c*) *Intravenous Infusions of* L-[^{35}S]*Cystine*

Three experiments were carried out in which small amounts (less than 10 mg) of L-[^{35}S]cystine were infused at a constant rate into the left jugular veins of three

sheep for about 3, 9, and 48 hr respectively. Blood samples (10–20 ml) were taken at intervals from the other jugular vein and the plasma obtained as described in Section III(*b*). The distribution of ^{35}S in each plasma sample was then determined. Another portion (2 ml) of each plasma sample was added to unlabelled L-cystine (400 μg in 0·1 ml 0·05N HCl) and allowed to stand at room temperature for 24 hr before precipitation of the proteins with TCA. This treatment was carried out to see if any of the ^{35}S bound *in vivo* could be removed by exchange with non-radioactive cystine.

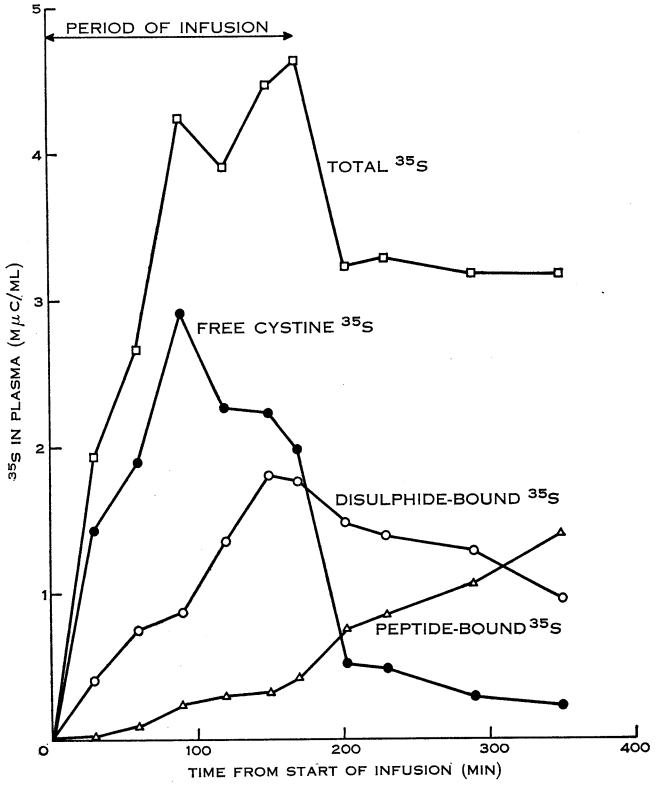

Fig. 3.—Distribution of ^{35}S in plasma during and after a 170-min intravenous infusion of L-[^{35}S]cystine at a constant rate (0·74 μc/min) into a sheep.

In the first experiment a dose of L-[^{35}S]cystine (125 μc; 10 mg) was infused at the rate of 0·74 μc/min for 170 min. Although there was some scatter in the results (Fig. 3), the concentration of free cystine ^{35}S became approximately constant during the last 2 hr of the infusion, while the concentration of P–S–^{35}S–Cy continued to increase until, at the end of the infusion, the concentrations of free and of disulphide-bound ^{35}S were about the same. Subsequently the concentration of free cystine ^{35}S fell rapidly, and that of P–S–^{35}S–Cy fell much more slowly, in agreement with previous results for intravenous doses (Downes 1961*b*). The concentration of peptide-bound ^{35}S was relatively small but increased progressively, presumably as newly synthesized

plasma proteins were released into the circulation by the liver. During the first 150 min of the infusion practically all of the ^{35}S in the plasma could be accounted for as free cystine plus TCA-precipitable (peptide-bound plus disulphide-bound) material. Later there was a small discrepancy presumably due to the presence of other labelled metabolites.

The treatment of the later plasma samples with unlabelled cystine for 24 hr before precipitating the proteins with TCA removed some of the disulphide-bound ^{35}S, which appeared as free cystine (Fig. 4). The plasma samples obtained during

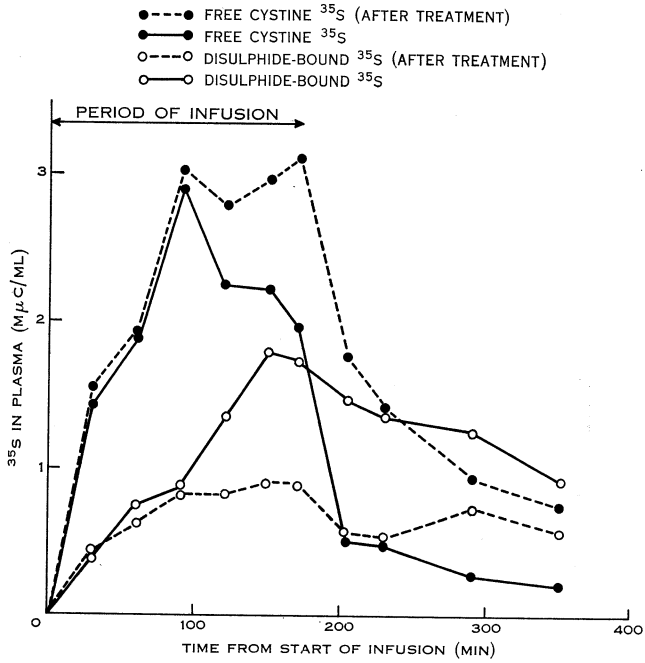

Fig. 4.—Effect of treatment of plasma samples from first infusion experiment (Fig. 3) with carrier L-cystine (200 μg/ml plasma) at room temperature for 24 hr and demonstration that the displaced ^{35}S was present as cystine. See text for full explanation.

the first 90 min of the infusion gave the same results for the specific activity of the proteins, whether they had been precipitated immediately or treated with unlabelled cystine for 24 hr before precipitation. However, this was probably due to the fact that the proportions of free and disulphide-bound ^{35}S were about the same *in vivo* during this period as the final proportions reached *in vitro* after adding the unlabelled cystine and allowing the thiol–disulphide exchange to proceed for 24 hr. In other words, under the experimental conditions used, the proportion of disulphide-bound ^{35}S *in vivo* had to be greater than about 22% before the displacement of the ^{35}S with more (unlabelled) cystine could be demonstrated.

The results of the second experiment, in which a dose of L-[^{35}S]cystine (88 μc; 3·7 mg) was infused over a period of 9 hr, showed that the concentrations of free

and of disulphide-bound [^{35}S]cystine were approximately constant during the last 5 hr of the infusion (Fig. 5). During this period 60–70% of the non-peptide [^{35}S]cystine was in the P–S–^{35}S–Cy form *in vivo*.

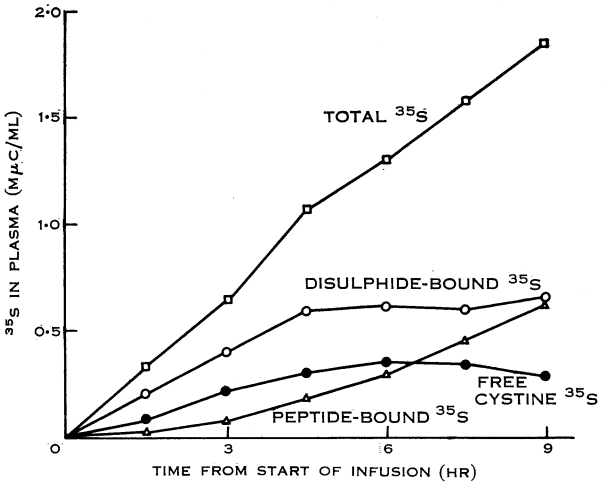

Fig. 5.—Distribution of ^{35}S in plasma during a 9-hr intravenous infusion (9·8 μc/hr) of L-[^{35}S]cystine into a sheep.

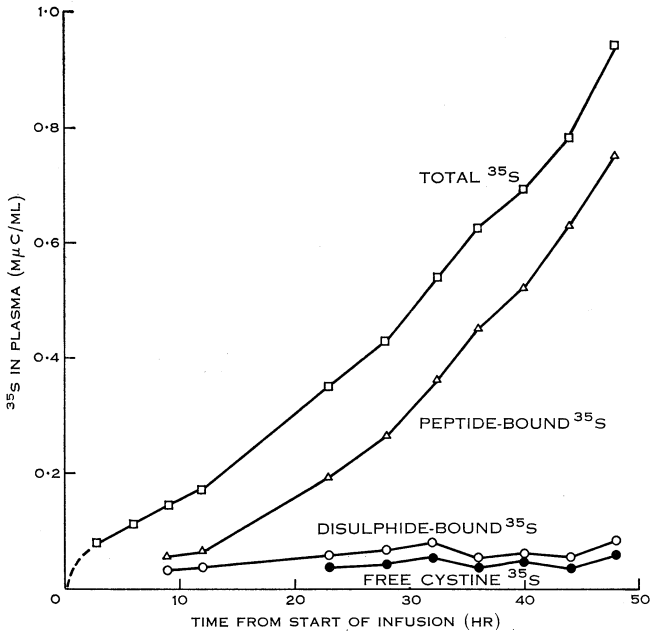

Fig. 6.—Distribution of ^{35}S in plasma during a 48-hr intravenous infusion (1·1 μc/hr) of L-[^{35}S]cystine into a sheep.

A portion (5 ml) of the plasma from the blood sample taken just before the end of the infusion was treated with unlabelled cystine (1 mg) for 24 hr at room temperature. The proteins were then precipitated with picric acid and the free amino

acids isolated and chromatographed on Amberlite IR120 as described by Stein and Moore (1954). No ^{35}S was detectable in the portions of the effluent corresponding to cysteic acid, glutathione, or the mixed disulphide of glutathione and cystine. 91% of the radioactivity applied to the column was recovered in the cystine region.

The third infusion (53 μc; 4·8 mg cystine), over a period of 48 hr, again showed that the concentrations of free and of disulphide-bound [^{35}S]cystine remained approximately constant while the peptide-bound ^{35}S increased (Fig. 6). Again the

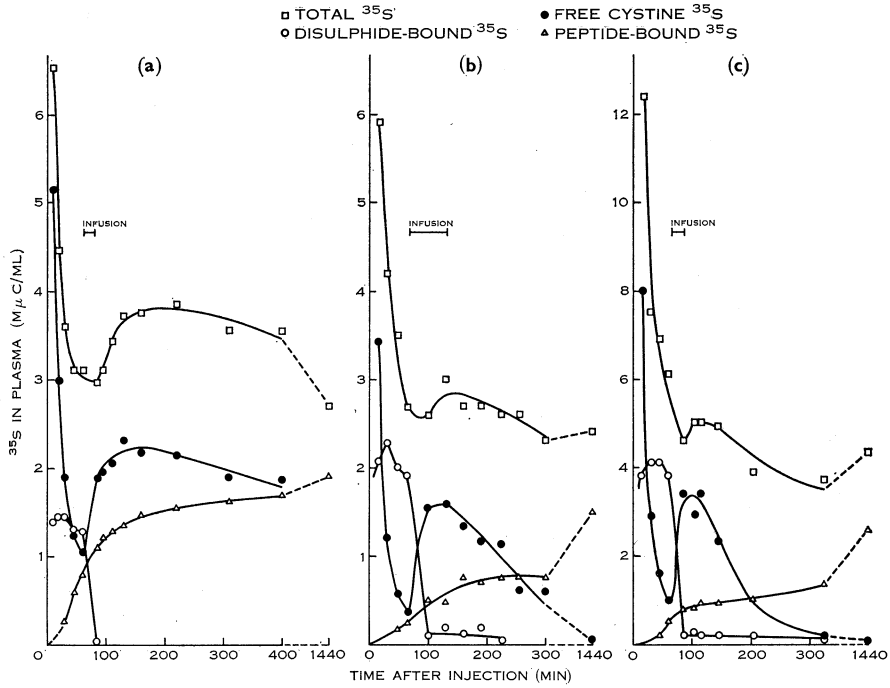

Fig. 7.—Effect of intravenous infusion of large mass of cyst(e)ine (as the hydrochloride) on the distribution of ^{35}S in plasma of sheep which had received an intravenous dose of L-[^{35}S]cysteine or L-[^{35}S]cystine. The period of each infusion is indicated by a horizontal line.

Dose at zero time	*Infusion*
(*a*) L-[^{35}S]cysteine (6·6 mg; 165 μc)	4 g L-cysteine + 4 g L-cystine
(*b*) L-[^{35}S]cystine (4·8 mg; 142 μc)	5 g L-cysteine + 5 g L-cystine
(*c*) L-[^{35}S]cystine (4·5 mg; 149 μc)	2 g L-cystine

disulphide-bound ^{35}S accounted for more than half (56–64%) of the non-peptide [^{35}S]cystine. Treatment of the plasma samples from both the second and third infusions with unlabelled cystine removed some of the disulphide-bound ^{35}S, which appeared as free [^{35}S]cystine, confirming the results shown in Figure 4.

(*d*) *Displacement of Disulphide-bound* [^{35}S]*Cystine* in vivo

In two of the experiments in Section III (*b*) and in four others in which sheep were given intravenous tracer doses of L-[^{35}S]cystine the dose was followed a short

time later by an intravenous infusion of a relatively large mass of unlabelled cystine or cyst(e)ine. At various time intervals blood samples were taken and the distribution of ^{35}S in the plasma determined. The blood samples all had about the same haematocrit value and there was no evidence of haemolysis, even though as much as 8 g of cyst(e)ine (as the hydrochloride) was injected over a 20-min period. In each case similar results were obtained, and are exemplified in Figure 7. Some of the ^{35}S became disulphide-bound *in vivo* as in earlier experiments. Most of this bound ^{35}S was displaced by the infusion of the massive dose of unlabelled cyst(e)ine, and was then present as free cystine. This confirmed that the ^{35}S designated as

TABLE 3

CONCENTRATION OF FREE CYST(E)INE IN SHEEP PLASMA

Sheep	Body Weight (kg)	Breed	Age (yr)	Daily Ration	Concentration in Plasma (mg/100 ml)	
					Cyst(e)ine*	Total α-Amino Nitrogen
MA52	42	Corriedale wether	6	800 g R9§	0·22, 0·24, 0·14, 0·24 (0·21)	8·2
MA52†	40	Corriedale wether	6	1400 g M2§	0·18, 0·14 (0·16)	3·3
MA51†	45	Corriedale wether	7	1400 g M2§	0·33, 0·37 (0·35)	4·7
2074‡	38	Merino ewe	6	1000 g M2	0·60, 0·63 (0·62)	5·8
2104	55	Merino ewe	6	1700 g M2	0·53, 0·47 (0·50)	5·4
2085	56	Merino ewe	6	2000 g M2	0·91, 0·73 (0·82)	6·7

* Each value represents a separate analysis.

† These sheep were penned together and received a total of 1400 g per day.

‡ Sheep 2074 and another sheep were penned together and received a total of 2000 g per day.

§ R9: 50% wheaten chaff, 50% lucerne chaff; M2: 50% lucerne chaff, 50% oats.

disulphide-bound must have been present as half-cystine residues. In most of these experiments, the infusion of the large mass of cyst(e)ine was followed by a return to the plasma of some of the ^{35}S which had previously been removed. This elevated concentration of ^{35}S lasted for several hours, possibly showing that the tissues had become saturated with cyst(e)ine and that the excess was carried around in the blood. There was a considerable variation in the extent of the rise in plasma ^{35}S after the infusion, but this is hardly surprising in view of the large number of variables.

(e) *Concentration of Free Cyst(e)ine in Sheep Plasma*

The results for the concentration of free cyst(e)ine in the plasma from five sheep are shown in Table 3. The concentration ranged from 0·16 to 0·82 mg/100 ml.

For comparison, values published for several other species are listed in Table 4. The concentration of total α-amino nitrogen, which is also shown in Table 3, ranged from *c.* 3–8 mg/100 ml.

IV. Discussion

The above results provide further evidence for the binding of half-cystine residues by sheep plasma proteins *in vivo*. The results obtained from the intravenous infusions of L-[^{35}S]cystine indicate that there is probably more disulphide-bound half-cystine than free cyst(e)ine in sheep plasma *in vivo*. No direct analyses of the amount of P–S–S–Cy have yet been made, and in view of the low total concentration of free cyst(e)ine, no attempt was made in the present experiments to measure the relative amounts of the oxidized and reduced forms.

Table 4

Comparison of free cyst(e)ine concentration in sheep plasma with values published for other species

Species	Concentration of Cyst(e)ine (mg/100 ml)	Reference
Sheep	0·16–0·82	Present work (Table 3)
Man	0·45–1·62	Iob, McMath, and Coon (1963)
	0·85–1·74	Brigham, Stein, and Moore (1960)
	0·96–1·20	Eagle, Oyama, and Piez (1960)
	1·08–1·30	Stein and Moore (1954)
	0·82–2·0	Johnson and Bergeim (1951)
	1·42–2·18	Schreier and Pluckthun (1950)
	0·71–1·14	Brown and Lewis (1941)
Dog	0·47–1·47	Hier (1947)
Horse	1·6	Numata (1940)
Pig	0·64–0·87	Brown and Lewis (1941)
Rabbit	0·93–1·11	
Rat	0·75–0·86	

It is not possible to state whether the disulphide-binding is of special significance for the sheep because, to our knowledge, there are no published data showing the relative amounts of free and disulphide-bound cystine in the plasma of other species. However, since a portion of the extravascular plasma protein is probably stored in the skin of sheep, as has been shown in the rabbit by Humphrey, Neuberger, and Perkins (1957), it is possible that the binding phenomenon provides a mechanism for the storage of some cystine (as half-cystine residues) in the plasma and in the skin. This cystine could be released, possibly by the thiol–disulphide exchange reaction with cysteine, to the follicles for incorporation into the growing wool fibres. Downes, Sharry, and Till (1964) have shown that some of the ^{35}S injected intradermally as albumin–S–^{35}S–Cy is incorporated into the wool growing at the site of the injection.

The results in Figure 1 show that the rate of equilibration of the plasma and extravascular albumin in sheep is about the same as in humans (Sterling 1951;

Takeda and Reeve 1963) and in dogs (Forker, Chaikoff, and Reinhardt 1952; Baker and Wycoff 1961). It therefore seems reasonable to attribute to the thiol–disulphide exchange reaction the much faster rate of disappearance from the circulation of P–S–^{35}S–Cy. Once displaced by this exchange reaction, most of the label would disappear at an even faster rate as the free amino acid (Downes 1961*b*). That the half-time for the exchange reaction in normal plasma *in vivo* is of the order of 1– 2 hr is supported by the fact that, during a constant infusion of L-[^{35}S]cystine, it took several hours for the disulphide-bound ^{35}S to reach a constant concentration in the plasma (Fig. 5). The disulphide-bound ^{35}S was, however, displaced much more rapidly by the infusion of large amounts of L-cyst(e)ine (Fig. 7), presumably through a mass action effect.

The results in Table 2, in spite of the large variations, show that much more disulphide-bound ^{35}S appeared after doses of L-[^{35}S]cystine than after doses of L-[^{35}S]cysteine. This is to be expected if the binding occurs by the reaction of a protein –SH group with a cystine molecule. Thus some of the ^{35}S would have to be converted to cystine, either by direct oxidation of the [^{35}S]cysteine or by the thiol–disulphide exchange:

$$\text{P–S–S–Cy} + \text{Cy}^{35}\text{SH} \rightleftharpoons \text{P–SH} + \text{Cy–S–}^{35}\text{S–Cy}$$

before any of the ^{35}S could appear as P–S–^{35}S–Cy. On the other hand Table 2 shows that the label from L-[^{35}S]cystine appeared more rapidly in the peptide chains of the plasma proteins than after doses of L-[^{35}S]cystine, presumably because cysteine, not cystine, takes part in plasma protein synthesis in the liver.

Great care is needed in the analysis of free cyst(e)ine in plasma, because the reaction of cystine with the protein thiol groups to form mixed disulphides can go to completion *in vitro* (Downes 1961*b*); Isles and Jocelyn 1963). In the present work the proteins were removed as quickly as possible after collecting the blood, under conditions in which no more than 10% of the free cyst(e)ine would be lost (Downes 1961*b*). The values (Table 3) obtained for the concentration of free cystine must therefore be close to the true values. In spite of this these values are in general lower than those obtained for plasma for other species. Although few samples of sheep plasma were analysed, the results suggest that in normal sheep the concentration of free cyst(e)ine in plasma is lower than for the other species studied, possibly because of the large demand for cystine by the follicles for wool growth or because more cystine is disulphide-bound *in vivo* in the sheep than in other species. The concentrations of total free α-amino nitrogen (3·3–8·2 mg/100 ml plasma) were of the same order as those obtained (4–6 mg/100 ml) by other workers in this laboratory by the method of Frame, Russell, and Wilhelmi (1943). The low cystine concentrations were therefore not associated with overall low concentrations of the other amino acids.

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VI. References

Baker, C. H., and Wycoff, H. D. (1961).—*Amer. J. Physiol.* **201**: 1159.
Brigham, M. P., Stein, W. H., and Moore, S. (1960).—*J. Clin. Invest.* **39**: 1633.
Brown, B. H., and Lewis, H. B. (1941).—*J. Biol. Chem.* **138**: 705.
Downes, A. M. (1961*a*).—*Aust. J. Biol. Sci.* **14**: 109.
Downes, A. M. (1961*b*).—*Aust. J. Biol. Sci.* **14**: 427.
Downes, A. M., and Lyne, A. G. (1961).—*Aust. J. Biol. Sci.* **14**: 120.
Downes, A. M., Sharry, L. F., and Till, A. R. (1964).—*Aust. J. Biol. Sci.* **17**: 945.
Eagle, H., Oyama, V. I., and Piez, K. A. (1960).—*J. Biol. Chem.* **235**: 1719.
Emiliozzi, R., Pichat, L., and Herbert, M. (1959).—*Bull. Soc. Chim. Fr.* **1959**: 1544.
Forker, L. L., Chaikoff, I. L., and Reinhardt, W. O. (1952).—*J. Biol. Chem.* **197**: 625.
Frame, E. G., Russell, J. A., and Wilhelmi, A. E. (1943).—*J. Biol. Chem.* **149**: 255.
Hier, S. W. (1947).—*J. Biol. Chem.* **171**: 813.
Humphrey, J. H., Neuberger, A., and Perkins, D. J. (1957).—*Biochem. J.* **66**: 390.
Iob, V., McMath, M., and Coon, W. W. (1963).—*J. Surg. Res.* **3**: 85.
Isles, T. E., and Jocelyn, P. C. (1963).—*Biochem. J.* **88**: 84.
Johnson, C. A., and Bergeim, O. (1951).—*J. Biol. Chem.* **188**: 833.
McFarlane, A. S. (1958).—*Nature* **182**: 53.
Moore, S., and Stein, W. H. (1954).—*J. Biol. Chem.* **211**: 907.
Numata, I. (1940).—*Biochem. Z.* **304**: 404.
Schreier, K., and Pluckthun, H. (1950).—*Biochem. Z.* **320**: 447.
Schwert, G. W. (1957).—*J. Amer. Chem. Soc.* **79**: 139.
Stein, W. H., and Moore, S. (1954).—*J. Biol. Chem.* **211**: 915.
Sterling, K. (1951).—*J. Clin. Invest.* **30**: 1228.
Takeda, Y., and Reeve, E. B. (1963).—*J. Lab. Clin. Med.* **61**: 183.
Till, A. R., and Downes, A. M. (1963).—*Lab. Prac.* **12**: 1006.