different sources is probably valid. The striking differences between the values of the (unsolvated) molecular weight M, shown in Table 2, are certainly real. The large values of the hydrodynamic specific volume V' are consistent with the random-coil form of particle, deduced for ox hyaluronic acid by Ogston & Stanier (1951) and, as would be expected, V' varies in parallel with M. Values of the ellipticity J are in some cases rather large for a simple random-coil, but it is doubtful whether any conclusion can be drawn from this. It should be noted that the values of V' and J given in Table 2 for ox hyaluronic acid differ from those of Ogston & Stanier (1952) because of the new choice of constants noted by Ogston (1953).

The reality of the differences of M are shown by the fact that considerable amounts of hyaluronic acid present in a human traumatic fluid passed through a glass filter which entirely prevented the passage of ox hyaluronic acid.

SUMMARY

1. Viscosity measurements have been made on a small series of traumatic and pathological synovial fluids from human knee joints; also on three samples from ankle joints of normal sheep.

2. Sedimentation measurements have been made on a few of these fluids.

3. Comparison of variation of viscosity with velocity gradient, with its value at zero velocity gradient, suggests the existence of inter-species and possibly intra-species differences in the specific properties of hyaluronic acid. Sedimentation measurements appear to confirm this and suggest that human hyaluronic acids have considerably lower particle weights ($1-4\times10^6$) than those of sheep ($5-6\times10^6$) or ox (10^7).

4. Specific properties of human synovial fluid hyaluronic acid cannot, at present, be satisfactorily compared on a basis of concentration estimated from weight of mucin.

We are much indebted to Mr R. G. Taylor of the Wingfield-Morris Hospital, and to the Director and Staff of the Rheumatism Research Unit, Canadian War Memorial Hospital, Taplow, for samples of human synovial fluids; to the Director of the Nuffield Institute for samples of sheep synovial fluids; and to the Oxford Co-operative Society for facilities at their slaughterhouse. This work was assisted by a research grant from the Medical Research Council.

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Metabolism of β-Mercaptoethylamine

1. IN MICE

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(*Received* 29 *June* 1954)

Bacq *et al.* (1953) have shown that 3 mg. of β-mercaptoethylamine injected immediately before irradiation protect mice against 700 r. of X-rays. The percentage of ^{35}S remaining in the body of a mouse at various times after intraperitoneal injection of 3 mg. [^{35}S]β-mercaptoethylamine has previously been determined (Verly, Bacq, Rayet & Urbain, 1954), but for the understanding of the duration of the very short-lived radioprotection, it is more interesting to measure the ^{35}S that is still present in β-mercaptoethylamine (cysteamine) or β:β′-diaminodiethyldisulphide (cystamine) molecules. This paper presents the results of such measurements.

* Associé du Fonds National Belge de la Recherche Scientifique.

EXPERIMENTAL

Synthesis of [^{35}S]β-*mercaptoethylamine hydrochloride*

This compound was prepared as follows (see also Mills & Bogert, 1940; Bestian, 1950). $Ba^{35}S$ was ground in a mortar with some water, then transferred to an H_2S generator where it was made to react with an excess of phosphoric acid.

The $H_2{}^{35}S$ liberated was dissolved in methanol cooled to −78° and ethyleneimine in methanol at −78° slowly added to the stirred solution containing an excess of $H_2{}^{35}S$. Stirring was continued while the solution was allowed to come to room temp., when the excess $H_2{}^{35}S$, then the methanol, were distilled off.

The residue was washed several times with anhydrous ether, then dried *in vacuo*. The —SH groups were titrated with I_2 giving a result equal to 100·5% of the theoretical for mercaptoethylamine.

The substance was dissolved in water and titrated with HCl to pH 6·5–7; the quantity of HCl was the theoretical required to bring an equal amount of mercaptoethylamine to the same pH (i.e. to neutralize 97·5% of the base).

Ampoules were then filled with the solution and sterilized but titration of —SH groups with iodine, after sterilization, gave only 85–88% of the theoretical for mercaptoethylamine.

Chromatograms of the sterilized solution, after complete oxidation with I_2, on Whatman no. 1 paper, developed with N-HCl-saturated butanol, or with a water-saturated mixture of collidine–lutidine (1:1, by vol.), showed, in every instance, only one radioactive spot, the R_F of which was that of cystamine dihydrochloride chromatographed under the same conditions.

It appears therefore that the sterilized solution contains exclusively cysteamine (85–88%) and cystamine (12–15%) hydrochlorides. It was used for intraperitoneal injection into mice.

Isolation of cysteamine–cystamine from tissues

At suitable times after injection the mice were killed by a blow on the neck and immediately put in dry ice. The frozen animals were pulverized in a meat grinder. The ground tissues from one mouse were added to 200 ml. of water containing an accurately weighed amount of cystamine dihydrochloride (about 300 mg.), and the suspension was quickly brought to the boiling point and kept boiling for 1 hr.

The cooled suspension was filtered through cotton wool, the filtrate acidified with HCl, an excess of I_2 added to oxidize any thiol to disulphide and the solution extracted with diethyl ether. The aqueous phase was filtered through a 25 ml. Amberlite IR-120(H) column and the resin washed with 200 ml. water; filtrate and washings, which contained the acid metabolites were discarded. The substances with basic groupings adsorbed on the ion-exchanger were eluted with 500 ml. of 3N-HCl.

The acid eluate was evaporated to dryness under reduced pressure. The residue, dissolved in water, was filtered through a 25 ml. Amberlite IRA-410(OH) column and the resin washed with 100 ml. of water. This filtrate contained the cystamine while ampholytes remained on the column.

The basic solution was evaporated to about 5 ml. under reduced pressure so that volatile bases distilled. HCl was then added and the now-acid solution evaporated to dryness.

The residue was extracted with ethanol; sparingly soluble mineral salts were removed by filtration and the filtrate was evaporated to dryness.

This final residue was taken up in 4–6 ml. of methanol, the solution filtered and cystamine dihydrochloride was precipitated by addition of 12 ml. of acetone. The precipitate stood a few hours in the refrigerator, then was centrifuged and the supernatant decanted; the precipitate was washed with acetone and dried.

The precipitate was twice crystallized in the following way: the solid was dissolved in a mixture of ethanol–water–conc. HCl (12:1:1, by vol.) (Eldjarn, 1954) with slight warming, the solution was filtered, and diethyl ether was added to the filtrate so that the precipitate that formed just disappeared on boiling. The crystals were allowed to form at 20°, then in the refrigerator, and were washed with ether, then dried *in vacuo* at 78°.

The m.p.'s of the cystamine dihydrochloride samples thus isolated are recorded in Table 1. During recrystallizations the specific activity increased slightly. Chromatograms of samples 1 and 2, on Whatman no. 1 paper, developed with a water-saturated mixture of collidine–lutidine (1:1, by vol.), showed, in each case, one radioactive spot the R_F of which was that of cystamine dihydrochloride chromatographed under the same conditions.

Determinations of radioactivity

A portion of the ^{35}S cysteamine hydrochloride solution was oxidized with Benedict's reagent and the sulphate formed was precipitated as benzidine sulphate (Fiske, 1921). Cystamine dihydrochloride was oxidized with HNO_3 in sealed tubes (Carius) and the resultant sulphate turned into benzidine sulphate (Niederl, Baum, McCoy & Kuck, 1940).

Measurements of radioactivity were performed on the benzidine sulphate precipitates using the Tracerlab windowless flow counter in the Geiger region. Results were corrected for background, selfabsorption and radioactive decay and are expressed in counts/min./total sample.

RESULTS

The sterilized solution of [^{35}S]β-mercaptoethylamine hydrochloride was found to be 0·0824M and 0·5 ml. ($5·37 \times 10^6$ counts/min.) was injected intraperitoneally into each of seven mice (C 57 black) which weighed 20 g.

Table 1. *Properties of the isolated cystamine dihydrochloride*

For details see text.

Time after injection (hr. min.)	0·15		1·00	6·00		24·00	
Mouse no.	1	2	3	4	5	6	7
Total radioactivities (counts/min. × 10^{-6})	3·87	3·96	2·00	0·48	0·30	0·11	0·09
Total activities expressed as % of injected activity	72	74	37	9	6	2	2
M.p.'s ° (corr.)	217·5–218·5	218–219	218·5–219	218–219	217·5–218·5	218–219	218·5–219

Two mice were killed after 15 min., one after 1 hr., two after 6 hr. and two after 24 hr. The total [^{35}S]β-mercaptoethylamine and β:β'-diaminodiethyldisulphide of each animal was measured with results recorded in Table 1.

DISCUSSION

The experimental procedure used for cysteamine–cystamine isolation has been devised to measure 'free' [^{35}S]cysteamine–cystamine. It is not known whether part of the 'bound' cysteamine (pantetheine, coenzyme A) may not also be involved, but this is not important if 'free' cysteamine cannot be bound and thus introduce radioactivity into such molecules as coenzyme A. It seems that such is actually the case, since the S of cysteamine is not utilized for cystine biosynthesis, and cysteamine does not seem to be directly used for pantetheine and coenzyme A biosyntheses (Brown & Snell, 1953; Pierpoint & Hughes, 1954).

Total ^{35}S (Verly *et al.* 1954) and [^{35}S]cysteamine–cystamine remaining in the mouse body after intraperitoneal injection of the same amount of labelled cysteamine are compared in Fig. 1, in which activities are expressed as percentages of the injected dose. Attention must be drawn to two facts: in the previous work (Verly *et al.* 1954) cysteamine benzoate was used in place of cysteamine hydrochloride, but it is unlikely that the anion has any influence on mercaptoethylammonium ion metabolism; also the total ^{35}S determinations by the Na_2O_2 method (Verly *et al.* 1954) on the whole mouse gave results which are 5–10 % low.

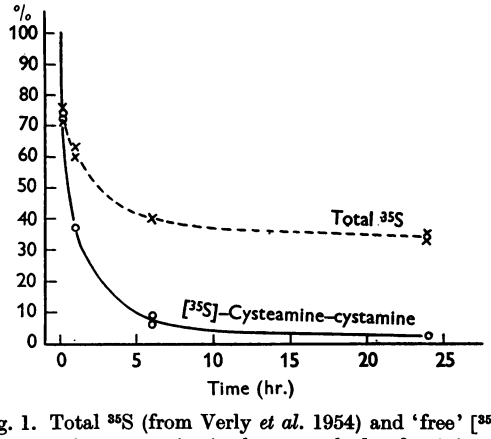

Fig. 1. Total ^{35}S (from Verly *et al.* 1954) and 'free' [^{35}S]-cysteamine–cystamine in the mouse body after injection of about 3 mg. of labelled cysteamine. The activities are expressed as percentage of the injected dose.

The curves of Fig. 1 show that 15 min. after the injection, most of the ^{35}S still in the organism is present as 'free' cysteamine–cystamine; after 24 hr. 'free' [^{35}S]cysteamine–cystamine is negligible although 34 % of the injected ^{35}S is still in the body of the mouse.

These facts could be explained if during the first minutes [^{35}S]cysteamine is excreted unchanged in the urine, but that later on the loss of labelled cysteamine is also dependent on tissue metabolism and the kidneys excrete a mixture of [^{35}S]cysteamine–cystamine and labelled metabolites, the latter become more and more predominant as time goes on.

SUMMARY

1. A method is described for the synthesis of [^{35}S]cysteamine–cystamine and for its determination in a whole mouse.

2. It was found that 50 % of 3 mg. of intraperitoneally injected labelled cysteamine disappear from the body of a mouse after about 40 min. After 24 hr., only 2 % are left.

The expenses of this work have been defrayed by grants from the Institut Interuniversitaire des Sciences Nucléaires and the Conseil Supérieur de la Sécurité Civile.

The authors wish to express their thanks to G. Espreux and A. Silvestre for their able assistance.

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