The above results indicate that the injection of BAL into rats produces an increased excretion of glucuronic acid in the urine; with rabbits, on the other hand, no such increase can be shown. This provides support for the view that the rat excretes BAL at least partially as a glucuronide, though in the absence of a constant ratio between the excess excretions of BAL and glucuronic acid, it is impossible to say definitely that the excreted compound is a BAL glucuronide.

In view of the large increases in glucuronic acid excretion in rats, the absence of any increased excretion in the rabbit following the injection of BAL is not easy to understand.

SUMMARY

1. Following the injection of BAL into rats, a sharp rise in the urinary excretion of neutral sulphur occurs in the first 24 hr. after injection, the increases

representing 40–60 % of the sulphur injected as BAL.

2. There is no increase in ethereal sulphate excretion after injection, so that rats do not conjugate BAL with sulphuric acid. However, a marked and persistent rise in inorganic sulphate excretion occurs. This appears to be due rather to increased breakdown of tissues caused by the injury produced by the injection of BAL, than to oxidation of BAL-sulphur to sulphate.

3. Injections of BAL cause increased excretions of glucuronic acid in the urine of rats in the first 24 hr. after injection, suggesting that BAL is excreted as a glucuronide by this species. No such increase was found in the rabbit.

I should like to express my thanks to Prof. R. A. Peters, M.C., F.R.S., Dr R. H. S. Thompson and Dr L. A. Stocken for their help and advice in connexion with this work.

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The Use of British Anti-Lewisite Containing Radioactive Sulphur for Metabolism Investigations

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Various methods have been used for investigating the metabolism of BAL. The nitroprusside reaction and iodine titration (Stocken & Thompson, 1941, 1946) showed that following the injection of BAL there was a rapid increase in the urinary excretion of thiol compounds. An examination of the coloured metallic derivatives suggested the presence of a dithiol, a view which was confirmed by enzyme protection experiments. On the other hand, the ultra-violet and visible absorption spectra of the excreted dithiol showed that this was not unmodified BAL (Spray, Stocken & Thompson, 1947). In the rat, but not in the rabbit, Spray (1947) obtained evidence

indicating the possibility of conjugation of the urinary thiol with glucuronic acid but not with sulphuric acid. At the same time, he found an increased excretion of inorganic sulphate which was presumed to be due to increased tissue catabolism caused by local damage at the site of injection. In an attempt to establish this last point with more certainty and to learn more about the distribution, BAL containing S³⁵ was tried. Further, since no satisfactory method has been found for the estimation of BAL in blood, it was considered that the 'tracer' technique could provide some information as to the rate of absorption, persistence and amount

in the circulation. The results we obtained were in general agreement with those of the earlier experiments and also with those found independently by Simpson & Young (1945), some of which appeared during the course of our work.

METHODS

The preparation of radioactive BAL

Reduction of BaSO₄. An intimate mixture of 125 mg. radioactive BaSO₄, 125 mg. BaSO₄ and 0.5 g. sugar charcoal was heated at 1000° for 1.25 hr. in a quartz tube. The tube was fitted with a ground-joint tap which was left open during the reduction. After cooling, the BaS was decomposed with the calculated quantity of N-HCl and the H₂S blown by a steady stream of nitrogen into a cooled (-20°) solution of 150 mg. sodium ethoxide in 5.0 ml. ethanol to give a solution containing radioactive sodium hydrosulphide.

Conversion of the radioactive NaSH into BAL. Ammonium hydrosulphide was prepared by leading dry H₂S and dry ammonia into anhydrous diethyl ether at -10°. The white crystals were quickly filtered off, pressed as free as possible from ether, and then dissolved in cold methanol. A volume of this solution (130 ml.), containing the equivalent of 8.06 g. H₂S, was mixed with the solution of radioactive NaSH and heated with a 2.3-dibromopropanol (10.9 g.) in a sealed glass bottle for 15 hr. at 90-95°. Excess H₂S and methanol were removed *in vacuo*, the residue made acid to Congo red with 0.1N-HCl and the solution saturated with ammonium sulphate. The product was exhaustively extracted with pure benzene and the extract dried over Na₂SO₄. The solvent was removed *in vacuo* and the residue distilled at low pressure. Yield 3.5 g. (56%) (cf. Stocken, 1947). 'SH' content by iodine titration, 53.1%. Calc. for C₃H₇OS₂: 53.2%.

Measurement of the radioactive sulphur. In view of the low energy of the β particles from S³⁵ (1.07×10^5 eV.) all the radioactivity measurements were made with a demountable counter of the type described by Collie & Morgan (1939) in which the radioactive preparation is held inside a movable glass cylinder which can be slid away from the counter for the purpose of determining the background count. After introducing the specimen, the counter was evacuated and

filled with a mixture of dry air (at 40 mm. Hg) and ethanol (at 10 mm. Hg). A satisfactory counter can be opened and filled indefinitely without changing its characteristics.

The overall efficiency of the counter was obtained by measuring the activity of the uranium X from a known amount of uranium nitrate precipitated on a ferric hydroxide carrier. It was found that 1/14th of the β particles released from the source were registered. It was shown by sliding the source up and down the counter that the sensitive area was sufficiently large for it to be unnecessary to make any special arrangements for locating the source exactly in order to obtain reproducible results.

For measurement, the material was evenly distributed over the inner surface of the cylinder by taking it up in a little water and rotating the cylinder on a horizontal axis while a stream of air was blown through it.

The BaSO₄ prepared from radioactive BAL and diluted with four times its weight of BaSO₄ was used as a standard. Its activity was repeatedly measured during the course of the work, but after allowing for the natural decay, no significant change in the count was observed.

No correction for absorption in the film was applied; since the thickness of the BaSO₄ film was not more than 0.2 mg./cm.² and the penetrating power of the rays in aluminium is several mg./cm.², the omission of this correction is justified.

EXPERIMENTAL AND RESULTS

Investigation of the radioactivity of urine sulphur fractions after BAL injection

Three groups of three male rats were used for the investigation of the urinary sulphur fractions. For intraperitoneal injections, the rats weighed 322, 332 and 265 g.; for intramuscular injections, 284, 289 and 247 g.; and for subcutaneous injections, 243, 281 and 261 g. Each rat received the equivalent of 63 mg./kg. body wt. of radioactive BAL (8% w/v in propylene glycol). The rats were fed outside the metabolism cages for 2.5 hr. (at 10.00-12.00 hr. and 17.00-17.30 hr.) each day. Treatment of the urine for the conversion of the three sulphur fractions to sulphate was carried out as described by Spray (1947).

The results given in Table 1 indicate that the mode of injection has little effect on the S³⁵ content

Table 1. *The excretion of S³⁵ in the sulphur fractions of urine by rats after injection of radioactive BAL*

Day	Fraction	Percentage of radioactivity of injected BAL in samples		
		Exp. 1 (Intraperitoneal injection)	Exp. 2 (Subcutaneous injection)	Exp. 3 (Intramuscular injection)
1	Inorganic SO ₄	3.6 (± 0.1)*	5.7 (± 0.13)	5.25 (± 0.1)
	Ethereal SO ₄	0.0	0.0	0.75 (± 0.02)
	Neutral S	41.6 (± 1.9)	40.9 (± 1.5)	40.2 (± 1.9)
2	Inorganic SO ₄	0.4 (± 0.05)	0.5 (± 0.08)	0.5 (± 0.04)
	Ethereal SO ₄	0.0	0.1 (± 0.02)	0.0
	Neutral S	1.5 (± 0.2)	2.0 (± 0.2)	1.5 (± 0.13)
3	Inorganic SO ₄	—	—	0.3 (± 0.03)
	Ethereal SO ₄	—	—	0.0
	Neutral S	0.4 (± 0.025) (total S)	0.9 (± 0.07) (total S)	0.9 (± 0.1)
4	Total S	0.25 (± 0.025)	0.3 (± 0.06)	0.3 (± 0.025)
5	Total S	—	0.2 (± 0.06)	0.3 (± 0.04)

* Figures in brackets are the probable error.

of the various fractions. During the first 22 hr. after injection (i.e. day 1) there was a large excretion of S^{35} in the neutral sulphur fraction. Some radioactivity, however, appeared in the inorganic and total sulphate fractions. This might have been due either to direct oxidation of some BAL sulphur to inorganic sulphate, or to exchange of S^{35} between BAL and some thiol compound which was subsequently oxidized to the inorganic sulphate. It is not yet possible to decide between these alternatives. The S^{35} content of the ethereal sulphate fractions was either zero or a very small fraction of the total.

Small amounts of S^{35} continued to be excreted for at least 5 days, indicating that there must be considerable retention of S^{35} , either as BAL or because

stand overnight. This caused the tissues to disintegrate, so avoiding the risk of explosion which sometimes occurs if heating is begun directly. To the mixture were added 5–10 ml. perchloric acid (60%) and the digestion carried out in the usual way, with the addition of a few drops of nitric acid from time to time. The sulphuric acid was precipitated as $BaSO_4$, which was collected, weighed and a convenient amount taken for the count. Almost theoretical yields of $BaSO_4$ were obtained by this method in a control experiment in which a known amount of BAL was oxidized in the presence of liver.

From Table 2 it can be seen that the kidney, liver and small intestine had a greater concentration of BAL calculated from the S^{35} content than the other organs. This distribution is probably due to rapid elimination in the urine and the bile.

Table 2. *The distribution of S^{35} and BAL in the body of a rat after intramuscular injection*

	Wt. of tissue (g.)	S^{35} content (% of amount injected)	BAL (μ g./g. tissue)
Eyes	0.33	0.02 (± 0.002)*	19.4
Brain	1.74	0.17 (± 0.002)	31.3
Stomach and contents	1.83	0.17 (± 0.002)	29.7
Small intestine and contents	7.17	3.6 (± 0.05)	161
Large intestine and contents	3.89	0.68 (± 0.007)	56.0
Heart	1.21	0.13 (± 0.003)	34.4
Lungs	1.39	0.21 (± 0.002)	48.4
Liver	9.08	2.8 (± 0.04)	98.6
Kidneys	2.46	2.1 (± 0.04)	274.0
Spleen	1.13	0.20 (± 0.002)	56.6
Testes	1.31	0.15 (± 0.003)	36.6
Skin	40.1	0.46 (± 0.01)	3.66
Muscle (remote)	3.07	0.30 (± 0.004)	31.2
Injection leg	29.8	20.1 (± 0.35)	21.6
Blood	4.77	—	55.0

* Figures in brackets are the probable error.

of exchange, in the body. It was not thought that determinations could usefully be carried on any longer than this, since it was not possible to measure the feeble radioactivity of the late samples with any accuracy. It was for this reason also that no radioactivity measurements were carried out on the inorganic and total sulphate fractions after the second or third day.

Distribution of S^{35} after injection of radioactive BAL

A large male rat (320 g.) after feeding on a synthetic diet for 7 days was starved for 24 hr. and then injected intramuscularly in the hind leg with 32 mg. radioactive BAL dissolved in 0.4 ml. propylene glycol. 1 hr. later it was killed by decapitation, and sufficient blood collected for estimation of the S^{35} content. Other organs and parts of the rat were dissected out. In this dissection great care was taken to avoid contamination of S^{35} between the various tissues. The skin was freed from subcutaneous fatty and connective tissues by scraping. Muscle was taken from the hind leg not used for injection.

The weighed tissues were placed in Kjeldahl flasks, covered with 10–20 ml. conc. nitric acid and allowed to

Persistence of S^{35} in the blood after injection of radioactive BAL

A large female rabbit (3100 g.) was given 160 mg. radioactive BAL dissolved in propylene glycol by intramuscular injection into the hind leg. At hourly intervals, blood was collected from the ear vein, and the S^{35} content estimated.

Table 3. *The distribution of S^{35} and BAL in rabbit blood after intramuscular injection*

Time after injection (hr.)	% of injected S^{35} in total blood wt.*	BAL (μ g./g. of blood)
1	5.4 (± 0.07)†	33.0
2	5.0 (± 0.12)	30.8
3	2.6 (± 0.05)	16.1
4	2.5 (± 0.04)	15.6
5	2.1 (± 0.04)	13.0
6	1.8 (± 0.07)	11.2
7	1.7 (± 0.04)	10.5

* Total wt. of blood is assumed to be 1/12th of body wt.

† Figures in brackets are the probable error.

From Table 3 it will be seen that the BAL content of blood reached a fairly high level during the first

2 hr. after injection, c. 5 % of the injected S^{35} being in the circulation; during the next hour it fell to about half this figure and then steadily declined over the next 5 hr.

In both this and the previous experiment, it must be remembered that the BAL content has been calculated from the amount of S^{35} found. The figures, therefore, necessarily give an upper limit to the BAL concentration, since there is the possibility of exchange and irreversible oxidation to non-thiol compounds.

DISCUSSION

There is general agreement in the distribution of S^{35} between the amounts obtained by Simpson & Young (1945) 6 hr. after injection of radioactive BAL and those found by us. With the exception of the small intestine where the percentages of S^{35} were approximately the same (3.7 and 3.61) the concentrations in the other tissues after 6 hr. were, as would be expected, consistently lower. In our experiments there is also fair agreement between the concentrations found in the blood of the rat and rabbit 1 hr. after injection; thus the rat which received twice the dose given to the rabbit had nearly twice the concentration of BAL in the blood.

From the therapeutic point of view it is desirable to determine the rate of absorption of BAL, the level of BAL in the blood stream and for how long an adequate concentration is maintained. The figures given in Table 2 show that 80 % of the injected BAL had already entered the circulation within 1 hr. of injection. At this time the maximal concentration

had been reached and was maintained for not less than 2 hr. (Table 3); assuming, therefore, that all the S^{35} is due to BAL itself, the data indicate that these doses repeated at intervals of 3 hr. are sufficient to ensure maximum therapeutic effect in rats. This frequency of dosage is consistent with the American recommendations embodying initial dosing of BAL at intervals of 4 hr. for patients suffering from arsenical dermatitis, a treatment which was based upon studies in rabbits (Eagle, 1943) and injection experiments in human volunteers (Sulzberger, Baer, Kanoff & Eagle, 1944).

A further point of therapeutic importance is the confirmation that a single dose of BAL is largely excreted within 6–24 hr. after injection; this means that any possible toxic effects of a single dose should be temporary.

SUMMARY

1. A method is given for the preparation of radioactive 2:3-dimercaptopropanol (BAL).
2. It has been shown that the S^{35} of radioactive BAL is largely excreted in the neutral sulphur fraction of post-injection urine.
3. The distribution and persistence in the circulation of S^{35} following injection of radioactive BAL has been determined.

The radioactive sulphur used in this work was obtained in the form of $BaSO_4$ through the good offices of the Chemical Defence Research Department of the Ministry of Supply from Dr F. C. Henriques of Harvard University, U.S.A., to whom our thanks are due.

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Oxidation of Phenol and Benzoic Acid by Some Soil Bacteria

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The utilization of aromatic compounds by certain bacteria is now well known, and numerous workers have described the isolation of micro-organisms from various sources, capable of metabolizing phenolic substances. Thus, Sen Gupta (1921) has studied quantitatively the disappearance of phenols

and cresols from soils of diverse origin, and showed clearly the biological nature of the process. Störmer (1908) had tested the effect on soil of a number of organic compounds, including toluene, xylene, phenol and *p*-cresol, and claimed to have isolated organisms that could destroy these compounds, but