Biochemical Studies of Toxic Agents

1. EXPERIMENTS WITH RADIOACTIVE 2:3-DIMERCAPTOPROPANOL (BRITISH ANTI-LEWISITE)

BY S. D. SIMPSON* AND L. YOUNG†
Department of Biochemistry, University of Toronto

(Received 12 December 1949)

During the period 1941–5, studies were conducted in this laboratory on the antidotal action of 2:3-dimercaptopropanol (BAL, British Anti-Lewisite) and other thiols in arsenic poisoning; for review see Young (1946). The investigations were undertaken after receiving early reports of the work of Peters and his co-workers on the value of 2:3-dimercaptopropanol as an antidote to arsenic. In 1945 Peters, Stocken & Thompson reviewed investigations on BAL which had been carried out at Oxford and elsewhere during the War, and, since the appearance of their article, numerous papers describing researches on BAL have been published.

The fact that no satisfactory method for determining BAL in biological material was available led us to synthesize BAL with radioactive sulphur (^{35}S) incorporated in the molecule (Simpson & Young, 1945*a*) and to use it in studies of the fate of BAL in the animal body (Simpson & Young, 1945*b,c*). A series of experiments was carried out on rats in which the absorption of BAL by the skin was allowed to proceed for a period of 6 hr. after application and the unabsorbed BAL was then removed. The absorption, distribution and excretion of ^{35}S were determined at 6, 12 and 24 hr. after the initial application (Simpson & Young, 1945*b*). A second series of experiments was conducted in which the distribution and excretion of ^{35}S were determined at 6, 12 and 24 hr. after the intramuscular injection into the rat of a solution of radioactive BAL in propylene glycol (Simpson & Young, 1945*c*).

An account of similar work with radioactive BAL, with results in general agreement with those described herein, has been published by Peters, Spray, Stocken, Collie, Grace & Wheatley (1947). These authors indicate that their work with radioactive BAL was in progress when reports describing some of the present investigations appeared.

EXPERIMENTAL

Analytical methods

The determination of S and the measurement of ^{35}S were carried out by the procedures described by Young, Edson & McCarter (1949) in which the material is oxidized by the

Carius method and the sulphate is precipitated as benzidine sulphate.

'SH' was determined by titration of a solution of the compound either with standard I_2 solution or with standard KIO_3 solution in the presence of HCl and excess KI (Simpson, Zbarsky & Young, 1944).

Synthesis of radioactive BAL

Dithiols are often prepared by the interaction of sodium hydrosulphide or ammonium hydrosulphide and the appropriate dihalide under pressure (cf. Simpson, 1947). The method can be used for the synthesis of 2:3-dimercaptopropanol with radioactive S incorporated in the molecule if H_2S containing H_2^{35}S is used to prepare the hydrosulphide. Radioactive BAL was prepared in the present work by allowing radioactive sodium hydrosulphide to react with 2:3-dibromopropanol in methanol solution.

Radioactive mustard gas was the only source of ^{35}S immediately available to us, and all that could be spared for the synthesis of radioactive BAL was 20 mg. This amount of radioactive mustard gas was converted to H_2S with negligible loss of ^{35}S by a modification of ter Meulen's method for determining S in organic compounds (ter Meulen, 1922; Masters, 1939). The mustard gas was heated in a stream of H_2 and the vapour passed over strongly heated platinized asbestos. The H_2S formed was collected in 0.5 ml. of aqueous NaOH solution. In five preliminary experiments, in each of which the H_2S formed by the decomposition of 20 mg. of non-radioactive mustard gas was collected in this manner, the recoveries of S (as determined iodometrically) varied from 97 to 101% (mean, 98%) of the theoretical value. The Na_2S was converted by the action of H_2S to sodium hydrosulphide, and this was used in the preparation of BAL. Several preparations of non-radioactive BAL were carried out in order to determine suitable conditions for the reaction between 2:3-dibromopropanol and sodium hydrosulphide. The results led to the use of methanol as solvent for the reagents in the presence of the least possible amount of water, and not less than four molecules of sodium hydrosulphide for each molecule of 2:3-dibromopropanol.

Procedure. Radioactive mustard gas (10 mg.) and an equal amount of non-radioactive mustard gas were placed in a platinum boat lined with filter paper. The boat was then introduced into a Vycor combustion tube (Corning Glass Works), which contained loosely packed platinized asbestos. The method of preparing the combustion train and the procedure used in the combustion were similar to those described by Masters (1939) for the determination of S in organic compounds and biological material. Pure H_2 was passed through the combustion tube for 5 min. to displace air. The platinized asbestos was then heated strongly and the portion of the combustion tube containing the platinum

* Present address: National Research Council of Canada, Atomic Energy Project, Chalk River, Ontario, Canada.

† Present address: Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E. 1.

boat was heated gently so that the mustard gas vaporized slowly and was carried through the platinized asbestos by the H_2 . The issuing gases were bubbled through a solution of 0.15 g. of NaOH dissolved in 0.5 ml. of water contained in a 15 ml. tapered centrifuge tube. The whole process was repeated with another 10 mg. sample of radioactive mustard gas and the H_2S was collected in another 0.5 ml. of NaOH solution. The two centrifuge tubes containing the radioactive Na_2S were set aside in an ice bath until required for the next stage of the synthesis.

Methanol (15 ml.) was placed in a 200 ml. glass pressure flask and 0.977 g. of metallic Na was added. The flask was placed in an ice bath and H_2S was bubbled through the methanol solution for 4 hr. The contents of the two centrifuge tubes were then transferred to the pressure flask. Each centrifuge tube was washed with a total of 5 ml. of cold methanol in small portions and these washings were added to the contents of the pressure flask. The flask was kept chilled in ice and H_2S was passed under pressure into the solution for 6 hr.

At the end of this period 0.0115 g.mol. of 2,3-dibromopropanol (Eastman Kodak Co.) was chilled and added to the radioactive sodium hydrosulphide (0.050 g.mol.) in the pressure flask. The flask was then sealed with a rubber stopper which was wired in position. The flask was kept at 30° for 80 hr. and was then chilled and opened. Concentrated HCl (2.4 ml.) was added slowly until the contents of the flask were acid to congo red. (The gas which was liberated during this process contained $H_2^{35}S$ and was collected as CdS.) The pressure flask and its contents were heated in a water bath at 50° and the methanol was then removed by evaporation under reduced pressure. The flask and its contents were cooled to room temperature and 10 ml. of water were added in order to dissolve the solid material in the flask. The contents of the flask were then extracted with five 10 ml. portions of peroxide-free ether, and the combined extracts were dried over anhydrous Na_2SO_4 . The ether was evaporated and a residue which weighed 1.629 g. was obtained. This material was distilled under reduced pressure and the fraction which boiled at 66° at 0.025 mm. pressure was collected. The weight of this fraction was 0.663 g. and represented a yield of BAL of 46% based on the amount of dihalide used. (Found: 'SH' (by iodine titration), 51.8; S, 50.7%. Calc. for $C_3H_2OS_2$: 'SH', 53.2; S, 51.6%.) The specific activity of the radioactive BAL was 64% of the maximum specific activity possible under the experimental conditions used.

RESULTS

Distribution of ^{35}S following the application of radioactive BAL to the skin of the rat

A study was made of the distribution of ^{35}S in the rat and its excreta at 6, 12 and 24 hr. after the application of radioactive BAL to the skin. Three experiments were performed in each of which 0.05 ml. of radioactive BAL was applied to the clipped skin of the back of a rat. In each experiment the site of application was covered with a glass and celluloid cap sealed to the back of the animal, and loss of BAL by evaporation and mechanical removal was thereby prevented. Absorption of BAL was allowed to proceed for 6 hr.,

and at the end of this period the BAL which remained on the skin was removed and its ^{35}S content was determined. After a known interval (0, 6 or 18 hr. after removal of the unabsorbed BAL, i.e. 6, 12 or 24 hr. after dosing) the rat was bled to death, dissected and analysed for ^{35}S . The urine and faeces were collected separately during the whole experimental period and were analysed for ^{35}S .

Procedure. Three male white rats (wt. 167, 157 and 173 g.) were used. The experiments were numbered as follows: rat no. 1, analysed at the end of the period of absorption of BAL (i.e. 6 hr. after dosing); rat no. 2, analysed 6 hr. after the end of the absorption period (i.e. 12 hr. after dosing); rat no. 3, analysed 18 hr. after the end of the absorption period (i.e. 24 hr. after dosing).

The hair in the central area of the back of each rat was clipped to the skin level by means of electric shears and scissors. A ring of glass with an inside diameter of 3.5 cm. and a depth of 1.5 cm. was sealed on to the clipped skin by means of glass cement (Ducco Cement, Canadian Industries Ltd.). The animal was anaesthetized with ether and 0.05 ml. of radioactive BAL (63 mg.) was measured by means of a micropipette (Young, 1941, 1947) on to the skin within the ring. The BAL was rubbed evenly over the skin within the glass ring for 5 min. with the flattened end of a glass rod. The site of dosing was then closed by sealing a circular piece of celluloid, 4.5 cm. in diameter, over the top of the glass ring. The BAL which remained on the glass rod was washed into a 25 ml. volumetric flask with ethanol and the solution was made to volume. In order to determine the BAL content of the solution, 10 ml. portions were diluted with water, acidified with HCl and titrated with 0.02N-KIO₃ solution in the presence of excess KI (Simpson *et al.* 1944). Hereafter the amount of BAL measured on to the skin minus the amount which adhered to the glass rod is described as 'radioactive BAL applied'.

After the animal had been dosed it was placed in a metabolism cage of a type which permitted the collection of urine separate from the faeces. Water was available to the rat while it was in the metabolism cage, but food was not provided. 6 hr. after the application of the BAL the rat was anaesthetized with ether in the metabolism cage (in these and other experiments the rat usually emptied its bladder during its struggles while being anaesthetized). The urine excreted during the 6 hr. period was analysed for ^{35}S . While the animal was still anaesthetized, the disc of celluloid covering the glass ring was removed and the dosed skin was wiped with three small pieces of cotton wool. The skin was next rubbed lightly with three pieces of cotton wool moistened with ethanol and after each rubbing it was wiped with dry cotton wool. All the pieces of cotton wool were dropped into ethanol and the mixture was stirred. The ethanol solution of the material removed from the skin was then washed into a 50 ml. volumetric flask through a funnel loosely plugged with cotton wool. The solution was made to volume and sample portions were analysed for their ^{35}S content. In the experiments in which the distribution of ^{35}S was studied at 6 and 18 hr. after the end of the absorption period, the celluloid disc was cemented over the glass ring after the BAL remaining on the skin had been removed. The rat was then returned to the metabolism cage. The dose of BAL applied minus the amount of BAL (based on the ^{35}S data) removed from the skin at the end

of the absorption period and that present on the cap and in the glass cement at the end of the experimental period is described hereafter as 'radioactive BAL absorbed'.

At the end of the experimental period each rat was anaesthetized in the metabolism cage and given an intravenous injection of 0.1 ml. of heparin solution (Connaught Laboratories, Toronto; 1000 units/ml.). It was then bled as completely as possible from the carotid artery. The blood was collected, its volume was measured, and 1 ml. samples were analysed for ^{35}S . The glass ring and the cement holding it to the back of the animal were removed and the amount of ^{35}S on them was determined. All the skin which had been covered by the cap was excised, weighed and analysed for ^{35}S . In order to avoid the possible transfer of ^{35}S from the dosed area to other parts of the animal, the dissecting instruments which were used in the above operation were set aside and others were used for the remaining dissection. Tissues removed from the animal were placed in stoppered glass weighing bottles, weighed, and duplicate samples were analysed for ^{35}S . The 'carcass' (i.e. all of the animal which remained after removal of the tissues which were analysed for ^{35}S) was weighed and its content of ^{35}S was measured.

Urine was collected and analysed for ^{35}S at the following times after dosing: rat no. 1, 6 hr.; rat no. 2, 6 and 12 hr.; rat no. 3, 6, 12 and 24 hr. The faeces were collected at the end of the experimental period and their ^{35}S content was measured.

The data in Table 1 shows the amounts of radioactive BAL applied and the amounts absorbed in the 6 hr. during which absorption took place. They also show the amounts of ^{35}S in the skin at the site of dosing, in the body of the rat and in the excreta at the end of the experimental period.

Table 1. *The distribution of ^{35}S at 6, 12 and 24 hr. after the application of radioactive BAL to the skin of the rat**

Rat no.	...	1	2	3
Time of killing after application of radioactive BAL (hr.)		6	12	24
Radioactive BAL applied (mg.)		62	58	60
Radioactive BAL absorbed in 6 hr. (mg.)		25	21	21
		% of ^{35}S applied as BAL		
		60	64	65
^{35}S not absorbed in 6 hr.		3	3	1
^{35}S in skin at site of dosing		10	3	3
^{35}S in body of rat (excluding skin at site of dosing)		14	22	25
^{35}S in urine and faeces		87	92	94
Total ^{35}S traced				

* Absorption of radioactive BAL by the skin was allowed to proceed for 6 hr.

The rate of absorption of BAL by the skin of the rat was low. In the three experiments just described, the amounts of BAL absorbed in 6 hr. were 25, 21 and 21 mg. In each experiment the area of dosed skin was 9.6 sq.cm. The rates of absorption in terms of BAL/sq.cm. of skin/hr. were therefore 0.43, 0.36 and 0.36 mg., and the mean value was 0.38 mg.

The fact that BAL or substances containing

sulphur derived from BAL did not accumulate in the dosed skin to any marked extent is shown by the relatively small amounts of ^{35}S in the skin at the end of the 6 hr. period of absorption. For example, in the case of rat no. 1, which was killed at the end of the absorption period, the amount of ^{35}S found in the skin corresponded to 1.9 mg. of BAL (i.e. 7.6 % of the BAL absorbed) and was distributed in 5.84 g. of skin.

In the blood and most of the tissues of rat no. 1, ^{35}S was present in low concentration. The concentrations of ^{35}S were equivalent to the following amounts of BAL (mg.)/g. of fresh tissue: blood, 0.07; liver, 0.07; kidney, 0.22; heart, 0.04; lung, 0.07; spleen, 0.06; brain, 0.03; stomach, 0.04; small intestine and contents, 0.12; testis, 0.03; muscle under dosed skin, 0.03; leg muscle, 0.03; skin remote from dosed area, 0.05; and carcass, 0.03. Only in the kidney and in the small intestine and its contents were the concentrations of ^{35}S appreciably higher than in the other tissues apart from the skin at the site of dosing, and this was probably related to the passage of radioactive material into the urine and the bile.

As a result of excretion of ^{35}S in the urine, the amount of ^{35}S in the organism decreased rapidly after the end of the period of absorption of radioactive BAL. At 6 hr. after the end of the absorption period the liver did not contain measurable amounts of ^{35}S , and the concentration of ^{35}S in the kidney had fallen to the equivalent of 0.04 mg. BAL/g. of fresh tissue. At 18 hr. after the end of the absorption period neither the kidney nor the liver contained a measurable amount of ^{35}S .

A striking feature of the data is the rate at which ^{35}S was excreted in the urine (see also Table 3). This is discussed later. Small amounts of ^{35}S were found in the faeces, but these may have originated from urine which came in contact with the faeces.

Distribution of ^{35}S following the intramuscular injection of BAL into the rat

A study, similar in many respects to that just described, was made with rats which were given radioactive BAL by intramuscular injection. The distribution of ^{35}S was studied at 6, 12 and 24 hr. after injection. In each of the three experiments performed, 20 mg. of radioactive BAL dissolved in propylene glycol were injected into the muscle of the left hind leg of a rat. The animal was then placed in a metabolism cage and at the end of the experimental period it was killed, dissected and analysed for ^{35}S . The urine and faeces were also collected and analysed for ^{35}S .

Procedure. Three male white rats, each of which weighed 185 g., were used in the experiments. The experiments were numbered as follows: rat no. 4 was analysed at 6 hr., rat no. 5 at 12 hr. and rat no. 6 at 24 hr. after the injection of radioactive BAL.

The hair on the left hind leg of each rat was clipped to the skin level. The animal was anaesthetized with ether and a freshly prepared solution of 20 mg. of radioactive BAL in 0.30 ml. of propylene glycol was injected into the muscle of the left hind leg. The point of withdrawal of the needle from the skin was clamped for 10 min. and was then sealed with glass cement.

The animal was then placed in a metabolism cage and was provided with water but not with food. At the end of the experimental period the rat was anaesthetized with ether in the metabolism cage and was given an intravenous injection of 0.1 ml. of heparin solution. It was then bled as completely as possible from the carotid artery and 1 ml. samples of blood were analysed for ^{35}S . The muscle of the left hind leg ('dosed muscle') was removed and analysed for ^{35}S . The remainder of the experiment was conducted in the manner described for those experiments in which rats were dosed by application of radioactive BAL to the skin.

The main findings are shown in Table 2.

Table 2. *The distribution of ^{35}S at 6, 12 and 24 hr. after the intramuscular injection of a solution of radioactive BAL in propylene glycol into the rat*

Rat no.	...	4	5	6
Time of killing after injection of radioactive BAL (hr.)		6	12	24
Radioactive BAL injected (mg.)		20	20	20
		% of ^{35}S injected as BAL		
^{35}S in dosed muscle		2	5	6
^{35}S in body of rat (excluding dosed muscle)		33	12	11
^{35}S in urine and faeces		48	69	82
Total ^{35}S traced		83	86	99

The fact that BAL or substances containing sulphur derived from BAL were not retained to any great extent at the site of injection is shown by the relatively small amounts of ^{35}S which were found in the dosed muscle. The maximum amount of ^{35}S found in the dosed muscle corresponded to 1.2 mg. of BAL (i.e. 6% of the dose) and this was present in 3.18 g. of muscle. Although the amount of ^{35}S in the dosed muscle appeared to increase with time, this may well have been due to slight differences in the rate of movement of the ^{35}S from the muscles of the different animals.

^{35}S was present in low concentration in the blood and a number of tissues 6 hr. after the injection of radioactive BAL (rat no. 4). The concentrations of ^{35}S were equivalent to the following amounts of BAL (mg.)/g. of fresh tissue: blood, 0.01; liver, 0.03; kidney, 0.06; heart, 0.04; lung, 0.03; spleen, 0.03; small intestine and contents, 0.12; skin, 0.03; carcass, 0.3. As in the case of rat no. 1, which was analysed 6 hr. after the application of radioactive BAL to the skin, the ^{35}S concentration of the small intestine and its contents was well above the average for the other tissues. In the kidney, however, the

concentration of ^{35}S was not much higher than the average, and in this respect rat no. 4 differed from rat no. 1.

At 12 hr. after injection, but not at 24 hr. after injection, detectable amounts of ^{35}S were found in the blood, liver and kidney. ^{35}S was excreted quite rapidly in the urine of the injected animals (see also Table 3). The amounts of ^{35}S found in the faeces were very small and may have originated from contamination of the faeces with urine.

Urinary excretion of ^{35}S after the administration of radioactive BAL to the rat

Table 3 shows the amounts of ^{35}S excreted in the urine in various periods after the administration of radioactive BAL to the rat by application to the skin or by intramuscular injection. The amounts of radioactive BAL which were absorbed when the

Table 3. *The urinary excretion of ^{35}S by the rat in 0-6, 0-12 and 0-24 hr. following the administration of radioactive BAL by application to the skin* or by intramuscular injection*

Rat no.	...	1	2	3	4	5	6
Radioactive BAL absorbed by the skin (mg.)		25	21	21	—	—	—
Radioactive BAL injected intramuscularly (mg.)		—	—	—	20	20	20
		% of ^{35}S absorbed as BAL		% of ^{35}S injected as BAL			
Urine collection period		35	43	43	47	50	46
0-6 hr. after administration		—	61	65	—	66	71
0-12 hr. after administration		—	—	72	—	—	81
0-24 hr. after administration							

* Absorption of radioactive BAL by the skin was allowed to proceed for 6 hr.

compound was left in contact with the skin for 6 hr. (25, 21 and 21 mg.) were not greatly different from the amount administered (20 mg.) in each of the experiments in which the compound was injected intramuscularly, and in both sets of experiments the rate of excretion of ^{35}S was approximately the same. This rate of excretion was such that the ^{35}S from about three-quarters of the dose of radioactive B was excreted in the 24 hr. following the administration of the compound.

Some interest attaches to the form in which ^{35}S is excreted in the urine of animals dosed with radioactive BAL. As a preliminary step in a study of this question the stability of BAL dissolved in urine was investigated. Aqueous solutions of BAL were prepared and added to normal rat urine so as to yield solutions which contained 1.0 mg. and 0.1 mg. of BAL/ml. After measured intervals of time, sample portions of these solutions were titrated with standard potassium iodate solution in the presence of hydrochloric acid and excess potassium iodide. A similar set of experiments was performed using

rat urine which had been made just acid to congo red by the addition of hydrochloric acid. The results obtained are given in Table 4, and they show that the thiol content of the solutions of BAL in urine decreased rapidly, but that this process occurred more slowly in urine which had been acidified.

Table 4. *The thiol content of normal rat urine and acidified rat urine at various times following the addition of BAL*

Time (min.)	Initial concn. of BAL in urine			
	1.0 mg./ml.		0.1 mg./ml.	
	Percentage of initial thiol concn.*			
	Normal urine	Acidified urine	Normal urine	Acidified urine
5	95	100	—	100
30	75	99	52	97
(hr.)				
1	63	98	43	95
3	22	96	20	85
6	10	93	6	73
12	4	88	—	60
24	—	76	—	33

* All values are corrected for the 'blank' thiol concentration of the urine.

An experiment was performed in which a male white rat (wt., 180 g.) was given an intramuscular injection of 20 mg. of radioactive BAL in 0.30 ml. of propylene glycol under the conditions already described. The animal was placed in a metabolism cage and its urine was collected in a flask which contained sufficient 2N-hydrochloric acid to render the urine just acid to congo red. The animal was anaesthetized in the metabolism cage at the end of 24 hr. after dosing and this caused it to empty its bladder. The total ^{35}S content of the urine was determined by the method described by Young *et al.* (1949). The ^{35}S content of the total sulphate fraction of the urine was determined as follows.

Urine (5 ml.) to which 5 ml. of 3N-HCl had been added was evaporated to dryness by heating on a water bath. Water (3 ml.) was added to the residue, and this yielded an acid solution containing a small amount of insoluble material. The mixture was centrifuged, the supernatant fluid was removed and the residue was washed with three 1 ml. portions of water and centrifuged after each washing. The supernatant fluid and washings (total vol., 6 ml.) were combined, and 4 ml. of 95% (v/v) ethanol and 4 ml. of benzidine hydrochloride reagent were added. The benzidine sulphate which formed was separated and its ^{35}S content was measured. The insoluble residue obtained after the hydrolysis of the urine was transferred to a Carius bomb, and its ^{35}S content was determined by the method of Young *et al.* (1949).

The amounts of ^{35}S (expressed as percentages of the ^{35}S injected as radioactive BAL) found in the various fractions of the urine were as follows: total

sulphur fraction, 74; total sulphate fraction, 3; insoluble fraction obtained after hydrolysis, 8%. Under the experimental conditions used, it appears unlikely that the insoluble fraction contained significant amounts of ^{35}S in the form of sulphate. Even if all the ^{35}S in the insoluble fraction was in the form of sulphate, 63% of the ^{35}S administered as BAL was present in the neutral sulphur fraction of the urine. It can be stated, therefore, that a large proportion (63–71%) of the ^{35}S injected as BAL was excreted in 24 hr. in the neutral sulphur fraction of the urine, and only a small proportion (3–11%) was excreted in the form of sulphate in this period. These findings are similar to those obtained by Peters *et al.* (1947) following the administration of a propylene glycol solution of radioactive BAL to rats by intraperitoneal, subcutaneous or intramuscular injection.

The presence of a large proportion of the ^{35}S in the neutral sulphur fraction of the urine made it appear possible that unchanged BAL was excreted in the urine.

In order to investigate this possibility three male rats (weighing 178, 180 and 185 g.) were each given an intramuscular injection of 20 mg. of radioactive BAL dissolved in 0.30 ml. of propylene glycol. The animals were placed in a metabolism cage over a glass funnel and the urine was collected for 6 hr. in a vessel which contained sufficient 2N-HCl to render the urine acid to congo red. Analysis of a sample of the urine showed that 46% of the ^{35}S administered as BAL was excreted during this period. At the end of the collection period an aqueous solution (5 ml.) of 0.20 g. of non-radioactive BAL was added to the urine. The urine was then shaken with several portions of peroxide-free ether. The ether extracts were combined and dried over anhydrous Na_2SO_4 . The ether was evaporated and the residue was distilled at low pressure. This yielded 0.172 g. of BAL; 'SH' content (by I_2 titration), 53.2%; calc. for $\text{C}_3\text{H}_5\text{OS}_2$, 53.2%. This material was converted to 2-phenyl-4-hydroxy-methyl-1:3-dithiolane by refluxing it on a water bath for 6 hr. with 10 ml. of benzene, 0.28 ml. of benzaldehyde and three drops of conc. HCl. The mixture was then evaporated to dryness in a current of air. The residue was dissolved in 1 ml. of benzene and precipitated by the addition of 2 ml. of light petroleum (b.p. 30–60°). This process was repeated and the crystalline material obtained was then extracted with boiling *n*-hexane. The *n*-hexane was removed by evaporation and the residue was recrystallized three times from benzene-light petroleum mixture. The dithiolane thus obtained weighed 0.200 g.; m.p. 66–68°. (Found: C, 56.4; H, 5.6; S, 30.5%. Calc. for $\text{C}_{10}\text{H}_{12}\text{OS}_2$: C, 56.6; H, 5.7; S, 30.2%.) When this compound was analysed for ^{35}S it was found that its radioactivity was too low to measure.

The conditions in the experiment just described were such that if 1% of the administered radioactive BAL had been present in the unchanged form at the end of the collection period and had then been converted to the dithiolane, this derivative would have contained measurable amounts of ^{35}S . The fact that this experiment yielded no evidence for the presence

of unchanged BAL in the urine does not rule out the possibility that the unchanged compound was excreted. While it seems unlikely that a major portion of the ^{35}S was excreted in the form of BAL it should be noted that the thiol content of a solution of BAL in urine decreases on standing even when the urine has been acidified, and small amounts of BAL, especially if they were excreted in the early part of the experimental period, might well have undergone chemical change by the end of the period during which urine was collected.

DISCUSSION

The present investigation has shown that BAL is absorbed slowly by the skin of the rat, for in the 6 hr. period following the application of radioactive BAL to the skin the average rate of absorption of ^{35}S corresponded to 0.38 mg. BAL/sq.cm. skin/hr. Values similar to this were obtained in experiments in which non-radioactive BAL was applied to the skin of the rat and the amount of unabsorbed BAL was determined by iodate titration (Simpson *et al.* 1944). It seems probable that the rate of absorption of BAL by human skin does not differ greatly from that found for the skin of the rat. In experiments in which radioactive BAL was applied to the flexor surfaces of the forearms of two human subjects and evaporation from the dosed areas was prevented by the method used in the experiments with rats, the average rate of absorption of ^{35}S during the 3 hr. period following dosing corresponded to 0.59 and 0.24 mg. BAL/sq.cm. skin/hr. The low rate of absorption of BAL is particularly interesting in view of the fact that the direct application of BAL to skin contaminated with lewisite is highly effective in preventing injury to the skin. Whether the rate of absorption of BAL by the skin is altered in the presence of lewisite has not been determined.

When the purpose of BAL administration is to combat systemic arsenical poisoning (or poisoning by other heavy metals for which BAL has been shown to be an antidote) it would appear desirable to administer the BAL by a method which makes it available within the organism more rapidly than would occur if it were applied to the skin. The mode of administration of BAL commonly employed for this purpose is intramuscular injection and the present work has shown that absorption of radioactive material takes place readily from muscle which has been injected with a solution of radioactive BAL in propylene glycol.

6 hr. after the administration of radioactive BAL to the rat by application to the skin or by intramuscular injection ^{35}S was found to be present in many tissues. It is not known, however, whether this ^{35}S was present as BAL or in the form of compounds incapable of exerting an antidotal action. Even if all the ^{35}S in the organism under these conditions was

in the form of BAL (and this seems unlikely) the total amount present was considerably less than that which had been absorbed from the skin or injected intramuscularly. The main reason for the falling off in the amount of ^{35}S in the organism was the passage of radioactive material into the urine, e.g. in the 6 and 12 hr. periods following the intramuscular injection of radioactive BAL 46–50 and 66–71 % respectively of the ^{35}S administered were excreted in the urine.

Most of the ^{35}S excreted in the 24 hr. period following the intramuscular injection of radioactive BAL into rats was present in the neutral sulphur fraction of the urine and relatively little was present in the total sulphate fraction. The nature of the ^{35}S -containing compound (or compounds) in the neutral sulphur fraction of the urine has yet to be established. Stocken & Thompson (1946) reported that the subcutaneous or intraperitoneal injection of BAL into rats or rabbits was followed by the excretion of an unidentified thiol (or thiols) in the urine, and Spray, Stocken & Thompson (1947) found that a considerable proportion of the thiol(s) present in urine obtained from catheterized rabbits after the intramuscular injection of BAL appeared to be present as a compound closely related to BAL, but not identical with it. In the present work no evidence was obtained for the presence of unchanged radioactive BAL in the urine of rats dosed with this compound. A negative finding under the conditions employed is not conclusive, however, for it is possible that some unchanged BAL was excreted and then underwent chemical change while the urine was being collected. It may be that BAL is excreted in the urine in conjugation with some other substance. This possibility has received some support from the work of Spray (1947), who found that there was an increased excretion of glucuronic acid in the urine of rats in the 24 hr. period following the injection of BAL.

SUMMARY

1. A description is given of the synthesis of 2:3-dimercaptopropanol (BAL) with radioactive sulphur (^{35}S) incorporated in the molecule.

2. The absorption, distribution and excretion of ^{35}S have been studied following the administration of radioactive BAL to rats by application to the skin or by intramuscular injection.

3. When radioactive BAL is applied to the skin of the rat it is absorbed slowly, the ^{35}S which is absorbed is distributed throughout the organism and most of it is excreted in the urine within the following 24 hr.

4. When radioactive BAL in propylene glycol is administered to rats by intramuscular injection, the ^{35}S passes almost completely from the dosed muscle in 6 hr. Radioactive material is present in most of

the tissues 6 hr. after the injection. A large proportion of the ^{35}S is excreted in the neutral sulphur fraction of the urine in the 24 hr. following the injection of radioactive BAL.

A short account of this work was presented at a meeting of the Biochemical Society held at St Thomas's Hospital Medical School, London, S.E. 1, on 18 February 1949 (Simpson & Young, 1949).

REFERENCES

- Masters, M. (1939). *Biochem. J.* **33**, 1313.
Meulen, H. ter (1922). *Rec. Trav. chim. Pays-Bas*, **41**, 112.
Peters, R. A., Spray, G. H., Stocken, L. A., Collie, C. H., Grace, M. A. & Wheatley, G. A. (1947). *Biochem. J.* **41**, 370.
Peters, R. A., Stocken, L. A. & Thompson, R. H. S. (1945). *Nature, Lond.*, **156**, 616.
Simpson, S. D. (1947). *Canad. J. Res. B*, **25**, 20.
Simpson, S. D. & Young, L. (1945*a*). Report to Directorate of Chemical Warfare, Canada.
Simpson, S. D. & Young, L. (1945*b*). Report to Directorate of Chemical Warfare, Canada.
Simpson, S. D. & Young, L. (1945*c*). Report to Directorate of Chemical Warfare, Canada.
Simpson, S. D. & Young, L. (1949). *Biochem. J.* **44**, xxv.
Simpson, S. D., Zbarsky, S. H. & Young, L. (1944). Report to Directorate of Chemical Warfare, Canada.
Spray, G. H. (1947). *Biochem. J.* **41**, 366.
Spray, G. H., Stocken, L. A. & Thompson, R. H. S. (1947). *Biochem. J.* **41**, 362.
Stocken, L. A. & Thompson, R. H. S. (1946). *Biochem. J.* **40**, 548.
Young, L. (1941). Report to Directorate of Chemical Warfare, Canada.
Young, L. (1946). *Science*, **103**, 439.
Young, L. (1947). *Canad. J. Res. E*, **25**, 137.
Young, L., Edson, M. & McCarter, J. A. (1949). *Biochem. J.* **44**, 179.