The Fate of Arsenical Vesicants in the Skin and the Effect of BAL (British Anti-Lewisite)

By A. F. GRAHAM, G. A. LEVY AND AVERIL C. CHANCE, *Departments of Biochemistry and Pharmacology, University of Edinburgh*

(Received 9 December 1946)

The work described in this paper was carried out on behalf of the Ministry of Supply, and preliminary accounts of it were circulated by the Ministry in 1943 and early in 1944. A quantitative investigation was made of various factors concerned in the disappearance of arsenic from the skin after the application of an arsenical vesicant. These factors determine the severity of the local and systemic effects caused by a given amount of a vesicant. Phenylchloroarsine and lewisite I (β -chlorovinyl-dichloroarsine) were studied, using rabbits as experimental animals. The amount of arsenic removed from the skin by absorption into the general circulation or evaporation of the vesicant, and the fraction of the residual arsenic which was fixed by combination with skin constituents, were determined at varying periods after applying the vesicant. The effect of varying the amount of vesicant applied to a given area of skin was examined. Stocken & Thompson (1946) had previously found that BAL (British Anti-Lewisite, 2:3-dimercaptopropanol) diminished the amount of arsenic remaining in the skin of rats 48 hr. after contamination with lewisite. Experiments were done to discover the way in which BAL modifies the fate of arsenical vesicants in the skin.

EXPERIMENTAL

General methods

The vesicants (obtained from the Ministry of Supply) were purified by distillation *in vacuo*. They were applied to the skin as freely falling drops, measured from the 'burette' devised by Lane (1946) or from the modification of this instrument developed by Levy (1946). The amounts of liquid to be measured were so very small that they could be delivered accurately only in the form of discrete drops. In using Lane's instrument, it was found convenient to retard the drop rate by applying a negative hydrostatic pressure to the column of vesicant. In experiments with phenylchloroarsine, two different drop sizes were employed (5 and 0.5 mg.), and in the case of lewisite I, three (4.5, 0.5 and 0.133 mg.). The exact weight of the drop applied in an individual experiment was calculated from the readings given by the instrument at the time, and this figure was used in working out the results. For drops of any one magnitude, the range from experiment to experi-

ment in the calculated figure for the weight was not more than 5% of the mean.

The rabbits used ranged in weight from 2 to 3 kg. and were of both sexes. For treatment with the vesicant, an area of skin on the back was freed from fur by clipping closely with scissors. One or more circles, approximately 3 cm. in diameter, were drawn in Indian ink on the skin, as far apart as possible if there were more than one. One drop of vesicant was allowed to fall on to each of these marked out 'spots'. In some cases the spot was immediately covered with a glass vessel, 2 cm. in diam. and 1 cm. in depth, which was firmly attached to the skin with adhesive plaster. Open glass cylinders of the same dimensions were secured to spots which were to be left uncovered so as to prevent the animal from removing the vesicant. The animal was kept in a warm room for a predetermined period and then killed. A piece of skin of 1.5-2 cm. radius around the point of impact of the drop of vesicant was excised, all underlying fatty tissue being left behind.

The excised skin was analyzed directly for arsenic or submitted to preliminary extraction with water or acetone. When arsenic was to be directly determined, the glass cover or guard was washed with benzene and arsenic in the washings was estimated separately or together with that in the skin, after removal of the benzene in presence of water. No appreciable amounts of arsenic were ever found in the benzene washings when analyzed separately. Extractions were carried out in a 50 or 100 ml. Soxhlet apparatus, the skin (plus glass cover or guard) being placed directly in the extraction chamber since paper thimbles were found to adsorb some of the arsenic. Arsenic was determined in the extract and residue, after the removal of acetone if it was initially present.

Portions of the extracts were dialyzed through 'Visking' sausage casing in the refrigerator. To prepare acetone extracts for dialysis, this solvent was replaced by evaporation *in vacuo* with at least 2 vol. water. Although this process resulted in the formation of a fat emulsion, the amount of arsenic in solution did not depart from the original figure.

Arsenic was determined by Levy's method (1943), using the small-scale digestion procedure. Whenever the amount of arsenic permitted, three or more portions of the skin digest or extract were analyzed. Control experiments were frequently done in which the arsenic in normal skin or filter paper treated with a drop of the vesicant was determined. This gave a check on the measurement of the vesicant and on the analytical procedure. Satisfactory results were obtained throughout. Normal rabbit skin was found to contain no detectable amounts of arsenic.

RESULTS

The absorption of vesicants from the skin

In these experiments the contaminated skin was covered to prevent loss of vesicant by evaporation. The absorption into the general circulation in a given period was found by determining the total residual arsenic. Results for the absorption of lewisite I are shown in Table 1 and of phenyldichloroarsine in

Table 1. *The disappearance of lewisite I from covered skin*

Time after application (hr.)	No. of experiments done	Average for arsenic absorbed as % applied
4.5 mg. drops		
0.25	1	21
0.5	2	21
1	2	24
2	3	37
3	1	58
4	3	56
6	1	76
10	1	72
16	2	83
24	3	78
30	1	89
48	2	90
96	1	91
168	1	85
0.5 mg. drops		
0.25	3	27
0.5	2	36
1	1	59
2	1	44
4	2	55
6	1	60
24	1	51
48	1	73
96	1	61
0.133 mg. drops		
1	1	40
24	1	48
72	1	50

Table 2. Some of the figures represent results for experiments with single animals whilst others are the averages for several animals. Since the rate of absorption of either vesicant appeared to be independent of the total number of drops applied, this factor has been ignored in compiling the tables. In most of the experiments the animal was treated with the vesicant at two points, in a few at one only and in a limited number at more than two.

It can be seen from Table 1 that absorption from 4.5 mg. drops of lewisite I ceased after about 24 hr., by which time 90 % had been transported from the skin; with 0.5 mg. drops, 60 % was absorbed in 1 hr. and there appeared to be little change thereafter. Absorption from 0.133 mg. drops showed little variation over the time range studied (1-72 hr.),

the average amount of arsenic removed being about 45 % of the total applied.

After 24 hr., the absorption of phenyldichloroarsine (Table 2) was about 45 % from 5 mg. drops and 35 % from 0.5 mg. drops. The divergence of the results for the two drop sizes can be accounted for from the initial delay of $\frac{1}{2}$ hr. before absorption from the smaller commenced. With both drop sizes, absorption, although slow, still appeared to be in progress after 6 or 7 days. Absorption of phenyldichloroarsine was not only slower than that of lewisite I, but in contrast to the latter never appeared to reach completion in the period studied.

With either vesicant, absorption must be influenced to a great extent by the rate of hydrolysis on and in the skin to the less penetrant arsenoxide.

Table 2. *The disappearance of phenyldichloroarsine from covered skin*

Time after application (hr.)	No. of experiments done	Average for arsenic absorbed as % applied
5 mg. drops		
0.5	3	13
1	3	9
1.5	1	12
2	1	21
3	1	22
4	6	32
6	1	39
10	2	21
18	1	40
24	3	43
30	1	50
48	1	49
96	1	47
168	1	56
0.5 mg. drops		
0.5	2	0
1	1	17
2	1	18
3	1	20
4	2	25
6	1	21
15	1	29
24	3	35
48	1	31
96	2	33
144	1	54

While hydrolysis of both compounds studied occurs extremely rapidly in water, it is probably greatly delayed in the outer layers of the skin. It seems certain, however, that all the arsenic transported from the skin in the blood will be in the form of the arsenoxide.

The evaporation of vesicants from the skin

It was considered that comparison of the rates of disappearance of arsenic from covered and from uncovered skin should reveal any loss of vesicant by evaporation. Table 3 shows the rate at which

lewisite I disappeared from uncovered skin. Each animal in these experiments was treated with either one or two uncovered drops of vesicant. As before, the number of drops applied to the animal did not affect the result. On comparing the results in Table 3 with those in Table 1, it seems that, if any loss of the vesicant by evaporation did occur, it did not exceed 10% of the total applied.

Table 3. *The disappearance of lewisite I from uncovered skin*

Time after application (hr.)	No. of experiments done	Average for arsenic lost by absorption and evaporation as % applied
4.5 mg. drops		
0.5	1	24
1	3	36
2	1	47
4	2	61
6	2	78
10	1	80
24	3	87
48	1	96
96	1	83
168	1	96
0.5 mg. drops		
0.5	2	59
4	3	67
24	1	79

A slightly different procedure was followed in studying the evaporation of phenyldichloroarsine from the skin. Each animal was treated with equal numbers of covered and uncovered drops of vesicant (usually one of each). The results for removal of arsenic from covered skin in these experiments have already been shown under the appropriate times in Table 2. Table 4 gives the corresponding figures for uncovered skin. Comparison of the two sets of results suggests that up to 25% of the vesicant applied may evaporate from the skin.

Table 4. *The disappearance of phenyldichloroarsine from uncovered skin*

Time after application (hr.)	No. of experiments done	Average for arsenic lost by absorption and evaporation as % applied
5 mg. drops		
1	3	18
4	6	55
10	2	48
24	3	49
0.5 mg. drops		
4	2	42
24	3	50

It should be pointed out that absorption and evaporation are probably mutually dependent pro-

cesses, each decreasing the amount of vesicant available for removal by the other. Experiments of the type described above can therefore be regarded as of qualitative value only.

To discover whether under ideal conditions evaporation of phenyldichloroarsine from the skin would proceed at all rapidly, dry filter paper was treated with 5 mg. drops and suspended in a current of dry air in an incubator at 38°. Only 60% of the arsenic disappeared in 4 hr. and 70% in 24 hr.

The fixation of arsenical vesicants in the skin

Arsenic which could not be extracted by fat solvents was considered to have been fixed by combination with skin constituents. In studying this, each animal was treated with the vesicant in two places. One piece of contaminated skin was extracted with a fat solvent, almost always acetone, while in the majority of experiments the other was extracted with water. All extractions were done for periods found to be more than ample for complete removal of arsenic extractable by the solvent.

Table 5 shows the free (acetone-soluble) and fixed (acetone-insoluble) arsenic in skin at varying periods after the application of lewisite I, while Table 6 gives the corresponding figures for phenyldichloroarsine. Since the fixed arsenic was never more than a small fraction of the total originally present, it is given as $\mu\text{g.}$ arsenic as well as in terms of percentage applied.

Table 5. *The fixation of lewisite I in the skin*

(Single experiments; skin extracted with acetone.)

Time after application (hr.)	Extract as % applied	Residue	
		$\mu\text{g. As}$	% applied
4.5 mg. drops, uncovered			
3	31	73	5
6	25	52	3
24	10	101	6
72	4	29	2
4.5 mg. drops, covered			
0.25	81	88	5
0.5	84	83	5
4	46	198	11
4.5	43	210	13
48	12	191	12
96	18	246	14
0.5 mg. drops, uncovered			
4	—	20	11
24	5	20	11
0.5 mg. drops, covered			
0.5	44	24	13
25	12	29	16
0.133 mg. drops, covered			
1	43	8	16
24	41	11	22
72	8	14	28

With 4.5 mg. drops of lewisite I, 5% of the arsenic applied was fixed at an early stage. When the skin was left uncovered, this figure remained constant for a considerable period. When the skin was covered, however, the arsenic fixed had risen 4 hr. after application to 12% of the total. With 0.5 mg. drops of lewisite I, the arsenic fixed was about 12% of the total applied, and the difference between covered and uncovered skin was less marked than with the larger drops. About 20% of the vesicant was fixed when it was administered as 0.133 mg. drops (covered). It should be noted that free arsenic was still present at periods when on the basis of former experiments absorption must have ceased.

When phenyldichloroarsine was applied to the skin, approximately 4% of the arsenic was fixed with 5 mg. drops (covered and uncovered), and at least 10% with 0.5 mg. drops. As expected from the results for the absorption of this vesicant, considerable amounts of free arsenic were present after 24 hr. On the whole it seems to be safe to say that with both vesicants the fraction fixed in the skin increased as the size of drop applied was made smaller.

Table 6. *The fixation of phenyldichloroarsine in the skin*

(Unless indicated to the contrary, skin extracted with acetone.)

Time after application (hr.)	Extract as % applied	Residue	
		$\mu\text{g. As}$	% applied
5 mg. drops, uncovered			
4	60	68	4
24	29	73	5
5 mg. drops, covered			
0.5	76	26	2
0.5	85	78	5
4	71	75	5
10	59	64	4
10*	47	55	3
24	37	42	3
24†	36	66	4
24	58	92	6
24‡	44	46	3
0.5 mg. drops, uncovered			
24	9	17	10
0.5 mg. drops, covered			
1	90	8	5
4	65	18	11
24	60	27	17

* Extracted with benzene instead of acetone.

† Skin desiccated before extraction with acetone.

‡ Extracted with acetone, benzene, ether and carbon tetrachloride.

In one of the experiments with phenyldichloroarsine shown in Table 6, the skin was dried *in vacuo* over H_2SO_4 for 14 days before extraction with acetone. This treatment

did not appear to affect the course of the extraction in any way. In other experiments listed in Table 6, the effects of extracting with fat solvents other than acetone were examined. Extraction with benzene took the same course as with acetone. When, after exhaustive treatment with acetone, the skin was extracted successively for 12 hr. with each of the following solvents, the amounts of arsenic in each extract were: acetone, 695; benzene, 5; ether, 6; carbon tetrachloride, 8 $\mu\text{g. As}$. It thus appears that the behaviour of the arsenic in skin towards extraction by a fat solvent is independent of the particular solvent used. The arsenic in such extracts probably corresponds to the free vesicant and its hydrolysis product.

All the arsenic in skin at any period after treatment with lewisite I or phenyldichloroarsine could be removed by extraction with water. In an experiment with phenyldichloroarsine, the skin after exhaustive extraction with acetone was extracted with water. All the acetone-insoluble arsenic was thereby removed.

The effects of BAL

The effects produced by dropping BAL (obtained from the Ministry of Supply) on to the contaminated skin on the absorption and fixation of lewisite I and phenyldichloroarsine are shown in Table 7. Each animal was treated with the vesicant at two points, both areas of skin being treated with BAL, and the skin was left uncovered in all experiments. Figures in the table for the dose of BAL refer to the amount applied to each contaminated area. After excision, one piece of skin was analyzed for total arsenic while the other was extracted with acetone. The results obtained by direct analysis for the absorption of arsenic are not quoted since they were in good agreement with the corresponding figures calculated from the total arsenic in extract plus residue (last col. in Table 7). It is at once apparent from the table that percutaneous administration of BAL reduced the arsenic fixed with either vesicant to, at the most, only a small fraction of its former value. Up to 6 hr. delay in applying the BAL did not affect the final result, showing that the fixation of arsenic was actually reversed. As regards absorption, comparison with Tables 3 and 4 reveals no appreciable hastening of this process. While the effect of BAL on the fixed arsenic might be expected to decrease the residue found at the end of any given period, the amount fixed is such a small fraction of the total applied that any change produced in the absorption will be masked by the very large animal variation.

In other experiments with lewisite I, BAL was given by injection either before or after applying the vesicant (Table 8). Subcutaneous injections were done about 4 cm. from each contaminated area, the dose shown in the table being divided between the two points of injection. Intravenous injections were made into the ear. All BAL solutions were made up immediately before use. Except in two

Table 7. *The effect of percutaneous administration of BAL on the fate of phenyldichloroarsine and lewisite I in the skin*

(Dose of BAL expressed as amount applied to each contaminated area.)

Time killed after con- tamination (hr.)	Time of BAL administration after con- tamination (hr.)	Amount of BAL (mg.)	Extract as % applied	Residue		Arsenic absorbed as % applied
				μg. As	% applied	
5 mg. drops of phenyldichloroarsine						
24	2	26	51	0	0.0	49
0.5 mg. drops of phenyldichloroarsine						
24	2	26	30	0	0.0	70
4.5 mg. drops of lewisite I						
2	0.1	28	55	0	0.0	45
4	0.5	25	45	12	0.7	54
4	2	28	25	0	0.0	75
6	4	28	25	11	0.7	74
7	0.1	28	40	6	0.4	60
7	2	28	42	2	0.1	58
24	0.1	28	15	17	1.0	84
24	0.5	28	33	6	0.3	67
24	2	28	8	4	0.2	92
24	4	28	12	12	0.7	87
96	2	26	11	16	1.0	88
96	6	26	6	5	0.3	94
0.5 mg. drops of lewisite I						
1	0.1	5	57	0	0.0	43
24	0.1	5	11	1	0.5	88
96	2	26	3	1	0.8	96
96	2	25	6	3	1.4	93
96	6	26	7	3	1.6	91

Table 8. *The effect of injection of BAL on the fate of lewisite I in the skin*

(Dose of BAL expressed as total amount injected into the whole animal. Unless indicated to the contrary, BAL injected subcutaneously in peanut oil.)

Time killed after con- tamination (hr.)	Time of BAL administration with respect to contamination (hr.)	Amount of BAL (mg.)	Extract as % applied	Residue		Arsenic absorbed as % applied
				$\mu\text{g. As}$	% applied	
4.5 mg. drops						
1	-1	200	30	59	4	66
2	-1	100	22	62	4	74
4*	+0.1	60	49	70	4	47
4†	0 and +0.5	50	18	153	9	73
24†	0, +0.5 and +4.5	75	5	127	8	87
96	0	200	2	9	1	97
0.5 mg. drops						
2	-1	100	38	9	5	57
96	0	100	5	15	8	87

* Subcutaneous injection of BAL in 0.9% NaCl.

† Intravenous injection of BAL in 0.9% NaCl.

experiments in which BAL was given intravenously, the contaminated skin was left uncovered. BAL did not affect the fixed arsenic in these experiments. While the results are not clear on this point, it is doubtful whether absorption was hastened.

Dialysis of water and acetone extracts of skin

In a great number of the experiments with both vesicants, samples of the extracts were submitted to dialysis. In every case, the arsenic in either type of

extract was completely dialyzable. This was also true for acetone extracts in the experiments with BAL.

DISCUSSION

From the results of the experiments described above, it is possible to formulate certain broad principles regarding the fate of lewisite I or phenyldichloroarsine applied to the skin of rabbits. With both vesicants, an increase in the size of drop applied favoured absorption into the general circulation at the expense of the arsenic (free and fixed) remaining at the site of contamination. Absorption of lewisite I rapidly reached a maximum (characteristic of the drop size) and then ceased. The fraction of a given amount of phenyldichloroarsine which was absorbed in 24 hr. was only about half the corresponding figure for lewisite I, but absorption, although very slow, still appeared to be in progress after much longer periods. While the experiments dealing with evaporation were unsatisfactory from a quantitative point of view, it appears that while the amount of lewisite I which volatilized from the skin was so small as to be negligible, appreciable amounts of phenyldichloroarsine did evaporate. A fraction of the arsenic applied could not be removed by extraction with acetone. This fraction was smaller the larger the drop applied, and there was no important difference between the two vesicants in the amounts of arsenic fixed.

The only effect of BAL administration on the arsenic in skin was to prevent or reverse the fixation of arsenic, and this effect was only seen when the BAL was applied to the contaminated area. The rate of absorption of arsenic was not noticeably increased by BAL. These experiments thus provide direct evidence for an *in vivo* reversal by BAL of a reaction between the arsenicals and skin constituents, and it appears likely that the fixed arsenic as measured by its insolubility in acetone is the fraction responsible for the local reaction. It is interesting to note that in unreported work it was found that 1 hr. after intravenous injection of phenylarsenoxide or *m*-

amino-*p*-hydroxyphenylarsenoxide into rabbits, none of the arsenic in liver could be extracted with acetone. After 24 hr., up to 30 % of the arsenic was extractable. Subcutaneous injection of BAL decreased the arsenic content, but did not affect the percentage extracted.

The explanation of the finding by Stocken & Thompson (1946) that the arsenic remaining in the skin of rats 48 hr. after treatment with lewisite was decreased by BAL would appear to be that in their experiments practically all the arsenic left in the skin after this period was fixed. In work subsequent to our own it was found by L. Young and his colleagues in Toronto that BAL did not affect the rate of disappearance of arsenic from the skin of rats treated with lewisite, even when applied directly to the contaminated area (private communication).

SUMMARY

1. Lewisite I (β -chlorovinyl-dichloroarsine) applied to the skin of rabbits was more rapidly absorbed into the general circulation than phenyldichloroarsine.
2. With both vesicants the percentage absorption was greater the larger the amount applied to the skin.
3. Evaporation from the skin appeared to be negligible with lewisite I, but appreciable with phenyldichloroarsine.
4. At any time after applying either vesicant, part of the arsenic in the skin could not be removed by extraction with acetone.
5. After treating the skin with BAL (2:3-dimercaptopropanol), all the arsenic could be extracted with acetone. Injection of BAL did not produce this effect.
6. BAL did not appear to affect the rate at which the vesicants were absorbed.

Permission from the Director General of Scientific Research (Defence), Ministry of Supply, to publish this work is gratefully acknowledged.

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