been studied when given by intravenous injection. In agreement with theory, increasing polarity of the molecule results in diminished toxicity. Mercaptosuccinate, acetylmercaptosuccinate, mercaptoadipate, dimercaptoadipate and glutathione cause no pathological symptoms in rabbits in dosage of up to 1 g./kg.

3. Many of these thiols when given by intravenous injection were able to prolong the life of rabbits given 3 LD₅₀ of lewisite.

4. The most effective therapeutic action was obtained with BAL-INTRAV, which prevented death in 100 % of the animals studied, even if not given until 6.5 hr. after administration of 3 LD₅₀ of lewisite (which in untreated animals kills in 10 to 20 hr.).

5. Details are given of the preparation of mercaptosuccinic acid, dimercaptoadipic acid, glucosylthiose, BAL-INTRAV (BAL glucoside), and a number of other thiol acids.

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Changes Undergone by Phenylarsenious Acid and Phenylarsonic Acid in the Animal Body

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It is believed that many of the most important biological properties of the arsonic acids result from their partial reduction in the body to the corresponding arsenious acids. The evidence that this reduction does occur is, however, entirely indirect. Arsonic acids display a negligible parasitocidal effect *in vitro* and are effective *in vivo* only after a lapse of several hours following injection. The corresponding

arsenious acids, on the other hand, are effective *in vitro*, and *in vivo* act immediately after injection (Voegtlin & Smith, 1920a; Yorke & Murgatroyd, 1930). Serum from animals injected with trypanamide has been found to be trypanocidal *in vitro*, but the serum shows maximal activity only if it is withdrawn a long time after the injection of the arsonic acid. The arsenic content of the serum when

the trypanocidal activity is greatest must be a small fraction of the original value. In contrast, the trypanocidal activity of serum from animals injected with reduced tryparsamide dithioglycollate is greatest when the arsenic content is at its peak (Murgatroyd, Russell & Yorke, 1934). *In vitro*, Thuret (1938 *a*) found that erythrocytes do not fix pentavalent, only trivalent, arsenicals. Following the injection of pentavalent arsenicals into the living animal, however, part of the blood arsenic is found attached to the erythrocytes (Thuret, 1938 *b*).

If reduction of arsonic acids occurs in the body, it is reasonable to assume that oxidation of arsenious acids will also take place prior to their excretion in the urine, since arsonic acids are more rapidly excreted (Voegtlin & Thompson, 1923). From results obtained in a study of the fate of arsenic in the body following injection of rabbits with phenylarsenious acid, mapharsen (*m*-amino-*p*-hydroxyphenylarsenoxide), and the arsonic acid corresponding to each, it was postulated that the rates of excretion of the trivalent compounds were largely determined by the rates at which they were oxidized in the body to the arsonic acids (Chance, Crawford & Levvy, 1945). Since there is some evidence to suggest that the arsenobenzenes owe their therapeutic action to oxidation in the body to arsenious acids (Voegtlin & Smith, 1920 *b, c*; Eagle, 1939; Rosenthal, 1932), information as to the fate of the latter is of interest also in connexion with the former class of compounds. In certain cases, at least, it is considered that the toxicity of organic arsenicals is partly due to the splitting off of inorganic arsenite or arsenate, but to what extent this occurs with compounds in common use in therapeutics it has been impossible to decide.

The lack of direct evidence regarding the changes undergone by organic arsenicals in the body results from the difficulty attending the separation in biological material of traces of the different compounds possibly formed. In a previous paper (Crawford, 1947), a method was described for the quantitative separation of phenylarsenious acid, phenylarsonic acid, arsenious acid and arsenic acid in urine. After separation of the different fractions, the total arsenic in each was determined by a microtitrimetric procedure (Levy, 1943). The method has now been applied to an investigation of the metabolism of phenylarsenious acid and phenylarsonic acid in rabbits. These compounds may be regarded as the simplest prototypes of compounds in use in therapeutics, and were chosen for investigation since it was considered that the absence of substituents in the benzene ring might facilitate the study of the changes undergone by the arsenic-containing part of the molecule. It should be noted that, in spite of the unfavourable chemotherapeutic index, phenylarsenious acid is a very effective

trypanocidal and treponemicidal agent (Eagle, Doak, Hogan & Steinman, 1940; Hawking, 1937). The initial assumption was made that any changes other than oxidation or reduction and decomposition to give arsenious or arsenic acids would be detected in the separation procedure. From the nature of the procedure, this assumption would appear to be sound.

METHODS

Solutions of phenylarsenious acid and phenylarsonic acid as their sodium salts were prepared and tested as described in an earlier paper (Chance, Crawford & Levvy, 1945). Male rabbits only were used. After administration of the arsenicals by intravenous injection, the rabbits were placed in metabolism cages and the urine collected in flasks, containing 10 ml. $N-H_2SO_4$ and 2 ml. chloroform, which were changed every 24 hr. Control experiments showed that, under the conditions of the urine collection, partial oxidation of phenylarsenious acid sometimes occurred even in the presence of chloroform unless acid was also present. At the end of each period of urine collection, the animal was catheterized and the urine thus obtained added to that already collected. Urine was stored in the refrigerator until it could be analyzed. Cages were well washed between successive periods of urine collection.

A measured volume of urine (40–200 ml.) was dialyzed against 200–310 ml. distilled water containing a few drops of chloroform for 24 hr. in the refrigerator. Experiments in which the urine was submitted to exhaustive dialysis showed all the As excreted to be dialyzable. Samples of dialysate were taken for fractionation according to the method already described (Crawford, 1947). In experiments with phenylarsenious acid, and in the later stages of the phenylarsonic acid experiments, the amounts of As excreted were so small that three days' urine was pooled for dialysis, and it was necessary to use more than 50 ml. of dialysate for each of the two parts of the fractionation procedure. Evaporation *in vacuo* of the dialysate could not be done since control experiments showed that such a procedure led to valency changes in the As. It was found, however, that the analytical method could be applied to volumes of fluid larger than 50 ml. provided corresponding increases were made in the amounts of the reagents. Increasing the amount of dithiol reagent proved unnecessary. In the phenylarsonic acid experiments the As contents of the urine dialysates were usually so high that the whole fractionation could be carried out in triplicate. As was determined in the sac contents at the end of the dialysis period so that the total in the urine could be calculated. To check the amounts found in various fractions in the separation, direct analysis of the original urine samples for total As was also done whenever material permitted.

RESULTS

The results obtained in the fractionation of urine from male rabbits injected with phenylarsonic acid (two doses) and phenylarsenious acid (one dose) are summarized in Table 1. The amounts of drug injected are expressed in mg. As/kg. body weight.

Table 1. *Nature of the urinary As after intravenous injection of phenylarsenious acid and phenylarsonic acid into rabbits*

Exp. no.	Wt. of rabbit (kg.)	Urine collection period (hr.)	Dose of arsenical (mg. As/kg.)	Fraction of dose excreted in urine (%)	Percentage urinary As as				
					Phenyl-arsenious acid	Phenyl-arsonic acid	Arsenious acid	Arsenic acid	Unidentified form
Phenylarsonic acid									
1	2.36	0-24	1.11	25	Tr.*	92	Nil	Nil	Nil
2	2.34	0-24	1.11	70	Tr.	93	Nil	Tr.	Nil
3	2.50	0-24	1.11	71	Tr.	93	Nil	Nil	Nil
4	2.30	0-24	5.56	70	9	91	Nil	Nil	Nil
5	1.92	0-24	5.56	79	8	92	Nil	Nil	Nil
6	2.48	0-24	5.56	80†	8	92	—	—	—
7	1.60	0-24	5.56	54†	Tr.	96	—	—	—
		24-72		2†	23	77	—	—	—
8	1.85	0-24	5.56	60†	8	92	—	—	—
		24-96		1	Tr.‡	73	Nil	Nil	Tr.‡
9	1.87	24-96	5.56	1	Nil	100	Nil	Nil	Nil
Phenylarsenious acid									
10	2.85	0-72	0.45	16	Nil	48	Nil	Nil	52
11	2.15	0-72	0.45	15	Nil	45	Nil	Nil	55
12	2.05	0-72	0.45	6	Nil	56	Nil	Nil	44
13	2.27	0-72	0.45	9	Nil	52	Nil	Nil	48

* Tr. = trace.

† Dialysate extracted with the dithiol reagent only (see text).

‡ The small amount of As not found in the phenylarsonic acid fraction was equally divided between the phenylarsenious acid fraction and the aqueous residue from the fractionation.

Amounts of As in each fraction are shown as percentages of the total As in the urine. The concentration of As in the first 24 hr. specimen of urine in the phenylarsonic acid experiments was sufficiently high to permit of analyses in triplicate and the figures shown in the table are the mean values. In all the other experiments a single fractionation only was possible. When the amount of As in the final determination, while outside the 'blank' range, was so small that the analytical figure had no quantitative significance ($> 4 \mu\text{g.}$), the result for the appropriate fraction is shown in the table as 'trace'.

No As was excreted in the urine as arsenious acid or arsenic acid after injection of phenylarsenious acid or phenylarsonic acid (Table 1). In only one experiment was any As found in the 'inorganic' fractions and, in that, the amount was so small as to be of little significance. After the injection of phenylarsonic acid, most of the As appeared in the urine in the form in which it was administered. Almost always, however, a small fraction of the As appeared in the phenylarsenious acid fraction. After allowing for possible entrainment of up to 5% of the phenylarsonic acid during extraction with the dithiol reagent, the results still suggest that phenylarsenious acid was present in appreciable amounts in many of the samples of urine. Since all As excreted after injecting phenylarsonic acid was found in early experiments to be in the phenylarsenious acid and phenylarsonic acid fractions, it was found possible to employ the 'dithiol reagent' (a solution of ethane-

1:2-dithiol in carbon tetrachloride, Crawford & Levvy, 1946) alone for fractionating the excretion products of this arsenical, and this was actually done in three of the later experiments. It was not possible, however, to simplify the fractionation procedure in this way after injecting phenylarsenious acid, since half the As present was in a form which did not react as arsenious acid, arsenic acid, phenylarsenious acid or phenylarsonic acid. All that one can say as to the nature of the unidentified As is that it was not extracted from acidified urine dialysate with ether after reduction or with the dithiol reagent before or after reduction. Attempts to convert it into arsenious acid, arsenic acid, phenylarsenious acid or phenylarsonic acid by incubating the urine dialysate at 38° at pH 10 for 3 hr. were unsuccessful. Qualitative studies were impossible with the minute amounts of material available. The largest amount of the unidentified arsenical present in three days' urine corresponded to only 106 $\mu\text{g.}$ As.

DISCUSSION

After the injection of rabbits with phenylarsenious acid, no detectable amounts of arsenious acid or arsenic acid were found in the urine; approximately half was probably present in a form which reacted as phenylarsonic acid and the remainder in an unidentified form. These results confirm to some extent the suggestion made in an earlier paper (Chance, Crawford & Levvy, 1945) that the rate of oxidation

of an arsenious acid to the corresponding arsonic acid determines the rate of its excretion in the urine, the latter in turn determining the toxicity of the arsenical (Voegtlin & Thompson, 1923). The presence and nature of the unidentified arsenical in urine from rabbits injected with phenylarsenious acid emphasizes the risk involved in assuming that any As not extracted by an agent reacting with a trivalent arsenical (Crawford & Storey, 1944; Crawford & Levvy, 1946; Graham, Crawford & Marrian, 1946) is the corresponding pentavalent compound without further examination.

Most of the As in the urine after injection of phenylarsonic acid was in a form which reacted as phenylarsonic acid. No arsenious acid or arsenic acid was detected but a small fraction (not more than 10%) of the As appeared to be present as phenylarsenious acid. It is interesting to note that the amount of phenylarsenious acid excreted was, however, sufficient to account for the toxicity of phenylarsonic acid on the theory that the latter must be reduced in the body before producing its lethal effects. In terms of elemental As the lethal dose of phenylarsenious acid is one-seventeenth that of phenylarsonic acid (phenylarsonic acid, LD₅₀ 5.94 mg. As/kg. (Hogan & Eagle, 1944); phenylarsenious acid, LD₅₀ 0.35 mg. As/kg. (Eagle *et al.* 1940). If the toxic effects of phenylarsonic acid are entirely secondary to the formation of phenylarsenious acid, it is difficult to explain the absence of the unknown arsenical, formed from the latter, from the urine of animals injected with the former. The absence of any detectable amounts of phenylarsenious acid in the urine after injection of this substance is not necessarily in contradiction with its presence after injecting the arsonic acid. If the ratio of phenylarsenious acid to phenylarsonic acid in the urine were the same in both cases, the trivalent fraction after injecting the arsenious acid would not be measurable since the total amount of As excreted was so small.

In the earlier paper (Chance, Crawford & Levvy, 1945), it was shown that As injected into rabbits as phenylarsonic acid was cleared almost exclusively in the urine, while in the case of phenylarsenious acid a small fraction of the As excreted was found in the faeces. Figures for the total As in the urine were in general similar to those given in Table 1.

In so far as they suggest that a trivalent arsenious acid and the corresponding pentavalent compound are interconvertible in the body, the results described above agree with those obtained by Storey (unpublished work), who applied the 'xanthate reagent' (a solution of sodium ethyl xanthate in carbon tetrachloride, Crawford & Storey, 1944) to a study of the valency of the As derivatives in the urine of rabbits injected with arsenious acid or arsenic acid. After injection of either compound, the bulk of the excreted As was not extracted by the 'xanthate reagent', but a small fraction was present in the trivalent state in both cases.

SUMMARY

1. The nature of the arsenicals excreted in the urine of rabbits injected with phenylarsenious acid and phenylarsonic acid has been studied.

2. No detectable amounts of arsenious acid or arsenic acid were present after the injection of phenylarsenious acid or phenylarsonic acid.

3. After injection of phenylarsonic acid, most of the arsenic in the urine appeared to be in the form in which it was administered. A small fraction (not more than 10%) was, however, in a form which reacted as phenylarsenious acid.

4. Of the arsenic excreted in the urine after injecting phenylarsenious acid, 50% appeared to be present as phenylarsonic acid and the remainder in the unidentified form.

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