stituents including an alcohol and probably sesquiterpenes. The oil contained about: 28 per cent levo alpha pinene; 24 per cent levo beta pinene; 7 per cent levo camphene; 8 per cent dipentene; 6 per cent geraniol partly as the acetate; a small amount of azulenogenic sesquiterpene. Traces of free acids were present, probably capric or caproic, while acetic acid was present in the combined state.

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THE EFFECT OF CYSTINE ON THE TOXICITY AND TRYPANOCIDAL ACTIVITY OF NEOARSPHENAMINE.*

BY A. E. JURIST AND W. G. CHRISTIANSEN.[1]

It is well known that neoarsphenamine injected intravenously in aqueous solution at times causes undesirable reactions of varied nature and severity. Consequently many attempts have been made to find some substance or substances which could be used either simultaneously with neoarsphenamine or during the course of the treatment to reduce both the frequency and severity of these reactions or eliminate them altogether. Particular attention has been paid to sulfur compounds owing to the effectiveness of sodium thiosulfate in arsenic poisoning reported first by Ravaut (1) and then by McBride and Dennie (2). Subsequently Raiziss (3), Groehl and Myers (4) and many others have pointed out that sodium thiosulfate might have a favorable effect in preventing neoarsphenamine reactions when given at the same time as the arsenical. At the present time, however, sodium thiosulfate is used in arsphenamine therapy, mainly for the treatment of the more severe occasional reactions such as dermatitis, jaundice; it hastens elimination of the arsenic.

More recently Sullivan (5) has pointed out that cystine is one of the amino acids most necessary to normal body growth and maintenance and plays an important rôle in nutrition. It has also been reported by Sullivan in a private communication that colloidal sulfur therapy in arthritis raised the cystine content of the nails from the subnormal level of 8.67% to 10.78%, a close approach to the normal value of 12%. Also cystine is a known constituent of the hair. Further, it has often been reported that in instances of arsenical poisoning arsenic has been found in the skin, hair and nails. It appeared possible, then, on the basis of a consideration of the chemistry of cystine in relation to the chemical reactions of arsphenamines in the body, to convert the excess arsenic present in the skin before

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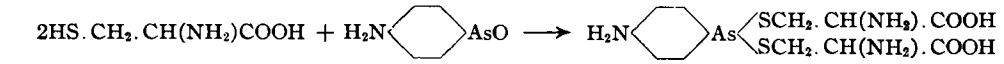
* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] Research Department of the Chemical & Pharmaceutical Laboratories, E. R. Squibb & Sons, Brooklyn, N. Y.

the onset of an arsenical dermatitis to a stable, less toxic form if the cystine or cysteine content of the epidermis could be raised to a sufficient level, thus averting the dermatitis.

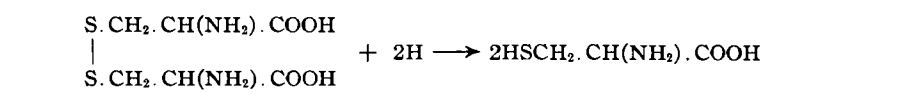
This possibility has its chemical basis in the generally accepted theory that the arsphenamines are converted in the body to "arsenoxide," 3-amino-4-hydroxyphenylarsenoxide, and owe their effectiveness to its formation. Voegtlin, Dyer and Leonard (6, 7) have shown that reduced glutathione, cysteine and other sulfhydril compounds cause a reduction in the trypanocidal action of "arsenoxide." This effect of sulfhydril compounds is due probably to the formation of a stable arsenical of the following type:



Arsenicals of the latter type have been prepared by Kharasch (8). They are more stable than arsphenamines and have been shown by Cohen, King and Strangeways (9, 10) to have a lower trypanocidal activity, in agreement with the observations of Voegtlin, et al. Also, they are clinically less effective as shown by the work of Becker and Obermayer (11), Connor, Shaw, Levin and Palmer (12), and Robinson and Moore (13) in their work on "Thioarsene," a compound of the above type obtained from 4-acetamidophenylarsenoxide and 4-mercaptobenzenesulfonic acid, and known chemically as disodium bis-(*p*-sulfophenyl)(acetamidophenyl)-dithioarsenite.

On the basis of the foregoing considerations it is not unreasonable to hope that if the arsenic present in the epidermis during arsphenamine therapy exists in the form of the "arsenoxide" it may be possible, by elevating the cysteine content of the epidermis, to so bind the arsenic in the form of such stable compounds as are described above as to render it less toxic and thus prevent the development of arsenical dermatitis. Therefore, it was decided to investigate this question. Before undertaking experimental work, however, it was necessary to select the sulfur compound to be used for this purpose.

The choice lay between cysteine and cystine because it was believed that if any effect at all was obtained it might be accomplished most efficiently with either cysteine itself or with cystine from which cysteine is obtained by reduction; *viz.*,



In view of the fact that cysteine is itself a sulfhydril compound and might directly reduce the effectiveness of an arsphenamine as shown by Voegtlin, Dyer and Leonard (*loc. cit.*), and in view of the fact that cysteine is less stable than cystine it was decided to utilize the latter in our experiments in conjunction with neoarsphenamine. It was believed that cystine would serve as a source of cysteine which could then react in the manner shown above.

Further, it is known that arsphenamines which are powerful reducing agents, will reduce cystine to cysteine. It was therefore thought undesirable to administer the cystine and neoarsphenamine simultaneously because the formation of cysteine by reduction of the cystine and the resultant oxidation of the neoarsphen-

amine to the corresponding arsenoxide derivative would permit the formation of a more stable, less effective dithioarsenite of the type discussed above. Since any possible reduction in the effectiveness of the arsenical was undesirable an attempt was made to avoid such a circumstance by administering the cystine orally on the day following the injection of the neoarsphenamine.

Our experiments showed that the toxicity of neoarsphenamine was not materially altered when one Gm. of cystine per Kg. of body weight was administered orally on the day following the injection of 350 mg./Kg. of neoarsphenamine in albino rats. On the other hand when doses of 250 mg. and 500 mg. of cystine per Kg. of body weight were administered orally on the day following the injection of from 5.0 mg. to 9.0 mg. of neoarsphenamine per Kg. of body weight in albino rats infected with *T. equiperdum*, the trypanocidal action of the arsenical was noticeably reduced. These results indicate that it is not advisable to administer cystine during the treatment of syphilis with neoarsphenamine and that in spite of planning the method of treatment so as to avoid any reduction in the activity of the arsenical this result was not accomplished.

If cystine reduces the effectiveness of neoarsphenamine by serving as a source of cysteine in the body and if the cysteine in turn combines with the arsenical in the manner described herein, then any sulfur compound which will raise the cystine content of the body will cause a reduction in the trypanocidal activity of neoarsphenamine. It is probable that the mechanism by which it was hoped to reduce the possibility of arsenical dermatitis with cysteine originating from the administration of cystine, namely, the formation of a dithioarsenite from cysteine and arsenoxide, is the one by which cystine reduced the trypanocidal activity of neoarsphenamine. However, in this case cystine, or more probably cysteine formed by its reduction in the body, reacts to form a stable, less trypanocidally effective dithioarsenite with the arsenoxide formed by oxidation of the neoarsphenamine in the blood stream, liver and other parts of the body of the infected animals used in these experiments.

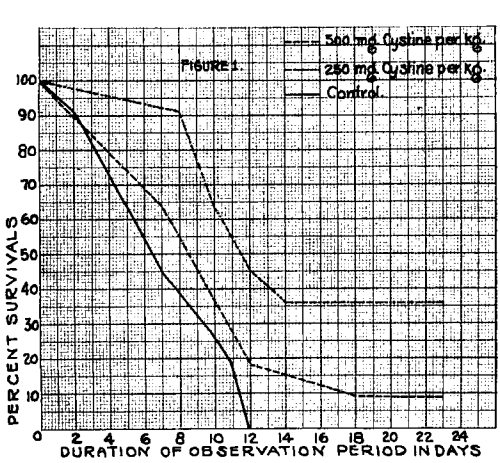
This does not, however, mean that there is no relation between the sulfur content of the skin and the possibility that arsphenamine therapy will produce dermatitis. It may be that patients suffering from a sulfur deficiency of the skin are more liable to have an arsenical dermatitis than those who do not. This question is being investigated.

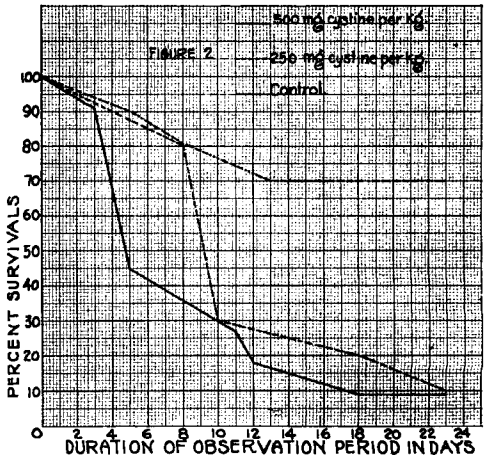
EXPERIMENTAL.

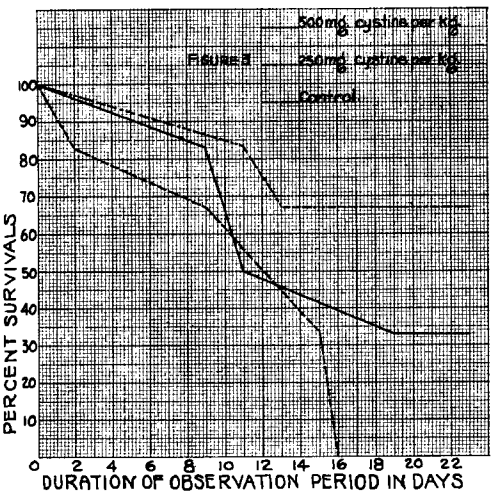
The toxicity of neoarsphenamine was determined on albino rats at 350 mg./Kg. Eleven rats were injected on one day with neoarsphenamine and on the next day, twenty-four hours after the injection, a dose of cystine equivalent to one Gm. per Kg. of body weight was administered orally to five of the rats; the other six were kept as controls. There were two deaths in the five animals which received both neoarsphenamine and cystine and two deaths in the six animals which received neoarsphenamine but no cystine. There was no material difference in the gross appearance of the kidneys of the surviving animals when they were killed at the end of the test period, irrespective of whether they had received cystine or not. The results show no material change in the toxicity of the neoarsphenamine due to the administration of cystine.

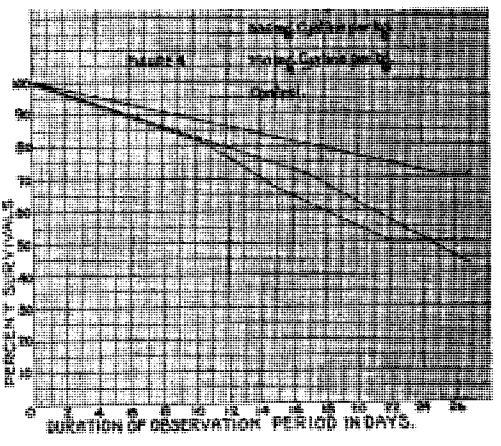
The effect of cystine on the trypanocidal action of neoarsphenamine was determined on albino rats infected with *T. equiperdum* using three different doses of neoarsphenamine, namely, 5.0 mg., 7.5 mg. and 9.0 mg. per Kg. of body weight and at each of these dosages the effect of both 250-mg. and 500-mg. doses of cystine was determined. In all instances two doses of neo-

arsphenamine were given intravenously and each was followed on the next day by a dose of cystine orally; the time between doses of neoarsphenamine varied from two to six days. At each neoarsphenamine dose level experiments were carried out with controls receiving no cystine. The trypanosome count just prior to the neoarsphenamine injection varied from 18,000 to 260,000 per cu. mm. of blood. In these experiments groups of six to eleven animals were used for each dose level of neoarsphenamine and cystine, making a total of 102 animals in all. All animals were observed for twenty-one days after the last injection unless death ensued in the meantime. The results obtained are given in the following series of curves:









Figures 1 and 2 give two sets of results obtained with two 5.0 mg. per Kg. doses of neoarsphenamine two days apart.

Figure 3 gives the results obtained with two 7.5 mg. per Kg. doses of neoarsphenamine two days apart.

Figure 4 gives the results obtained with two 9.0 mg. per Kg. doses of neoarsphenamine six days apart.

At the 5.0 mg. per Kg. dose of neoarsphenamine as in Figs. 1 and 2 the results show a lower percentage of survival for the infected animals receiving 500 mg. per Kg. of cystine than for those

which received 250 mg. per Kg. of cystine, while the controls show a still higher percentage of survival at the end of the twenty-three day period of observation. With 7.5 mg. per Kg. of neoarsphenamine the results from the two cystine experiments are anomalous but here again the controls show a definitely higher percentage of survival at the end of the observation period. At the 9.0 mg. per Kg. dose the percentage of survivals is not very different for animals receiving 250 mg. per Kg. of cystine and the controls are somewhat higher for those receiving 500 mg. per Kg. of cystine. However, since the 9.0 mg. per Kg. dose of neoarsphenamine is almost the Minimal Sterilizing Dose in rats infected with *T. equiperdum* it would be expected that the effect of cystine would be less noticeable. Further, the number of animals tested at this dosage was smaller than at 5.0 mg. and 7.5 mg. per Kg. greatly increasing the possibility of error.

The results show clearly that the trypanocidal effectiveness of neoarsphenamine is materially reduced by the administration of cystine during the period of neoarsphenamine therapy.

We gratefully acknowledge the assistance of the Biological Laboratories of E. R. Squibb & Sons in conducting the biological tests reported herein.

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THE CHEMICAL NATURE OF IODOBISMUTHIC ACID AND ITS RELATION TO THE CHEMISTRY OF THE ALKALI IODIDE. COMPOUNDS OF BISMUTH IODIDE.*

BY A. E. JURIST AND W. G. CHRISTIANSEN.[1]

Recently Motard (1), François (2), Delwaulle (3) and François and Delwaulle (4) have identified a number of alkali metal iodide compounds of bismuth iodide and antimony iodide. While studying the chemistry of Iodobismitol, which is a solution of sodium iodobismuthite and sodium iodide in a glycol, preferably propylene glycol, the structural characteristics of the compound sodium iodobismuthite have been investigated. A compound which might be an iodobismuthic acid, having the formula BiI_3, HI, $3\,H_2O$, has already been isolated by Arppe (5) but no relation between it and the alkali metal iodide-bismuth iodide compounds has yet been shown.

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* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] Research Department of the Chemical & Pharmaceutical Laboratories, E. R. Squibb & Sons, Brooklyn, N. Y.