Those with concomitant amœbic infection were given routine army treatment which consisted of emetine parenterally for 6 days, retention enema (8 oz. of 2½ per cent yatren) from the 5th to the 14th day, emetine bismuth iodide 3 gr. orally from the 7th to the 12th day, and amœbiarson 1 tablet (0.25 gm.) twice a day from the 13th to the 25th day.

After six weeks' treatment every case was given one month's leave. The patients were then disposed of under three classes: (*a*) duty; (*b*) categorized 'C' permanent, *i.e.* fit for duty in India only, with no parade and P. T.; and (*c*) categorized 'E' invalidment. These were examined at intervals and the relapses invalided. The results of treatment are given in table II.

TABLE II

Analysis of results

Cured, discharged to duty ..	129	(53.4%)
Improved, categorized 'C' permanent.	22	(9.0%)
Improved, categorized 'C' permanent and relapsed—therefore invalided out of service.	67	(36.8%)
Not improved and invalided out of service.	22	
Died	2	(0.8%)
	242	(100.0%)

Acknowledgment

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APPENDIX

Time	Diet No 1. 2nd and 3rd weeks	Diet No. 2. 4th to 6th weeks
0600 hours	Sprue tea (*i.e.* milk tea), 8 oz.	Sprue tea (*i.e.* milk tea), 8 oz.
0800 „	Milk 8 oz., toast 2 oz., eggs 2.	Milk 8 oz., toast 2 oz., eggs 2.
1000 „	Milk 8 oz., vegetables 2 oz., vegemite ½ oz.	Milk 8 oz., vegetables 4 oz., vegemite ½ oz.
1200 „	Milk 8 oz., toast 2 oz., juice from 8 oz. liver.	Milk 8 oz., toast 2 oz., juice from 8 oz. liver.
1400 „	Kher 6 oz., or rice 4 oz. with milk or porridge 8 oz.	Kher 6 oz., or rice 4 oz. with milk or porridge 8 oz., minced meat 4 oz.
1500 „	Fruit juice 6 oz.	Fruit juice 6 oz.

APPENDIX—*concld.*

Time	Diet No. 1. 2nd and 3rd weeks	Diet No. 2. 4th to 6th weeks
1600 hours	Milk 8 oz., tea.	Milk tea 8 oz., toast 2 oz.
1800 „	Milk 8 oz., toast 2 oz., juice from 8 oz. liver, khichri 4 oz.	Milk 8 oz., toast 2 oz., juice from 8 oz. liver, khichri 4 oz.
2000 „	Milk 8 oz. with Horlicks.	Milk 8 oz., chicken soup ¾ pints.
2200 „	Milk 8 oz., vegemite ½ oz.	Milk 8 oz., vegemite ½ oz.

Lassi of dahi 2 lb. between 0900 to 1600 hours.
Lemon water 2 pints when required.

ON THE TOXICITY OF SOME ORGANIC ANTIMONIAL DRUGS USED FOR THE TREATMENT OF KALA-AZAR

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DERIVATIVES of antimony have proved very useful remedies in the treatment of both schistosomiasis (bilharziasis) and leishmaniasis (particularly kala-azar). Both these diseases are widespread. Some indication of the value of antimony is the fact that without treatment the death rate from kala-azar is over 90 per cent, whereas with treatment in Indian kala-azar it is reduced to below 10 per cent.

Although tartar emetic was found of value in the treatment of kala-azar, its considerable toxic effects made it imperative to obtain other derivatives which should be better tolerated while having, if possible, a greater therapeutic effect. The introduction of a pentavalent organic antimony derivative, urea stibamine, by Brahmachari in the year 1922, was an important development in the history of chemotherapy of kala-azar. Urea stibamine is obtained by the interaction of *p*-aminophenylstibinic acid with urea. The exact chemical nature of this important drug is still not definitely known. Since its discovery, attempts have been made by various workers in different laboratories to produce an ideal drug for the treatment of kala-azar. Most of the organo-antimonials that have had clinical trials have been derivatives of *p*-aminophenylstibinic acid. Besides urea stibamine, neostibosan (the diethylamine salt of *p*-aminophenylstibinic acid) has also proved effective in the treatment of kala-azar.

The carbon-to-antimony bond in organic antimonials is much weaker than the analogous carbon-to-arsenic bond and, as a result, many antimonials tend to decompose in solution. Because of the nature of antimony chemistry, the factors affecting administration, absorption

and toxicity are important. Moreover, the preparation of these compounds in commercial quantities is difficult, as evidenced by the many reports of variations between samples of the same compound. In kala-azar, secondary effects are very undesirable owing to the much longer period of treatment. Urea stibamine, although a very potent drug for the treatment of kala-azar, has been reported to show at times toxic reactions. The cause of such toxic manifestations deserves further investigation.

It is not known exactly what determines the toxicity of organic pentavalent antimony compounds. It is surmised that, in the body, the pentavalent forms slowly decompose into trivalent antimony compounds, which ultimately produce the therapeutic action of the drugs. It has been frequently observed that different batches of the same antimony preparation, though prepared under apparently identical conditions, differ markedly in their toxicity. Moreover, the different derivatives of *p*-aminophenylstibinic acid also vary widely in their toxicity. It has been, therefore, considered to be of considerable interest to study the properties of some derivatives of *p*-aminophenylstibinic acid, with a view to ascertaining the factors underlying the development of their toxicity.

The present communication deals with a study on the toxicity of some derivatives of *p*-aminophenylstibinic acid in relation to their antimony contents. The recorded data are collected from the results of experiments with some of the typical samples, though the conclusions are based essentially on the analyses of more than a hundred samples of urea stibamine and other derivatives.

Experimental

Study on the toxicity of urea stibamine.—Our first object was to study the influence of the total antimony content on the toxicity of urea stibamine. For this purpose, several batches of the drug (primarily prepared in this laboratory, and a few samples obtained from the market) have been subjected to acute toxicity tests and chemical analyses with regard to total antimony contents. With some samples (*, *vide* table I), the antimony content was artificially lowered by mixing mechanically with some quantity of anhydrous glucose, in order to find the effect of the lowering of the antimony content on the production of toxicity. All the toxicity tests were carried out on white mice (weight varying from 14 to 16 grammes), according to the procedure described by Burn. Observations of mortality were made up to a period of 96 hours. The total antimony content was determined according to the method of Gray (1926). All these results are summarized in table I. Samples (1 to 10) were prepared in this laboratory and samples (11 and 12) were obtained from the market.

Samples were dried at ordinary temperature in a vacuum desiccator over fused calcium chloride till the weight was constant.

TABLE I

Toxicity of different samples of urea stibamine of different antimony content

Sample number	Total antimony content, per cent	Intravenous dose of the drug, mg./kg.	Reaction in 30 minutes	Mortality in 96 hours among 10 white mice
1	41.3	250	Slight	0
2	38.5	250	„	2
3	42.0	225	Severe	6
*3*a*	35.0	225	„	4
4	43.6	225	„	8
*4*a*	39.0	225	„	6
5	40.2	225	„	4
6	29.9	225	Moderately severe.	6
7	26.0	225	„	6
8	20.0	250	Slight	0
9	20.1	250	Severe	3
10	41.9	275	Moderate	1
11	40.3	225	Severe	5
12	40.0	225	„	4

The above results indicate that the toxicity of urea stibamine does not depend solely upon the total antimony content. Even with an appreciably low antimony content, a sample may possess undue toxicity.

It is, however, known that antimonic and antimonious acids are formed, though in small quantities, during the preparation of urea stibamine. In order to see if the presence of these acids has any influence on the production of toxicity, the antimony content (in various states, such as the inorganic state or in organic combination, trivalent or pentavalent) of some samples were estimated. These estimations have been carried out according to the method of Gray (1926) and other standard methods as quoted by Datta, Ghosh and Bose (1945), a blank experiment having been carried out in each case. The antimony content present in organic combination in the pentavalent state was deduced by subtracting the percentage of antimony (present as antimonic acid) from that of total antimony content present in the pentavalent state. The results obtained are summarized in table II. This table indicates that toxicity is related to the amount of antimonious acid present.

Acute toxicity of other pentavalent organic antimony compounds

In order to have an idea of the toxicity of other pentavalent organic antimony compounds in comparison with that of urea stibamine, several samples of the diethylamine salt and of tri-*iso*-propylamine salt of *p*-aminophenylstibinic acid, prepared in this laboratory (compare Ghosh, Bose and Mitra, 1945), have been

TABLE II

Toxicity of urea stibamine and the state of the antimony

Sample number	Percentage of antimony					Intravenous dose of the drug mg./kg.	Mortality in 96 hours among 10 white mice	Ratio of (*c*) : (*e*)
	Total (*a*)	Total pentavalent (*b*)	Present as antimonious acid (*c*)	Present as antimonic acid (*d*)	In pentavalent organic combination (*e*)			
1	39.4	38.2	1.15	1.05	37.15	225	1	1 : 32.3
2	40.2	39.34	0.88	1.08	38.26	225	0	1 : 43.4
3	42.5	41.04	1.5	1.2	39.84	225	2	1 : 26.5
4	38.4	36.2	2.3	1.3	34.9	225	4	1 : 15.1
5	34.9	33.0	2.1	1.25	31.75	225	3	1 : 15.1
6	40.4	38.0	2.5	1.5	36.5	225	4	1 : 14.6
7	36.0	33.1	2.86	1.6	31.5	225	4	1 : 11.0
8	37.5	36.5	1.02	1.01	35.49	225	0	1 : 34.7
9	41.8	40.91	0.92	1.3	39.61	225	1	1 : 43.0
10	39.8	38.8	1.05	1.1	37.7	225	1	1 : 35.9

TABLE III

Samples prepared in this laboratory were dried in a vacuum desiccator over fused calcium chloride till the weight was constant

Amine used	Sample number	Percentage of total antimony	Dose of the drug mg./kg.	Mode of administration	Reaction in 30 minutes	Mortality in 96 hours among 10 white mice
Diethylamine, manufactured by Schering-Kahlbaum (obtained in sealed tubes).	1	42.5	250	Intravenous	Severe	10
	2	42.5	250	„	„	6
	2	42.5	300	Subcutaneous	Slight	3
	2	42.5	350	„	Severe	10
	3	43.6	300	„	„	10
Diethylamine, from Schering-Kahlbaum, once distilled here.	4	42.1	250	Intravenous	Moderate	1
	4	42.1	300	„	Severe	2
	4	42.1	300	Subcutaneous	*Nil*	0
	4	42.1	350	„	Moderate	2
	4	42.1	400	„	Severe	4
Diethylamine, from Schering-Kahlbaum, thrice distilled here.	5	42.1	300	Intravenous	Moderate	0
	5	42.1	350	Subcutaneous	*Nil*	0
	5	42.1	400	„	*Nil*	0
	6	41.8	400	„	*Nil*	0
	6	41.8	300	Intravenous	*Nil*	0
Bayer's neostibosan	7	42.1	250	„	*Nil*	0
	7	42.1	300	„	*Nil*	0
	7	42.1	300	Subcutaneous	*Nil*	0
	7	42.1	350	„	*Nil*	1
	7	42.1	400	„	*Nil*	1
Tri-*iso*-propylamine, manufactured by Eastman Kodak Co.	8	36.3	350	„	Moderate	6
	9	36.5	350	„	„	5
Tri-*iso*-propylamine, from Eastman Kodak Co., thrice distilled under reduced pressure.	10	36.7	300	Intravenous	Moderate	0
	10	36.7	350	„	Severe	4
	10	36.7	350	Subcutaneous	*Nil*	0
	10	36.7	400	„	*Nil*	0

subjected to acute toxicity tests (intravenous and subcutaneous) and chemical analyses with regard to their antimony contents. For comparison, Bayer's 'neostibosan' (diethylamine salt of *p*-aminophenylstibinic acid) has also been tested side by side. As usual, the toxicity tests were carried out on white mice (weight varying from 14 to 16 grammes). The results obtained are summarized in table III.

Discussion

The exact composition of urea stibamine has not as yet been settled, though the recent

studies of Gray *et al.* (1931) and of Datta *et al.* (1945) have thrown some light on the possible composition of the drug and the difficulties underlying this problem. A compound whose chemical identity is not established, or one whose relative constituents are not known, is naturally liable to show variation in its animal toxicity during preparation of different batches. For the preparation of a drug, all lots of which should conform to a fixed standard of toxicity, it is essential to ascertain the causes of variation in toxicity. Recently, it has been observed by Datta *et al.* (1945) that the complete removal of free uncombined urea from urea stimabine leads to an increase in its toxicity. Thus, the presence of some trace of urea seems to be necessary to reduce the toxicity of the drug within certain limits.

From the observations recorded in this paper, it is evident that, although the toxicity of urea stibamine somewhat increases with the increase in the total antimony content of the drug, still the latter is not the sole cause for the generation of toxicity. Sometimes a sample containing less antimony is found more toxic than one with a higher antimony content. Artificial reduction of antimony content of such a sample by the addition of anhydrous glucose does not bring down its toxicity within the normal limits. At the same time, it is found that urea stibamine containing 41 to 42 per cent antimony can be prepared in such a way as to pass the toxicity test in an intravenous dose of even 275 mg./kg. It is, therefore, evident that the toxicity of urea stibamine does not depend solely on the total antimony content but is influenced to a great extent by the presence of antimonious acid, as is shown by a scrutiny of the ratio of the percentage of antimony present as antimonious acid to that present in pentavalent organic combination (*vide* table II). It is observed that increase in this ratio above a certain limit (1 : 26) tends to bring about a definite increase in the toxicity of the drug.

It would be interesting to note here that Bose, Iyengar and Mukerji (1945) have found the LD50 of urea stibamine to be 215 mg./kg. with a sample whose antimony content present as antimonious acid was found to be definitely high (2.7 per cent). All these observations suggest that, apart from the percentage of total antimony content, other factors (such as, the presence of antimonious acid) are responsible for the development of toxic reactions ; and that, prepared carefully under certain standardized conditions, urea stibamine can be made to satisfy much higher standards of toxicity tests than those accepted at the present time (namely, 200 to 225 mg./kg. intravenously ; compare Guha, Dutta and Mukerji, 1943).

In the case of the secondary and tertiary amine salts of *p*-aminophenylstibinic acid, it has been observed that unless specifically prepared, the derivatives are likely to give rise to more toxic reactions than expected. In order to obtain these salts devoid of undue toxicity, these amines must be in a state of high purity, and should be absolutely free from any other complex amine*.

From an analysis of the data regarding the toxicity of urea stibamine and the diethylamine salt of *p*-aminophenylstibinic acid, it appears that though the percentages of antimony in these two compounds do not vary markedly, the variation in their relative toxicities is, however, very well marked. Considering that all these compounds are derived originally from *p*-aminophenylstibinic acid, this lowering of toxicity in the cases of diethylamine and tri-*iso*-propylamine salts appears to be a function of the nature of salt formation. Here, antimony acts as a secondary factor, allowing its toxic properties to be modified according to the virtue of the reaction base.

With regard to the diethylamine salt of *p*-aminophenylstibinic acid, some experimental evidence has been obtained which indicates that the toxicity of this salt can be further lowered by incorporating a minute trace of a suitable reducing agent. Further work on this point is in progress.

Summary

1. The toxicity of a large number of samples of urea stibamine and other pentavalent organic antimony derivatives has been studied in relation to their antimony contents.

2. It is found that the toxicity of urea stibamine does not depend solely upon the total antimony content but is influenced by the presence of antimonious acid.

3. It is found that urea stibamine, if carefully prepared, can be made to satisfy much higher toxicity limits than those accepted at the present time (namely, 200 to 225 mg./kg.).

4. The toxicity of the diethylamine and tri-*iso*-propylamine salts of *p*-aminophenylstibinic acid depends to a considerable extent on the purity of these amines used.

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*It has been previously observed in this laboratory by one of us (A. N. B.) during the preparation of tri-*iso*-propylamine salt of bismuth that, unless the amine is repeatedly distilled and used in a very pure state, the salt, though satisfying the chemical tests, gave rise to a severe type of gangrene of the limbs of the injected animals.