Classics in Indian Medicine

Sir UPENDRANATH BRAHMACHARI 1875–1946

KB, Kaiser-I-Hind (Gold), MA, MD, PhD, FRASB, FSMF (Bengal), FNI

He was born into a Hindu Brahmin family from Bengal. He had his initial education in the Hughli College and later in the Presidency College, Calcutta. During his medical education in the Medical College of Bengal he won many honours including the University and Coates Medals and the Griffith Memorial Prize of the Calcutta University. He was later awarded the Mente Medal of the Calcutta School of Tropical Medicine and Hygiene and the Sir William Jones Medal of The Asiatic Society of Bengal. He began his career as a House Physician in the Medical College Hospital, Calcutta, in 1899. In 1901 he joined the Dacca Medical School as a teacher of materia medica. From 1905 to 1923 he taught medicine at the Campbell Medical School, Calcutta and then served as a physician in the Medical College Hospital, Calcutta, till 1927. Later he was appointed Professor of Tropical Medicine in the Carmichael Medical College, Calcutta, and a physician in the Chittaranjan Hospital, Calcutta. During his illustrious career he held many important positions. He was president of the Indian Science Congress in 1936, president of the Indian Chemical society, the Indian Society of Microbiology and the Society of Biological Chemists of India. He was the vice-president of the Physiological Society of India, Head of the Biochemistry Department of the University of Calcutta and the founder of the Brahmachari Research Institute, Calcutta, a member of the Court of the Indian Institute of Science, a Fellow of the Royal Society of Medicine and the Royal Society of Tropical Medicine and Hygiene, London, a Fellow of the University of Calcutta and an Honorary Fellow of the State Medical Faculty, Bengal.

His most important achievement was the discovery of ureastibamine for the treatment of Kala-azar. He also published numerous papers on infantile biliary cirrhosis in India.

CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION.

Part I.

THE TOXICITY OF ANTIMONYL TARTRATES—THE INFLUENCE OF THE BASIC RADICLE OF AN ANTIMONYL TARTRATE UPON ITS TOXICITY—SOME ARYL PENTAVALENT ANTIMONIAL COMPOUNDS—*P*-AMINO-PHENYL STIBINIC ACID AND SOME OF ITS DERIVATIVES—THEIR TOXICITY—THE THERAPEUTIC VALUE OF AMMONIUM ANTIMONYL TARTRATE AND UREA STIBAMINE.

BY

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[Received for publication, January 3, 1922.]

The Symptoms of Antimony Poisoning in the Guinea-pig and the Rat after Injection of Antimonyl Tartrates.

THE symptoms of acute antimony poisoning in the guinea-pig and the white rat are not always constant. Vomiting and purging which are frequently observed in man after intravenous injections are not at all common in guinea-pigs even after toxic doses, and it is not rare to find solid fæces in the large intestines even when the animal dies of severe acute symptoms.

The symptom complex of the intoxication produced by the various antimonyl tartrates enumerated below does not vary much whatever may be the salt used. They are divisible into two groups of phenomena.

(1) Nervous.

(2) Nutritional.

In some severe cases of acute poisoning, the animal passes into a state of prostration with complete paralysis of the central nervous system within ½ to 1 hour after injection. In less severe cases the symptoms come on more gradually. Marked tremor and chattering of the teeth are sometimes very characteristic features of intoxication and then the animal passes into a state of coma and in the comatose state may develop spasmodic movements of groups of muscles coming on at intervals of ½ to 1 minute. In fatal cases the breathing is hurried and pulse quick. In some cases, the animal lies in a comatose condition for some hours before death. Sometimes the animal shows marked muscular tremors and incoordination when disturbed. Salivation has been observed in many cases but is not a constant symptom. In some cases a few minutes after intravenous injection the animal exhibits a very marked spasmodic movement of the whole body at frequent intervals. In some cases spasmodic contraction of diaphragm resembling hiccough in man has been observed. In many cases, soon after the injection of a fairly large dose, the animal frequently scratches its mouth with its front legs. Sometimes even after sublethal doses the animal appears to be ill and faint for a short time. It is unsteady, the gait is staggering and the animal may roll about. The animal is less active and takes its food less freely than usual. If it survives for 10 or 12 hours after the injection, then there is development of a peculiar bloated appearance of the face in fatal cases. In cases that survive, this phenomenon is slightly or not at all marked.

There may be marked emaciation in cases that survive 2 or 3 days after injection but frequently the animal regains in weight in a week's time.

In some cases, the animal progressively loses in weight. It takes very little food, remains dull and dies on the 7th or 8th day. Such cases are rare and generally it may be stated that if recovery takes place, it comes on within two or three days and is complete.

Period at which death takes place after doses within the toxic range.

The earliest period at which death took place in guinea-pigs after minimum lethal doses was 4 hours. In some cases, the animals took 12 to 18 hours to die after injection of the minimum lethal doses. With smaller doses, but still within the toxic range, the animals, that did not survive, died 24 to 36 hours after injection. Sometimes the animals survived for eight to ten days, and after death they showed symptoms of antimony poisoning. Cases of delayed antimony poisoning will be described in another series.

PATHOLOGY OF THE INTOXICATION

The pathological changes produced in the animal may be studied under the following heads :—

(1) Local effects.

(2) Systemic effects.

It may be stated that, generally speaking, the local effects produced after intramuscular injections of the antimonyl tartrates are much less marked in the case of the guinea-pig and the rat than in the case of man. There may be some irritation and swelling at the seat of injection but necrosis or destruction of tissues is rarely met with—a phenomenon frequently met with in the case of man.

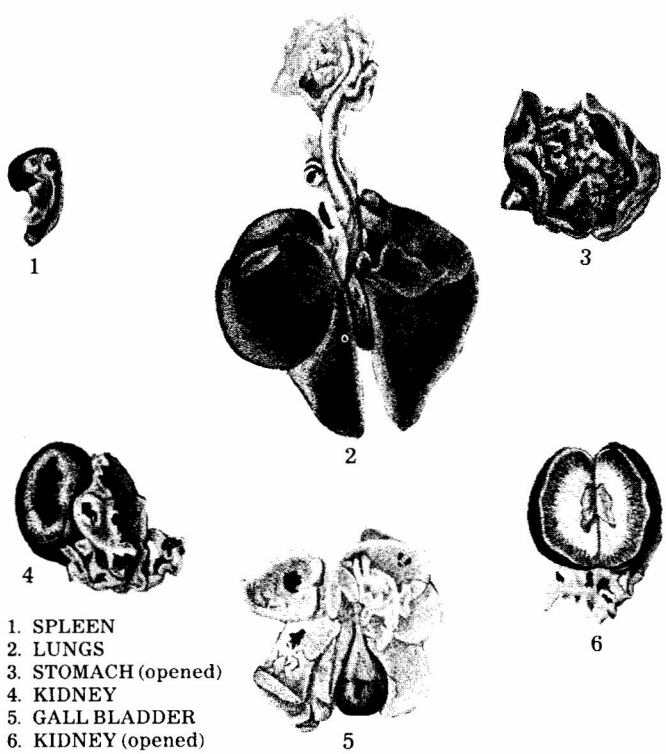
Systemic effects.

The effects produced by the antimonyl tartrates upon the animal as a whole, when given in toxic doses, are very marked. In general, after toxic doses, the pathological lesions consist of hæmorrhages into the internal organs and necrosis of their cellular elements. The organs in which the changes are most marked are (1) Lungs, (2) Kidneys and (3) Liver. Marked changes in the gastro-intestinal tract are not frequently met with. Sometimes there is congestion of or even hæmorrhage or ulceration in the gastric mucosa. There may be hæmorrhages into the substance of the spleen. Among the ductless glands that have been studied marked pathological changes may take place in the adrenals and pituitary. No change has been observed in the thyroid. I shall now describe these changes in the different organs in detail :—

(1) *Lungs.*—In about 90 per cent of the cases that die of acute poisoning, the whole lungs are in a state of extreme congestion with hæmorrhages into their substance and alveoli. On section, blood pours out freely from the cut surfaces. On microscopic examination, extensive hæmorrhages into the substance of the lungs with destruction of the parenchyma with round celled infiltration and exudation of necrosed material into the alveoli of the lungs are met with (see Plates XII, XIII and XIV). In one animal there was evidence of lobar pneumonia in one lung, but this might have been accidental.

(2) *Kidneys.*—In fatal cases, marked destructive changes are met with in the kidneys. In acute cases, the kidneys are slightly enlarged. There may be hæmorrhages into their capsules. The congestion is sometimes most marked in the boundary zone and frequently extends outwards along the medullary rays towards the capsular surface. Sometimes there is cloudy swelling and sometimes necrosis of the kidney epithelium. Hæmorrhage which may sometimes be very extensive may be seen in the interstitial tissues of the kidney. The tubules of the kidneys may be blocked with granular debris. (See Plates XII and XV.)

(3) *Liver.*—In some cases, the liver presents a pale, yellowish appearance which is indicative of extensive fatty change. On the surface there may be spots of hæmorrhage, sometimes very extensive. In other cases the liver presents a deeply congested appearance with hæmorrhages into its substance. The latter is observed in those cases in which the animal dies within a few hours after injection and the former



1. SPLEEN
2. LUNGS
3. STOMACH (opened)
4. KIDNEY
5. GALL BLADDER
6. KIDNEY (opened)

PLATE XIII.

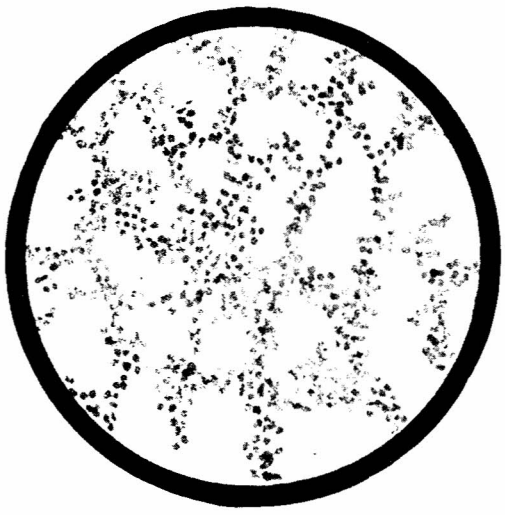


PLATE XIII. Section of Lung showing haemorrhage into the interstitial tissue.

PLATE XIII. Magnification, No. I, Eye-piece, objective Zeiss D.

PLATE XIV. Section of Lung showing haemorrhage round cell infiltration and blocking of alveoli with debris.

PLATES XIV to XXII. Magnification, No II, Eye-piece, objective Zeiss D.

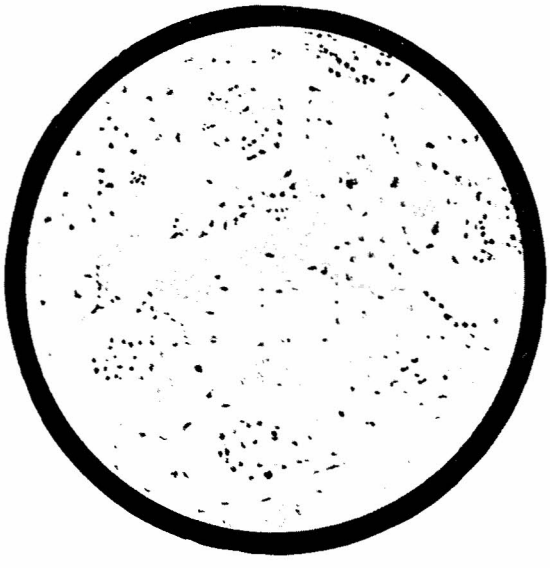


PLATE XV. Section of Kidney showing haemorrhage into the interstitial tissue, cloudy swelling and destruction of the kidney epithelium, and exudation of granular material into the kidney tubules.

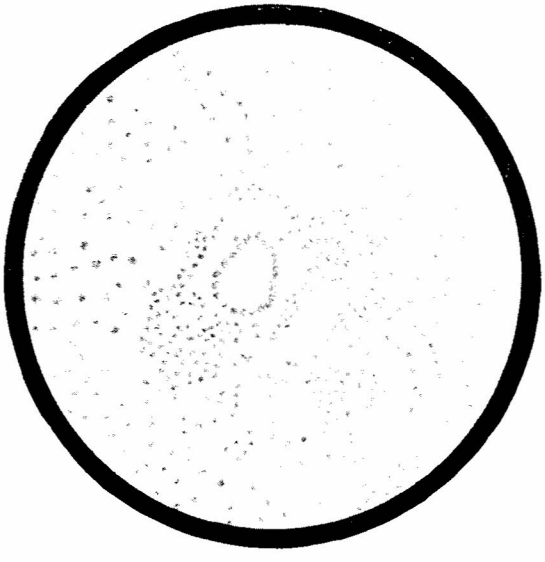


PLATE XVI. Section of Liver showing round cell infiltration round the portal system, fatty degeneration, cloudy swelling and haemorrhage.

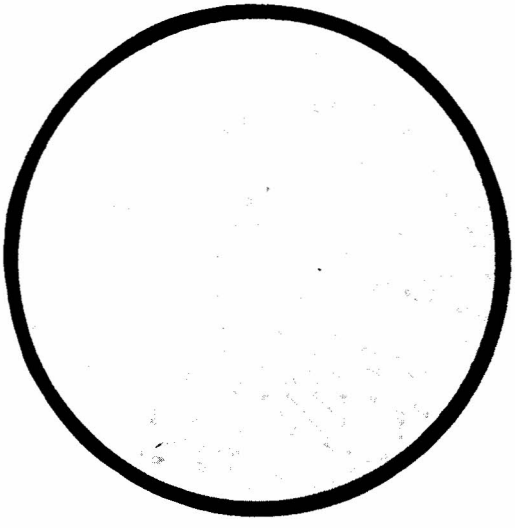


PLATE XVII. Section of Spleen showing destruction of Spleen Pulp.

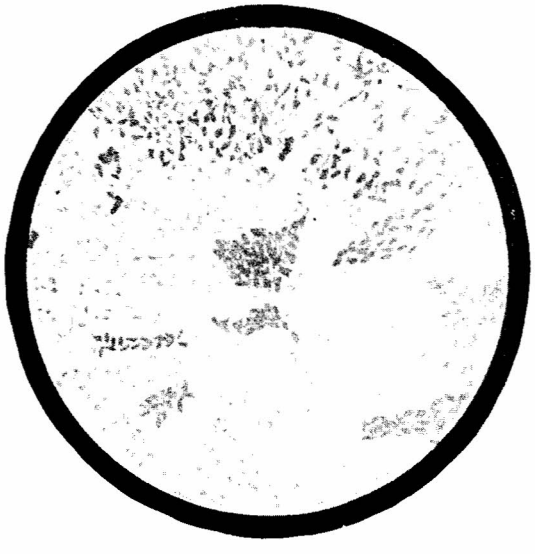


PLATE XVIII. Section of Suprarenal gland showing haemorrhage in zona reticularis and medulla.

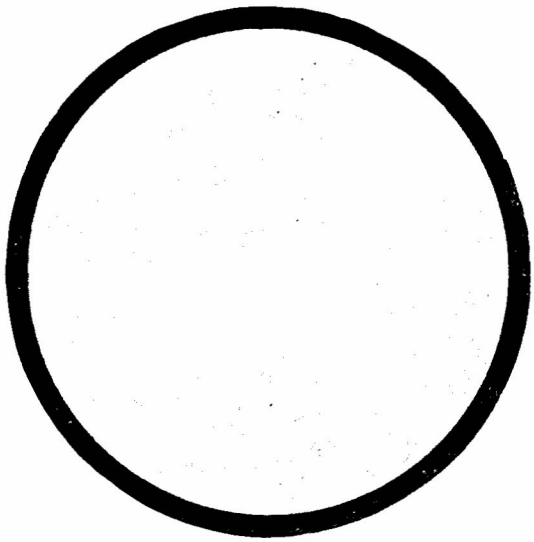


PLATE XIX. Fig. 1 Section of Pituitary, pars anterior normal.

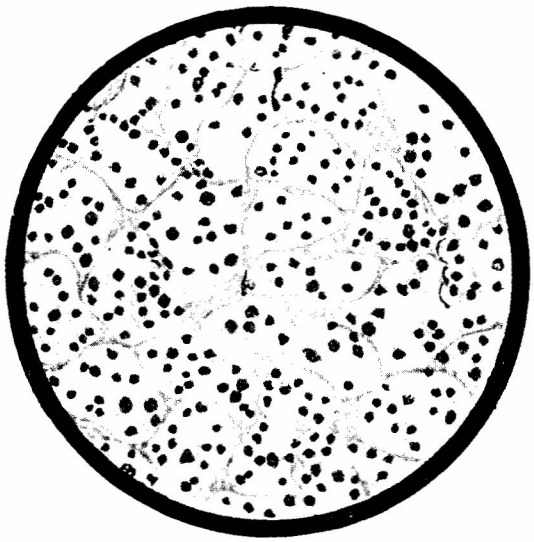


PLATE XIX. Fig. 2 Section of Pituitary, pars anterior showing diminution of eosinophile staining, contracted appearance of cells and haemorrhage. Death 12 hours after tartar emetic injection.

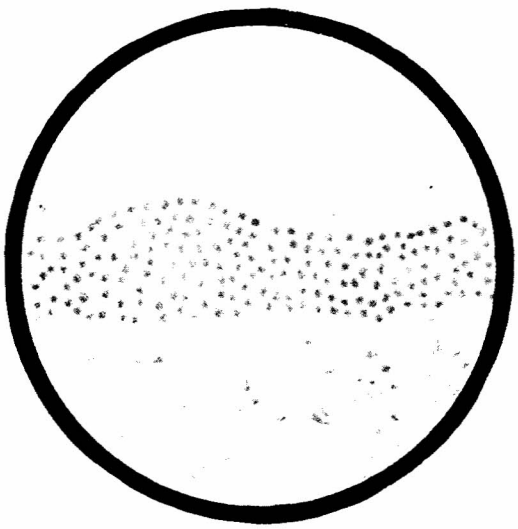


PLATE XX. Fig. 1 Section of Pituitary, pars posterior normal.

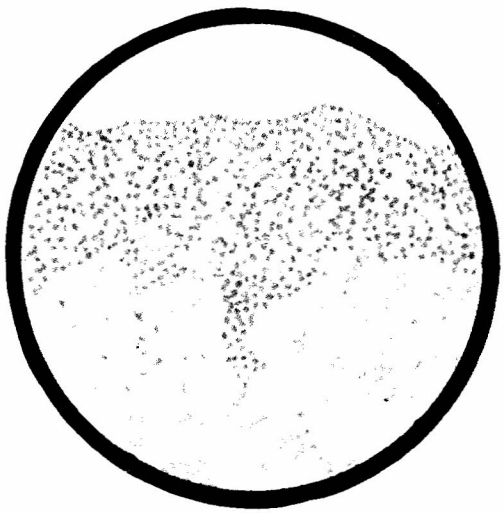


PLATE XX. Fig. 2 Section of Pituitary, pars posterior. Death from tartar emetic poisoning, the animal died seven days after injection. The section shows marked diminution of eosinophile staining of the cells, nuclei of the cells contracted.

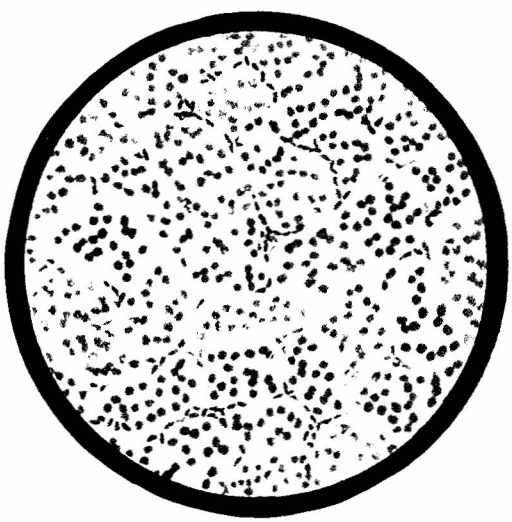


PLATE XXI. Section of Pituitary, pars anterior, showing marked diminution of eosinophile staining of the cells, the nuclei of the cells contracted, and interstitial haemorrhage. Death took place seven days after tartar emetic injection

PLATE XXII. Section of Parotid gland showing degeneration of glandular cells and haemorrhage.

when the animal dies after more than 24 hours after injection. There may be hæmorrhages into the substance of the gall bladder. Bile may be blood-stained. (See Plate XII.)

On microscopic examination, the following changes are noticeable :—(See Plate XVI).

(*a*) Round celled infiltration around the portal system and hæmorrhage into the interstitial tissue.

(*b*) Necrosis and extensive fatty degeneration of the hepatic cells.

(*c*) Blocking of bile capillaries with granular debris.

(4) *Spleen.* There may be hæmorrhages into the substance of the spleen. Necrosis of the splenic pulp may be observed. (See Plate XVII.)

(5) *Gastro-intestinal Tract.*—In some cases there may be signs of acute congestion with patches of ulceration in the stomach, but this is not constantly met with. Sometimes the small intestines are deeply congested and there may be hæmorrhages into their peritoneal coating. The large intestines frequently escape and there may be solid fæces inside them.

(6) *Salivary Glands.*—There may be extensive destruction of the secreting cells of the Parotids with hæmorrhage and round celled infiltration. (Plate XXII.)

(7) *Ductless Glands.*—(1) *Thyroid.* No changes have been observed in the thyroid.

(2) *Adrenals.* Hæmorrhages into the substance of the adrenals are not infrequently observed in the acute cases. The cortical vessels may be swollen. There may be marked decrease in the cortical pigmentation. Degenerative changes may be observed in the cortex and medulla. (Plate XVIII.)

(3) *Pituitary.* The changes in the pituitary may be divided into two classes :—(See Plates XIX, XX and XXI).

(1) Changes that take place in the gland after death from severe acute poisoning.

(2) Changes that take place in the gland after death from subacute poisoning.

In the former, there may be hæmorrhages into the substance of the anterior portion of the pituitary with diminution in the eosinophile staining of the cells. In the latter, marked increase in the basophile staining of the cells with slight hæmorrhages is the characteristic change and there may be shrinking of the protoplasm and the nuclei of the cells with prominence of the interstitial tissue. The same changes may be more or less present in the posterior portion of the pituitary.

The Toxicity of Antimonyl Tartrates—The Influence of the Basic Radicle of an Antimonyl Tartrate upon its Toxicity.

Since the discovery of antimony as a specific in the treatment of leishmaniasis, no systematic work has been done to determine the toxicity of the antimonyl tartrates. It is at the same time evident that such an investigation should be of the highest importance as the antimonyl tartrates are the compounds that are still most commonly used in the treatment of the various forms of leishmaniasis.

In the present paper, the toxicity of the following antimonyl tartrates has been investigated :—

(1) Ammonium antimonyl tartrate, (2) Urea antimonyl tartrate, (3) Aniline antimonyl tartrate, (4) Potassium antimonyl tartrate, (5) Sodium antimonyl tartrate.

Urea Antimonyl Tartrate.

In the process of my chemotherapeutic investigations, I have succeeded in preparing a new antimonyl tartrate, the urea antimonyl tartrate, the preparation and properties of which have been described by me in the Journal and Proceedings, Asiatic Society of Bengal (New Series, Vol. XVI, 1920, No. 8). The therapeutic value of this antimonyl compound in Kala-azar has been recorded by me in the *Journal of Tropical Medicine and Hygiene*, August 15th, 1921. Since then, with the help of one of my chemists, I have succeeded in preparing this compound in another way, which is described as follows :—

1 gram of urea is gently heated with a watery solution of 5 grams of tartaric acid for about half an hour. This gives a solution of acid urea tartrate which is subsequently concentrated by gentle heating. To the concentrated solution of the acid urea tartrate, a small weighed quantity of Sb_2O_3 is added and the mixture gently boiled till the Sb_2O_3 goes into solution. This process is repeated till 4·8 grams of Sb_2O_3 are dissolved. The solution is then filtered and concentrated to a syrupy consistency and then allowed to crystallize. In 24 to 48 hours, beautiful crystals separate which are removed and dried on a porous plate and purified by repeated crystallization. Yield=8 grams.

The salt originally prepared by me corresponded to the following formula :—$CO(NH_2).(C_4H_5SbO{\cdot}O_6)_2.5H_2O$. Prepared in the above way it contains $\frac{1}{2}H_2O$ as water of crystallization.

Composition.

Calculated for $CO(NH_2)_2.(C_4H_5SbO{\cdot}O_6)_2.\frac{1}{2}H_2O$ Sb=37·55%, N=4·38%, C=16·9%, H=2·34%.

Found Sb=37·55%, N=4·28%, C=16·8%. H=2·2%.

It thus appears that in urea antimonyl tartrate, urea combines with two equivalents of antimonyl tartaric acid, being therefore different from other salts of urea, in which only one of the amino groups in the urea is neutralized by the carbonyl group.

Ammonium Antimonyl Tartrate.

It is best prepared by the interaction of acid ammonium tartrate with Sb^2O_3.

6·7 grams of acid ammonium tartrate mixed with 5·8 grams of Sb^2O_3 are digested with about 50 c.c. of water till all the Sb_2O_3 goes into solution. The solution is filtered and concentrated gently on the water bath. On cooling, crystals of ammonium antimonyl tartrate separate. Yield=11 grams. The salt is purified by repeated crystallization. It contains $\frac{1}{2}$ molecule of water of crystallization and its antimony content= 38·58% on theoretical calculation. Found Sb=38·1%

Aniline Antimonyl Tartrate.

It is best prepared by heating two gram-molecular weights of acid aniline tartrate and one gram-molecular weight of antimony trioxide in the presence of water. 7·5 grams of tartaric acid are dissolved in water. 4·7 grams of aniline are added to this and the mixture boiled for quarter of an hour. The solution is filtered and crystallized yielding 10 grams of acid aniline tartrate.

4·9 grams of acid aniline tartrate are digested with 2·9 grams of antimony trioxide in the presence of water, till all the antimony trioxide goes into solution. The solution is then allowed to crystallize. Yield= 5·1 grams.

Composition.

Calculated for $C_6H_5NH_2.C_4H_5SbO{\cdot}O_6$, Sb=31·75%.

Found Sb=31·75%.

It has been prepared in other ways by previous workers which need not be described here.

Purity of the salts used :—

(1) The sodium and potassium antimonyl tartrates were specially prepared for me as chemically pure by Messrs. Martindale & Co.

(2) The ammonium, urea and aniline antimonyl tartrates were prepared and purified in my laboratory by repeated crystallization.

The antimony content of the salts used, as estimated by actual calculation.

(1) Ammonium antimonyl tartrate, $NH_4.C_4H_4.SbO{\cdot}O_6.\frac{1}{2}H_2O$. Sb=38·1%.

(2) Urea antimonyl tartrate, $CO(NH_2)_2.(C_4H_5SbO{\cdot}O_6)_2.\frac{1}{2}H_2O$. Sb=37·55%.

(3) Aniline antimonyl tartrate, $C_6H_5NH_2.C_4H_5{\cdot}SbO{\cdot}O_6$. Sb=31·75%.

(4) Tartar emetic, $KC_4H_4SbO{\cdot}O_6.\frac{1}{2}H_2O$, Sb=36·1%.

(5) Sodium antimonyl tartrate, $NaC_4H_4SbO{\cdot}O_6.2\frac{1}{2}H_2O$. Sb=34·1%.*

Lethal doses :—

In the following tables and the subsequent portions of this paper the abbreviations used are explained as follows :—

(1) M. L. D. the minimum lethal dose, *i.e.*, the minimum dose in grams per kilo of body weight which killed all the animals used.

* The following notes are quoted from Wenyon's "*Leishmaniasis: A Review of*

(2) Maj. L. D, the majority lethal dose, *i.e.*, the dose in grams per kilo of body weight which killed 66 per cent only of animals used.

(3) M. T. D, the maximum tolerated dose, *i.e.*, the maximum dose in grams per kilo of body weight which was tolerated by all the animals used.

(4) Maj. T. D, the majority tolerated dose, *i.e.*, dose in grams per kilo of body weight which was tolerated by only 66 per cent of the animals used.

(5) T. R, toxic range, *i.e.*, the range between the minimum lethal dose and the maximum tolerated dose.

Experiments on Guinea-pigs.

Method of administration and measurement of doses.

The toxicity experiments on guinea-pigs with the above compounds will be first described in the present paper. The drugs were administered intramuscularly, the injections being given in the outer part of the thigh. The strength of the solution was two per cent in distilled water. In all these experiments, each time the solution was freshly prepared and an old or stock solution was never used. The smaller doses were always measured by means of a tuberculin syringe graduated in hundreds of a centimetre.

Table I.

Lethal effects obtained from the administration of a 2 per cent solution of ammonium antimonyl tartrate to guinea-pigs by intramuscular injection.

Dose in gram per kilo of body weight.	Number of guinea-pigs used.	Number died.	Remarks.
·06	6	6	M. L. D.
·055	4	3	..
·05	4	3	..
·045	6	4	Maj. L. D.
·035	6	2	Maj. T. D.
·03	6	*Nil.*	M. T. D.

Table II.

Lethal effects obtained from the administration of a 2 per cent solution of urea antimonyl tartrate to guinea-pigs by intramuscular injection.

Dose in gram for kilo of body weight.	Number of guinea-pigs used.	Number died.	Remarks.
·055	4	4	M. L. D.
·05	2	1	..
·045	4	2	..
·04	6	3	..
·035	5	2	..
·03	3	1	Maj. T. D.
·025	4	*Nil.*	M. T. D.
·02	1	*Nil.*	..

Recent Literature " published in Tropical Diseases Bulletin, Vol. 19, No. 1, 1922 :—

" Stibacetin.—Sodium paraacetylaminophenylstibinate. $C_{24}H_{25}O_{10}N_2Sb_2Na$, contains theoretically 40·12 per cent of antimony. The form of ' Stibacetin ' sold as Stibenyl in this country in May, 1920, contained 34·85 per cent of antimony, and that now on sale, which is advertised as Von Heyden's, contains 33·16 per cent. The different batches contained 32·54 and 33·79 = mean 33·16. Sodium antimonyl tartrate, $C_4H_4O_7SbNa$, $\frac{1}{2}H_2O$, contains 38·06 per cent of antimony, and commercial salt as made for use in medicine is pure. Potassium antimonyl tartrate, $C_4H_4O_7SbK$, $\frac{1}{2}H_2O$, contains 36·14 per cent of antimony, and the commercial salt is pure.

All the foregoing percentages are expressed as percentages of metallic antimony for the formulæ as given, including water of crystallization where shown in the formulæ." The formula given above for Stibacetin corresponds to a compound consisting of condensation of one molecule of $C_8H_9O_4N$ SbNa. and two molecules of $C_8H_{10}O_4N$ Sb—2 H_2O. The percentage of antimony present in the compound prepared in our laboratory corresponds to the formula $C_{16}H_{19}O_8N_2Sb_2Na$, the exact analogue of Atoxyl without any water of crystallization. Further observations on this subject will be made later on.

The difference in the antimony contents of some of the antimonyl tartrates as estimated in my laboratory from those quoted above, is due to their containing different molecules of water of crystallization. It is a well known fact that the water of crystallization may vary in an antimonyl tartrate. See Watt's Dictionary of Chemistry.

Table III.

Lethal effects obtained from the administration of a 2 per cent solution of potassium antimonyl tartrate to guinea-pigs by intramuscular injection.

Dose in gram per kilo of body weight.	Number of guinea-pigs used.	Number died.	Remarks.
·055	4	4	M. L. D.
·05	3	2	Maj. L. D.
·045	4	2	..
·04	8	5	..
·035	6	3	..
·025	6	3	..
·02	6	2	Maj. T. D.
·015	3	*Nil.*	M. T. D.

Table IV.

Lethal effects obtained from the administration of a 2 per cent solution of sodium antimonyl tartrate to guinea-pigs by intramuscular injection.

Dose in gram per kilo of body weight.	Number of guinea-pigs used	Number died.	Remarks.
·055	4	4	M. L. D.
·05	3	2	Maj. L. D.
·045	4	2	..
·04	7	5	..
·035	6	3	..
·025	4	2	..
·02	5	1	..
·015	3	*Nil.*	M. T. D.

Table V.

Lethal effects obtained from the administration of a 2 per cent solution of aniline antimonyl tartrate to guinea-pigs by intramuscular injection.

Dose in gram per kilo of body weight.	Number of guinea-pigs used.	Number died.	Remarks.
·055	4	4	M. L. D.
·05	4	3	..
·045	6	4	Maj. L. D.
·04	5	3	..
·03	4	2	..
·025	7	*Nil.*	M. T. D.

Represented graphically, the values obtained for the minimum lethal doses and the maximum tolerated doses of the various antimonyl tartrates for guinea-pigs will form a curve shown in the accompanying diagram (Plate XXIII).

The Influence of the Base of an Antimonyl Tartrate upon its Toxicity.

The toxicity of a drug when administered to the same species of animals as determined from its minimum lethal dose is *inversely proportional* to its minimum lethal dose. From this the toxicity of the antimonyl tartrates and of their antimony content can be expressed as follows :—

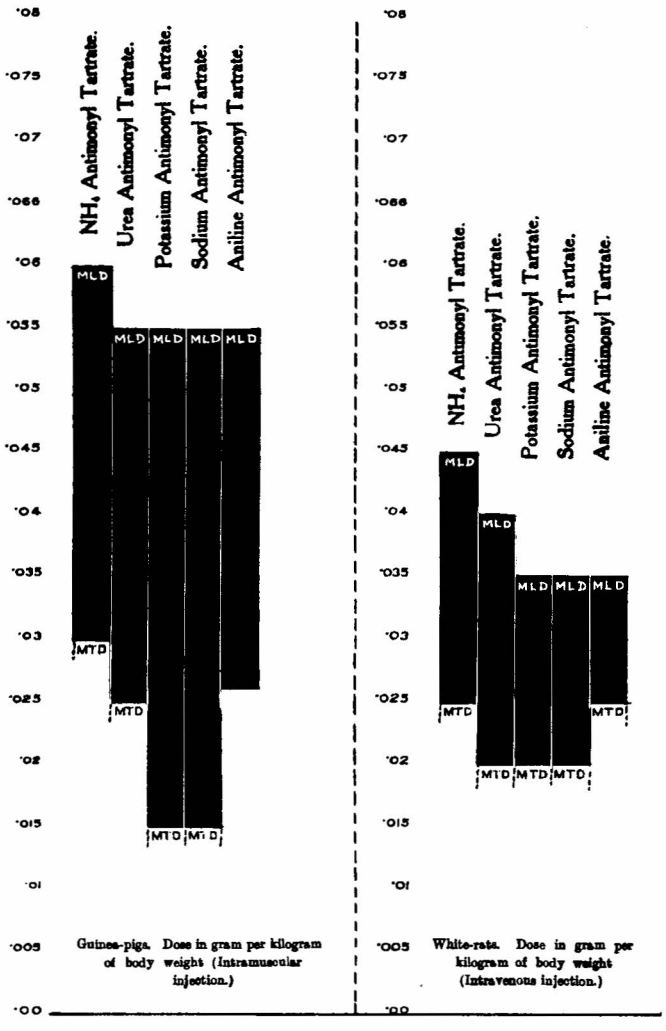


PLATE XXIII.

GUINEA-PIGS.

(1) Toxicity of ammonium antimonyl tartrate .. $= \frac{K}{\cdot 06}$

(2) Do. of urea antimonyl tartrate .. $= \frac{K}{\cdot 055}$

(3) Do. of potassium antimonyl tartrate .. $= \frac{K}{\cdot 055}$

(4) Do. of sodium antimonyl tartrate .. $= \frac{K}{\cdot 055}$

(5) Do. of aniline antimonyl tartrate .. $= \frac{K}{\cdot 055}$

(1) Toxicity of the antimony content of ammonium antimonyl tartrate $= \frac{K}{\cdot 06 \times 38 \cdot 1}$

(2) Do. do. of urea antimonyl tartrate $= \frac{K}{\cdot 055 \times 37 \cdot 55}$

(3) Do. do. of potassium antimonyl tartrate $= \frac{K}{\cdot 055 \times 36 \cdot 1}$

(4) Do. do. of sodium antimonyl tartrate $= \frac{K}{\cdot 055 \times 34 \cdot 1}$

(5) Do. do. of aniline antimonyl tartrate $= \frac{K}{\cdot 055 \times 31 \cdot 75}$

If T (NH_4), T(Urea), T(K), T(Na), T(Aniline) represent the toxicity of the above tartrates respectively, we then have :—

$$\frac{T(NH_4)}{T(Urea)} = \frac{T(NH_4)}{T(K)} = \frac{T(NH_4)}{T(Na)} = \frac{T(NH_4)}{T(Aniline)} = \frac{55}{60} \text{ or } \frac{11}{12}.$$

If T. Sb (NH_4), T. Sb (Urea), T.Sb (K), T. Sb(Aniline), T. Sb (Na) represent the toxicity of the antimony content of the above tartrates we have :—

$$\frac{T.Sb(NH_4)}{T.Sb(Urea)} = \frac{41}{46} \quad \frac{T.Sb(NH_4)}{T.Sb(K)} = \frac{40}{46} \quad \frac{T.Sb(NH_4)}{T.Sb(Na)} = \frac{38}{46} \quad \frac{T.Sb(NH_4)}{T.Sb(Aniline)} = \frac{35.}{46.}$$

Therefore in the case of the guinea-pigs, ammonium antimonyl tartrate is the least toxic, then comes the urea salt, then the sodium and potassium salts which are equally toxic and then the aniline salt.

The maximum tolerating capacity of the same species of animals for a drug is *directly proportional* to its maximum tolerated dose.

We thus have :—

(1) Maximum tolerating capacity of guinea-pigs treated with ammonium antimonyl tartrate $= K^1 \times \cdot 03$

(2) Do. do. do. Urea antimonyl tartrate $= K^1 \times \cdot 025$

(3) Do. do. do. Potassium antimonyl tartrate $= K^1 \times \cdot 015$

(4) Do. do. do. Sodium antimonyl tartrate $= K^1 \times \cdot 015$

(5) Do. do. do. Aniline antimonyl tartrate $= K^1 \times \cdot 025$.

From this we conclude that of all the antimonyl tartrates used in the case of the guinea-pigs, their maximum tolerating capacity is with ammonium antimonyl tartrate.

EXPERIMENTS ON WHITE RATS.

Method of administration and measurement of doses.

In the case of white rats, the drugs were administered intravenously, the injection being given into one of the prominent veins of the tail. The strength of the solution was one per cent in distilled water. Whenever the injection was given, the time taken in injecting a given volume of the solution was always the same, being at the rate of $\frac{1}{2}$ c.c. per minute. The injections were given by means of a tuberculin syringe and the solutions were always freshly made.

TABLE VI.

Lethal effects obtained from the administration of a 1 per cent solution of ammonium antimonyl tartrate to white rats by intravenous injection.

Dose per kilo.	Number of rats used.	Number died.	Remarks.
·045 grm.	4	4	M. L. D.
·04 „	6	4	Maj. L. D.
·035 „	6	4	....
·03 „	8	2	....
·025 „	4	*Nil.*	M. T. D.

TABLE VII.

Lethal effects obtained from the administration of a 1 per cent solution of urea antimonyl tartrate to white rats by intravenous injection.

Dose per kilo.	Number of rats used.	Number died.	Remarks.
·04 grm.	5	5	M. L. D.
·035 „	7	5	Maj. L. D.
·03 „	2	1	....
·025 „	3	1	Maj. T. D.
·02 „	4	*Nil.*	M. T. D.

TABLE VIII.

Lethal effects obtained from the administration of a 1 per cent solution of potassium antimonyl tartrate to white rats by intravenous injection.

Dose per kilo.	Number of rats used.	Number died.	Remarks.
·04 grm.	7	7	....
·035 „	7	7	M. L. D.
·03 „	7	4	....
·025 „	5	3	....
·02 „	8	*Nil.*	M. T. D.

TABLE IX.

Lethal effects obtained from the administration of a 1 per cent solution of sodium antimonyl tartrate into white rats by intravenous injection.

Dose per kilo.	Number of rats used.	Number died.	Remarks.
·04 grm.	3	3	....
·035 „	10	10	M. L. D.
·03 „	6	4	Maj. L. D.
·025 „	7	4	....
·02 „	6	*Nil.*	M. T. D.

TABLE X.

Lethal effects obtained from the administration of a 1 per cent solution of aniline antimonyl tartrate into white rats by intravenous injection.

Dose per kilo	Number of rats used	Number died.	Remarks.
·04 grm.	5	5	....
·035 „	6	4	M. L. D.
·03 „	4	2	....
·025 „	5	*Nil.*	M. T. D.

Represented graphically the values obtained for the minimum lethal doses and the maximum tolerated doses of the various antimonyl tartrates for rats will form a curve shown in the accompanying diagram (Plate XXIII).

It will be seen from the above tables that the toxic range (T. R) is not so great in the case of white rats as in the case of guinea-pigs and the difference in the toxicity of the various antimonyl tartrates is more marked in the case of white rats.

SOME ARYI PENTAVALENT ANTIMONY COMPOUNDS—*p*-AMINO-PHENYL STIBINIC ACID AND SOME OF ITS DERIVATIVES—THEIR TOXICITY.

Before proceeding to the study of the aromatic antimonial compounds dealt with in the present paper, I give here a brief summary of the principles of chemotherapy which have been followed in preparing them.

(1) Phenyl stibinic acid should be more toxic than *p*-amino-phenyl stibinic acid, just as phenyl arsinic acid is more poisonous than *p*-amino-phenyl arsinic acid.

(2) The sodium salt of *p*-amino-phenyl stibinic acid is the antimony analogue of atoxyl which is of marked therapeutic value in protozoal diseases. I have observed that the urea salt is more stable and less toxic than the sodium salt.

(3) With the idea of reducing the toxicity of *p*-amino-phenyl stibinic acid and its salts, acyl substitution compounds may be prepared by the introduction of various acidic radicles into the amino group of *p*-amino-phenyl stibinic acid to form secondary amines. Those that have been prepared are described as follows :—

(*a*) Acetyl-*p*-amino-phenyl stibinic acid and its sodium salt. The latter is identical with "Stibenyl" of Allen and Hanbury.

(*b*) Benzene-sulphonyl-*p*-amino-phenyl stibinic acid and its sodium salt. This latter is allied to Hectine of Mouneyrat.

(*c*) N-phenyl-gycine- amide-*p*-stibinic acid and its sodium salt. The above mentioned acid is allied to N-phenyl-glycine-amide-*p*-arsonic acid of Jacobs and Heidelberger which has been found by Pearce and Brown to have low toxicity but marked therapeutic properties in experimental trypanosomiasis. (*Journal of Experimental Medicine*, 1919).

(*d*) The urethano derivative of *p*-amino-phenyl stibinic acid has been found useful in fowl spirillosis. This compound may be described as carbethoxy-*p*-amino-phenyl stibinic acid.

(4) Allyl thio-carbamino-*p*-stibanilic acid allied to allyl thiocarbamino-*p*-arsanilic acid has been prepared with the idea of having the therapeutic action of allyl and stibinic compounds without the toxic character of the latter.

(5) To reduce the toxicity of the compound phenol-*p*-stibinic acid, carboxy-methylene group may be introduced into this compound to replace the H of the OH present in the para-position of the phenolic compound giving rise to carboxy-methylene-oxyphenyl-4-stibinic acid. The corresponding arsenic compound possesses such trypanocidal power that it can cure animals infected with highly resistant strains of trypanosomes.

(6) That the introduction of acidic groups into the molecule of a compound may markedly diminish the therapeutic value of a drug has been taken into consideration.

(7) When an antimonial compound has to be used for therapeutic purposes $\frac{C}{T}$ *i.e.*, $\frac{\text{Curative dose (Dose sufficient to kill all parasites)}}{\text{Toxic dose (Maximum dose which patient can tolerate)}}$ should be satisfactory, which, according to Ehrlich, is $\frac{1}{3}$ or less.

It will be seen, later, that (7) can only be determined indirectly in the case of Kala-azar. In this paper the term *effective dose* of a drug will be used to denote *a dose per day* by which the best effect appears to be obtained in the treatment of Kala-azar, when that dose is given for a sufficient length of time. It is impossible to produce *therapia sterilisans magna* in Kala-azar with a single dose of any antimonial preparation known up to the present time.

We have now to investigate how far some of the above principles of chemotherapy which are based on theoretical considerations, are borne out by actual experiments.

The starting material in the preparation of the new aromatic antimonial compounds dealt with in the present paper is acetyl-*p*-amino-phenyl stibinic acid. The sodium salt of this compound is sometimes known as stib-acetin. Kala-azar and other forms of leishmaniasis have been successfully treated by its administration (G. Caronia, 1916, Pediatria. Also Khahina-Marinucii). More recently, the same compound has been used by Manson-Bahr in the treatment of Kala-azar under the name of "stibenyl." (*Lancet*, Vol. II, 1920.)

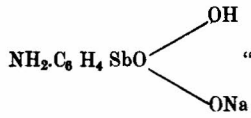
The successful use of this compound in the treatment of Leishmaniasis naturally leads one to attempt to prepare derivatives of this compound allied to those of *p*-arsanilic acid. The present paper contains a description of some new pentavalent antimony analogues of such derivatives of *p*-arsanilic as have been found to be of definite therapeutic value.

Acetyl-*p*-amino-phenyl-stibinic acid, $CH_3.CO.NH.C_6H_4SbO.(OH)^2$ was prepared in my laboratory by the action of sodium antimonite upon diazo-solution in a way somewhat analogous to Bart's reaction. By diluting the sodium antimonite solution it was found that the yield was greater than that obtained by following the method described in Morgan's work on Organic Compounds of Arsenic and Antimony, which is the method of Von Heyden. By the latter method, the preparation is difficult and the yields are low (Percy May). The percentage of antimony in $C_8\ H_9\ O_4\ N\ Sb\ Na$, the sodium salt of the above acid is 36·8. By actual calculation it was found to be 36·1.

This compound yields on hydrolysis 4-amino-phenyl-stibinic acid

(Von Heyden D. R. P. 270, 488). The sodium salt of this acid is the antimony analogue of atoxyl and has been described in the German patent (Von Heyden D. R. P., 254, 421).

For the sake of simplicity I have called this sodium salt



"Stibamine", from its analogy to the corresponding salt of arsenic which is also known, as ars-amine (*Journal of Tropical Medicine and Hygiene*, August 15, 1921). Stibamine as prepared in my laboratory, is an amorphous powder fairly soluble in water. Its solution must be perfectly neutral. The presence of alkali or acid in its solution helps its decomposition. The solution should be freshly prepared immediately before use.

Composition :—

Calculated for $C_6 H_7 O_3 NSb Na$, Sb=42·25%, N=4·93%.

Found Sb=42·10%, N=4·88%.

(1) *Urea-stibamine.*

$CO(NH_2)_2 . H_2 N C_6 H_4 SbO . (OH)_2$.

This is carbamide salt of *p*-amino-phenyl-stibinic acid.

EXPERIMENTAL.

2·3 grams of *p*-amino-phenyl-stibinic acid suspended in water, are treated with solid urea until a clear solution is obtained on slight heating. The solution is then concentrated on the water bath. To the concentrated solution absolute alcohol is added in excess, when a precipitate forms. The mixture is heated for a few minutes to dissolve any excess of urea. The precipitate is then filtered and thoroughly washed with absolute alcohol to dissolve the last traces of uncombined urea. It is then dried on porous plate. The yield is about 1·5 grams. I propose to call this compound, urea-stibamine.

The salt is fairly soluble in water and is amorphous.

Composition :—

Calculated for $C_7 H_{12} O_4 N_3 Sb$, Sb=37·26%, N=13·04%.

Found Sb=36·95%, N=12·52%.

The toxic and therapeutic properties of this compound will be described in the present paper.

(2) *Benzene-sulphonyl-P-amino-phenyl-stibinic acid.*

$C_6 H_5 . SO_2 . NH . C_6 H_4 Sb O_3 H_2$.

The sodium salt of this compound is the antimony analogue of Hectine of Mouneyrat, which is sodium-benzene-sulphonyl-*p*-amino-phenyl arsinate. This latter compound is of marked reputed value in spirochaete infection.

EXPERIMENTAL.

Benzene-sulphonyl-P-amino-phenyl-stibinic acid.

·5 gram of stibamine is dissolved in 2 c.c. of $\frac{N}{1}$ sodium hydroxide and treated with ·5 gram of benzene-sulphonyl chloride. The mixture is warmed on water bath at 60°C and shaken from time to time. The alkali is replenished as soon as it is found to be exhausted. After an hour and a half the reaction is found to be complete. The solution is filtered and conc. HCl is added to it drop by drop until it is distinctly acid. The sulphonyl compound is precipitated and is then filtered. For purification, the precipitate is suspended in water and carefully dissolved in $\frac{N}{1}$ sodium hydroxide and again precipitated by hydrochloric acid. The process is repeated three times and the precipitate is filtered and carefully washed with water and dried on a porous plate. The yield is ·33 gram.

The sodium salt is a fairly stable compound and is freely soluble in water. It has not yet been obtained in a crystalline form. I propose to call it "Stib-hectine."

Composition :—

Calculated for $C_{12} H_{12} O_5 NS Sb$, Sb=29·85%, N=3·5%. Found Sb=29·3%, N=4·06%.

(3) *Urethane derivative of P-amino-phenyl-stibinic acid.*

Carbethoxy--P-*amino-phenyl-stibinic acid.*

EXPERIMENTAL.

2·9 grams of *p*-amino-phenyl-stibinate of sodium and ·6 gram of 35 per cent caustic soda solution are dissolved in 10 c.c. of water. The mixture is treated with 1·3 grams of ethyl chlorocarbonate and 1·2 grams of 35 per cent caustic soda solution and stirred. After about half an hour the mixture is filtered. From the filtrate, urethano-derivative is precipitated with dilute hydrochloric acid. The precipitate is purified by dissolving it in caustic soda solution and then precipitating with dilute hydrochloric acid.

Composition :—

Calculated for $C_9 H_{12} O_5 N Sb$, Sb=35·92%, N=4·19%.

Found Sb=35·74%, N=3·96%.

(4) *Carboxy-methylene-oxyphenyl-4-stibinic acid.*

$CO OH . CH_2 . O . C_6 H_4 . Sb O_3 H_2$.

EXPERIMENTAL.

It is prepared by adding an alkaline solution of monochloracetic acid to the solution of sodium-phenol-*p*-stibinate.

Preparation of *p*-hydroxy-phenyl-stibinic acid :—The diazo-solution obtained from a mixture of 2·2 grams of *p*-amino-phenol, 3 grams of sulphuric acid in 20 c.c. of water, and 1·4 grams of sodium nitrite is added with stirring, to a sodium antimonite solution. The latter is obtained by mixing a solution of antimony trichloride, prepared by dissolving 2·88 grams of antimony trioxide in 12 c.c. of hydrochloric acid (D=1·123) and an aqueous solution of sodium hydroxide (12 grams.) in 120 c.c. water. When the decomposition is complete, the excess of sodium hydroxide is almost neutralised with dilute sulphuric acid. The mixture is saturated with carbon dioxide and filtered repeatedly to remove any traces of antimony trioxide, *p*-hydroxy-phenyl-stibinate of sodium is then precipitated by saturating the solution with sodium chloride, *p*-hydroxy-phenyl-stibinic acid is precipitated from this by dilute sulphuric acid. It is then filtered and dried on porous plate.

Preparation of Carboxy-methylene-oxyphenyl-4-stibinic acid :—It is prepared by adding successively monochloracetic acid (1·88 grams) in 3 c.c. of water and 4 grams of 35 per cent caustic soda solution to the solution of 2·63 grams of *P*-hydroxy-phenyl-stibinic acid and ·4 gram of caustic soda in 5 c.c. of water. The mixture is heated at 60°C. for about three hours. When cooled, the mixture is carefully acidified with hydrochloric acid. The precipitated acid is purified by dissolving in sodium hydroxide solution and precipitating with dilute hydrochloric acid.

(5) *N-phenyl-glycine-amide-P-stibinic acid.*

$NH^2 . OC . H_2 C . NH . C_6 H_4 SbO (OH)^2$.

We have tried to investigate whether amino-phenyl-stibinic acid possesses the property of giving rise to compounds of the following type $RHN . CO . CH_2 NH . C_6 H_4 SbO (OH)_2$, which are similar in constitution to those prepared by Jacobs and Heidelberger from *p*-arsanilic acid (*Journal of American Chemical Society*, 1919). Of these glycine compounds of antimony, we have prepared N-phenyl-glycine-amide of stibinic acid which is allied to N-phenyl-glycine-amide of arsonic acid of the above authors. This latter compound has given very remarkable results in the treatment of experimental trypanosomiasis and spirochæte infection in the hands of Pearce and Brown (*Journal of Experimental Medicine*, 1919). It is therefore expected that the corresponding antimony compound prepared in my laboratory should exhibit similar results in the treatment of leishmaniasis. Its toxicity and therapeutic properties have not yet been studied by me.

EXPERIMENTAL.

N-(Phenyl-4-stibinic acid)-glycine-amide or N-Phenyl-glycine-amide-P-stibinic acid.

·8 gram of stibamine is dissolved in 4 c.c. of $\frac{N}{1}$ sodium hydroxide solution. After adding ·74 gram of chloracetamide the mixture is warmed on water bath under a reflux condenser for about two hours. During warming a reddish-brown precipitate is gradually formed and settles at the bottom, the flask being shaken from time to time. After the operation the crude product is allowed to cool. ·14 c.c. of concentrated

hydrochloric acid is added to the cold mixture to hold any unchanged stibamine in solution. During this treatment the portion of the amido-glycine-compound which was retained in solution by the alkali is precipitated. The substance is then filtered off and carefully washed with cold water. For purification it is suspended in sufficient water to form a thin paste and carefully treated with stirring, with sodium hydroxide solution until the acid is dissolved. It is filtered from the undissolved product, and is then treated with a little excess of dilute acetic acid, whereupon the substance separates as a white precipitate. After filtering and washing thoroughly it is quickly dried on a porous plate and kept in a sealed tube. The yield obtained was ·2 gram. The acid is purified by its repeated solution in alkali and precipitation by acetic acid.

Sodium Salt.—The pure acid is suspended in enough water to form a thick paste and carefully treated with 25 per cent sodium hydroxide solution, until completely dissolved and the solution reacts neutral to litmus. Two volumes of alcohol are then added, the pure sodium salt quickly separating as a white powder. After filtering and washing with 85 per cent alcohol it is quickly dried on a porous plate.

The acid is sparingly soluble in cold water. It dissolves more easily in hot water. The sodium salt is freely soluble in water. It has not yet been obtained in a crystalline form and is less soluble in water than the corresponding arsenic compound. I propose to call this compound "Stib-Glycine-Amide."

Composition :—

Calculated for $C_8 H_{10} O_4 N_2 Sb Na$, Sb =35·19 %, N=8·2 %.

Found Sb=35·41 %, N=7·9 %

(6) *Allyl-thio-carbamino-P-amino-phenyl-stibinic acid.*

$C_3 H_5 NH. CS. NH. C_6 H_4 SbO (OH)_2$.

The above compound is prepared by treating stib-amine with allylthiocarbamide in methyl alcohol.

Experimental.

·2 gram stibamine is dissolved in 3 c.c. methyl alcohol and to the mixture ·08 grm. oleum synapis (containing 90 per cent allylthocarbamide) is added. The mixture is kept at ordinary temperature for 24 hours, and then filtered. The filtrate is diluted with a little water and treated with a few drops of concentrated hydrochloric acid, which precipitates the allylthiocarbamino derivative. The crude product is filtered and washed with water and dried in a desiccator. The dried substance is finally washed with ether, to free it from oil. The compound obtained is a yellowish white amorphous powder, soluble in sodium hydroxide solution, but not soluble in sodium carbonate. The yield is ·2 gram.

Composition :—

Calculated for $C_{10} O_3 H_{13} N_2 S. Sb$, Sb=33·2%, N=7·7%

Found Sb=33·4%, N=7·8 %.

In the present paper, the toxicity and therapeutic properties of urea-stibamine will be described. The toxicity of some of the other aryl antimonial compounds dealt with in the present paper will also be described here.

Toxicity Experiments with Phenyl Stibinate of Sodium, Stibamine, Urea-Stibamine, etc.

Method of administration.—The drugs were administered into guinea-pigs intramuscularly, the injections being given in the outer part of the thigh. The strength of the solution was 2 per cent in distilled water. In all these experiments, each time the solution was freshly prepared and an old solution was never used.

Table XI.

Lethal effects obtained from the administration of a 2 per cent solution of phenyl stibinate of sodium to guinea-pigs by intramuscular injection.

Dose per kilo.	Number of guinea-pigs used.	Number died.	Remarks.
·2 grm.	2	2	....
·1 „	2	2	....
·05 „	3	3	....
·025 „	4	*Nil.*	....

Table XII.

Lethal effects produced from the administration of a 2 per cent solution of acetyl-p-amino-phenyl-stibinate of sodium to guinea-pigs by intramuscular injection (Stibenyl).

Dose per kilo.	Number of guinea-pigs used.	Number died.	Remarks.
·7 grm.	3	3	M. L. D.
·6 „	5	3	....
·5 „	4	2	....
·45 „	4	2	....
·4 „	6	1	....
·35 „	2	*Nil.*	M. T. D.

Table XIII.

Lethal effects produced from the administration of a 2 per cent solution of urea stibamine to guinea-pigs by intramuscular injection.

Dose per kilo.	Number of guinea-pigs used.	Number died.	Remarks.
·7 grm.	4	4	M. L. D.
·65 „	3	2	Maj. L. D.
·6 „	4	2	....
·5 „	2	1	....
·45 „	4	1	....
·4 „	4	1	....
·35 „	4	*Nil.*	M. T. D.

Table XIV.

Lethal effects produced from the administration of a 2 per cent solution of stibamine to guinea-pigs by intramuscular injection.

Dose per kilo.	Number of guinea-pigs used.	Number died.	Remarks.
·5 grm.	4	4	M.L.D.
·45 „	4	3	....
·4 „	4	3	....
·35 „	4	2	....
·3 „	8	2	....
·2 „	6	*Nil.*	M.T.D.

Table XV.

Lethal effects produced from the administration of 2 per cent solution of stib-hectine to guinea-pigs by intramuscular injection (Compound made in my laboratory).

Dose per kilo.	Number of guinea-pigs used.	Number died.	Remarks.
·6 grm.	4	4	....
·5 „	4	4	M.L.D
·4 „	3	2	....
·3 „	3	2	....
·2 „	2	1	....

Table XVI.

Lethal effects produced from the administration of a 2 per cent solution of stib-hectine to guinea-pigs by intramuscular injection (Compound supplied by Chemisch. Fabrik. von Heyden).

Dose per kilo.	Number of guinea-pigs used.	Number died.	Remarks.
·5 grm.	3	3	....
4 „	5	5	M.L.D.
·3 „	4	3	....
·25 „	2	1	....

Symptoms of poisoning after intramuscular injection of aryl antimonials into guinea-pigs.—These symptoms are, generally speaking, similar to those following toxic doses of the antimonyl tartrates. The pathological changes in the organs after toxic doses of the aryl antimonials are also similar to those obtained after administration of toxic doses of antimonyl tartrates. In one case in which the optic nerve was examined, I did not find such degenerative changes in the optic nerve as have been observed to have followed the use of the aryl arsonates.

Having determined the toxicity of stibamine and urea stibamine, I give a summary of their physical and chemical properties:

Physical and chemical properties of stibamine and urea-stibamine.
Properties of stibamine:—

(1) Stibamine is a brown amorphous powder soluble in water, the solution being of a reddish-yellow colour.

(2) The solution of stibamine is easily decomposed, giving rise to a precipitate containing antimony, in the presence of an acid or alkali.

(3) The filtrate after separation of the above precipitate also contains antimony.

Properties of urea stibamine:—

(1) It is a brown amorphous powder like stibamine and is soluble in water giving rise to a reddish solution. It is insoluble in alcohol.

(2) Unlike 'stibamine,' its solution is not so easily decomposed by boiling for a few minutes. Its solution can be sterilized by boiling.

(3) It is more stable than 'stibamine' when kept in solution.

(4) It is an 'additive' compound of urea and liberates N_2 when treated with solution of sodium hypobromite.

The therapeutic value of ammonium antimonyl tartrate and urea stibamine.

Having proved that ammonium antimonyl tartrate is the least toxic of the five antimonyl tartrates investigated in the present paper, I now pass on to describe its effects when administered to man for therapeutic purposes. Only a few clinical cases will be described here. It is however, beyond the scope of the present paper to give the comparative value of the various antimonyl tartrates in the treatment of kala-azar.

Treatment of kala-azar with intravenous injection of ammonium antimonyl tartrate.

(1) Patient K., aet 30, was admitted into hospital suffering from kala-azar. The spleen extended 5½″ below the costal margin. On spleen puncture L. D. bodies were found. At the time of admission the body weight was 5 stone. Patient was treated with intravenous injections of ammonium antimonyl tartrate twice a week, the doses being increased from 2 c.c. to 8 c.c. of a 2 per cent solution. Altogether 16 injections were given. As a result of the treatment, the fever of the patient completely stopped, the spleen could not be felt below the costal margin and on spleen puncture no L. D. bodies could be found after the 16th injection. Patient increased one stone in weight during the treatment.

Result of blood examination:—

(1) RBC-2,300,000, WBC-2000, Hb-32 per cent on 15-6-1921 before treatment.

(2) RBC-4,300,000, WBC-7000, Hb-55 per cent on 12-9-1921 after treatment

(2) Patient N, aet 16, was admitted into hospital suffering from kala-azar. The spleen extended 4″ below the costal margin. On spleen puncture many L. D. bodies were found. At the time of admission the body weight was 3 stone. Patient was treated with intravenous injections of ammonium antimonyl tartrate twice a week, the doses being increased from 1 c.c. to 6 c.c. of a 2 per cent solution. Altogether 25 injections were given. As a result of treatment, the fever of the patient completely stopped, the spleen could not be felt below the costal arch after the 20th injection, and at the time of discharge no L. D. bodies could be found on spleen puncture. Patient increased 1 stone in weight during the treatment.

Result of blood examination:—

(1) RBC-3,200,000, WBC-2600. Hb-44 per cent on 3-6-1921 before treatment.

(2) RBC-4,500,000, WBC-7000, Hb-60 per cent on 1-6-1921 after treatment.

(3) Patient A, aet 21, was admitted into hospital suffering from kala-azar. The spleen extended 7″ below the costal margin. At the time of admission the body weight was 6 stone. Patient was treated with intravenous injections of ammonium antimonyl tartrate twice a week, the doses being increased from 3 c.c. to 9 c.c. of a 2 per cent solution. Altogether 14 injections were given. As a result of treatment, the fever subsided, the spleen could just be felt below the costal marign after the 15th injection. and at the time of discharge no L. D. bodies could be found on spleen puncture. Patient increased 1 stone in weight during the treatment.

Result of blood examination:—

(1) RBC-2,900,000, WBC-1200, Hb-42 per cent on 30-6-1921 before treatment.

(2) RBC-4,200,000, WBC-11400, Hb-55 per cent on 5-11-1921 after treatment.

Each of the above cases appeared to be cured. It will be seen that the highest dose given up to now was 9 c.c. of a 2 per cent solution. Symptoms of vomiting and purging were not great after these injections. A series of cases which could not bear treatment with tartar emetic, on account of severe reactions, such as, high fever, vomiting and purging, are now being treated with ammonium antimonyl tartrate with less marked reactions. The intramuscular injection of the compound is painful, and may give rise to local reaction, which is not so marked as in the case of tartar emetic.

The treatment of kala-azar with intravenous injection of urea stibamine.

In the following cases of kala-azar the effects of intravenous injection of urea stibamine are briefly recorded.

(1) Name Manu, aet 10 years. L. D. bodies found on spleen puncture. Dose=·15 gram given twice a week.

Effect of treatment:—

RBC	WBC	Hb.
4,200,000	3200	50 per cent on admission.
3,100,000	4800	40 ,, after 5 injections.
3,400,000	4800	48 ,, ,, 16 ,,
3,200,000	10400	46 ,, ,, 20 ,,

Spleen reduced from 3½″ to 1½″ below the costal arch. No L. D. bodies found after 20 injections. Patient free from fever for one month.

(2) Tofu, aet 30 years. L. D. bodies found on spleen puncture before treatment.

$$\text{Dose} = \begin{cases} (1)\ 10 \text{ c.c.} - 2 \text{ injections} & (0{\cdot}2 \text{ gram}). \\ (2)\ 12\tfrac{1}{2} \text{ c.c.} - 13 \text{ ,,} & (0{\cdot}25 \text{ gram}). \\ (3)\ 15 \text{ c.c.} - 3 \text{ ,,} & (0{\cdot}3 \text{ gram}). \end{cases}$$

Injections given twice a week.

Effect of treatment:—

RBC	WBC	Hb.
2,900,000	1800	38 per cent on admission.
2,800,000	4200	38 ,, after 3 injections.
3,200,000	5200	42 ,, ,, 14 ,,
4,700,000	10400	52 ,, ,, 18 ,,

Spleen reduced from 2½″ to *nil* beneath costal arch. Body weight increased from 5 st. 2½ lb. to 6 st. 3 lb. No L. D. bodies found on spleen puncture after 18 injections. Patient free from fever for one month.

(3) Abdul, aet 30 years. Spleen 6″ below the costal arch in the left nipple line and 2″ away from mid-line to the right side. L. D. bodies found on spleen puncture before treatment.

Dose=·2 gram at each injection. Injections given twice a week.

Effect of treatment:—

RBC	WBC	Hb.
1,900,000	1800	28 per cent on admission.
3,000,000	2600	44 ,, after 7 injections.
3,900,000	3600	50 ,, ,, 13 ,,

Spleen slightly felt below the costal arch and body weight increased from 6 st. 6 lb. to 7 st. 4 lb. Patient discharged at his own request. Patient free from fever for one month.

(4) Anath Bondhu, aet 14 years. L. D. bodies found on spleen puncture before treatment.

Dose=·2 gram twice a week.

Effect of treatment :—

RBC	WBC	Hb.
2,600,000	1200	38 per cent on admission.
2,800,000	4200	42 ,, after 5 injections.
3,800,000	8000	50 ,, ,, 12 ,,

Spleen reduced from 6″ to almost *nil* below the costal arch. Body weight increased from 4 st. 21b. to 5 st. 6 lb. Patient free from fever for one month.

(5) Abdul, aet 12 years. Spleen 4½″ below the costal arch. L. D. bodies found on spleen puncture before treatment.

Dose=·15 gram twice a week.

Effect of treatment :—

RBC	WBC	Hb.
2,900,000	1400	44 per cent on admission.
3,700,000	2400	48 ,, after 10 injections.
4,200,000	2400	50 ,, ,, 14 ,,
3,900,000	5200	42 ,, ,, 16 ,,

Patient absconded from hospital.

(6) Mosafar, aet 30 years. L. D. bodies found on spleenpuncture before treatment.

Doses :—1st 2½ c.c., 2nd 5 c.c., 3rd 10 c.c. and the last 6 injections. 12½ c.c. of a 2 per cent solution. Injections given twice a week.

Effect of treatment :

RBC	WBC	Hb.
2,400,000	1600	32 per cent on admission.
2,400,000	7000	36 ,, after 9 injections.

Patient absconded from hospital.

(7) Horoz, aet 10 years. L. D. bodies found on spleen puncture before treatment. Spleen extended 5½″ below the costal arch before treatment.

Dose=·05 to ·15 gram every alternate day.

Effect of treatment :—

RBC	WBC	Hb.
2,300,000	2000	36 per cent before treatment.
3,400,000	3800	40 ,, after 3 injections.
4,200,000	13800	46 ,, ,, 16 ,, and same after 27 injections.

Spleen just felt below costal arch, Patient free from fever for one month.

No L. D. bodies found on spleen puncture after 20 injections.

(8) Abdul, aet 25. L. D. bodies found on spleen puncture before treatment.

Dose=·25 gram twice a week

Effect of treatment :—

RBC	WBC	Hb.
3,000,000	2400	40 per cent on admission.
4,300,000	7000	50 ,, after 7 injections

Spleen reduced from 7″ to almost *nil* below the costal arch. Body weight increased from 6 st. to 7 st. 4 lb.

Patient left hospital before treatment was completed.

REMARKS.

In the present paper, the toxicity of some of the antimonyl tartrates and of some new aromatic antimonials has been described.

In his observation on the Treatment of Oriental Sore, Greig has come to the following conclusions with regard to the use of tartar emetic in the treatment of the disease :—

'It is not desirable to exceed 12 to 13 c.c. (1 per cent solution) at one time as toxic symptoms become more marked above this limit. Hence we see that the $\frac{C}{T}$-dose ratio of antimonium tartaratum is not very satisfactory, the organo- and parasito-tropic properties are not in the correct proportion. .....The ideal drug for the destruction of *Leishmania tropica* in the tissues has still to be sought.' (*Indian Journal of Medical Research*, October 1917.) The same also holds good in the use of the drug in the treatment of kala-azar. The average minimum effective dose of tartar emetic in the case of an adult man in the treatment of kala-azar may be taken as 6 c.c. of a 2 per cent solution (=·12 gram). The average minimum effective dose of urea stibamine used for the same purpose is ·25 gram. If C and C¹ denote these minimum effective doses respectively, we have $\frac{C}{C^1}=\frac{\cdot 12}{\cdot 25}$ or $\frac{1}{2}$ approximately.

From the toxicity experiments described in the present paper it will be seen that the maximum tolerated doses per kilo of body weight in the case of the guinea-pig are ·015 gram of tartar emetic and ·35 gram of urea stibamine. If T and T′ represent these tolerated doses we have :—

$$\frac{T}{T'}=\frac{\cdot 015}{\cdot 35}\text{ or }\frac{1}{23}\text{ nearly.}$$

Though it does not necessarily follow that the minimum tolerated dose for the human being can be reckoned weight for weight by rule of three with mathematical accuracy from observations on the guinea-pig still it is evident from the above figures that urea stibamine is a much safer antimonial for use in the treatment of kala-azar than tartar emetic. The fact also holds good in the case of the other antimonyl tartrates. The effective dose of urea stibamine in the treatment of kala-azar is $\frac{1}{23}$th the tolerated dose for the guinea-pig, while in the case of tartar emetic, it is 8 times the tolerated dose for the same animal.

CONCLUSIONS.

(1) After the administration of a toxic dose of an antimonyl tartrate, the pathological changes are most markedly seen in the lungs, the kidneys, the liver, pituitary and adrenals. These consist chiefly of hæmorrhages into the substance of these organs and destruction of their cellular elements. Similar pathological changes are also observed after toxic doses of the aromatic antimonial compounds.

(2) Ammonium antimonyl tartrate is the least toxic of all the antimonyl tartrates used.

(3) The toxicity of the *antimony content* of an antimonyl tartrate is least marked in the case of the ammonium salt.

(4) The presence of N in the basic radicle of an antimonyl tartrate diminishes the toxicity of some of them.

(5) Ammonium antimonyl tartrate is of marked therapeutic value in the treatment of kala-azar.

(6) The low toxicity of ammonium antimonyl tartrate and its high antimony content lead to the conclusion that of all the antimonyl tartrates dealt with in the present paper, ammonium antimonyl tartrate is the best for use in the treatment of kala-azar.

(7) A series of new organic aromatic antimonials have been discovered, the preparations of which have been described in the body of the paper.

(8) The toxicity of the following aromatic antimonials has been estimated in the case of the guinea-pig :—(1) Phenyl stibinic acid, (2) Acetyl-*p*-amino-phenyl stibinic acid, (3) Stibamine, (4) Urea stibamine, (5) Stib-hectine.

(9) The acetyl derivative of *p*-amino-phenyl stibinic acid is less toxic than stibamine.

(10) Urea stibamine is less toxic than stibamine.

(11) Urea stibamine has been found useful in the treatment of kala-azar.

(12) Urea stibamine is a much safer antimonial for use in the treatment of kala-azar than tartar emetic or other antimonyl tartrates.

(13) Symptoms, such as, vomiting and purging, are much less marked after intravenous injection of an effective dose (=·25 gram) of urea stibamine than that of tartar emetic or sodium antimonyl tartrate (=·12 gram).