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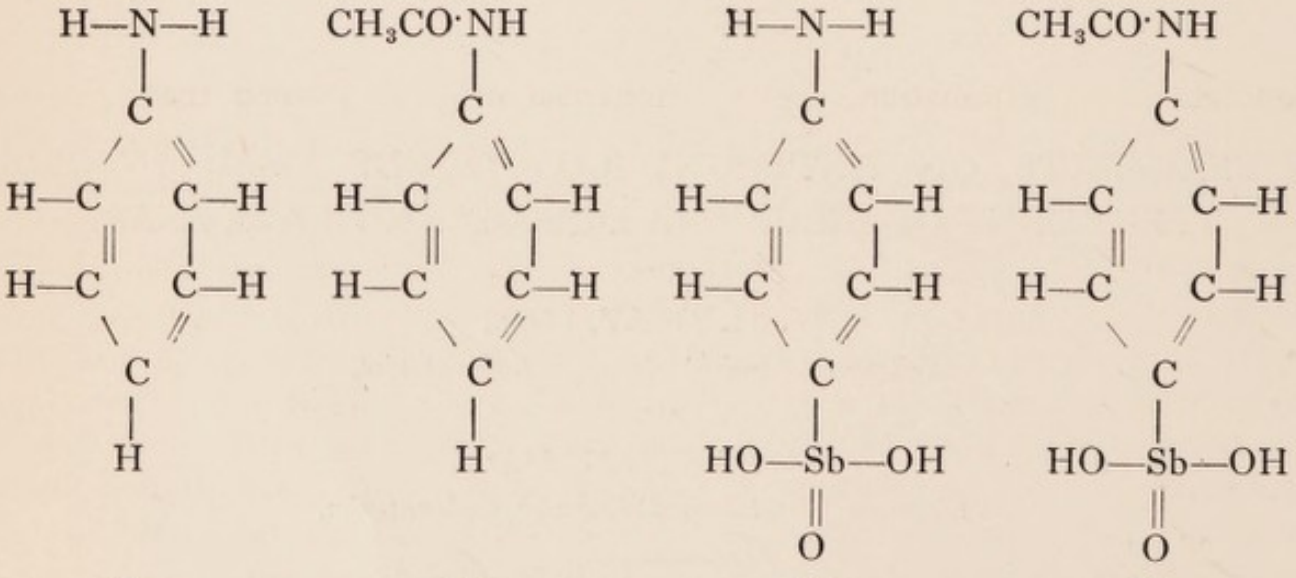
The history of the treatment of kala-azar is divided into three periods. Prior to 1915, many drugs of various kinds were tried without showing any promise of affecting the 90 per cent. death rate of the untreated disease. This state of affairs was almost reversed by the discovery that tartar emetic cured 80 per cent. of the cases treated with it (Di Cristina and Caronia, 1915; Rogers, 1915; Muir, 1915). Introduction of the organic compounds

of antimony, in which antimony is attached directly, and not as in the " emetic " type of substance through oxygen, to carbon, marked the third and most successful stage. These do not give rise to the pneumonia, thought to be due to the liberation of antimony trioxide by the serum alkali, which is the most frequent cause of death during treatment with tartar emetic ; the death rate has been reduced by their use to less than 5 per cent., and the period of treatment shortened from the eight to ten weeks required with tartar emetic to a few days.

The first of them to achieve clinical success in India was " urea stibamine," prepared by BRAHMACHARI (1922) by the action of urea upon *para*-amino-phenylstibinic acid (stibanilic acid). Its nature, however, was the subject of controversy. The progress of chemotherapy depends upon exact knowledge of the chemical constitution of drugs which prove to be clinically useful, and for this reason the authors (1931) thought it desirable to investigate " urea stibamine," and have shown that it is a mixture and identified its constituents. The problem involved the chemical and pharmacological examination of " urea stibamine " itself, and comparison of its trypanocidal activity with that of those constituents prepared by other methods in a pure condition.

Stibanilic acid (III) is the parent substance of all the organic compounds of antimony which have come into use in medicine. It is a derivative of aniline (I), formed by substituting the antimonic acid residue-$SbO(OH)_2$ for a hydrogen atom :—

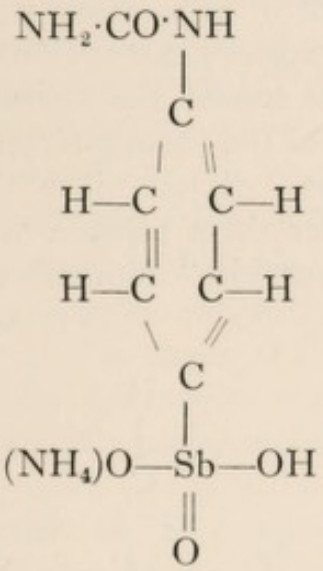


I. II. III. IV.

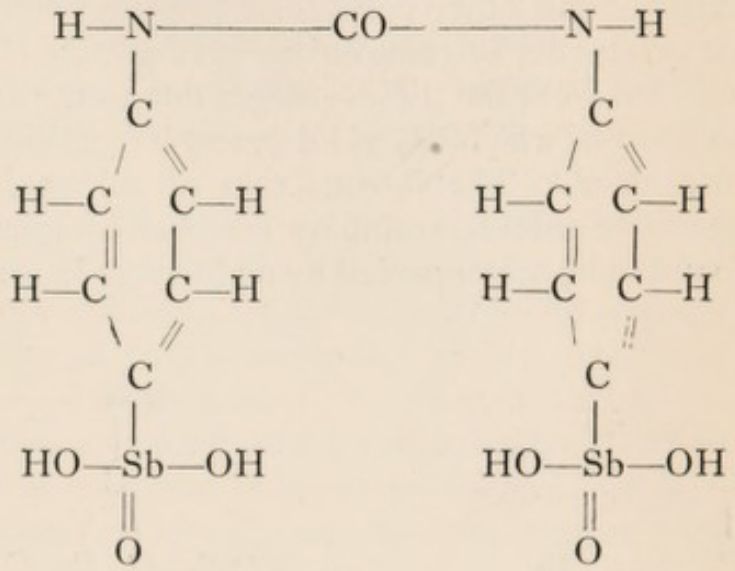
Introduction of an acetyl group, $CH_3 \cdot CO$-, as in the formation of acetanilide (II) from aniline, decreases the toxicity in both instances and gives the acid (IV), of which the sodium salt, known as " stibacetin," was the first organic antimony compound to be tried clinically. With this, good results were obtained in the treatment of kala-azar in Italy, but not in India. A chlorinated modification,

known as " stibosan," was more successful. Two derivatives of stibanilic acid of pronouncedly low toxicity remain to be mentioned, " neostam " (stibamine glucoside), in which a glucosyl group takes the place of the acetyl group present in stibacetin, and " neostibosan," which is stated to be a diethylamine salt of stibanilic acid. With these two substances the infection can be cured in a few days, and they are thus within measurable distance of achievement of the " *therapia sterilisans magna* " visualised by EHRLICH, but unfulfilled, as yet, by any other artificially prepared chemotherapeutic remedy.

" Urea stibamine " was stated by BRAHMACHARI (1924) to be the ammonium salt of the carbamyl derivative of stibanilic acid, (V),



V.

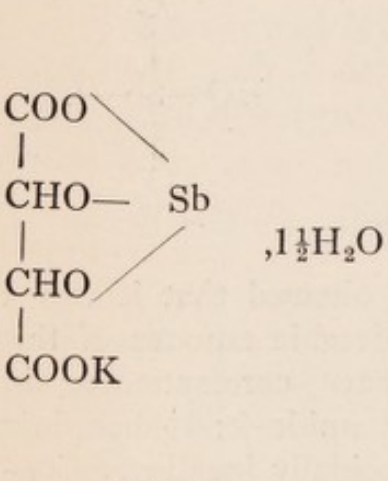


VI.

but chemical examination of the commercial material showed that it was a mixture which could at most contain only an inconsiderable amount of this substance, the principal constituents being a substance corresponding in composition to the di-substituted urea, *sym*-diphenylcarbamide-4 : 4′-distibinic acid (VI), and antimonic acid, the latter being trypanocidally inactive. Confirmation was obtained in two ways : the di-substituted urea was prepared in a pure state, by means of carbonyl chloride, and found to have a high trypanocidal activity corresponding to that of " urea stibamine," and the monosubstituted urea, (V), in a pure state, to be less active than " urea stibamine,"* also, the action of urea upon stibanilic acid was studied under various conditions ; when these were chosen theoretically to favour the formation of the di-substituted urea, analysis of the resulting product and its relatively high trypanocidal activity confirmed the presence of this substance. Less than 5 per cent. of urea was found, so that the statement of GHOSH and others (1928) that " urea stibamine " is a complex salt of urea, from which this constituent may readily be removed by washing, is not borne out.

* SCHMIDT (1930) has also prepared ammonium *p*-carbamidophenylstibinate by another method, and reports that it is inactive in hamster kala-azar.

HANS SCHMIDT and his collaborators (1930) to whom was due the discovery and early development of the organic antimonials, have more recently turned their attention to a class of substances in which the instability and toxic properties of tartar emetic are avoided in a different way from that recounted above, and these have found favour in the mass treatment of bilharziasis. The molecular formula of tartar emetic is still under discussion, but the most satisfactory expression yet put forward is VII (REIHLEN and HEZEL, 1931). The new compounds of this type are complex salts of antimony, chemically related to tartar emetic, in which more powerfully complex-forming agents than tartaric or similar acids have been used, namely the aromatic hydroxy-compounds, catechol and other polyhydric phenols. Specific treatment of bilharziasis with tartar emetic, introduced by MCDONAGH (1915) and CHRISTOPHERSON (1918), suffered from the disadvantages due to the acidity of tartar emetic, the aqueous solution of which has pH 4·5, and its instability at the pH of the tissues, already mentioned. The introduction of sulphonic acid groups, $-SO_3H$, into the sparingly soluble antimony catechol compounds, to render them soluble, was found to be accompanied by an increase in stability as well, and in " antimosan " (VIII),



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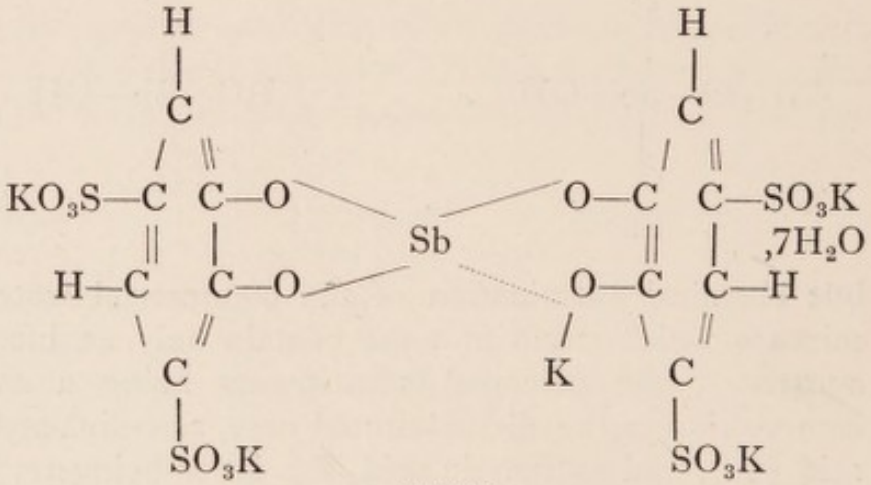

VIII.

a soluble and stable neutral compound was obtained which was preferable to tartar emetic, particularly for mass treatment. Still better results were obtained when all the potassium atoms in " antimosan " were replaced by sodium, and a 6·3 per cent. aqueous solution of the sodium salt is now in routine use in the Egyptian hospitals under the name of " Fuadin."

The trypanocidal activity of " urea stibamine " and its constituents was worked out by intravenous injection into mice infected with *Trypanosoma equiperdum*. We attempted to decrease the effect of the variability of the mice and of the condition of the strain of trypanosomes by the following procedure :—

1.—All experiments were made with control infected groups of mice

injected with a dose of an organic antimony compound, stibamine glucoside (" neostam ") as a control.

2.—Groups of eight to twelve mice for each drug and for the controls were injected. The mice were selected so that each animal in the group injected with the substance under examination had a litter mate in the group injected with the control.

3.—Simple statistical tests were used to determine whether the differences observed were significant or not. Experiments were repeated until doses of the drug and the standard were found which gave mean survival times not differing by a statistically significant amount.

4.—For the comparison of results we found that it was immaterial whether the duration of the freedom of the blood of the treated mice from infection or the survival time after treatment was used. The latter was chosen as being more convenient. The mice only died very rarely from other causes and death was always preceded by a rapid increase in the number of trypanosomes in the blood.

We have found that examination of the blood for trypanosomes between treatment and death is unnecessary. If the blood of the animal is examined for trypanosomes within eighteen hours of death, it can be established whether or not the death of the animal is due to trypanosomiasis. Such a procedure greatly diminishes the labour and so enables a larger number of tests to be done. An occasional mouse survives for a period much longer than that of its comrades in the same group. For the purposes of statistical comparison such animals are best regarded as surviving one day longer than the longest lived of the remainder of the group.

We have found that the intensity of the infection can be varied within wide limits without affecting the *comparison* between the drugs. Very heavy infections are undesirable as the mice die before the drug produces any apparent effect on the infection. Very light infection inconveniently prolongs the time of survival. Counting of the number of trypanosomes in the blood of the mice was not done. The degree of accuracy obtainable is illustrated by the following figures. Two experiments comparing " urea stibamine " with stibamine glucoside were carried out. In the first, 20 animals were used. The mean survival time in one group injected each with 1·3 mgms. of " urea stibamine " for 20 grams body weight was 9·1 days. For the other groups injected with three times as much stibamine glucoside the survival time was 6·3 days. From the values for the survival times of the individual mice the standard deviation of the difference of the mean survival time was calculated. The ratio of the difference of the survival time to its standard deviation was 2·13 and Fisher's tables (FISHER, 1928) show that such a difference of survival time could be expected to arise by chance only once in twenty-five times. " Urea stibamine " is, therefore, with a considerable degree of certainty more active than three times its weight of stibamine glucoside. In the second experiment 0·75 mg.

of " urea stibamine " was compared with 3·0 mgm. of stibamine glucoside. The mean survival time was in this case longer for the group injected with the glucoside and the probability was only 1 in 500. So that " urea stibamine " is less active than four times its weight of stibamine glucoside. The weight of " urea stibamine " equivalent to one mgm. of stibamine glucoside is therefore less than 0·33 mgm. and greater than 0·25 mgm. with a mean of 0·29 mgm. The range of uncertainty is ± 14 per cent. The figures given in the first part are all obtained in a similar manner.

The practical value of these tests remains to be discussed. ROEHL has made the statement that the assessment of the clinical value of antimonial preparations cannot be carried out on trypanosomiasis in the mouse and recommends the use of hamsters infected with leishmania. We have done no work with hamsters, but what clinical reports on the relative activity of "urea stibamine " and stibamine glucoside exist do not suggest that there is any difference of the order indicated by the results detailed above. It is difficult to say how far the clinical reports do really assess the *relative* efficiency of the two drugs. It seems possible that the doses of the drugs used have been too large to show a difference which might exist. It is possible that a difference may exist which would only be shown if the dose was reduced to a point where one or both drugs failed to cure a fair proportion of the cases under treatment. It is a point on which closer and more direct association between the laboratory worker and the physician, both imbued with a suitable reverence for statistical method, is urgently needed.

Whether the results can be transferred directly to human medicine or not there is, however, a directly practical application which is of some importance. The chemical characterisation of these organo-metallic compounds is extremely difficult and if the effect of the drug on trypanosomiasis can be determined with an accuracy of the order indicated it can serve the chemist directly, in adding one very specific quantitative " constant " to the few he has at his disposal for the identification of the compound.

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