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# OBSERVATIONS ON REPUTED DYSENTERY REMEDIES

BY

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## OBSERVATIONS ON REPUTED DYSENTERY REMEDIES.

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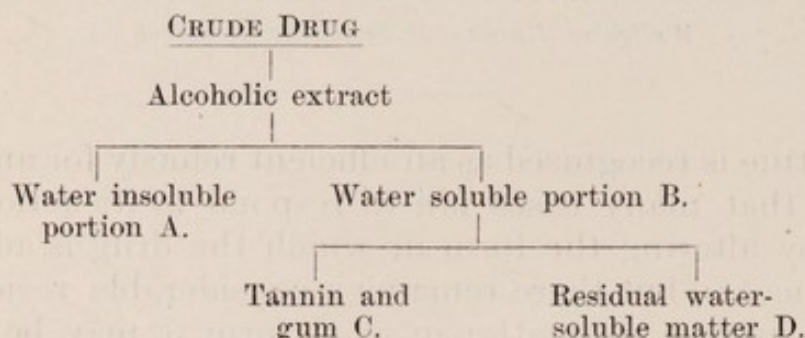
Though emetine is recognised as an efficient remedy for amœbic dysentery, it is well known that many cases fail to respond to its action. Attempts to deal with these by altering the form in which the drug is administered have met with some success, but there remains a considerable residuum of cases in which emetine is useless, no matter in what form it may be used<sup>1</sup>. In considering the possibility of finding a new remedial agent, the first line of enquiry to suggest itself was whether, using emetine as a basis, some new product could not be developed from this alkaloid which would be worth trial. Unfortunately, little is yet known regarding the chemistry of emetine, and trials of the alkaloids related to it have shown that these are less effective amœbicidal agents than emetine itself, so that it is not possible at present to find a basis in this direction for the synthesis of products likely to be of value in the treatment of amœbic dysentery. In these circumstances it seemed to the authors desirable to examine various drugs which enjoy, in the countries in which they occur, some reputation as remedies for this disease, in the hope of finding among these a useful drug, or at least discovering some clue to the kind of molecular structure, which might be promising for further work.

Among these drugs are *Monsonia ovata* and *Rhyncosia adenodes*, both used in South Africa, and two species of *Brucea*, viz., *B. abyssinica* and *B. sumatrana*, employed in Abyssinia and Malaya respectively. These four drugs have been examined chemically already without disclosing the presence of any constituent to which their alleged amœbicidal value could be ascribed.

Thus MABERLEY<sup>2</sup> isolated from *M. ovata* a product "entericin" to which he attributed the anti-dysenteric action of the drug, but "entericin" is so ill-defined that it would be difficult for anyone to prepare it again. In the case of *Brucea* species, POWER, LEES and SALWAY<sup>3</sup> isolated amorphous bitter substances, but trials of these on free-living protozoa in the course of the present work showed that they were inactive either alone or in the presence of alkali. Similarly, the present authors have exhaustively examined the bark of *R. adenodes* without finding any well-defined active substance other than the tannin to be referred to later. The authors have, therefore, tried to combine biological and chemical methods in the hope of being able to select, from the considerable number of such drugs available, any which seemed promising enough for detailed chemical examination. The combined method used is as follows:—

The finely ground drug was exhausted with boiling alcohol, the extract

concentrated *in vacuo*, and the thick syrup diluted with water to precipitate fatty and resinous matters, which form preparation A. The liquor from this precipitate, after further concentration *in vacuo* to remove all the alcohol, constituted preparation B. The latter was then treated with lead acetate to remove tannin and gum, which, after recovery from the lead precipitate, gave preparation C, and the residual liquor, after removing the excess of lead added, yielded preparation D. The scheme may be represented diagrammatically as follows:—



The strength of each of these preparations was adjusted so that 1 c.c. represented a definite quantity of the original drug.

All four preparations were then tested on *Glaucoma*, an actively motile ciliate, cultures of which are easily obtained in hay infusions; the method employed is that already described<sup>4</sup> and may be briefly summarised as follows:—

The culture of the protozoon was first filtered through coarse muslin and then washed twice in tap water by centrifuging it at slow speed, the supernatant fluid being removed on each occasion. One volume of this washed suspension of the organism in tap water was then taken and thoroughly mixed with one volume of distilled water and an equal quantity of the varying dilutions of the reagent under investigation. This mixture was placed on a slide and examined under a  $\frac{2}{3}$ in. objective. A 15-minutes' time limit was used throughout.

In all the cases tried, preparations A and D proved to be non-toxic, and B and C toxic to *Glaucoma*, and, since C and D together form B, the activity must be due to one of the components of C, that is, to one of the substances precipitated by lead acetate. The degree of activity may be illustrated by the case of *R. adenodes*.

TABLE I.

Drug.	Character of Preparation.	Strength.	Toxicity to <i>Glaucoma</i> .	
			Kills at dilution.	Fails at dilution.
<i>Rhyncosia adenodes</i> .	A	1 c.c. of extract = 3 grams of crude drug.	Inactive.	Inactive.
	B		Equivalent to 0·2 gram of crude drug.	Equivalent to 0·16 gram of crude drug.
	C		Equivalent to 0·25 gram of crude drug.	Equivalent to 0·20 gram of crude drug.
	D		Inactive.	Inactive.

It seems clear, therefore, that the toxic substance is included in the components precipitated by lead acetate.

The materials removed by lead acetate from such plant extracts are usually gums, tannins, colouring matter and acids, and of these the only substances likely to possess protozoocidal properties are the tannins. It is interesting to note in this connection that many of the drugs which have been used for the treatment of unspecified dysenteries are rich in tannin, and it has been assumed generally that such drugs owe their value merely to their astringency and not to any toxic action of the tannins on the parasites. In view of these results it was considered advisable to try the action of a number of pure tannins on *Glaucoma*. Unfortunately, the chemistry of the tannins has not yet made much progress, and though these products can be isolated fairly easily from plants, it is usually not possible to say whether the material obtained is a single substance or a mixture of various tannins. Only one tannin, that of galls (gallotannin) is readily obtainable commercially in a pure state, but a number of substances, which are known to be closely related to the tannins, have been obtained crystalline, and there can be no doubt as to their purity. Among these are ellagic acid, chebulinic acid\* (from myrabolans), catechin (from Gambier catechu), and acer tannin\*. These five pure products have been tried, and, in addition, the authors have isolated, by the following process, the tannins present in a number of well-known tanning materials and astringent drugs:—

The finely-ground plant was extracted with boiling light petroleum to remove wax and resin; and then with boiling dry ether, which in some cases removed a little more resin. The residue was next extracted with boiling acetone. Where the tannin was removable with difficulty by acetone, ethyl acetate was used, and where this failed absolute alcohol was employed. The solvent was distilled from the extract *in vacuo*, the residue dissolved in water, the solution filtered and then precipitated (fractionally, if necessary, to remove colouring matter) with lead acetate. The lead acetate precipitate was de-leaded by suspension in water and passage of sulphuretted hydrogen, and the filtrate from the lead sulphide concentrated *in vacuo* to a thick syrup, absorbed in sawdust, which had been extracted previously with solvents to remove all soluble matter, dried thoroughly by heating below 100° *in vacuo*, and then re-extracted with dry ether to remove any traces of fat or resin left, and finally with acetone, ethyl acetate or absolute alcohol. This treatment was repeated if necessary until a product, completely soluble in water or alcohol and giving all the reactions of tannins and free from gum, so far as could be judged by the application of tests, was obtained. Solutions of definite strength of the tannins in water were then made, and their action on *Glaucoma* determined by the method already described. The results are summarised in the following table:—

\* For specimens of these two substances the authors are much indebted to Prof. A. G. PERKIN, F.R.S., of Leeds University.

TABLE II.

Substance.	Strength of Preparation, per cent., found.	
	(a) Toxic to Glaucoma.	(b) Not Toxic to Glaucoma.
Ellagic acid ... ..	Inactive	...
Acer tannin ... ..	"	—
Catechin ... ..	"	—
Chebulinic acid ... ..	"	—
Gallotannin ... ..	0·23	0·20
Myrabolans tannin ... ..	0·20	0·16
Wattle bark tannin ... ..	0·33	0·30
Catechu tannin ... ..	0·50	0·33
Monsonia tannin... ..	0·08	0·06
Rhyncosia tannin ... ..	0·06	0·05
Sant tannin ... ..	0·06	0·03
Bael fruit tannin ... ..	0·06	0·03
Sweet flag tannin ... ..	0·10	0·06
Combretum tannin (a) ... ..	0·10	0·06
Combretum tannin (b) ... ..	0·66	0·50
Ipecacuanha tannin* ... ..	0·06	0·03
Kurchi bark tannin* ... ..	0·65	0·52

\*In these cases special care was taken to remove all the alkaloids from the extracts before isolating the tannins.

The sources of these tannins are all well known, with the possible exception of sant pods, combretum and kurchi. Sant pods consist of the fruit pods of *Acacia arabica*, a tree better known as one of the sources of gum arabic. The pods and the bark (babool bark) of the plant have long been used in India, chiefly for tanning, but also in medicine as astringents. *Combretum pilosum* has been recommended recently by RAMSAY<sup>5</sup> as an anthelmintic, and is now under examination from that point of view; it contains gallic and ellagic acids and two species of tannin, (a) readily soluble in cold water, and (b) soluble in boiling water but almost insoluble in cold water. Kurchi bark, derived from *Holarrhena antidysenterica*, is well known in India as a remedy for dysentery, and one of the present authors<sup>4</sup> has shown that its reputed value in amœbic dysentery is probably due to the alkaloid conessine, which is very toxic to protozoa. Other species of *Holarrhena* occur in West Africa, and these have also a local reputation as remedies for dysentery. From one of them, *H. congolensis*, PYMAN isolated conessine,<sup>6</sup> identical with the alkaloid found in the Indian species, and from a second West African species, *H. Wulfsbergii*, the present authors have isolated the same alkaloid.

As already stated, the first four substances in the foregoing list are all crystalline and sparingly soluble in water; they are closely related to the tannins, and can, so far as their structure is known, be regarded chemically as tannins, but acer tannin, catechin and ellagic acid do not combine with skin substance to produce leather,<sup>7</sup> and in this respect their absence of toxicity towards Glaucoma corresponds with their lack of real tanning properties. The remaining thirteen substances are all toxic to Glaucoma and fall into two

well-marked groups. The first group is toxic in solutions varying in strength from 0.1 to 0.6 per cent., and the second in solutions varying from 0.06 to 0.1 per cent. Though but little progress has been made in knowledge of the chemistry of tannins, they can be roughly classified into at least three groups, which are more or less characterised by the products formed on hydrolysis, and, less satisfactorily, by colour reactions with iron, alum, etc., and it would be interesting if the two groups into which these thirteen tannins are separated by their relative toxicity to Glaucoma, corresponded with this rough chemical grouping, which is all that is possible with the present meagre knowledge of the subject. To a certain extent this correspondence does exist; thus, gallotannin, and the tannins of myrabolans, and *Combretum* undoubtedly belong to the pyrogallol group, and these are placed by their action on Glaucoma in the less toxic first group. *Rhyncosia*, wattle-, kurchi- and catechu-tannins, on the other hand, have all the characters of catechol or phlobaphene-forming tannins, but as regards their behaviour towards Glaucoma, *Rhyncosia* belongs to the more toxic and the other three to the less toxic group. The catechol group of tannins may differ among themselves in the nature of the second product they yield on hydrolysis, and in this connection it is of interest to mention that three of the most active tannins in the foregoing list, viz., *Monsonia*, sant and *Rhyncosia* either yield phloroglucinol on hydrolysis or show evidence of the presence of a phloroglucinol residue in their molecule; but this is probably also true of wattle- and catechu-tannins, which belong to the less toxic group. Phloroglucinol itself (see Table III) proved to be only slightly toxic.

Allusion has already been made to the difficulty of isolating single tannins, and it seemed possible that, by carefully fractionating the tannins prepared, as already described, and trying the various fractions on Glaucoma, useful evidence on two points might be obtained: (1) that the toxicity of these preparations is an inherent property of the tannins themselves and not due to the occurrence with them of traces of some very toxic substance, and (2) that the lower toxicity of the first group referred to above is not due to the inclusion of considerable quantities of inert material. The fractionation was made by means familiar to chemists and need not be stated in detail here. The results showed that, in the case of wattle and sant tannins, no inert material could be separated, but with myrobolans, catechu and *Monsonia* tannins small amounts of sparingly soluble fractions separated first which were inert or of low toxicity, but the increase in toxicity due to their removal was never very marked except in the case of catechu-tannin, where the most soluble fraction was four times as toxic as the original tannin, a change not sufficient, however, to remove it from the less toxic group.

In view of this it seems clear that such action as these substances have on Glaucoma is due to the tannins themselves and not to some adhering substance, which can be concentrated by fractionation. The latter serves to eliminate sparingly soluble inactive substances in the first fraction from myrabolans, *Monsonia* and catechu tannins, but on the whole has very little effect on the relative activities of the different tannins.

It has been tacitly assumed, so far, that toxicity to Glaucoma might be

taken to indicate amoebicidal activity. The authors are fully aware that this assumption may not be justified, but they would point out that the method of investigation now used has already given good results in their hands, since it was on the basis of a similar bio-chemical investigation of conessine, the alkaloid of kurchi bark, that preparations of the latter were tried by WILLMORE<sup>1</sup> in resistant cases of amoebic dysentery and found useful. A similar investigation of quinine<sup>8</sup> also led to trials of this drug by WILLMORE<sup>1</sup> in amoebiasis, with results which, though promising at first, have proved not to be lasting, probably due to the difficulty of administering the quinine in a suitable form.

With this assumption in mind, it must, however, be pointed out that these tannins are not very promising amoebicidal agents; thus the best of them, sant tannin, is toxic to Glaucoma at 1 in 1,660 which compares unfavourably with the toxicity of conessine (1 to 120,000\* approx), emetine (1 in 30,000\* approx), and quinine (1 in 6,000\* approx). In view of this it seems unlikely that any of these tannins will prove useful in resistant cases of dysentery, though they may have their value in mild cases and as adjuncts to other drugs; and the authors hope to have clinical trials carried out to ascertain whether they are likely to be serviceable in the latter direction.

These observations are chiefly of interest as affording a possible explanation of the continued use of such drugs as *M. ovata* and the preference displayed by some authorities<sup>9</sup> for an extract of the whole of the soluble matter of ipecacuanha, in place of the pure, active, alkaloid emetine. Even de-emetinised ipecacuanha, if it still contains the tannin, may have some slight value.

Since these tannins proved to be generally toxic to Glaucoma, it became a matter of some interest to investigate the action on this protozoon of substances from which the tannins can be regarded as built up. These results are shown in the following table:—

TABLE III.

Substance.	Strength of Preparation, per cent., found.	
	(a) Toxic to Glaucoma.	(b) Not Toxic to Glaucoma.
Catechol ... ..	0·33	0·30
Quinol ... ..	0·02	0·01
Resorcinol ... ..	0·66	0·33
Pyrogallol ... ..	0·66	0·33
Phloroglucinol ... ..	1·66	1·33
Protocatechuic acid (sodium salt)	1·66	1·33
Protocatechuic aldehyde ...	0·16	0·13
Gallic acid ... ..	0·83	0·33

It will be seen that these substances have on the whole the same order of activity as the tannins (Table II), but one of them, quinol, is more active

\*These figures are for the free base in presence of N/600 alkali.

than any of the tannins, possibly because of the ease with which it is converted into the very active substance quinone (see Table IV), and the two acids, protocatechuic and gallic, are much less active than most of the tannins. In view of this, it seemed worth while to try a few more phenolic substances and their derivatives, and with these a few typical constituents of essential oils have been included, since nutmeg<sup>10</sup> and chenopodium (worm-seed) and other essential oils have from time to time been suggested for use in amoebic dysentery. The results of these trials are given in the following table. The oils had to be used in the form of emulsions with solution of gum acacia; a similar emulsion with olive oil was used as a control and had no action on Glaucoma, even after two hours' exposure.

TABLE IV.

Substance.	Strength of Preparation, per cent., found.	
	(a) Toxic to Glaucoma.	(b) Not Toxic to Glaucoma.
p-tert Butylphenol ... ..	0·010	0·009
p-iso Amylphenol .. ..	0·004	0·003
Thymol ... ..	0·016	0·013
Eugenol (from clove oil) ...	0·030	0·003
Anethole (from anise oil) ...	0·030	0·003
Myristicin (from nutmeg oil) ...	0·030	0·003
Pulegone (from pennyroyal oil)	0·300	0·030
Ascaridole (from wormseed oil)	0·130	0·100
Citral (from lemon oil) ... ..	0·030	0·003
Sweet flag oil ... ..	0·030	0·003
Quinone ... ..	0·0016	0·0003
Thymoquinone ... ..	0·030	0·016

These results are interesting, as showing that phenolic substances have a comparatively high protozoocidal activity, which is retained, though in a lessened degree, by their ethers, *e.g.*, myristicin and anethole, the chief components of nutmeg and anise oils respectively. This toxicity is not characteristic of essential oil components as a whole, since in pulegone, the active constituent of pennyroyal oil, it is much diminished. Quinone and thymoquinone were tried because MORGAN and COOPER<sup>11</sup> have shown that, although presumably closely allied in constitution, the former is much more active as a bactericidal agent than the latter, and this is also true of their respective toxicities to free-living protozoa.

Finally, mention may be made of three other drugs used for the treatment of dysentery, which have been examined by the method described above. *Ispaghul* (*Plantago ovata*) probably owes its value as a soothing agent in acute dysentery to the large amount of mucilage it contains. Examined by the method described above, which excludes the mucilage, it contains no agent toxic to protozoa.

*Bael Fruit*.—Preparation B (page 378) made from this substance was

markedly toxic to Glaucoma, but owing to the large amount of gum present it proved difficult to get a satisfactory preparation of the tannin of the plant (see Table II), but even in the impure form used, the tannin belongs to the more active group. Bael fruit extract has long been used as a dysentery remedy in India.

*Sweet Flag*.—This drug has been used successfully in unspecified dysentery by EVERS.<sup>12</sup> Clear evidence was obtained (see Table II) that it contains a moderately active tannin, but, as in the case of bael fruit, there is so much gum present that the tannin was difficult to isolate in a reasonably pure state. The drug also contains an essential oil, which is somewhat toxic to Glaucoma.

#### SUMMARY OF RESULTS.

It is shown that a number of the drugs which continue to be used in various parts of the tropics as remedies for dysentery may owe their activity to the tannins they contain, since these tannins are toxic to Glaucoma. A number of tannins have been investigated from this point of view, and it has been found that they belong to three classes, (a) the crystalline, sparingly soluble tannins, which are non-toxic, (b) the moderately toxic tannins, typified by gallotannin, and (c) the toxic tannins, of which the best example is the tannin of sant or babool pods. These three classes do not correspond with the three classes into which tannins may be arranged chemically. The usual decomposition products of tannins, such as gallic acid, resorcinol and catechol, are toxic to Glaucoma but not markedly so. Certain of the higher alkyl phenols, such as *isoamylphenol*, are markedly toxic, and this is also true, to a less extent, of the phenolic and phenolic ether constituents of some essential oils which have been used in amœbic dysentery.

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