the greater amount of carbonic oxide by maintaining a longer layer of the cigar red-hot.

It is of interest to compare the proportions of carbonic oxide in straight coal gas, made by simply distilling coal, with that in tobacco smoke.

A cigar of average size was smoked under such conditions that the volume of smoke formed could be measured. Four-fifths only of the cigar was smoked in order to make allowance for the portion of cigar usually discarded. It was found that 0.303 cubic foot of smoke was produced, of which 7 per cent. was carbonic oxide. The quantity of straight coal gas containing an equivalent amount of carbonic oxide is 0.25 cubic foot—that is, 1 cubic foot of straight coal gas contains an amount of carbonic oxide equal to that in the smoke from four cigars; the proportion of carbonic oxide in South Metropolitan gas, it may be added, is about that of average cigar smoke.

As to the effects of smoke, the cigarette smoker often has the habit of inhaling; but as a rule pipe and cigar smokers do not; friends tell me that pipe and cigar smoke are nauseous when inhaled: apparently volatile products of distillation are carried forward much more than in cigarette smoking and these protect the cigar smoker notwithstanding the larger production of carbonic oxide. The blood of the cigarette smoker often shows signs of carbonic oxide absorption. A friend, who is an excessive smoker, is said to have 5 per cent. of his blood out of action; another who is an equally great smoker and inhales constantly shows no sign of absorption. The latter is an exceptionally vigorous person, who eats and drinks freely; apparently his is scarcely a case of tolerance: if carbonic oxide enter his blood either it is swept out without difficulty by gaseous exchange or is worked up in some way. Apparently there is room for further study of the conditions regulating the natural exchange of carbonic oxide for oxygen in the blood. Smoking has its interesting side, even for the non-smoker.

We have to thank Mr. Pickard and Miss Bucknell for the very skilful aid they have given and the special interest they have taken in this little experimental excursion.

OBSERVATIONS ON THE AMOEBICIDAL ACTION OF CONESSINE.

BY

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THE bark and seeds of *Holarrhena antidysenterica* have been held to rank amongst the most important medicines of the Hindu materia medica for many centuries; in fact Garcia de Orta, who wrote in 1563, speaks of their great merit in the treatment of dysentery. The plant is a small deciduous tree found throughout India and Burma, belonging to the Apocynaceae; according to Sir George Watt,[1] it has been confused with another species, *Wrightia tinctoria*, belonging to the same family.

From *Wrightia antidysenterica*, Stenhouse,[2] in 1864, isolated an amorphous base forming amorphous salts. Hains[3] had previously obtained the same alkaloid and described it under the name of "conessine."

The alkaloid conessine is now a commercial product prepared by Merck, and the formula $C_{12}H_{20}N$ is given to it. In the United States *Dispensatory*, nineteenth edition, it is stated that the medicinal properties of this substance have apparently not been investigated.

In 1880 Baboo Rakhal Das Ghose[4] described *Holarrhena antidysenterica* (Wall) as being synonymous with *Wrightia antidysenterica*, and from the seeds he obtained an alkaloid, which was isolated by Baboo Ram Chandra Datta, which the latter called "kurchicine." He found it useful in cases of chronic dysentery, and used it in doses of 2 to 5 grains.

Burn[5] gives an account of the physiological action of conessine, holarrhene, and oxyconessine, and Pyman[6] in 1919 described the chemical properties of holarrhenine and conessine. Apart from the study of the physiological action of the alkaloid by Burn no attempt has apparently been made to study the action upon the causative agents in dysentery.

The remedy is chiefly used in Northern India in the form of an infusion of kurchi, this being the Bengali name for the plant. The efficacy of this infusion in certain cases of dysentery is, in the opinion of those who have used it, little short of miraculous, and it seems strange that although conessine has been isolated for more than half a century the amoebicidal action of this drug has apparently not been tested. I have seen two cases in which its effect was very striking, in one of them dramatic. It may be that, as Burn pointed out, although the alkaloid possesses the properties of a local anaesthetic it cannot be used as such, at any rate by the hypodermic route, owing to the production of a necrotic area at the site of the inoculation; the necrosis is apparently quite independent of secondary contaminating organisms and is produced even with doses of 10 milligrams.

The preparation with which the following experiments were made was in the form of a neutral solution of conessine sulphate, originally isolated by Dr. Pyman from a sample of *Holarrhena congolensis* obtained from the Belgian Congo; a regular supply has been sent to me through the kindness of Dr. Henry, Director of the Wellcome Chemical Research Laboratories.

In the first place an attempt was made to ascertain whether the alkaloid had any specific bactericidal action on bacilli of the dysentery group. It was found that although the drug did exert some specific action *in vitro* such large doses had to be used as to place any chance of cure of bacillary dysentery by this means outside the bounds of probability, and the rest of the investigation was directed to the action of conessine upon the growth of amoebae.

As it was not found possible to obtain suitable material from an acute case of amoebic dysentery with which kittens might have been infected and the strain maintained, the action of the alkaloid was studied upon cultures of free-living amoebae which had been isolated from pond water by Dr. Wenyon; for these cultures, and for his valuable suggestions and guidance, I wish to express my best thanks. The cultures of this strain of amoebae were maintained upon an agar medium (Walker's medium) upon which the amoebae grew luxuriantly at room temperature.

There is a great deal of discrepancy in the published statements as to the results obtained by different observers when studying the amoebicidal action of emetine upon material from the intestinal contents in which *Entamoeba histolytica* was present. For instance, Leonard Rogers[7] states that when a small piece of mucus containing these active amoebae was placed in saline solutions of emetine of the strength of 1 in 10,000 the organism was immediately killed, and that even in a 1 in 100,000 dilution they were rendered inactive and apparently killed after only a few minutes' immersion. Dale and Dobell,[8] however, found that certain of their strains of *Entamoeba histolytica* survived the action of 1 in 1,000, and even 1 in 100 emetine for periods up to one hour. Further, Wenyon and O'Connor[9] showed that amoebae in pus treated with a 6.5 per cent. solution of emetine could remain alive for several minutes.

Of course it is not possible to say that the conditions of the test in each case were exactly comparable, and it appears that the relative differences in the amount of intestinal mucus present may be a variable factor in the experiments. This intestinal mucus is capable of interfering with the action of emetine, as is shown in the following experiments.

Experiment I.

A 1 per cent. solution of conessine sulphate and a 1 per cent. solution of emetine hydrochloride were made. Of these 5 c.cm. was placed in two small centrifuge tubes, then to each tube an equal amount of intestinal mucus was added, the quantity of mucus being approximately 1 c.cm. The mucus was then beaten up for five minutes and the tubes centrifuged for five minutes. From the supernatant fluid varying dilutions were made and then 1 c.cm. of these dilutions was added to 9 c.cm. of the melted agar. These tubes were then autoclaved, the contents poured into Petri dishes, and the surfaces planted with a saline suspension of amoebae.

Emetine Hydrochloride concentration in the Medium.	Bacterial Growth.	Amoebic Growth.	Conessine Sulphate concentration in the Medium.	Bacterial Growth.	Amoebic Growth.	Control.
0.1 %	++	0	0.1 %	++	0	
0.01 %	++	0	0.01 %	++	0	Bacterial growth ++
0.001 %	++	++	0.001 %	++	+	Amoebic growth ++
0.0001 %	++	++	0.0001 %	++	++	-

Experiment II.

This experiment was repeated as above, only in this case a four hours' contact was allowed between the mucus and the reagents, with the result shown in the following table.

Emetine.	Bacterial Growth.	Amœbic Growth.	Conessine.	Bacterial Growth.	Amœbic Growth.	Control.
0.1 %	++	+	0.1 %	++	0	
0.01 %	++	++	0.01 %	++	++	Bacterial growth ++
0.001 %	++	++	0.001 %	++	++	Amœbic growth ++
0.0001 %	++	++	0.0001 %	++	++	

From the above two experiments certain deductions can be made. In the first place it is clear that the amoebicidal action of these alkaloids is considerably lessened if the solutions of the alkaloids are brought in contact with intestinal mucus. The time of contact is an important factor, and one sees that after four hours' contact the amoebicidal action of the alkaloids is practically destroyed. Another point here shown is that the conessine is distinctly antagonistic in its action to the growth of amoebae and that this property is not affected by contact with intestinal mucus quite to the same extent as in the case of emetine.

The diminution of the amoebicidal action of these drugs by contact with mucus is better seen when we compare the results of the above two experiments with those obtained by incorporating varying dilutions of the two reagents without any admixture with mucus.

Results without Admixture of Mucus.

Emetine Hydrochloride concentration in the Medium.	Bacterial Growth.	Amœbic Growth.	Conessine Sulphate concentration in the Medium.	Bacterial Growth.	Amœbic Growth.	Control.
0.1 %	+	0	0.1 %	+	0	
0.01 %	++	0	0.01 %	+	0	Bacterial growth ++
0.001 %	++	0	0.001 %	++	0	Amœbic growth ++
0.0001 %	++	0	0.0001 %	++	0	
0.00001 %	++	++	0.00001 %	++	++	

From these experiments it is evident that conessine is, if not actually amoebicidal, at any rate capable of inhibiting the growth of free-living amoebae when planted on media containing even a concentration of 1 in 1 million, and that this property is equal to that of emetine. Further, it is clear that the inhibitory action of these drugs on the growth of the amoebae is not due primarily to a deprivation of food supply of the amoebae by the inhibition of bacterial growth, for it is shown that the amoebic growth is affected before there is any change in the amount of bacterial growth. Another point that is manifest is that the inhibitory action of both emetine and conessine upon the growth of the amoebae is not affected by heat, for the media containing these alkaloids had been subjected to a temperature of 115° C. for twenty minutes in the autoclave. It might be argued that this strain of free-living amoebae was very sensitive to the action of alkaloids in general, and in order to see whether this was the case the inhibitory action of conessine was compared with that of quinine bihydrochloride, with the following result.

Comparison of Conessine and Quinine.

	Conessine Sulphate.				Quinine Bihydrochloride.			
	0.1 %	0.01 %	0.001 %	0.0001 %	0.1 %	0.01 %	0.001 %	0.0001 %
Amoebic growth	0	0	0	++	0	++	++	++
Bacillary growth	0	+	++	++	+	++	++	++

The conditions of the test in this case were identical with those the results of which have been recorded above, with the exception that the medium in this case had a reaction of P_H 7.5 and in the former cases it was P_H 7.9.

It will be noticed that complete inhibition of growth when using a medium of P_H 7.9 occurred in a concentration of 0.0001 per cent., whereas with a medium of P_H 7.5 total inhibition does not occur until a concentration of 0.001 per cent. of conessine is reached. This is interesting in that it agrees with the finding of Acton[10] that the toxicity of the cinchona alkaloids on paramœcium varies with the P_H of the culture. They are much more toxic in alkaline than in acid cultures.

Mode of Action of Emetine on Amoebae.

That this cannot be looked upon as due to a direct amoebicidal action is held by Dale and Dobell, who consider that the host must in some way co-operate in the effect.

In order to see whether the serum of a patient receiving large doses of emetine had any action on free-living amoebae, the serum of a man who had received twelve daily doses of bismuth emetine iodide was allowed to interact for varying time limits up to two hours with an equal volume of a saline suspension of free-living amoebae. A control with normal serum was used. After interaction at room temperature a loopful was planted on Walker's medium, but the reading after forty-eight hours showed no difference in the amoebic growth in the case when the emetinized serum was used as compared with that when using normal serum.

Comparative Toxicity of Emetine and Conessine.

Burn (1915) states that comparatively large does of conessine may be given subcutaneously to cats, rabbits, and guinea-pigs without any danger to life, but in every case, even when such a small dose as 10 mg. was given to guinea-pigs, there was considerable local necrosis and induration, although carried out under strictly aseptic precautions. The smallest subcutaneous dose which he found lethal to a 300 gram guinea-pig was 250 mg. This necrosis might possibly have been due to the reaction of the sample used, but when even a neutral solution was employed there was some degree of necrosis when 50 mg. was injected subcutaneously into guinea-pigs. Whether this can be obviated requires further work.

As regards the oral method of administration, one guinea-pig of 600 grams was given 1 c.cm. of a 10 per cent. solution and showed no symptoms, whereas a guinea-pig of 680 grams which received 5 c.cm. of a 10 per cent. solution died in forty-eight minutes, death being preceded by paralysis of the hind limbs. The toxicity of emetine and conessine is shown in the following observations.

Intravenous Administration.

Emetine Hydrochloride (0.1 per cent. solution):
Mouse 25 grams, 0.1 c.cm. ... No symptoms.
Mouse 25 grams, 0.3 c.cm. ... Severe symptoms; recovered.
Mouse 20 grams, 0.3 c.cm. ... Died in two minutes.

Conessine Sulphate (0.1 per cent. solution):
Mouse 27 grams, 0.4 c.cm. ... No symptoms.
Mouse 22 grams, 0.4 c.cm. ... Severe symptoms; recovered.
Mouse 22 grams, 0.45 c.cm. ... Died in about one minute.

Subcutaneous Administration.

Emetine Hydrochloride (1 per cent. solution):
Mouse 29 grams, 0.1 c.cm. ... No symptoms.
Mouse 24 grams, 0.3 c.cm. ... Died during night.

Conessine Sulphate (1 per cent. solution):
Mouse 22 grams, 0.2 c.cm. ... No symptoms.
Mouse 24 grams, 0.4 c.cm. ... Died during night.

Hence the subcutaneous M.L.D. of conessine for a mouse is approximately 3 mg., and of emetine 2 mg. The intravenous M.L.D. is 0.45 mg. and 0.3 mg. respectively, or, in other words, as far as these observations go emetine is approximately 50 per cent. more toxic than conessine.

SUMMARY.

1. Conessine, an alkaloid having the formula $C_{12}H_{20}N$, which has been isolated from several members of the family of Apocynaceae, is here shown to exert a very strong inhibitory action upon the growth of free-living amoebae; in fact, exactly equal to that of emetine.

2. Infusions of the seeds of these plants have for many years been used with marked success in cases of chronic dysentery.

3. Although when administered subcutaneously conessine produces an area of necrosis at the site of inoculation, it can yet be administered by the mouth or intravenously in suitable doses without producing symptoms.

4. It is approximately 50 per cent. less toxic than emetine.

5. A diminution of the inhibitory action on free-living amoebae of solutions of emetine and conessine which have been in contact with intestinal mucus is shown.

6. The serum of patients receiving full and repeated doses of emetine has apparently no amoebicidal action; hence it is unlikely that the presence of conessine in blood serum could be detected by its effect on amoebae.

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