down to 57½ pounds, as her œdema had almost subsided. The character of her stools was still white and greasy.

On account of her giardia infection with steatorrhœa, she was put on crinodora (a substitute for atebrin), 0.1 gramme thrice daily for 5 days, and the acid mixture was continued, the dose of dilute hydrochloric acid being increased to one drachm.

With this treatment, her diarrhœa stopped completely, and the stools became normal in colour. She had no more pain in the abdomen. The stools were again examined for 6 consecutive days; no *Giardia intestinalis* was found.

Subsequently, owing to presistent pain in the legs, she was given a course of vitamin B_1 injections, and her teeth were attended to. This helped her very much, and she could now walk comfortably.

During convalescence she had slight diarrhœa for 2 days; this was readily controlled by a few doses of sodium sulphate mixture.

She was discharged cured on 11th May, when her weight was 67½ pounds.

Summary and conclusions

A case is reported with symptoms suggestive of sprue or 'para-sprue'. Steatorrhœa, macrocytic anæmia, pseudo-achlorhydria and giardiasis were the main findings. The general condition improved with liver therapy and acid mixture, but the steatorrhœa persisted. Eradication of giardia infection coincided with the cessation of steatorrhœa.

It is difficult to estimate the exact rôle played by the *Giardia intestinalis* in causing fatty diarrhœa, but since there was no evidence to account for this condition, it is possible that this infection deranged the function of the small intestinal epithelium interfering with the absorption of fat. The clinical improvement immediately after the specific treatment does suggest fairly strongly that the presence of giardia was more than a fortuitous association.

Acknowledgment

I should like to express my grateful thanks to Dr. L. E. Napier, for his frequent advice on the investigation and treatment of this patient and for his kind permission to report the case. My thanks are also due to Dr. J. P. Bose who carried out the biochemical investigations.

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THE SEARCH FOR AN ANTI-MALARIAL DRUG IN THE INDIGENOUS MATERIA MEDICA

PART II—*Cæsalpinia bonducella*, Fleming

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Introduction

Cæsalpinia bonducella (N. O. Leguminosæ), commonly known as 'bonduc nut' or 'fever nut' in English, as 'nata' or 'natakranja' in Bengali, and as 'katkaranj' in Hindi, has long been known to the Hindu and Mohammedan physicians for its medicinal properties. The root, bark, leaves and the seeds have all been used in indigenous medicine, but the seeds or their yellowish-white kernels are generally considered as possessing well-marked anti-periodic properties, and are largely used by practitioners of indigenous systems of medicine in acute and chronic fevers as a substitute for quinine. For this purpose, the seeds or seed kernels are pounded with black pepper, and a dose varying from 5 to 30 grains is administered by mouth. In 1868, the seeds were made official in the Pharmacopœia of India, a dose of 15 to 30 grains of the powdered seeds being recommended as a safe anti-pyretic, and their use was favourably reported upon by several medical officers. An investigation of the anti-malarial properties of these nuts was therefore undertaken with a view to finding out if there is any action in experimental and clinical malaria.

Chemistry, pharmacology, etc.

Heckel and Schlagdenhauffen (1886) first attempted to isolate the constituents of *Cæsalpinia bonducella* nuts. They obtained a non-alkaloidal bitter principle from the kernel, in the form of a white powder (bonducin) to which they gave the formula $C_{14}H_{15}O_5$; they attributed the physiological properties of the seeds to this constituent. Bacon (1906) isolated the bitter principle 'bonducin' from the seed kernel, and gave evidence to suggest that it was a mixture of complex resinous bodies. He failed to detect any glucoside or alkaloid in the alcoholic extract of the kernels. Bhaduri (1912) claimed to have separated an alkaloid from the kernel, and suggested the name 'natin' for it, but no details were given in support of his findings.

Godbole, Paranjpe and Shrikhande (1929) isolated the bitter principle from the alcoholic extract of the kernel, and concluded that it was a sulphur-containing glucoside. Chopra, Ghosh and Dutta (1929) attempted to separate the active principle from the seeds, but found, on careful examination, the presence of only a bitter principle of non-glucoside nature, insoluble in

water but soluble in alcohol. The presence of an alkaloid as reported by Bhaduri could not be confirmed. Katti (1930) isolated a bitter principle of a complex resinous character in the petroleum ether extract. Ghatak (1934) reported the presence of a non-crystalline bitter glucoside (bonducin) in the kernel of the seeds, having a molecular formula $C_{20}H_{28}O_8$, and being insoluble in water but soluble in alcohol, acetone, pyridine, chloroform and carbon tetrachloride.

Chopra *et al.* (*loc. cit.*) studied the pharmacological action of the non-glucoside bitter principle, but found it to be devoid of any activity, as far as general pharmacological tests were concerned.

Experimental

(*a*) *Alcoholic extract of* C. bonducella.—As the present work was primarily designed for the evaluation of the anti-malarial property of the drug, no attempt was made to work out in detail the complicated chemistry or to isolate the individual components of the seeds. Previous experience appeared to indicate that an alcoholic extract of the seed kernels freed from fat would contain all the active principles likely to possess anti-malarial properties. For experimental trials, an alcoholic extract was, therefore, prepared in the following manner.

Two hundred grams of the dried powdered kernel (constituting about 45 per cent of the entire seeds) were extracted with petroleum ether in a small percolator. The residue was taken out, dried in air and more finely powdered, and again extracted with petroleum ether (B.P. 40° to 60° cg.) until it was free from fatty matter. The petroleum ether extract, on evaporation, left a greenish yellow thick oil with a disagreeable odour. The oil gave no reaction for alkaloids or glucosides. Finally, the powdered drug was extracted with rectified spirit in a Soxhlet apparatus until completely exhausted. The alcoholic extract, on evaporation in vacuum, left a residue which was found to be very bitter in taste, but it gave no reaction for the presence of alkaloids. The yield was found to be approximately 15 per cent.

(*b*) *Pharmacological studies.*—Detailed pharmacological investigations of the alcoholic extract could not be undertaken, as a soluble preparation of the active principle suitable for parenteral injection could not be obtained. The toxicity, when administered by the oral route, of the alcoholic extract (triturated with 2 c.cm. water to form an emulsion) was however roughly determined on cats and rabbits. The toxicity was found to be on the low side, as a dose of nearly 1 gm. of the extract (after evaporation of alcohol) per kilo body-weight was well tolerated in both these animals. This dose being much higher than the therapeutic dose recommended of the bonducella nut powder (15 to 30 grs. for a person weighing 60 kilo body-weight), it was not considered worth while for purposes of this investigation to determine accurately the toxicity of the alcoholic extract. All animal experiments were conducted with a dose larger than the recommended therapeutic dose, but smaller than 1 gm./kg. body-weight of the alcoholic extract.

(*c*) *Chemotherapeutic studies.*—Unlike the study reported previously by the authors (1942) on monkey malaria with the total alkaloids of *Alstonia scholaris*, this investigation was carried out with fowl malaria. The difficulty in feeding monkeys with a definite dose of the alcoholic extract through a stomach tube could not be easily overcome, and it was not possible to prepare an injectable preparation of the active principles. Domestic fowls, on the other hand, could be comparatively easily handled, and the drug was given in small quantities by mouth, care being taken to see that no regurgitation took place.

Fowl malaria experiments were chiefly conducted by one of us (L. B. S.). Domestic fowls, weighing on an average from 350 to 400 gm., were inoculated either intravenously or intramuscularly with ½ c.cm. of citrated blood from fowls showing a normal infection with the strain of *P. gallinaceum*, Br., originally isolated from a jungle fowl by Brigadier H. E. Shortt, I.M.S., in Madras. The experimental fowls were treated with the alcoholic extract of *C. bonducella* after parasites had been observed in the peripheral blood. The drug was administered by the oral route in daily doses of 2 c.cm. of a 5 per cent (100 mg./2 c.cm), 10 per cent (200 mg./2 c.cm) and 20 per cent (400 mg./2 c.cm.) emulsion. Control birds were treated with intramuscular injections of mepacrine hydrochloride (synthesized in Calcutta by two local firms) in a dosage of ½ to 1 mg. per 20 gm. of body-weight dissolved in ½ c.cm. of distilled water. The anti-malarial properties of the drugs were judged by their effects on the infection rate of the blood cells from day to day, and on the morphology of the parasites. The number of infected cells was counted in totals of 1,000, 500 or 100 red blood cells, according to whether parasites were very scanty, readily observed or extremely numerous. A negative result was recorded only when parasites could not be detected in a thin film after a full three-minute search.

The results of the experiments are represented in three columns in tabular form (*see* table).

In the first experiment, two fowls (nos. 400 and 404) were treated with three daily doses each of a 5 per cent and a 10 per cent emulsion of bonducella extract respectively, while a third control fowl (no. 402) was left untreated. In all three birds, the progressive increase in the numbers of parasites was manifest in the rising infection rate of the red cells, the highest values in the case of treated birds being recorded after treatment with three doses. The comparatively low peaks of infection of 8 to 26 per cent were undoubtedly due to the early death of both treated and untreated birds from an unknown cause. No evidence of degeneration of the parasites was obtained in the treated birds.

TABLE

Showing lack of action of alcoholic extract of C. bonducella (*'Nata'*) *in fowl malaria* P. gallinaceum, *Br.*)

Expt.	Treated fowls					Untreated control fowls			Remarks
	Bird number	Treatment	Percentage infection of r.b.c.			Bird number	Percentage infection of r.b.c.		
			Before treatment	After treatment	Peak		First positive examination	Peak	
I	400	Three doses 'nata' 5%.	Less than 0.1	8	8	402	Less than 0.1	26	All three birds died early. Cause of death doubtful.
	404	Three doses 'nata' 10%.	1.4	22	22				
II	440	(*i*) Three doses 'nata' 10%.	0.6	80	80	439	0.2	55	No. 439 was later treated with 3 doses of mepacrine hydrochloride; infection fell to 2 per cent. In both birds, degeneration of parasites observed after treatment with mepacrine hydrochloride.*
		(*ii*) Further treated with 3 doses (10 mg. each) of mepacrine hydrochloride.	80	1	..				
III	442	Six doses 'nata' 20%.	1	22	80	441	1.4	87	No. 443 became negative 2 days after the last dose of mepacrine hydrochloride.†
	443 (control).	Three doses of 10 mg. of mepacrine hydrochloride.	4	1	4				

* Both birds died later showing infection of endothelial cells of the brain capillaries.
† All three birds died showing infection of the endothelial cells of brain capillaries.

In the second experiment, another fowl (no. 440) was treated with three doses of a 10 per cent solution of *C. bonducella* extract. A control bird (no. 439) was kept untreated for a period of five days, during which time the infection rate had risen to 55 per cent. In fowl no. 440, the infection rate rose from 0.6 per cent to 80 per cent in the three days during which the drug was given. The parasites were normal in every way. Both birds were then treated with mepacrine hydrochloride and an immediate response was observed in the form of degenerating parasites. The infection rates dropped precipitously to 1 per cent in fowl no. 440, and 2 per cent in fowl no. 439. The birds died seventeen to twenty-one days after the inoculation, showing infection of the endothelial cells of the brain capillaries with the remarkable exo-erythrocytic forms which are known to be resistant to anti-malarial drugs.

In the final experiment, one fowl (no. 442) was treated with six doses of 20 per cent solution of *C. bonducella* extract, while of two controls, one was treated with mepacrine hydrochloride (0.5 mg. per 20 gm. approximately of body-weight) and the other not treated. The infection rate in no. 442 rose from 1 to 22 per cent during the six days' treatment, and two days later reached the quite normal figure of 80 per cent. The untreated bird (no. 441) showed similar findings. The bird treated with mepacrine hydrochloride never showed an infection rate higher than that seen before treatment, namely 4 per cent, although it lived for nineteen days after inoculation. Degenerating parasites were observed in this bird. All three birds died showing exo-erythrocytic schizonts in the brain capillaries.

(*d*) *Clinical studies.*—The wide popularity and reputation of the so-called 'fever nuts' led the Indigenous Drugs Committee of Madras (1924) to give clinical trials of the seed powder in several hospitals (Chopra, 1933). Though the recorded findings do not definitely justify the conclusions arrived at (there being no data of regular blood examinations and demonstration of parasites in cases diagnosed as malaria), the Committee recommended the use of the drug as a valuable anti-periodic, febrifuge and effective tonic.

(*Concluded on next page*)

LETHAL ACTION OF POTASSIUM PERMANGANATE ON VIBRIOS

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POTASSIUM permanganate pills were used by Rogers (1913) in cholera cases to neutralize the toxins of the cholera vibrio, and permanganate is commonly used to disinfect clothes, well water and tank water, cholera stools, etc., during an epidemic. Hands also are often washed with permanganate solutions after attending cholera cases. There are various old references to potassium permanganate in dilutions as high as 1 in 500,000 being lethal to the cholera vibrio, but not to other organisms, and therefore the matter needs reinvestigation in the light of our present knowledge.

In the present paper are reported the results of a study of the effect of various dilutions of potassium permanganate on the cholera vibrio and on some other organisms. Standard numbers of organisms were treated for varying times with potassium permanganate in varying dilutions.

Permanganate is well known as an oxidizing agent of organic matter. The chemical is weakened and its colour is discharged in the presence of an excess of organic matter. Hence to demonstrate the full effect of the drug, extraneous organic matter was excluded as far as possible by washing the vibrios three times in normal saline.

The deposit present after centrifugation of the permanganate-treated suspension was again suspended in saline, and the number of vibrios was estimated by matching with opacity tubes. These procedures were carried out within the shortest time possible.

Recently isolated virulent strains were used in the experiments. The dilutions of potassium permanganate were made in sterile flasks containing 100 c.cm. of sterilized pyrogen-free, redistilled water at pH 6.8. The required number of vibrios in a small quantity of inoculum was then added to the solutions, and the suspension was well shaken and kept at room temperature. Subcultures were made at specified intervals with 0.5 c.cm. spread on nutrient agar, and, if no growth was seen in 24 hours, 1 c.cm. of the suspension, after vigorous shaking, was seeded into 10 c.cm. of peptone water to confirm its sterility. A control test was put up without the addition of permanganate. It was found that the discharge of pink colour was not necessarily an indication of multiplication and growth of vibrios. In high dilutions of the permanganate, the colour was barely visible, but still the solution showed bactericidal properties. After mixing and incubation, the colour was discharged in some, but vibrios were still found killed.

The following tables show the results of the experiments.

It will be seen from the tables below that potassium permanganate in a high dilution such as 1 in 10^6 with hardly any visible colour, exerts a bactericidal effect on Inaba and Ogawa subtypes of *Vibrio choleræ* in ½ to 20 hours. A still higher dilution of 1 in 10^9 kills the non-agglutinating vibrios. In contrast to its lethal action on *V. choleræ*, potassium permanganate in a much higher concentration fails to kill even a smaller number of *Bact. typhosum*.

As vibrios are generally present in nature in association with organic matter, it was felt necessary to see how the organisms would react to permanganate in the presence of organic matter. Washed vibrios were suspended in

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(*Continued from previous page*)

Due to the paucity in Calcutta of definitely proved malaria cases untreated with other antimalarial drugs during the winter months, our attempts to give the alcoholic extract of *C. bonducella* a thorough clinical trial were not successful. However, its administration in one proved case was far from encouraging. Further clinical trials might be undertaken during the malaria season but are perhaps not justifiable.

Summary and conclusions

An alcoholic extract of *C. bonducella* nuts (fat-free powder prepared from the kernels) when fed in a dose up to 400 mg. per gm. body-weight failed to arrest the normal multiplication of *P. gallinaceum* in domestic fowls; moreover the parasite showed no change in morphology. Mepacrine hydrochloride (atebrin) synthesized by local manufactures in Calcutta produced both these effects. These results do not encourage tests with 'nata' on other malarial infections—animal or human. It appears unlikely that 'nata' has any specific action in malaria.

Acknowledgment

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