The Action on Avian Malaria of the Alkaloids of Cinchonas from the Cameroons and the Belgian Congo

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During the course of the war it has become increasingly important to seek efficient and cheap remedies against malaria among the alkaloids of the cinchonas.

Numerous experiments on cinchona febrifuges have been carried out in several countries, e.g. Ejercito & Santos [1937], Field [1934], Fourneau, Trefouel, Trefouel, Stefanopoulo, Benoit, Lestranger & Melville [1930], Hicks & Chand Diwan [1935], Kingsbury [1937], League of Nations [1934; 1937], Manon, Perez & Russell [1935], Miyahara [1938], Parvulescu & Boeriu [1934], Sergeant, Sergeant & Vogt [1925], Sinton [1929], Slatineanu, Ciuca, Balteanu, Alexa, Francke & Rugina [1934], Wijerana [1939], Yao & Yung [1934]. From all these experiments it appears that in the treatment of ordinary cases of malaria cinchona febrifuges are equal to, if not of slightly superior value to, quinine. Nevertheless these cinchona febrifuges vary largely in composition, in origin and in the mode of preparation, and it seems desirable at the present time to obtain more detailed and accurate information about the nature of the total alkaloids from different cinchonas, and to examine their therapeutic action, before considering their intensive cultivation and the establishment of small plants for the purpose of extracting these alkaloids on the spot by simple processes.

The initial experiments to be described are confined to the action on avian malaria of alkaloids from the barks of *C. succirubra* and *C. Ledgeriana* from Dschang (French Cameroons) and Kivu (Belgian Congo). Some experiments on avian malaria have already been carried out by several authors: Bishop [1942]; Buttle, Henry & Trevan [1934]; Buttle, Henry, Solomon, Trevan & Gibbs [1938]; Giemsa [1933]; Goodson, Henry & Macfie [1930]; Sergeant, Sergeant & Vogt [1923; 1924; 1925]. The alkaloids used in the present study were either extracted by the classical laboratory method or by industrial processes. The samples were in all cases prepared in our laboratories and were practically pure (95-97%), containing the same percentage of quinine as the laboratory product. Some small differences may exist in the relative proportions of the amorphous alkaloids present.

EXPERIMENTAL

The malaria parasites used in this work were a strain of *Plasmodium relictum* obtained from Dr Keilin of Cambridge to whom we wish to offer our thanks. The birds used were all canaries; owing to difficulties in obtaining supplies these were not all of a constant strain, but gave a uniform response to the infection.

The malaria was transmitted by direct blood inoculation from bird to bird. Blood was obtained from the vein running down the inside of the leg of an acutely infected bird and was collected in an equal volume of citrate-saline (NaCl 0.8%, Na citrate 2%). Each bird to be inoculated received 0.25 ml. diluted blood, injected into the pectoral muscle. Usually the examination of the blood of infected birds was begun on the fifth day, the blood films being treated with Giemsa's stain. At least 30 fields were examined on each slide.

The alkaloids were administered orally to the birds in the form of small pellets, which were prepared by mixing 1 part of alkaloid with 9 parts of corn flour, together with a little H₂O. Each pellet thus contained the amount of alkaloid (in form of insoluble base) required for a test on a single bird. The pellets were readily swallowed, and experience has shown that this method was more convenient than administration by catheter [Fourneau *et al.*, 1930].

The routine method of testing compounds was as follows: 6 hr. after a bird had been inoculated, a dose of alkaloid was given and five further doses were given at 24-hr. intervals. There were two untreated control birds in each experiment.

The birds chosen for each test were of approximately equal weight (to within 2 g.) and uniform doses were administered. It was found that under these conditions, the appearance of parasites in the blood is not dependent on the weight of the bird, either in untreated controls or in treated birds.

Most of the experiments were carried out with a daily dose of 3.5 mg. of total alkaloid, an amount which gave a good therapeutic effect without toxic symptoms.

RESULTS

The therapeutic effect of the alkaloids is measured in terms of the retardation of infection in the different groups. The results of experiments are summarized in Tables 1 and 2. In only one bird of the whole series (30 mg. of alkaloid from *C. succirubra*) was the onset of malaria indefinitely retarded.

the Cameroons) exerted a more powerful therapeutic action than quinine.

The alkaloids from *C. Ledgeriana* in doses of 2.5 and 3.5 mg. were more effective than the same amount of quinine.

In all the experiments no differences were observed between the industrial and laboratory preparations from the different sources.

(ii) *Total alkaloids minus quinine*. Three of the

Table 1. Influence of alkaloids per os on the time of onset of avian malaria

Alkaloid	Source of alkaloid		Method of extraction	Influence of alkaloid on onset of malaria in canaries		
	Country	Bark		Daily dose given (mg.)	No. of birds	Period of delay in onset of malaria (days)
Quinine (base)	—	—	—	2.5	4	5.25
				3.5	6	9.40
				5.0	4	11.00
Total	Cameroons	<i>C. succirubra</i>	Laboratory	2.5	4	4.25
				3.5	9	7.35
				5.0	4	>15.20
Total	Cameroons	<i>C. succirubra</i>	Industrial	2.5	3	3.70
				3.5	2	7.50
Total	Cameroons	<i>C. Ledgeriana</i>	Laboratory	3.5	5	10.20
Total	Cameroons	<i>C. Ledgeriana</i>	Industrial	2.5	3	8.00
Total (Sample R)	Belgian Congo	<i>C. succirubra</i>	Laboratory	2.5	4	8.50
Total (Sample M)	Belgian Congo	<i>C. succirubra</i>	Industrial	2.5	4	9.00
Total minus quinine (Sample E)	Belgian Congo	<i>C. Ledgeriana</i>	Industrial	2.5	2	4.50
Total minus quinine	Cameroons	<i>C. succirubra</i>	Industrial	2.5	1	4.50
Total minus quinine	Cameroons	<i>C. Ledgeriana</i>	Industrial	2.5	2	4.00

Table 2. Composition of the total alkaloids tested

Source and method of extraction	Total alkaloid in barks (%)	Composition (%) of total alkaloid				
		Quinine base	Cinchonidine	Cinchonine	Amorphous alkaloids	Impurities
<i>C. succirubra</i> (Dschang). Industrial	6.40	33	27	14	26	3
<i>C. Ledgeriana</i> (Dschang). Industrial	7.50	62	—	—	—	1
<i>C. succirubra</i> ; sample R (Kivu). Laboratory	8.35	22	—	—	—	—
<i>C. succirubra</i> ; sample M (Kivu). Industrial	7.00	13	—	—	—	5
<i>C. Ledgeriana</i> ; sample E (Kivu). Industrial	7.25	59	—	—	—	—

DISCUSSION

(i) *Total alkaloids (including quinine)*. The results indicate that the total alkaloids from all the sources examined have an effect similar to that of quinine in retarding the onset of avian malaria. The effect appears to vary largely according to the origin of the barks—the Belgian *C. succirubra* (M and R) gave a mixture of alkaloids which, although less rich in quinine, seemed to be more effective than the mixture extracted from the French bark.

The alkaloids of *C. succirubra*, when given in doses of 3.5 mg./day, were somewhat less effective than the same amount of quinine base, but on increasing the dose to 5 mg./day, these alkaloids (from

industrial preparations have been tested after the removal of their contained quinine. In a dosage of 3.5 mg./day they produced a retardation of avian malaria for 4–5 days, compared with the figure 7.35–10.5 days observed with the similar dose of alkaloids including quinine. The explanation of this difference may lie in a synergistic action of small amounts of quinine and other alkaloids from cinchonas, and experiments are in progress to elucidate this very important question.

SUMMARY

1. The total alkaloids from *Cinchona succirubra* and *C. Ledgeriana* from both the Cameroons and the Belgian Congo, have been tested for therapeutic action on avian malaria.

2. In all cases the results indicate that the total alkaloids have an effect similar to, or even better than, that of quinine.

3. Industrial and laboratory preparations are of equal value.

4. The total alkaloids from which the quinine has been extracted have a poor therapeutic effect, and the difference between these and the results with the total alkaloids may be due to the synergistic action of small amounts of quinine and other alkaloids.

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The Reducing Properties of Fibrinogen

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The protein, fibrinogen, is characterized by (1) its low solubility in moderately strong salt solutions, (2) its conversion to fibrin by thrombin and (3) its marked instability and ease of denaturation. The last property has made difficult its purification and study but it is now possible [Florkin, 1930] to prepare fibrinogen free from other proteins and without any evidence of denaturation. Study of the clotting of this purified fibrinogen by thrombin [Jaques, 1938] could not be continued until a method was found to stop the reaction quickly in order to obtain samples for analysis. The methods commonly used in enzyme chemistry for this purpose (heat, etc.) are inapplicable because they affect the solubilities of fibrinogen and fibrin. Dilute iodine, potassium permanganate and potassium dichromate, tested for this purpose, were found to give a heavy precipitate with fibrinogen. As this phenomenon was not found with the serum proteins and does not

appear to have been reported before, it was investigated. Secondary reactions took place with $K_2Cr_2O_7$ and $KMnO_4$, as shown by re-solution of the precipitate. The study was therefore limited to the action of iodine and H_2O_2 .

EXPERIMENTAL

Materials

Fibrinogen. Preparation by repeated precipitation with concentrated salt solutions is not very satisfactory in practice. The principle of Florkin [1930], who found it necessary for preparation of homogeneous solutions of the protein that all operations should be made at 2° and at pH 6.0–7.0, has been followed. Fibrinogen is rapidly denatured by acid even at low temperatures [Fay & Hendrix, 1931].

Florkin used NaCl as the precipitant, but the resulting 'salt of fibrinogen' tends to form gels and it is difficult to