Edenburgh University

31st March 1920

This thesis entitled

The action of Quinine on the defence mechanism of the body.

is also entered for the Christison Prize in Materia Medica. (Cunning Victoria Jubilee Prize)

F. G. Macnaughton

THE ACTION OF QUININE

ON THE

DEFENSE MECHANISM OF THE BODY.

By

F.G. MACNAUGHTON, M.B., D.T.M. & H.

- PREFACE. -

On two occasions during the war the writer has encountered epidemics of Pneumonia, and in each epidemic an increased death rate amongst cases receiving large doses of Quinine was observed. In an endeavour to explain this increased death rate the writer has made his first steps into the fields of research.

QUININE is often looked upon not only as a panacea but as a prophylactic for all the ills that flesh is heir to. Why Quinine should ever be used as a prophylactic is indeed puzzling: it failed to prevent malaria in Salonika, and in the treatment of Malaria Quinine stands unchallenged. Its popularity probably lies in its disgusting taste which engenders a virtuous feeling of "a painful duty nobly done".

The extensive use of Quinine throughout the recent influenza epidemics probably did little harm, since it was usually administered in small doses, but large doses have become fashionable as a result of war experience with Malaria. Some of the dangers of large doses of Quinine are set out in this thesis.

Throughout/

Throughout the work described in Part II, the main research has been carried out on the action of Quinine on the leucocytes and the defensive properties of the serum.
Several side issues have been touched on, and these are recorded in the hope that more capable hands may take them up.

The writer wishes to express his gratitude to Professor Ritchie for his kindness in giving him facilities to carry out the research.
To Professors Cushny and Meakins for much helpful advice, and to Dr. McCartney for his assistance and his skill in drawing off blood for experiment.

I N D E X.

PART I.

PART II.

P A R T I.

(1). CLINICAL OBSERVATIONS AT N'GUNG & STOBHILL.

(2). SOME NOTES ON THE CLINICAL ADMINISTRATION OF QUININE.

PART I.

CLINICAL NOTES.

N'GUNG SERIES.

While serving with the Cameroon Expeditionary Force in 1915-16 I was stationed for about two months at N'Gung on the Yaundi Road Column Lines of Communication.

N'Gung was the first stopping place for convoys on the Intim plateau, which is situated about a hundred miles inland and rises about a thousand feet above sea level.

This plateau lies about 2° North of the equator and the daily variation of temperature is extreme; varying from 130° - 140° F. in the sun in the early afternoon to (on some occasions) below freezing point at night.

This great variation of temperature was probably responsible for the high incidence of pneumonia amongst the carriers: since many carriers arrived from the low country without any clothing beyond a loin cloth and had to pass the night without covering.

During the first three weeks at N'Gung I was associated with an officer of the West African Medical Service who advised me on the treatment of the cases which passed through my hands.

The means of treatment available were poor. A large palm leaf shed acted as a hospital, and the supplies of/

of drugs and medical comforts were very incomplete since everything which came up had to be carried by carriers.

In consequence, whenever a case could stand moving it was evacuated by hammock to Wumbiagas, the first post below the plateau, Wumbiagas was in communication by motor transport with the advanced base at Edea.

At N'Gung between the 29th December 1915 and the 25th of January 1916 I took into Hospital and treated 40 cases of Pneumonia.

All cases received a standard treatment except -

From 29.12.15 to 5.1.16. all cases received 5 grains of Quinine Bisulphate orally twice a day.

From 5.1.16 to 17.1.16. at the suggestion of the Advising M.O. the dose of Quinine Bisulphate was increased to 15 grains twice a day.

From 17.1.16, after the departure of the Advising M.O., to 25.1.16. the administration was stopped.

Of 12 cases treated in the first group 3 died and
9 recovered and were evacuated.

Of 10 cases treated in the second group 8 died and
2 recovered and were evacuated.

Of 18 cases treated in the third group 1 died and
17 recovered and were evacuated.

The case which died in this latter group received one day's treatment with quinine bisulphate 15 grs. twice a day: he died 2 days after quinine was stopped.

All/

All cases were well marked on admission, which was usually about the 3rd day of the disease, since the carriers would not report sick, being terrorised by their headmen. Three of my cases I cut out of a convoy which was reported fit to start.

Unfortunately submarine activity has deprived me of records of these cases, except the following extracted from A. B. 153 in which I recorded deaths and evacuations. However, it shows the grouping of the cases. The dates of the groups were obtained from a private diary in which movements of Officers were entered.

The Standard Treatment:-

Counter irritation with Tinct. of Iodine and Ung. Capsici, and a cough mixture. During day carried out and laid on a stretcher in the sun if not too ill to move.

Diet - Tinned milk and Lemco with rice added to it.

GROUP I.

EVACUATED.					DIED.				
2.1.16.	625	Carrier	BANNAKI	Pneumonia	8700	Carrier	AOYBIE	Pneumonia
	1077	:	MOMO MANJAMBA	:				
	2392	:	JOE MENDIE	:				
	170	:	MAVERNO	:				
	2707	:	MENSAH	:				
	2304	:	KOSHI	:				
3.1.16.	2747	:	RAHLEA	:				
					2741	:	CANDY	:
5.1.16.	2	Headman	MORGAN	:	64	:	CORFO	:
	8917	Carrier	UMBAQUAI	:				

GROUP II/

GROUP II.

	EVACUATED.		DIED.	
7.1.16.			176 Carrier OCABO	Pneumonia
8.1.16.	543 Carrier JOE MENSA	Pneumonia	Unknown Carrier	:
	2775 : TORI GUMMAH	:		
12.1.16.			(O.N. ~~176~~)Name unknown local BACOCO Carrier	:
15.1.16			1333 Carrier HORSA	:
			Unknown BACOCO Carrier	:
16.1.16.			180 Carrier OVOH	:
17.1.16.			1712 : ADANA	:
			166 : CURSHI	:

GROUP III/

GROUP III.

	EVACUATED.			DIED.
19.1.16.				8570 Carrier WACHUCULO Pneumonia
22.1.16.	Sgt.Major BCHU, D.C.M., 2nd N.R.		Pneumonia	
23.1.16.	495 Carrier	HOWA	:	
	364 :	KOSSI	:	
	2907 :	DIO	:	
	359 :	WIGA	:	
	2959 :	MOMO KANEMA	:	
24.1.16.	8506 :	MOMO	:	
	47 :	KORIE	:	
	451 :	OUVAY	:	
	456 :	AVOA	:	
	4 :	MOMO KANO	:	

GROUP III CONTD.

EVACUATED.

24.1.16.	7385	Carrier	JOSEPH	Pneumonia
25.1.16.	609	Pte. 2nd N.R.	ALLI BELONGA	:
	2101	Carrier	AMADU BARGALA	:
	-	Boy	JOSEPH	:
26.1.16.	494	Pte.	MARMIDA KETA Gambia Coy.	:
27.1.16.	345	Carrier	OCONGO	:

STOBHILL SERIES.

During the influenza epidemic of 1918 I was attached to Ward 13 of 3rd Scottish General Hospital under Professor Stockman of Glasgow. This ward was reserved for severe medical cases and also took in Malarial cases. All together 197 cases of influenza and influenzal pneumonia were admitted. 8 of these were admitted as malaria and according to routine orders received 30 grains of Quin. Sulphate from the Orderly Officer on admission.

(These were all local cases who had had malaria at Salonika or Gallipoli, and on reporting sick stated that they were suffering from a relapse).

Of these cases one had malaria (Plasmodium Vivax being found in the blood) and developed influenza in Hospital.

The above 197 cases are divided into two groups:-

(1)189 cases which did not receive quinine.

(2) 8 cases which did receive quinine.

	Cases	Developed Pneumonia	Died
Group (1)	189	35*	7*
		18% of cases.	Slightly under 4% of cases & 21% of cases developing pneum-onia.
Group (2)	8	7	6
		87% of cases	75% of cases & 86% of cases developing pneumonia.

*Including one case of Septic Bronchitis.

No Americans are included in these statistics since they were admitted under totally different conditions. They had been exposed to most inclement conditions on transports, and in many cases were moribund on admission.

Cases of Pneumonia Treated in Ward 13

from 5.6.18 - 30.11.18 /

ADMITTED	NUMBER	UNIT	RANK & NAME	DISEASE	DIS-CHARGED	RESULT.
5.6.18.	43475	3rd S.R.	Pte. Barrowman	Pneumonia	4.7.18	R.
5.6.18.	587124	Lab. Corps	Pte. Hessian	Septic Bronchitis	26.7.18.	D.
17.6.18.	188679	R.F.A.	Gr. Hagan	Broncho-Pneumonia	15.7.18.	R.
17.6.18.	5221	13th Sussex	Pte. Andrews	:	10.7.18.	R.
17.6.18.	240101	1/6 H.L.I.	Pte. Robertson	:	10.7.18.	R.
1.7.18.	256655	16th S.R.	Sgt. Carson	Pneumonia	15.7.18.	R.
7.7.18.	15525	16th H.L.I.	Cpl. Duncan	:	9.8.18.	R.
11.7.18.	59373	R.F.A.	Dvr. Ford	Broncho-Pneumonia	1.8.18.	R.
16.7.18.	133281	R.H.A.	Gr. Taylor	:	1.8.18.	R.
16.7.18.	023882	A.S.C.	Farrier Riley	:	15.8.18.	R.
16.7.18.	582085	Lab.Corps	Pte. McRae	:	7.8.18.	R.
16.7.18.	148075	A.S.C.	Pte. Morris	:	7.8.18.	R.
16.7.18.	60066	16th S.R.	Pte. Shaw	:	1.8.18.	R.
18.7.18.	763404	Can. Inf.	Pte. Goodman	:	26.7.18.	D.

ADMITTED	NUMBER	UNIT.	RANK & NAME	DISEASE	DIS-CHARGED	RESULT
21.7.18.	265045	7th S. R.	Pte. Atkinson	Broncho-Pneumonia	13.8.18.	R.
23.7.18.	31119	16th S. R.	Pte. Orr	:	13.8.18.	R.
6.8.18.	56610	M.G.C.	Pte. Croll	:	24.8.18.	D. Admitted as Malaria.
8.8.18.	6552	Aust.A.S.C.	Dr. Reimers	:	11.9.18.	R.
10.8.18.	57511	R.F.A.	Cpl. Madden	:	30.10.18.	R. Admitted as Malaria
18.8.18.	15919	R.F.A.	Cpl. Hall	:	7.9.18.	R.
18.8.18.	52803	15 Worcester	Pte. Clifford	:	9.9.18.	R.
24.8.18.	16191	R.I.E.	Pte. Johnson	:	21.9.18.	R.
11.9.18.	88261	M.G.C.	Pte. McGinlay	:	1.10.18.	R.
15.9.18.	47513	R.F.A.	Dvr. Raich	:	17.9.18.	D. Admitted as Malaria
29.9.18.	24	R.N.	E.R.A.Henderson	:	15.11.18.	R.
2.10.18.	280872	7th H.L.I.	Pte. Dormar	Lobar Pneumonia	6.10.18.	D. Admitted as Malaria
5.10.18.	397302	Lab.Corps	Pte. Stewart	Broncho-Pneumonia	12.11.18.	R.
7.10.18.	30656	16th S.R.	Pte.Roundhill	:	27.10.18.	R.

ADMITTED	NUMBER	UNIT	RANK & NAME	DISEASE	DIS-CHARGED	RESULT.
11.10.18.	40051	1st R.D.F.	Pte. Denvers	Broncho-Pneumonia	15.10.18.	D. Admitted as Malaria.
11.10.18.	9898	R.G.A.	L.Cpl.McDermid	:		R.
21.10.18.	3282	Lab.Corps	Pte. McLeod	:	12.11.18.	R.
23.10.18.	80368	R.D.C.	Pte. Anderson	:	2.11.18.	D.
25.10.18.	17803	R.A.F.	Sgt.Singleton	:	2.11.18.	D.
27.10.18.	68499	1stRes.H.L.I.	Pte. Walker	:	18.11.18	R.
31.10.18.	63434	4th R.S.	Pte. Neil	:	8.11.18.	D. Admitted with Malaria. P.Vivax in blood.
2.11.18.	1031	Aust.F.C.	2nd A.M.Kimpton	:	18.12.18.	R.
4.11.18.	404339	Can. Inf.	Pte. Hughie	Pneumonia	21.11.18.	R.
20.11.18.	7/1990	N.Z.F.A.	T.Bomb.Collison	Broncho-Pneumonia	27.11.18.	D. Admitted as Malaria.
21.11.18.	318549	R.A.M.C.	Pte.Averall	:	26.11.18.	D.
24.11.18.	836052	A.O.C.	Pte. Rankin	:	24.11.18.	D.
26.11.18.	S/4365	10th Gordons	Sgt. Cavers	:	30.11.18.	D.
28.11.18.	2247.	1st Aust. Sig. Coy.	Spr. Pearce	:		R.

D = Died.
R = Recovered.

In both series quinine was administered orally.

I was struck with the high rate of mortality of the Pneumonia cases treated with Quinine at N'Gung, and in consequence I was not surprised to find the same occurring in the Bronchopneumonia cases at Stobhill.

Goodall[1] thus records similar experience in Macedonia - "A complication of cerebral malaria which "almost always precluded recovery was pneumonia. "Here one faced the dilemma that untreated the "patient would die, and that on the other hand an "intravenous injection of quinine would almost "certainly be fatal". and again - "One gives an intravenous injection" (of quinine) "to a pneumonia patient with fear and trembling".

The Stobhill cases which received quinine im--pressed me as making a poor fight with the disease. The only case which recovered was 81 days in hospital before being fit to send to an Auxiliary Hospital as a convalescent. (There were no suggestions of tuberculosis in this case and so far as it was clinically possible T.B. was excluded). The other cases, never rallying, sank and died. These cases did not die of acute heart failure after receiving quinine, nor were there any symptoms of hyper--sensitiveness or drug anaphylaxis.

The/

The average time in hospital from admission till death was 7 days.

Goodall[1] cites a similar case which died 7 days after Quinine treatment for malaria, of double lobar pneumonia.

The two groups, N'Gung and Stobhill, present one common factor. - High mortality following large doses of quinine, each group being adequately controlled by many cases treated (except for the administration of Quinine) in the same manner and in the same surroundings and at the same time without the excessive mortality.

It would appear that the quinine had in some way interfered with the patient's normal defense mechanism with a deleterious result.

In an attempt to elucidate this deleterious action of quinine, the research to be described in Part II was undertaken.

The defense of the body rests to a great extent with the blood, both serum and leucocytes being involved.

No previous work on the action of quinine on the defensive properties of the blood serum can be traced; while the action of quinine on the leucocytes is far from definitely known. Thus Smale & Russell[2] quoting Binz state that quinine 1.20000 destroys the amoebic movement/

movement of White Blood Corpuscles. While Manson[3] suggests that quinine acts in malaria by stimulating the phagocytes, the natural enemies of the parasite. Castellani & Chalmers[4] state "In order that it may "kill off asexual forms of the malarial parasite "it is judged that the quinine must be present in "the blood in a strength of 1.20000".

Binz[5] first showed that quinine 1.400 paralised infusoria at once and that 1.20000 rendered them motionless in 2 hours.

While Krunkenberg[6] stated that a 1.10000 solution killed Polycelis in 30-40 minutes and a 1.100000 solution killed Turbellaria in a few hours.

Binz[5] referring to leucocytes states -"If quinine "is added to the blood in as small a proportion "as 1.20000 their amoeboid movements are noticeably "checked and if a larger quantity is added they "become almost instantaneously coarsely granular "and perish".

This, however, is not Binz's own work though he is often credited with it. Engelmann[7] was the investigator.

The same authority quotes Golgi as having noticed that administration of quinine produced a decline or impairment of the phagocytic process in the blood.

In favour of this latter, and in opposition to Manson's/

Manson's views, it is of interest to state that in personal observations of malarial blood, inclusions of malarial pigment in leucocytes have never been observed after quinine treatment has been established although they have been observed in several cases before treatment was begun.

The observations of Binz, Engelmann & Castellani offer what at first appears to be a possible ex--planation of the high mortality in my series of cases. Assuming that 1.20000 concentration of quinine in the blood is required to cause the disappearance of the asexual stage of the malarial parasite, as Castellani affirms, and assuming that quinine in a dilution of 1.20000 will diminish the amoebic action of the Leucocyte, as Engelmann states it does, it would follow that this high mortality in both the Stobhill and the N'Gung series was due to the action of Quinine on the leucocytes: since in a normal case 30 grains of quinine daily will rapidly remove the asexual stage of the malarial parasite.

McGilchrist,[8] investigating the minimal thera--peutic dosage of Quinine in Benign Tertian Malaria necessary to remove the asexual form of the parasite, shows doses totalling from 1.2 gms - 0.75 gms., and averaging 0.925 gms. of Quinine Sulphate administered by the mouth, per 70 kilos body weight to effect this purpose. Now 0.925 grams of Quinine Sulphate represents/

represents approximately 0.74 gms. of Quinine Alkal--oid. Taking Haldane & Lorrain Smith's figure that "5% of body weight is represented by the blood", then since .74 gms. in the blood of a man of 70 kilos would represent a 1.5000 solution, it follows that a quarter of the total quinine administered circulat--ed in the blood.

Now McGilchrist's doses were given 8 hourly, and Kerner[9] has shown that 30% of quinine administer--ed is excreted in the urine within 12 hours, so a quarter of the total quinine is an underestimate of the amount that must be available for circulation in the blood.

Taking a man weighing 130 lbs. 2.56 grains would represent a 1.20000 solution in total blood. Hence any dose of quinine containing 10 grains of quinine alkaloid, i.e. 12.5 grs. Quin. HCl. ought to produce a condition in which the amoebic movement of white blood corpuscles is diminished and in consequence phagocytosis much impaired.

From the number of persons who have taken such doses for considerable periods without markedly ill effects it would appear that some error had arisen in the work of Engelmann & Binz, and Castellani, Ramsden Lipkin & Whitley[10] confute the suggestion that a 1.20000 solution of quinine is necessary to remove/

remove the asexual stage of the malarial parasite by showing that a 1.60000 solution produces intolerable symptoms in man, and that indeed the concentration of quinine in the blood in man seldom exceeded 1.200000, regardless of the dosage employed, the concentration varying with the individual rather than the dosage.

These observers showed, however, that quinine is taken up by various organs of the body in different amounts, and that the ratio between the concentration of quinine present in the tissues and the concentration present in the blood varies enormously. Thus their highest estimation of the tissue blood ratio (i.e. $\frac{\text{Tissue Quinine}}{\text{Blood Quinine}}$ in equal bulks of tissue and blood) for the suprarenal was 700, for the kidney 35, for the liver 12, for the bone marrow 7, for the lung 9.1, etc.

These results suggest that a strength of quinine not present in the blood may come into action in the tissues, and accordingly affect the defense mechanism in the tissues while not affecting it in the blood.

The $\frac{\text{Tissue Q.}}{\text{Blood Q}}$ ratio of 9.1 in the lung might easily produce a concentration of quinine in that organ as high as 1.20000. (Hence investigations of the action of quinine in such concentrations were carried out by the writer and are recorded in Part II of this thesis).

These/

These observers also show that large amounts of Quinine are destroyed in the liver, kidneys and intestinal walls and probably also in the pancreas, and confirm Kerner's finding as regards the early excretion of Quinine by the kidney, though how the latter recovered 80 - 96% of the Quinine administered from the urine, in view of the amount destroyed in the liver, needs further investigation.

As no satisfactory explanation of the high mortality in both the N'Gung and the Stobhill series seemed to be available, an investigation into the action of Quinine on the defense mechanism of the body was undertaken and is recorded in Part II of this thesis.

Part I is concluded with some notes on the administration of Quinine.

NOTES ON THE ADMINISTRATION OF QUININE.

ORAL ADMINISTRATION -

The writer (presumably healthy) took 5 grs. of quinine bihydrochloride orally t.i.d. for two days and then increased the dose to 15 grs. t.i.d. for two days for the purpose of experiments recorded in Part II of this thesis.

After 3 doses of 5 grains there were marked general symptoms, - headache, deafness and ringing in the ears and considerable depression: this latter symptom passed off to some extent until the doses of 15 grs. were started, when the symptoms became more marked and a general malaise, with marked intestinal symptoms - nausea, diarrhoea and colic, were added.

It cannot be desirable to add these symptoms to any disease from which a patient is suffering unless there is a distinct indication for the exhibition of quinine.

It is of interest to offer an explanation of the value of a purge - especially a mercurial purge - before the oral administration of quinine, which anyone experienced in the administration of oral quinine always prescribes, unless contra indicated. It is a well known clinical fact that the immediate after-effect of a mercurial purge is an increased absorption/

absorption of intestinal toxins, owing to a temporary breaking down of the toxin resisting power of the intestinal wall and possibly of the toxin destroying power of the liver.

Now Ramsden, Lipkin & Whitley[10] have shown that the intestinal wall and the liver destroy quinine. It is probable therefore that the quinine is absorbed and escapes destruction to some extent, in a similar manner to the intestinal toxins, when it is ad--ministered after a mercurial purge.

HYPODERMIC ADMINISTRATION -

HYPODERMIC QUININE has recently been extolled by several French authorities. The writer had personal experience of one case only. - An enthusiastic student administered 10 grains of Quinine bihydrochloride hypodermically to stimulate the uterus in a labour case:- when the slough separated it left a punched out ulcer which had not healed after a month.

INTRAMUSCULAR ADMINISTRATION -

INTRAMUSCULAR QUININE has been fully dealt with by Dudgeon[11] who shows that every intramuscular injection of quinine of therapeutic value causes necrosis and destruction of muscle and any other tissue/

tissue that may be involved in the injection, although the quinine is rapidly and almost completely absorbed. This necrosed tissue makes a suitable starting place for organismal infection and many cases of tetanus following intramuscular quinine have been recorded. Bahr[12] confirms Dudgeon's work and records a case of streptococcal infection of the necrosed tissue, probably of hæmic origin.
He is of the opinion that extensive necrosis is more common when the ilium is impinged on.
Intramuscular injection of quinine is a most painful method of administration and is most unpopular with patients.

INTRAVENOUS ADMINISTRATION -

Although Binz first experimented with the intravenous administration of quinine, it is only of late years that this method has become popular, or the technique studied.
The essential points in the technique are - apart from asepsis - slow injection and small doses.
A useful formula for the rate of injection is a minute for each grain of quinine bihydrochloride; although the writer prefers 15 minutes for 10 grains and does not advise the use of a larger dose.
The injection is always followed by a drop in blood pressure: if the injection is too rapid this fall in/

in blood pressure becomes excessive and there are symptoms of pulmonary embarrassment. This fall of blood pressure may be explained in three ways:-

(1) Direct action of quinine on the heart.

(2) The slow injection might suggest a practical desensitisation for anaphylaxis. The symptoms of falling blood pressure, pulmonary embarrassment, and the well known action of quinine in causing contraction of the non-striped muscle of the uterus might well be considered anaphylactic symptoms.

(3) Ramsden Lipkin & Whitley have shown that the suprarenals absorb enormous quantities of quinine: this might well interfere with their function and, by inhibiting the production of adrenalin, cause a fall in blood pressure.

Experiments on these points are recorded in Part II of this thesis.

The writer has had no experience of the rectal ad--ministration of quinine.

VAGINAL ADMINISTRATION -

In 1915 the writer was consulted about the wife of an officer who complained of slight dispareunia. She had been using pessaries containing 10 grains of quinine bihydrochloride. On douching an epi--thelial cast of the vagina was produced. On two subsequent/

subsequent occasions after the use of single pessaries similar casts were produced. The use of the pessaries was stopped and no further symptoms occurred, nor were further casts produced. The casts were evidently due to superficial necrosis of the vaginal epithelium by the quinine.

The general symptoms following the administration of quinine vary greatly with the individual to whom it is administered, and it is always advisable to test the susceptibility of a patient to quinine before putting him on the large doses which have recently been advocated.

PART II.

A RECORD OF EXPERIMENTAL WORK CARRIED OUT WITH REFERENCE TO THE ACTION OF QUININE ON THE DEFENSE MECHANISM.

PART II.

EXPERIMENTAL WORK on the Action of Quinine on the Defense Mechanism of the body, consisting of the Action of Quinine on -

(1) BLOOD CORPUSCLES.

(2) SERUM.

(3) BLOOD OXYGEN.

(4) NON PROTEIN NITROGEN IN BLOOD, and THE ACTION OF QUININE ON THE KIDNEYS, and

(5) ACTION OF QUININE ON SUPRARENALS.

(6) ACTION OF QUININE IN COMBINATION WITH A TOXIN (RICIN).

(7) ATTEMPTS TO PRODUCE QUININE ANAPHYLAXIS.

Throughout this work Quinine Hydrochloride dissolved in Ringer's solution has been used, except when Quinine was taken orally by the writer, in which cases Quinine bihydrochloride was used, because the latter salt is very easily soluble and hence is easily absorbed, and because from experience the writer has found that Quinine bihydrochloride produces less gastric irritation than any other salt of Quinine which he has taken.

The/

The solutions of Quinine Hydrochloride have always been made up in units of alkaloidal quinine not in units of Quinine Hydrochloride.
Thus, when a solution of 1 in 1000 was used, it represented 1 part of quinine alkaloid in 1000 parts, and contained 1.25 parts of quinine Hydrochloride. Quinine Hydrochloride was used since it is soluble 1.32 in water and a 1.1000 solution in Ringer's solution is alkaline to litmus.

Ringer's solution was used on the advice of Professor Cushny.

THE ACTION OF QUININE ON WHITE BLOOD CORPUSCLES.

Appearance of Corpuscles in Stained Slides.

Wilson[13] in describing the appearance of leucocytes treated with quinine solutions stronger than 1. 20000, states that "there is a marked absence "of granules, the contour is rugged and they are "often vacuolated. There is a marked diminution of staining power."

During the experiments to be described many films treated with strengths of quinine varying from 1:3000 to 1:240000 were examined.

In those treated with a solution of 1. 3000 there were several well marked features .

In those treated with solutions of lesser strengths no definite differences from normal could be made out.

In blood films made from blood containing quinine in a dilution of 1. 3000, and kept at 37°C. for 15 minutes before making the films, the following appearances were noticed:-

Films stained with Leishman's Stain .

No abnormal appearances could be made out in Red Blood Corpuscles.

White Blood Corpuscles. Polymorphs.

(1)/

(1) NEUTROPHYL - Form round, and smaller than in control slides. They have a granular appearance: the granules appear larger than normal and have a tendency to basophylic staining.
The lobes of the nucleus lie huddled together and take up the stain deeply. Vacuolation is not a constant feature.

(2) EOSINOPHYLS - Beyond a tendency to increased fragility no variation from normal could be detected.

(3) BASOPHYLS. A sufficient number have not been studied to make out any characteristic change.

LYMPHOCYTES. Large and small showed no differentiation from normal. The fragility of large hyaline corpuscles rendered their study very difficult.
Their fragility was more marked in 1.3000 quinine than in controls with Ringer's Solution.

THE EFFECT OF QUININE ON THE AMŒBOID MOVEMENTS OF WHITE BLOOD CORPUSCLES.

Galritchevesky[14] showed that Quinine 1. 200 has a negative chemiotactic influence. Such a solution, however, is unimaginable in the human body and this observation is of academic rather than practical interest.

Ikeda[15] observed that when quinine was injected subcutaneously it reduced leucocytic emigration at the point of local injury.

This has been confirmed by Dudgeon[11] who found no evidence of a leucocytosis round the necrosed tissue following intramuscular injections.

In the first part of this thesis reference is made to the work of Engelmann in which it is stated that Quinine in a dilution of 1. 20000 produced a marked diminution in the amœboid movement of leucocytes.

These results might explain the high mortality rate in cases of pneumonia treated with quinine at N'Gung and Stobhill, since the quinine by inhibiting amœboid movement would interfere with a possible phagocytosis. It therefore seemed of importance to confirm Engelmann's results.

The following experiments were performed. - Human blood was mixed with an equal volume of quinine solution and observed on a warm stage kept at 40°C. A control experiment, using Ringer's solution in place of the solution of Quinine was always carried out.

TECHNIQUE - Thoroughly clean slides and cover glasses must be used. They should be washed in distilled water and carefully dried, immediately before use. Blood is obtained by pricking the finger. The Quinine solutions and Ringer's solution are put out in covered and marked watch glasses. The mixture of blood and the diluent can be made either with a capillary pipette or a platinum loop. The latter method was employed because of its more easy application and because it gave a suitable volume, since the mixture can be made directly on the slide. It was found to give results equally as accurate as those obtained when measurement was done by a pipette. The mixture is made as follows. - A loopful of the diluent is placed on the slide and a loopful of blood is added and well mixed: the preparation is covered with a coverslip and put at once on the warm stage, from which it is not removed until the observations have been completed, thus avoiding errors introduced by change in temperature. Any trace of alcohol on the coverglass vitiates the experiment: evaporation from the preparation is avoided by the use of vaseline smeared round the edges.

are these equal

All experiments were observed with 1/6 objective.

The following are typical protocols of the experiments:-

EXPERIMENT I.

	Ringer	Strength of Quinine Soln. Used	Quinine Solution	Blood	Strength of Quinine.
A.	1	-	-	1	Control
B.	-	1.10000	1	1	1.20000
C.	-	1.5000	1	1	1.10000
D.	-	1.1000	1	1	1.2000

OBSERVATIONS -

	On Putting Up.	After 15 minutes
A.	Actively motile	Actively motile
B.	: :	: :
C.	: :	: :
D.	Sluggish movement	No movement observable.

EXPERIMENT II. To confirm D of Experiment I.

	Ringer	Quinine Soln. 1.1000.	Blood	Strength of Quinine.
A.	1	-	1	Control
B.	-	1	1	1.2000

OBSERVATIONS -

	On Putting Up.	After 45 minutes
A.	Actively motile	Actively motile
B.	Slightly motile movement ceased in 10 minutes & leucocytes became round and granular.	non motile, leucocytes round granular and shrunken.

It/

It appears from these experiments that a solution of Quinine of the strength of 1.2000 inhibits the amoeboid movement of leucocytes. From Experiment I it would appear that a dilution of Quinine of 1.10,000 does not immediately inhibit amoeboid movement in leucocytes, so experiments to discover the critical solution were carried out.

EXPERIMENT III.

TO FIND THE CRITICAL SOLUTION OF QUININE.

	Ringer	Strength of Quinine Soln. Used	Quinine Solution	Blood	Strength of Quinine
A.	1		-	1	Control
B.	-	1.1000	1	1	1.2000
C.	-	1.2000	1	1	1.4000
D.	-	1.4000	1	1	1.8000
E.	-	1.8000	1	1	1.16000

OBSERVATIONS

	On Putting Up	After 30 minutes
A.	Actively motile	Actively motile
B.	Slight motility	Contracted: granular: non motile
C.	Actively motile	Actively motile
D.	: :	: :
E.	: :	: :

From this experiment it would appear that a 1.4000 dilution of quinine does not inhibit amœboid movement of leucocytes in 30 minutes.

It was thought to be of the utmost importance to confirm this and to extend observations to a period of an hour.

EXPERIMENT IV/

EXPERIMENT IV.

TO CONFIRM THE OBSERVATION THAT QUININE IN A DILUTION OF 1.4000 DID NOT INHIBIT AMŒBOID MOVEMENT OF LEUCOCYTES.

	Ringer	Strength of Quinine Soln. Used	Quinine Solution	Blood	Strength of Quinine
A.	1		-	1	Control
B.	-	1.1000	1	1	1.2000
C.	-	1.2000	1	1	1.4000
D.	-	1.2000	1	1	1.4000
E.	-	1.2000	1	1	1.4000

OBSERVATIONS -

	On Putting Up	After 1 hour
A.	Actively motile	Actively motile
B.	Slight motility ceasing in 7 minutes.	Non motile: round: granular.
C.	Actively motile	Actively motile
D.	: :	: :
E.	: :	: :

This so far confirms Experiment III. It was felt, however, that further confirmation was advisable and the experiment was repeated as follows:-

EXPERIMENT V/

EXPERIMENT V.

(REPETITION OF EXPERIMENT IV).

Ringer	Strength of Quinine Soln. Used	Quinine Solution	Blood	Strength of Quinine
A. -	1.1000	1	1	1.2000 (Control)
B1 -	1.2000	1	1	1.4000
B2 -	:	1	1	:
B3 -	:	1	1	:
B4 -	:	1	1	:
B5 -	:	1	1	:
C. 1		-	1	Control

OBSERVATIONS -

	After 5 minutes	After 1 hour
A.	non-motile: round granular	non-motile round granular.
B1	Actively motile	Actively motile
B2	: :	: :
B3	: :	: :
B4	: :	: :
B5	: :	: :
C.	: :	: :

From these experiments it appears that Quinine in a dilution of 1.4000 does not inhibit amœboid movements of leucocytes, while quinine in a dilution of 1.2000 does inhibit amœboid movement of leucocytes. The action of Quinine in a dilution of 1.3000 remained to be tested: this was done as follows -

EXPERIMENT VI/

EXPERIMENT VI.

THE EFFECT OF QUININE IN A DILUTION OF 1.3000 ON THE AMŒBOID MOVEMENT OF LEUCOCYTES.

	Ringer	Quinine 1.1000	Blood	Strength of Quinine
A.	2	-	1	Control
B.	1	1	1	1.3000
C.	1	1	1	1.3000
D.	1	1	1	1.3000

OBSERVATIONS -

A. Leucocytes actively motile after 1 hour.

B. On putting up were motile but no motile leucocytes after 15 minutes.

C. : : : were motile but no motile leucocytes after 5 minutes.

D. : : : were motile but no motile leucocytes after 10 minutes.

In B. C. & D. careful search revealed no motile leucocytes, but many were seen in a round condition: granularity was nor marked.

From these experiments it would appear that a dilution of 1.3000 is the critical dilution of quinine with respect to the inhibition of amœboid movement of leucocytes.

It was considered of interest to investigate this action of quinine: whether it is a direct action/

action of quinine on leucocytes themselves, or an action in which the blood serum is involved.

Washed white blood corpuscles were obtained by Wright's method from 10 c.cs. of citrated blood, and mixed with an equal volume of Ringer's solution. This mixture was used in place of blood in an experiment otherwise identical to Experiment IV.

EXPERIMENT VII

ACTION OF QUININE ON WASHED LEUCOCYTES.

	Ringer	Strength of Quinine Soln. Used	Quinine Solution	Washed W.B.Cs.	Strength of Quinine.
A.	1		-	1	Control
B.	-	1.1000	1	1	1.2000
C.	-	1.2000	1	1	1.4000
D.	-	1.2000	1	1	1.4000
E.	-	1.2000	1	1	1.4000

OBSERVATIONS -

	On Putting Up	After 1 hour
A.	Actively motile as soon as warm,	Actively motile
	Motile when warmed.	
B.	Motility ceased within 10 minutes, became round and granular.	Non-motile, round granular.
C.	Actively motile as soon as warm	Actively motile.
D.	:	: :
E.	:	: :

When/

When it was observed that after 1 hour the leucocytes in Experiment C were still actively motile, Experiments D and E were carried out for confirmation. At this stage evidence of the efficiency of the technique is introduced: with blood the difficulty due to clotting on the one hand, or the possibility of introducing an unknown factor by using citrate to prevent clotting on the other, made the use of known measurements undesirable, but with washed leucocytes these troubles do not arise so a confirmatory test was carried out in small agglutination tubes. Using the moist chanber to be described in experiments on phagocytosis, and measuring amounts with a 1 c.c. pipette graded to .01 c.c.

EXPERIMENT VIII.

	Ringer.	Strength of Quinine Soln. Used	Quinine Solution	Washed W.B.Cs.	Strength of Quinine.
A.	0.15c.c.		-	0.15 cc	Control
B.	-	1.2000	0.15 cc	0.15 cc	1.4000
C.	-	1.1000	0.15 cc	0.15 cc	1.2000

Incubated in moist chanber at 37°C.

Preparations were made after 15 minutes and after 1 hour, from each tube and examined on the warm stage.

OBSERVATIONS/

OBSERVATIONS -

After 15 minutes	Leucocytes in	A	actively motile
	:	B	actively motile
	:	C	non-motile, round granular.
After 1 hour	:	A	actively motile
	:	B	actively motile
	:	C	non-motile round granular.

This experiment confirms the results of Experiment VII, and shows the value of the platinum loop technique.

The action of Quinine in a dilution of 1.3000 on washed leucocytes was investigated in the same manner as the action on whole blood in Experiment IV.

EXPERIMENT IX/

EXPERIMENT IX .

Ringer	Quinine 1.1000	Washed W.B.Cs.	Strength of Quinine .
A. 2	-	1	Control
B. 1	1	1	1.3000
C. 1	1	1	1.3000
D. 1	1	1	1.3000

OBSERVATIONS -

	On Putting up	After 1 hour
A.	Actively motile as soon as warm.	Actively motile
B.	Slight motility when warmed, non-motile within 12 mins. and became round.	Non-motile round.
C.	Only one leucocyte show--ing amœbic movement seen, this stopped within a few minutes and became round.	: :
D.	Slight motility when warmed, stopped within 10 minutes and became round.	: :

Again, as in Experiment IV, granularity was not marked.

It appears therefore that the inhibition of amoeboid movement of leucocytes by Quinine is due to a direct action on the leucocyte itself and that the presence of blood serum is unnecessary for this action/

action, and that the presence of blood serum does not inhibit the action.

These Experiments show:-

(1) That a dilution of Quinine of 1.4000 will not inhibit the amœboid movements of leucocytes so far as can be observed during 1 hour on a warm stage at 40° C.

(2) That a dilution of Quinine of 1.3000 will inhibit the amœboid movements of leucocytes.

(3) That the action of the Quinine in inhibiting amœboid movement in leucocytes is a direct action on the leucocytes themselves and is not affected by blood serum.

From these results it is at once apparent that the amœboid movement of leucocytes as they exist in the blood stream cannot be affected by any therapeutic dosage of quinine, and it is extremely doubtful if they will be so affected in the tissues (with the possible exception of the suprarenal bodies where, from the work of Ramsden, Lipkin & Whitley[10] such a concentration as 1.3000 might conceivably exist).

No confirmation can be given to Engelmann's work on the effect of quinine on the amœboid movement of white blood corpuscles.

THE EFFECT OF QUININE ON PHAGOCYTOSIS BY LEUCOCYTES.

In Part I of this thesis reference was made to Manson's view, that quinine stimulated the leucocytes to increased phagocytic activity. T.M. Wilson[13] investigating the effect of Quinine sulphate on Phagocytosis, came to the conclusion that dilutions of Quinine stronger than 1.20000 diminished phagocytosis. No confirmation of this work could be found, and it seemed of interest to confirm it, since Quinine, when administered in large doses, may well be present in the lungs and other tissues in such a concentration: this would then be a possible explanation of the high death rate in the N'Gung and Stobhill series.

It was decided to use Staphylococcus aureus as the organism on which to test the phagocytic index.

Before commencing this work it appeared advisable to investigate the action of Quinine on the organism itself, since Quinine is reputed to have a considerable antiseptic power.

Culture/

Culture tubes were prepared as follows -

Contents of Tubes	Ordinary Broth	Ringer	Quinine Sol. 1.1000	Strength of Quinine
A.	5 c.c.	5 cc	-	Control
B.	5 c.c.	-	5 cc	1.2000
C.	5 c.c.	2.5 cc	2.5 cc	1.4000
D.	5 c.c.	3.75 cc	1.25 cc	1.8000

These tubes were then sterilised.

The experiments were carried out as follows - 1 tube of Class A (as a control), 3 tubes of Class B, 3 tubes of Class C and 3 tubes of Class D were all inoculated with a loopful of a broth culture of the strain of staphylococcus aureus and incubated.

After 24 hours -

A showed well marked turbidity and film preparations showed the presence of many staphylococci.

B1) B2) B3) showed no turbidity, and no organisms were found in film preparations.

C1) C2) C3) showed marked turbidity (not so marked as in A): film preparations showed the presence of many staphylococci.

D1) D2) D3) Showed a turbidity as well marked as in A, film preparations showed the presence of many staphylococci.

1 c.c. of the contents of each tube was then diluted.

In the case of the B series 10 times

In all the other cases 100 times

.1 c.c. of each dilution was plated out on an agar plate and incubated for 24 hours.

Plates prepared from B1 had 423 colonies

B2 : 621 colonies

B3 : 215 colonies

Plates from A, C1, C2, C3, D1, D2, D3 All had an almost universal growth, estimation of colonies being impossible.

After 1 c.c. had been removed from each tube for the above plating out, the tubes were re-incubat-ed. After 5 days - all showed an abundant growth and tubes could not be recognised from each other by their turbidity.

From this experiment it appears that Quinine in a dilution of 1.2000 considerably delays the growth of the Staphylococcus used but in weaker solutions the Staphylococcus grows well.

TECHNIQUE USED IN ESTIMATING THE ACTION OF QUININE ON THE PHAGOCYTIC INDEX OF THE BLOOD.

This was based on Leishman's original method.

Equal parts of Blood, Emulsion of Staphylococci, and Ringer's solution alone, or with varying strengths of Quinine in solution were taken by means of a platinum loop and thoroughly mixed on a clean slide, covered with a clean coverslip, and incubated for the desired time at 37°C in a moist chamber within an incubator.

The moist chamber was formed by covering over an agglutination tube rack with blotting paper and then moistening the paper and setting it to stand in a dish of water of such a depth that the water reached the blotting paper without wetting the lower perforated plate of the rack on which the slides were laid.

After the desired time of incubation the coverglass was removed and the mixture spread over the slide and stained with Leishman's stain.

An average of cocci included in leucocytes was then struck from 100 or 150 leucocytes. This method, which at first seems rather crude, has several advantages over Wright's method. -

(1) The leucocytes have a large surface to spread on and in consequence their phagocytic capacity is greater than in a capillary tube.

(2)/

(2) In these experiments it was not desired to separate leucocytes from serum.

(3) Had whole blood been used in capillary tubes, the resulting clotting would have caused much trouble.

The accuracy that can be obtained by this method is demonstrated by the following result. It was known that in one experiment two slides had been made that the same solution of quinine: on counting over the slides the one slide was found to give an index of 3.10 while the other slide gave an index of 3.14.

This accuracy is further shown in the experiments on low dilutions of quinine.

TYPICAL PROTOCOLS OF EXPERIMENTS.

EXPERIMENT I.

Experiment I was only of a tentative nature, saline was used instead of Ringer's solution and 50 leucocytes only were counted.

	Saline	Emulsion of Staphylococci.	Blood	Quinine Solution	Strength of Quinine Sol.
A.	1	1	1	-	Control
B.	-	1	1	1	1.1000

Incubated 15 minutes.

	Index	Strength of Quinine in mixture.
A.	3.24	Control
B.	0.34	1.3000

This/

This showed that a 1.3000 solution of quinine apparently reduced the phagocytic index: this is to be expected since this strength rapidly inhibits amoeboid movement in leucocytes: it, however, gave encouragement to carry on the investigation by the technique evolved.

EXPERIMENT II.

	Ringer	Emulsion of Staphylo--cocci.	Blood.	Quinine Solution	Strength of Quinine Sol.
A.	-	2	2	2	1.1000
B.	-	2	2	2	1.2000
C.	-	2	2	2	1.4000
D.	2	2	2	-	Control

Incubated for 10 minutes.

	Index.	Strength of Quinine in mixture.
A.	1.1	1.3000
B.	2.4	1.6000
C.	4.5	1.12000
D.	5.2	Control

It is of interest to notice the progressive increase in the phagocytic index accompanying the decrease in the strength of the quinine solution.

This experiment was repeated and amplified.

EXPERIMENT III/

EXPERIMENT III.

	Ringer	Emulsion of Staphylococci.	Blood	Quinine Solution	Strength of Quinine Sol
A.	1	1	1	0	-
B.	-	1	1	1	1.1000
C.	-	1	1	1	1.2000
D.	-	1	1	1	1.4000
E.	-	1	1	1	1.8000

Incubated 15 minutes.

	Index	Strength of Quinine in mixture.
A.	10.5	Control
B.	0.3	1.3000
C.	3.35	1.6000
D.	5.1	1.12000
E.	7.15	1.24000

This experiment confirms Experiment II. It seemed necessary, however, to confirm the fact that Quinine in a dilution of 1.24000 decreased the phagocytic index.

The three following experiments were performed at weekly intervals (for physical reasons, - counting the organisms produced severe headaches). A number of small boils may have had some effect on the differences of the indices.

EXPERIMENT IV/

EXPERIMENT IV.

	Ringer	Emulsion of Staphylo--cocci.	Blood	Quinine Solution	Strength of Quinine Sol
A.	1	1	1	0	-
B.	0	1	1	1	1.12000

Incubated for 10 minutes.

	Index.	Strength of Quinine in mixture.
A.	3	Control
B.	1.1	1.24000.

EXPERIMENT V. (Repetition of Ex. IV)

	Ringer	Emulsion of Staphylo--cocci.	Blood	Quinine Solution	Strength of Quinine Sol
A.	1	1	1	0	-
B.	0	1	1	1	1.8000

Incubated for 7 minutes.

	Index.	Strength of Quinine in mixture.
A.	2.6	Control
B.	1.6	1.24000.

EXPERIMENT VI. (Second repetition of Ex.IV)

	Ringer	Emulsion of Staphylo--cocci.	Blood	Quinine Solution	Strength of Quinine Sol
A.	1	1	1	1	-
B.	0	1	1	1	1.8000

Incubated for 15 minutes.

	Index	Strength of Quinine in mixture.
A.	8.14	Control
B.	4.2	1.24000

From these experiments it appears that a 1.24000 dilution of Quinine decreases the phagocytic index of the leucocytes. Such a concentration might easily occur in the body tissues, e.g.- lung, and this must have a deleterious action in a disease in which phagocytosis has a part to play in the defense mechanism.

It was considered of interest to see if this decrease of the phagocytic index might be produced by dilutions of quinine known to exist in the blood stream, so Experiment II was repeated, using very dilute solutions of quinine.

EXPERIMENT VII.

	Ringer	Emulsion of Staphylococci.	Blood	Quinine Solution	Strength of Quinine Sol.
A.	1	1	1	-	
B.	-	1	1	1	1.20000
C.	-	1	1	1	1.40000
D.	-	1	1	1	1.80000

Incubated for 15 minutes.

	Index.	Strength of Quinine in mixture.
A.	13.8	Control
B.	13.42	1.60000
C.	14.18	1.120000
D.	14.23	1.240000

This Experiment was repeated.

EXPERIMENT VIII/

EXPERIMENT VIII.

	Ringer	Emulsion of Staphylo--cocci.	Blood	Quinine Solution	Strength of Quinine Sol
A.	1	1	1	-	-
B.	-	1	1	1	1.20000
C.	-	1	1	1	1.40000
D.	-	1	1	1	1.80000

Incubated for 10 minutes.

	Index.	Strength of Quinine in mixture.
A.	7.72	Control
B.	7.65	1.60000
C.	7.38	1.120000
D.	7.32	1.240000

From these results it is apparent that Quinine in any dilution likely to occur in the blood does not affect phagocytosis, although concentrations capable of affecting phagocytosis may easily exist in the tissues.

The results of Experiments VII and VIII show the accuracy of the platinum loop method of making the mixtures.

ACTION OF QUININE ON THE OXYDASE REACTION OF THE GRANULES OF THE LEUCOCYTES.

The Oxydase reaction of the granules of the leucocytes was investigated when the leucocytes had been acted upon by a 1.2000 solution of Quinine.

A mixture of equal parts of blood and 1.1000 solution of Quinine was made on four slides. Two were covered and placed in the moist chamber in the incubator for 15 minutes and then spread into films and fixed with 2% osmic acid. The other two slides were spread and fixed at once. No difference in the staining properties of the granules could be made out either between the slides themselves, or on comparison with control films of equal parts of Ringer and blood or plain blood films. The reaction was done by the method outlined by Dunn[16].

The reagents,(viz. Dimethylparaphenylenediamine and a. Naphthol)were kindly supplied by Dr. McCartney.

THE ACTION OF QUININE ON THE NUMBER OF LEUCOCYTES IN THE PERIPHERAL BLOOD.

Cullen[18] and Cushny[17] are of the opinion that Quinine produces a leucopœnia while Gulland and Goodall[19] are of the opinion that Quinine may produce a polymorph leucocytosis.

On two occasions the writer subjected himself to the administration of quinine: blood counts at different periods during the administration were made.

Results are an average of three counts and differentials are averages of 1,200 cells counted. All counts taken at 4 p.m. in afternoon.

EXPERIMENT I.

Before Commencing Quinine -

Red Blood Corpuscles - 5,500000:
Haemoglobin - 105%: Colour Index .96
White Blood Corpuscles 5,600

Differential Count - Polymorphs 58%: Eosinophils 3%

Small Lymphocytes 34%: Large Lymphocytes 1%

Large Hyaline 4%.

After 2 days treatment 5 grs. Quinine bihydrochloride t.i.d. -

Red Blood Corpuscles - 5000000
Haemoglobin - 100%: Colour Index 1.
White Blood Corpuscles 5,400

Differential Count -Polymorphs 53%:Eosinophils 1%:

Small Lymphocytes 36%: Large Lymphocytes 7%:

Large Hyaline 3%.

After/

After 2 days further treatment with 15 grs. Quinine bihydrochloride, t.i.d. -

Red Blood Corpuscles - 5,000,000
Hæmoglobin 101%: Colour Index 1
White Blood Corpuscles - 5,200.

Differential Count - Polymorphs 58%:Eosinophyls 1%
Small Lymphocytes 34%: large Lymphocytes 4%:
Large Hyaline 2%.

EXPERIMENT II.

Before Quinine -

Red Blood Corpuscles - 5,260,000
Hæmoglobin 98: Colour Index .92
White Blood Corpuscles 7,600

Differential Count - Polymorphs 62%:Eosinophyls 2%:
Small Lymphocytes 29%: Large Lymphocytes 4%:
Large Hyaline 4%.

After 2 days treatment Quinine bihydrochloride gr. xv t.i.d. -

Red Blood Corpuscles - 5,520,000
Hæmoglobin 95: Colour Index 86%
White Blood Corpuscles - 8000

Differential Count - Polymorphs 65%:Eosinophyls 0.5%
Small Lymphocytes 24%: Large Lymphocytes 6%:
Large Hyaline 4%: Mast 0.5%.

The calculation of hæmoglobin was made with a different scale in the 2nd Experiment: it appears to be defective.

From these results no definite sudden increase or decrease in leucocytes can be made out.

THE ACTION OF QUININE ON BACTERICIDAL BODIES IN THE BLOOD SERUM.

No previous work on this subject can be traced, so the whole method of work had to be evolved. This research has taken some nine months to perform. The first point was choice of an organism to use for estimating the bactericidal action of the serum. The coli-typhoid group contains many organisms which are susceptible to bacteriolytic influences in the serum, and being easily cultivated it appeared that an organism of this group would probably be suitable.

Several strains of B. coli were tested against the writer's serum and finally a strain isolated from the writer's fæces was found to be destroyed by the writer's serum. This strain was used throughout the experiments.

It was found to be of the utmost importance that the serum should be absolutely fresh. Little bactericidal power was shown by serum that had been obtained by allowing blood to clot overnight in an ice chest.

The estimation of the bactericidal power of the serum in various concentrations of Quinine was estimated by a modification of "the method of Neisser and Wechsberg" for estimating the bacteri-cidal power of sera.

TECHNIQUE -

The experiments were carried out in sterile Wassermann tubes.
The volume of the Quinine solution to be used (see protocols of Experiments) and the required amount of Ringer's solution to make the final volume up to 3 c.c. were pipetted into the tubes.
The tubes were then plugged and sterilised.
When cool the required amount of serum for the experiment was added and finally 1 c.c. of a very dilute young broth culture of B. coli, and the whole incubated at 37°C for 3 hours. After incubation 0.5 c.c. of the contents of each tube was plated out on agar: the plates incubated overnight and the colonies counted next morning.

The fresh serum was obtained by drawing off 5 c.c. of blood into 5 c.c. of Ringer's solution and centrifugalising immediately, everything used being sterile.
The B. coli culture used was an 18 hours broth culture diluted so that 1 c.c. of the dilution contained 1/10000 of a c.c. of the broth culture.
The dilution was made with sterile Ringer's solution.

It is evident that uniform results between experiments could not be expected from such a method: since an unknown amount of B. coli is present in the broth culture.

The/

The only comparison that can be made must be made between the plates of each experiment and not between plates of two different experiments.

The inhibiting action of Quinine on the growth of the Staphylococcus used for estimating phagocytosis suggested that its action on the strain of B. coli used in these experiments should be investigated. The investigation was carried out with the same technique as was used for the staphylococcus. It was found that the strain of B coli used grew freely in a 1.2000 dilution of Quinine.

The following are Protocols of Experiments -

EXPERIMENT I.

In this experiment, which was a preliminary one, the action of Quinine in dilutions of 1.3000, 1.6000, 1.12000 and 1.24000 was tested against varying doses of serum, since it was impossible to predict any result, and the possibility of quinine increasing the bactericidal power of the serum had to be borne in mind.

SERIES A. (Controls without Quinine)	Ringer	Quinine Sol. 1.1000	Serum in Ringer	Dil. Broth Cult. of B. coli.
1.	1.6 cc	-	.4 cc	1 cc
2.	1.8 cc	-	.2 cc	1 cc
3.	1.9 cc	-	.1 cc	1 cc
4.	1.95 cc	-	.05 cc	1 cc

SERIES B/

SERIES B. (Containing Quinine 1.3000)

	Ringer	Quinine Sol. 1.1000	Serum in Ringer.	Dil. Broth Cult. of B. coli
1.	0. 6 cc	1 cc	.4 cc	1 cc
2.	0. 8 cc	1 cc	.2 cc	1 cc
3.	0. 9 cc	1 cc	.1 cc	1 cc
4.	0.95 cc	1 cc	.05cc	1 cc

SERIES C. (Containing Quinine 1.6000)

	Ringer	Quinine Sol. 1.1000	Serum in Ringer	Dil. Broth Cult. of B. coli
1.	1. 1 cc	.5 cc	.4 cc	1 cc
2.	1. 3 cc	.5 cc	.2 cc	1 cc
3.	1. 4 cc	.5 cc	.1 cc	1 cc
4.	1.45 cc	.5 cc	.05cc	1 cc

SERIES D. (Containing Quinine 1.12000)

	Ringer	Quinine Sol. 1.1000	Serum in Ringer.	Dil. Broth Cult. of B. coli.
1.	1.35 cc	.25 cc	.4 cc	1 cc
2.	1.55 cc	.25 cc	.2 cc	1 cc
3.	1.65 cc	.25 cc	.1 cc	1 cc
4.	1.70 cc	.25 cc	.05cc	1 cc

SERIES E/

SERIES E. (Containing Quinine 1.24000)

	Ringer	Quinine Sol. 1.1000	Serum in Ringer	Dil. Broth Cult. of B. coli .
1.	1.475 cc	.25 cc	.4 cc	1 cc
2.	1.675 cc	.25 cc	.2 cc	1 cc
3.	1.775 cc	.25 cc	.1 cc	1 cc
4.	1.825 cc	.25 cc	.05 cc	1 cc

All incubated for 3 hours, and 0.5 c.c. of each tube plated out.

Colonies counted after incubation of plates.

	A. Controls	B. Quin. 1.3000	C. Quin. 1.6000	D. Quin. 1.12000	E. Quin. 1.24000
1.	7	1200*	2000*	3200*	1640*
2.	370	x	x	x	x
3.	960*	x	x	x	x
4.	x	x	x	x	x

* = Number estimated

x = practically universal growth.

In no subesquent experiment was this action of Quinine on the bactericidal power of the serum so well marked as it was in the tubes numbered (1) in each series.

It will be noted that tubes bearing the same number in each series form an experiment on the action of different dilutions of Quinine on the same amount of serum.

It/

It was decided to use the quantities of serum and quinine used in tubes A1, B1, C1, D1 and E1 for further experiment and in that form to repeat the experiment several times.

Protocol of EXPERIMENTS II, III and IV.

	Ringer	Quinine Sol. 1.1000	Serum	Dil Broth Cult. of B. coli.
1.	1. 6 cc	-	.4 cc	1 cc
2.	. 6 cc	1 cc	.4 cc	1 cc
3.	1. 1 cc	1 cc	.4 cc	1 cc
4.	1.35 cc	1 cc	.4 cc	1 cc
5.	1.475cc	1 cc	.4 cc	1 cc

This form was repeated three times with the following results -

	Expr.II	Expr.III	Expr. IV.
1.	141 colonies	153 colonies	344 colonies
2.	902 :	841 :	1380 :
3.	804 :	364 :	1658 :
4.	736 :	611 :	2460 :
5.	987 :	428 :	1984 :

Photographs of the series of plates of Experiment IV are presented.

These results all show that fewer colonies develop from tubes in which there was serum alone than from tubes in which quinine was present.

The/

The larger numbers of colonies are therefore due to the presence of Quinine.

The Quinine has therefore inhibited the bactericidal action of the serum, and this inhibition has been accomplished by Quinine in a dilution of 1 in 24000.

It was considered of interest to try the effect of weaker dilutions of Quinine on the bactericidal action of the serum, i.e.- such dilutions as might be expected in the blood stream. Experiment II was therefore modified by using a dilution of Quinine of 1.20000 instead of 1.1000 and repeated three times but dilutions were not taken below 1.240000.

Protocol of EXPERIMENTS V, VI, VII.

	Ringer	Quinine Sol. 1.20000	Serum.	Dil. Broth Cult. of B. coli.
1.	1. 6 cc	-	.4 cc	1 cc
2.	.6 cc	1 cc	.4 cc	1 cc
3.	1. 1 cc	0.5 cc	.4 cc	1 cc
4.	1.35 cc	0.25cc	.4 cc	1 cc

Colonies Counted after Incubation of Plates

	Expr. V	Expr. VI	Expr. VII
1.	3	19	None
2.	None	5	None
3.	23	None	None
4.	None	4	None

It appears therefore that Quinine in dilutions of 1.60,000, 1.120,000 and 1.240,000 did not inhibit the bactericidal action of the serum.

PLATES OF EXPERIMENT IV.

No. 1. No Quinine.

344 colonies.

No. 5. Containing Quinine 1.24000

1984 colonies.

No . 2. Containing Quinine 1.3000 . 1380 colonies.
Colonies very small: only seen on close examination.

No. 3. Containing Quinine 1.6000 . 1658 colonies.

No. 4. Containing Quinine 1.12000 . 2460 colonies .
Many small colonies only seen on close examination.

Following on the discovery of the inhibition of the bactericidal power of the serum by Quinine it was considered of interest to investigate the action of Quinine on Complement and on agglutinins.

Experiments on the possibility of the DEVIATION OF COMPLEMENT BY QUININE.

It was considered of importance to carry through these experiments with different concentrations of Quinine. Varying doses of complement and quinine 1.1000 in Ringer's solution were tested.

TECHNIQUE. - The desired doses of Quinine and Complement were measured out and sufficient Ringer's solution added to bring the final volume up to 3 cc. The whole were incubated at 37°C for 1½ hours. Then 3 M.H.Ds. of I.B. were added and 1 c.c. of a 1% emulsion of appropriate R.B.Cs., and the presence of free Complement investigated by further incubation for 1½ hours.

Protocols of Experiment.

EXPERIMENT I.

In this experiment Ox blood corpuscles and an appropriate I. B. were used.

The M.H.D. of Complement was 0.1 c.c. of a 1.10 dilution of the Complement used.

The M.H.D. of I. B. was o.1 c.c. of a 1.100 dilution. The whole was suitably controlled.

	Tubes	Quinine 1.1000	Complement	Ringer	I.B.	R.B.Cs.
A. Strength of Quinine in Volume 1.5000	1.	.5 cc	.4 cc	.8 cc	.3cc	1 cc
	2.	.5 cc	.3 cc	.9 cc	.3cc	1 cc
	3.	.5 cc	.2 cc	1 cc	.3cc	1 cc
	4.	,5 cc	.1 cc	1.1cc	.3cc	1 cc
B. Strength of Quinine in finalvol 1.10000	1.	.25 cc	.4 cc	1.05cc	.3cc	1 cc
	2.	.25 cc	.3 cc	1.15cc	.3cc	1 cc
	3.	.25 cc	.2 cc	1.25cc	.3cc	1 cc
	4.	.25 cc	.1 cc	1.35cc	.3cc	1 cc
C. Strength of Quinine in final volume 1.25000	1.	.1 cc	.4 cc	1.2 cc	.3cc	1 cc
	2.	.1 cc	.3 cc	1.3 cc	.3cc	1 cc
	3.	.1 cc	.2 cc	1.4 cc	.3cc	1 cc
	4.	.1 cc	.1 cc	1.5 cc	.3cc	1 cc
D. Strength of Quinine in final volume 1.50000	1.	.05cc	.4 cc	1.25cc	.3cc	1 cc
	2.	.05cc	.3 cc	1.35cc	.3cc	1 cc
	3.	.05cc	.2 cc	1.45cc	.3cc	1 cc
	4.	.05cc	.1 cc	1.55cc	.3cc	1 cc
Controls without Quinine	1.	-	.4 cc	1.3cc	.3cc	1 cc
	2.	-	.3 cc	1.4cc	.3cc	1 cc
	3.	-	.2 cc	1.5cc	.3cc	1 cc
	4.	-	.1 cc	1.6cc	.3cc	1 cc
Control of Quinine	5.	.5 cc	-	1.5cc	.3cc	1 cc
Control of I.B. & Quinine.	6.	.5 cc	-	1.2cc	-	1 cc

Series/

Series A.B.C.D. and the Controls without Quinine all gave the same result.
Complete Haemolysis in tubes 1 and 2 and partial Haemolysis in tubes 3: no haemolysis in tube 4.
Controls 5 & 6 gave no Haemolysis.

EXPERIMENT II - Repeated 3 times.

In these experiments Table A of Experiment I was repeated with the controls, but sheeps R.B.Cs. and a suitable I.B. were used.

The M.H.D. of Complement was found to be .1 cc of a 1.10 dilution of the complement, and the M.H.D. of I.B. was found to be .1 cc of a 1.100 dilution.

	Tubes	Quinine 1.1000	Complement	Ringer	I.B.	R.B.Cs
Strength of Quinine in final volume 1.5000	1.	.5 cc	,4 cc	.8 cc	.3cc	1 cc
	2.	.5 cc	.3 cc	.9 cc	.3cc	1 cc
	3.	.5 cc	.2 cc	1 cc	.3cc	1 cc
	4.	.5 cc	.1 cc	1.1.cc	.3cc	1 cc
Controls without Quinine	1.	-	.4 cc	1.3 cc	.3cc	1 cc
	2.	-	.3 cc	1.4 cc	.3cc	1 cc
	3.	-	.2 cc	1.5 cc	.3cc	1 cc
	4.	-	.1 cc	1.6 cc	.3cc	1 cc
Control of Quinine.	5.	.5 cc	-	1.5 cc	-	1 cc
Control of Quinine & I.B.	6.	.5 cc	-	1.2 cc	.3cc	1 cc

The experiment was put up three times.

In/

In each case the Experiment and Controls gave the same results - Complete Haemolysis in tubes 1, 2, and 3: no Haemolysis in tube 4: no Haemolysis in Control tubes 5 & 6.

These experiments show that Quinine does not deviate Complement, and suggest that the action of Quinine on the bactericidal bodies of the serum is not due to a deviation of Complement.

THE ACTION OF QUININE ON AGGLUTININS.

This was tested on B. Para Typhosus B. since this organism and a suitable agglutinating serum were available.

The experiments were carried out macroscopically using the usual technique as carried out in the Bacteriological laboratory in Edinburgh University, the tubes being kept in the water bath at 55°C for 2½ hours. In the Control experiment the dilution of the agglutinating serum was carried out with Ringer's solution.

In the Quinine Experiment the dilution was carried out with 1.1000 solution of Quinine.

Protocols of Experiment/

EXPERIMENT I.

In this Experiment the agglutinating serum was diluted 1.80 before commencing.

The known titre of the serum was 1 in 3000.

		Control Experiment			Quinine Experiment		
Tubes	Dil. of agglutin-ating serum.	Vols. of Ringer	Vols.of Emuls.of Para B.	RESULT	Vols.of 1.1000 Quinine	Vols.of Emuls.of Para B.	RESULT.
1.	1. 160	1	1	A	1	1	A.
2.	1. 320	1	1	A	1	1	A
3.	1. 640	1	1	A	1	1	A
4.	1.1280	1	1	A	1	1	A
5.	1.2560	1	1	A	1	1	A
6.	1.5120	1	1	-	1	1	-

A - Agglutination.

From this Experiment it appears that Quinine does not affect the agglutination of B. Para. B.

EXPERIMENT II.

In this Experiment, which was repeated three times, the serum was diluted 1.40 times.

	Control Experiment				Quinine Experiment -repeated 3 times			
Tubes	Dil. of agglutinating serum.	Vols.of Ringer	Vols.of Emuls.of Para B.	RESULT.	Vols.of 1.1000 Quinine	Vols.of Emuls.of Para B.	RESULT.	
1.	1.80	1	1	A	1	1	A A A	
2.	1.160	1	1	A	1	1	A A A	
3.	1.320	1	1	A	1	1	A A A	
4.	1.640	1	1	A	1	1	A A A	
5.	1.1280	1	1	A	1	1	A A A	partial
6.	1.2560	1	1	-	1	1	- - -	
7.	1.5120	1	1	-	1	1	- - -	

A - Agglutination.

This Experiment was carried out with another sample of the same serum as was used in Expr. I, but a different strain of B.Paratyphosus B. was used. This Experiment confirms Experiment I.

From these Experiments it would appear that Quinine in a dilution of 1.2000 does not act on the agglutinins of a high titre agglutinating serum. These Experiments also confirm the independence of agglutinins and bacteriolysins in serum.

Reviewing the actions of Quinine on the blood serum - It would appear that the action of Quinine in diminishing the bactericidal power of the serum is a direct action on the bactericidal bodies and is not an action on the serum as a whole, since agglutinization is not affected, and it is not due to the deviation of Complement.

THE ACTION OF QUININE ON THE OXYGEN CARRYING POWER OF THE BLOOD.

PRELIMINARY EXPERIMENTS on this subject were carried out by Professor Meakins while the writer was taking Quinine for the investigation of its action on the non protein blood nitrogen in man.

A distinct diminution in the oxygen carrying power of the blood was apparent. On each of the two occasions on which the writer pushed the administration of Quinine until symptoms of cinchonism were felt, the oxygen saturation percentage of the blood remained constant, as also did the percentage of hæmoglobin.
This fall in the oxygen carrying power of the blood was also found in a guinea-pig which received intraperitoneal injections of Quinine.

This communication is made with the permission of Professor Meakins.

It is of interest to note that Cullen[18] records an increase in the number of Red Blood Corpuscles in the blood following the administration of Quinine: this suggests a compensatory reaction to make up for the lessened oxygen carrying power of the blood.

THE ACTION OF QUININE ON THE OXIDIZING POWER OF THE BLOOD.

With reference to the action of Quinine on the Oxidizing Power of the Blood Cushny[17] states -

"Thus the oxidizing action of drawn blood was shown "to be diminished in several experiments performed "by Binz.

"For example it fails to form the blue oxidation "product of guaiac, or to decolorize indigo when "it is applied to it along with quinine.

"The well-known guaiac experiment is performed as "follows - A fresh solution of guaiac resin in "alcohol, to which some peroxide of hydrogen has "been added, is divided into two parts. To one "a minute quantity of Quinine is added, and one or "two drops of blood are allowed to flow into each "part. The one containing the quinine remains "uncoloured, while the other assumes a blue tint "from the oxidation of the guaiac by the unpoisoned "blood".

It was considered that this reaction might be of use for detecting the presence of quinine in the blood of patients who had been receiving large doses of quinine (i.e. the blue tint would not be produced by blood containing quinine).

The/

The experiment was therefore attempted with the blood of a patient who had received 10 grains of Quinine bihydrochloride intramuscularly 4 hours before.
The blue colouration appeared when the blood was added. The reaction was tried again but before adding the blood, 1 grain of quinine bihydrochloride was added to the guaiac peroxide of hydrogen mixture.
The blue colouration appeared when the blood was added.
It then appeared of interest to test this reaction, and repeat Binz's experiments. Binz's technique, as described by Cushny, was followed but the mixture of guaiac and peroxide of hydrogen was divided into three parts. The first was used as a control.
To the second 1 grain of quinine bihydrochloride was added.
To the third 10 grains of quinine bihydrochloride were added.
On the addition of blood all three gave the blue colouration.
The method of performing the test was altered, the hydrogen peroxide being added last, the other ingredients being well shaken together before it was added. The experiment performed in this manner still gave the blue colouration.
This last method was repeated but ozonic ether was run on to the surface of the mixture instead of adding peroxide of hydrogen.
A well marked blue ring was formed.

The above experiments were repeated using Quinine Hydrochloride and Quinine Sulphate in place of Quinine Bihydrochloride: these salts could not all be got into solution in the amounts used, hence free crystals of quinine were present, but the blue colouration appeared in every case.

In all, the experiments recorded above were carried out 6 times, varying the source of the ingredients, and in all cases the blue colouration appeared. In one series the quinine and blood were allowed to remain in contact for 1 hour before the peroxide of hydrogen was added: when it was added the blue colouration was produced.

This led to the investigation of the power of the blood to decolourize indigo. Binz's method of performing this experiment was as follows: -
Render a solution of Indigo Sulphate alkaline by adding sodium bicarbonate; on the addition of blood to the alkaline indigo solution the colour changes from blue to green: if ozonic turpentine is then added the green colour turns to a clear yellow within a few minutes: Quinine if present will delay this change from green to yellow.
This experiment was performed, using ozonic ether in place of ozonic turpentine. It was found that when Quinine was present in a dilution of 1.100 the colour change had not taken place after 24 hours, while

when Quinine was present in a dilution of 1.10000 no difference between the rate of disappearance of the colour in the Quinine experiment, and the colour in the control experiment, could be made out. The rate of disappearance of the colour varied greatly with the amount of ozonic ether added.

THE ACTION OF QUININE ON THE AMOUNT OF NON PROTEIN NITROGEN IN THE BLOOD.

Prior[20] showed that the administration of Quinine diminished the amount of urea and especially the amount of uric acid in the urine. This he brought forward as evidence that metabolic change in the body was diminished by Quinine.

Recently Cornwall[21] has shown that prolonged administration of Quinine produced a state of chronic nephritis in rabbits.

It appeared to the writer that the diminution in the excretion of nitrogen might be due to its being held up in the blood owing to damage to the secreting tubules of the kidney by Quinine.

Investigation of the amount of non-protein nitrogen in the blood before and during the administration of quinine were carried out. The writer lived on a diet with a fixed nitrogen intake, and on the third day the non protein blood nitrogen was estimated.

For the next two days Quinine bihydrochloride 5 grains t.i.d. were taken, and then a second estimation of the non-protein nitrogen was made.

For the next two days the dose of Quinine bihydrochloride was raised to 15 grains t.i.d. and then a further estimation of the non protein blood nitrogen made.

Results were as follows /

Normal Blood -

28.46 Mgms. of non-protein nitrogen in 100 gms. of blood.

After 5 grains of Quinine bihydrochloride t.i.d. -

30.96 Mgms. of non-protein nitrogen in 100 gms. of blood.

After 15 grains of Quinine bihydrochloride t.i.d -

37.98 Mgms. of non-protein nitrogen in 100 gms. of blood.

Attempts to confirm this finding, however, were not successful.

An experiment on a rabbit which received 0.025 gms. of Quinine (calculated as alkaloidal Quinine) intraperitoneally daily for three days showed a lower non-protein blood nitrogen after the quinine than before.

Rabbit Before Quinine -

36.23 Mgms. of non-protein blood nitrogen in 100 gms. of blood.

Rabbit After Quinine -

33.89 Mgms. of non-protein blood nitrogen in 100 gms. of blood.

A similar result to that obtained from the rabbit was obtained when, again on constant diet, the writer took 15 grains of Quinine bihydrochloride by the mouth for two days.

Before Quinine -

31.35 Mgms. of non-protein blood nitrogen in 100 gms. of blood.

After Quinine -

27.20 Mgms. of non-protein blood nitrogen in 100 gms. of blood.

The estimations were carried out by Miss Christina Hawick, B.Sc., Folin's method being used.

No conclusions can be drawn from the above experiments. Possibly if Quinine were administered over longer periods some more definite results might be obtained.

THE ACTION OF QUININE ON THE KIDNEY.

The action of the prolonged administration of Quinine on rabbits' kidneys has been investigated by Cornwall?[21]

During experiments on attempts to produce anaphylaxis to Quinine in guinea-pigs, and other experiments, several guinea-pigs died of Quinine poisoning. It was thought of interest to examine their kidneys. Slides of these Kidneys are presented with this thesis. A section of the same tissue from a reputedly healthy guinea-pig killed by mechanical means is also presented for contrast.

The Kidney of Guinea-pig No. 9 -

This guinea-pig was killed with 0.1 gramme of Quinine hydrochloride in Ringer's solution injected intraperitoneally: this was found to correspond to 2 minimum lehtal doses.

Description -

Very marked acute toxic change is found in all the functional elements of the kidney: these are more especially marked in the convoluted tubules and glomeruli. In the glomeruli the majority of the nuclei are very faintly staining with hæmatein. In the convoluted tubules there is an almost complete disappearance of the nuclei: the cells are considerably swollen, very granular and almost completely fill the lumen of the tubules.

In/

In the conducting tubules there is again severe damage, especially to the nuclei which are very faintly staining and in many cases have disappeared. There are numerous small hæmorrhages throughout the substance of the kidney.

The Kidneys of Guinea-pigs Nos. 1 and 2 -

These guinea-pigs were killed with 0.05 gramme of Quinine hydrochloride in Ringer's solution injected intraperitoneally - this is 1 minimum lethal dose.

Description -

There is severe damage to collecting and convoluted tubules. The glomeruli appear to be little damaged.

The convoluted tubules show extreme cloudy swelling: the nuclei stain faintly and some have even disappeared. In the collecting tubules the nuclei are also affected, their staining reaction being faint.

There are small hæmorrhages throughout the kidney substance.

In general in guinea-pigs killed with Quinine, the toxic action seems to be more marked on the nuclei in the secreting tubules.

SECTIONS FROM KIDNEYS OF GUINEA-PIG 27/

SECTIONS FROM KIDNEYS OF GUINEA-PIG 27.

This guinea-pig was used in the investigation of the action of Quinine on the oxygen carrying power of the blood.
It received daily injections of 1.100 solution of Quinine in Ringer's solution intraperitoneally during a period of 8 days. On the 1st, 4th and 8th day it received 2. 5 c.cs.: on the other days it received 1 c.c.
It was killed on the 8th day.

Report on Section -

There is evidence of an acute nephritis specially affecting the convoluted tubules. The condition has extended beyond the stages shown by guinea-pigs 1, 2 & 9. A granular degeneration of the secreting epithelium is shown, with well marked catarrhal features. There is evidence of small hæmorrhages though these are not well marked.
Small areas of round celled infiltration can be made out.

This is in accordance with the appearances shown by sections of kidney from guinea-pigs receiving lethal doses of Quinine.

THE ACTION OF QUININE ON SUPRARENALS.

Ramsden, Lipkin & Whitley have recently shown that for its weight the Suprarenal takes up enormous quantities of Quinine.

It has been suggested in this thesis that the sudden fall of blood pressure on the intravenous injection of Quinine might be due to some action in which the suprarenal and the quinine were connected.

A single experiment on this subject is recorded: it was carried out on the writer: the sensations were so unpleasant that its repetition has not been considered.

7.5 Minims of 1.1000 Adrenalin Chloride were injected hypodermically into the writer and records of systolic and diastolic blood pressure were taken along with the pulse rate and pulse tracings.
The tracings of the experiments are presented.
The observations were made by Dr. McCartney and very carefully checked.

A TYCOS Sphygmomanometer, known to be accurate, was used to take the blood pressures: these were taken by auscultation: the systolic controlled by palpation, and the diastolic by the maximum excursion of the indicator.

The following day the writer took 15 grains of Quinine bihydrochloride by the mouth and the injection of adrenalin was repeated in the same amount and at

the same locality. 2½ hours after the quinine had been taken, similar observations were made -

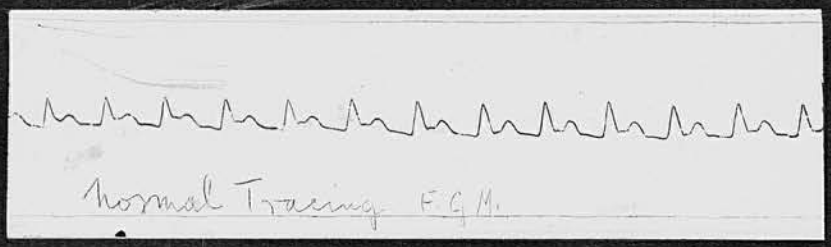
TABLE OF OBSERVATIONS.

BEFORE ADRENALIN -	Without Quinine	With Quinine
Systolic pressure	142	145
Diastolic pressure	104	120
Pulse Rate	86 Tracing taken	83 Tracing taken.
AFTER ADRENALIN -		
(5 minutes after injection)		
Systolic Pressure	146	166
Diastolic Pressure	105	84
Pulse Rate	76	102
(10 minutes after injection -		
Systolic Pressure	152	176
Diastolic Pressure	105	72
Pulse Rate	76 Tracing taken	93 Tracing taken.
20 minutes after injection -		
Systolic Pressure	155	176
Diastolic Pressure	104	72
Pulse Rate	75	90
25 minutes after injection		Tracing taken.

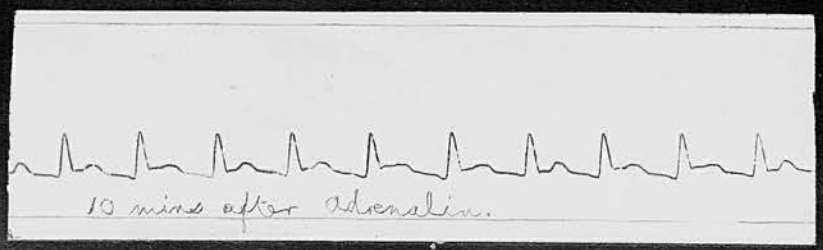
TABLE OF OBSERVATIONS, CONTD.

BEFORE ADRENALIN	Without Quinine	With Quinine.
30 minutes after injection -		
Systolic Pressure	146	176
Diastolic Pressure	104	74
Pulse Rate	69	88
35 minutes after injection	Tracing taken	Tracing taken
40 minutes after injection -		
Systolic Pressure	145	170
Diastolic Pressure	103	72
Pulse Rate	65	80
50 minutes after injection		
Systolic Pressure		170
Diastolic Pressure		80
Pulse Rate		86
60 minutes after injection -		
Systolic Pressure		160
Diastolic Pressure		88
Pulse Rate		78

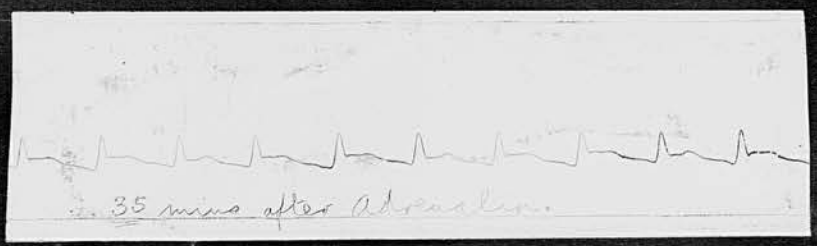
Pressures are given in M.ms. of mercury.



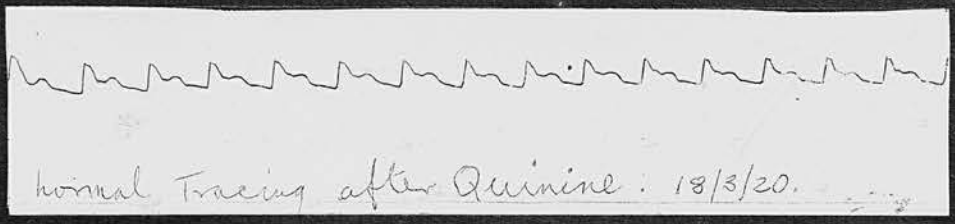
1. Normal.



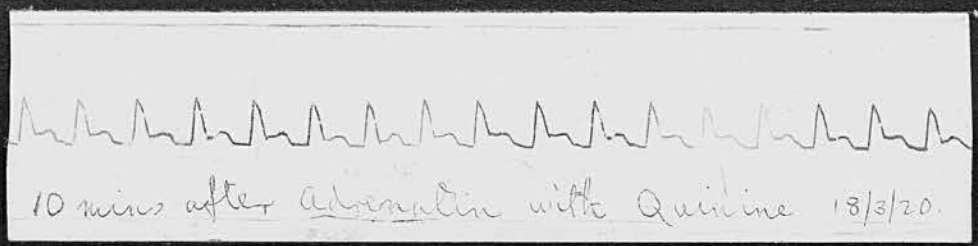
2. Shows slowing of pulse rate.



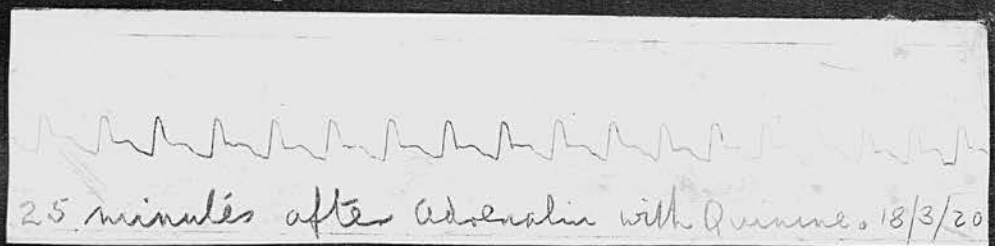
3. Shows slowing of pulse rate.



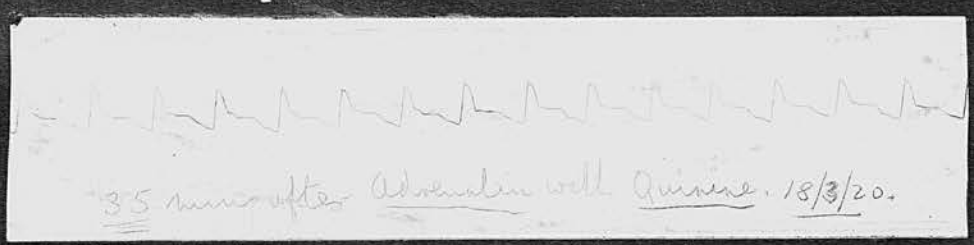
1. Normal after Quinine.
   Pulse of fair tension.



2. Shows increase of pulse rate and rise in systolic with fall in diastolic pressure.



3. Shows increase of pulse rate and rise in systolic with fall in diastolic pressure.



4. Shows increase of pulse rate and rise in systolic with fall in diastolic pressure.

In the experiment with Quinine the action of the adrenalin was more marked than in the experiment without Quinine. The writer felt very uncomfortable.

The systolic pressure rose much higher and the fall in the diastolic pressure was much greater. At one time there was a difference of over 100 m.m. of mercury between the two pressures.

This was quite noticeable on palpation: (a marked water hammer pulse being produced), and this is also shown in the pulse tracings. The altered pressures took a longer time to reach normal in the experiment with Quinine.

No explanation of this experiment is offered. It suggests an action between Quinine and adrenalin.

Sections which were made from the suprarenals of guinea-pigs which were killed by Quinine are presented, as well as a section of reputedly normal tissue for contrast.

Section of Suprarenal of Guinea-pig No. 9, killed with 2 minimum lethal doses of Quinine - Report -

Very severe destruction of all elements: nuclei stain very faintly, or have disappeared. There/

There is fatty degeneration in cells of cortex.
Numerous haemorrhages throughout cortex and medulla.

Section of Suprarenal of Guinea-pig No. 2, killed with 1 minimum lethal dose of Quinine -

Report -

Tissue shows some damage: many nuclei are faintly staining, and some have disappeared: cloudy swelling of cortex and medulla.

SUPRARENAL OF GUINEA-PIG No. 27 -

This guinea-pig had received daily doses of Quinine for 8 days. On 1st, 4th and 8th day 2.5 cc, on other days 1 cc of 1.100 Quinine in Ringer's solution, injected intraperitoneally.

Report on Sections -

Haematein & Eosin Section -

There is evidence of fatty change in the cortex.

The medulla shows the presence of pigment, of a yellowish brown colour, both in large and small granules.

The nature of this pigment was investigated.
Except for several small granules (which evidently contain iron) the pigment does not show the Prussian blue reaction, and therefore is evidently not haemosiderin.
This is further confirmed by the fact that the granules do not stain black with the Ammonium sulphide method: nor do they bleach by this method, which/

which excludes the possibility of their being 'melanin pigment'.

Frozen sections stained with Sudan III show the granules taking up this stain, from which it would appear that they are of the nature of a lipochrome.

The Sudan III shows marked fatty change in the cortical cells.

These sections suggest that Quinine has a deleterious action on the suprarenal bodies.

This action is more marked in the cortex than in the medulla.

The large amount of lipochrome pigment in the medulla of the suprarenal of Guinea-pig 27 is of interest, but scarcity of animals has prevented further investigation.

THE ACTION OF QUININE IN COMBINATION WITH A TOXIN (RICIN).

It has been shows in Part I of this thesis that Quinine in large doses has an unfavourable influence on Pneumonia.

In Part II experimental work has shown that Quinine in a concentration of 1.20000 inhibits the bactericidal power of serum.

Following on this it was intended to investigate what action, if any, Quinine had on the natural and acquired immunity of the guinea-pig to Ricin.

Unfortunately the scarcity of animals has prevented the carrying out of this work.

One experiment only was conducted but it has proved to be so interesting that it is here recorded.

The minimum lethal dose of Ricin for a guinea-pig of about 350 gms. was found to be 2.5 cc of a 1.100000 dilution of the sample of Ricin used, when the dilution had been made up just before use.

Actually it was found that 2.5 cc killed a guinea-pig of 380 gms. within 46 hours, while 2 cc did not kill a guinea-pig of 370 gms. when injected hypodermically.

In a similar way it was found that 5 ccs. of a 1.100 solution of Quinine in Ringer's solution was the minimum lethal dose for a guinea-pig of approximately the same size, since 5 c.cs. killed

and/

and 4 c.cs. did not kill guinea-pigs of this size when injected intraperitoneally.

EXPERIMENT I.

TO INVESTIGATE THE ACTION OF QUININE ON THE NATURAL IMMUNITY OF THE GUINEA-PIG TO RICIN.

It was decided to use a large though non-lethal dose of ricin, and accompany it with an injection of Quinine of half its minimum lethal dose, since it has been found that such a dose is well tolerated by the guinea-pig on several successive days.

The inoculation was performed as follows -

Guinea-pig.	Weight.	Ricin 1.100000	Quinine 1.100	
I.	Control 350	1.5 cc	-	
II.	Control 370	-	2.5 cc	Dose of Quinine repeated in 24 hours.
III.	315	1.5 cc	2.5 cc	
IV.	380	1.5 cc	2.5 cc	
V.	360	1.5 cc	2.5 cc	
VI.	345	1.5 cc	2.5 cc	

Guinea-pig VI died during the night of the 6th day.

Guinea-pigs III, IV & V died on 7th day.

Unfortunately these guinea-pigs died while the writer was absent, since a negative result to this experiment was assumed when no deaths had occurred during the first four days.

On/

On post mortem examination of the bodies of the guinea-pigs, mild signs of ricin poisoning were observed, in addition, in all cases, the livers were very markedly congested.
The condition of the bodies was such that any hope of obtaining material useful for microscopic examination had to be given up.
The controls with Ricin alone and Quinine alone did not die, and while estimating minimum lethal doses, the amounts of Quinine or Ricin given, when given independently, were found not to be lethal.

Unfortunately this experiment could not be confirmed. It is of interest to recall that cases of pneumonia treated with Quinine at Stobhill lived on an average for 7 days after receiving the Quinine.
This experiment, though unconfirmed, suggests that Quinine decreases the natural immunity of the Quinea-pig to Ricin.

ATTEMPTS TO PRODUCE QUININE ANAPHYLAXIS.

Hypersensitiveness to Quinine has lately received a considerable amount of attention, and several authorities now consider this hypersensitive--ness to be of the nature of anaphylaxis.
Boermer[22] found that he himself was extremely sensitive to Quinine, and discovered that he gave a skin reaction similar to Von Pirquet's test for tuberculosis, towards Quinine. He experimented with his serum on guinea-pigs: in his published description, however, he states that his control guinea-pigs were killed by the same dose of quinine as he gave his experimental guinea-pigs.
It is interesting to note that his lethal dose was 0.5 cc of a 1.100 solution of Quinine bisulphate in normal saline.
The writer found the minimum lethal dose of quinine reckoned as alkaloid to be 5 cc of a 1.100 solution in Ringer's solution for a 350 gram guinea-pig.
Recently O'Malley & Richey[23] recorded two cases of "Quinine Idiosyncracy" in which Boermer's skin reaction was positive. Complete desensitization was accomplished in one case, and partial de-sensitization in the other by administering repeated small doses of Quinine.
In both cases sensitising doses were traced.

Attempts/

Attempts were made to produce Anaphylaxis in guinea-pigs.

All the guinea-pigs used in working up to the minimum lethal dose of Quinine were kept for three weeks and then received ½ minimum lethal dose. Eight guinea-pigs were tested in this way and none gave any anaphylactic symptoms.

The serum of a guinea-pig which had received 1/5 minimum lethal dose of Quinine 28 days before, was collected and 2.5 cc injected intravenously into a rabbit: no symptoms followed the intravenous injection of Quinine after a suitable interval.

Manoiloff[24] claims to have produced a passive anaphylaxis in rabbits and guinea-pigs, by injecting 3 - 5 cc of serum from three cases which showed marked intolerance to Quinine.

The animals injected with the serum died very rapidly on the injection of Quinine, whilst control animals injected with normal serum had no symptoms when injected with Quinine.

While the writer was taking Quinine in the experiments on non-protein blood nitrogen, he had an interesting experience. Prior to the first experiment he had taken no quinine for some years. During the first experiment he pushed Quinine until symptoms of cinchonism were established, when the quinine was stopped, the last dose being taken on the 19th of February. The second experiment was started/

started on the 8th of March, 18 days afterwards. Before going to bed on that day the writer took 15 grains of Quinine bihydrochloride by the mouth. About 1½ hours after going to bed he was seized with severe rigors and chilliness: this was followed by marked pulmonary embarrassment of an asthmatic type, and followed later by a profuse expectoration. During the rigors the pulse was hardly perceptible.

The following day the dosage was continued, and beyond having symptoms of cinchonism, no further trouble arose. A dose of 15 grains of Quinine bihydrochloride which was taken 8 days afterwards produced no effect beyond ringing in the ears.

It might be suggested that the first experiment produced a sensitization: the first dose of the second experiment an anaphylactic condition, and desensitization was produced by continuing the dosage of Quinine during the second experiment

- S U M M A R Y. -

(1) Quinine in large doses can diminish the natural resistance of the body to disease.

(2) Quinine in a dilution of 1.20000 will decrease the phagocytic capacity of leucocytes, and also inhibit the bactericidal action of the serum.

(3) Quinine in a solution of 1.4000 or weaker does not inhibit the amoeboid movements of leucocytes so far as can be observed on a warm stage during the period of one hour.

(4) The action of Quinine on the bactericidal power of the serum is a direct action on the bactericidal bodies in the serum, since Quinine does not affect the agglutination of bacteria by a high titre serum, nor deviate complement.

(5) There is evidence that Quinine may decrease the oxygen-carrying capacity of the blood: have a deleterious action on the kidneys, especially on the secreting epithelium of the convoluted tubules, and affect the suprarenal bodies and their secretion.

(6)/

(6) There is also some evidence that Quinine may produce a condition of Hypersensitiveness, and that that condition is of the nature of an Anaphylaxis.

(7) The high mortality in the N'Gung and Stobhill series amongst the cases treated with Quinine may well be due to the above actions of Quinine, since Quinine in a concentration of 1.20000 or higher may exist in the lung tissue, although such a concentration is not found in the blood stream.

(8) The use of Quinine in Pneumonia can only be justified on its antipyretic action, since Brown[25] has shown that a concentration of Quinine of 1.1000 is required to kill the pneumococcus, while Ramsden, Lipkin & Whitley[10] have shown that such a con-centration in the lung tissue does not occur.

(9) Quinine in large doses is a source of danger to the recipient as it decreases the efficiency of his defense mechanism, and large doses should only be administered when there is a real indication for their use (i.e.- when the patient is suffering from Malaria).

REFERENCES.

1.	GOODALL.	Edin. Med. Journal. March 1919. Malaria in Macedonia.
2.	SWALE & RUSSELL.	Lancet. May 17th 1919. P.841. Quinine as an Abortificiant.
3.	MANSON.	Tropical Diseases. 6th Edit. Manson. P. 127.
4.	CASTELLANI & CHALMERS.	Manual of Trop. Med. 3rd Edit. 1919. P 1201.
5.	BINZ.	Lectures on Pharmacology Binz. Vol. II. The New Sydenham Society Translation 1897. P 228.
6.	KRUNKENBERG.	Verteichend Physiol. Studien an der Kusten der Adria 1888 L S 8
7.	ENGELMANN.	Engelmann's letter to Binz. Arch. f. Physiol. Bonn. Bd. ii S 293.
8.	McGILCHRIST.	Indian Journal of Med. Vol III. Quin.
9.	KERNER.	Arch. f.d. ges Physiol. Bd III. S 123 & 160.
10.	RAMSDEN, LIPKIN, & WHITLEY.	Ann. of Trop. Med. & Parasit-ology. Vol. XI p. 434 Vol. XII p. 233 Vol. XIII p. 149
11.	DUDGEON.	Journal of Hygiene. Vol. 18. No. 3 October 1919.
12.	BAHR.	Lancet. Jan. 10th 1920. p. 84.
13.	WILSON.	American Journal of Phys. Vol. XIX p. 445.
14.	GALRITCHEVESKY.	Ann. de L'Inst. Pasteur. 1890 (4) 346.
15.	IKEDA.	Journ. Pharmacol. 1916 (8) 137.
16.	DUNN	Journal of Pathology. 1911. P.21.
17.	CUSHNY.	Pharmacology & Therapeutics 5th Edit. p. 412-13.

REFERENCES, CONTINUED.

18. CULLEN. Thesis for Graduation 1911. Edin. University Library.

19. GULLAND & GOODALL. The Blood. 2nd Edit. P. 76.

20. PRIOR. Ueben des Einfluss des Chinins auf den Stoffinechsel des gesunden Organismus Arch. f.d. ges. Physiol 1884. Bd. XXXIV. S. 237.

21. CORNWALL. (The Pharmaco-Dynamics of Quinine. (Indian Journal of Med.Research July 1919.

22. BOERMER. Journal of American Med. Assoc. LXVIII. p. 907. 1917.

23. O'MALLEY & RICHEY. Arch. Int. Med. October 1919. record of 2 cases of Quinine Idiosyncracy.

24. MANOILOFF. Zeitschr. f. Immunitats. I. Orig. XI. p. 425. 1911.

25. BROWN. Proc. Biol. Chem. 2. 70. 1912.