VOLUME 1

MEPACRINE AND FALCIPARUM MALARIA

A Study of the Chemotherapeutic, Pharmacological, and Toxic Properties of the Drug.

By

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INTRODUCTION

Atebrin and Malaria. - Atebrin* was prepared in Germany in 1932 and was found to have marked anti-malarial properties.[1] In 1937 it was generally agreed that the drug was effective in the treatment of falciparum, tertian, and quartan malarias[2] and its superiority over quinine as a preventive of malaria had also been clearly established,[3,4,5,6,7] though it did not supplant quinine because of sporadic reports of serious effects following its use as a prophylactic[7] and therapeutic agent.[8] More data on the toxicity of atebrin were required before it could be recommended on a large scale for malaria prevention. However, a decision was forced by Japan's entry into the war at the end of 1941. This event resulted in the loss of natural sources of quinine and made it certain

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*Atebrin or atebrin-like compounds have different names in almost every country in which they are produced. Synonyms are:- Atebrin - German, Crinodora - Italian, Quinacrine - French, Acriquine - Russian, Quinacrine (previously Atabrine) - American, Mepacrine - British.

that armies would have to be maintained in highly malarious parts of the world. Since atebrin could be prepared synthetically and quinine could not, the risk of toxic effects from atebrin had to be weighed against its advantages as a malarial preventive. Experience in the 1914-18 world war was decisive: the risk of atebrin toxicity was worth taking.[9] Investigations on the synthesis of atebrin were made and methods for large scale production were developed. A drug, named mepacrine, was prepared that was claimed to be the same as German atebrin.

The investigations to be dealt with in this thesis started in 1943. Their object was to assess, under controlled conditions, the value of mepacrine as a preventive of falciparum malaria* and to determine the best methods of employing the drug for this purpose. Falciparum malaria was chosen for experimental infection because this was the type of malaria most likely to cause a military disaster. The work has entailed the following:- employment

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* Synonyms are malignant tertian, and tertian malaria.

of healthy volunteers who were willing to be infected with malaria; provision of large numbers of anopheline mosquitoes carrying sporozoites; hospital beds for observation and treatment; and laboratory facilities for microscopic and biochemical examinations.

In previous work of this kind, dose of drug has been employed as the reference index. Earlier workers have sought to define, more or less by trial and error, the dose that would give a desired therapeutic effect without causing toxic reactions. The greater the difference between minimal therapeutic dose and minimal toxic dose - the greater the therapeutic latitude in the employment of the drug. But an inevitable serious drawback to this approach is the variation in clinical response of different individuals to the same dose of drug. Large numbers of observations usually had to be made and years had to elapse before the therapeutic limits of a new drug could be properly defined. This method of investigation

was not suitable for the needs of the Army in 1943 because time was of great importance. The great advantage of the new approach to sulphonamide chemotherapy over the older empirical method outlined above, was pointed out by Shannon[10] and was largely responsible for the method of investigation employed in this work. James[11] considered that the morphological changes in malaria parasites observed soon after administration of mepacrine was evidence of a direct action of the drug on parasites. Shannon concluded that if mepacrine acted directly on parasites, then this action probably depended on the concentration of the drug in the body. This conception offered a new approach to the study of antimalarial drugs and the introduction of accurate methods for estimating minute amounts of mepacrine in body fluids[12,13] provided a unique opportunity to investigate the chemotherapeutic action of mepacrine in relation to its concentration in body fluids and tissues. The

methods introduced were sufficiently sensitive to allow ranges of concentration to be estimated that were much lower than had ever been attempted before in chemotherapeutic investigations.

Under the new scheme the value of mepacrine for malaria prevention has been assessed in the first place by giving varying doses of the drug to volunteers who had never previously been exposed to malaria, and then comparing their reactions to experimental malarial infection with the reactions of non-immune controls who were similarly infected but received no drug. The chemotherapeutic action of mepacrine in relation to the concentration of the drug in the blood has been examined by comparing: (1) - the blood mepacrine levels of volunteers who took mepacrine but developed malaria with (2) - the levels of similar volunteers who escaped the disease. If the blood mepacrine should prove to be an index of the chemotherapeutic action of the drug, then it was hoped to define the minimum concentration that would prevent malaria; and, by concurrent

investigation of the pharmacology and toxicity of mepacrine, to define the best methods of maintaining an effective blood level.

The investigations are described in five parts. Part 1 deals with the chemotherapeutic action of mepacrine in falciparum malaria; part 2, with the pharmacology of the drug; part 3, with its toxicity; part 4, with the application of the results in the prevention and treatment of malaria with mepacrine; and part 5, with detailed results in the form of tables, graphs, and illustrations. For ease of reference the work is presented in two volumes. The first volume includes parts 1, 2, 3, and 4; the second volume, part 5.

PART 1

THE CHEMOTHERAPEUTIC ACTION OF MEPACRINE IN FALCIPARUM MALARIA*

Fifty-five healthy volunteers taking different doses of mepacrine were experimentally infected with sporozoites of falciparum malaria to investigate the following:-

1. - The reactions of volunteers taking mepacrine to experimental malarial infection.

2. - The relation between dose of mepacrine and the value of the drug as a malarial preventive.

3. - The relation between blood mepacrine and the chemotherapeutic action of the drug in the prevention of falciparum malaria.

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* This part of the investigation was carried out in collaboration with K.Mellanby, D.Sc., W.D.Nicol, F.R.C.P., and P.G.Shute, F.R.E.S.

The majority of the volunteers were members of the Friends' Ambulance Unit and Pacifists' Service Unit and were recruited and organised by Dr.Mellanby. P.G.Shute prepared batches of infected mosquitoes, infected the volunteers, and examined all blood films for parasites at the Ministry of Health Laboratory, Horton. Dr.W.D.Nicol made clinical observations and was responsible for the treatment of the first three groups of volunteers who were infected. The writer was responsible for blood-mepacrine estimations throughout, and for clinical observations and treatment of the fourth and fifth groups of volunteers.

Methods of Infection. - Seven volunteers were infected with an Italian strain of falciparum malaria, and 48 with a Roumanian strain. Fifty volunteers were infected by exposure, on 1 to 4 occasions, to the bites of mosquitoes whose salivary glands contained numerous sporozoites. The other five volunteers were infected by intravenous injection of a sporozoite-gland suspension prepared by dissecting the salivary glands of infected mosquitoes, and after rupture, suspending them in Locke's solution. Immediately after biting, the number of mosquitoes that had fed on a volunteer was counted by observing the presence or absence of fresh blood in the mosquitoes; large numbers of sporozoites were demonstrated in the salivary glands of the majority of mosquitoes that had actually fed. Similarly, only glands that contained numerous sporozoites were used to prepare the sporozoite-gland suspension for intravenous injection.

To ensure that the mosquito bites and the sporozoite-gland suspensions were able to induce

malaria, an untreated control volunteer who had never been exposed to malaria was infected along with each group of volunteers taking mepacrine. Without exception the controls developed malaria 7 to 14 days after sporozoite infection; so that it was reasonable to attribute absence of malaria in the volunteers taking mepacrine to the action of the drug.

Reactions to Sporozoite Infection

Thirteen of the 55 volunteers developed malaria. Twenty others had fever ranging from 100 to 103°F., but parasites were not found in their blood despite careful search of thick and thin films. The malarias and the fevers appeared 9 to 21 days after the first or last sporozoite infection. Fever with parasitaemia was of one day's duration in 15 volunteers and was characterised by a rapid rise of temperature and an equally rapid fall. In the other 6 volunteers the fever was remittent or intermittent, and 2 or 3 temperature peaks were recorded over a period of 2 to 6 days. Representative temperature charts of

fevers and malarias are shown in Graph 1 (Vol.2 p35). The symptoms that accompanied the fever without demonstrable parasitaemia varied in severity from minor effects that did not require bed treatment to incapacitating reactions that were indistinguishable clinically from an acute attack of malaria. The minor effects were mainly malaise and headache, often diagnosed by the men themselves as the "start of a cold in the head", but a nasal discharge, the usual sign of acute coryza, did not appear. The severe reactions were characterised by sensations of cold followed by feelings of warmth accompanied by occipital headache, pain behind the eyes, and pains in the neck, back, and limbs. All grades of severity between minor and severe reactions were encountered.

The incidence of malaria, and of fever without parasitaemia, in five groups of volunteers over a period of a year is shown in Table 1 (Vol.2 p1). Fever without parasitaemia appeared in all groups although they were infected with sporozoites at

different times of the year. Its incidence was no higher in December 1943 when influenza was prevalent than in June 1944 when the risk of contracting intercurrent infection was minimal.

When first encountered, fever without demonstrable parasitaemia was regarded as an incidental event, probably due to an intercurrent infection, because complete reliance was placed on prolonged examination of blood films to disclose parasites in all patients with malaria. But this view had to be revised when fever kept reappearing in volunteers taking mepacrine and other antimalarial drugs after experimental infection with sporozoites of falciparum malaria. Fever is now considered to be a sequel to malarial infection. This view has been strengthened by the successful transmission of malaria to another individual by injecting the blood of a volunteer when fever without obvious parasitaemia developed.

Dose of Mepacrine and Falciparum Malaria

The volunteers who remained at work, took

mepacrine in the presence of a non-medical witness and a signature in a diary was obtained for each dose taken. There was no reason to believe that the drug was not taken as prescribed. Two mepacrine-dosage regimes were employed:-

(1) a daily dose of 0.1g. mepacrine

(2) a loading dose of 1.0g. in three consecutive days, (0.1g. on day 1, 0.3g. on day 2, and 0.6g. on day 3) followed by 0.1g. daily for variable periods up to 25 days (see Table 2 Vol.2 p2).

A loading dose of 1.0g. in 3 days, followed by 0.1g. on the day of infection and for 24 days thereafter, was found to be most effective in preventing malaria. None of the volunteers treated in this way developed a frank attack of the disease. When a daily dose of 0.1g. was given for 21 and 35 days before infection, and for 28 and 24 days after infection, 1 in 25 infected volunteers developed malaria. On the other hand when dosing with 0.1g. daily for 20 days started on the day of infection, 2 of 6 infected volunteers developed malaria. Similarly, when a

loading dose of 1.0g. was given for 3 days before infection, followed by 0.1g. on the day of infection, and for the next three days, all 5 volunteers developed malaria.

Fever without demonstrable parasitaemia occurred in volunteers taking each regime so that no relation could be established between dose of mepacrine and this condition.

These findings suggest that, in general, dose of mepacrine is directly related to prevention of frank malaria but that assessment of results on a dosage basis has serious limitations. In the first place the number of volunteers is too few to make sound conclusions; secondly, no explanation can be given why some individuals develop malaria while others taking the same doses of mepacrine escape the disease; and thirdly, fever without demonstrable parasitaemia appears to bear no relation to dose. For these reasons, blood mepacrines were employed in the hope that they would provide a better index to assess more precisely the chemotherapeutic action

of mepacrine.

Blood Mepacrine and Falciparum Malaria

The relation of blood mepacrine to the chemotherapeutic action of the drug in falciparum malaria has been examined by giving volunteers different doses of mepacrine, infecting them with sporozoites of falciparum malaria, and then comparing the blood mepacrines of the volunteers who developed malaria, with the blood mepacrines of those who escaped the disease. First, the method of estimating blood mepacrine will be outlined; second, the findings will be recorded separately for the occurrence of malaria and fever without parasitaemia.

Blood-mepacrine Estimation. - When the investigations started, three methods of estimating mepacrine in tissues and body fluids had been developed: the Masen method,[13] and the single- and double-extraction methods of Brodie and Udenfriend.[12] These methods required extraction agents of a high standard of purity. Suitable extraction

agents for Masen's method and for the single extraction method of Brodie and Udenfriend were obtainable. In both these methods small blank values were obtained. The single extraction method of Brodie and Udenfriend was selected because it required only one extracting agent and because it had been shown to combine high specificity with excellent extraction recoveries over the range of concentration likely to be encountered when small doses of mepacrine are given. Estimations had to be made on whole blood as the only instrument available for measuring low fluorescent intensities was a Hilger Fluorimeter. After modification it was capable of reading a concentration of mepacrine equivalent to about 20 ug. per litre of blood. It was therefore suitable for whole blood but not for plasma estimations, in men taking the small doses of the drug employed in the prevention of malaria. The following is a brief account of the method. Blood is laked with distilled water. An alkali buffer and ethylene dichloride are added and the

mixture is shaken. The mepacrine plus the degradation products are selectively extracted into the ethylene dichloride. The ethylene dichloride is then separated and washed in a strong alkali to remove mepacrine degradation products. The aqueous phase is removed by aspiration and the ethylene dichloride containing pure mepacrine remains. After transference to a clean test tube a small amount of glacial acetic acid is added to stabilise and enhance the fluorescence. The blood mepacrine is determined by comparing the fluorescent intensity of the acidified ethylene dichloride extract of blood with that of a standard prepared by the same extraction procedure from a stock aqueous solution of known strength. Small but fairly constant reagent blank readings are obtained with this method. They are always subtracted from the standard and unknown.

Later, a Coleman Fluorimeter was available and its performance was compared with that of the Hilger Fluorimeter. Both instruments had adequate

sensitivity for the range of concentration encountered in whole blood. The Coleman was much more sensitive but it tended to lose sensitivity over a period of a year, whereas the sensitivity of the Hilger remained more or less constant. But even then the Coleman was more sensitive than the Hilger. The loss of sensitivity was not due to discharge of the batteries. It may have been due to changes in the photocell or in the amplifier.

The reproducibility of blood mepacrines, gauged by comparing triplicate estimations from the same sample of blood, are shown in Table 3 (Vol.2 p3).

The difference in triplicate estimations from the same sample of blood was insignificant over a concentration range of 66 to 286 ug. per litre. This finding, together with the high degree of specificity and the excellent extraction recoveries reported by Brodie and Udenfriend,[12] suggests that the method is reliable.

Blood Mepacrine and Malaria. - Blood was withdrawn for mepacrine estimation immediately before the

daily dose of drug was given, so that minimal daily levels were determined. The incubation period of malaria in the volunteers taking mepacrine varied from 9 to 21 days, but it was reasonably constant in each group of volunteers exposed to the same type and number of infections. The relation between blood mepacrine and the occurrence of malaria has therefore been examined by comparing the blood levels of the malarias in each group within 24 hours of the onset of malaria, with the levels of the other volunteers in the same group at the same time.

The blood mepacrine values of 12 volunteers who developed malaria, and those of comparable volunteers who escaped the disease, are shown in Table 4 (Vol.2. p4). If the blood mepacrines are compared group by group, it will be seen that the levels in the malarias were always lower than the minimum levels of the others. The blood mepacrines of the 12 malarias ranged from 10 to 77 ug. per litre, the values of the others ranged from 100 to 680 ug.

per litre. It would therefore appear that a minimal daily blood-mepacrine level of 100 ug. per litre just before the onset of malaria was necessary to prevent the disease.

Typical blood mepacrine curves of 4 volunteers throughout the incubation period are shown in Graph 2 (Vol.2 p36). Two volunteers received a total dose of 1.0g. mepacrine in 3 days at different times in the incubation period; one developed malaria, the second had no reaction. The other two volunteers were given 0.1g. mepacrine daily for 20 days beginning on the day of infection; one developed malaria, and the second had a temperature of 100°F. 12 days after infection but parasites were not found in the blood and the fever did not recur. The blood mepacrines of the two malarias were less than the blood mepacrines of the other two at the onset of the disease. The levels on the day of infection and for 6 days thereafter did not appear to have much influence on the subsequent course of events. The blood mepacrine

just before the onset of malaria decided whether or not the disease appeared.

These results indicate that the prevention of malaria with mepacrine depends entirely on the concentration of the drug in the blood.

Blood Mepacrine and Fever without Parasitaemia. - The relation of blood mepacrine to fever without parasitaemia has been examined by contrasting the blood mepacrines of the same volunteer before the onset of fever and after it had subsided. Blood for mepacrine estimations was taken when the temperature was normal. The results are shown in Table 5 (Vol.2 p5).

The numbers are small, but in general, the closer to the onset of fever that estimations were made the lower were the blood mepacrines, and conversely, the closer to the day of subsidence of fever that estimations were made the higher were the blood mepacrines. These findings suggest that fever without parasitaemia may have some influence on blood mepacrine. Evidence supporting this view

has been found in three other volunteers who developed malaria after mepacrine administration had been stopped:-

Volunteer 1. - When the temperature returned to normal after the first peak of fever the blood mepacrine was 130 ug. per litre. Before starting treatment with mepacrine and after the temperature had reached a second and much higher peak, the blood mepacrine had risen to 500 ug. per litre. The spontaneous rise in blood mepacrine coincided with the fever.

Volunteer 2. - A first relapse of M.T. malaria was treated with mepacrine (3.4g. in 7 days). A second relapse occurred 12 days after the last dose of mepacrine. The blood mepacrine at the onset of the second relapse was 13 ug. per litre, and the plasma mepacrine was 6 ug. per litre. Twelve hours later the temperature was 104°F., the blood mepacrine was 390 ug. per litre, and the plasma mepacrine was 40 ug. per litre, though no mepacrine had been given.

Volunteer 3. - Two attacks of fever without parasitaemia were followed by an afebrile period that preceded the onset of a frank attack of malaria. The blood mepacrine curve throughout the incubation period, together with data on infection, doses of mepacrine, and fever, are shown in Graph 3 (Vol.2 p37). Fever was observed on three occasions. Parasites were not found in the blood despite careful search of thick and thin films during the first two attacks, but they were found the third time that fever appeared. Blood, taken from the volunteer at the time of the first fever when no parasites were found, caused malaria after injection into another individual. Spontaneous rises in the blood mepacrine were found that coincided with the first two attacks of fever, and following these rises, fever subsided. But after the blood mepacrine had fallen to a very low level, a frank attack of malaria developed.

A pronounced rise in blood mepacrine associated with fever was observed in three volunteers.

This rise was spontaneous and occurred long after mepacrine dosing had been stopped so that drug administration was not responsible for the change. The relatively high mepacrine content of tissues and cells suggests that the spontaneous rise in blood mepacrine may have resulted from:-

(1) a transference of mepacrine from tissues to the blood.

(2) an increase in the cellular elements of the blood, particularly leucocytes.

The magnitude of the rise, the simultaneous increase in the plasma mepacrine of one volunteer, and the fact that leucopenia is the usual finding during malarial fever, suggest that the increase in blood mepacrine was mainly due to transference of mepacrine from the tissues. The cause of the rise is uncertain, but it may result from the diminution in alkali reserve that accompanies fever, as a small change in pH has been found to alter the partition of mepacrine in a simple protein-water system in this way (Vol.2 p50). But apart from the precise

cause of the rise in blood mepacrine, the nature of the change explains the occurrence of fever without parasitaemia. If parasites are present in the blood when the concentration of mepacrine has fallen below the parasiticidal level, then the parasites may be able to proliferate and cause fever. The increase in the blood mepacrine resulting from the fever may raise the level sufficiently to kill the parasites and so abort the attack of malaria. Thus while cure of the attack of malaria ultimately depends on the tissue mepacrine, the prevention of fever depends entirely on the blood mepacrine.

Summary. - The chemotherapeutic action of mepacrine in falciparum malaria has been found to be related to some extent to dose of mepacrine but more precisely to the blood concentration of the drug. Dose was an inferior chemotherapeutic index because it offered no explanation why some individuals developed malaria while others taking the same dose escaped the disease. Blood mepacrine estimations on the

other hand, showed that those who developed malaria invariably had lower blood levels than the others who escaped the disease, and they also provided a possible explanation for the occurrence of fever without parasitaemia. For these reasons blood mepacrine has been employed to define the aim of the preventive treatment of falciparum malaria with mepacrine.

A frank attack of falciparum malaria did not develop in volunteers experimentally infected with sporozoites if the minimal daily blood mepacrine was 100 ug. per litre or more towards the end of the incubation period. The aim of preventive treatment may therefore be defined as the maintenance of a minimal daily blood-mepacrine level of 100 ug. per litre throughout the period of exposure to infection, and for one month thereafter to deal with infection contracted just before leaving the malarious area. Possible methods of maintaining this minimal daily blood-mepacrine level are dealt with in the pharmacological and toxicity investigations.

PART 2

THE PHARMACOLOGY OF MEPACRINE

Mepacrine is a yellow basic acridine dye that is soluble in water to the extent of about 5 g. per 100 ml. at 37°C.. It is stable in powder form but in aqueous solution it is slowly broken down to another compound that is almost insoluble in water.

The dye-properties of mepacrine are responsible for its unusual distribution in the human body. The mepacrine concentration of tissues and cells has been found to be much higher than the concentration in plasma. For example, the concentration in leucocytes was more than 400 times that of plasma and even in plasma 80 to 90 per cent is probably bound to protein.[10] As a consequence of this distribution Shannon argued, that if mepacrine acts directly on malaria parasites, the free plasma water mepacrine is of fundamental importance

chemotherapeutically and pharmacologically, because the plasma water mepacrine is in equilibrium with tissues, cells, proteins, and malaria parasites whether they are intracellular or in the plasma water.

Since it is not practical to estimate plasma water mepacrine, blood mepacrine was taken as a possible index to investigate the chemotherapeutic action of the drug. Two important observations were made that have an important bearing on the pharmacology of the drug. They were:-

1. - The limitations of dose and the advantage of blood mepacrine as a chemotherapeutic index of the action of mepacrine in falciparum malaria.

2. - The rise in blood mepacrine during fever, possibly resulting from the transference of the drug from tissues to the blood.

The limitation of dose as a chemotherapeutic

index has made it necessary to define the relationship of blood mepacrine to dose, route of administration, and period of administration. Preliminary investigations on the degradation of mepacrine in the body have also been carried out and the relation of blood mepacrine to urinary mepacrine and plasma mepacrine has been investigated.

The rise in blood mepacrine observed during fever in volunteers infected with falciparum malaria may result from the transference of mepacrine from tissues to the blood. This implies an alteration in the tissue:blood partition of the drug. Intrinsic factors apart from dose that alter blood mepacrine may therefore be of great importance chemotherapeutically. Direct investigation of the effect of such factors on the tissue:blood partition was not practical but the basic principles regulating the partition of mepacrine in a simple protein:water:mepacrine system have been studied.

The relation of blood mepacrine to dose, route of administration, and period of administration;

and the relation of urinary mepacrine to dose and period of administration will first be described. Then degradation of mepacrine in blood, plasma, urine, and faeces will be considered and will be followed by an examination of the factors that influence the protein:water partition of the drug. Finally the interrelations of blood, plasma, and urinary mepacrine will be discussed.

Blood Mepacrine and Dose. - The relation of blood mepacrine to dose has been investigated by giving groups of healthy volunteers different doses of the drug by mouth, over the same period, and estimating the blood mepacrines at comparable intervals. The volunteers were all healthy soldiers taking the same diet and were confined to bed during the five days of the investigation. Mepacrine was given in tablet form and a half pint of water was taken with each dose. The dosage regimes and the number of individuals employed were as follows:-

Regime	Dose of Mepacrine	No.of Volunteers
1	Day 1 - Initial dose 0.6g. Days 2 to 5 inclusive 0.3g. daily Total dose 1.8g. in 5 days	8
2	Day 1 - Initial dose 0.2g. 0.3g. at 4 hrs. and 0.5g. at 12 hrs. Days 2 to 5 inclusive 0.6g. daily Total dose 3.4g. in 5 days	7
3	Day 1 - 0.2g. 4 hrly, 6 doses Day 2 - 0.2g. 6 hrly, 4 doses Days 3 to 5 inclusive 0.2g. 12 hrly, 2 doses Total dose 3.2g. in 5 days	8

The frequency of dosing and the total amount of mepacrine given in 5 days was different in the three regimes. In regime 1, single daily doses were given throughout. In regime 2, increasing daily doses were given in the first 12 hours, thereafter single daily doses were given on days 2 to 5. The single daily doses in regime 2 were twice the daily doses given in regime 1. In regime 3 an attempt was made to give approximately the same total daily dose as in regime 2, but in small doses given more frequently throughout the

24 hours.

Regime 1 (1.8g. in 5 days). - Blood mepacrines were estimated at 4, 12, 24, 48, 72, 96, and 120 hours after the first dose. There were marked variation in the levels of different individuals taking the same dose, but the group mean values provided a good index of the trend in each individual. The group mean blood mepacrines at the above times were:- 213, 173, 84, 179, 225, 245, and 264 ug. per litre.

Regime 2 (3.4g. in 5 days). - Blood mepacrines were estimated at 4, 12, 25, 48, 72, 96, and 120 hours after the first dose. As in regime 1, marked differences in the individuals' values were observed, but the group mean level reflected the trend in each volunteer. The group mean values at the above times were:- 105, 246, 384, 415, 560, 679, and 873 ug. per litre of blood.

Regime 3 (3.2g. in 5 days). - Estimations were

made at 8, 16, 29, 53, 77, 101, and 125 hours after the initial dose. The corresponding group mean blood mepacrines were:- 131, 363, 387, 618, 604, 665, and 858 ug. per litre. As in the previous two regimes, these values were indicative of the trend in each individual.

Detailed results are given in Table 6 (Vol.2 p6).

Group mean blood mepacrines for the three regimes are shown in Graph 4 (Vol.2 p38). In regime 1, a total dose of 1.8g. mepacrine was given in 5 days and the group mean blood mepacrine curve 24 hours after each dose was uniformly lower than the curves for regimes 2 and 3 in which total doses of 3.4 and 3.2g. mepacrine were given respectively. This suggests a direct relationship between blood mepacrine and dose in the initial days of dosing.

In regimes 2 and 3 approximately the same total dose was given in 5 days but the frequency of dosing was different. The values for both regimes 24 hours after total doses of 3.4 and 3.2g.

were 873 and 858 ug. per litre of blood respectively. That is, for practical purposes the blood mepacrines were the same, and since the differences in the mid-parts of the curves are accounted for by differences in the total dose given, frequency of dosing would seem to be less important than the total dose in determining the minimal daily blood mepacrine level. However, frequency of dosing may be important in the first day of dosing when it is desirable that a particular blood-mepacrine level should be quickly attained and then maintained. In regime 1, a single dose of mepacrine was given on the first day, the group mean blood mepacrine at 4 hours was 213 ug. per litre and at 24 hours it had fallen to 84 ug. per litre. In the other two regimes, more frequent doses were given and the levels progressively increased during the first 24 hours.

Urinary Mepacrine and Dose. - The total amount of mepacrine excreted in the urine of the volunteers taking mepacrine dosage regimes 1, 2, and 3

has been estimated. In regime 1 (1.8g. in 5 days), urinary estimations were made on samples collected 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after the first dose and thereafter at intervals of 24 hours until the end of the investigation on day 5. In regime 2 (3.4g. in 5 days), estimations were made on samples collected at 0 to 8, 8 to 16, and 16 to 24 hours after the initial dose and then at 24 hour intervals until day 5. In regime 3 (3.2g. in 5 days), urinary mepacrine was estimated in samples at 0 to 4, 4 to 8, 8 to 12, 12 to 16, 16 to 20, and 20 to 29 hours after the initial dose and then at intervals of 24 hours until the end of the investigation on day 5.

The results are shown in Tables 7, 8, and 9 (Vol.2 p7,8 & 9) and have been expressed as the total amount of mepacrine excreted in each period. The variation in urinary mepacrines of individuals taking the same doses of the drug was considerable in each of the three regimes, but the group mean urinary mepacrine provided a reliable index of the

general trend in each individual. The group mean urinary mepacrine curves are shown in Graph 5 (Vol.2 p39). The curves for regimes 2 and 3, in which the total dosage was approximately the same, were almost identical. Both were much higher than the curve for regime 1 in which the total dosage was a little more than half that of the other two regimes. Urinary mepacrine would therefore seem to be related to the amount of drug given in the early days of dosing, and because of the similarity in urinary mepacrines of regimes 2 and 3, total dose would also appear to be more important than frequency of dosing in determining the urinary excretion of the drug.

Comparison of Tables 6 to 9, and of Graphs 4 and 5, reveal that the minimal daily blood mepacrine is directly related to the amount of mepacrine excreted in the urine in the previous 24 hours. In each regime, the range of variation in blood mepacrine and in urinary mepacrines was considerable, but individuals with high blood mepacrines usually had high urinary mepacrines and

vice versa. This strongly suggests that the same cause is responsible for the variation in the blood and urinary mepacrines of individuals taking the same doses of the drug.

Blood Mepacrine and Route of Administration. - The blood mepacrine after administration of the drug by different routes has been investigated. The same dose was given as a solution containing 0.2g. mepacrine hydrochloride in 10 ml. sterile distilled water to groups of 4 to 6 healthy volunteers by mouth, and by rectal, subcutaneous, intramuscular, and intravenous injection. Blood mepacrines were estimated at intervals during the following 24 hours. In addition, two 0.1g. mepacrine hydrochloride tablets were given by mouth with half a pint of water to another group of volunteers to compare blood mepacrines after oral administration of tablets and mepacrine in solution. The results are shown in Tables 10 to 15 (Vol.2 p10 to 12).

The blood mepacrines after rectal, subcutaneous, intramuscular, and intravenous injections

were for practical purposes the same. The blood mepacrines after oral administration of tablets, differed from parenteral and rectal administration in three important respects:-

(1) in the time required to reach a peak concentration.

(2) in the magnitude of the peak.

(3) in the variation between individual blood mepacrines.

The group mean values in Graph 6 (Vol.2 p40) show the difference between parenteral and oral dosing in two respects - namely, in the time required to reach a peak and in the magnitude of the peak attained. Group mean peak concentrations of 565 and 617 ug. per litre were reached in 15 minutes after intramuscular and subcutaneous injections respectively. A group mean peak of 632 ug. per litre was reached in 45 minutes after rectal injection, and a group mean peak level of 226 ug. per litre was attained in the same time after oral administration of mepacrine in solution. A group

mean peak of only 162 ug. per litre was reached in 2½ hours after oral administration of tablets.

The blood-mepacrine levels fell, rapidly at first then more slowly, after attainment of peak concentrations by mepacrine injections. Minimal values were usually observed 24 hours after the dose. The group mean blood mepacrines at 24 hours after tablets by mouth, rectal injection, subcutaneous injection, intramuscular injection, and intravenous injection were 54, 47, 60, 92, and 102 ug. per litre respectively. That is, the post absorptive blood mepacrines were higher after intravenous and intramuscular injection than after oral administration of tablets.

The variation in the individual blood-mepacrine values was more pronounced after tablets by mouth than after oral administration of a mepacrine solution and injection by the other routes.

The delay in reaching a peak concentration following the administration of tablets by mouth is partly explained by the time required for

solution of the tablets, but as the tablets employed were not coated, and as they disintegrated readily in water, it is not likely that incomplete solution of the tablets was responsible for the differences in blood mepacrines that were observed. Diminished absorption due to other causes seems a more probable explanation. But apart from consideration of the cause of the difference in blood mepacrine following oral and parenteral administration, the observation may have a practical application in the treatment of malaria, particularly in seriously ill patients in whom a rapid chemotherapeutic effect is desired. With this possibility in view, the blood mepacrines after repeated parenteral injections, and after combined oral and parenteral administration have been defined in healthy volunteers who remained in bed during mepacrine administration.

Repeated Parenteral Injections. - Four healthy volunteers were given an initial subcutaneous injection of 0.2g. mepacrine hydrochloride in 10 ml. distilled water followed by two 0.1g. injections by

the same route, two hours and five hours later. The blood mepacrines at intervals throughout the 24 hours after the first injection are shown in Table 16 (Vol.2 p13).

Peaks in the group mean blood mepacrine of 688, 947, and 606 ug. per litre were observed fifteen minutes after each injection. A group mean value of more than 200 ug. per litre was maintained during the first 9 hours and at 24 hours the level had fallen to 117 ug. per litre.

Combined Oral and Parenteral Administration. - Another eight volunteers were given an initial dose of 0.2g. mepacrine hydrochloride intramuscularly and 0.2g. (2 tablets) by mouth. Six hours, 14 hours, and 24 hours later, single doses of 0.4g. (4 tablets) were given by mouth. A total dose of 1.6g. mepacrine was given to each individual in 24 hours. The resulting blood mepacrines at intervals during the 72 hours after the initial dose are shown in Table 17 (Vol.2 p14).

The initial 0.2g. oral dose did not have

much influence on the group mean blood mepacrine during the first six hours (Cf. Tables 15 and 17 Vol.2 p12 & 14), but at 12 hours, after a total dose of 0.8g. mepacrine, the group mean value reached the very high level of 1798 ug. per litre and then fell relatively slowly to 325 ug. per litre at 72 hours.

Maintenance of a steady blood mepacrine by parenteral injection alone is difficult, because of the rapid rise to, and the rapid fall from peak levels. However, the rate of fall is influenced by combined oral and parenteral treatment, but before this action comes into play very high temporary levels may be reached. Parenteral injection seems in some unexplained way to potentiate absorption of mepacrine from the gut. This may explain why serious toxic effects have been so frequently reported after combined oral and parenteral administration.[2]

Blood and Urinary Mepacrine and Duration of Dosing. - The blood and urinary mepacrines have been defined

after administration of the drug for 25, 76, and 86 days to three groups of healthy volunteers who were carrying out light duties. Dosage regimes were chosen to investigate the effect on blood and urinary mepacrine of a preliminary loading dose of the drug, and also to compare daily doses of 0.1g. with weekly doses of 0.5g. The doses given and the number of volunteers employed were as follows:-

Regime	Dose of Mepacrine	No. of Volunteers	Period days
4	0.1g. daily	11	86
5	Day 1 0.1g. Day 2 0.3g. Day 3 0.6g. Days 4 to 25 0.1g. daily	8	25
6	Day 1 0.1g. Day 2 0.2g. Day 3 0.3g. Day 4 0.4g. Day 5 0.5g. Day 11 0.5g. and then 0.5g. at weekly intervals until Day 76.	6	76

In regime 4 (0.1g. daily for 86 days), the taking of mepacrine was carefully supervised from

Monday to Saturday each week. On Sundays each individual was responsible for taking the drug himself and all but one volunteer forgot to take the drug on 1 to 5 occasions during the investigation. In regime 5, it was possible to ensure only that each individual took the drug in the presence of a witness who attested in a diary that the dose had been taken. There was no reason to believe that the drug was not taken as prescribed. In regime 6, dosing was carefully supervised throughout the investigation.

Regime 4. (0.1g. daily). - Blood samples were taken for mepacrine estimation about every third or fourth day. Samples were withdrawn immediately before the daily dose so that minimum daily blood mepacrine levels were estimated. Corresponding urine samples were collected for mepacrine estimation over the three hours immediately preceding the daily dose. The individual results and the group mean blood and urinary mepacrines are shown in Graphs 7 and 8 (Vol.2 p41 & 42). Mepacrine dosing

and blood and urinary estimations were continued for a total period of 140 days in 4 of the 11 volunteers.

The group mean blood mepacrine increased to 167 ug. per litre on the 15th day of dosing, then it fell to 109 ug. per litre on the 25th day. The fall was followed by a sharp rise to 215 ug. per litre on the 29th day. The level again fell, slowly to 195 ug. per litre on the 39th day and then more rapidly to 94 ug. per litre on the 53rd day. Thereafter the blood mepacrine was much more stable and varied between 100 and 120 ug. per litre until the end of the main investigation on day 86. The same level was maintained until day 140 in 4 of the 11 volunteers in whom observations were continued.

The group mean urinary mepacrine curves expressed either as a concentration (mg. per litre) or as the total amount excreted in 3 hours (mg.), were for practical purposes the same. Both progressively increased to reach peak values during

the first 15 days of dosing. Then, after considerable fluctuations, the general tendency was for the urinary mepacrine to decrease progressively until day 39 when a relatively stable level was reached, and maintained.

The changes in blood and urinary mepacrine after prolonged administration of the drug were essentially the same. In general there was a build up to peak concentrations, then a fall to stable levels that were about half the peak values (Graph 9 Vol.2 p43).

Regime 5. (Loading dose 1.0g. then 0.1g. daily until day 25). - Unlike the other volunteers those employed in this investigation were billeted in another hospital and other arrangements had to be made for collection of blood and urinary samples. Blood for mepacrine estimation was taken 20 to 24 hours after a dose and the mepacrine concentration in the corresponding night urine was determined. Estimations were made over a period of 121 days, that is for 96 days after the last dose of mepacrine.

Detailed results are shown in Tables 18 and 19 (Vol.2 p15 & 16). The group mean blood and urine mepacrines are shown in Graph 10 (Vol.2 p44).

The group mean blood mepacrine reached a peak of 516 ug. per litre on day 4 - the day after the 0.6g. dose. It then fell to 328 ug. per litre on the day 11 and remained at this level until day 17. Then the level rose to 479 ug. per litre on day 24. On day 29, that is 4 days after dosing had stopped, the level was 387 ug. per litre and on day 37 it had fallen to 151 ug. per litre. An appreciable rise to 270 ug. per litre was recorded on day 58, and thereafter the level fell progressively to 41 ug. per litre on day 121.

The concentration of mepacrine in the night urine reached a peak value on day 11, and then fell sharply. A second peak was reached on day 29 and then the level progressively fell until the end of the investigation on day 121.

In general the changes in blood and urinary mepacrine were of the same nature throughout the

investigation. The persistence of mepacrine in blood and urine long after dosing had stopped is noteworthy.

Regime 6. (Loading dose of 1.5g. in 5 days, then 0.5g. weekly). - The blood mepacrine was estimated 24 hours after the daily dosing period and then immediately before each weekly dose so that minimum blood levels were determined. The urinary mepacrine was estimated on two consecutive days each week. The first estimation was made on the urine passed during the three hours immediately preceding the weekly dose, and the second estimation on a three hours' sample of urine from the 21st to the 24th hour after the weekly dose. The intention was to determine the post absorptive minimum and maximum urinary mepacrines each week. The results are given in Tables 20 and 21 (Vol.2 p17 & 18). The group mean blood and urinary mepacrines are shown in Graph 11 (Vol.2 p45).

The group mean blood mepacrine was 184 ug. per litre on day 6 after a loading dose of 1.5g.

in the previous 5 days. It then fell to 62 ug. per litre on the 27th day and rose to 117 ug. per litre on the 34th day. Thereafter the level remained comparatively stable at about 100 ug. per litre.

The urinary mepacrines, estimated 21 to 24 hours after the weekly dose (post-absorptive maximum level), increased from day 14 to day 42, and then stabilised. The urinary mepacrines, estimated on a three hours' sample immediately preceding the weekly dose (post-absorptive minimum level), increased from day 13 to day 27, and then decreased to reach a stable level on day 34. The difference in post-absorptive maximum and minimum levels was slight after the first weekly dose. It was greater after the third weekly dose and continued to increase until the 5th weekly dose. Thereafter both urinary levels were relatively stable.

When the group mean minimum and maximum urinary mepacrines were low and when the difference between them was slight, the group mean blood

mepacrine fell. When the difference in the urinary levels increased, the blood mepacrine started to rise. When the difference in urinary levels was pronounced but constant, the blood level was stable. These findings suggest that only a small amount of the first three weekly doses of mepacrine was absorbed, or that the rate of degradation of mepacrine within the body had temporarily increased when the first three weekly doses were given.

Blood and urinary mepacrines after administration of different doses of the drug for 25, 76, and 86 days have been defined. In the two regimes that the drug was given for prolonged periods, three distinct phases in the group mean blood and urinary mepacrines have been observed:-

1. - an initial phase that may be termed the build up period, in which blood and urinary mepacrines ran in parallel curves to reach peak values. The duration of the build up period, and the peak levels attained, depended on the

amount of mepacrine given. When 0.1g. mepacrine was given daily with and without a preliminary loading dose, a higher peak was reached in a shorter time with the loading dose than without it (Cf. Graphs 9 and 10 Vol.2 p43 & 44).

2. - an intermediate phase in which the blood and urinary mepacrines both fell from the peak levels to minimum values. During this time marked fluctuations in blood and urinary mepacrines, even from day to day, were observed; and though the general tendency was for both levels to fall, the parallelism that had previously been observed was not always apparent. The fall in both blood and urinary mepacrine occurred in spite of continuous mepacrine dosing, so that dose had little or no influence on the changes during this temporary unstable phase. (Graphs 9 and 11 Vol.2 p43 & 45).

3. - a later phase in which the blood and urinary mepacrine curves were similar and

reached comparatively stable levels that were approximately half the peak values. Fluctuations in both blood and urinary mepacrines were not so pronounced as in the previous phase and there was much less variation in individual results. The difference in the group mean blood mepacrine after 0.1g. daily and 0.5g. weekly was slight, suggesting that total dose is more important than frequency of dosing in maintaining the subsequent stable level.

The simultaneous fall in blood and urinary mepacrine after the peak levels had been reached was the most important change. That it occurred in two investigations, carried out at different times with different regimes, suggests that it was more than a chance observation. It seems highly probable that a powerful factor regulating blood and urinary mepacrine comes into play when the drug has been given for prolonged periods. Moreover, the changes in blood and urinary mepacrine were essentially the same, it therefore seems

likely that they are due to the same cause. Variation in absorption from the gut or variation in the rate of degradation within the body seemed the most likely explanations. An attempt to determine their relative importance had been made by estimating faecal mepacrines of groups of volunteers during the build up period, and during the later stable phase when blood and urinary mepacrines were about half the peak values. It would have been preferable to have estimated faecal mepacrines in the same group at different times, but the supply of volunteers to take the drug for long periods was limited and so observations had to be made on two different groups. Before reporting these investigations it is necessary to refer to the method of estimating mepacrine in faeces.

Estimation of Faecal Mepacrine. - The stool was collected in a tared litre beaker and weighed. Anhydrous sodium sulphate was added to dry the faeces, and the beaker + faeces + sodium sulphate was weighed again. The contents of the beaker were then turned into a large mortar and ground and mixed for

20 minutes. The resultant mixture was like a very fine dry sand. 5.0g. of the mixture were placed in a stoppered graduated 100 ml. cylinder and distilled water added to the 100 ml. mark. The flask was stoppered and shaken vigorously for 5 minutes and then placed on the bench to allow the insoluble materials to separate.

Five ml. of the supernatant solution were taken and the mepacrine concentration estimated in the same way as in urine.

Two major factors required investigation with this method of dealing with faeces. One, whether 5.0g. of the faeces - anhydrous sodium sulphate mixture was a representative sample of the whole mixture; and two, whether extraction recoveries were adequate and were influenced by the time faeces were allowed to stand before estimation. These problems were examined by adding known amounts of mepacrine to the faeces of individuals who had never taken the drug. The mepacrine content of duplicate and triplicate samples of the stool was then estimated by the method outlined above. This practice was carried out at intervals of 3, 24, 48, and 72 hours after

the addition of 50 mgm. mepacrine to the stools of three individuals who had never taken the drug. The results are shown in Table 22 (Vol.2 p19).

The difference in the estimations of duplicate and triplicate samples from the same stool was sufficiently small to indicate that the mixing procedure was adequate and that 5g. of the sodium sulphate-faeces mixture was a representative sample.

The rate of disappearance of mepacrine from the three stools was rapid and was approximately the same for each stool. At 3 hours, an average of 70% of the added mepacrine was recovered; at 24 hours, 48%; at 48 hours, 18%; and at 72 hours, 14%. That is, the more mepacrine present the more rapid the rate of disappearance. Disappearance of mepacrine has been interpreted as being mainly due to degradation of the drug by faeces. This striking finding made it necessary to estimate the mepacrine content of faeces as soon as possible after they had been passed, but

even then no indication of the amount of mepacrine degraded within the bowel could be obtained. However, since the rate of degradation of mepacrine in different specimens of faeces seemed to be fairly constant, it was considered worth-while estimating faecal mepacrines of two groups of volunteers during the build up of blood mepacrine, and during the later stable phase provided that the faecal mepacrine estimations of both groups were treated in the same way.

Faecal Mepacrines. - In view of the rapid disappearance of mepacrine from faeces after they have been passed, faecal mepacrines were estimated whenever possible within 3 hours of the time of defaecation. This was not always practicable and a few estimations were made 12 - 16 hours after defaecation.

The faecal mepacrines of four volunteers, during the first 6 days of taking 0.1g. mepacrine daily were estimated and compared with those of seven other volunteers who had taken the same dose

of the drug for the previous 62 days. The seven volunteers belonged to the group of 11 men whose blood and urinary mepacrines were defined over a period of 86 days. At the time the observations were made the group mean blood and urinary levels were stable and had fallen to about half the peak values.

The total mepacrine in the faeces of the four men during the first six days of dosing was 8.5, 11.8, 20.0, and 41.1mg. The total faecal mepacrines of the other seven men for a similar period were:- 114.6, 119.4, 131.8, 146.3, 167.0, 167.0, and 172.2mg.. These values do not represent absolute faecal mepacrines, but as the investigations were carried out under comparable conditions, the uniformity of the individual results and the marked difference in faecal mepacrines of the two groups strongly suggest that more mepacrine is excreted in the faeces during the late stable phase in blood and urinary mepacrine, than during the build up period. The simultaneous fall in both

blood and urinary mepacrine to about half the peak levels, together with an increase in faecal mepacrine suggests that diminished absorption of mepacrine from the gut is at least partly responsible for the fall. The cause of diminished absorption is still obscure, but an important clue was obtained in the development of a suitable method for estimating mepacrine in faeces. In this work mepacrine added to stools rapidly disappeared. It is reasonable to presume that similar changes occur within the gut, so that diminished absorption may be in part due to degradation by intestinal contents. The differences in faecal mepacrines at different times in the administration of the drug may be accounted for by the fact that during the build up period tissue mepacrine is progressively increasing until a peak value is reached. The attainment of a peak tissue mepacrine coincides with peak blood and urinary values; and then tissue, blood, and urinary mepacrines are in equilibrium with the dosage regime. Doses of

mepacrine given after this equilibrium come in contact with an intestinal mucosa with which they are already in equilibrium, so that less mepacrine is absorbed and a greater proportion of the dose remains in the gut. But the longer that mepacrine remains in the gut the greater is the amount degraded by the intestinal contents. The fall in blood and urinary mepacrines to stable levels of about half the peak value may therefore be largely due to diminished absorption resulting from this chain of events.

Mepacrine Excretion. - Blood and urinary mepacrines were estimated in addition to the faecal mepacrines of the 7 men who had taken daily doses of 0.1g. mepacrine for 62 days before the investigation. The results are shown in Tables 23 to 29 (Vol.2 p20 & 21).

In two volunteers it was not possible to estimate the 24 hours' urine every day but in the remaining five the total mepacrine excreted in the urine in 6 days by each individual varied from 15.6 mg. to 32.1 mg. The group mean urinary mepacrine for the

6 day period was 24 mg.. The total faecal mepacrine of the same volunteers in 6 days ranged from 119.4 mg. to 151.2 mg. and the group mean was 138.mg. That is, the total group mean mepacrine excreted in 6 days in urine and faeces was 162 mg.. But the total dose given in this period was 600 mg., and the group mean blood mepacrine was practically constant, so that the remaining 438 mg. probably represents the total amount of mepacrine degraded in 6 days.

Similar investigations were carried out on another volunteer taking weekly doses of 0.5g. mepacrine. Blood, urine, and faecal mepacrines were estimated for 3 days before and for 5 days after the weekly dose. Experimental conditions were particularly good because stools of approximately the same weight were obtained about the same time each day throughout the investigation and estimations were made immediately after they had been passed. The results are shown in Table 30 (Vol.2 p22).

The blood mepacrine was fairly stable

throughout the investigation. The total mepacrine excreted in the urine in one week was about 14 mg. and the total faecal mepacrine in the same period was 146 mg.. That is, 160 mg. of the total weekly dose of 500 mg. were excreted in urine and faeces, but since the blood level was practically constant the remaining 340 mg. was probably degraded.

The relationship between the total urinary mepacrine and the total faecal mepacrine over the period of investigation is noteworthy. In the 5 men taking 0.1g. mepacrine daily from day 63 to 67 inclusive, individuals with a low total urinary mepacrine had low total faecal mepacrines, and vice versa. In the volunteer taking 0.5g. weekly the relationship was even more striking. The curves for urinary and faecal mepacrine were practically parallel (Graph 12 Vol.2 p46). These findings suggest that when the blood mepacrine is stable, the urinary and faecal mepacrines are proportional to one another.

Blood, Urine, and Faecal Mepacrines. - The relation between blood, urine, and faecal mepacrines has been examined in the 7 men taking 0.1g. mepacrine daily for the previous 62 days. Complete results were obtained in 4 men over the 6 days' balance period. The mean blood mepacrine of each man during this period has been compared with the total urinary and faecal mepacrine over the same period. The following results were obtained:-

	Mean Blood Mepacrine ug./litre	Total Urinary Mepacrine mg.	Total Faecal Mepacrine mg.
1	82	15.6	119.4
2	99	22.9	139.0
3	111	22.9	146.0
4	156	32.1	151.2

The urinary and faecal mepacrine tended to increase as the blood mepacrine increased. In other words the blood mepacrine, and by inference the tissue mepacrine, controls the excretion of the drug in urine and faeces after prolonged dosing.

Mepacrine Degradation. - When blood and urinary mepacrines were stable after daily doses of 0.1g.

of the drug, an average of about 30% of the total dose given in 6 days was recovered from urine and faeces, the remaining 70% was unaccounted for. But as the blood level was constant, the tissue level may also be presumed to be constant, so that 70% of the dose was probably degraded. Degradation of mepacrine may therefore be important pharmacologically.

When mepacrine degradation is considered, it is necessary to distinguish clearly between degradation within the bowel, and degradation in the tissues of the body, because mepacrine degraded in the intestine has had no opportunity to exert a chemotherapeutic action whereas mepacrine degraded in the body has had this opportunity. An indication of the importance of degradation in the bowel is probably provided by the rate of degradation of the drug in faeces, but it is still impossible to measure degradation of the drug in the body. Investigations of the rate of degradation in incubated blood, plasma, and urine were carried out to explore possible methods

of assessing degradation of mepacrine in the body.

Degradation of Mepacrine in Blood. - A volunteer was given 1.5g. mepacrine in 3 days. A large volume of blood was taken 24 hours after the last dose of the drug and was divided into 5 ml. samples that were placed in glass stoppered extraction bottles. Precautions were taken to avoid bacterial contamination of the blood. The extraction bottles were placed in an incubator at 37°C. Blood mepacrine of duplicate samples was estimated soon after withdrawal of blood, and then at 24 hour intervals for the next 8 days. The results are shown in Table 31 (Vol.2 p23) and in Graph 13 (Vol.2 p47).

The initial mean blood mepacrine was 575 ug. per litre. The mean values at 24 hour intervals for the next 8 days were 410, 320, 250, 200, 200, 160, 160, and 130 ug. per litre. As with faecal mepacrines, the rate of degradation was rapid at first and then it slowed down as the total amount of mepacrine diminished.

Degradation of Mepacrine in Plasma. - Blood from a volunteer who had been given 1.5g. mepacrine in 3 days was taken 24 hours after the last dose of mepacrine and the plasma was separated immediately. It was then divided into 5 ml. samples in sterile extraction bottles and incubated at 37°C. Care was taken to avoid bacterial contamination during the separation and sampling of the plasma. Plasma mepacrine estimations were made soon after withdrawal of blood and at intervals during the next week. The results are shown in Table 32 (Vol.2 p24). The initial plasma mepacrine was 120 ug. per litre and the final value 7 days later was 80 ug. per litre.

Degradation of Mepacrine in Urine. - A known amount of mepacrine in solution was added to the urine of a healthy subject. Five ml. samples were then added to sterile glass stoppered extraction bottles that were placed in an incubator at 37°C. Urinary mepacrine was then estimated at 24 to 48 hour intervals during the following week. The results are shown in Table 32 (Vol.2 p24). The

initial urinary mepacrine was 10.2 mg. per litre; one week later it was 9.5 mg. per litre.

Degradation of Mepacrine in Faeces. - When 50 mg. mepacrine were added to faeces, the amount of mepacrine recovered during the next three days progressively fell. The fall was considered to be mainly due to degradation of the drug in faeces (p.53). Further investigations after adding 10 to 500 mg. mepacrine to the stools of two other healthy volunteers have confirmed the previous findings, but it has also been found that degradation depends on the amount of mepacrine in the faeces. Degradation was minimal when 10 mg. mepacrine were added to stools, it was maximal when 50 mg. were added; and it was minimal again when 100 mg. to 500 mg. were added. (Table 33 Vol.2 p25). That is, the general shape of the curve for degradation of mepacrine in faeces in relation to the amount of drug added resembles an inverted "U", the tips of the two limbs representing 10 mg. and 100 mg. respectively, and the highest point in the bend

corresponding with 50 mg. This suggests that degradation of mepacrine results from enzyme action. Small amounts of mepacrine may not be degraded because of strong physico-chemical affinities of the mepacrine-faeces mixture. Large amounts may not be degraded because of "blockage" of the enzyme system responsible for degradation of the drug.

Mepacrine is degraded by blood, plasma, urine, and faeces. The rapid rate of degradation in blood and faeces supports the view that degradation of mepacrine is important in determining the blood and tissue mepacrine levels after the drug has been given for prolonged periods. But as the excretion of mepacrine is dependent on the blood and tissue mepacrine levels, the amount of mepacrine degraded is therefore proportional to the amount of mepacrine absorbed, if constant blood and tissue levels are maintained. In other words, if absorption of mepacrine is constant, degradation of the drug may be assessed by changes in the blood and tissue mepacrine. An attempt to test this hypothesis

has been made by giving a volunteer 1.5g. mepacrine in 3 days and then stopping administration of the drug. Twenty four hours after the last dose of mepacrime, blood was taken for mepacrine estimation and further estimations were made at 24 hour intervals in the next 8 days. During this time no mepacrine was given so that absorption was nil and therefore constant. If the previous argument is correct then the rate of fall in blood mepacrine would be expected to be mainly due to degradation of the drug within the body. For comparison, the rate of degradation in incubated blood was also estimated. A large sample of blood was taken from the volunteer 24 hours after the last dose and was divided into 5 ml. samples that were placed in sterile glass stoppered extraction bottles. Duplicate estimations of blood mepacrine were made soon after the withdrawal of blood, and then at 24 hour intervals for the next 8 days on both circulating and incubated bloods. The results are shown in Table 34 (Vol.2 p26).

The blood mepacrines of the incubated and

circulating blood were almost identical. That is, the rate of degradation in incubated blood was the same as the rate of fall in circulating blood mepacrine. Such a close correlation was not expected. To eliminate the possibility of a chance observation, similar investigations were made on circulating and incubated blood mepacrines of another 4 men who were given different doses of mepacrine before the test. The results, shown in Table 35 (Vol.2 p27) were practically the same as those obtained with the first volunteers. But in addition to the parallelism between the rate of fall in circulating blood mepacrine and the rate of degradation in incubated blood mepacrine, both rates were proportional to the amount of mepacrine originally present in the blood. When the blood levels were high, the rate of fall and the rate of degradation were more rapid than when the initial levels were low. (Graph 14 Vol.2 p48). These findings are of fundamental importance in appreciating the relationship between blood and tissue

mepacrine on the one hand; and dose, absorption, degradation, and excretion of the drug on the other.

If the rate of fall in circulating blood mepacrine equals the rate of degradation of mepacrine in incubated blood then the rate of fall in circulating blood mepacrine may be considered to be equivalent to the rate of degradation of mepacrine in circulating blood. This relationship could only hold if rate of degradation in tissues was proportional to rate of degradation in circulating blood. That is, the rate of degradation in incubated blood equals the rate of degradation in circulating blood, and both are proportional to the rate of degradation in tissues. But the rates of degradation in incubated and circulating blood, within the limits described, are also proportional to the concentration of mepacrine in the blood. The rate of degradation in tissues is therefore proportional to the concentration of mepacrine in tissues, that is:-

$$\frac{\text{rate of degradation in blood}}{\text{rate of degradation in tissues}}$$

$$= \frac{\text{concentration in blood}}{\text{concentration in tissues}}$$

$$= \text{a constant}$$

or

$$\frac{\text{rate of degradation in blood}}{\text{concentration in blood}}$$

$$= \frac{\text{rate of degradation in tissues}}{\text{concentration in tissues}}$$

$$= \text{a constant}$$

Two important relationships may be deduced from the equations:-

1. - Blood mepacrine in health is a reliable index of tissue mepacrine, as the ratio of the two is constant.

2. - Changes in the blood mepacrine, and therefore in tissue mepacrine, are attributable to variation in absorption because (a) the blood mepacrine level was previously found to depend on the ratio, - absorbed mepacrine : degraded mepacrine; and (b) because the rate of degradation in blood and tissue mepacrine is constant for a particular blood or tissue level.

The full implications of these findings will be discussed later.

When a possible cause for degradation of mepacrine within the body is considered, it is probably significant that tissues, circulating blood, and incubated blood, in addition to having proportional rates of degradation are also alike in having relatively high oxygen tensions and rich supplies of oxidative enzymes. This similarity is all the more significant when it is appreciated that mepacrine in aqueous solution is degraded by bubbling oxygen through it and by adding oxidising agents like hydrogen peroxide. It is therefore probable that the first stage in the degradation of mepacrine within the body is an oxidative process.

Mepacrine Partitions. - The investigations so far described have dealt with blood mepacrine in health and the possible factors that may influence it. When the chemotherapeutic action of the drug was examined, evidence was found suggesting that certain pathological changes may influence blood mepacrine

profoundly. The rise in blood mepacrine that accompanied fever was attributed to transference of mepacrine from tissues to blood. In other words the partition of mepacrine between tissues and blood was altered by fever. Possible factors controlling the partition of mepacrine in a protein: water:mepacrine system have been examined because investigation of tissue:blood partition in man was not practical. It was considered that qualitative changes in mepacrine partition in this simple system probably also apply to the more complex mepacrine partitions within the human body.

Denatured egg albumen and mepacrine in aqueous solution were employed. The effect of time, concentration of the mepacrine solution, pH, temperature, and electrolyte concentration on the protein mepacrine : water mepacrine partition has been examined. The results of the individual investigations are not comparable with one another because the same preparation of denatured egg albumen was not used throughout. This was probably

of little importance as the object was to define qualitative rather than to determine exact quantitative changes. The results are shown in Graphs 15 to 17 (Vol.2 p49 to 51).

Staining Time. - The partition of mepacrine between protein and water over a period of 160 minutes has been investigated. At 2½ minutes mepacrine was almost equally divided between protein and water, at 20 minutes the protein mepacrine:water mépacrine ratio was as 3 : 1, and at 160 minutes it was as 6 : 1. (Graph 15 Vol.2 p49).

Concentration of Mepacrine Solution. - Mepacrine solutions ranging from 8 to 1000 mg. per litre were employed. When the original solution contained 8 mgm. mepacrine per litre of water, the protein mepacrine:water mepacrine ratio was as 1 : 5; when it contained 16 mg. mepacrine per litre, the ratio was as 1 : 1; when it was 125 mg. per litre, the ratio was as 3 : 1; and when it was 1000 mg. per litre, the ratio was as 11 : 1. That is, as the mepacrine concentration increased, the protein :

water mepacrine ratio also increased. (Graph 16 Vol.2 p50).

Hydrogen Ion Concentration. - The partition of mepacrine over a pH range of 2 to 12 was examined. From pH.2 to pH.8 the protein mepacrine : water mepacrine ratio increased from 5 : 1 to 19 : 1. Over the range pH.8 to pH.12 the ratio remained between 19 : 1 and 18 : 1. The most pronounced change was observed between pH.6 and pH.8. (Graph 17 Vol.2 p51).

Temperature. - The effects of temperatures between 15°C and 70°C has been investigated and though the protein mepacrine : water mepacrine ratio increased from 1.7 : 1 to 2.8 : 1, the change was insignificant in comparison with the previous observations (Graph 17 Vol.2 p51).

Electrolyte Concentration. - The protein mepacrine : water mepacrine ratio was as 19 : 1 when the electrolyte concentration was 0.85g. sodium chloride per 100 ml.. It fell to 17.: 1 when the electrolyte concentration was 28g. sodium chloride per 100 ml..

Like temperature, electrolyte concentration has little influence on the protein : water partition of mepacrine (Graph 17 Vol.2 p51).

The staining time, the mepacrine concentration, and the pH of the system were found to be important in determining the protein : water partition of mepacrine. They probably also play an important part in the pharmacology of the drug. The complete dependence of the partition on the mepacrine concentration and the marked change in partition that may accompany small pH changes between pH.6 and 8 are noteworthy. The flattening out of the curve at pH.8 to 12 may be related to the lower solubility of the drug in an alkali solution.

When these findings are translated to the administration of mepacrine to man, the staining time and mepacrine concentration are represented by dose and frequency of dosing; the effect of pH depends on the pH of tissues and body fluids. In health, pH changes in tissue and blood of men in sedentary occupations vary within narrow limits so

that the mepacrine partition between tissues and body fluids would be expected to be fairly constant. In disease, greater variation in pH as evidenced by changes in the alkali reserve may be found. Fever diminishes the alkali reserve, so that the tissue: blood, or tissue:plasma partition of mepacrine may be expected to decrease. In other words the blood and plasma mepacrines may be expected to rise at the expense of tissue mepacrine. The rise in blood mepacrine, that was observed at the time of fever without obvious parasitaemia in volunteers experimentally infected with malaria, may therefore be due to the diminution in alkali reserve associated with fever.

Hydrogen ion concentration may also be partly responsible for the striking difference in blood mepacrines after oral and parenteral administration of the drug. The acid gastric juice by decreasing the partition of mepacrine between the stomach wall and the gastric contents may allow greater amounts of the drug to remain in solution within the stomach,

the rate of absorption after oral administration may therefore be expected to be slower than after parenteral injection.

Blood, Plasma, and Urinary Mepacrines in Health. - In ordinary circumstances a discussion of the relations between blood, plasma, and urinary mepacrines would be superfluous in view of the previous findings. But Shannon's conclusion regarding the respective merits of blood and plasma mepacrine in patients suffering from malaria has unfortunately been extended by others to apply to healthy individuals.[16] The result has been that blood mepacrine is considered, on the best interpretation, to be inferior to plasma mepacrine; and on the worst interpretation, to be valueless as a pharmacological index. It is significant that this deduction has not so far been substantiated by comparative figures of blood and plasma mepacrine in health. Nevertheless, so much confusion has been caused by the opposed views on the respective merits of blood and plasma mepacrine in health,

that an attempt to decide the issue by direct comparison has been undertaken.

All are agreed that plasma water mepacrine and tissue mepacrine are theoretically the best indices of pharmacological properties of the drug. But since the one is directly proportional to the other, either would be acceptable. Unfortunately no reliable method exists for estimating plasma water mepacrine, and tissue estimation as a routine procedure is impracticable. A decision on the respective merits of blood mepacrine and plasma mepacrine, as an index of the plasma water mepacrine or the tissue mepacrine, would therefore seem to be impossible for lack of a basis for comparison. But as urine in health contains only traces of non-diffusible constituents it seems reasonable to presume that urinary mepacrine is closely related to plasma water mepacrine. Urinary mepacrine has therefore been tentatively employed to compare blood and plasma mepacrines of two groups of volunteers taking the drug. In one group estimations were

made during the period of dosing; in the other group, after dosing had stopped. The single extraction method of Brodie and Udenfriend was employed for all estimations and fluorescent intensities were read on a Coleman Fluorimeter.

The first group of seven volunteers were given an initial dose of 0.6g. mepacrine followed after 24 hours by 0.3g. daily for 4 days. Blood and plasma mepacrines were estimated on the same samples of blood taken 4 hours after the first dose and at 24 hours after each dose. Urinary mepacrines were estimated daily from 24 hour collections. Detailed results are shown in Table 36 (Vol.2 p28). The group mean blood, plasma, and urinary mepacrines showed essentially the same changes during the dosing period. (Graph 18 Vol.2 p52). When the relation between blood mepacrine and urine mepacrine on the one hand, and between plasma mepacrine and urinary mepacrine on the other, was examined by plotting the blood and plasma mepacrines of each individual against the urinary mepacrine, the

variation in blood:urinary mepacrine ratios was less marked than the plasma:urinary mepacrine ratios. (Graphs 19 & 20 Vol.2 p53).

The second group of ten volunteers were given 3.4g. mepacrine in seven days. Daily blood and plasma mepacrines were made on the same samples of blood for 27 days starting estimations 24 hours after the last dose. Daily urinary mepacrines were made on the 3 hours' urine immediately preceding the daily blood sample. Detailed results are shown in Table 37 (Vol.2 p29). The group mean blood, plasma, and urinary mepacrines from the 2nd to the 6th day after dosing were practically parallel. Thereafter blood and urinary levels continued to run parallel, but the plasma mepacrines showed wide fluctuations that had no relation to either the blood or urinary mepacrine values. (Graph 21 Vol.2 p54). The correlation between blood and urinary mepacrines was closer than between the plasma and urinary mepacrines (Graphs 22 & 23 Vol.2 p55).

In these two investigations a relatively

wide range of blood, plasma, and urinary mepacrine has been covered and a reasonable correlation was found to exist between blood, plasma, and urinary mepacrines when plasma values were more than 20 ug. per litre. But below this level, plasma mepacrines bore no constant relation either to blood mepacrine or to urinary mepacrine though blood and urinary levels continued to run in parallel. Over-all, blood mepacrine was more closely and more consistently related to urinary mepacrine than plasma mepacrine. For this reason it was considered to be a better pharmacological index in health. Independent observations on the degradation of mepacrine have resulted in the conclusion that blood mepacrine is directly proportional to tissue mepacrine. But as tissue mepacrine and plasma water mepacrine are directly related to one another it may be concluded that the basis of comparison employed in this investigation was sound.

The previous generalisation on the value of blood mepacrine and plasma mepacrine may therefore

be reversed. Plasma mepacrines in health, estimated by single extraction methods are almost valueless for reasonably accurate determination of concentrations less than 20 ug. per litre. That is, in the range of concentration resulting from suppressive doses of mepacrine. This conclusion is amply supported by the erratic results of triplicate estimations of plasma mepacrine that have been reported[14] (Cf. with triplicate estimations of whole blood mepacrines Table 3 Vol.2 p3). But it is not incompatible with Shannon's original observation on patients exhibiting wide variation in leucocyte count. The important fact is that physiological variation in leucocyte count is not sufficient to vitiate results. A somewhat similar conclusion was arrived at independently by the American workers at Fort Knox who compared plasma mepacrine and cellular mepacrine of the blood calculated from blood estimations and leucocyte counts. They found that the cellular mepacrine of healthy soldiers bore a more consistent relationship

to dose than plasma mepacrine, and on this account they advocated reconsideration of the whole problem of the best index to employ in pharmacological investigations.[15]

The Pharmacology of Mepacrine: Conclusions. - Tissue mepacrine may be regarded as the dominant factor in the pharmacology of the drug. It controls blood mepacrine, the amount of drug excreted, and the amount absorbed and degraded. The specific relations of each of these factors to tissue mepacrine will now be considered.

Blood mepacrine in health is directly proportional to tissue mepacrine but the actual partition between the two may vary with the pH of the tissues and blood. In healthy volunteers engaged in sedentary work the partition remains fairly constant, but in disease characterised by a diminution in the alkali reserve, a decrease in

the tissue : blood partition is expected and the blood mepacrine increases at the expense of the tissue mepacrine.

Mepacrine is mainly excreted in the faeces and urine. The excretion of the drug has been found to be directly proportional to the blood level and so to the tissue mepacrine. In other words, excretion of mepacrine is determined by the blood and tissue levels of the drug.

Blood and tissue mepacrine levels are determined by the ratio - absorbed mepacrine : degraded mepacrine. When absorbed mepacrine exceeds degraded mepacrine the blood and tissue mepacrines rise. When absorbed mepacrine is less than degraded mepacrine, the blood and tissue levels fall. When absorbed mepacrine equals degraded mepacrine, the blood and tissue mepacrines are constant. These three phases in blood and tissue mepacrine may be identified with the build up, the fall away from the peak, and the late stable blood mepacrines that have been observed after prolonged administration of the same doses of

the drug. The fall away from the peak blood mepacrine and the assumption of a final stable blood-mepacrine level that is about half the peak level requires special consideration. The doses of mepacrine given to prevent malaria are likely to be rapidly degraded in the bowel. A little more than half the original amount of drug was recovered from stools 24 hours after the addition of 50 mg. mepacrine to 50 g. faeces. The longer that mepacrine remains in the bowel, the less will be available for absorption owing to degradation by intestinal contents. During the build up phase absorption is rapid so that degradation within the bowel is minimal. When equilibrium between tissues and dose is reached, absorption is slower, and so the opportunity for degradation within the bowel is increased. The nett result is that only a portion of the dose is now available for maintenance of a tissue : dose equilibrium that was determined in the first place by the whole dose. The consequence is that a new tissue : dose equilibrium has to be

established. This will necessarily be lower than the original peak level - hence the fall in blood mepacrine to a final level of about half the peak.

Degradation of mepacrine in faeces, tissues, and blood is probably an oxidative process in the first place. The rate of degradation in blood and faeces has, within limits, been found to be directly proportional to the amount of drug present. This means that the rate of degradation is constant for a particular blood, or faecal mepacrine level. But it has also been found that the ratio of the rates of degradation in tissues and blood, is equal to the ratio of the total mepacrine in tissues and blood. Changes in the blood and tissue mepacrine levels may therefore be considered to be mainly due to variation in absorption.

An indication of the relative importance of the various factors concerned in maintaining a stable blood mepacrine level after prolonged administration of the drug, and an estimate of the total tissue mepacrine may be obtained by calculations based

on known data and experimental results. In these calculations two assumptions have been made, (1) that no mepacrine is degraded in the bowel, and (2) that maximal degradation in the bowel is the same as the maximal degradation in faeces. In this way, an estimate of largest and smallest amounts of mepacrine in the body has been made. The calculations are based on the following data:-

Daily dose of mepacrine	=	100 mg.
Average daily faecal mepacrine	=	25 mg.
Average daily urinary mepacrine	=	5 mg.
Daily blood mepacrine	=	100 ug./litre
Rate of degradation of 100 mg. mepacrine in 100g. faeces	=	50 mg./day
Rate of degradation of mepacrine in blood; initial concentration 100 ug./litre.	=	20 ug./day

If the quantity of mepacrine degraded within the bowel each day is assumed to be the same as that degraded by faeces in the same time, then the total mepacrine lost each day amounts to 80 mg. of the daily dose of 100 mg. This includes 50 mg. lost by degradation within the bowel, 25 mg. in the faeces, and 5 mg. in the urine. The remaining 20 mg. may

be presumed to be necessary to maintain the stable blood and tissue mepacrine level, and so are equivalent to the total amount degraded each day. But the rate of degradation in blood is about 20 ug. per litre per day when the original concentration is 100 ug. per litre. This is equivalent to a total degradation of 100 ug. per day by the blood if the blood volume is assumed to be 5 litres. The total amount of mepacrine degraded each day was considered to be 20 mg., and the ratio - rate of degradation in tissues : rate of degradation in blood is a constant, therefore the ratio of the daily rates of degradation in tissues and blood is, as (20mg. - 100 ug.) : 100 ug., or as 199 : 1. But this ratio is equal to the ratio of the amount of mepacrine in tissues and in blood, and the total blood mepacrine is 500 ug. with an assumed blood volume of 5 litres. The total tissue mepacrine during the late stable phase in blood mepacrine is therefore 199 x 500 ug., that is 99.5 mg.. This figure is based on the probable maximum degradation

in the bowel that could occur. If it is now assumed that no mepacrine is degraded in the bowel then only 30 mg. would be lost daily - 25 mg. in faeces and 5 mg. in the urine. The amount degraded each day would therefore be equivalent to 70 mg. and the ratio of the rates of degradation in tissues and blood would be as 699 : 1, and the total tissue mepacrine 349.5 mg. The true tissue mepacrine during the late stable blood mepacrine level probably lies between the two extremes, but it is probably nearer 99.5 than 349.5 mg.

When mepacrine is given by intravenous, intramuscular, subcutaneous, and rectal injection, very high blood mepacrine levels are obtained soon after the injection. The blood levels after oral administration of the same dose are much lower. Even when allowance is made for differences in the circulating system at the different sites of absorption, it is considered that the slow rise in blood mepacrine and the relatively low levels attained after oral administration are due to diminished absorption

from the pH effect of the acid gastric contents and then to degradation of the drug in the intestine.

An initial intramuscular injection of mepacrine combined with oral administration of the drug seems to increase absorption of subsequent doses given by mouth. The blood levels attained may be very high. The mechanism of this change is not understood but it is of interest that a similar finding has been reported with another antimalarial drug. Howie and Murray-Lyon[18] found that no quinine appeared in the urine of certain patients taking large doses of the drug by mouth. After intravenous injection of quinine, large amounts of the drug appeared in the urine of these patients, and most important, large amounts continued to appear in the urine when subsequent doses of the drug were given by mouth.

PART 3

MEPACRINE TOXICITY

Headache, vomiting, diarrhoea, abdominal colic, muscle cramps, epilepsy, and psychosis have been attributed to the administration of large doses of mepacrine to patients with malaria. Unfortunately it is not possible to decide to what extent mepacrine, and to what extent the disease, was responsible. Undesirable effects that were most probably due to mepacrine have been encountered in a few healthy volunteers who were taking the drug. The relation of these toxic manifestations to blood and urinary mepacrine has been examined by comparing the blood and urinary values of volunteers who developed undesirable effects, with the values of symptom-free volunteers who took the same doses of the drug at the same times. If a definite correlation could be established between toxic effects and blood and urinary mepacrine, then it was hoped to define the minimum blood and urinary levels at which toxic effects

were encountered.

In addition to acute toxic effects encountered during the administration of large doses of mepacrine, the possibility of cumulative chronic toxic effects resulting from prolonged administration of small doses of mepacrine had also to be borne in mind, because soldiers operating in malarious areas may have to take the drug continuously for 1 to 2 years, or perhaps even longer. Both aspects of mepacrine toxicity have been studied and they will be dealt with separately.

Acute Toxicity. - The acute toxic symptoms that have arisen are conveniently divided into three classes:-

(1) Mild symptoms such as headache, nausea, epigastric discomfort, and minor visual disturbances that were entirely subjective and were not sufficiently severe to interfere with ordinary duties.

(2) Alimentary symptoms like vomiting, colic, and diarrhoea that were temporarily incapacitating.

(3) Severe systemic effects characterised by marked lassitude and depression and sometimes accompanied by neuralgic pains that were wholly incapacitating.

Mild Symptoms. - Headache, nausea, epigastric discomfort, and minor visual disturbances were most frequently encountered. They occurred after administration of large doses of mepacrine (0.5g.) and also after small doses (0.1g.). But when small doses continued to be taken, symptoms disappeared and did not recur. Because of the mild nature of the symptoms and their complete dependence on the subjective sensations of the individual, it was not possible to classify them satisfactorily. No further investigation was therefore possible.

Alimentary Symptoms. - The effect of fluid intake on the appearance of toxic effects from mepacrine has been investigated. Two groups of volunteers, each containing 6 healthy men, were given the same doses of mepacrine at the same times. The fluid intake of one group was restricted for 3 days before, and on the day of taking mepacrine. The fluid intake of the other group was liberal. Both groups were confined to bed in different wards on

the day mepacrine was given. The doses employed were as follows:-

Initial dose	0.2g. mepacrine
After 5 hours	0.3g. mepacrine
After 10 hours	0.4g. mepacrine
After 15 hours	0.5g. mepacrine.
Total dose	1.4g. mepacrine

The urinary output of the restricted fluid intake group ranged from 240 to 900 ml. on the day of test; that of the other group ranged from 1,700 to 2,400 ml. on the same day (Table 38 Vol.2 p30).

Five of the six men taking a restricted fluid intake had abdominal colic and loose watery stools. Movement of the bowels relieved the colic. These manifestations did not appear in the men taking a liberal fluid intake.

Blood samples for mepacrine estimations were taken at $\frac{1}{4}$ hour, $\frac{3}{4}$ hour, $2\frac{1}{2}$ hours, 5 hours, 10 hours, $14\frac{1}{2}$ hours, and $23\frac{1}{2}$ hours after the initial dose. The total mepacrine excreted in the urine in the 24 hours after the initial dose was also estimated. The results are shown in

Tables 39 and 40 (Vol.2 p31 & 32). The group mean blood mepacrines of the two groups did not differ markedly. For practical purposes they may be regarded as the same. The urinary mepacrines on the other hand were much lower in the restricted fluid intake group than in the liberal fluid intake group. That is, colic and diarrhoea were not related to blood mepacrine but appeared to be associated with low urinary mepacrines. The only explanation that can be offered for the similarity in blood mepacrines of the two groups and the disparity in urinary mepacrines is that the tissue: blood partition of the drug was lower in the restricted fluid intake group than in the liberal fluid intake group. In consequence it seems reasonable to suppose that less mepacrine was absorbed by the restricted fluid intake group and therefore more was left in the alimentary tract. Diarrhoea and colic may therefore be due to direct action of mepacrine on the bowel. The hinge on which this argument is based, namely,

that the tissue:blood partition of the drug was different in the two groups, is supported by the fact that dehydration is usually associated with a diminished alkali reserve; and a small change in pH has already been found to alter the drug partition in the manner described.

Severe Systemic Effects. - Six medical officers took a single daily dose of 0.5g. mepacrine for 5 days whilst carrying out sedentary duties. Two developed severe systemic toxic manifestations, the other four were unaffected.

Blood mepacrines of the six men were estimated immediately before and 24 hours after the fifth and last dose. Then estimations were made every other day for the next week. Twenty four hour urinary mepacrines were estimated during the 5 days of dosing, and during the 6 days after dosing. Details of the toxic effects were as follows:-

Lieut.I. had severe headache a few hours after the first two doses of mepacrine. The headache lasted 11 hours on the first day and 3 hours on the second

day. Immediately after the 3rd, 4th, and 5th doses he was perfectly fit and had no complaints, but 18 hours after the 5th dose at 3 a.m. on the 6th day, he wakened with pain in both loins. He was thirsty, had pain in his tongue, his mouth was dry, and he was sweating profusely. He quenched his thirst but could not sleep as he felt very excited. At 9 a.m. on the same day he was much improved and reported for examination when he complained of excessive salivation, a bitter taste in his mouth, and thirst. His urine was albumen-free and contained no macroscopic blood. He looked tired and felt out of sorts but carried out his duties until the afternoon of the 7th day when he had to return to bed. He was fairly comfortable until evening when pain behind the eyes, pain in the maxilla, and pain in the lumbo-sacral region radiating down the left leg developed. His pulse rate was 120 per minute and his temperature was 101°F.. The muscles of both thighs felt tight and spasmodic twitching of bundles of muscle fibres was observed. He was given two Codeine Phos.Co. tablets and slept fairly well. On the morning of the 8th day he noticed an erythematious rash on the chest and flexor aspects of the arms. He felt weak, but was comfortable in bed. On the morning of the 9th day he was allowed up and walked with a perceptible limp. The rash was still present and small erythematious patches were observed on the chest and on the flexor aspects of the arms (Illustration Vol.2 p56). On the 10th day the rash was fading and had disappeared on the 11th day. On the 12th day he was able to resume duties.

Capt.G. - During the first 5 days of mepacrine dosing this officer complained of epigastric discomfort, nausea, thirst, headache, and mental confusion, but was able to continue with his duties. He was perfectly fit on the 4th and 5th days but on the forenoon of the 6th day he felt very weak, depressed, generally out of sorts, and had to retire to bed. Symptoms persisted on the 7th day, but on the 8th day he was fit enough to resume his duties.

The blood mepacrines of the two volunteers who had severe reactions after mepacrine administration, and the values of the other four men who were unaffected, are shown in Table 41 (Vol.2 p33). The urinary mepacrines are given in Table 42 (Vol.2 p34). The blood mepacrine of the individuals with incapacitating toxic effects were higher than the values of the others at the time symptoms were present. The urinary mepacrines were no different from those of the symptom-free volunteers. Severe systemic toxic effects would therefore seem to be associated with a high post absorptive blood mepacrine. A level of about 800 ug. per litre 24 hours after the dose was not associated with symptoms but a level of more than 1,000 ug. per litre was. Severe toxic manifestations would therefore appear to be associated with a high tissue concentration of the drug.

Investigations of acute mepacrine toxicity are necessarily incomplete because toxic effects develop accidentally and therefore few volunteers

were available for study. But the results obtained suggest that alimentary symptoms by themselves, are due to local action of mepacrine on the bowel; and severe systemic effects associated with a high blood mepacrine are due to a high tissue concentration of the drug. From the nature of the symptoms, the nervous system would appear to bear the brunt of the toxic action. The maximum safe post absorptive blood mepacrine is tentatively defined at 800 ug. per litre.

Dehydration favours the occurrence of alimentary symptoms. A liberal fluid intake with each dose of mepacrine will therefore prevent or at least minimise vomiting, colic, and diarrhoea.

Chronic Toxicity.* - Experiments on laboratory animals have shown that large doses of mepacrine cause necrosis of the liver.[19,20] Isolated reports of liver necrosis in human subjects taking mepacrine have neither proved nor disproved that

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* Published in The Lancet 2, 107, 1945. in collaboration with Lt.Col.W.R.M.Drew.

mepacrine was responsible.[7] The yellow staining of the skin of white men that results from taking mepacrine may have lent support to the belief that mepacrine was a liver poison because of the early difficulty in distinguishing it from jaundice.

At the time this problem was being discussed, British troops who had taken mepacrine as a malarial suppressive for periods ranging from 4 to 18 months, were returning home from West Africa. It was decided to examine a number of them to discover whether or not liver damage or any other significant disability had developed during their stay in the tropics. In these men positive findings would not necessarily implicate mepacrine, but negative findings would provide good evidence that mepacrine, taken in suppressive doses for prolonged periods, did not cause serious ill effects.

Investigations. - One hundred and two men, still taking 0.1g. mepacrine daily were available for study. Investigations were carried out on the following lines:-

Careful inquiry was made about the regularity with which mepacrine had been taken, about possible symptoms from taking the drug, and about attacks of malaria and other diseases during the period of service in West Africa.

Complete clinical examination was undertaken. Particular attention was paid to the size of liver and spleen. Liver function was assessed by the hippuric acid synthesis test.[20] If the test was abnormal or if there was any other reason to suspect liver involvement - such as hepatic or splenic enlargement - a further series of liver function tests was carried out. These included estimations of serum bilirubin, plasma protein, and serum phosphatase,[21] and the sucrose tolerance test.[22]

Histological examination of the liver was also carried out by liver puncture biopsy[23] on volunteers with hepatic or splenic enlargement or an abnormal hippuric acid synthesis test.

Other investigations were:- blood-counts,

including differential leucocyte count and an examination of thick and thin films for malaria parasites; faecal cultures for pathogenic bacteria; microscopic examination of stools for parasitic cysts, eggs, and worms; examination of the urine for abnormal constituents; and assessment of renal function by the urea-clearance test. The results are most conveniently considered under these headings.

Mepacrine Administration. - The 102 soldiers examined had been in West Africa for periods ranging from 8 to 36 months. All had been under orders to take mepacrine during the last 4 - 18 months of their stay. Thirteen had been ordered 0.2g. twice weekly for an initial period of 1 - 3 months; after this period all were ordered 0.1g. daily on six days of each week. The taking of the drug was supervised with a degree of thoroughness that varied considerably in different units.

Five men had complained of vomiting during the first week on mepacrine; seven others reported insomnia and nightmares after the drug had been

taken for several weeks; and two stated that these symptoms were especially noticeable when tablets were taken without water. These symptoms were never bad enough to interfere with duty or compel the men to stop taking the drug.

Incidence of Malaria and Other Diseases. - All the men had been exposed to malarial infection from 2 to 31 months before mepacrine was taken. During this time quinine was taken in a daily dose of grains 5 to reduce the incidence of malaria; on quinine suppression 70 men had 250 attacks of malaria and one had blackwater fever. For a comparable period on suppressive mepacrine, 40 men had 79 attacks of malaria and none had blackwater fever.

Next to malaria, dysentery was the commonest cause of illness. Sixteen men had dysentery - eight bacillary, five amoebic, and three of unspecified origin. The only other important diseases were jaundice and syphilis. Four of the 102 men had jaundice; attributed in two to infective

hepatitis, in one to yellow fever inoculation, and in the fourth to arsenical treatment for syphilis.

Clinical Findings. - Yellow staining of the skin from mepacrine was visible in all the men. The intensity of staining varied in different individuals, being well marked in 26, moderate in 65, and slight in 11. Colouration was most marked on the exposed parts of the body, and this may have been due to mepacrine deposition plus natural tanning. There were no skin lesions. Two men who showed staining of the conjunctivae had serum bilirubin values of 0.4mg. and 0.5mg. per 100 c.cm. blood. The edge of the liver was about two fingers-breadth below the costal margin on full inspiration in 9 men; the spleen was palpable in thirteen.

Liver Function Tests. - The hippuric acid synthesis test was abnormal in 9 of the 102 men. The abnormality was of moderate degree in 7 and marked in two. The other liver function tests carried out on these 9 men on the following day were all within normal

limits. The hippuric acid synthesis test was repeated after four months on 8 of the 9 men whose first test had shown abnormal values. During these four months they took no mepacrine. On this second test normal results were recorded in 7 men, and an abnormal value persisted in only one.

Liver Puncture Biopsy. - Ten men, who had taken mepacrine regularly for 6 to 17 months, were transferred to the British Post-Graduate Medical School for liver puncture to be performed by Dr.J.McMichael and Dr.S.P.V.Sherlock. Of these, seven had slight hepatic or splenic enlargement or an abnormal hippuric acid synthesis test. Clinical and biochemical findings were normal in the other three and they served as controls. No ill effects resulted from the liver puncture.

Professor J.H.Dible, who kindly examined the sections, reported as follows:-

> "The livers on the whole show no departure from normal, with the exception of some haemozoin pigment in the reticulo-endothelial cells, and slight excess of

> iron in about one-half of the cases. The former, I imagine, is only to be expected in men who have suffered from malaria in the not very distant past. The latter may be associated with anaemia. Apart from these observations, the livers appear healthy or show only slight variations such as one would consider to be within physiological limits."

Blood, Stool, and Urine Examinations. - The red-cell count ranged from 3.6 to 5.5 millions per c.mm.. The leucocyte count varied from 4,000 to 15,000 per c.mm. and averaged 6,000. The differential leucocyte count was within normal limits in 100 men. Two men showed an eosinophilis, one of 25% and one of 11%. Parasites of benign tertian malaria (Plasmodium vivax) were found in only one thick blood film.

Cysts of Entamoeba histolytica were found in the stools of three men. No pathogenic bacteria were isolated on culture from the stools. No abnormal constituents were found in the urine, except a trace of albumen on one occasion. The urea-clearance tests were all within normal limits.

Conclusions. - Falciparum malaria was by far the

most frequent disease encountered in West Africa. Eighty-four men had 329 attacks in 8 - 36 months. Eighteen men escaped a recognised attack of the disease.

At first sight this may appear to be a high incidence of malaria, but it must be remembered that in a hyperendemic area like West Africa, the incidence of malaria would probably be at least 100% per month if no anti-malarial precautions were taken. It should also be realised that the malarial incidence varied enormously with the quality of unit anti-malarial discipline, including the administration of suppressive drugs.

Slight enlargement of the liver and spleen has also been found in Australian soldiers taking suppressive mepacrine after experimental infection with falciparum malaria.[16]

The finding of a moderately abnormal hippuric acid synthesis test in seven men, and a markedly abnormal test in two men is not considered to have much significance, since other liver function tests

and the histological appearance of the liver were within physiological limits. At most, the defective hippuric acid tests might indicate a temporary derangement in liver function, as normal values were found in all but one man when the test was repeated later. Moreover, this upset cannot be attributed to mepacrine; for other factors, such as malaria were involved.

Other findings worthy of note were: a lowered red-cell count in an appreciable number of men, an unexplained eosinophilia in two men, and cysts of E.histolytica in the stools of three men. In 30 men the red-cell count ranged from 3.6 to 4.5 millions per c.mm. blood; but this slight anemia, which is probably a legacy of malaria, rapidly disappears after a therapeutic course of mepacrine.

Residence of from 8 to 36 months in West Africa need not of itself cause serious impairment to health. The greatest hazard is falciparum malaria, and its most serious effects may be

effectively prevented by strict observance of all anti-malarial precautions including the regular taking of mepacrine. In a proportion of men exposed for a long time to malarial infection slight hepatic and splenic enlargement may result while mepacrine is being taken. Liver function tests and histological examination indicate that no change in liver function or structure results from taking the drug. In short, there is no evidence that mepacrine in suppressive doses taken for 4 - 18 months has any cumulative toxic action on the liver, kidney, or blood-forming tissues. On the contrary, there is good reason to claim that the drug can eliminate severe and fatal forms of malignant tertian malaria, as well as serious complications like blackwater fever and much chronic ill health resulting from frequent attacks of malaria.

Since this investigation, a skin disease resembling lichen planus has been reported in a small proportion of individuals who have taken

mepacrine for prolonged periods; but whether the disease is incidental or related to the taking of mepacrine is still uncertain.

PART 4

THE PREVENTION AND TREATMENT OF MALARIA WITH MEPACRINE

Malaria Prevention. - The prevention of malaria with mepacrine has been found to be related to the amount of the drug in the blood. The minimum effective post absorptive level has been defined as 100 ug. per litre. When the blood mepacrine falls below this level, malarial fever may be expected in individuals infected with the disease. Whether fever is self-limiting or whether it develops into a frank attack of malaria depends on the tissue reserves of mepacrine and on the magnitude of the rise in blood mepacrine that accompanies the "acidosis" of fever.

The blood mepacrines after prolonged administration of daily doses of 0.1g., and weekly doses of 0.5g. of the drug preceded by a loading dose, have been defined. With both regimes stable

group mean blood mepacrines of about 100 ug. per litre were reached after 40 to 50 days, so that at any time after the sixth week about half the number of volunteers could be expected to have blood mepacrines below 100 ug. per litre. If these men were being continuously infected with sporozoites in a hyperendemic malarious area they would be expected to develop a short term, self-limiting fever, that would not usually be associated with parasitaemia. It is difficult to decide the practical importance of this fever because of lack of information on the natural rate of infection - and because of the absence of reliable figures on the incidence of short term "Pyrexias of Unknown Origin" in the field. But in 25 volunteers taking 0.1g. mepacrine daily, who were experimentally infected with sporozoites of falciparum malaria, 12 volunteers developed fever within 14 days of infection. The fever was of 1 day's duration in 8 men, 2 days' duration in 3 men, and of 3 days' duration in the remaining volunteers. That is,

in 14 days about half the volunteers were fevered for an average period of about 1½ days. If it is assumed that the rate of infection in a hyper-endemic area is 100% per month, then with 0.1g. mepacrine daily, about half the number of men would have fever for 1½ days every month. Short term pyrexia therefore deserves careful attention because it will tend to undermine health and reduce physical fitness rather than cause an acute disaster. It will be difficult if not impossible to recognise its essential connection with malaria in the field, because other causes of P.U.O. have to be considered. But its occurrence in such a large proportion of volunteers infected with malaria in this country is most significant. The fever occurred regularly at about the same time after infection in different groups of volunteers who were taking mepacrine and other antimalarial drugs. Subinoculation of another individual, with blood from a volunteer with fever but no demonstrable parasitaemia, resulted in the successful transmission of the disease.

Subsidence of fever coincided with a spontaneous rise in blood mepacrine. These findings, together with the fact that fever could not be attributed to any other cause, make it almost certain that it is a malarial manifestation. Many P.U.Os have been reported from hyperendemic malarious areas and it is likely that the majority are malarial in origin.

The real incidence of short term P.U.O. in hyperendemic malarious areas has not been determined because statistics have mainly been prepared from the returns of hospital admissions, and it is the usual practice to allow 48 hours to elapse before an admission is recorded. If the patient is fit within this time, no admission is registered, and since it may take several days to reach hospital many of the short term malarial P.U.Os will have cured themselves and will not have been reported. For these reasons a careful check of the real incidence of P.U.O.

in a hyperendemic malarious area is advocated so that its effect on manpower may be properly assessed. Moreover, as there is evidence to suggest that a higher group mean blood mepacrine may prevent short term fever, trials with larger doses of mepacrine are also recommended. In this connection it is interesting to note that daily doses of 0.5g. mepacrine have been used by small units in Burma without toxic effects, and with success in the prevention of malaria.

It should be made perfectly clear that assessment of the value of 0.1g. mepacrine daily has been made under very stringent conditions because the issues to be decided were of vital importance and did not permit a more elastic interpretation. It may be argued that the employment of other protective measures like nets, repellants, and insecticides, may so reduce the rate of infection that short term fever will be of little importance. This may be true under static conditions and during quiet periods when these measures may be properly

carried out, but it has not been the usual experience under conditions of stress. Both in Burma and Sicily when active fighting was in progress malaria rates increased. The other protective measures were largely discarded by fighting soldiers because they were alleged to interfere with military efficiency. In an emergency reliance has to be placed almost entirely on mepacrine. It is the most valuable drug we possess at present for the prevention of malaria. Nothing that has been said is intended to detract from this statement. It has effectively checked falciparum malaria, a disease that if left uncontrolled would have made sustained military operations in hyperendemic malarious areas impossible; it has undoubtedly prevented many deaths from malaria, and reduced chronic disability from serious complications of the disease. But, because it may be used to even greater advantage the view that 0.1g. mepacrine daily will prevent malaria is considered to be misleading. It is for practical purposes true, if parasites have to be demonstrated in a patient's

blood before malaria is diagnosed. Unfortunately failure to demonstrate parasites does not alter the fact that an individual with fever is not fit. Furthermore, the statement has been taken at its face value and the onset of malaria has been considered to be evidence of a breach in mepacrine discipline, and therefore, a punishable offence. It is difficult on the evidence available, even on grounds of expediency, to justify this procedure. An open mind and further work are still necessary before a final decision on the ultimate value of mepacrine as a preventive of malaria and its manifestations can be made. But the indications are that if a group mean blood level of about 200 ug. per litre can be maintained, short term fever as well as frank malaria may be prevented.

Treatment of an Attack of Malaria. - A post absorptive blood mepacrine of 100 ug. per litre has been found to prevent the occurrence of falciparum malaria after sporozoite infection.

Lower levels are not effective. It is also known that a more rapid clinical response is obtained with large doses of mepacrine than with small doses. The upper safe post absorptive blood mepacrine has been tentatively defined as 800 ug. per litre. The aim of treatment of malaria with mepacrine is therefore to raise the blood mepacrine to a post absorptive level of more than 100 ug. per litre, and as near to 800 ug. per litre, as quickly as possible. This may be effected by parenteral or by oral administration of the drug. But as very high levels may be attained with combined parenteral and oral treatment, the combination is not recommended for general use until further observations have been made. Only in dangerously ill patients should parenteral treatment be given. In these circumstances an initial dose of 0.2g. mepacrine followed by three 0.1g. doses may be given by intramuscular injection in the first 24 hours. Subcutaneous injection should be avoided. No

ill effects resulted after injection of the drug by this route into healthy volunteers, but when 0.2g. in 10 ml. distilled water was given by subcutaneous injection to a patient with malaria, a large ulcer developed at the site of injection that took fully 6 weeks to heal (Photograph Vol.2 p57).

The maximal total dose that may be given by mouth to a healthy adult male without the risk of incurring serious toxic effects is considered to be 3.0g. mepacrine in 5 to 7 days. The following dosage regime is recommended for trial:- a maximum total of 1.0g. in the first 24 hours; divided into 3 doses of 0.3g., 0.3g, and 0.4g. at six-hour intervals; followed by 0.5g. daily for the next four days, either as single doses or preferably divided into twice daily doses of 0.2g. in the morning and 0.3g. in the evening. A liberal fluid intake should be prescribed and at least half a pint of water should be given with each dose if the alimentary

symptoms due to mepacrine are to be avoided. These symptoms will tend to be aggravated by the dehydration associated with malaria.

Before concluding, it may be useful to discuss briefly the methods of investigation employed in this work to decide whether they have a more general application in testing other drugs. The first departure from the usual practice was to undertake concurrent investigations on chemotherapy, pharmacology, and toxicity. The second, was to employ concentration of the drug at its probable site of action instead of dose as a reference index for these investigations. As a result, it may fairly be claimed that the value of mepacrine as an antimalarial agent has been defined in a shorter time, and with more precision than would have been possible by the older empirical method. Advances in biochemical technique, and the introduction of sensitive fluorimeters and colorimeters have made it possible to estimate ranges of concentration of drugs in biological

fluids that were hitherto impossible. This has enabled the greatest possible benefit to be derived from a combined study of chemotherapy, pharmacology, and toxicity.

Before the therapeutic value of a drug is known, three properties have to be defined:-

1. - The minimum concentration of the drug at the site of action that will give a maximum therapeutic response.

2. - The concentration of the drug at its site of action in relation to dose.

3. - The maximum concentration of the drug at the site of action that is free from undesirable toxic effects.

It is considered that the same technique as has been employed for mepacrine may also be applied to almost all diseases in which drugs have a beneficial or curative action.

The advantages of this approach over the older method of assessing the value of a drug are

considerable. In addition to being a quicker, more precise, and more logical approach, it is less likely to be associated with toxic effects from overdosing, or protracted illness from underdosing - the usual accompaniments of new drugs soon after their introduction. These effects may be avoided because treatment need not start and finish with the administration of drugs, it may be controlled throughout the course of the disease by appropriate estimation of drug concentration in biological fluids. These are the obvious advantages, but there are others that are probably even more important. In the work on mepacrime; observations on the chemotherapeutic action of the drug; on the partition of the drug between blood and tissues; on the absorption, degradation, excretion, and toxicity of the drug; have all been leading to the same end, namely, the identification of a particular biological reaction with the physical and chemical properties of mepacrine. Further investigation along these lines is still necessary,

but it is reasonable to hope that therapeutic, pharmacological, and toxic properties of drugs may eventually be associated with particular physical and chemical properties. When this has been achieved it may be possible to introduce new remedies for the treatment of disease that are not, as at present, wholly dependent on more or less fortuitous discoveries in the general field of biology.

SUMMARY

1. - Prevention of falciparum malaria in volunteers experimentally infected with sporozoites has been found to depend on the concentration of mepacrine in the blood. When the blood mepacrine is 100 ug. per litre or more, malaria does not appear. But, when the blood mepacrine is less than 100 ug. per litre, a fever develops that is almost certainly malarial in origin, though parasites are not usually found in the blood. Whether this fever is self-limiting, or whether it develops into a frank attack of malaria, depends on whether enough mepacrine is transferred from tissues to the blood to raise the blood mepacrine to a parasiticidal level. The transference of mepacrine from tissues to the blood is considered to be due to a diminution in the tissue : blood partition of the drug resulting from the change in pH of tissues and blood that accompanies fever.

2. - The dye properties of mepacrine are most

important in determining the distribution of the drug in the body. The drug tends to concentrate in tissues rather than in internal body fluids. For this reason tissue mepacrine is the dominant pharmacological factor. It is largely responsible for the persistence of the drug in the body long after dosing has been stopped. Blood mepacrine is directly proportional to tissue mepacrine, and the partition of the drug between the two is determined by the pH of tissues and blood. In healthy men engaged in sedentary occupations, the tissue : blood partition is practically constant. In disease associated with diminution in pH of tissues and blood, the partition may be markedly diminished, with the result that blood mepacrine increases at the expense of tissue mepacrine.

The blood- and tissue-mepacrine levels of healthy individuals depend mainly on dose. For each dose, an equilibrium between dose and blood- and tissue-mepacrine, is eventually reached. The greater the dose, the higher the blood- and

tissue-mepacrines.

Absorption of mepacrine is inversely proportional to the blood- and tissue-levels of the drug. That is, when blood- and tissue-levels are low, maximal absorption takes place; when blood- and tissue-levels are in equilibrium with dose, absorption is minimal and is determined by the amount of drug degraded in the tissues.

Degradation of mepacrine which occurs in tissues, blood, and alimentary tract is probably an oxidative process in the first place. Evidence has been found suggesting that the rate of degradation in blood is proportional to the rate of degradation in tissues, and that both are proportional to the respective concentration in blood and tissues. Degradation by alimentary contents is considered to be mainly responsible for the fall in blood mepacrine from the initial peak, to the late stable level that has been observed after prolonged administration of the drug.

Mepacrine is mainly excreted in urine and

faeces, and the total excretion of the drug is determined by the blood- and tissue-mepacrine levels. When these are high, greater amounts are excreted than when they are low.

After tablets by mouth, the blood mepacrine is much lower than after parenteral or rectal injection of the same dose. The difference is due, partly to the time required for solution of the tablets, partly to the pH effect of the gastric contents, and partly to diminished absorption resulting from degradation of mepacrine in the bowel. Combined parenteral and oral administration may result in very high blood-mepacrine levels. Parenteral injection seems, in a way that is not understood, to increase subsequent absorption from the bowel.

3. - Toxic effects from mepacrine may arise soon after administration of the drug. They may be mild, temporarily incapacitating, or wholly incapacitating. Mild symptoms; like headache, nausea, epigastric discomfort, and minor visual disturbances - are most frequent, and occur with large and small

doses of the drug. They pass off if administration of small doses is continued. Temporary incapacitating symptoms - such as vomiting, colic, and diarrhoea are considered to be mainly due to local action of mepacrine on the bowel. They may be avoided, or at least minimised, by ensuring a liberal fluid intake. Wholly incapacitating effects - characterised by lassitude and depression, and sometimes by localised neurological symptoms - are associated with high blood- and tissue-mepacrines, and arise only when large doses of the drug are given. The nervous system seems to bear the brunt of the initial toxic action.

Prolonged administration of mepacrine in doses of 0.1g. daily does not appear to cause damage to the liver, kidneys, or blood-forming tissues. A skin lesion, resembling lichen planus, has been reported after prolonged administration of the drug, but its specific connection with mepacrine is still uncertain.

4. - Doses of 0.1g. mepacrine daily will prevent

frank malaria in the majority of individuals taking the drug. But, if infection is heavy, a considerable proportion of those infected may be expected to develop a short-term, self-limiting fever that is essentially malarial in origin. Available evidence suggests that this fever may also be prevented by maintaining a higher, stable blood-mepacrine level. For this reason a careful assessment of the incidence of "Pyrexia of Unknown Origin" in hyperendemic regions is advocated, as well as preliminary trials with larger doses of the drug.

5. - The aim of the treatment of malaria with mepacrine is to raise the blood mepacrine above 100 ug. per litre, and near to 800 ug. per litre, as quickly as possible. A dosage regime that will give maximal safe blood mepacrines has been put forward for trial. Treatment by parenteral injection of mepacrine should, at present, be reserved for seriously ill patients.

6. - Advantage has been taken of improvements

in biochemical technique to investigate the chemotherapeutic, pharmacological, and toxic action of mepacrine concurrently. The concentration of the drug in the blood has been employed as the reference index instead of dose. The work so far completed, and investigations in progress, suggest that mepacrine acts directly on malarial parasites. This action may depend on blocking oxidative enzyme systems within the parasite. Toxic action in man may also depend on the same mechanism.

The advantages of the methods employed in this work over the older methods of assessing the value of a drug have been discussed. The same technique may be usefully employed to assess the therapeutic value of almost any drug that has a curative action in disease.

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VOLUME 2

TABLES GRAPHS AND ILLUSTRATIONS

PART 5

Table 1.

MONTH OF INFECTION AND REACTION TO INFECTION.

Group	Total No. of Volunteers	Month of Infection	No. of Volunteers developing:- Malaria	Fever	No Reactions
1	25	Sept.- Oct.1943	1	11	13
2	7	Nov.- Dec.1943	0	2	5
3	6	Feb.- March 1944	3	2	1
4	12	June 1944	6	4	2
5	4	Sept.1944	2	1	1

Groups 1, 2, 3, and 4 were infected by mosquito bites. Group 5 was infected by intravenous injection of a sporozoite gland suspension.

Table 2.

DOSE OF MEPACRINE AND REACTION TO SPOROZOITE INFECTION.

Group	Total No. of Volunteers	Mepacrine Dosage	Infection(s)	No. of Volunteers developing:- Malaria	Fever	No Reactions
I A	25	0.1g. daily for 49 to 69 days.	21 to 35 days after starting dosing.	1	11	13
I B	6	0.1g. daily for 20 days.	First day of dosing.	2	2	2
II A	7	Loading dose of 1.0g. in 3 days then 0.1g. daily till day 25.	Fourth day of dosing.	0	2	5
II B	5	Loading dose of 1.0g. in 3 days and 0.1g. daily for 4 days.	Fourth day of dosing.	5	0	0

Group IIA was infected with Italian M.T. malaria.
The others were infected with Roumanian M.T. malaria.
Group IA was infected on 4 occasions, the others on one occasion.

Table 3.

TRIPLICATE ESTIMATION OF BLOOD MEPACRINE.

(Single Extraction Method of Brodie and Udenfriend).

Sample No.	Blood Mepacrine ug./litre Estimation:- 1	2	3	Mean
1	71	71	66	69
2	70	65	73	69
3	67	80	67	71
4	75	71	79	75
5	80	80	93	84
6	93	93	80	88
7	87	91	87	88
8	84	100	84	89
9	84	107	84	92
10	131	138	123	130
11	137	133	137	135
12	280	291	286	285

Table 4.

BLOOD MEPACRINE AND MALARIA.

Group	Total No.of Volun-teers	Malarias		Others	
		No.	Blood Mepacrine ug./litre	No.	Blood Mepacrine ug./litre
A	6	1	77	5	150, 230, 254, 225 and 337
B	3	2	53 60	1	113
C	8	6	10 20 20 40 40 64	2	100, 120
D	3	1	20	2	220, 260
E	2	2	26 70	0	
F*	5	0	-	5	220,360,300,316,286 (7 days) - 480,448,240,520 (14days) 248,680 - 420,408 (20days)

* Group F had no malarias but the blood levels at 7 days, 14 days, and 20 days after infection are shown for comparison with the others.

Table 5.

BLOOD MEPACRINE AND FEVER.

Volunteer No.	Blood Mepacrine			
	Before Fever		After Fever	
	*No.of Day(s)	ug./L.	*No.of Day(s)	ug./L.
1	1	15	5	100
2	1	52	2	228
3	-	-	2	928
4	3	150	1	288
5	5	224	2	760

* No. of Day(s) before or after fever that the blood mepacrine estimations were made.

Table 6.

BLOOD MEPACRINE AND DOSE.

Regime No.	Total Dose mg.	Volunteer No.	Blood Mepacrine (ug. per litre) Hours after initial dose. 4	12	24	48	72	96	120
1	1.8	1	220	205	100	200	300	266	395
		2	190	160	102	235	235	200	295
		3	280	185	117	160	250	225	280
		4	190	125	37	155	185	175	185
		5	220	135	67	160	200	-	200
		6	-	170	67	145	200	210	185
		7	235	285	135	240	235	330	395
		8	160	120	65	140	200	310	180
Group Mean:-			213	173	86	179	225	245	264
2	3.4	9	52	115	185	190	225	265	450
		10	115	185	355	450	725	750	1115
		11	160	390	530	525	760	715	1085
		12	124	185	280	275	385	665	850
		13	68	325	400	570	570	850	700
		14	76	270	535	490	705	680	1000
		15	144	275	410	410	550	830	915
Group Mean:-			105	249	385	415	560	679	873
			Hours after initial dose. 8	16	29	53	77	101	125
3	3.2	16	132	410	340	590	544	815	665
		17	144	360	425	670	690	580	965
		18	150	490	455	750	660	700	1030
		19	94	295	265	490	525	562	835
		20	164	540	540	880	640	800	915
		21	144	255	450	645	500	600	915
		22	136	300	300	410	690	600	665
		23	88	255	330	510	585	-	880
Group Mean:-			131	363	388	618	604	665	858

Table 7.

URINE MEPACRINE AND DOSE.

Regime 1.

Volunteer No.	Urinary Mepacrine (mgm.) Period (hours)								
	0-4	4-8	8-12	12-24	0-24	24-48	48-72	72-96	96-120
1	0.11	0.65	1.26	4.50	6.52	9.56	-	-	-
2	0.18	0.33	0.96	3.41	4.88	8.53	10.05	8.11	5.18
3	0.07	0.38	1.02	2.25	3.72	6.56	6.95	8.99	9.02
4	0.12	0.33	0.44	1.31	2.20	6.23	5.01	5.30	6.44
5	0.08	0.51	0.79	2.69	4.07	6.72	10.40	13.55	10.52
6	0.06	0.21	0.41	1.93	2.61	5.18	6.88	7.62	6.51
7	0.16	0.36	2.10	3.70	6.42	10.43	10.98	11.34	10.93
8	0.05	2.26	0.80	2.05	5.18	5.45	6.36	6.28	6.54
Mean	0.10	0.63	0.97	2.73	4.45	7.33	8.09	8.74	7.86

Table 8.

URINE MEPACRINE AND DOSE.

Regime 2.

Volunteer No.	Urinary Mepacrine (mgm.) Period (hours)							
	0-8	8-16	16-24	0-24	24-48	48-72	72-96	96-120
9	0.60	0.84	1.87	3.31	9.90	14.0	20.0	21.20
10	0.45	0.58	3.36	4.49	25.86	36.40	21.30	37.0
11	0.28	0.63	4.74	5.65	23.90	29.54	30.0	43.9
12	0.75	0.23	1.54	2.52	11.87	19.0	30.50	27.10
13	1.24	0.54	9.56	11.34	27.90	29.60	21.20	23.40
14	0.49	5.36	4.95	10.80	21.0	15.70	14.20	33.80
15	0.50	1.36	2.75	4.61	18.0	26.20	32.20	46.50
Mean	0.61	1.36	4.11	6.10	19.77	24.35	24.2	33.3

Table 9.

URINE MEPACRINE AND DOSE.

Regime 3.

Volunteer No.	Urinary Mepacrine (mgm.) Period (hours)										
	0-4	4-8	8-12	12-16	16-20	20-29	0-29	29-53	53-77	77-101	101-125
16	0.13	0.53	0.58	0.73	0.90	0*	2.67	31.60	25.45	37.40	21.80
17	0.01	0.60	0.60	0.56	1.34	4.53	7.64	20.75	30.80	22.0	72.0
18	0.08	0.12	0.30	0.51	2.15	4.80	7.96	22.34	31.07	17.6	21.80
19	0.30	0.74	0.22	1.35	1.90	3.60	8.11	24.0	22.50	11.50	63.60
20	0.19	0.51	0.73	0.95	4.19	10.50	17.07	19.4	24.70	22.50	27.80
21	0.08	0.41	0.28	0.73	0.58	13.03	15.11	25.1	27.60	25.90	30.0
22	0.30	0.26	0.58	1.37	0.71	3.50	6.72	13.0	17.50	18.70	16.20
23	0.11	3.48	1.44	1.50	1.80	5.18	13.51	19.40	14.40	24.80	18.40
Mean	0.15	0.83	0.59	0.96	1.70	5.64	9.85	21.95	24.25	22.55	33.95

* No urine excreted.

Table 10.

BLOOD MEPACRINE AFTER ORAL ADMINISTRATION OF TABLETS.

Dose 0.2g. Mepacrine Hydrochloride

Volunteer No.	Blood Mepacrine (ug./litre)					
	¼ hr.	¾ hr.	2½ hrs.	5½ hrs.	10½ hrs.	24 hrs.
16	33	33	76	73	46	33
17	26	83	275	66	33	46
18	73	93	180	46	46	86
19	43	80	73	73	53	53
20	130	126	207	80	60	-
Mean	61	83	162	67	47	54

Table 11.

BLOOD MEPACRINE AFTER ORAL ADMINISTRATION OF MEPACRINE IN SOLUTION.

Dose 0.2g. Mepacrine Hydrochloride.

Volunteer No.	Blood Mepacrine (ug./litre)			
	¼ hr.	¾ hr.	2½ hrs.	5½ hrs.
21	150	208	193	140
22	150	350	153	187
23	183	217	153	233
24	175	183	127	147
25	167	183	253	173
26	175	217	133	187
Mean	166	226	168	178

Table 12.

BLOOD MEPACRINE AFTER RECTAL INJECTION.

Dose 0.2g. Mepacrine Hydrochloride.

Volunteer No.	Blood Mepacrine (ug./litre)					
	¼ hr.	¾ hr.	2½ hrs.	5½ hrs.	10½ hrs.	24 hrs.
27	240	500	133	120	-	50
28	283	1200	86	95	-	55
29	530	365	116	95	100	45
30	335	465	120	140	140	40
Mean	347	632	113	112	120	47

Table 13.

BLOOD MEPACRINE AFTER INTRAVENOUS INJECTION.

Dose 0.2g. Mepacrine Hydrochloride.

Volunteer No.	Blood Mepacrine (ug./litre)					
	⅓ hr.	¾ hr.	2½ hrs.	6 hrs.	12 hrs.	24 hrs.
31	290	140	150	127	67	87
32	340	200	138	120	87	107
33	335	165	-	107	87	107
34	400	190	215	127	67	107
35*	450	330	465	107	140	73
Mean	341	173	125	120	77	102

* Part of the dose given subcutaneously. Results excluded in calculating the mean blood mepacrine.

Table 14.

BLOOD MEPACRINE AFTER INTRAMUSCULAR INJECTION.

Dose 0.2g. Mepacrine Hydrochloride.

Volunteer No.	Blood Mepacrine (ug./litre)					
	¼ hr.	¾ hr.	2½ hrs.	6 hrs.	12 hrs.	24 hrs.
36	650	365	185	133	93	113
37	385	365	165	107	87	107
38	735	350	165	133	120	80
39	385	200	235	127	93	87
40	665	235	150	120	100	73
Mean	564	303	180	124	98	92

Table 15.

BLOOD MEPACRINE AFTER SUBCUTANEOUS INJECTION.

Dose 0.2g. Mepacrine Hydrochloride.

Volunteer No.	Blood Mepacrine (ug./litre)					
	¼ hr.	¾ hr.	2½ hrs.	5½ hrs.	10½ hrs.	24 hrs.
41	812	480	153	93	91	53
42	692	286	206	140	133	55
43	195	746	140	93	91	56
44	746	233	106	106	108	53
45	640	313	385	153	91	83
Mean	617	412	198	117	102	60

Table 16.

BLOOD MEPACRINE AFTER REPEATED SUBCUTANEOUS INJECTIONS.

Volunteer No.	Blood Mepacrine after the Initial Injection. (ug. per litre)						
	¼hr.	2hrs.	2¼hrs.	5hrs.	5¼hrs.	9hrs.	24hrs.
1	600	455	1030	265	800	325	145
2	800	430	830	185	-	265	115
3	665	465	1000	180	485	215	85
4	-	430	930	200	535	260	125
Group Mean.	688	440	947	207	606	266	117

Subcutaneous injections:- 0.2g. at zero hour.
0.1g. at 2 hours and at 5 hours.

Table 17.

BLOOD MEPACRINE AFTER COMBINED INTRAMUSCULAR AND ORAL ADMINISTRATION.

Volunteer No.	Blood Mepacrine after the First Dose. (ug. per litre)								
	¼ hr.	¾ hr.	1½ hrs.	3 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.
1	930	565	400	315	165	5440	960	475	416
2	1300	465	305	135	85	345	440	-	292
3	1130	300	200	135	90	415	440	383	267
4	665	635	245	150	165	985	560	666	332
5	800	435	245	185	125	2080	445	333	367
6	330	500	270	150	115	2720	320	300	267
7	365	335	255	150	100	825	665	383	332
8	535	900	335	315	135	1570	495	336	332
Group Mean.	757	517	282	191	122	1798	540	411	325

Mepacrine Dosage:- 0.2g. by intramuscular injection and
0.2g. by mouth at zero hour.
0.4g. by mouth at 6, 14, and 24 hours.

Table 18.

BLOOD MEPACRINE AFTER PROLONGED ADMINISTRATION.

Dosage Regime - No.5 Day 1, 0.1g.; day 2, 0.5g.; day 3, 0.6g.; Days 4 to 28 inclusive 0.1g. daily.

Volunteer No.	Blood Mepacrine (ug. per litre).									
	Day 4	Day 11	Day 17	Day 24	Day 29	Day 37	Day 44	Day 58	Day 82	Day 121
58	408	320	260	240	380	148	232	200	30	20
59	548	392	-	860	928	-	-	-	84	-
60	300	316	240	420	400	100	140	140	-	88
61	900	272	200	-	216	208	272	420	-	-
62	388	300	288	288	240	90	180	160	64	20
63	512	440	480	-	760	200	420	264	58	60
64	648	286	520	408	-	160	200	440	54	20
Group Mean	515	332	328	443	487	151	240	270	58	41

Volunteers No. 59, 60, 61, and 64 were infected with sporozoites of M.T. malaria (Italian strain) on day 4. Nos 60 and 64 had no reaction to infection. Nos 59 and 61 had fever without parasitaemia, starting on days 20 and 14 respectively.

Table 19.

URINE MEPACRINE AFTER PROLONGED ADMINISTRATION.

Dosage Regime - No.5	Day 1, 0.1g.; day 2, 0.3g.; day 3, 0.6g.; Days 4 to 25 inclusive 0.1g. daily.									
Volunteer No.	Urinary Mepacrine (Night urine mg. per litre).									
	Day 4	Day 11	Day 17	Day 24	Day 29	Day 37	Day 44	Day 58	Day 82	Day 121
58	7.0	22.2	4.8	7.7	11.2	1.2	3.4	3.9	0.07	0
59	10.0	7.5	-	3.3	8.0	1.5	-	-	0.10	-
60	2.8	6.1	4.5	2.0	7.3	2.5	1.5	0.8	0.13	-
61	7.2	9.0	2.0	-	3.8	2.4	0	-	-	-
62	4.4	1.6	0.8	4.8	4.6	0.3	0	0.4	0.03	0
63	18.2	21.0	16.1	-	16.0	7.3	1.1	0.2	0.20	0
64	7.4	12.2	7.0	12.2	4.6	4.7	3.3	2.0	0.13	0
Group Mean	8.1	11.4	5.8	6.0	7.9	2.8	1.6	1.3	0.11	0

Volunteers No. 59, 60, 61, and 64 were infected with sporozoites of M.T. malaria (Italian strain) on day 4. Nos 60 and 64 had no reaction to infection. Nos 59 and 61 had fever without parasitaemia, starting on days 20 and 14 respectively.

Table 20.

BLOOD MEPACRINE AFTER PROLONGED ADMINISTRATION.

Dosage Regime No.6:- Day 1, 0.1g.; day 2, 0.2g.; day 3, 0.3g.; day 4, 0.4g.; day 5, 0.5g.; and then 0.5g. at weekly intervals until day 76.

Day	Blood Mepacrine (ug./litre). Volunteer No:-						
	65	66	67	68	69	70	Group Mean.
3	40	44	30	16	64	56	41
6	188	168	260	188	132	168	184
13+	120	168	160	56	64	80	108
20+	132	120	160	100	80	100	115
27+	-	64	-	80	48	56	62
34+	160	100	116	132	116	80	117
41+	120	80	160	116	116	64	109
48+	132	80	-	116	80	100	101
55+	168	132	-	-	-	100	
69+	168	64	168	80	80	-	112
76+	-	116	168	80	64	-	107

+ Weekly doses of 0.5g. given immediately after blood and urine samples were collected.

Table 21.

URINE MEPACRINE AFTER PROLONGED ADMINISTRATION.

Dosage Regime No.6:- Day 1, 0.1g.; day 2, 0.2g.; day 3, 0.3g.; day 4, 0.4g.; day 5, 0.5g.; and then 0.5g. at weekly intervals until day 76.

Day	Urinary Mepacrine before and after the Weekly Dose. (mgm. per 3 hours). Volunteer No:- 65	66	67	68	69	70	Group Mean.
13+	0.36	0.11	0.20	0.28	0.37	0.82	0.35
14	0.38	0.49	0.44	-	-	0.61	0.48
20+	1.0	0.66	0.42	0.65	0.18	0.73	0.61
21	-	-	0.64	-	-	-	
27+	-	0.42	0.48	0.35	0.55	0.63	0.49
28	-	0.66	0.94	0.82	0.89	0.85	0.83
34+	0.24	0.28	0.34	0.21	0.31	0.50	0.31
35	1.50	1.24	2.11	1.0	1.53	1.10	1.41
41+	0.34	0.33	0.29	-	-	0.28	0.31
42	2.70	2.30	2.40	1.45	1.69	1.19	1.95
48+	0.34	0.15	-	-	-	0.24	0.24
49	2.11	2.0	-	1.53	1.94	1.63	1.84
55+	0.27	0.26	-	-	-	0.25	0.26
56	-	2.33	-	-	-	1.64	
69+	0.63	0.14	0.36	0.19	0.22	-	0.31
70	-	-	-	-	-	-	-
76+	-	0.30	0.66	0.17	-	-	0.38
77	-	1.34	2.56	-	-	-	

+ Weekly dose of 0.5g. given immediately after blood and urine samples were collected.

Table 22.

RECOVERY OF MEPACRINE ADDED TO FAECES.

50 mgm. mepacrine added to each stool at zero hour.					
Stool	Sample	Mg. Mepacrine recovered at:- 3hrs.	24hrs.	48hrs.	72hrs.
A	1	33.8	28.8	9.5	7.5
	2	31.2	24.0	8.0	7.0
B	1	34.6	23.0	10.1	
	2	38.6	22.7	9.6	
	3	36.2	22.3	8.7	
C	1	33.6	22.1		
	2	37.6	25.5		
	3	40.0	24.7		
Mean Recovery Mgm.		35.7	23.9	9.1	7.2
Per cent.		71.4	47.8	18.2	14.4

Tables 23 - 29.

BLOOD URINE AND FAECAL MEPACRINES AFTER PROLONGED ADMINISTRATION.

Table	Volunteer No.	Blood, Urine and Faecal Mepacrines.	Dosage Regime 0.1g. mepacrine daily. Day of Dosing 63	64	65	66	67	68
23	71	*BM.ug/L	160	160	132	160	168	160
		UM.mg/L	3.9	3.9	2.2	1.2	2.6	2.1
		UM.mg/24hrs	5.7	10.1	3.2	4.2	5.1	3.8
		FM.mg/24hrs	18.5	51.5	42.6	-	23.0	35.6
24	72	BM.ug/L	80	100	120	132	100	64
		UM.mg/L	2.0	4.7	1.4	0.9	2.2	1.5
		UM.mg/24hrs	5.8	6.0	2.7	1.9	2.9	3.6
		FM.mg/24hrs	10.7	34.3	40.0	18.7	-	33.3
25	73	BM.ug/L	80	-	132	116	80	116
		UM.mg/L	3.5	4.7	1.9	-	-	-
		UM.mg/24hrs	11.9	7.6	3.5	-	-	-
		FM.mg/24hrs	17.3	52.6	38.3	33.3	22.2	14.3
26	74	BM.ug/L	80	64	100	80	100	72
		UM.mg/L	1.7	2.2	1.1	1.3	1.2	1.3
		UM.mg/24hrs	8.2	2.1	3.9	1.5	3.7	2.1
		FM.mg/24hrs	-	30.1	30.0	20.0	24.0	15.3

* BM. = Blood Mepacrine.
UM. = Urinary Mepacrine.
FM. = Faecal Mepacrine.

Continued on next page.

Tables 23 - 29 (continued).

Table	Volunteer No.	Blood, Urine and Faecal Mepacrines.	Dosage Regime 0.1g. mepacrine daily. Day of Dosing 63	64	65	66	67	68
27	75	BM.ug/L	116	80	160	116	116	80
		UM.mg/L	2.4	5.5	1.4	1.1	2.0	1.3
		UM.mg/24hrs	4.4	4.6	2.9	3.3	4.7	3.0
		FM.mg/24hrs	2.7	35.6	15.1	34.4	20.0	38.5
28	76	BM.ug/L	80	80	120	80	-	72
		UM.mg/L	-	-	1.1	1.2	1.8	1.1
		UM.mg/24hrs	-	-	2.9	2.9	4.9	4.3
		FM.mg/24hrs	10.5	-	51.6	16.4	18.4	17.7
29	77	BM.ug/L	100	72	120	-	-	80
		UM.mg/L	2.6	3.9	1.9	1.0	2.9	2.4
		UM.mg/24hrs	4.5	11.0	2.7	1.6	5.0	3.5
		FM.mg/24hrs	9.3	19.0	-	54.5	22.0	29.0

Table 30.

BLOOD URINE AND FAECAL MEPACRINE AFTER PROLONGED ADMINISTRATION.

Day	Volunteer No.82 Weekly dose of mepacrine 0.5g. on day 79			
	Blood Mepacrine ug./L.	Urinary Mepacrine mg./L.	mg./24hrs	Faecal Mepacrine mg./24hrs
77	160	-	-	7.4
78	116	0.07	0.14	5.8
79	160	0.03	0.06	6.1
80	168	2.70	4.0	81.6
81	160	3.0	5.0	24.3
82	160	1.90	2.3	23.0
83	132	1.1	2.2	9.2
84	-	0.06	0.15	7.0
85	-	0.08	0.17	-

Table 31.

DEGRADATION OF MEPACRINE IN INCUBATED BLOOD.

Days after withdrawal of blood.	Blood Mepacrine ug./litre.		
	Estimation:-		
	No.1	No.2	Mean
0	600	550	575
1	420	400	410
2	320	320	320
3	260	240	250
4	200	200	200
5	200	200	200
6	160	160	160
7	160	160	160
8	120	140	130

Table 32.

DEGRADATION OF MEPACRINE IN PLASMA AND URINE

Days after withdrawal of blood.	Plasma Mepacrine ug./litre	Days after addition of mepacrine to urine.	Urinary Mepacrine mg./litre
0	120	0	10.2
1	120	1	9.8
2	120	2	-
3	100	3	10.2
4	100	4	-
5	-	5	9.8
6	80	6	9.5
7	-	7	9.5

Table 33.

DEGRADATION OF MEPACRINE IN FAECES.

Volunteer.	Mepacrine added to 50g. faeces mg.	Mepacrine recovered after 24hrs mg.	% Recovery after 24hrs.
"D"	10	10.1	101
	50	25.7	51
	100	81.3	81
	250	284.5	114
	500	504.5	101
"E"	10	10.4	101
	50	20.5	41
	100	91.0	91
	250	279.6	112
	500	521.4	104

Table 34.

DEGRADATION OF MEPACRINE IN INCUBATED BLOOD AND RATE OF FALL IN CIRCULATING BLOOD.

Days after withdrawal of blood	Blood Mepacrine ug./litre	
	Circulating Blood	Incubated Blood
0	575	575
1	380	410
2	320	320
3	240	250
4	220	200
5	200	200
6	160	160
7	160	160
8	140	130

Table 35.

DEGRADATION OF MEPACRINE IN INCUBATED AND CIRCULATING BLOOD.

Days after withdrawal of blood	Blood Mepacrine ug./litre							
	Volunteer 1.		Volunteer 2.		Volunteer 3.		Volunteer 4.	
	*C B.	I B.	C B.	I B.	C B.	I B.	C B.	I B.
0	-	-	600	600	360	360	-	-
1	630	600	530	540	290	300	100	140
2	420	400	410	460	280	260	100	80
3	350	360	340	400	230	240	80	60
4	290	320	310	280	180	180	60	60
5	160	140	200	200	160	160	40	40
7	120	120	180	150	120	100	40	40

* C B. = Circulating Blood.
I B. = Incubated Blood.

Table 36.

BLOOD PLASMA AND URINARY MEPACRINES DURING DOSING.

Dosage Regime :- 0.6g. mepacrine day 1.
0.3g. " days 2 to 5.

Volunteer No.	Blood Mepacrine ug./litre Hours after initial dose:- 4	24	48	72	96	120
1	220	100	200	300	266	395
2	190	102	255	235	200	295
3	280	117	160	250	225	280
4	190	37	155	185	175	185
5	220	67	160	200	-	200
6	-	67	145	200	210	185
7	255	135	240	255	330	395
8	160	65	140	200	310	180
	Plasma mepacrine ug./litre.					
1	25	27	35	33	40	41
2	26	17	-	40	43	29
3	44	22	36	25	40	58
4	26	17	30	27	53	23
5	38	22	30	40	-	29
6	44	21	40	33	33	23
7	44	15	36	33	33	44
8	32	22	36	33	30	23
	Urinary Mepacrine mg./24hrs.. Hours:- 0-24	24-48	48-72	72-96	96-120	
1	6.52	9.56	-	-	-	
2	4.88	8.53	10.05	8.11	5.18	
3	3.72	6.56	6.95	8.99	9.02	
4	2.20	6.23	5.01	5.30	6.44	
5	4.07	6.72	10.40	13.55	10.52	
6	2.61	5.18	6.88	7.62	6.51	
7	6.42	10.43	10.98	11.34	10.83	
8	5.18	5.45	6.56	6.28	6.54	

Table 37.

BLOOD PLASMA AND URINARY MEPACRINES AFTER DOSING.

Dosage:- 3.4g. mepacrine in the previous 7 days.

Volunteer No.	Blood Mepacrine ug./litre. Days after last dose of mepacrine. 1	2	3	5	6	9	12	14	16	20	27
1	285	262	302	165	159	109	74	57	86	69	34
2	239	216	267	142	130	91	51	40	40	46	28
3	240	256	246	97	109	86	51	34	34	63	34
4	262	273	256	171	165	126	45	51	51	74	28
5	228	246	259	105	115	75	34	34	46	69	28
6	353	273	252	69	97	80	34	28	40	40	28
7	307	234	240	120	142	86	63	57	51	63	34
8	210	177	194	103	126	80	45	45	51	63	34
9	253	194	216	80	97	51	34	51	28	46	28
10	220	205	200	115	86	63	28	34	40	51	28
	Plasma Mepacrine ug./litre.										
1	40	46	74	11	17	44	14	11	23	34	17
2	51	40	40	11	11	43	2	7	6	23	17
3	40	51	28	17	11	40	5	7	6	28	23
4	23	36	57	12	17	41	11	11	6	28	17
5	17	23	46	22	6	40	11	5	6	23	17
6	23	23	23	22	11	40	13	2	11	23	11
7	-	40	51	17	6	21	13	4	0	23	17
8	17	46	37	11	11	27	14	7	6	28	23
9	31	31	46	12	11	14	13	8	6	28	17
10	28	28	28	11	11	27	14	7	6	28	23
	Urinary Mepacrine mg./3 hrs.										
1	1.0	1.50	0.93	0.79	0.73	0.40	0.31	0.22	0.21	0.16	0.08
2	0.70	0.49	1.50	1.0	0.70	0.24	0.27	0.19	0.11	0.13	0.09
3	0.76	0.84	0.30	0.48	0.36	0.07	0.13	0.15	0.12	0.10	0.33
4	1.30	1.02	1.32	0.71	1.0	0.22	0.40	0.28	0.28	0.24	0.10
5	1.20	1.20	0.82	0.85	0.56	0.13	0.14	0.19	0.31	0.12	0.09
6	0.91	0.44	0.18	0.50	0.15	0.32	0.17	0.16	0.46	0.07	0.27
7	0.70	0.55	0.39	0.65	0.30	0.24	0.42	0.13	0.13	0.15	0.07
8	1.20	0.53	0.85	0.89	0.55	0.28	0.32	0.05	0.26	0.14	0.08
9	1.10	0.67	0.70	0.15	0.20	0.17	0.12	0.15	0.48	0.41	0.07
10	0.12	0.27	1.04	0.58	0.72	0.14	0.28	0.28	0.35	0.16	0.05

Table 38.

DAILY URINARY OUTPUT.

Volunteers taking Restricted Fluids	Daily Urinary Output ml. Day-3	Day-2	Day-1	Day of Test
1	800	600	60	240
2	1040	500	220	440
3	840	600	360	720
4	900	870	540	900
5	940	720	100	760
6	880	840	880	900
Mean				660
Volunteers taking Liberal Fluids				
7	-	-	-	2100
8	-	-	-	2360
9	-	-	-	1800
10	-	-	-	1700
11	-	-	-	2080
12	-	-	-	2400
Mean				2070

Table 39.

BLOOD MEPACRINE AND FLUID INTAKE.

Volunteer	Restricted Fluid Intake Group Blood Mepacrine (ug./litre)						
	¼ hr.	¾ hr.	2½ hrs	5 hrs	10 hrs	14½ hrs	23½ hrs
1	133	217	167	433	233	333	250
2	150	217	133	187	150	283	317
3	133	192	180	233	258	517	767
4	141	192	260	173	125	300	433
5	133	242	120	193	233	583	633
6	150	208	127	193	167	333	383
Group Mean	140	211	164	235	194	392	463
	Liberal Fluid Intake Group						
7	150	208	193	140	167	450	633
8	150	350	153	187	167	333	433
9	183	217	153	233	158	267	467
10	175	183	127	147	158	267	467
11	167	183	253	173	83	200	317
12	175	217	133	187	158	367	600
Group Mean	166	226	169	178	148	314	486

Table 40.

URINARY MEPACRINE AND FLUID INTAKE.

Volunteer	Restricted Fluid Intake Group Urine		
	Volume /24hrs ml.	Mepacrine mg/L	mg/24hrs
1	240	3.6	0.9
2	440	5.3	2.3
3	720	6.6	4.7
4	900	10.3	9.3
5	760	2.6	1.9
6	900	11.0	9.9
Group Mean	660	6.6	4.8
	Liberal Fluid Intake Group		
7	2100	8.6	18.1
8	2360	9.6	22.6
9	1800	8.6	15.5
10	1700	6.6	11.2
11	2080	6.0	12.5
12	2400	7.1	17.1
Group Mean	2073	7.7	16.1

Table 41.

BLOOD MEPACRINE AND SEVERE TOXIC EFFECTS.

Dosage Regime:- 0.5g. mepacrine daily for 5 days						
Day	Blood Mepacrine ug./litre					
	*Severe Toxic Effects		No Symptoms			
	I.	G.	C.	K.	Cn.	F.
5	670	480	692	288	220	206
6	1116	1076	787	768	558	440
7	750	-	-	-	-	-
8	-	487	228	281	618	187
9	838	-	-	-	-	-
10	-	-	-	-	-	-
11	310	214	470	230	247	164
13	224	213	197	213	262	180

* I. Severe toxic effects from days 6 to 10.
G. Severe toxic effects on days 6 and 7.

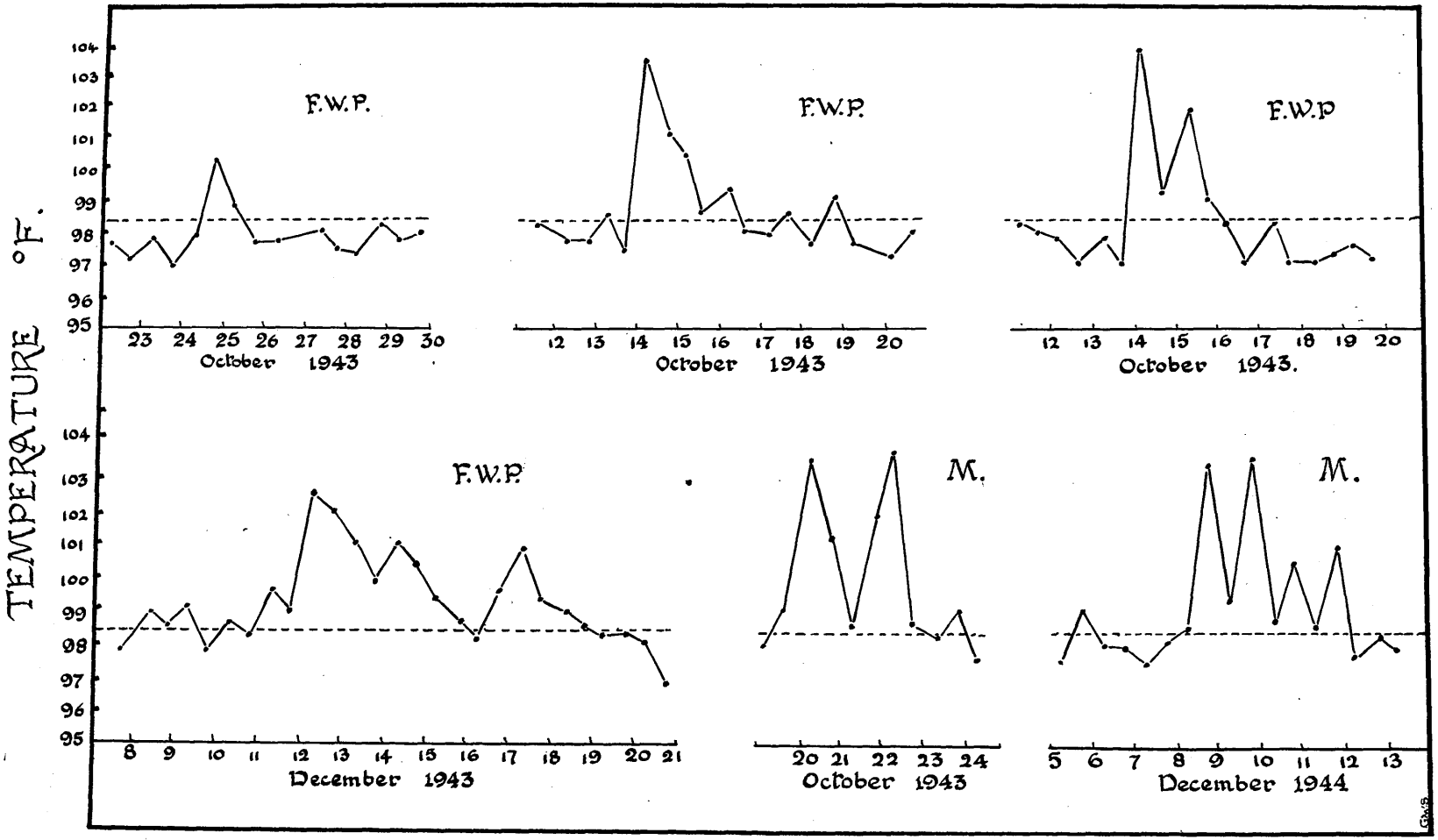
Table 42.

URINARY MEPACRINE AND SEVERE TOXIC EFFECTS.

Dosage Regime:- 0.5g. mepacrine daily for 5 days.						
Day	Urinary Mepacrine mg./24 hours					
	Severe Toxic Effects		No Symptoms			
	I.	G.	C.	K.	Cn.	F.
1	4.3	2.1	3.1	3.3	4.9	0.5 +
2	3.7	4.2	2.1	3.8	4.5	10.3
3	8.7	5.0	9.5	12.2	4.0	9.3
4	16.2	12.5	18.3	14.1	17.1	16.6
5	20.9	21.5	29.0	20.1	17.8	21.3
6	16.9	14.6	22.7	25.2	4.3	-
7	16.3	12.3	20.6	8.3	13.0	-
8	15.2	2.7	14.8	5.7	5.3	9.8
9	9.2	5.3	14.0	12.9	6.8	3.8
10	10.7	6.0	10.9	7.7	2.5	7.3
11	7.5	3.9	7.0	10.5	4.3	5.7
12	5.2	4.7	5.8	5.6	6.0	6.9

GRAPH 1.

REPRESENTATIVE TEMPERATURE CHARTS OF THE FEVERS AND THE MALARIAS

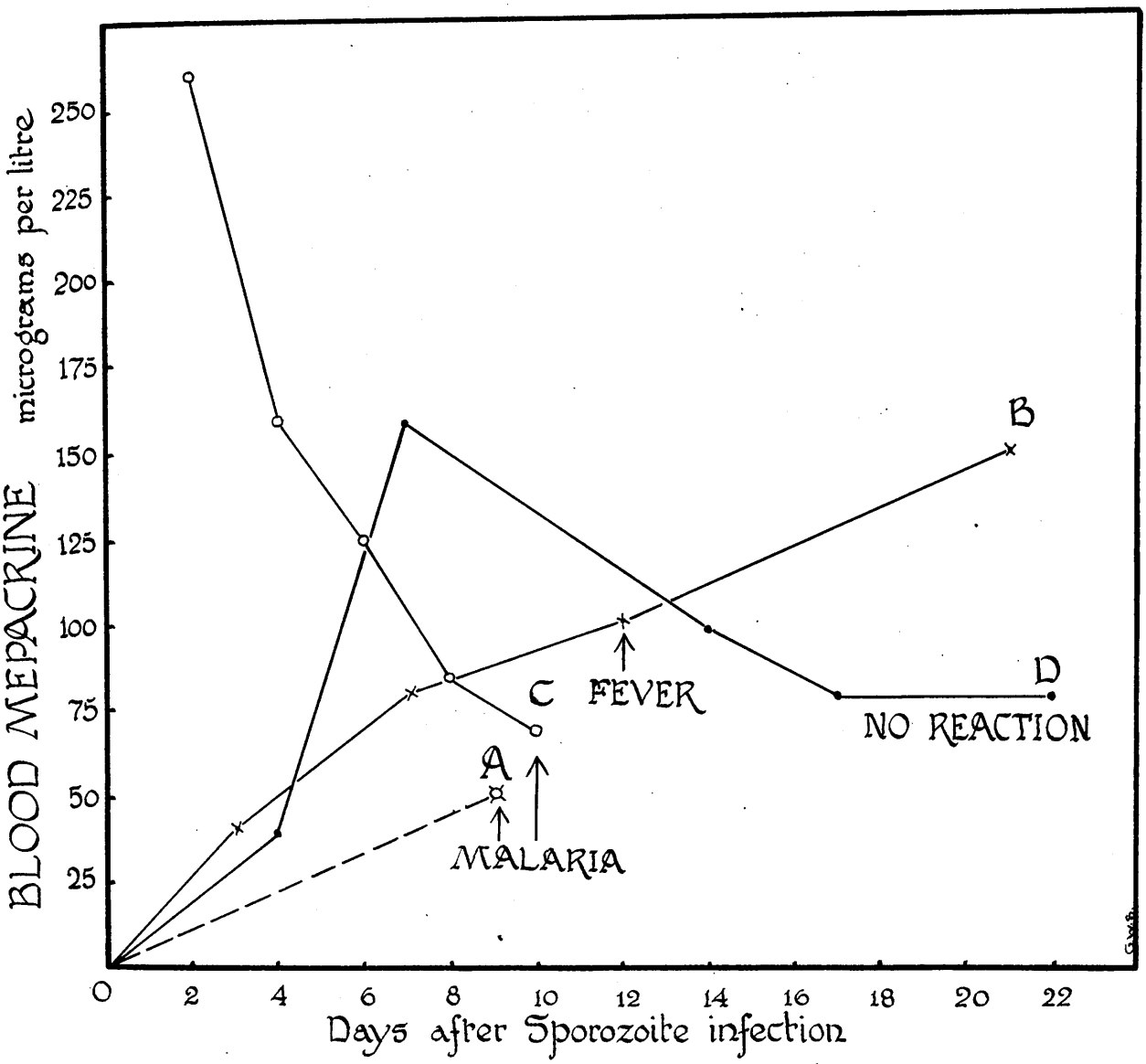


F.W.P. = Fever without demonstrable parasitaemia in volunteers infected with sporozoites who were taking mepacrine.

M = Malaria in similar volunteers.

GRAPH 2.

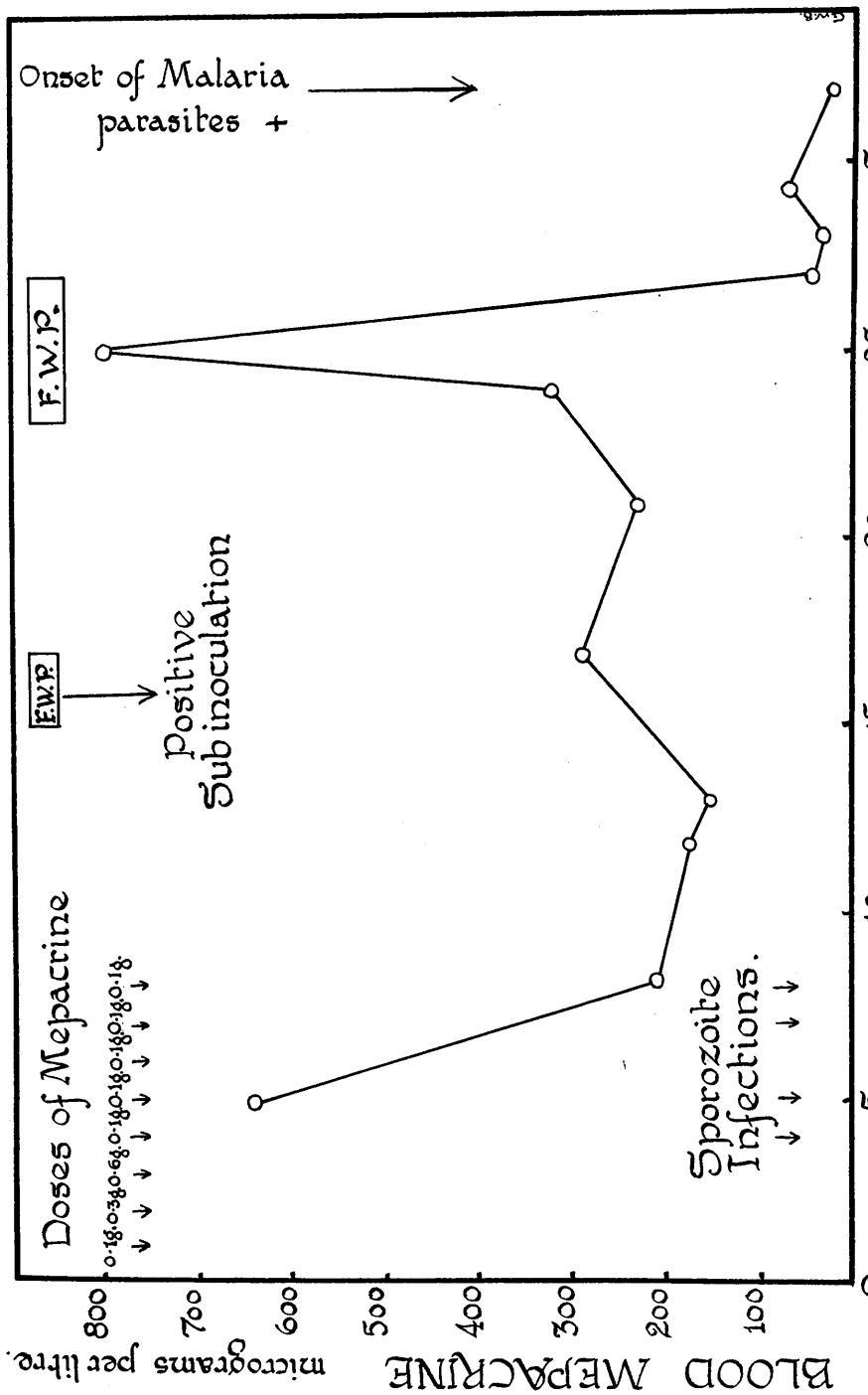
BLOOD MEPACRINE AND MALARIA



Volunteers A, B, C, after Sporozoite infection on one occasion with sporozoites of M.T. [illegible].
A and B received 0.1g. mepacrine daily for 20 days and began dosing on the day of infection. A developed malaria; B had a mild fever of a few hours duration (peak temperature 100°F.) but parasites were not found in the blood.
C and D received 1.0g. mepacrine in 3 days. C was given 0.1g. two days before infection, 0.3g. one day before infection and 0.6g. on the day of infection. D received 0.1g. 3 days after infection, 0.3g. four days after infection and 0.6g. five days after infection.
C developed malaria; D had no reaction.

GRAPH 3.

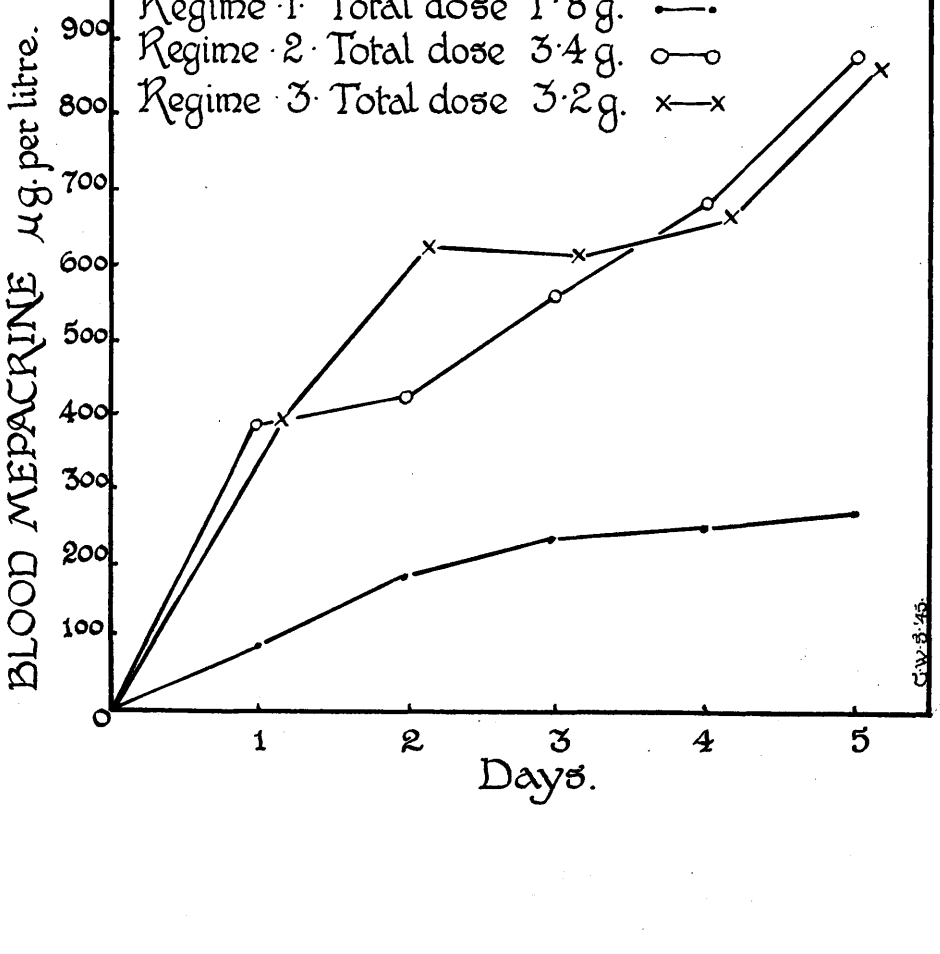
BLOOD MEPACRINE AND FEVER



F.W.P. = Fever without demonstrable parasitaemia. Positive sub-inoculation means that blood taken from the volunteer at the time indicated caused malaria after injection into another individual.

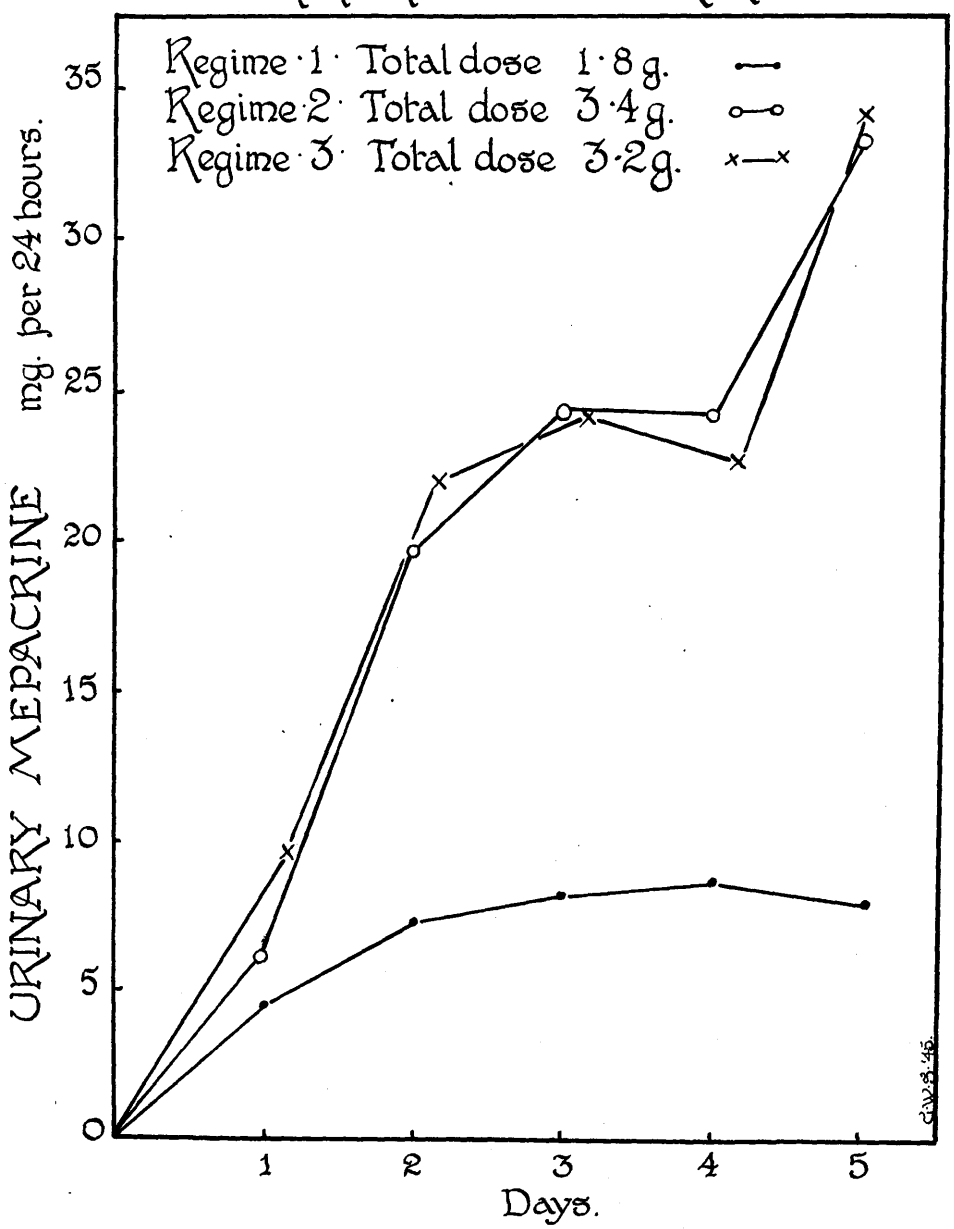
GRAPH 4.

DOSE AND GROUP MEAN BLOOD MEPACRINE.



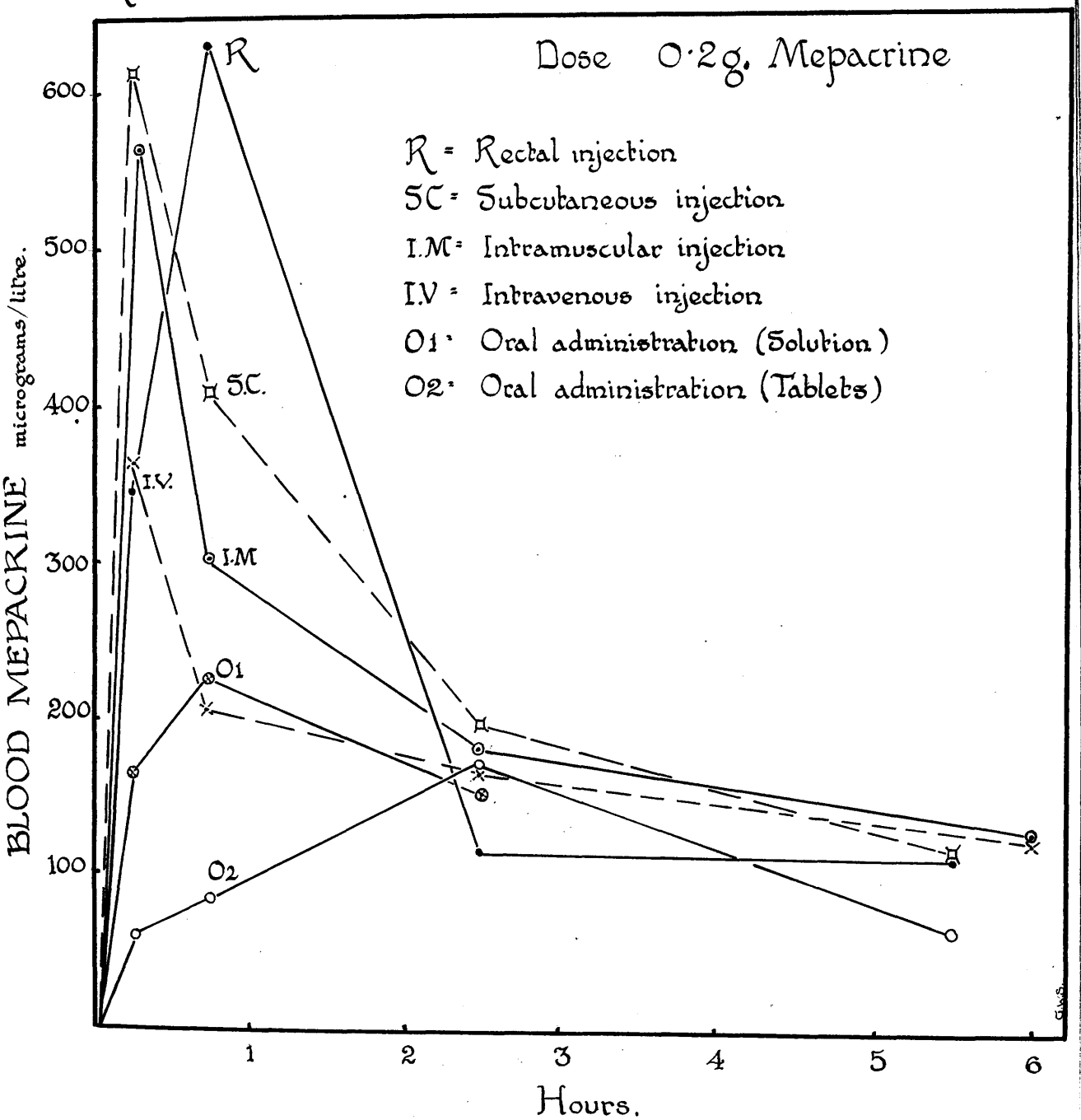
GRAPH 5.

DOSE AND GROUP MEAN URINARY MEPACRINE.



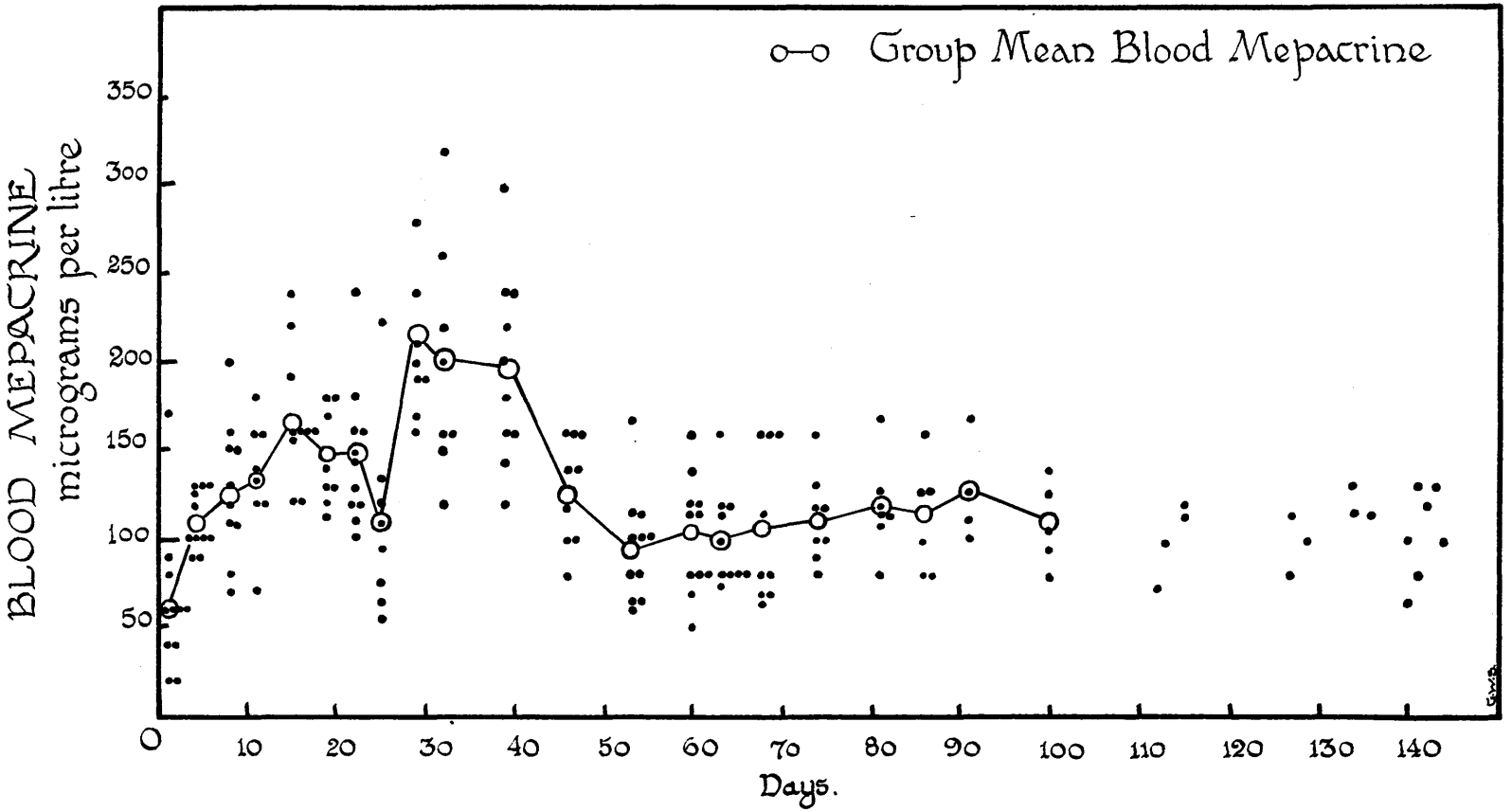
GRAPH 6.

ROUTE OF ADMINISTRATION AND BLOOD MEPACRINE



GRAPH 7.

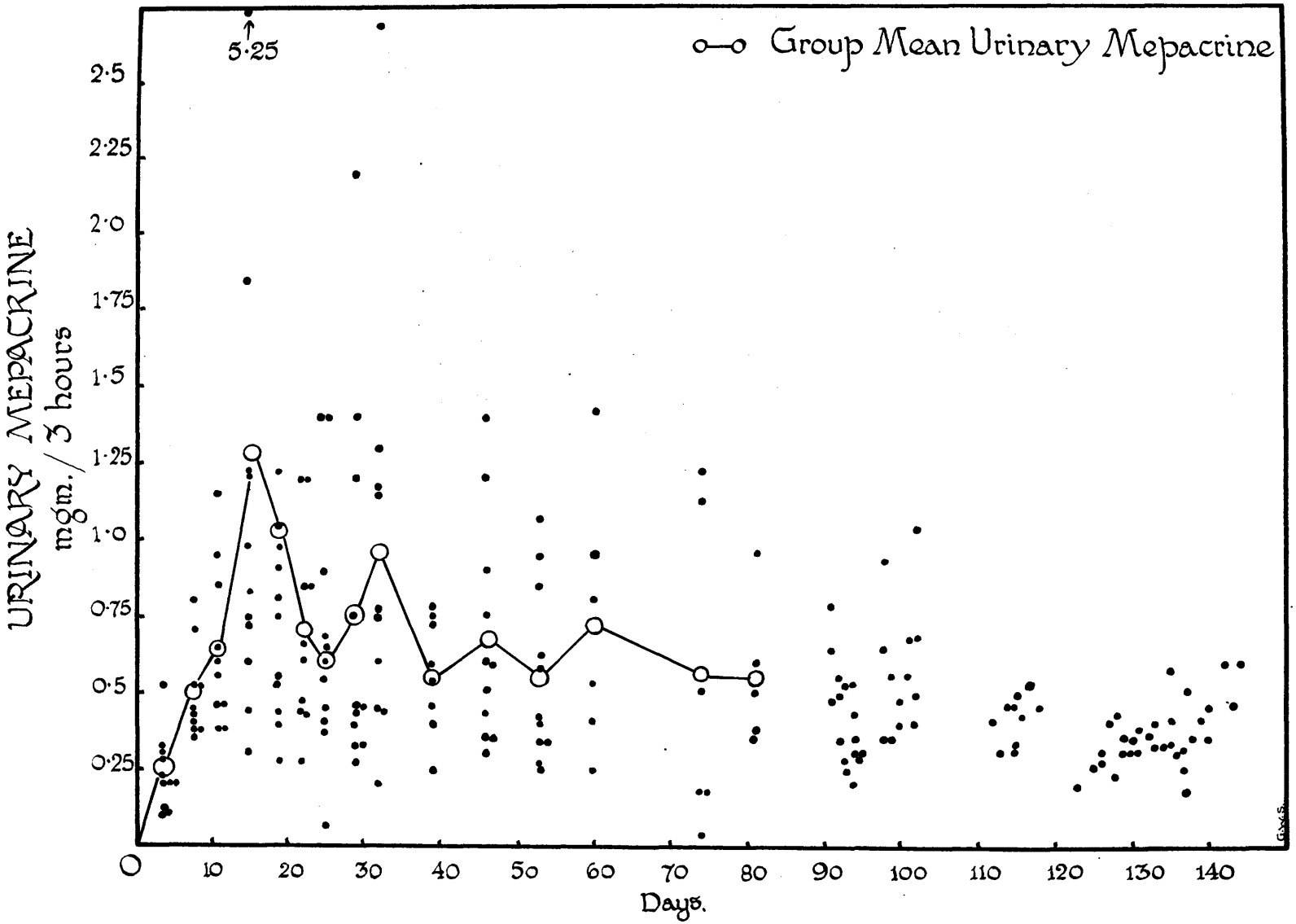
INDIVIDUAL VARIATION IN BLOOD MEPACRINE AFTER 0·1g. DAILY



Blood mepacrine estimations were made on 4 volunteers after day 100. When similar levels were obtained in two or more individuals on the same day they were plotted alongside one another.

GRAPH 8.

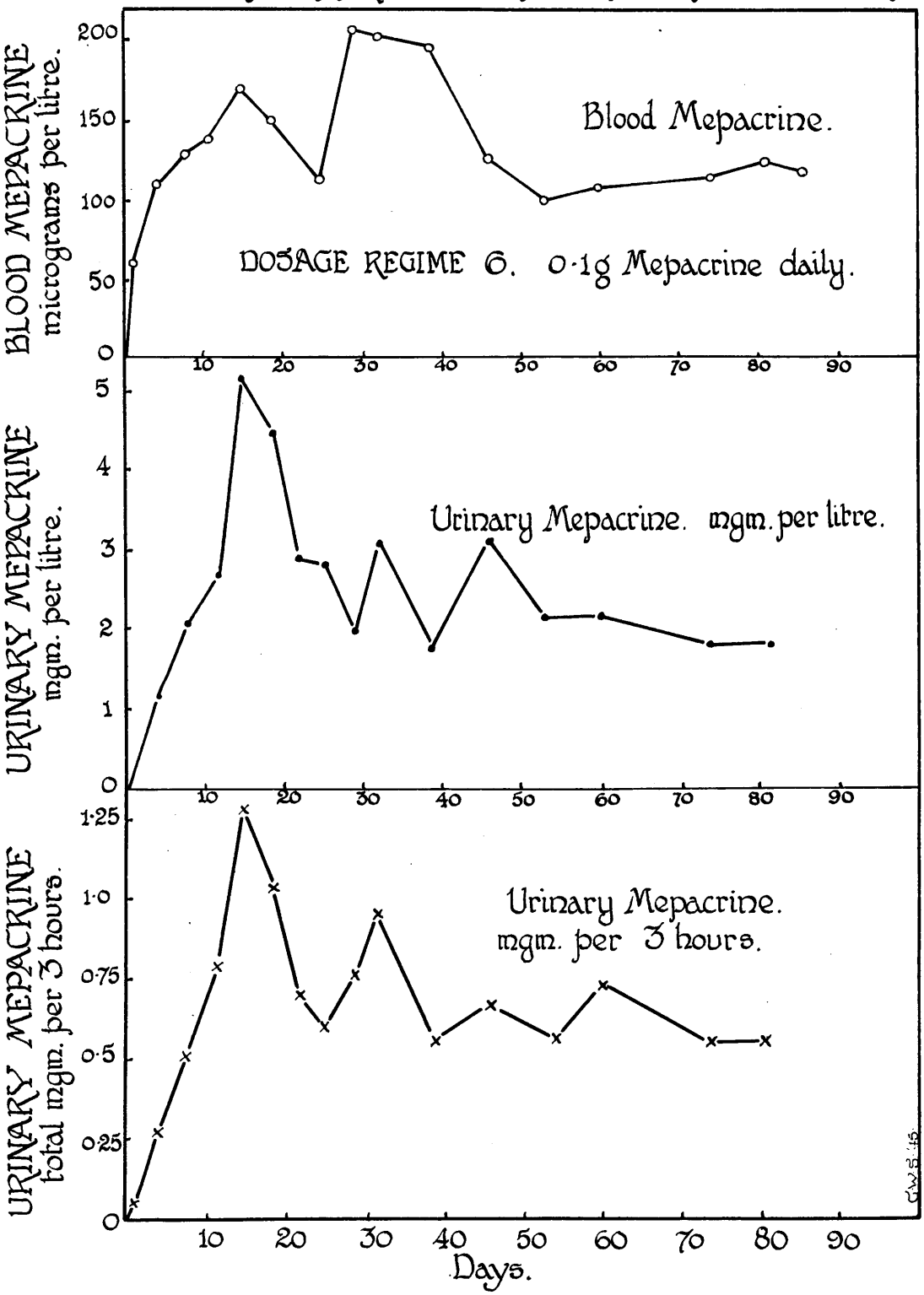
INDIVIDUAL VARIATION IN URINARY MEPACRINE AFTER 0·1g DAILY



Urinary mepacrine estimations were made on 4 volunteers after day 80.

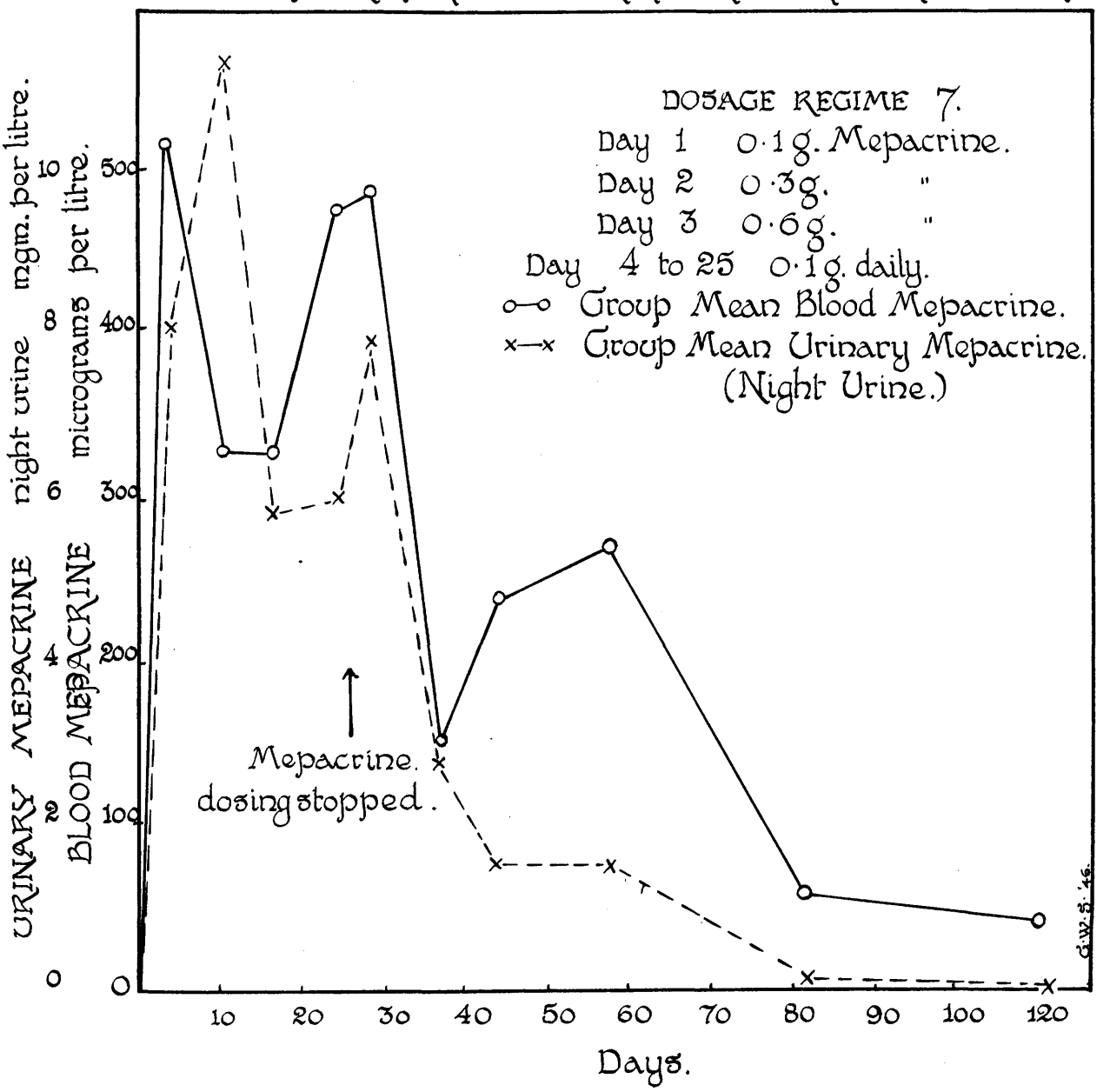
GRAPH 9

BLOOD AND URINARY MEPACRINE AND DURATION OF DOSING.



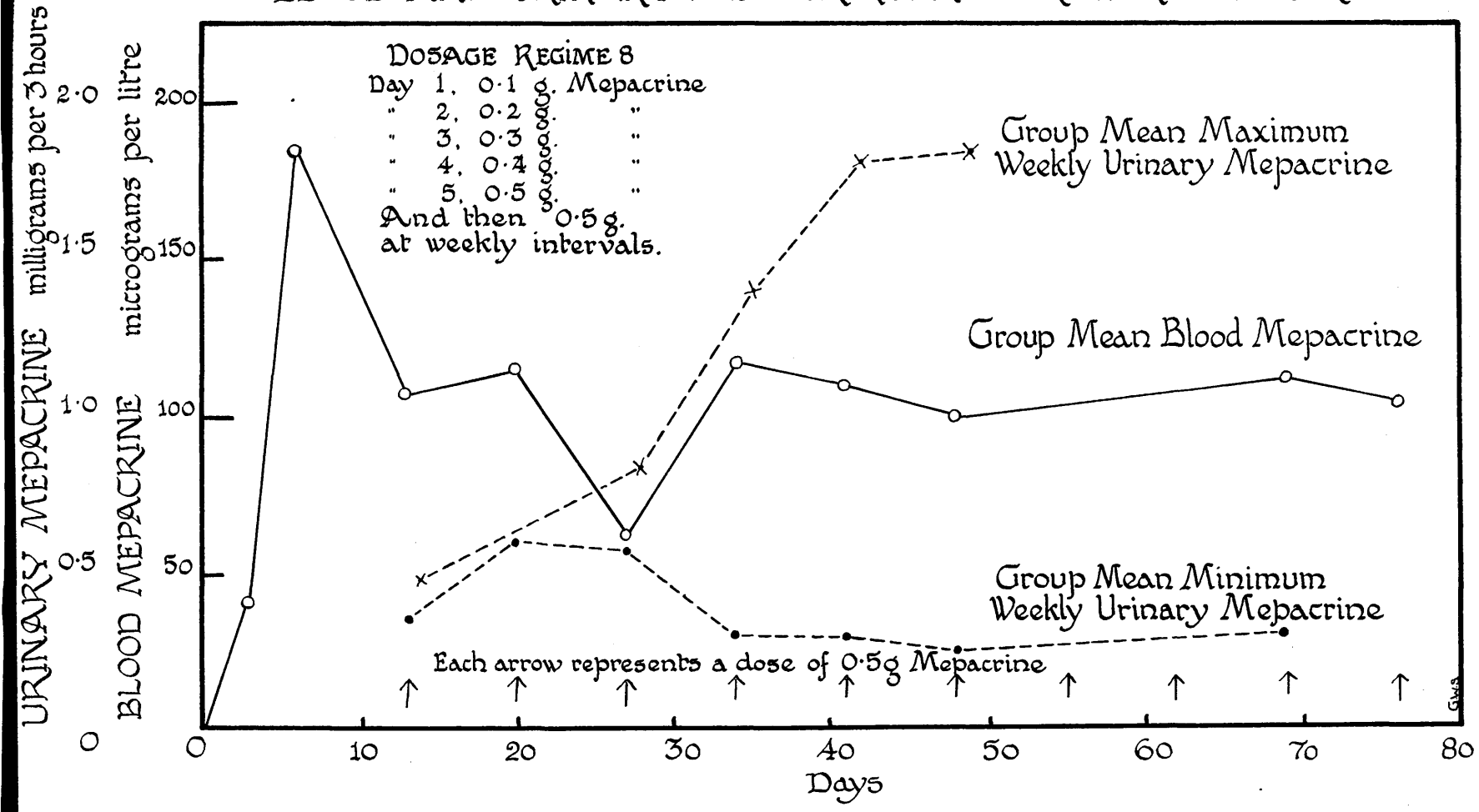
GRAPH 10

BLOOD AND URINARY MEPACRINE AND DURATION OF DOSING.

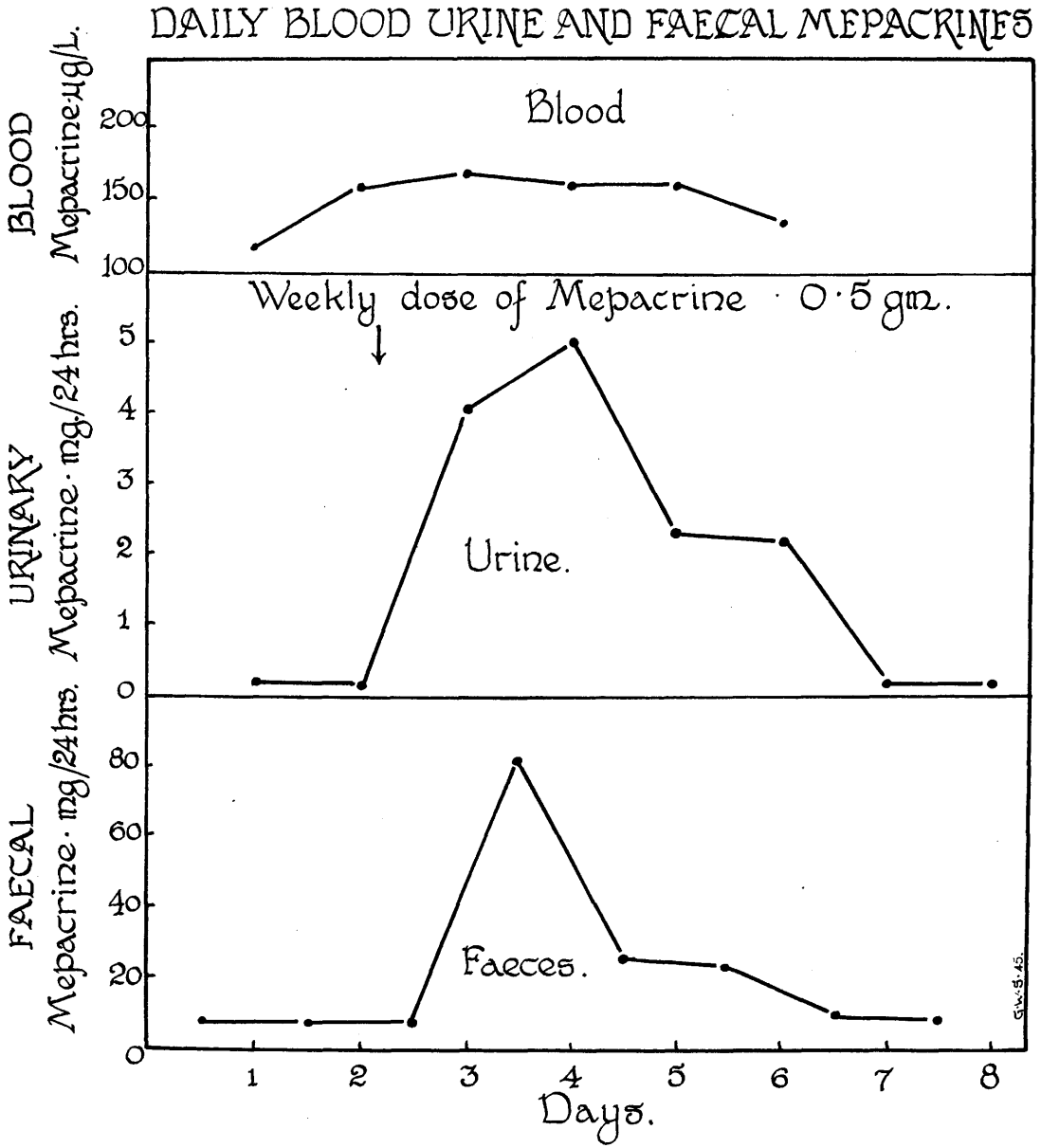


GRAPH 11.

BLOOD AND URINARY MEPACRINE AND DURATION OF DOSING

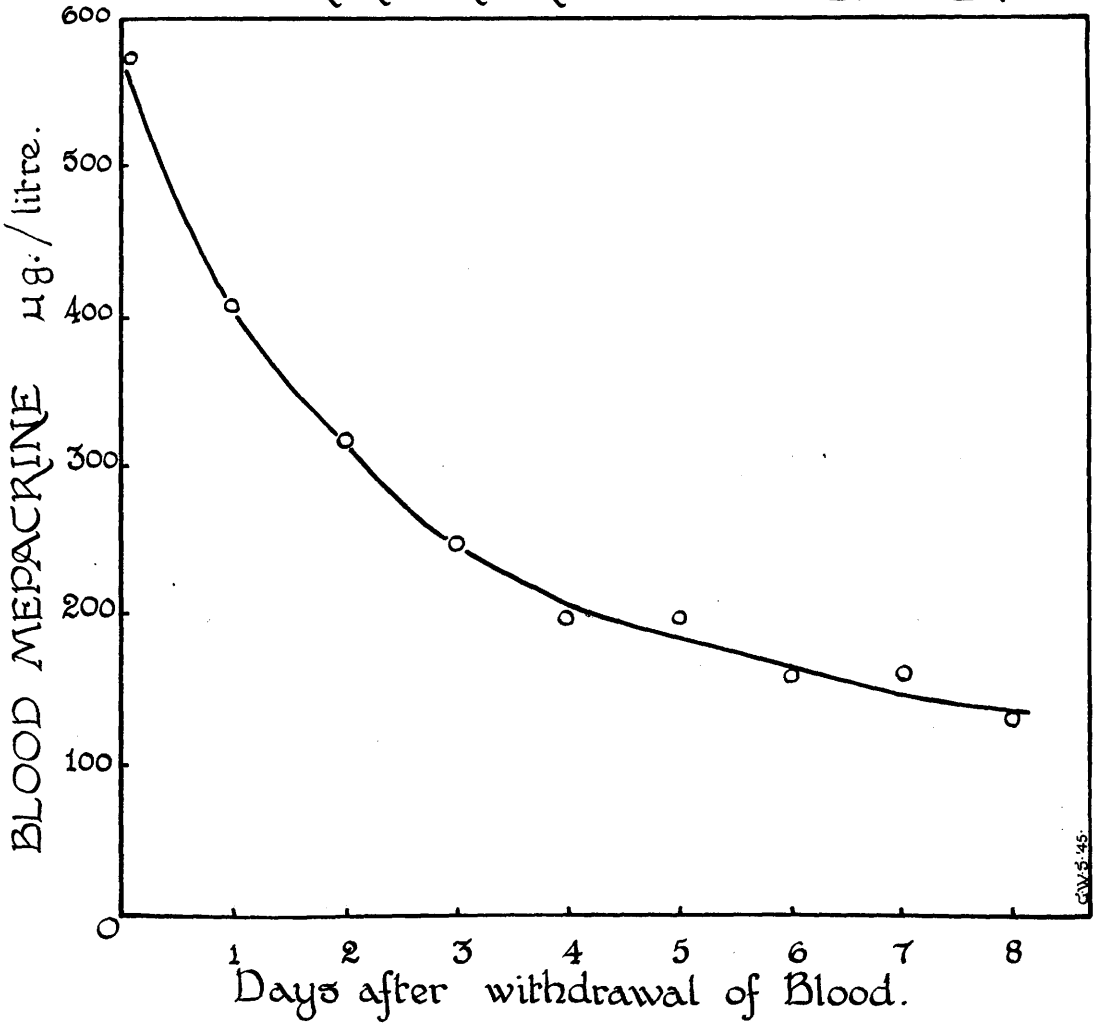


GRAPH 12.


DAILY BLOOD URINE AND FAECAL MEPACRINES
BLOOD
Mepacrine:ug/L.
200
150
100
Blood
Weekly dose of Mepacrine · 0·5 gm.
URINARY
Mepacrine · mg./24 hrs.
5
4
3
2
1
0
Urine.
FAECAL
Mepacrine · mg/24 hrs.
80
60
40
20
0
Faeces.
1
2
3
4
5
6
7
8
Days.
G.W.S. 45.

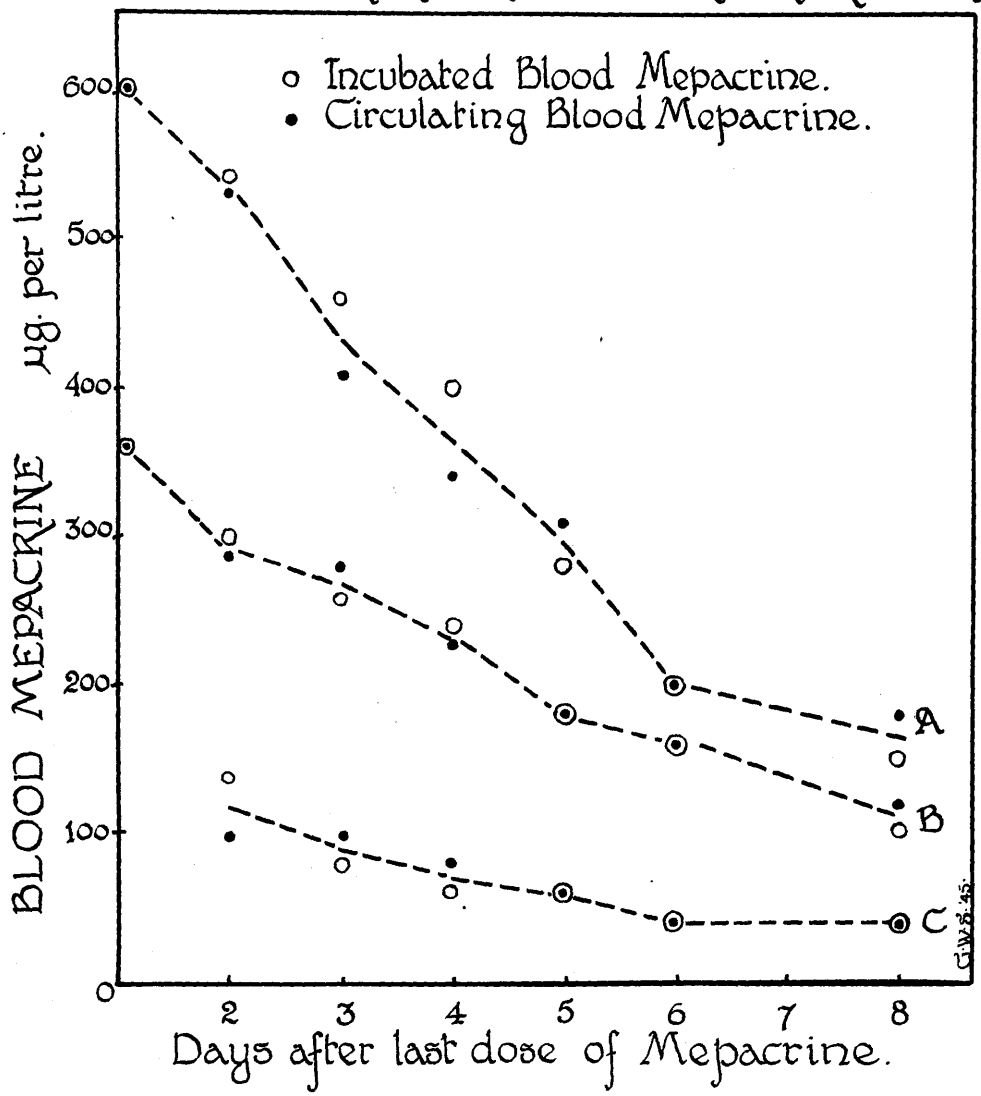
GRAPH 13.

RATE OF DEGRADATION OF MEPACRINE IN INCUBATED BLOOD.



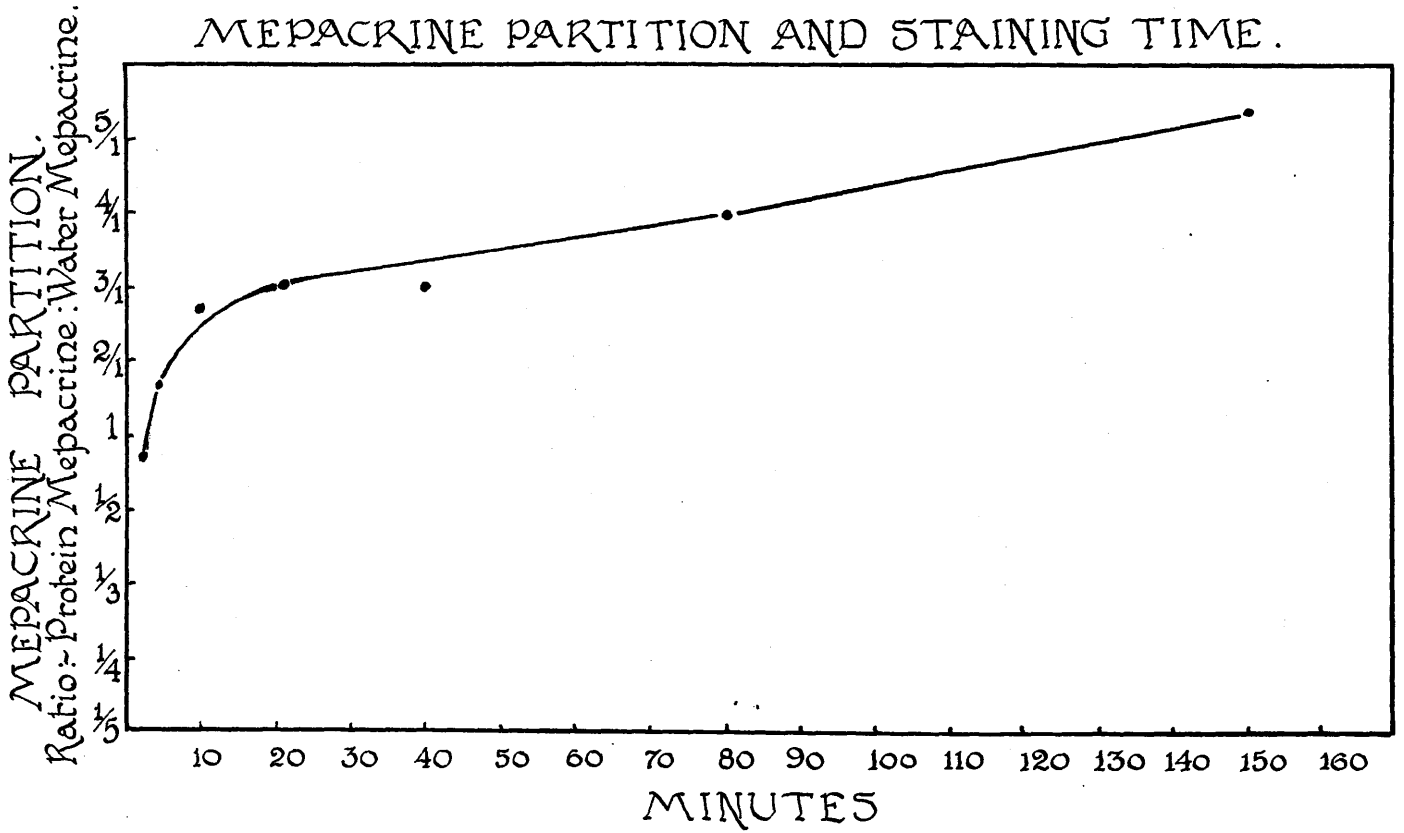
GRAPH 14.

RATE OF DEGRADATION OF MEPACRINE IN BLOOD OF THREE VOLUNTEERS A, B and C WITH DIFFERENT INITIAL CONCENTRATIONS.



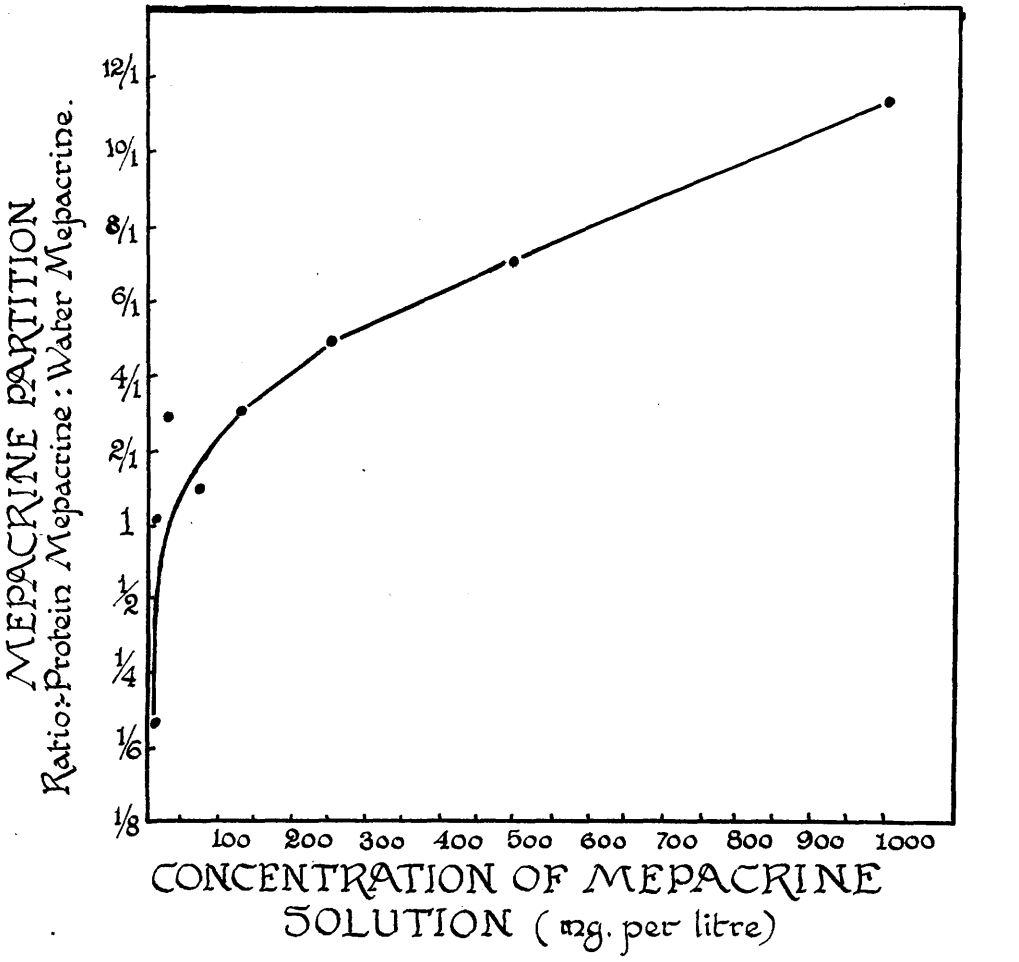
GRAPH 15

MEPACRINE PARTITION AND STAINING TIME.



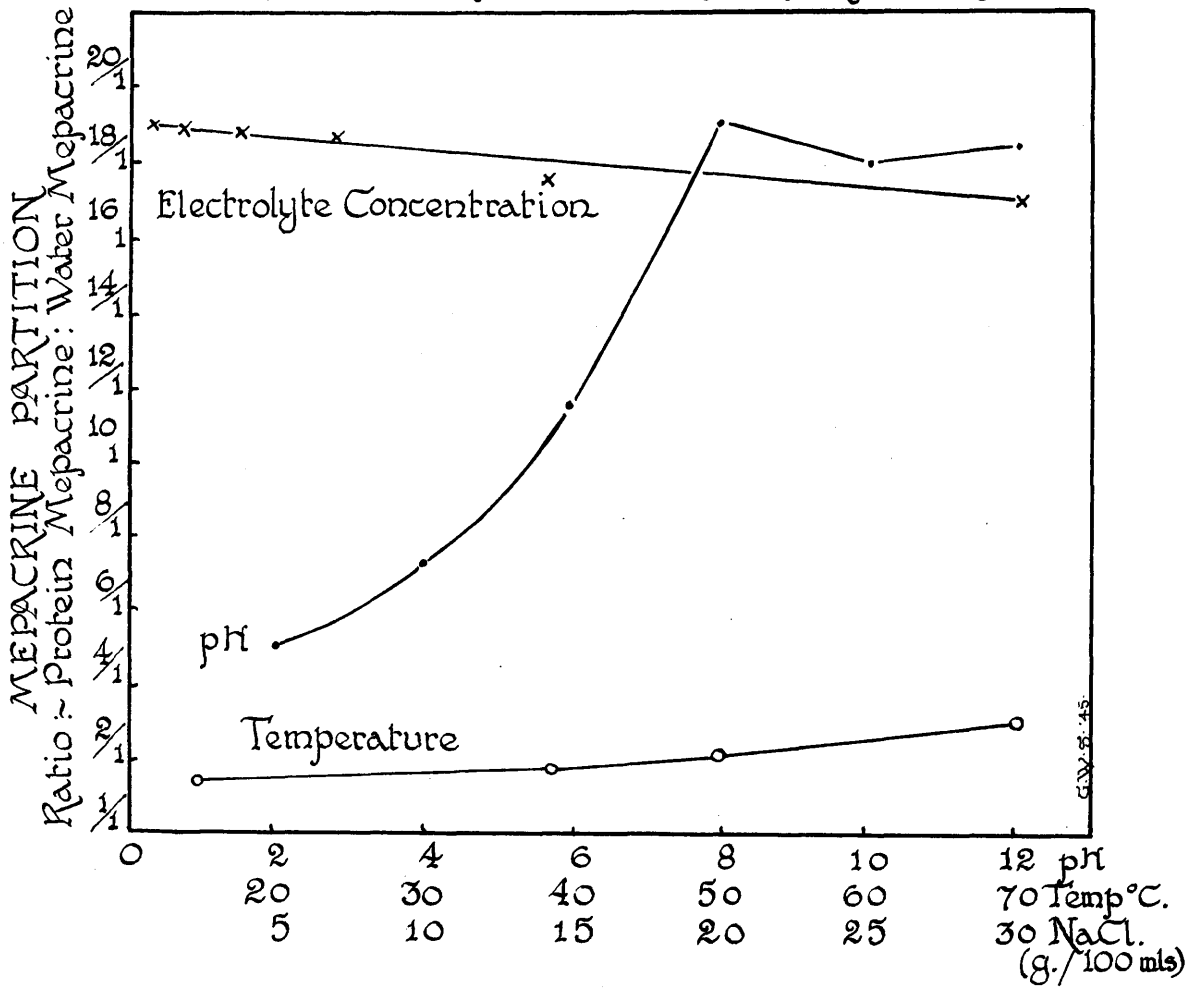
GRAPH 16.

MEPACRINE PARTITION AND CONCENTRATION OF MEPACRINE SOLUTION



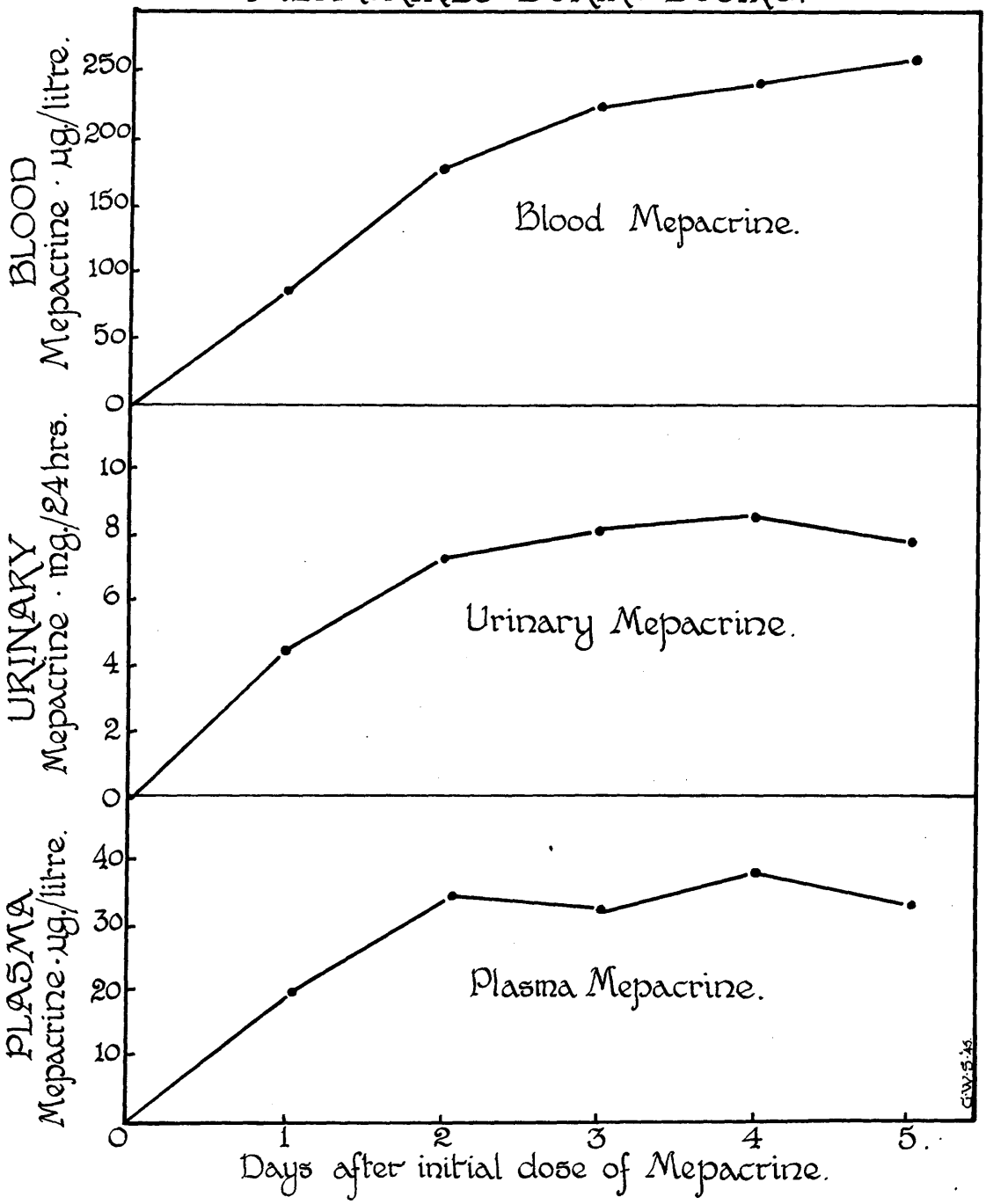
GRAPH 17.

MEPACRINE PARTITION AND pH TEMPERATURE AND ELECTROLYTE CONCENTRATION



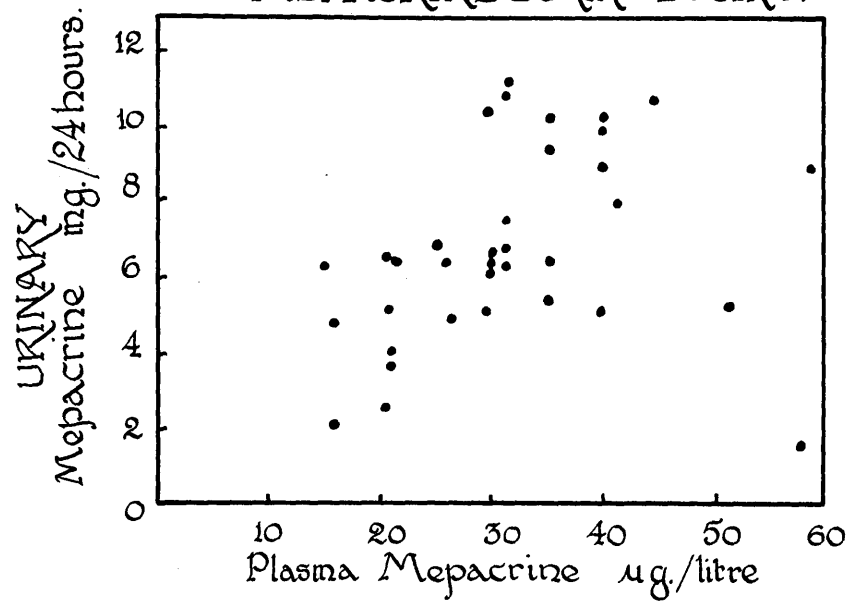
GRAPH 18.

GROUP MEAN BLOOD URINE AND PLASMA MEPACRINES DURING DOSING.



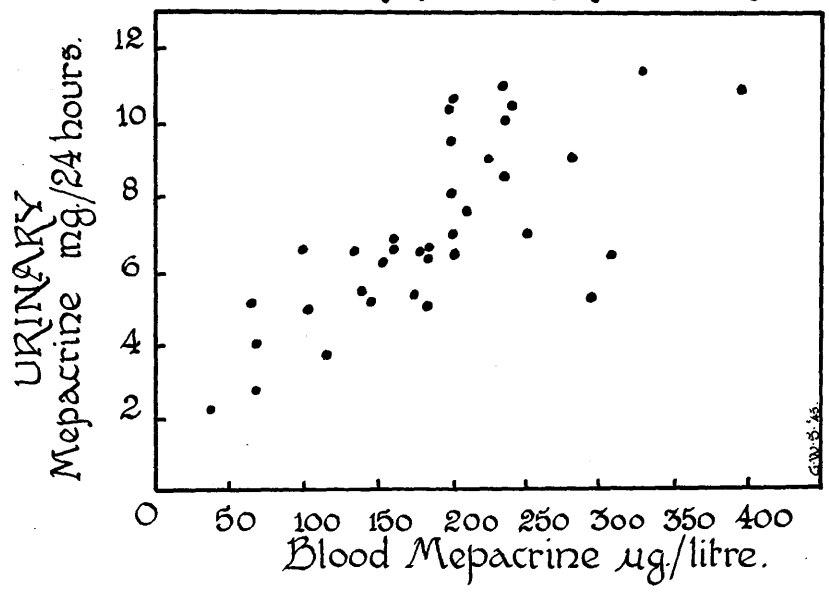
GRAPH 19.

PLASMA MEPACRINE AND URINARY MEPACRINE DURING DOSING.



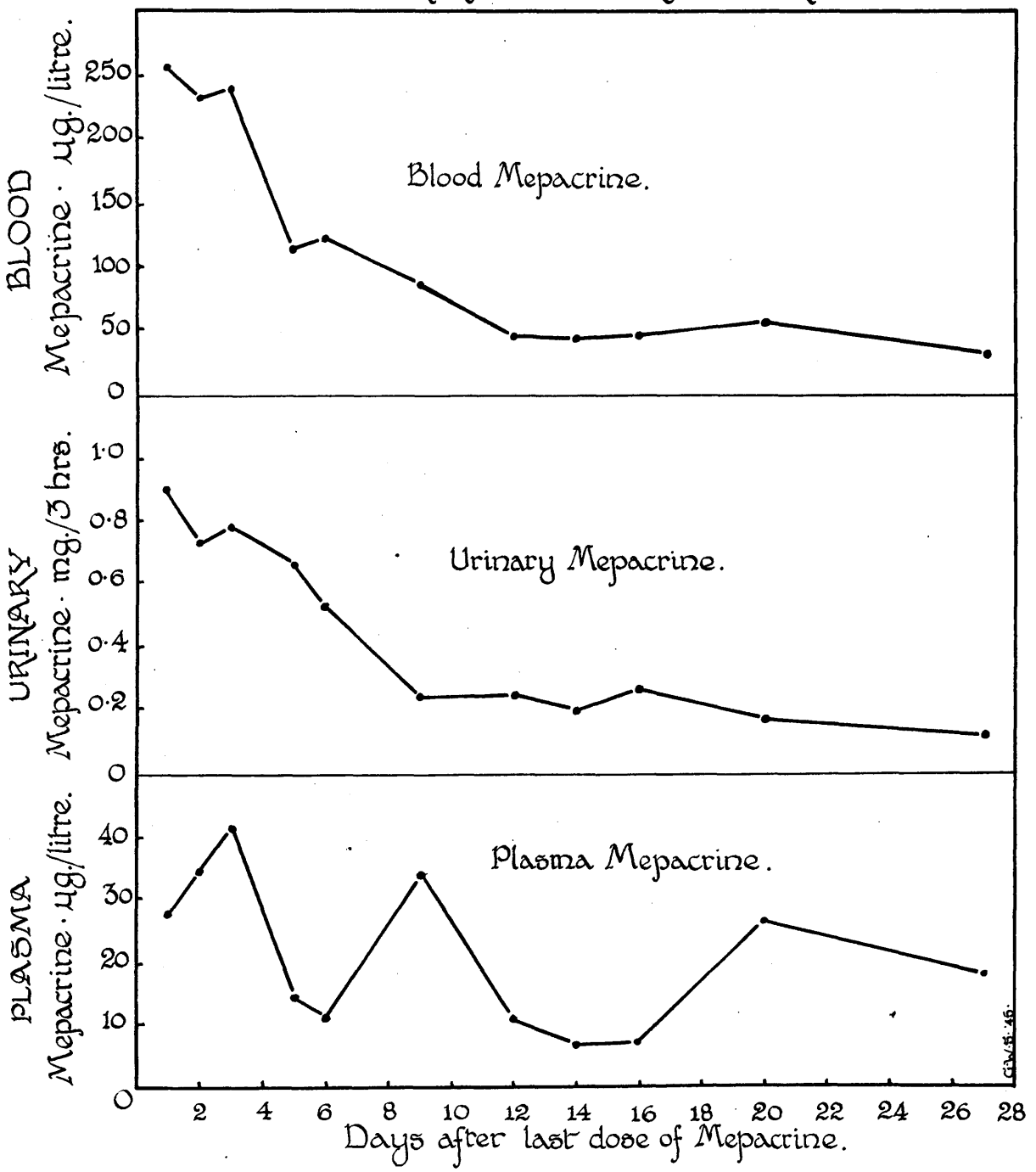
GRAPH 20

BLOOD MEPACRINE AND URINARY MEPACRINE DURING DOSING.



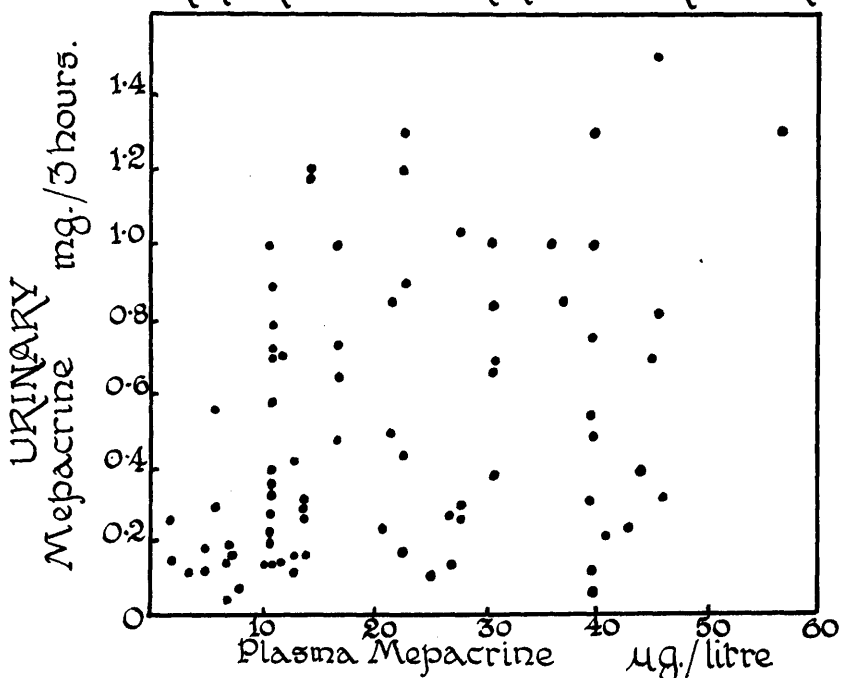
GRAPH 21.

GROUP MEAN BLOOD URINARY AND PLASMA. MEPACRINES AFTER DOSING.



GRAPH 22

PLASMA MEPACRINE AND URINARY MEPACRINE AFTER DOSING.



GRAPH 23

BLOOD MEPACRINE AND URINARY MEPACRINE AFTER DOSING.

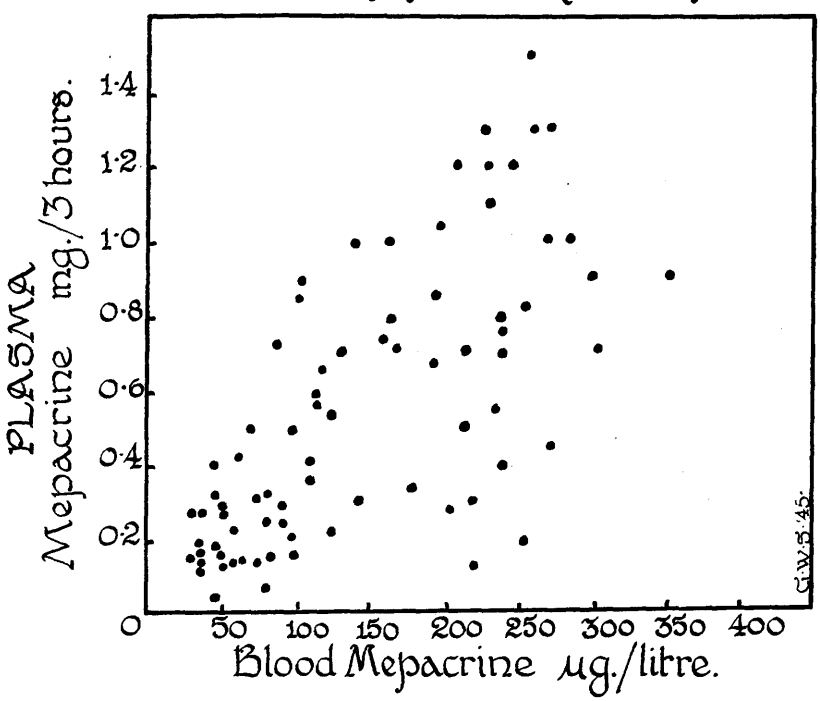
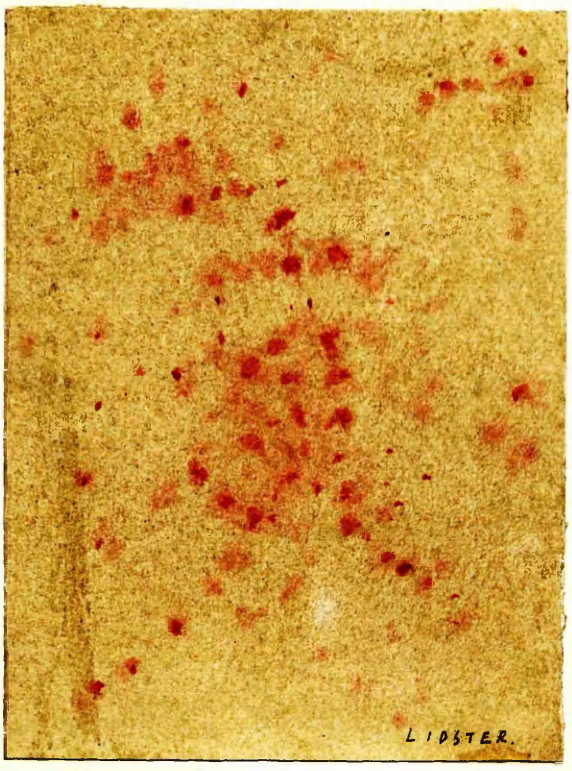


ILLUSTRATION OF THE RASH ON THE INNER ASPECT OF THE FOREARM THAT APPEARED AFTER LARGE DOSES OF MEPACRINE



PHOTOGRAPH OF AN ULCER ON THE BUTTOCK CAUSED BY INJECTING 0.2g. MEPACRINE SUBCUTANEOUSLY