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STUDIES ON THE CHRONIC TOXICITY OF CHLOROQUINE (SN-7618)¹

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Chloroquine, 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline, is an antimalarial drug of the 4-amino quinoline series. It has been shown to have antimalarial activity similar to and superior to that of quinacrine in avian malaria, and in trophozoite- and sporozoite-induced *vivax* and *falciparum* malaria in man (1, 2, 3, 4).

The present study was undertaken for the purpose of establishing whether chloroquine can be administered for prolonged periods as a suppressive drug without causing serious toxicity. To accentuate toxic manifestations and establish the margin of safety, larger dosages than those necessary for suppressive treatment were administered. Normal inmate volunteers at the Illinois State Penitentiary at Stateville served as subjects for these investigations.

PROCEDURES AND METHODS

The subjects under study were selected on the basis of history and physical examination to exclude any volunteer with physical disability or neurosis. The following observations and tests were made prior to initiating treatment and were repeated at frequent intervals during the early

course of medication and later at gradually increasing intervals:

<i>General</i>	<i>Kidneys</i>
Subjective symptoms	Urinalysis
Oral temperature	Blood non-protein nitrogen
Weight	Phenolsulfonphthalein excretion
Physical appearance	<i>Nervous System</i>
<i>Cardiovascular System</i>	Visual acuity
Pulse rate	Diplopia
Blood pressure	Visual accommodation (monocular)
Electrocardiogram	Nystagmus
<i>Blood</i>	Extraocular movements
Erythrocyte count	Tremor
Leucocyte count, total and differential	Coordination and gait
Hemoglobin	Handwriting
<i>Liver</i>	Romberg test
Urine urobilinogen	Deep reflexes
Serum bilirubin	
Cephalin-cholesterol flocculation	

Toxicity studies were begun simultaneously on two groups of 20 subjects each. The first group was placed on a weekly-dosage schedule of 0.5 gram of chloroquine base, administered orally once a week. The second group was given a total daily dosage of 0.3 of the base in two doses (0.1 and 0.2 gram) for 77 days. At the end of this period the volunteers in the latter group joined those on the weekly-dosage regime at 0.5 gram (base) once a week, and both groups continued on this schedule for a total time of one year. Placebos were given for one week before and for two months after the year of chloroquine administration. During the periods of placebo administration no change was made in the routine of observation.

Because of parole, discharge, or for administrative reasons, 10 men failed to complete the full year.

Chemical method. The drug was determined in plasma by the method of Brodie *et al.* (5) with the following modifications: 30 ml. of heptane and 2 ml. of ethyl alcohol (charcoal treated and redistilled until non-fluorescent) were placed in a 60-ml. glass stoppered bottle. Ten ml.

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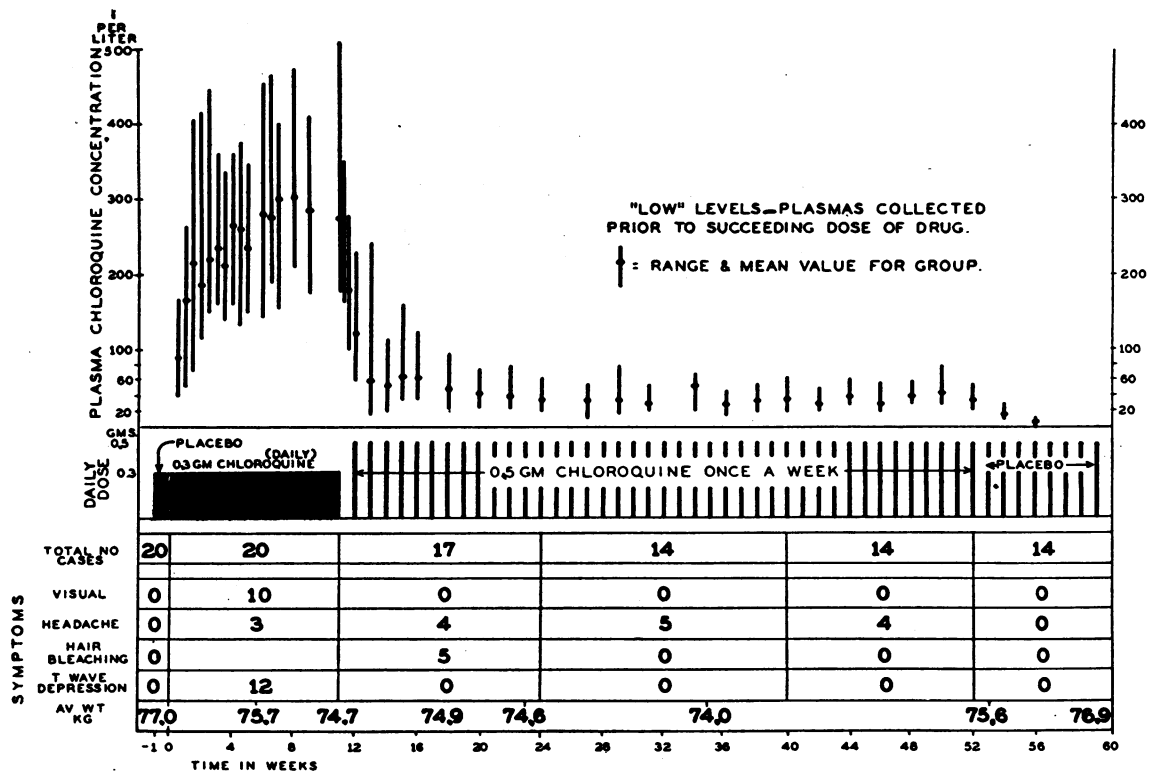


FIG. 1. TOXICITY OF CHLOROQUINE

Daily-dosage study. Plasma concentrations represent range and mean of the "low" levels; plasmas were drawn prior to the succeeding dose of the drug. The body weights are the average of only 12 cases, those lacking complete records being excluded. Drug dosages are expressed in terms of the base.

of plasma and 5 ml. of 0.2 N NaOH were added and the mixture shaken for 15 minutes. After the two phases separated, the water phase was aspirated and the heptane layer was poured into a 125-ml. glass-stoppered bottle containing 50 ml. of 0.1 N NaOH and shaken for 10 minutes. After the phases separated, the water phase was aspirated. Washing with 0.1 N NaOH was repeated three times. Prior to the third washing, 1 ml. of the non-fluorescent ethyl alcohol was added. After the separation of layers, and the aspiration of the water phase, 20 ml. of the heptane phase were transferred by means of a pipette, just previously rinsed with the non-fluorescent alcohol, to a 40-ml. glass-stoppered pointed centrifuge tube containing 6 ml. of 0.05 N HCl. The mixture was then shaken for 10 minutes. The heptane layer was removed by aspiration and 5 ml. of the acid phase were transferred to a tall fluorometer tube containing 0.25 ml. of a 5 per cent cysteine solution. Two and one-half ml. of buffer solution (buffer: 6 volumes of 0.6 N sodium hydroxide and 5 volumes of 0.6 M boric acid in 0.6 N potassium chloride) were then added.

All tubes (blanks, standards and unknowns) were placed in an irradiator for two hours' treatment with ultra-violet light emitted by an H-4 mercury arc lamp. Since the distance from the light to all tubes was not

uniform, the tubes, after one hour of irradiation, were moved to the opposite side of the irradiator for the second hour of treatment.

The intensity of fluorescence of the samples was determined in a Coleman Photofluorometer, Model 12 A, using B-1-S and PC-1 filters.

RESULTS

Concentration of drug in the plasma. In the group of volunteers receiving 0.3 gram of chloroquine daily the drug plasma concentrations, prior to the morning dose, increased for the first four weeks and remained on a plateau thereafter (Figure 1). There was a wide individual range. The level usually varied inversely with the body weight of the subject. When the dosage was reduced (after 77 days), 11 weeks elapsed before the plasma concentrations in this group fell to the levels of the group in which subjects had received 0.5 gram weekly from the start of the experiment.

In the weekly-dosage studies the individual "low" values, collected just prior to the next dose,

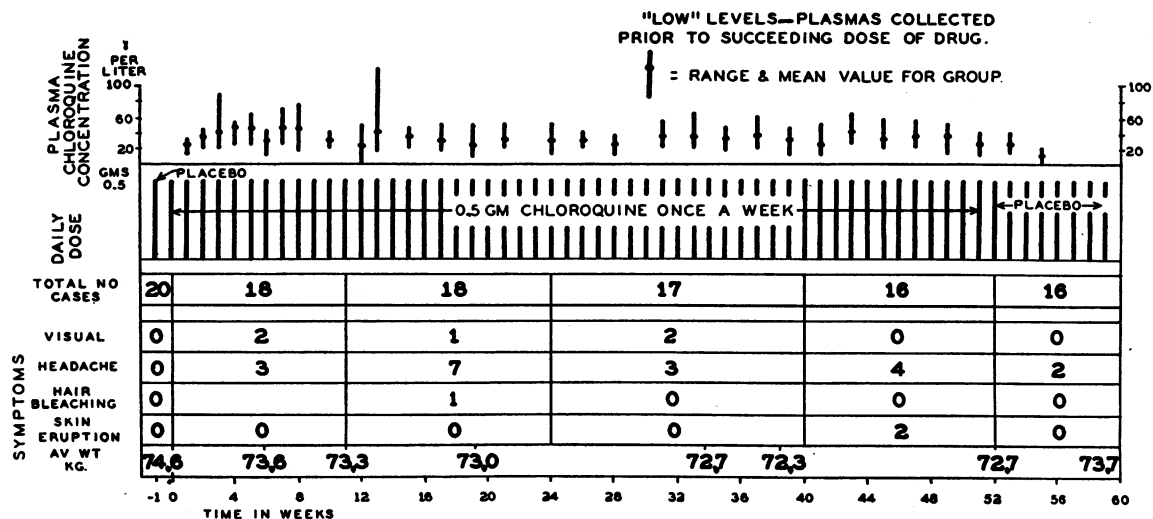


FIG. 2. TOXICITY OF CHLOROQUINE

Weekly-dosage study. Plasma concentrations represent range and mean of the "low" levels; plasmas were drawn prior to the succeeding dose of the drug. Body weights represent the average of those 14 individuals who completed the experiment. Drug dosages are expressed in terms of the base.

rarely fell below 20 gamma per liter and the means of these weekly values were usually between 25 and 40 gamma per liter (Figure 2).

During the first seven weeks of drug administration in the weekly-dosage group a record of the highest plasma concentrations was made by col-

lecting samples five hours after the weekly dose of 0.5 gram. The peak concentrations ranged from 44 to 346 gamma per liter. The mean peak concentrations for the group rose week by week, as follows: 89, 135, 176, 154, 197, 252, and 215 gamma per liter.

The rate of decline of the plasma concentration after one of the weekly doses is shown in Figure 3. In 48 hours the mean concentration (94 gamma per liter) was less than half of the six-hour level (215 gamma per liter). At seven days the mean had fallen to 29 gamma per liter.

Toxicity. The toxic phenomena encountered in the daily-dosage study were visual difficulties, bleaching of the hair, and electrocardiographic changes. Skin eruptions occurred in the weekly-dosage study. Some individuals in both groups lost weight and had headaches (Figures 1 and 2).

Visual symptoms were noted by half the subjects in the daily-dosage study but by only an occasional subject in the weekly-dosage group. These symptoms consisted of a difficulty in changing focus quickly from a near to a far object. The tests for visual acuity, power of monocular accommodation, and diplopia failed to demonstrate objective abnormality. However, no tests for speed of accommodation were performed. The visual symptoms in the daily-dosage group disap-

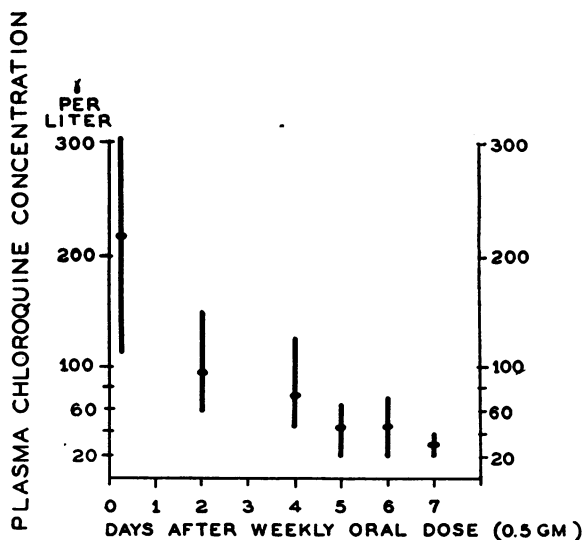


FIG. 3. PLASMA CHLOROQUINE CONCENTRATION

Means and ranges of plasma concentrations during seven days following termination of oral administration of 0.5 gram of chloroquine base to each of 18 individuals.

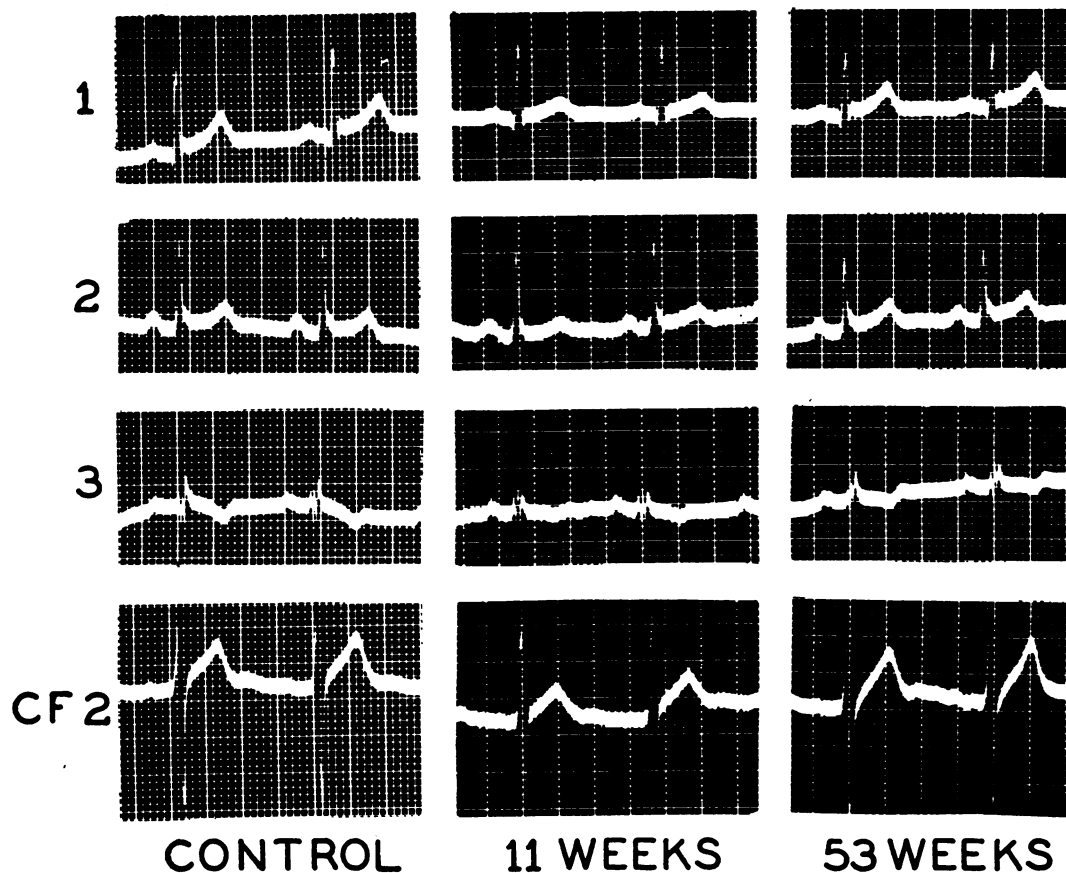


FIG. 4. TOXICITY OF CHLOROQUINE

Electrocardiograms taken after 11 weeks of chloroquine at 0.3 gram of base daily. Normal curves returned in spite of continued dosage at 0.5 gram of base weekly.

peared when the dosage was lowered to 0.5 gram weekly.

Shortly after the conclusion of the daily-dosage study, it was noticed that the hair of one of the blond subjects was lighter near the roots. Examination of others showed that bleaching had occurred in all blond subjects in the daily-dosage experiment and possibly in one of the two blond subjects in the weekly-dosage test. The hair gradually regained its normal color after reduction of the dosage in the former group.

Electrocardiographic changes were noted in 12 of the 20 men in the daily-dosage study. The changes consisted of a concordant diminution of the height of the T-waves in some or all of the leads. No other evidence of cardiovascular abnormality was observed, and the T-waves regained their original amplitudes after the dosage was reduced (Figure 4).

In two individuals in the weekly-dosage group, mild skin eruptions developed during the last few months of drug administration. The eruptions occurred over the flexor surfaces of the extremities and on the trunk. In one subject the lesions consisted of reddish violaceous papules and annular macules with a ring of papules about a paler center. The appearance resembled lichen planus, but no single lesion was typical of that disease. Histological studies showed only chronic inflammation. In the other subject there were reddish macules of similar distribution which on histological section were suggestive of lichen planus. The case histories of the two individuals with skin eruptions are presented elsewhere (6).

Both groups lost weight. The weight loss was slight but probably significant. At 38 weeks, the average weight had fallen 2.6 kg., most of which was regained in the placebo period. Of 26 indi-

viduals with complete weight records, 23 lost weight. Most of the weight loss in the daily-dosage group occurred during the 77 days when dosage was high and was not regained until the placebo period.

The appraisal of headaches was difficult. In the daily-dosage studies, headaches, different from those previously experienced, were noted by six of the 20 subjects. During the course of the weekly-dosage experiments, headaches were occasionally reported. Sometimes they recurred for several weeks in the same individual 6 to 12 hours after the drug was administered. Occurring in the occipital or frontal areas, or both, the headaches were usually mild, but lasted several hours and were occasionally still present the following morning. Only those headaches which seemed to be attributable to the drug by temporal relationships and history were included in Figures 1 and 2. Two subjects, however (Figure 2), continued to have headaches during the second placebo period.

The other tests and observations failed to reveal abnormalities.

DISCUSSION

The amount of chloroquine *base* recommended for the treatment of an attack of *vivax* or *falciparum* malaria is 1.5 grams in three days, and for suppression, 0.3 gram a week (1). The daily-dosage group received 23.1 grams in 77 days and the weekly-dosage schedule was 0.5 gram a week. In both studies, therefore, the dosage used was considerably in excess of that required for antimalarial therapy or suppression. This difference is further emphasized by a comparison of the plasma chloroquine concentrations attained in these studies with the concentrations required for antimalarial suppression. In the daily dosage studies mean "low" plasma concentrations in excess of 200 gamma per liter were maintained for 10 weeks while a plasma chloroquine concentration of 10 gamma per liter is adequate for antimalarial suppression (7). Individual "low" values in excess of 500 gamma per liter occurred without unusual symptoms.

The toxic manifestations in the group on the daily-dosage schedule were in fact reversible and caused no incapacity. There were unequivocal visual disturbances similar to those described by other observers (7, 8, 9, 10). The T-wave de-

pression in the electrocardiograms was similar to that reported as a result of drugs unrelated to chloroquine (11, 12, 13, 14, 15, 16). It was probably non-specific and without clinical significance. The bleaching of the hair gradually disappeared after the dosage was reduced, as it did in cases described by Butler (9).

The toxic symptoms which occurred in the subjects on the weekly-dosage regime were milder. It should be emphasized that the difficulty in accommodation was a common complaint in subjects who received 0.3 gram daily but it was very rare in those who received 0.5 gram once a week. The skin eruption which developed in two individuals was mild but similar to that reported during suppressive therapy with quinacrine. More extensive experience with chloroquine will be necessary to determine its incidence and seriousness. In both cases the rash faded rapidly after the year of treatment was completed.

The temporal relationship of headaches to medication suggested an etiological relationship, but their unpredictability in incidence, location, and severity made it impossible accurately to appraise this symptom even after a year of close observation. The loss of body weight was small and disappeared with discontinuance of medication.

The special tests performed gave no clue to the mechanism of the toxic effect of chloroquine.

SUMMARY AND CONCLUSION

1. Two groups of inmate volunteers of 20 each were given chloroquine (SN-7618) orally for one year in greater dosages than those required for antimalarial therapy or suppression, in order to detect and evaluate any toxic reactions.

2. The first group took 0.3 gram (base) daily for 77 days and 0.5 gram (base) once weekly thereafter. On the higher dosage, visual disturbances, headache, bleaching of the hair, electrocardiographic changes, and slight weight loss were observed. These changes caused no incapacity and diminished or disappeared when the dosage was decreased.

3. The second group, which received 0.5 gram (base) weekly from the beginning of investigations, had occasional headaches, slight weight loss, and, in two cases, a skin eruption resembling lichen planus.

4. Under the conditions of this investigation, it can be concluded that chloroquine is a safe anti-malarial compound when given in the recommended dosage.³

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