THE EFFECT OF HOOKWORM DAMAGE ON LEVELS OF QUININE
ATTAINED IN BLOOD AND URINE OF DOGS FOLLOWING
SINGLE DOSES OF QUININE SULFATE

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The refractoriness of many cases of malaria to quinine therapy has been
ascribed, in some clinical circles, to the complication of that disease with hook-
worm. Few references to the coexistence of these two conditions are to be found
in the literature, although many such cases exist (1). While numerous papers on
quinine levels in blood and urine have appeared in the literature (2-7) there are
no references, to the authors' knowledge, concerning the effect of hookworm
damage upon the quinine levels attainable in blood and urine. Although little
information is available as to the chemotherapeutic action of quinine, it is
generally held that the rapid attainment and the maintenance of maximal sub-
toxic concentrations of the drug in the blood is desirable for effective therapy.
The present studies were undertaken to ascertain whether or not damage to the
small intestine by hookworm infection alters the concentration of quinine in
blood and its excretion in the urine following its ingestion by mouth.

METHODS. The work reported below was carried on with two male dogs of 12 and 20 kg.
weight respectively, the former (A) a mongrel of very irritable disposition and the latter
(B) a bird dog, quite easy to handle. The criteria used in the selection of these animals
were their apparent state of good nourishment and the mildness of their initial intestinal
parasite infections, as shown by fecal egg counts. It is practically impossible to find adult
dogs which have not at some time in their lives had a hookworm infection. These dogs
were freed from intestinal parasites by means of a dose of 10 cc. of tetrachloretylene and
15 cc. of saturated magnesium sulfate and were given a period of four to five days for healing
of the intestine.

The dose administered in each experiment was 20 mg. of Merck's U. S. P. quinine sulfate
per kilogram of body weight. This amount represents approximately the sum of the doses
per kilogram of body weight given in 24 hours to human malaria patients and about three
times the amount of any single dose. Because of its common employment in malaria
therapy, quinine sulfate was used instead of more soluble salts.

Gelatin capsules were used, in most cases, for administration of the dose. In two
experiments on hookworm free dogs, a stomach tube was used without any detectable differ-
ence in results. The dissolving of the capsule evidently causes no noticeable delay.

Blood samples (6 cc.) were taken from the jugular vein and oxalated. Urine was col-
clected by catheterization. At other times during an experiment the dogs were kept in
clean, dry metabolism cages for the collection of any urine voided. Both blood and urine
samples were stored in a refrigerator until analyses could be made. It has been shown in
this laboratory that blood and urine samples to which known amounts of quinine have been
added may be kept under refrigeration conditions for as long as two weeks without diminu-

1 All larvae cultures and egg counts were made by Mr. W. W. Taylor of the School of
Public Health of this University.
tion in the amount recovered. All determinations of quinine were made as described by Kyker, Webb and Andrews (8).

In each experiment, the dogs were fasted from 12 to 18 hours before quinine was administered and were not fed again until after the 24-hour blood and urine samples were taken. The only exception made to this procedure was in those cases in which the animals were greatly weakened by heavy hookworm infection. In such cases a small meal was given 12 hours after quinine dosage.

After control samples of blood and urine were taken the quinine sulfate was administered. Both blood and urine samples were taken at the following intervals after quinine administration: 30 minutes, 1, 2, 4, 6, 12, and 24 hours. In addition a blood sample was taken at 15 minutes.

**Results.** The figures (Series I) show representative curves of blood level and of urinary excretion obtained on dogs A and B. Urinary excretion is expressed both in terms of concentration of quinine in the urine and in terms of cumulative excretion. The method used does not reliably detect amounts of quinine less than 0.7 mg. per liter. Figures of this magnitude and less therefore represent blank determinations.

**Reaction of dogs to quinine.** In 15 to 30 minutes after administration of the dose both these dogs, as well as some others used in preliminary work, showed signs of cinchonism. Nervousness and a marked increase in respiratory rate were evident. The latter returned to normal in about 40 minutes.

**Infection with hookworm.** After 3 such experiments were made on each dog while free from hookworm, the animals were infected with dog hookworm by spraying about 2000 larvae into the mouth. According to Chandler (9) larvae so administered develop in the alimentary tract without passing through the lungs. They attach themselves to the intestinal mucosa in about 6 days and reach maturity in about 2 weeks.

Experiments run on both dogs at intervals of 1, 2 and 3 weeks after infection showed no marked changes in either blood or urine curves. However, since fecal egg counts showed that only a light infection had been produced, the dogs were four weeks later reinfected by subcutaneous injection of 6,000 larvae each into the groin. The degree of infection attained by the second treatment is shown by the fact that the Stoll count (eggs per gm.) for nearly the whole month ranged from a few hundred to over 10,000. This would be considered a moderate hookworm infection. This second infection also produced considerable local swelling, inflammation and, in one case, a draining ulcer. Fecal worm counts made at a later date, following administration of the anthelmintic, showed for Dog A, 88 worms and for Dog B, 75 worms.

Quinine administration was repeated with these dogs at intervals of 1, 2, 3, and 6 weeks after reinfection. The figures (Series II) show curves obtained two weeks afterwards, and are typical of those obtained under conditions of moderate infection. At seven weeks after reinfection the dogs were given an anthelmintic (10 cc. tetrachlorethylene and 15 cc. saturated MgSO4) by stomach tube, their feces collected and the worms counted. The results of this count were those given above. Repeated fecal examinations made one week later showed negative results for both dogs, after which the quinine administration was again repeated. The results are also shown in the figures. (Series III)
FIGS. 1 AND 2. COMPOSITE CURVES FOR DOGS A AND B

Series I. Concentration of quinine before hookworm infection (blood and urine).
Series II. Concentration of quinine after moderate hookworm infection (blood and urine).
Series III. Concentration of quinine following anthelmintic, when dog was free of hookworm (blood and urine).

Series I. Cumulative excretion of quinine in urine before hookworm infection. (Weight of quinine shown by figures on left).
Series II. Cumulative excretion of quinine after moderate hookworm infection. (Weight of quinine shown by figures on left).
Series III. Cumulative excretion of quinine following anthelmintic, when dog was free of hookworm. (Weight of quinine shown by figures on left).
Comparison of the blood level curves obtained under different conditions from either dog shows that moderate intestinal damage due to hookworm infection has little or no effect on either the maximum blood level of quinine attained or in the length of time elapsing between administration of the dose and the attainment of that maximum. This statement is based on the results of a number of separate experiments on quinine administration other than those recorded herein. In general it may be said that almost all of the maximum levels of blood quinine attained lay between 3 and 4 mg. per liter whereas the time required for attainment of these levels was from one-half to two hours. These levels are comparable with those obtained by Kaiser (7) although, in our experiments, they were never reached in as short a time (8 minutes) as reported by him.

From the standpoint of the length of time over which comparatively high levels of quinine are maintained in the blood stream, a difference is sometimes apparent between the curves of hookworm-free animals and those of dogs with moderate hookworm infection. In the latter the blood quinine sometimes reaches higher levels and maintains them for a longer period of time than the former. This difference is not always observed and usually requires a determination of the area under the curve to make it evident, but we have encountered it in several isolated experiments. In several such cases we have measured the areas under the curves of quinine concentration in blood and have found them, under conditions of hookworm infection, to be often as much as double those produced by normal animals. In the curves herein reproduced, little difference is found in these areas.

It should be emphasized that the apparent concentration of quinine in blood, as indicated by our determinations, represents the difference between the rate of income from the small intestine versus the outgo. The outgo is, in turn, roughly divisible under two heads: excretion of quinine by way of the kidney into the urine, and metabolic destruction. In each series there are presented curves for urinary excretion corresponding to those representing blood levels. It will be noted that each curve represents both the concentration of quinine in the urine and the cumulative weight excreted. In terms of total percentage of the original dose excreted in 24 hours, our data show that in the experiments with Dog A, the recoveries were, respectively, 5.9, 3.4, and 3.7%. The same series for Dog B gives (in the same order) 4.4, 5.3 and 4.3% recovery. Continued collection and analysis for a second period of 24 hours in some cases doubled these figures, in others showed little or no additional quinine. In general, it can be said that total recoveries in the urine varied from 4 to 12% of the dose administered. It should furthermore be emphasized that the method of Kyker, Webb and Andrews undoubtedly does not distinguish between quinine and other similar cinchona derivatives. The above figures represent some substance, expressed in terms of quinine, to which the silicotungstate method responds. As far as the writers are aware, the literature contains no reliable report of the isolation, from dog urine after quinine administration, of any substance of correct composition, crystalline form, optical activity etc., to make possible the unequivocal demonstration that any unaltered quinine has been excreted. Pre-
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Preliminary attempts at such a demonstration in this laboratory have been thus far unsuccessful. We must conclude therefore that only a comparatively small percentage, if any, of the quinine administered by mouth reaches the urine. In this we are in agreement with earlier workers in this field.

The fact that such a large percentage of the dose is changed to products which do not respond to the analytical method employed raises interesting speculations as to the reason for any differences in form of the blood curves between hookworm-free and hookworm-infected animals. The occasional finding that in the latter case the apparent quinine concentration in the blood remains elevated for a longer period, might imply impairment of whatever mechanism is involved in its metabolic decomposition. These higher blood curves therefore may possibly be ascribed to delay in the process of metabolic decomposition. This laboratory is engaged in experimental studies designed to answer some questions of this type.

CONCLUSIONS

1. The curves of blood concentration and urinary excretion of quinine, after administration of a single dose by mouth as the sulfate, have been studied on two dogs under normal conditions, under two different degrees of hookworm infection, and after normal intestinal conditions were reestablished.

2. Hookworm infection, with the resulting damage to the small intestine, does not decrease the maximum quinine level attainable in the blood, nor does it materially change the time required to attain that maximum.

3. The shape of the curves obtained in hookworm infection sometimes differs from the normal in the occurrence of a plateau following the initial peak, thus increasing the area underneath the curves.

4. The highest concentrations of quinine in the urine paralleled those in blood. The percentage recovery of the total dose in the urine varied from about 4 to 12% of that administered.

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REFERENCES