

THE CHRONIC TOXICITY OF QUINACRINE (ATABRINE)¹

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Although the recent extensive use of quinacrine in the therapeutic and prophylactic treatment of malaria has focused attention on its possible chronic toxicity, no study of its lifetime effects on laboratory animals has been reported. Experiments (1, 2) conducted for shorter periods of time have shown that quinacrine can cause extensive pathological lesions in animal tissues, especially when relatively large amounts are administered. Early experiments (3, 4, 5, 6, 7) suggested and later work (8, 9, 10, 11) established the fact that an accumulation of quinacrine occurs in animal tissues. These observations indicate the possibility of damage from small dosages. Furthermore, if quinacrine is to be administered therapeutically or prophylactically over long periods of time, dietary considerations are likely to be important. Recently Seudi and Hamlin (12) reported that rats maintained on a high protein, low fat diet resisted the toxic action of quinacrine to a greater degree than those maintained on a low protein diet or a diet high in both protein and fat. Hegsted, McKibbin and Stare (13) found that the addition of various vitamins, yeast and protein did not prevent changes that were produced by quinacrine in animals on an already adequate diet. In the present investigation we have demonstrated differences in response to the toxic action of quinacrine when administered for the approximate lifetime of albino rats on high and low protein diets.

PART I. HIGH AND LOW PROTEIN DIETS. Experimental. Groups of 18 weanling rats each were selected at random with respect to litter mates from our colony of Osborne-Mendel strain. Six rats, 3 of each sex, were chosen from a litter and assigned to 10 different diets as required for balanced incomplete blocks of 3 with 10 treatments. The design was replicated as a whole 3 times, giving a total of 180 rats. Five groups were placed on a high Protein diet and 5 groups were placed on a low protein diet. One group on each diet, was given 100 ppm. of quinacrine; a second 200 ppm.; a third 400 ppm.; a fourth 800 ppm.; and a fifth, which was used as control, received the diet without the added quinacrine. The rats on the 100 and 200 ppm. were started at these levels. Those on the 400 ppm. were started at 200 ppm. and raised to 400 ppm. between 10 days and 2 weeks. Those on the 800 ppm. were started on 200 ppm. and in a like manner raised to 400 ppm. and then after another week raised to 800 ppm. The rats were kept in individual cages in a room with the temperature and humidity controlled for the duration of the experiment. The rations used had the following composition: The low protein diets contained dextrose 75%, casein 6%, corn oil 6%, brewer's yeast 5%, salt mixture (U. S. P. XII No. 2) 4%, whole liver powder 2%, and cod liver oil 2%. In the high protein diet the casein was increased to 30% with a corresponding reduction in dextrose.

¹ Trade name quite commonly used.

The effect on growth. The first noticeable effect of quinacrine is an apparent dislike for the food containing it with a retardation of growth which may later be overcome by an adjustment to the distastefulness. Since many animals on the low protein diet with 800 ppm. of quinacrine died early, the interval of the first 12 weeks on the experimental diet was selected as the first period of study for a comparison of growth data from all groups. At this period the retarding effect

TABLE 1
Mean gain in weight of rats fed diets containing quinacrine

| TIME IN MONTHS | DOSAGE OF QUINACRINE | SEX | LOW PROTEIN DIET | | HIGH PROTEIN DIET | | |
|----------------|----------------------|-----|------------------|---------------------|-------------------|---------------------|--------------|
| | | | No. of Animals | Mean gain in weight | No. of Animals | Mean gain in weight | |
| 3 | 0 | M | 8 | 177.4 ± 12.0 | 8 | 334.2 ± 13.6 | |
| | | F | 10 | 148.4 ± 12.1 | 9 | 217.0 ± 8.5 | |
| | 100 | M | 7 | 162.7 ± 8.4 | 9 | 315.0 ± 10.5 | |
| | | F | 9 | 112.3 ± 6.1* | 9 | 199.1 ± 5.7 | |
| | 250 | M | 8 | 157.1 ± 7.3* | 9 | 289.4 ± 12.8* | |
| | | F | 9 | 113.8 ± 6.7)* | 9 | 192.1 ± 8.9' | |
| | 400 | M | 9 | 105.9 ± 7.0† | 8 | 250.4 ± 13.7† | |
| | | F | 9 | 101.2 ± 7.6† | 9 | 159.5 ± 6.4† | |
| | 800 | M | 7 | 91.9 ± 6.4† | 9 | 17.1 ± 9.7† | |
| | | F | 9 | 73.7 ± 4.1† | 9 | 132.8 ± 6.9† | |
| | 12 | 0 | M | 7 | 461.6 ± 26.9 | 6 | 488.3 ± 26.9 |
| | | | F | 10 | 288.8 ± 15.6 | 7 | 293.0 ± 13.8 |
| 100 | | M | 7 | 425.4 ± 13.5 | 8 | 495.2 ± 19.4 | |
| | | F | 8 | 232.9 ± 11.1† | 8 | 294.6 ± 8.9 | |
| 200 | | M | 7 | 387.6 ± 15.0* | 8 | 478.8 ± 20.4 | |
| | | F | 8 | 235.5 ± 9.5† | 8 | 293.3 ± 10.5 | |
| 400 | | M | 6 | 260.0 ± 4.1† | 7 | 384.9 ± 21.5* | |
| | | F | 4 | 208.3 ± 25.5† | 6 | 222.7 ± 12.3† | |

● $p < .05 \rightarrow .01$.

† $p < .01$.

of quinacrine on growth increases with the concentration of quinacrine (table 1). The early growth rate was retarded by all dosages used; however, the degree of retardation was not significant in all cases. At concentrations of 100 ppm. quinacrine retarded significantly the growth rate only of the female animals on the low protein diet. At the 200 ppm. level all values for retardation are significant except that for the male animals on the low protein diet.

In order to study the effect of quinacrine on growth for a longer period a second interval of the first year on the experimental diet was selected. By the end of the year all rats on the 800 ppm. of quinacrine had died. An analysis (table 1) for the groups on the low protein diet shows that there was a retardation at all concentrations; however, the value for the group of male animals on 100 ppm. of quinacrine remains nonsignificant. On the high protein diet quinacrine only at the dosage level of 400 ppm. caused significant retardation. The growth rate of the rats on the 200 ppm. of quinacrine had increased so that, the value for the retardation of growth changed from significant at 3 months to nonsignificant at 12 months.

The effect on mortality. The mortality rate of the rats on the higher concentrations of quinacrine was related to the dosage of quinacrine and to the kind of diet (table 2). Quinacrine at 800 ppm. in the diet was very toxic. All animals on the low protein diet at this concentration died within 6 months. On the high protein diet the early mortality was less, but all rats died within a year. At the 400 ppm. level all rats in the low protein group died within 18 months and in the

TABLE 2
Per cent mortality of rats fed diets containing quinacrine

| DOSAGE OF QUINACRINE ppm. | LOW PROTEIN DIET | | | HIGH PROTEIN DIET | | |
|-------------------------------------|------------------|---------|---------|-------------------|---------|---------|
| | 6 mos. | 12 mos. | 18 mos. | 6 mos. | 12 mos. | 18 mos. |
| 0 | 5.5 | 5.5 | 3.3 | 11 | 27.5 | 44 |
| 100 | 11 | 17 | 39 | 5.5 | 11 | 39 |
| 200 | 5.5 | 17 | 61 | 0 | 11 | 55 |
| 400 | 17 | 39 | 100 | 11 | 27.5 | 83 |
| 800 | 100 | 100 | 100 | 11 | 100 | 100 |

high protein groups only 1 was living at the end of the experiment. In the groups on 200 ppm. of quinacrine fewer rats were living at the end of the experiment than in the control groups, but the difference is not significant ($p = 0.15$).

Hematology. At intervals of 6 weeks blood studies were made on 6 rats from each group until too few survivors remained in a group to continue the studies. Table 3 gives the observations at 6 month intervals. The outstanding change was a leukocytosis, predominantly neutrophilic. It was marked in the groups on 800 ppm. of quinacrine, less striking in the groups on 400 ppm. and scarcely noticeable in those on 200 ppm. A slight increase in hemoglobin concentration and erythrocyte counts was noted in the groups on 800 ppm. of quinacrine.

Confirming the observations of Seigel and Mushett (1) the lymphocytes showed rounded basophilic granules within the cytoplasm (figure 1). In the earlier weeks of the experiment as many as one-half of the lymphocytes in the 800 ppm. groups contained these granules. Smaller percentages were seen in the groups on the lower dosages of quinacrine. In later observations the percentages gradually decreased until by the end of the first year the granules were rarely seen.

Pathology. Microscopic examination was made of 114 of the 180 rats. Lung, heart, liver, spleen, pancreas, stomach, small intestine, colon, kidney, adrenal and testis were sectioned routinely; thyroid, parathyroid, leg muscles, bone and marrow of tibia and femur, lymph node, brain, salivary glands, uterus and ovary

TABLE 3
Mean hematological effects of quinacrine at intervals of 6 months

| WEEKS ON EXPT. | QUINACRINE IN DIET | NO. OF ANIMALS | HEMOGLOBIN (KLETT METHOD) | ERYTHROCYTES | LEUKOCYTES | NEUTROPHILS |
|-------------------|--------------------|----------------|---------------------------|-----------------------------|------------------------------|-----------------|
| High protein diet | | | | | | |
| | <i>ppm.</i> | | <i>grams</i> | <i>millions per cu. mm.</i> | <i>thousands per cu. mm.</i> | <i>per cent</i> |
| 25 | None | 6 | 17.1 ± .48* | 7.8 ± .25 | 13.6 ± 2.2 | 23.3 ± 4.5 |
| 50 | | 6 | 18.4 ± .57 | 8.4 ± .57 | 12.8 ± 1.2 | 27.6 ± 3.3 |
| 76 | | 6 | 16.8 ± .30 | 9.0 ± .70 | 10.6 ± 2.2 | 38.3 ± 6.4 |
| 25 | 200 | 6 | 17.7 ± .53 | 8.6 ± .17 | 14.2 ± 1.4 | 28.3 ± 5.2 |
| 50 | | 6 | 18.7 ± .14 | 8.9 ± .39 | 14.6 ± 1.4 | 29.5 ± 1.1 |
| 76 | | 5 | 16.6 ± .61 | 9.5 ± .42 | 14.2 ± 3.4 | 39.8 ± 2.0 |
| 25 | 400 | 6 | 18.7 ± .34 | 8.7 ± .13 | 15.6 ± 2.0 | 28.3 ± 2.3 |
| 50 | | 6 | 16.4 ± .53 | 7.6 ± .26 | 26.6 ± 3.1 | 42.1 ± 5.6 |
| 76 | | 3 | 15.6 ± .47 | 7.5 ± .37 | 14.4 ± 2.3 | 35.6 ± 1.5 |
| 25 | 800 | 6 | 17.8 ± .53 | 8.8 ± .24 | 28.4 ± 5.7 | 48.5 • 8.9 |
| 44 | | 3 | 21.0 ± 1.90 | 10.2 ± 1.40 | 21.7 ± 9.9 | 47.3 ± 4.1 |
| 75 | | No survivors | | | | |
| Low protein diet | | | | | | |
| 25 | None | 6 | 17.1 ± .34 | 8.6 ± .28 | 10.9 ± 1.0 | 30.0 ± 4.7 |
| 50 | | 6 | 18.9 ± .42 | 8.4 ± .18 | 17.6 ± 1.8 | 29.3 ± 3.9 |
| 76 | | 6 | 16.3 ± .53 | 9.7 ± 1.00 | 14.6 ± 3.4 | 34.8 ± 7.2 |
| 25 | 200 | 6 | 16.7 ± .26 | 7.9 ± .14 | 11.2 ± 0.5 | 25.5 ± 2.0 |
| 50 | | 6 | 18.2 ± .27 | 8.4 ± .23 | 14.5 ± 2.5 | 34.0 ± 3.6 |
| 76 | | 6 | 14.5 ± .30 | 7.2 ± .35 | 11.3 ± 1.5 | 43.1 ± 4.2 |
| 25 | 400 | 6 | 16.2 ± .56 | 8.2 ± .35 | 21.7 ± 1.6 | 32.6 ± 3.5 |
| 50 | | 6 | 17.0 ± .44 | 7.9 ± .21 | 22.9 ± 1.9 | 40.6 ± 2.7 |
| 76 | | No survivors | | | | |
| 13 | 800 | 5 | 11.1 ± .70 | 7.4 ± 1.52 | 69.9 ± 7.1 | 67.6 ± 8.2 |
| 25 | | No survivors | | | | |

* The number following each mean is the standard error.

were sectioned less frequently. In addition to the usual hematoxylin-eosin stains on paraffin sections of formalin-fixed material, Perls' acid-ferrocyanide reaction with a basic fuchsin counterstain was used to indicate the nature of various pigments, and a Sudan IV stain on frozen sections was used for demon-

strating fat. A small amount of work with other fixatives and stains was done for the purpose of noting the reactions of the peculiar basophilic granules to be mentioned later.

Brief notes on the lesions seen in our rats following the feeding of high levels of quinacrine have been published (14, 15). Since many of the details of similar lesions have been described in 2 papers dealing primarily with the structural changes, namely, those of Wright and Lillie (2) and of Seigel and Mushett (1), many changes in our rats will be only briefly described, with more attention to those points not mentioned in the previous publications. Seigel and Mushett (1) found "slight adrenal and myocardial alterations" in rats given 1% of the LD50 for 15 months. They also noted moderate myofibrosis and basophilic granulation in various locations in rats given 2% of the LD50 for 4 to 6 months. The next higher dose, 5% of the LD50, killed the animals after 6 to 12 weeks, while still higher doses caused death after various shorter periods. Hegsted,

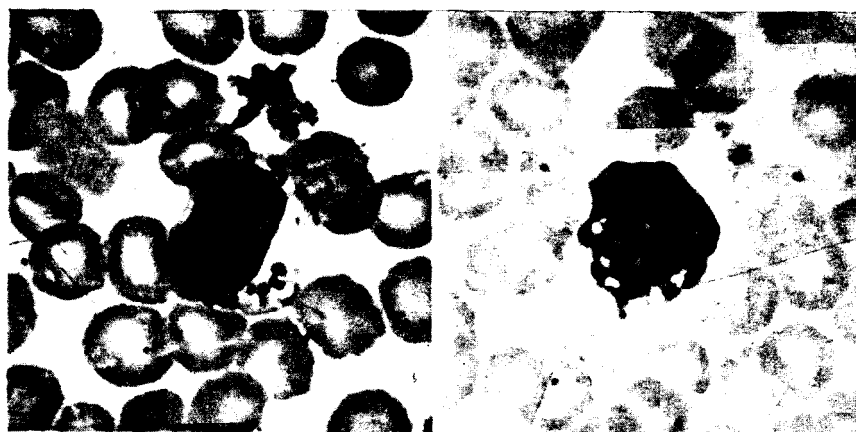


FIG. 1. BASOPHILIC GRANULES IN LYMPHOCYTES (X 1700; WRIGHT'S STAIN)

McKibbin and Stare (13) examined microscopically "a few rats" which had received quinacrine at levels of 25 and 40 mgm./100 grams of diet for 6 months and found only minimal changes, essentially limited to basophilic granulation; no necrotic changes were detected. Barlow, Auerbach and Rivenburg (9) briefly discuss the pathology in connection with pharmacological studies in rats which received quinacrine for 70 days. They found that 5% of the LD60 given daily by stomach tube for 49 days caused insignificant findings at necropsy. Wright and Lillie (2) continued their experiments for 50 days. All of the above named workers except Hegsted et al. used the weight of the animal in computing the dosage level while our levels were computed as percentages of the diet, making a direct comparison of mortalities at a given dosage level somewhat difficult. The 40 mgm./100 grams of diet used by Hegsted et al. corresponds to our 400 ppm.

Among our rats fed 800 ppm. quinacrine in the diet, those on the low protein diet were severely affected much sooner than those on the high protein diet. The

first death in the low protein group occurred after 6 weeks with changes essentially those of inanition. All but 1 of the remaining 17 rats died or were sacrificed because of poor condition after 13 to 19 weeks, while the last rat lived 21 weeks. In addition to a generalized yellow discoloration, all 17 rats showed at autopsy large irregular areas of necrosis of the liver, and thin fibrous adhesions more or less completely gluing together the contents of the cranial half of the abdomen, including the diaphragm and the cranial half of the right kidney. The necrosis as seen with the unaided eye involved one-fourth to one-half of the liver in 2 instances, half to three-fourths in 13, and over three-fourths in the remaining 2. Ascites was present in 4 rats, and pulmonary edema in 6. Microscopically there were massive areas of hepatic necrosis, with the viable portions of the liver generally showing a mixture of regenerative hyperplasia and (from the incidental slight or moderate degree of inanition) a lesser degree of atrophy. Varying amounts of loose to dense fibrous tissue were present around the large areas of necrosis and irregularly in the viable portions of the liver.-- In the meshes of the fibrous tissue were generally numerous foamy macrophages, and smaller numbers of these macrophages were present in the sinusoids. The macrophages, and to an equal or greater extent the hepatic cells, contained moderate numbers of small basophilic granules of a type not previously seen in any of our experimental animals of any species. Blood vessels both inside and outside the liver in the vicinity of the large necrotic areas often contained thrombi, sometimes organized and canalized; the vessels generally appeared to be parts of the portal venous system, rarely of the hepatic arteries. Slight or moderate bile duct proliferation in the liver was seen frequently. Macrophages containing hemosiderin were generally present in the fibrous tissue gluing the cranial surface of the liver to the diaphragm, and to a lesser extent around the large areas of necrosis and elsewhere in the liver. Focal myocardial necrosis with varying degrees of replacement by fibrous tissue was frequent, but was usually slight in degree in contrast to the more extensive examples noted after longer periods of feeding, and at lower dosage levels. Nearly all the rats whose leg muscles were sectioned showed focal necrosis of the muscle fibers, generally moderate in degree, with more or less fibrous replacement. The lungs frequently contained foci of large agranular foamy macrophages, tending to occur subpleurally and peribronchially and making up from 25 to 35% of the area of the microscopic section. These macrophages, together with the somewhat less frequent edema fluid and a small alveolar and septal cellular exudate which was chiefly mononuclear (smaller and more solid macrophages, together with some polymorphonuclear leukocytes), made a rather characteristic picture in this and other groups on the higher dosage levels. Basophilic granules similar to those in the liver were present in renal glomerular and tubular epithelial cells, and in lesser concentration in masses of foamy macrophages in the villi of the small intestine, in scattered macrophages in the splenic pulp and lymph nodes, in the cells of the adrenal cortex, in myocardial and pulmonary venous muscle cells, and in the endothelial cells of arteries, chiefly those of the lung, heart, liver and spleen. They were very rare in arterial muscle cells, which were frequently slightly en-

larged and contained weakly oxyphilic granules. Thirteen spleens in this group were slightly enlarged; this enlargement was accounted for microscopically by a slight pulp hyperplasia plus the presence of the macrophages.

To aid in studying the pathogenesis of the changes resulting from quinacrine administration and to determine what fraction of the changes resulted from the moderate degree of inanition present, a special group of 24 rats, not forming a part of the 180 rats on the 2-year experiment, was divided into 2 groups, 12 rats receiving 800 ppm. quinacrine in the diet and 12 rats receiving no quinacrine but with each animal having its dietary intake restricted to that of its paired litter mate. Because all of the rats in the first series had with one exception lived at least 13 weeks, it was intended to sacrifice 1 male and 1 female of the second series together with their paired feeding controls, at 3, 5, 7, 9, 11 and 13 weeks. However, after the first 2 pairs had been sacrificed at 3 and 5 weeks respectively, the remaining 8 treated rats either died or were sacrificed in extremis at from 5 to 8 weeks. A slightly faster rate of bringing the animals to the 800 ppm. level than that used for the first group may have been responsible for the earlier deaths. The microscopic changes were essentially identical with those described in the preceding paragraph, with even greater portions of the liver being necrotic in the last 8 treated rats. The pair sacrificed at 3 weeks showed a slight to moderate hydropic degeneration of the liver, while the pair sacrificed at 5 weeks showed a moderate fatty degeneration. Small numbers of foamy macrophages in the lung and small intestine had already made their appearance. The male of each of the above pairs showed focal necrosis of the myocardium; leg muscles were negative. In the remaining animals the leg muscles showed focal necrosis similar in degree to that in the first group; hepatic necrosis and other histopathologic changes were also similar with the exception that the myocardium was slightly less damaged. The bone marrow of the rats receiving quinacrine was generally slightly hyperplastic while that of the paired feeding controls was slightly hypoplastic. The treated rats showed slight reduction of new bone formation at the epiphyseal lines and sometimes slight osteoporosis beyond the changes of these types resulting in the control rats from reduced dietary intake.

Returning now to the main experiment, at the same dosage level of 800 ppm. but in a high protein diet, the first rat of the group of 18 was sacrificed because of poor condition after 21 weeks of feeding. Twelve of the remaining 17 died or were sacrificed because of poor condition between the 26th and 32nd weeks, while the last rat died after 50 weeks. The gross and microscopic changes in the liver were more variable than in the low protein group, some showing the extensive changes described above, others less, with almost no macroscopic change in some instances, and the microscopic lesions consisting of slight to moderate focal necrosis and additional minor changes. In 9 of the 18 rats there were upper abdominal adhesions and macroscopic liver necrosis; the mean degree of these changes was less than in the low protein group. Cardiac damage, however, was greater than in the low protein group. Moderate dilatation was generally present, and in the microscopic sections there was usually marked myocardial fibrosis, best seen in the left ventricle. Small numbers of muscle fibers showed recent

necrosis, **which** together **with** the appearance of variable age in the **fibrous** tissue indicated that the nature of the process was that of continuing necrosis of muscle fibers, small in number at any given time. **Nine** rats showed a lesion **not** seen in the low protein group, namely, thrombus formation in the left atrium of the heart; in a tenth rat there was a mural thrombus in the left ventricle. The atrial thrombi were usually 6 or 7 mm. in diameter and **were whitish** in color. Microscopically there was usually slight peripheral organization in the thrombi. **Ascites** was not present in this group; edema of the lungs was noted in 9 animals and hydrothorax in 1. The spleen was slightly enlarged in 1 animals. **Basophilic** granulation, foamy macrophages, low grade pulmonary consolidation, focal necrosis of leg muscles, splenic and bone marrow hyperplasia and other changes were similar to those in the **800** ppm. low protein group.

At a dosage level of 400 ppm. in either a high or low protein diet, the majority of the rats had died or had been **sacrificed because** of poor condition at the end of 50 to **60** weeks of feeding. There was little of the massive macroscopic necrosis of the liver seen in the 800 ppm. groups but the lesions were still of high grade, especially in the microscopic sections. Whitish thrombi in the left cardiac atria of the same size as in the previous group were present in 7 rats of the high protein group, and in 2 of these ventricular thrombi were also present, once each in the right and left ventricles. In the low protein group there were 6 atrial thrombi, plus left ventricular thrombi in 3 of these 6 rats. Edematous lungs were noted 8 and 5 times respectively, and hydrothorax once in each group. **Ascites** was **seen only** once, in a low protein rat other than that **having hydrothorax**. Both groups showed the dilatation of the heart seen in the **800** ppm. high protein group, and in addition distinct hypertrophy was evident. Only **1** rat in the high protein group showed a macroscopic area of necrosis in the liver, while these, together with upper abdominal adhesions, were seen 3 times in the low protein group. Of the remaining livers, about half were grossly negative, while the other half showed slight changes such as a nutmeg appearance, roughening of the surface, or slight disproportions and distortions of the **architecture**. The spleen often showed a minimal enlargement, but **definite** enlargement **was** noted only once in the high and twice in the low protein group. **Microscopically**, in both the high and low protein groups, approximately equal numbers of animals showed slight, moderate and marked liver damage, consisting chiefly of necrosis, with slight regenerative changes. The necrosis was most often **centrolobular**, less often patchy, and only in a few instances in massive blocks. Myocardial scarring (old and recent necrosis of muscle fibers with replacement fibrosis) was usually moderate in degree in the high protein group, while in the low protein group it **was** either moderate or marked. Hypertrophy and rarefaction of the muscle fibers of the left ventricle and large pulmonary veins were frequent, but **basophilic** granules in the muscle fibers were rarely seen. The atria and right ventricle of the heart showed the hypertrophy and rarefaction to a lesser degree than did the left ventricle; the same was true of the necrosis and scarring. **Basophilic granules** in hepatic and renal epithelial cells and in **macrophages** in the liver, small

intestine and spleen were present in small to moderate numbers, showing a moderate reduction from the number present when 800 ppm. were fed. The granules were now rare in arterial endothelial cells. In general, leg muscles showed slight focal necrosis and/or fibrosis, and the bone marrow and splenic pulp were slightly hyperplastic. The lungs were about as described for the 800 ppm. level. The adrenal showed a mild degree of damage not seen at other levels; in about one-fourth of the animals the inner portion of the cortex had a very slight to moderate moth eaten appearance probably from previous damage with replacement by loose fibrous tissue, and in a few adrenals small foci of frank necrosis were present. Another change noted only at this level was the rather frequent presence of small amounts of pigment, chiefly nonferrous, in the renal convoluted tubular epithelium. In summary, in the 400 ppm. groups damage to the heart seemed to be as great a factor in causing death as was damage to the liver which is contrary to the findings in the groups on higher dosage. A high protein level in the diet had only a slight sparing effect on the development of anatomical changes and on mortality as compared to the marked effect seen when a diet containing 800 ppm. quinacrine was fed.

TABLE 4
Numbers of rats with microscopic changes of moderate or marked degree

| GROUP | MICROSCOPICALLY EXAMINED | HEART | LIVER | KIDNEY |
|------------------|--------------------------|-------|-------|--------|
| 200 ppm. | 2 | 3 | 9 | 7 |
| 100 ppm. | 27 | 2 | 2 | 4 |
| Control. | 16 | 0 | 0 | 0 |

At a dosage level of 200 ppm. the treated animals did not begin to die in greater number than their controls until after about 18 months of feeding. Microscopically, the visible changes at this level were slight. The viscera showed a slight yellow staining, and a minority of the livers had such changes as slight enlargement, slight roughening of the surface, a fine dark orange-and-red mottling, and some rounding of the edges. Pulmonary edema was present in 3 animals, 2 in the high protein group. In the one in the low protein group, which died after 20 months, hydrothorax and the only cardiac atrial thrombus at this level were also present. A few hearts were slightly dilated and hypertrophied and a few spleens very slightly enlarged. In the 100 ppm. groups the gross changes were on the whole very slight, although one rat on the low protein diet, evidently unusually susceptible, showed extensive hyperplasia and architectural distortion of the liver when sacrificed after 15 months. Microscopically, lesions attributable to quinacrine in the 200 and 100 ppm. groups, whether on a high or low protein diet, were essentially limited to the liver and heart and to a lesser extent the kidney. The mild kidney damage appeared late in the experimental period. The actual numbers of lesions graded as moderate or marked in the microscopically examined rats are given in table 4; slight or very slight degrees of most of these changes

were present in many of the control animals (as they are in any group of rats two years of age) and in more of the test animals. The cardiac lesion was focal myocardial fibrosis, greatest in the left ventricle, and the renal lesion consisted of focal cortical tubular atrophy and dilatation, the presence of hyaline tubular casts, and some glomerular atrophy. In these two organs the lesions caused by quinacrine at such low levels of dosage were not specific histologically and differed only in degree and numerical incidence from the "spontaneous" lesions in control animals of this species; this was not true for the liver. Lesions of the liver were never more than moderate in degree except in the one unusually susceptible animal and included (in addition to accentuation of the common "spontaneous" slight hepatic cell atrophy and bile duct proliferation seen in old rats) hepatic cell hyperplasia, disarrangement of normal architecture, centrolobular necrosis, and rarely focal vacuolar degeneration of hepatic cells. The basophilic granules and macrophages regularly seen in the 400 ppm. groups were seen infrequently and in small numbers at the 200 ppm. level and only once at 100 ppm. Slight focal necrosis and/or fibrosis of leg muscles was seen infrequently at 200 ppm. and not at 100 ppm.; slight hyperplasia of the splenic pulp was seen occasionally in both groups, and the adrenal and bone marrow were negative in both.

The thyroid deserves a word of mention since we were unable to find a reference to it in previous reports. Twenty-one thyroids scattered among the various treated groups were examined microscopically and the only hyperplasia noted was a minimal or questionable one in three glands. Another gland showed a marked depletion of colloid, interpreted as recent. These four glands were all in rats on a high protein diet at levels of 200 and 400 ppm. quinacrine; otherwise, the glands were similar to those of the controls. Parathyroids were included in most of the thyroid sections and were all negative. An unpublished study of a relatively large series of dogs given 2 and 5 mgm./kgm./day of quinacrine for 2 years showed only minimal changes in the thyroid (16).

The following remarks on the pathological changes apply to most or all of the rats in this study. The large areas of necrosis in the liver had an infarct-like appearance and as stated thrombi were frequently found in microscopic sections. However, certain groups showed rather frequent examples of multiple small foci of necrosis or larger areas of an earlier or lower grade of necrosis, without the presence of thrombi. Considering the entire series, it was our impression that, in general thrombosis was secondary to the attainment of a large area of high grade necrosis, or at least that such an area was not primarily an infarct suddenly following thrombosis. although exceptions were not uncommon, the right side of the liver was more susceptible to necrosis; Seigel and Mushett (1) noted the same tendency. The clustered foamy macrophages in the lung were larger than the foamy macrophages located elsewhere, were easily ruptured, and contained very few basophilic granules. The macrophages, endothelial cells and muscle cells containing basophilic granules frequently contained in addition poorly defined oxyphilic and fewer neutrophilic granules; whether these represented additional types of ingested material or were transitional forms of the basophilic

material is not known. In the small intestine multiple levels were frequently **sectioned**, and showed an increasing gradient of macrophages and basophilic **granules** from the upper to the lower end; these were not seen in the stomach or **colon**. In spite of their frequent presence in renal glomerular epithelial cells, **basophilic** granules were never noted in the adjacent endothelial cells. Among the renal tubular epithelial cells, basophilic granulation and its frequent accompaniment of hypertrophy and some rarefaction of the cell were essentially limited to the distal portions of the looped tubules and less often the proximal thin portions; they were rarely seen in convoluted or **collecting** tubules. The granules had the same appearance following Bouin or Zenker fixation as they did after formalin. The granules were not metachromatic when stained with toluidine blue, and they did not stand out when an unstained paraffin section or a smear of liver was examined with polarized light. Granules **often** remained basophilic in necrotic hepatic cells. We assume that these basophilic granules, colorless in unstained sections, are some quinacrine-protein compound, but we have no exact knowledge of their nature. The macrophages containing them often contained sudanophilic material in the liver, but not in other locations; the pulmonary macrophages were not sudanophilic. In the most severe examples of myocardial fibrosis, there was about the same amount of fibrous as of muscular tissue in the left ventricular wall. The cardiac valves never showed damage. In the foci of myocardial necrosis of short duration (**800** ppm. low protein group) there was often slight calcification. **Ascites**, hydrothorax and pulmonary edema were more frequent in the animals found dead than in those sacrificed because of poor condition. The mechanism of formation and the chemical characteristics of the fluids were not investigated. A chronic passive congestion of the liver, often accompanying such fluid formation in man, was **not** seen. Voluntary muscles other than those of the leg were occasionally sectioned, and showed the same focal **necrotizing** changes as did the leg muscles. The adrenals of the rats on the higher dosage levels were enlarged, as noted by Seigel and Mushett (1) and by Barlow et al. (9). Splenic hemosiderosis was not a result of quinacrine administration; the treated rats had no more than the moderate amount, of pigment present in the controls. Neither was there an excess of the usual justamedullary cortical pigment in the adrenal. **As** already noted, hemosiderin was present to a certain extent in the liver; lymph node hemosiderosis varied as in untreated rats from almost-none to moderate, and a small or very small amount of hemosiderin was often present in the deeper portion of the lamina propria of the small intestine, underneath the **foamy** macrophages in the villi. The uteri and ovaries of **7** rats scattered among the various treated groups were sectioned and showed nothing of note; the same was true of 4 brains and **4** each of submaxillary and sublingual salivary glands. Testicular atrophy from quinacrine was only slight when the factors of inanition and of late "spontaneous" atrophy in the controls were considered; **a** possible slight degree of atrophy of the pancreas belongs in the same category. In both treated and control groups on a low **protein** diet, calcified tubular casts around the renal corticomedullary junction were frequently seen, while **they** were scarce in the high protein groups. These renal calcified tubular

casts are a typical result of a low protein diet, with accentuation by inanition, in our rats, and there is surprisingly little tubular atrophy proximal to these casts.

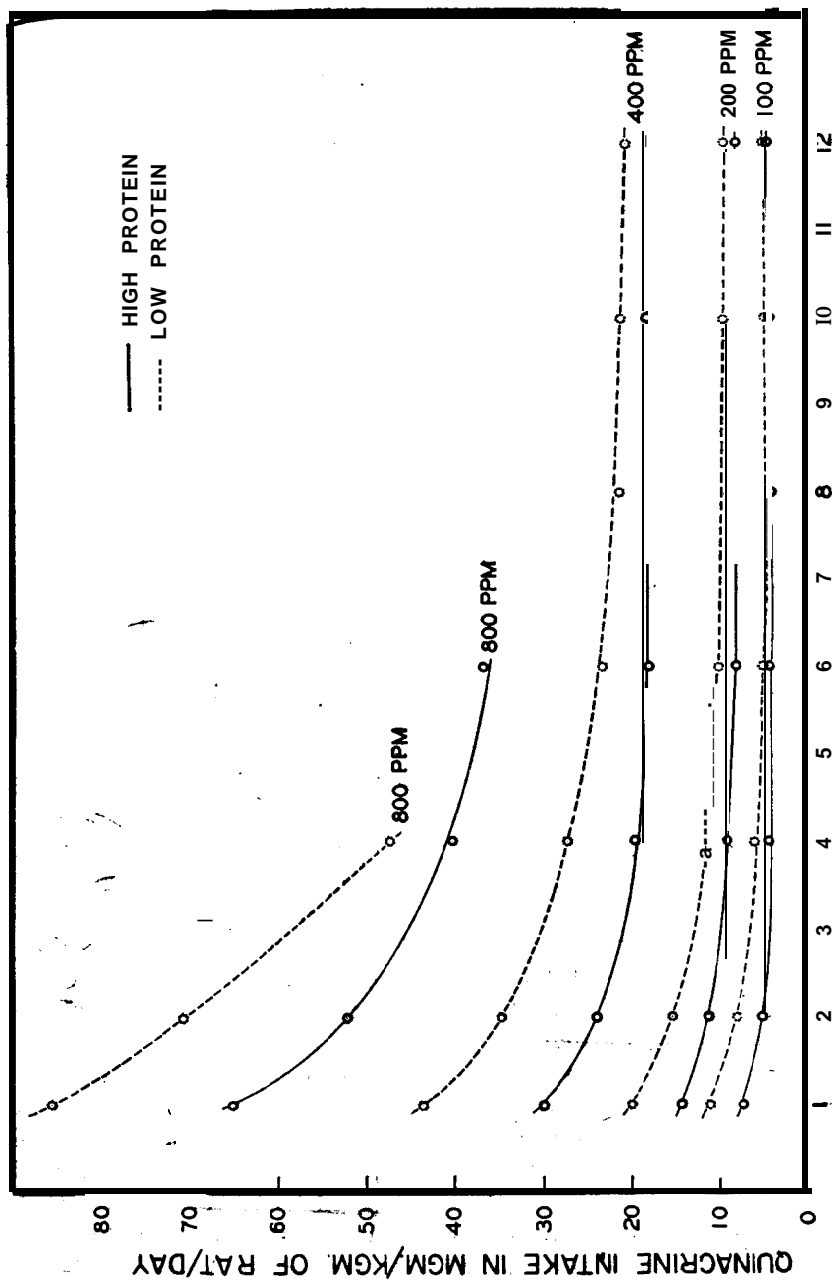
In view of the destructive effect of quinacrine on the liver, its possibilities as a cirrhotogenic and carcinogenic agent in prolonged administration must be considered. It is true that several of the rats discussed in this paper had livers that both microscopically and grossly showed nodularity and portal fibrosis to such an extent that were hepatic cirrhosis the primary object of this study these livers would have been considered to show positive results. On the other hand, these results did not appear in the rats on low dosage levels and living for 2 years. Only 1 liver tumor was seen in the group, an adenoma 2.2 x 2.0 x 1.1 cm. composed of hepatic cells, in a rat that received 100 ppm. in a high protein diet for 2 years; this is a normal incidence of this type of tumor in our rats.

PART II. THE EFFECT OF STARTING AGE ON THE TOXICITY OF QUINACRINE.

Ten litters of 4 male rats each were placed on an adequate diet containing 800 ppm. of quinacrine and allowed to remain on this ration until they died. A rat from each of the litters was placed on the quinacrine-containing diets at weekly intervals from 3 weeks of age until all rats had been placed on their diets.

An analysis of the data showed that there was no significant difference in mortality in the 4 groups of rats. The older and therefore the larger rats died in about the same number of days on the experimental diet as the younger rats. An analysis of variance, however, brought out the fact that there was a significant difference in the mortality rate between litters. A rat in any given group which resisted the toxic action of quinacrine for a longer time had litter mates in the other 3 groups which also resisted the toxic action of quinacrine.

Discussion. The many toxic effects produced by quinacrine in these experiments compels a consideration of the relationship of the dosage received by the rats and the human dose in the treatment of malaria. According to Circular Letter No. 22, Office of the Surgeon General, War Department (17) the therapeutic dose for malaria in man is 300 mgm. given daily for a week and the prophylactic dose is 200 mgm. given semi-weekly. Chart 1 shows the calculated quinacrine intake per day in terms of the body weight of the rat. These values were calculated from the weekly food intake of the rats at the various monthly intervals. The quinacrine intake of the rats at any given dosage level decreased rapidly during the first 2 months and then became almost constant as shown by the straight line (Chart 1) at about 6 months on the experimental diet. This fact is accounted for by the change in the growth rate of the rats from the fast growing period to the plateau period, while at the same time their daily food intake remained almost constant. Part of the increased toxicity observed in the rats on the low protein diet is undoubtedly caused by the greater amount of quinacrine consumed than by rats with a similar dosage level on the high protein diet. As shown in the chart the dosage of 100 ppm. of quinacrine corresponds to about 4 mgm./kgm. of body weight, per day for the rat. The dosage level is the borderline at which slight damage may occur to some rats given quinacrine for 2 years and to others no harm occurs. Taking the length of time into consideration, therefore, the amount of quinacrine that will produce toxic effects in rats is above the therapeutic or prophylactic dose for man.



MONTHS ON EXPERIMENT
CHART 1. CALCULATED QUINACRINE INTAKE IN MG/M./KGM. OF BODY WEIGHT IN RATS RECEIVING VARIOUS LEVELS OF QUINACRINE IN THE DIET

In another experiment, involving 59 rats, 400 and 800 ppm. of quinacrine in an adequate diet produced the same effects as those reported above for the rats at similar concentrations in a high protein diet.

SUMMARY AND CONCLUSIONS

1. Quinacrine at concentrations of 100 ppm. or more in the diet of rats produces toxic effects. At the 100 ppm. level the effects are slight, at higher levels they become progressively more severe.

2. On the low protein diet there was a significant retardation of growth at all concentrations of quinacrine used in this experiment; however, on the high protein diet there was no significant retardation below the 200 ppm. level. Because of a difference in food intake the rats on a low protein diet consumed more quinacrine per kgm. of body weight than those with a similar dosage level on a high protein diet.

3. A concentration of 800 ppm. of quinacrine produces early death in rats and one of 400 ppm. significantly increases the death rate; lower dosages do not affect mortality significantly.

4. The outstanding hematological change is a leukocytosis. This is marked in the groups on 800 ppm., less striking in the groups on 400 ppm. and scarcely noticeable in those on 200 ppm. of quinacrine. There is an increase in the hemoglobin concentration and erythrocyte counts in the rats on the 800 ppm. of quinacrine.

5. At a dosage level of 800 ppm. of quinacrine in the diet, any small group of our rats showed all and any individual rat showed most of the following changes: Generalized yellow discoloration; high grade necrosis of the liver; regenerative changes in the remaining viable liver; upper abdominal adhesions; focal necrosis and/or fibrosis of the myocardium and voluntary muscles; basophilic granules in hepatic, renal and other cells; foamy macrophages with or without similar basophilic granules in several locations; slight hyperplasia of the splenic pulp and bone marrow; and a low grade pulmonary consolidation.

6. At a dosage level of 800 ppm. a high level of protein in the diet considerably delayed and reduced the severity of liver damage as compared to that seen with a low level of protein; at 400 ppm. this delay and reduction was only slight, and at lower levels it was not apparent.

7. At 400 ppm. and lower levels of quinacrine, myocardial fibrosis and other cardiac changes, a more cumulative type of damage than that in the liver, became a factor equal to or greater than liver damage in causing the death of the animal.

8. After 18 to 24 months of feeding, a longer period than previously reported, rats on 200 ppm. of quinacrine in either a low or high protein diet frequently showed distinct anatomical lesions as a result of the medication. Even at 100 ppm. there was a small amount of damage.

9. The percentage of rats in which quinacrine caused cirrhosis of the liver was so small that we do not class quinacrine as a distinctly cirrhogenic agent. Only 1 liver tumor was seen, an adenoma which was probably incidental.

10. The age of young rats at the beginning of the experimental period had no

effect on the toxicity of quinacrine. There was found, however, a significant effect between different litters of rats. This fact demonstrates the importance of choosing litter mates for chronic feeding experiments.

11. In relation to the body weight of the rat the lowest dosage of quinacrine which produced slight toxic effects in some animals corresponds to approximately 4 mgm./kgm./day for 2 years.

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