Table 1. Transmission of TM from tumor-bearing to tumor-free hamsters by *Aëdes aegypti*. In blood of normal hamsters the white cell count is about 6000.

Donor		Recipients		
No.	White cell count	Total No.	No. developing tumors	Time tumors observed (days)
1	146,850	3	0	
2	111,000	3	0	
3	45,000	12	0	
4	122,000	3	0	
5	65,000	11	2	13,20*
6	82,000	10	2	20,23†
7	159,000	8	1	23‡

* Mosquitoes caged with recipients 35 minutes and 60 minutes, respectively. † Mosquitoes caged with recipients 90 minutes and 50 minutes, respectively. ‡ Mosquitoes with recipient 30 minutes.

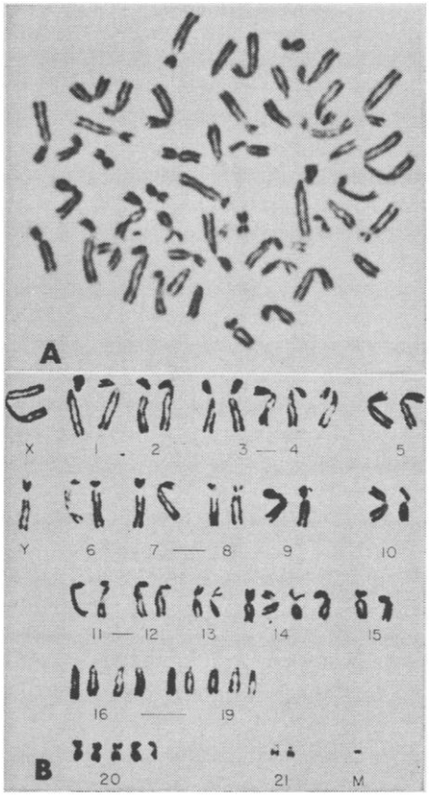

Fig. 1. Metaphase plate (*A*) and karyotype (*B*) of typical cells from tumor arising in a hamster bitten by mosquitoes fed previously on a TM-bearing hamster. The karyotype is identical to that previously described for TM (*2*). The minute marker chromosome (*M*) is shown, X-chromosome, extra chromosome in group 3-4, extra chromosome in group 16-19, two extra chromosomes in group 14-15, and three extra chromosomes in group 20. Total chromosome number is 51. The numbers given in the karyotype correspond to the pairs of chromosomes of the normal Syrian hamster karyotype, which contains 44 chromosomes.

transferred to a cage containing a tumor-free recipient weanling hamster, similarly shaven, anesthetized, and strapped to a board. At least three, and usually five or six mosquitoes, were allowed to feed on the recipient. The time that the last mosquito was introduced into the recipients' cage and the time the mosquitoes were removed were recorded. Seven hamsters were used as donors and 50 as recipients. The results are shown in Table 1.

Within 23 days five of the 50 recipients developed tumors which resembled histologically those of the donors. The first tumor that we observed developed in a hamster that had been bitten by mosquitoes 13 days previously. Two tumors appeared as subcutaneous nodules with metastases, one over the sternum and one over the right abdomen. In three other hamsters, there were no skin or subcutaneous tumors but there was extensive visceral involvement which included mesenteric fat and lymph nodes, kidneys and retroperitoneal nodes, thymus, diaphragm, lungs, and liver. In one hamster, the tip of the sternum was infiltrated.

Chromosome studies were performed on one of the tumors arising from a mosquito bite (*2*, *3*). The karyotype of the cells of this tumor (Fig. 1) was identical to that previously described for TM by Cooper *et al.* (*2*). Of 19 cells examined, 17 contained 51 chromosomes, including a single X chromosome, three extra chromosomes in group 20 (Fig. 1), two extra chromosomes in group 14-15, and one extra chromosome in each of groups 3-4, and 16-19 (*4*). A characteristic minute marker chromosome was also present.

The tumor, TM, used in these experiments has been examined repeatedly for virus by means of tissue culture, passage of cell-free material in animals, and electron microscopy, but no virus has been found. Cooper *et al.* (*2*) have shown that TM has a very consistent and highly specific karyotype, differing from the normal pattern for hamster cells. This karyotype is maintained if the tumor is transplanted to other animals, is induced by feeding tumor tissue, or is passed by caging tumor-bearing and tumor-free animals together (cannibalism). The study of Cooper *et al.* thus indicates that transmission is the result of implantation of tumor cells. In the present study, since the same specific karyotype was maintained in the recipient, the transmission of the tumor by the mosquito is considered to be the result of transfer of viable cells by the mosquito from one animal to the other.

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Quinine-Resistant Plasmodium berghei in Mice

Abstract. *During induction of chloroquine resistance,* Plasmodium berghei *developed resistance to quinine administered in doses near the maximum amounts tolerated by mice. Resistant parasites did not form malarial pigment. Normal sensitivity to both quinine and chloroquine returned and pigment formation resumed during serial passage of the parasites through untreated mice.*

The chemotherapy of human malaria is being complicated by increasing evidence (*1*, *2*) of resistance by parasites to the main groups of synthetic suppressive drugs: 4-aminoquinolines, acridines, biguanides, and pyrimethamine. In contrast, unequivocal resistance to quinine has not been demonstrated in human malaria. Although variable amounts are required to cure *Plasmodium falciparum* malaria, quinine has proved in controlled studies (*2*) to be effective against several strains of *P. falciparum* that show resistance to one or more of the synthetic drugs. Hence quinine is regaining, at least for certain strains, the prominent position it filled before the

era of synthetic antimalarial drugs. But resistance to such a wide range of drugs focuses attention on the possibility of resistance to quinine. The older literature on the capacity of plasmodia to develop resistance to quinine contains a report of a twofold resistance acquired by *P. gallinaceum* in chicks (*3*). While our work was under way, mention was made, without elaboration or documentation, of high resistance to quinine by a chloroquine-resistant strain of *P. berghei* in mice (*4*). We now report development of strong resistance to quinine by two lines derived from the same parent strain of *P. berghei*, in both instances during induction of resistance to chloroquine.

Resistance to chloroquine was induced by serial passage of parasites in mice given partially suppressive doses of the drug; the parasites were passed weekly by injecting parasitized blood intraperitoneally, and the drug was administered daily by subcutaneous injection. The dosage of chloroquine was raised slowly from an initially suppressive 1.0 mg/kg per day, commensurately with the tolerance gradually acquired by the parasites.

Drug-sensitivity tests were conducted by infecting mice with parasitized cells (15×10^6) intraperitoneally, treating them subcutaneously (or orally, as indicated) twice daily for 4 days (on the 2nd through 5th days of infection), and examining them on the 6th day for the percentage of parasitized cells in Giemsa-stained blood smears. Drug doses are expressed in terms of the base. Groups of ten mice were used; the treated and parent lines were examined in parallel, and the controls were given sham doses. The treated line of parasites was kept continuously in treated mice except for one or two passages immediately before infecting test mice.

The first line of parasites developed approximately 30-fold resistance to chloroquine during 47 weeks. The line was maintained for 2 weeks longer in mice given chloroquine daily at 30 mg/kg, and its sensitivity to quinine was then determined. Quinine doses of 50 mg/kg daily were used for the parent strain, doses known to be near the minimal strongly suppressive amounts. Parasitemias in the control and treated mice of the parent strain were 30.9 and 5.5 percent, respectively. The resistant line was treated daily with quinine doses of 50 and 200 mg/kg; parasitemias in these mice were as follows: 10.0 percent in the controls, 8.6 percent in mice treated with 50 mg/kg daily, and 8.5 percent in mice treated with 200 mg/kg daily. In terms of dosage required for appreciable suppression, the chloroquine-resistant line thus appeared to be more than four times more resistant to quinine than the parent line.

Table 1. Effect of quinine against the parent strain and the second chloroquine-resistant line in mice.

Daily dose (mg/kg)	Parent strain		Chloroquine-resistant	
	Deaths, mice	Cells parasitized (mean %)	Deaths, mice	Cells parasitized (mean %)
	Subcutaneous treatment			
400			0/10	3.8
200			0/10	6.1
100	0/10	0.1	0/10	6.1
50	0/10	4.8	0/10	6.5
25	0/10	15.6		
12.5	0/10	24.4		
0	1/10	44.4	0/10	10.8
400			2/10	2.3
200			0/10	4.8
100	0/10	0.2	0/10	4.5
50	0/10	2.0	0/10	5.1
25	0/10	6.6		
12.5	0/10	6.7		
0	0/10	9.0	0/10	3.3
	Oral treatment			
800	6/10	0	7/10	0.8
400	1/9	0	0/10	0.1
200	0/10	0	0/10	2.0
100	0/10	0.1	0/10	3.2
50	0/10	2.4	0/10	5.5
25	0/10	4.3	0/10	5.8
0	0/10	7.4	0/10	3.8

Repetition of the test was needed for full acceptance of the results. The decision was made at this time to maintain the resistant line of parasites in untreated mice. After seven weekly passages in untreated mice, the line unexpectedly reverted to normal sensitivity to chloroquine. Consequently, work was started immediately to develop a second chloroquine-resistant line and continue the studies. Such reversion by chloroquine-resistant *P. berghei* in untreated mice has been subsequently reported verbally from several laboratories.

Approximately 57 weeks were required for the second line to acquire 30-fold resistance to chloroquine. Results of three sensitivity tests are summarized in Table 1. Administered subcutaneously, quinine strongly suppressed the parent strain in daily doses of 100 or 50 mg/kg but had less effect against the resistant line in doses of 400 mg/kg. Administered orally, daily doses of 100 mg/kg greatly suppressed the parent strain, but 400 to 800 mg/kg were required for comparable suppression of the resistant line.

The stability of acquired resistance to both chloroquine and quinine was then determined in two experiments with the second resistant line. In both instances, sublines from the resistant lines were maintained by serial passage through untreated mice weekly for 7 weeks, while the resistant parasites were kept in mice receiving chloroquine (30 mg/kg per day). Sensitivity tests were conducted, at various dosages (subcutaneous), on the parent, resistant, and "chloroquine-removed" lines. Both experiments showed that the line removed from treatment with chloroquine completely reverted to normal sensitivity to chloroquine and quinine, but that the treated line remained resistant to both drugs.

The loss of malarial pigment in chloroquine-resistant parasites reported by Peters (*4*) also was confirmed in the second line; moreover, pigment formation was resumed upon loss of chloroquine resistance.

The treated lines produced high parasitemias less rapidly than normal lines, both while resistant and after loss of resistance.

Thus parasites treated with chloroquine developed at least four- to eightfold resistance to quinine; such resistance approaches the maximum obtainable, inasmuch as the largest doses of quinine were not fully effective even though they equaled or approached the toxic dose. Resistance was lost fairly rapidly when the parasites were passed serially through untreated mice.

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