A SIMPLIFIED *IN VIVO* DRUG SENSITIVITY TEST FOR MALARIA IN THE FIELD

Myint Lwin[1], Soe Aung[2], Myat Phone Kyaw[1], Htin Aung[2], Nyan Sint[2], Ye Win[2], Hla Oo[2], Hla Thein[2] and Soe Myat Tun[2]

[1]Department of Medical Research, No. 5 Ziwaka Road, Yangon, Myanmar; [2]Department of Health, Ministry of Health, Yangon, Myanmar

Abstract. The study was intended to develop a simple and reliable *in vivo* field test for monitoring of sensitivity of *P. falciparum* to antimalarials. The test is to be used as a built in sustainable monitoring system and applied at regular frequencies to provide guidance in developing a country-wide antimalarial drug policy. The study was conducted as a hospital based study in Mon State in Mudon, Kamawet and Pa-auk hospitals. The criteria matched malaria patients were treated with standard dosages of chloroquine, sulfadoxine-pyrimethamine and mefloquine and blood films were taken on days 0, 2, 3, 4, 7, 14 and 28. The assessment of the *in vivo* drug response of *P. falciparum* on days 2, 3 and 4 were compared with WHO standard 28 days and 7 day tests.

The following successful tests were carried out for 7 days with different antimalarials: 171 tests with chloroquine and sulfadoxine-pyrimethamine and 167 tests with mefloquine. Tests were also carried out for 28 days: 59 tests with chloroquine, 77 tests with sulfadoxine-pyrimethamine and 78 tests with mefloquine. The results found that 3 day tests, taking blood films on days 0 and 3, can be reliably used as an adjunct to 28-day tests. Since the test is simple and can be used extensively and sustainably throughout the country and the results are applicable to be used for epidemiological purposes, the method is suggested for use as a built-in monitoring method for the malaria control program.

INTRODUCTION

In Myanmar, *Plasmodium falciparum* resistant to antimalarials has been observed since 1969 with gradually increasing degree of resistance level. Chloroquine resistance has been widely distributed to almost all the states and divisions. An *in vitro* study conducted in eastern, southern and central areas of Myanmar in 1990 showed that *P. falciparum* isolates were 100 % sensitive to mefloquine, 92 to 100 % sensitive to quinine and 65 to 86 % sensitive to sulfadoxine-pyrimethamine (SP). (Myint Lwin, 1991). However hospital based 28-day *in vivo* studies in Tharyarwady and Mingaladon showed *P. falciparum* was 72 % to 100 % resistant to 4 amino-quinolines (chloroquine and amodiaquine), 16 % resistant to quinine, 63 to 65 % resistant to sulfadoxine-pyrimethamine, 7 to 14 % resistant to mefloquine and 15 % resistant to mefloquine sulfadoxine-pyrimethamine combination. Malaria control people are using a 7-day *in vivo* test. Because of the increasing problem of multi-resistant falciparum malaria, it is essential to monitor the increase in degree and spread of the resistant isolates in Myanmar.

WHO has divised an extended 28-day *in vivo* field test, which is not suitable to conduct in the field in malaria endemic areas. Also 7-day *in vivo* test is not simplified enough to be performed by the malaria control teams and basic health workers of the rural health centers. So it is aimed to produce an easy, rapid, feasible and sustainable method so as to use it as a built in monitoring method for the malaria control program.

MATERIALS AND METHODS

Study design

The study is a descriptive study and is conducted as a hospital based study. *P. falciparum* infected patients from the three hospitals, Mudon township hospital, Pa-auk and Kamawet station hospitals, were selected according to the WHO drug testing selection criteria.

Selection of patients

(a) Inclusion criteria: The patients of both sexes

above 5 years admitted to the hospitals were screened on the admission day. The *P. falciparum* infected patients who had not taken antimalarials during the last week were selected. The persons who would stay in the hospital or in the town proper for 28 days were included for the 28-day test and 7 days for the 7-day test.

(b) Exclusion criteria: The *P. vivax* or mixed infections and those with concomittant illness were excluded from the study. The pregnant women, severe and complicated malaria patients or the patients who do not give the consent were also excluded from the study.

(c) Deletion criteria: Those who underwent severe and complicated malaria and those who did not wish to participate anymore in the study and those who were judged as no longer feasible for the study.

Blood film examination

Thick and thin blood films were collected on the same slide from the finger tip of each patient. The slides were stained in 10% Giemsa for 30 minutes and examined under the microscope at 1,000 × magnification. Parasite counting was done against 300 WBC and the result was expressed as number of parasites per cubic milimeter of blood.

Urine test for antimalarials

Dill and Glazko urine test for 4-aminoquinolines and Lignin test for sulfanamides were carried out according to WHO (1966). Urine of the patient was checked for the presence of antimalarials on day 0 before giving any antimalarials for assurance that the subject had not taken any antimalarial prior to the treatment. Urine was again checked for the second time on day 1 or 2 for assurance of absorption of the drug.

Antimalarials

Chloroquine, sulfadoxine-pyremethamine (SP), and mefloquine were used for *in vivo* drug sensitivity testing. The antimalarials were supplied by the Vector Borne Diseases Control Program of WHO. The drug dosages were according to the WHO recommendations (WHO, 1985).

Chloroquine dosage

Chloroquine is given as a single dose orally on each of the three successive days (a total of 1.5g of base for a 60kg adult) according to the following schedule:

Day 0:- 10mg/kg (600 mg of base for a 60 kg adult)
Day 1:- 10mg/kg (600 mg of base for a 60 kg adult)
Day 2:- 5 mg/kg (300 mg of base for a 60 kg adult)

Sulfadoxine-pyrimethamine dosage

Adult dosage = 3 tablets single dose orally and children were given age related dosage (1 tablet = sulfadoxine 500mg + pyrimethamine 25mg)

Mefloquine dosage

Adult dosage = 3 tablets single dose orally and age related dose for children. (1 tablet = 250 mg base)

In vivo drug testing

WHO 28-day *in vivo* extended filed test was used for the study. Blood films were collected on days 0, 2, 3, 4, 7, 14 and 28 and parasite counts were done accordingly. The patients were admitted to the hospital for at least 7 days and follow up were done on days 14 and 28. The assessment were made by comparing the *in vivo* sensitivity response of the parasite on days 2 or 3 with the response of the 7-day and 28-day tests.

RESULTS

Malaria situation of the area in 1992

Mudon Township Hospital had 50,548 out patients and 2,807 (5.5%) clinically suspected malaria (CSM) cases with 5.6% malaria positivity rate. There were 1,212 malaria inpatients with a slide positivity rate of 29.6% and malaria mortality rate of 6.1%. Pa-auk Station Hospital had 8,343 out patients with 629 clinically suspected malaria cases and a slide positivity rate of 7.5%. There were 342 malaria inpatients with a mortality rate of 1.46%. Kamawet Station Hospital admitted 135 malaria inpatients with a slide positivity rate of 18.5% and malaria mortality rate of 3.7%.

Drug testing

Both the 28-day and 7-day tests were used for

the study using chloroquine, SP and mefloquine antimalarials. The 28-day test was done in Mudon hospital during the low malaria transmission season, the blood films were also collected on the same patients on days 2, 3 and 7 for comparison. Also 7-day tests were carried out at Mudon, Pa-auk and Kamawet hospitals, where blood films were also monitored on days 2 and 3 to be compared with the result of the day 7-test.

Statistical analysis

Spearman Rank Sum Correlation test was done to find out the relationship of the different day tests. The ranks were 0 for S, 1 for RI, 2 for RII and 3 for RIII.

***In vivo* tests with chloroquine**

28-day test: Two seperate 28-day tests consisting of 59 patients were carried out in Mudon hospital

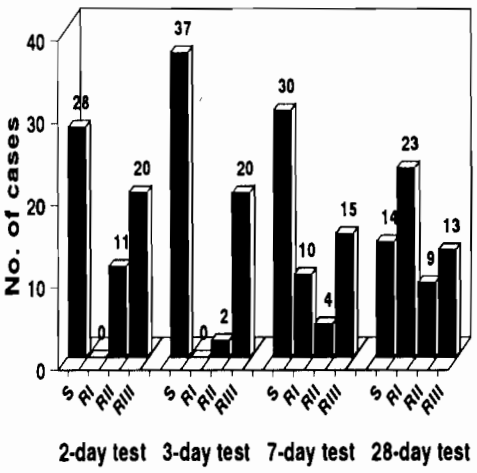
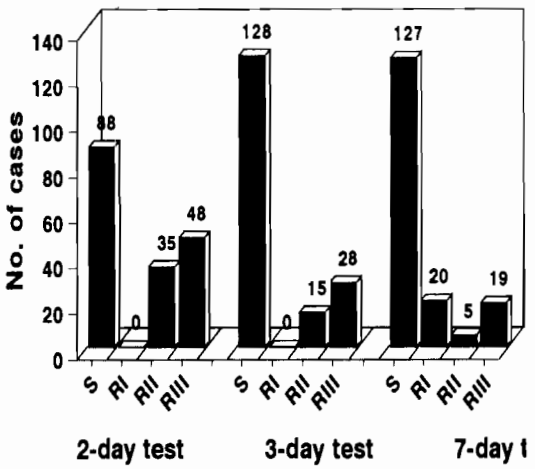


Fig (1)



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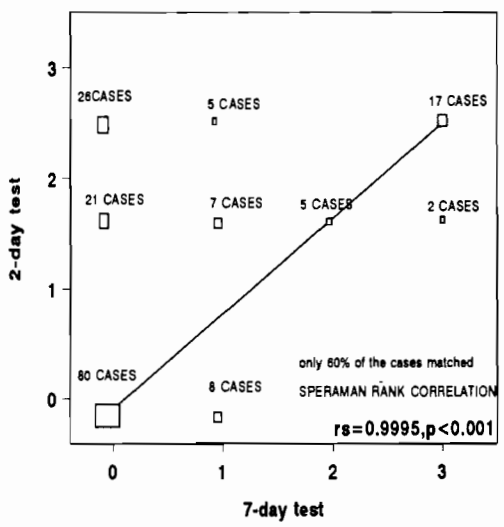

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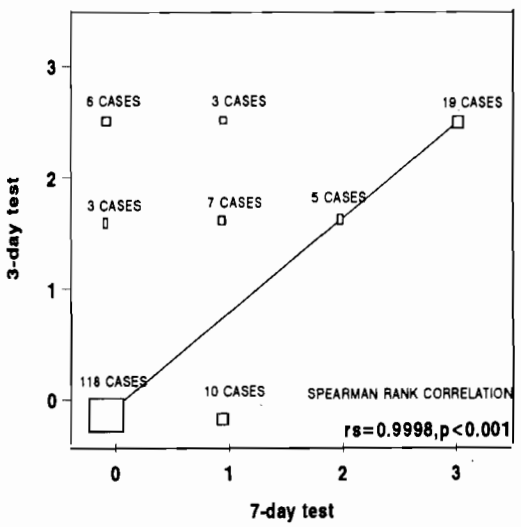
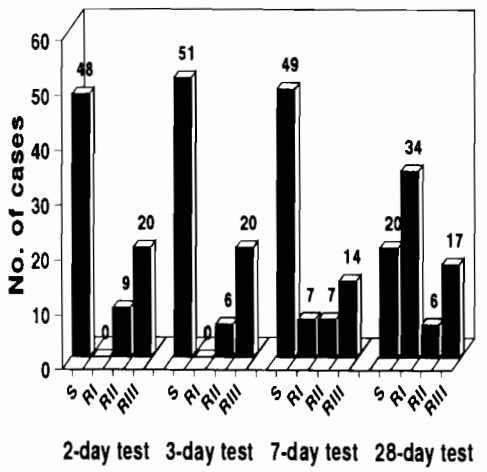


Fig (4)

Fig 1-4–Comparison of chloroquine sensitivity to *P. falciparum* using 2, 3, 7 and 28-day tests.

(Fig 1). The study showed that there were 14 sensitive (S), 23 RI (total SRI 37) 9 RII and 13 RIII resistant cases. The results interpreted on day 7 (7-day test) showed 30 S, 10RI, (*ie* 40 SRI) 4RII and 15RIII. However, the results on day 3 (3-day test) gave 37S, 2RII, 20 RIII and on day 2 (2-day test) revealed 28S, 11RII and 20RIII.

7-day test: The study consited of 6 individual studies with 171 observations carried out in Mudon Township Hospital, Pa-auk and Kamawet Station Hospitals. The results denoted that using the 7-day test there were 127S, 20RI, 5RII and 19RIII. However 3-day tests on the same observation showed 128S, 15RII, 28RIII and 2-day tests gave 88S, 35 RII and 48RIII (Fig 2). The relationship of the 3-day or 2-day versus 7-day tests are also shown in Figs 3-4. Day 3 tests were found to be highly correlated with 7-day tests.



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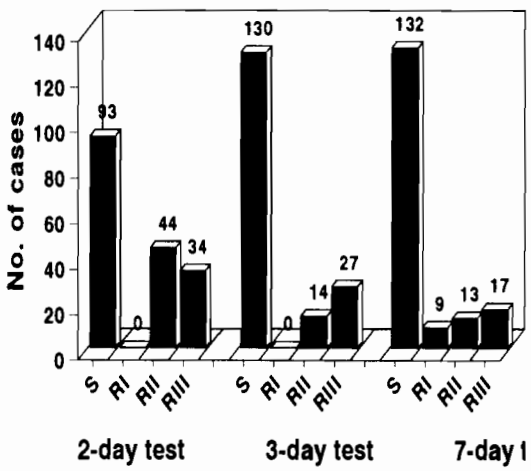

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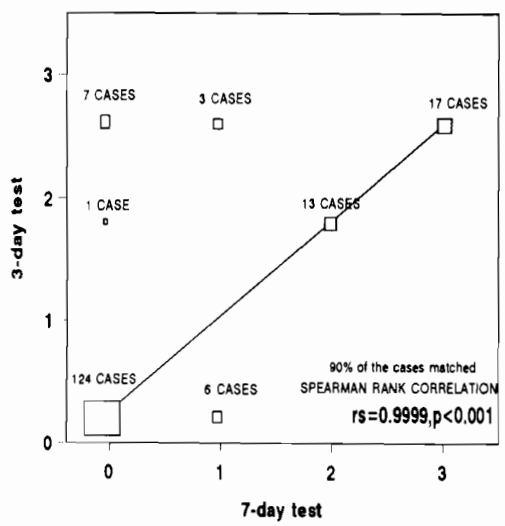

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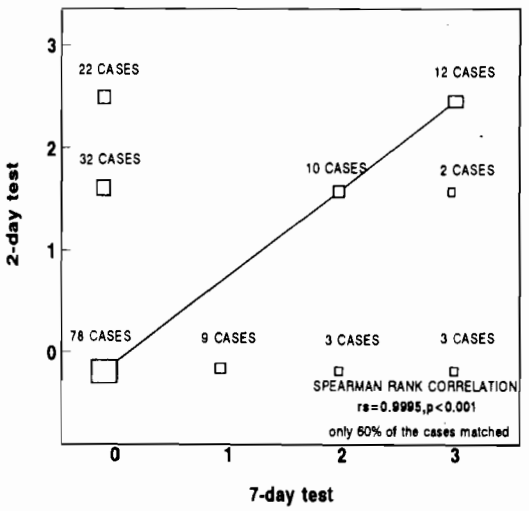


Fig (8)

Fig 5-8–Comparison of sulfadoxine/pyrimethamine sensitivity to *P. falciparum* using 2, 3, 7 and 28-day tests.

In vivo tests with SP

28-day test: Two different studies were carried out in Mudon Hospital on 77 inpatients and the result showed 20S, 34RI (SRI, 54), 6RII, 17RIII and 7-day tests interpreted on the same observations gave 49S, 7RI (SRI, 56), 7RII and 14RIII. However 3-day tests on the same observations showed 51S, 6RII, 20RIII and 2-day tests gave 48S, 9RII and 20RIII (Fig 5).

7-day test: The studies were carried out in the above hospitals on seven occasions on 171 subjects. The 7-day tests showed 132S, 9RI, 13RII and 17 RIII. Two-day tests showed 93S, 44RII and 34RIII and the 3-day tests showed 130S, 14RII and 27RIII (Fig 6). The correlation between the 2 day or 3 day with that of the 7-day tests are also shown in Fig 7-8. The results of 3-day and 7-day tests were highly correlated.

In vivo tests with mefloquine

28-day test: Two seperate 28-day tests were carried out in Mudon Hospital on 78 patients and showed 66S, 12RI (SRI 78) and 7-day tests interpreted on the same observations gave 78S. Interpretation of the three day tests on the same study showed 76S and 2RII and 2-day tests gave 27S and 6RII (Fig 9).

7-day test: The study was carried out in the same hospitals on 168 patients. Each study consisted of 45 to 60 patients. There were 161S, 4RI, 1RII and 1RIII in day-7 tests and for day-3 tests 161S, 5RII and 1RIII. The day-2 tests showed 126S 40RII and 1RIII (Fig 10). The correlation between the 2-day and 7-day tests and 3-day and 7-day tests are shown in Figs 11-12. The 3 day test and 7-day tests were highly correlated.

DISCUSSION

In 1967 the WHO Scientific Group on Chemotherapy of Malaria proposed an arbitrary grading system based on the response to the normally recommended dosage of chloroquine. Three tests are available at present for the *in vivo* sensitivity of chloroquine: The standard field test consists of an observation period of 7 days after giving standard chloroquine treatment. The same test with the observation period extended over a total of 28 days has been used as an extended test. The alternative test with a single dose of chloroquine (10 mg/kg base) has also been used in high endemic areas (WHO, 1986). The above *in vivo* tests designed for the detection of chloroquine resistance to *P. falciparum* have been adapted to other antimalarials with some assumptions. The criteria used in the WHO extended 28-day tests which was originally developed for chloroquine were not applicable to be used for the slow acting schizonticide, Quinine (Myint-Lwin *et al*, 1985). Quinine has a prolonged parasite clearance time of up to 8-15 days in 15 out of 39 patients (Marlar Than *et al*, 1992), which will not be able to show some sensitive cases according to the 28-day test. Also the test has to be modified to a 42-day test for mefloquine sensitivity testing because of its long half-life (Harinasuta *et al*, 1983). Some scientists used a 14-day test for drug sensitivity testing (Karbwang *et al*, 1991). In fact there is no fixed rule for which test should be used. The *in vitro* tests are also available but need special equipment and expertise and the results are not correlated with the *in vivo* data.

The study was intended to introduce a simplified field test which will be practically feasible to be applied in many areas of the country. Since the majority of antimalarials have parasite clearance times of more than 48 hours, the day-3 test was chosen. The parasite clearance times were 50 ± 18 hours and 43 ± 20 hours when treated with mefloquine 100mg single dose or split doses on 43 subjects (Ye-Thwe *et al*, 1991). Even with the most potent antimalarial, oral artesunate, the parasite clearance time is up to 52 hours (Karbwang *et al*, 1994).

The results of the different tests pointed out that, there were some differences in sensitivity pattern in between any two tests. For chloroquine tests, there were 14S for the 28-day test and 30S for the 7-day test. The 3-day test cannot detect RI cases as do the 7-day and 28-day tests. So when interpreting the 3- day test one should think of possible RI cases in S. The 3-day test results are similar to the 7-day test for all the drugs tested. Chloroquine gave 128S with the 3-day test and 127 S with the 7-day test (Fig 2). Also SP gave 130S and 132S with 3-day and 7-day tests, respectively (Fig 6). The study showed 3-day test results were strongly correlated with 7-day test results in all three drugs tested, chloroquine being the highest, sulfadoxine-pyrimethamine being the medium and mefloquine be-

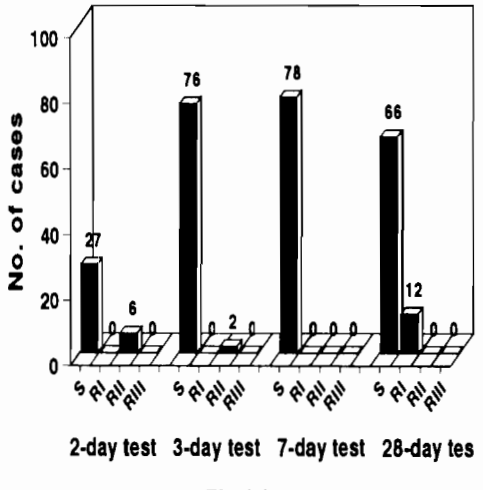
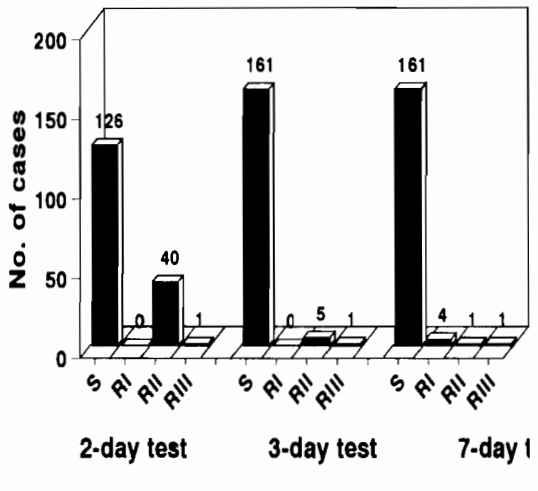


Fig (9)



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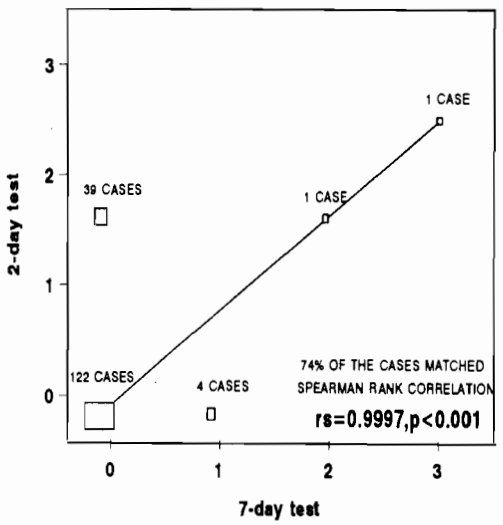

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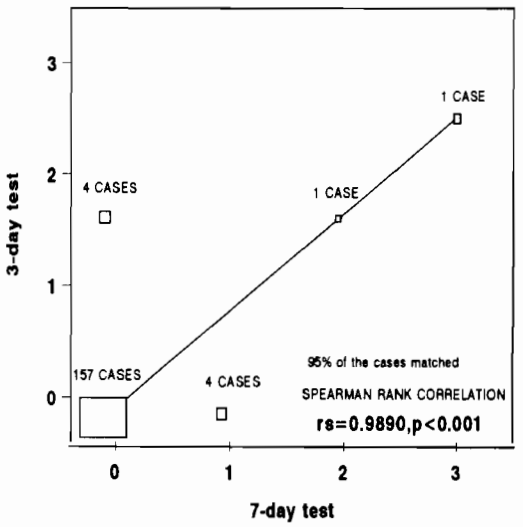

Fig (12)

Fig 9-12–Comparison of mefloquine sensitivity to *P. falciparum* using 2, 3, 7 and 28-day tests.

ing the least resistant drug for *P. falciparum*.

Though the 7 day test was originally meant for the field usage, it is not simple to perform in areas where transportation is difficult as in the Rural Health Centers and in the sub-centers. The VBDC in Myanmar is adopting the 7-day test for their program. For the 7-day test, a special team have to go to the area of study for follow up of the patient. For emergency situation the test can be performed without the microscope. When the staff treating the malaria suspected patient he has to take the blood films on day 0 (before treatment) and on day 3 and treatment noted. The slides are to be sent routinely. At the center the microscopist will screened the day 0 slide, select if *P. falciparum* positive and will proceed to day 3 slide for the interpretation of the result. Hence, the 3-day test is simple enough to perform in any area of the country and may be able to carry out in large number

without much expenses and involvement of many health staff. For the epidemiological purpose the 3-day test can be used to monitor the spread and increase in resistance of *P. falciparum* to anti-malarials in different geographical areas of Myanmar. The test can be used as a built in system in the malaria control division of the country, VBDC. The test may also be carried out in hospitals for check up of the patient response for the management of malaria cases.

ACKNOWLEDGEMENTS

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