- Kinsell, L. W., Michaels, G. D., Li, C. H., and Larsen, W. E. (1948). *J. clin. Endocrinol.*, 8, 1013.
- Li, C. H., Evans, H. M., and Simpson, M. E. (1945). *J. biol. Chem.*, 159, 353.
- (1948). *Science*, 108, 624.
- Lister, J., Nash, J., and Ledingham, U. (1951). *British Medical Journal*, 1, 376.
- Long, D. A., Miles, A. A., and Perry, W. L. M. (1951). *Lancet*, 1, 1085, 1392; 2, 902.
- Long, J. B., and Favour, C. B. (1950). *Bull. Johns Hopk. Hosp.*, 87, 186.
- Lynch, J. (1950). *J. Amer. med. Ass.*, 144, 921.
- McArthur, J. W., Sprague, R. G., and Mason, H. L. (1950). *J. clin. Endocrinol.*, 10, 307.
- Marks, H. P., and Young, F. G. (1940). *Lancet*, 1, 493; 2, 710.
- Mering, J. V., and Minkowski, O. (1890). *Arch. exp. Path. Pharmacol.*, 26, 371.
- Miller, H. C., Hurwitz, O., and Kuder, K. (1944). *J. Amer. med. Ass.*, 124, 271.
- Mirsky, I. A. (1939). *Endocrinology*, 25, 52.
- Moon, H. D., Simpson, M. E., Li, C. H., and Evans, H. M. (1950). *Cancer Res.*, 10, 364.
- Natrass, F. J., and Latner, A. L. (1951). *British Medical Journal*, 2, 790.
- Priestley, J. T., Sprague, R. G., Walters, W., and Salassa, R. M. (1951). *Ann. Surg.*, 134, 464.
- Reeder, W. H., and Mackey, G. S. (1950). *Dis. Chest, Chicago*, 18, 528.
- Robertson, J. D., and Kirkpatrick, H. F. W. (1951). *Lancet*, 2, 54.
- Robinson, A. M., and Norton, J. M. (1951). *J. Endocrinol.*, 7, 321.
- Selye, H. (1946). *J. clin. Endocrinol.*, 6, 117.
- Sevitt, S. (1951). *British Medical Journal*, 2, 791.
- Simpson, S. L. (1948). *Major Endocrine Disorders*, 2nd ed. Oxford Univ. Press.
- (1951a). *Proc. roy. Soc. Med.*, 44, 453.
- (1951b). *Bull. N.Y. Acad. Med.*, 27, 723.
- Dennison, M., and Korenchevsky, V. (1934). *J. Path. Bact.*, 39, 569.
- Sprague, R. G., Priestley, J. T., and Dockerty, M. B. (1943). *J. clin. Endocrinol.*, 3, 28.
- Summers, V. K., and Sheehan, H. L. (1951). *British Medical Journal*, 2, 564.
- Suzman, M. M., and Rudolph, J. A. (1951). *Lancet*, 1, 660.
- Szego, C. M., and White, A. (1949). *Endocrinology*, 44, 150.
- Thorn, G. W., Forsham, P. H., Frawley, T. F., Wilson, D. L., Renold, A. E., Fredrickson, D. S., and Jenkins, D. (1951). *Amer. J. Med.*, 10, 595.
- Van Cauwenberge, H. (1951). *Lancet*, 2, 374.
- Wilder, R. M. (1950). *J. Amer. med. Ass.*, 144, 1234.
- Young, F. G. (1941). *British Medical Journal*, 2, 897.
- (1951). *Ibid.*, 2, 1167.

DARAPRIM IN TREATMENT OF MALARIA

A STUDY OF ITS EFFECTS IN FALCIPARUM AND QUARTAN INFECTIONS IN WEST AFRICA

BY

IAN A. MCGREGOR, L.R.C.S., L.R.F.P.S., D.T.M.&H.
Member of Scientific Staff, Human Nutrition Research Unit,
Medical Research Council

AND

DEAN A. SMITH, M.D., D.T.M.&H.

Lecturer, Department of Human Nutrition, London School
of Hygiene and Tropical Medicine

(From the Medical Research Council Field Research
Station, Fajara, Gambia)

During 1949–50 a large series of substituted 2 : 4-diaminopyrimidines was synthesized by Falco *et al.* (1951) and tested against laboratory infections of *Plasmodium gallinaceum* in chicks, of *P. berghei* in mice, and, in certain cases, of *P. cynomolgi* in monkeys. The most powerful schizonticide of the series proved to be 2 : 4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (compound number 50–63), now known as "daraprim." Clinical trials in cases of quiescent human malaria were conducted in Lagos by Archibald (1951) on children aged 5 to 10 years; promising results were obtained and no toxic effects were observed. A supply of daraprim having been made available in August, 1951, a clinical trial on subjects having overt attacks of malaria was undertaken at the Medical Research Council's Field Research Station, Fajara, Gambia, between August and November, 1951.

Subjects and Methods

Subjects were selected who showed high levels of parasitaemia and fever and other manifestations of clinical malaria. They were divided into four trial groups consisting of 18

infants (group I), 7 older children (group II), and 4 adults (group III) infected with *P. falciparum*, and 3 small children (group IV) infected with *P. malariae*. Unlike the children investigated by Archibald (1951), these subjects showed considerable variation in the degree of acquired malarial immunity which they possessed. The infants in group I had little or none; the adults in group III all exhibited substantial immunity; the resistance of the children in groups II and IV varied between these two extremes. The characteristics of the groups are summarized in Table I.

TABLE I.—Details of the Four Groups of Subjects Treated with Daraprim and of Their Parasitaemia and Pyrexia

	Group I	Group II	Group III	Group IV
No. { Male	9	5	3	1
Female	9	2	1	2
Total	18	7	4	3
Age { Mean	14 months	3.75 years	36 years	3.25 years
Range	4–24 "	2.5–5 "	29–45 "	1.5–4.5 "
Weight { Mean	8.0 kg.	13.1 kg.	55 kg.	10 kg.
Range	5–12 "	11–15 "	45–60 "	5–13 "
Infecting parasite	<i>P. falcip.</i>	<i>P. falcip.</i>	<i>P. falcip.</i>	<i>P. malariae</i>
Mean parasite density before treatment:				
Proportion of red cells infected	4.7%*			
Parasites per field of thick film		49	13	1
Pyrexia before treatment (Temperature (°F.):)				
Mean	102.3	103.1	All reported	100
Range	100–106	101–104.5	fever	Irregular
No. in whom spleen was:				
Not palpable	6	2	4	0
Just palpable	4	2	0	0
Palpable up to 3.5 cm. below costal margin	7	2	0	1
Palpable 5 cm. or more below costal margin	1	1	0	2
No. in whom liver edge was:				
Not palpable	5	3	4	0
Palpable up to 3.5 cm. below costal margin	7	3	0	2
Palpable more than 3.5 cm. below costal margin	6	1	0	1

* 5 cases in this group had peripheral schizogony.

together with their average levels of parasitaemia and pyrexia before treatment. Among the children a number had other diseases which almost certainly contributed to their fever, so that changes in temperature cannot be wholly attributed to malaria (cf. notes to Table II). Thus five had enteritis, four had pulmonary infections, and one each had trypanosomiasis, exfoliative dermatitis, septic gingivitis, cancrum oris, and whooping-cough. The presence of these complications makes the results of daraprim treatment the more striking.

Parasitaemia was investigated by the use of both thick and thin films. Thick films were prepared by the standard method of Field (1948) and stained by Field's rapid method. Thin films were stained with Giemsa. In estimating density of infection two methods were used. In very heavily infected subjects thin films were examined and the percentage of parasitized red cells was ascertained by counting at least 1,000 cells. In less severe infections the average number of parasites per field of thick film was counted, using an oil-immersion 1/12 objective and ×8 eye-pieces in a binocular microscope. Two hundred fields of thick film were always examined. Blood films were taken at least six-hourly for the first two days after treatment and thereafter twice daily.

The drug was administered by mouth, under strict supervision by one of us, in a single dose calculated in relation to body weight. In the early cases 10-mg. tablets of daraprim were ground up with pulv. tragacanth. in a measured volume of glycerin and water to make a uniform suspension, and a volume proportional to the calculated dose was given; later 5% lactic acid was substituted for water, as the drug is said to form a stable solution in weak acids; eventually a syrup prepared by Messrs. Burroughs, Wellcome and Co. containing 5 mg. of daraprim per ml. was used exclusively.

The drug is quite tasteless, and little or no difficulty was experienced in getting even very ill babies to swallow their dose.

Results

Eight babies in group I received the drug at a dosage level of 0.25 mg. per kg. of body weight, as did all the adults. The remaining cases received 0.5 mg. per kg. The results are summarized in Table II. It will be seen that in

TABLE II.—Results of Treatment with Daraprim in the Four Groups of Subjects

	Group I		Group II		Group III	Group IV
Parasite	<i>P. falcip.</i>		<i>P. falcip.</i>		<i>P. falcip.</i>	<i>P. malariae</i>
No. of subjects . . .	18		7		4	3
No. given 0.25 mg. per kg.	8		1		4	3
No. given 0.5 mg. per kg.	10		6			
No. in whom asexual parasites were still present after:						
24 hours	8	10	1	6	4	3
48 "	7	8	1	3	1	3
72 "	1	1	0	0	0	3
96 "	1	1	0	0	0	0
More than 5 days . .	1*	1†	0	0	0	0
No. in whom temperature was still raised after:						
24 hours	6	9	1	5		3
48 "	2	5	0	1		1
72 "	1	1	0	1	0	1
96 "	1	1	0	0		1
More than 5 days . .	0	1‡	0	0		1§

* Case 11. See discussion. † Case 10. See discussion. ‡ Case 5. Pulmonary infection and enteritis. § Case 31. Septic gingivitis.

all *P. falciparum* infections except two asexual parasites had disappeared from the peripheral blood within 72 hours of treatment and that in most cases the temperature had reached normal by that time. The rate of disappearance of parasites from the blood seemed to be unrelated to previously acquired immunity. In two instances parasites did not disappear from the peripheral blood. In the first (Case 11), a child of 13 months who received 0.25 mg. per kg., the parasitaemia was reduced to a level of 1 parasite in 200 fields on the fifth day; thereafter parasite density increased to a level of 8 per field in 10 days. A second dose of the drug was given 15 days after the first at the rate of 0.5 mg. per kg.; parasites disappeared from the blood within 72 hours and did not return. In the second case of failure (Case 10), a child of 10 months, there had been a seemingly satisfactory response to 0.25 mg. per kg. when the child was first treated, but she was brought back 14 days after discharge with a heavy parasitaemia, which did not respond to dosages of 0.25 mg. per kg. given on two occasions and of 0.5 mg. per kg., given once at approximately weekly intervals.

In five of the *P. falciparum* cases, in which treatment had been given very shortly after the first appearance of parasites, no gametocytes were ever seen in the peripheral blood. In all the remaining cases gametocytes appeared in variable numbers, usually reaching a peak on the fifth day after treatment and persisting on an average 12 days. The longest period for which gametocytes were observed to persist was 26 days. The gametocytes appeared normal, and in two cases apparently normal exflagellation was observed. We were not able to test the fertility of gametocytes after treatment by observing their development in mosquitoes.

In the three quartan cases asexual parasites persisted in diminishing numbers beyond 72 hours, but were always absent 96 hours after treatment. Gametocytes were seen in all three cases, and persisted for six, eight, and twelve days respectively.

Relapses.—In this hyperendemic area, at a season when transmission is high, the chances of reinfection within a short time after discharge from hospital are considerable. Six patients returned with parasites in the blood after intervals of 37 to 60 days. The nature of these parasitaemias was more suggestive of reinfection than of relapse, but we have no evidence on which the occurrence of relapse can be excluded or established.

Effects of Daraprim on the Morphology and Development of Parasites

In 18 of the 29 cases of *P. falciparum* malaria no change was discernible in the morphology and development of ring-form trophozoites before they disappeared from the blood. However, from the moment of administration, invasion of red cells by merozoites ceased abruptly. From the 24th to the 48th hour after treatment trophozoites in the blood of 10 subjects showed abnormalities. There was retardation or cessation of growth, the chromatin became pyknotic and stained deeply, and the cytoplasm became pale, attenuated, and extremely fragmented. Similar changes were seen in one case within the first 24 hours.

In the five cases with late-stage solid trophozoites and schizonts in the peripheral blood, treatment caused rapid disappearance of mature schizonts. A small number of immature segmenting forms were seen in which chromatin division was most irregular—the daughter fragments varying greatly in size and staining properties—and in which the cytoplasm stained faintly and showed vacuolation.

The main factor governing the diminution in parasitaemia after treatment seemed to be the age of the trophozoites at the time the drug was given. Except in the one case, in which there appeared to be a direct and immediate effect on the trophozoites, reduction of parasitaemia did not occur until the time at which the parasites would normally have been expected to withdraw from the peripheral circulation to undergo schizogony in the internal organs. Thereafter no asexual parasites were seen in the blood.

If at the time of drug administration the bulk of the trophozoites were well grown, a pronounced fall in density was observed during the 24 hours after treatment—the precise time coinciding with the estimated time for normal withdrawal—but when the treatment was given shortly after schizogony, most of the trophozoites being very young, no noticeable decline in parasitaemia was seen until after the 24th hour.

All three cases of quartan malaria showed asynchronous infection, with parasites at all stages of development. After administration of daraprim, mature schizonts disappeared rapidly from the blood and no further infection of red blood cells with merozoites took place. Parasites reaching the stage of schizogony were the first to show abnormal morphological changes. In some instances the chromatin failed to divide. It persisted in a single mass and became pale-staining and diffuse. Chromatin division, when it occurred, was irregular and gave rise to daughter fragments of varying size around which the cytoplasm did not condense. The cytoplasm stained poorly and often showed vacuolation. Occasionally extrusion of some pigment from the cytoplasm was noted. Growing ring trophozoites were normal in appearance until the stage of schizogony was reached, when the changes described above became apparent, but their persistence in the blood for some hours beyond the normal 72 hours of the quartan cycle suggests a growth-retarding effect of the drug.

Toxicity of Daraprim

In two cases vomiting occurred after administration of the drug. An infant suffering from whooping-cough vomited immediately after its dose; a second dose was given and retained. A child, who on the previous day had undergone a liver-function test and vomited severely after ingestion of sodium benzoate, vomited 40 minutes after the administration of daraprim. The dose was not repeated. As already noted, a number of children had enteritis, but in no case could this be attributed to daraprim. No skin rashes were seen and, although detailed urinary investigations were carried out, there was no evidence of toxic renal damage. Regular blood counts were performed, but no depression of haemopoiesis was found; indeed, all cases showed swift regeneration of blood with rapidly rising counts and haemoglobin levels, often with considerable reticulocytosis, after

treatment. In no case was there a significant lowering of the white-cell count. A rising eosinophilia, reaching a peak at an average of five days after treatment, was noted in seven cases: in three of these the proportion of eosinophils rose from 1% to 5%; in two cases it reached 13%; and in two it exceeded 25%. No toxic phenomena unequivocally attributable to daraprim were noted.

Discussion

Daraprim is clearly a powerful schizonticide. A single dose of 0.5 mg. per kg. of body weight causes the disappearance of asexual forms of *P. falciparum* and *P. malariae* from the blood in the great majority of cases, at a rate which is not dependent on the degree of immunity possessed by the host. While in a minority of cases there is some evidence of action upon ring trophozoites, the drug seems to exert its effect mainly at the stage of plasmodial development, when the chromatin is in active division. The evidence presented suggests that after a single dose the concentration of the drug in the blood remains high enough to be lethal to all the asexual forms, even if some do not reach the vulnerable phase of schizogony for 72 hours after treatment, as in the quartan cases.

In two cases asexual parasites persisted in the blood stream. It seems reasonable to attribute the first of these failures (Case 11) to underdosage, since a second treatment at a higher dosage (0.5 mg. per kg.) was rapidly effective. The cause of the second failure (Case 10) is more obscure. In this case the infant on admission had a severe generalized exfoliative dermatitis, and on examination the edge of a very hard liver was palpable a hand's breadth below the costal margin. A considerable degree of metabolic disorder, including impairment of liver function, may be inferred. Should the antimalarial activity of daraprim depend upon the disintegration of the drug into some metabolite, it may be that the impaired liver function interfered with this process and prevented the establishment of an adequate concentration of the active substance in the blood. However, the possibility that the failure was due to the presence of a naturally resistant strain or to the establishment of drug resistance by previous inadequate treatment—in this case at a dosage level of 0.25 mg. per kg.—cannot be excluded.

Our experience is that daraprim promises to prove a valuable antimalarial drug. Its action seems to parallel closely that of proguanil ("paludrine"), while the rate at which it eliminates parasitaemia seems to be intermediate between that of proguanil and chloroquine. Its small effective dosage and tasteless character may well make this the drug of choice in the treatment of malaria in infants and children. It certainly merits extended trials, both therapeutic and prophylactic, against the known strains of plasmodia infecting man.

Summary

Cases of clinical malaria with high parasitaemia and pyrexia, due in 29 cases to *P. falciparum*, and in three to *P. malariae*, were treated with daraprim in single oral doses of 0.25 or 0.5 mg. per kg. of body weight.

The drug has a powerful destructive action on dividing schizonts and a lesser and inconstant effect on trophozoites. The blood was free of asexual parasites within 72 hours in all but two cases of *P. falciparum* infection and within 96 hours in all cases of *P. malariae* infection. No toxic effects were observed.

The drug seems to act just as swiftly in non-immunes as in subjects who possess considerable immunity.

As daraprim is tasteless it is particularly suitable for the treatment of malaria in infants and children.

Daraprim well merits extended trial as a therapeutic drug effective in very small single doses, and its efficacy as a suppressive deserves investigation.

We gratefully acknowledge our indebtedness to Brigadier J. S. K. Boyd, Director of the Wellcome Laboratories of Tropical Medicine, for his help and advice throughout this investigation and for making available to us supplies of daraprim and early information about its properties; and to Professor B. S. Platt, Director of the Medical Research Council Field Research Station, Gambia, for making it possible for us to do this work and for permission to publish this paper.

REFERENCES

- Archibald, H. M. (1951). *British Medical Journal*, 2, 821.
Falco, E. A., Goodwin, L. G., Hitchings, G. H., Rollo, I. M., and Russell, P. B. (1951). *Brit. J. Pharmacol.*, 6, 185.
Field, J. W. (1948). *Stud. Inst. med. Res. Kuala Lumpur*, No. 23, p. 82.

DARAPRIM (B.W. 50-63)—A NEW ANTIMALARIAL

TRIALS IN HUMAN VOLUNTEERS

BY

L. G. GOODWIN, M.B., B.S., B.Sc., B.Pharm.

(From the Wellcome Laboratories of Tropical Medicine,
183-93, Euston Road, London, N.W.1)

2:4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine, "B.W. 50-63," or "daraprim," is a new antimalarial drug of high potency. Laboratory tests have shown it to be 60 times as active as proguanil against *Plasmodium gallinaceum* in chicks and 200 times as active against *P. berghei* in mice (Falco *et al.*, 1951). A single dose of 5 mg. was found by Archibald (1951) to clear *P. falciparum* schizonts from the blood of Lagos schoolchildren. Daraprim worked rather more slowly than mepacrine, but the 5-mg. dose was approximately equivalent to a dose of 300 mg. of mepacrine tested under the same conditions. Toxicity tests in the laboratory showed that the drug was less toxic than proguanil to mice; doses of 5 mg. per kg. of daraprim given twice daily to a rhesus monkey for five days every week for six weeks had no effect on the general health, appetite, or the blood picture. We estimated that if it could be assumed that human malaria parasites were as sensitive to daraprim as *P. cynomolgi*, an effective suppressive dose for a 70-kg. man would be of the order of 5 mg. once weekly. This is not, of course, a justifiable assumption, but we thought it reasonable to expect that a dose of 25 mg. once weekly would be effective as a suppressant in human malaria. The present paper is an account of a personal trial of the drug as a malaria suppressant, and of toxicity studies in a group of human volunteers.

A Personal Trial

From January to June, 1951, I was working in Africa, and therefore had the opportunity of making personal trials with daraprim. The aims of the trial were (a) to take the drug at a dose level higher than was necessary for the suppression of malaria and watch carefully for any toxic effects; and (b) to ensure that I came into contact with infected mosquitoes.

Dose of Drug.—A beginning was made by taking 50 mg. of daraprim twice weekly. This dosage was continued from December 5, 1950, until February 11, 1951, and no ill effects of any kind were observed. The blood picture and the urine were normal throughout. As I was spending a good deal of time in the bush and it needed an effort to remember which days to take the drug, I changed to a higher total dose of 25 mg. daily, which was continued from February 12 until April 4. Again no ill effects were observed. On February 5 I had diarrhoea after an expedition to Kankara, in Katsina Province; this responded rapidly to treatment with sulphaguanidine. I had two boils, which appeared on