Gmail

Christine Massey <cmssyc@gmail.com>

Your CDC FOIA Request #23-00174-FOIA

MNHarper@cdc.gov <MNHarper@cdc.gov> Fri, Nov 4, 2022 at 9:37 AM
To: cmssyc@gmail.com

November 4, 2022

Request Number: 23-00174-FOIA

Dear Ms. Massey:

This is regarding your attached three Freedom of Information Act (FOIA) requests of October 31, 2022, for:

All studies and/or reports in the possession, custody or control of the people who work at/for the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and are responsible for the CDC's claim that parasites cause malaria, that describe controlled experiments using any purified parasite to prove causation of malaria.

All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of the alleged "HTLV-I" aka "Human T-Lymphotropic Virus Type I" or the alleged "HTLV-II" aka "Human T-Lymphotropic Virus Type II".

All studies and/or reports in the possession, custody or control of the people who work at/for the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and are responsible for the CDC's claim that bacteria can cause pneumonia, that describe controlled experiments using purified Mycoplasma pneumoniae, or any other type of purified bacteria, to prove causation of pneumonia..

Please see the attached letter.

Sincerely,
CDC/ATSDR FOIA Office
770-488-6399

4 attachments

Revised Acknowledgement- 23-00174 for three requests.pdf
167K

FOI to CDC re_ proof of any parasite causing malaria.msg
201K

FOIA request to CDC re_ scientific proof of _HTLV I or II__ or purification.msg
103K

FOI to CDC re_ proof of any bacteria causing pneumonia.msg
202K

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

November 4, 2022

Christine Massey
[redacted]
Via email: cmssyc@gmail.com

Dear Ms. Massey:

The Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) received your attached three Freedom of Information Act (FOIA) requests dated October 31, 2022. Your request assigned number is 23-00174-FOIA, and it has been placed in our complex processing queue.

In unusual circumstances, an agency can extend the twenty-working-day limit to respond to a FOIA request.

We will require more than thirty working days to respond to your request because we reasonably expect that two or more CDC centers, institutes, and offices (C/I/Os) may have responsive records.

To process your request promptly, please consider narrowing the scope of your request to limit the number of responsive records. If you have any questions or wish to discuss reformulation or an alternative time frame for the processing of your request, you may contact the analyst handling your request Mark Harper at 770-488-8154 or our FOIA Public Liaison, Roger Andoh at 770-488-6277. Additionally, you may contact the Office of Government Services (OGIS) to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services; National Archives and Records Administration; 8601 Adelphi Road-OGIS; College Park, Maryland 20740-6001; e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

Because you are considered an "Other requester" you are entitled to two hours of free search time, and up to 100 pages of duplication (or the cost equivalent of other media) without charge, and you will not be charged for review time. We may charge for search time beyond the first two hours and for duplication beyond the first 100 pages. (10 cents/page).

If you don't provide us with a date range for your request, the cut-off date for your request will be the date the search for responsive records starts.

You may check on the status of your case on our FOIA webpage https://foia.cdc.gov/app/Home.aspx and entering your assigned request number. If you have any questions regarding your request, please contact me at 770-488-8154 or via email at wzj6@cdc.gov.

We reasonably anticipate that you should receive documents by January 21, 2023. Please know that this date roughly estimates how long it will take the Agency to close requests ahead of your request in the queue and complete work on your request.

Gmail

Christine, of the Massey family <cmssyc@gmail.com>

Your CDC FOIA Request #23-00174-FOIA

MNHarper@cdc.gov <MNHarper@cdc.gov> Mon, Dec 5, 2022 at 10:51 AM
To: cmssyc@gmail.com

December 5, 2022

Request Number: 23-00174-FOIA

Dear Ms. Massey:

This is regarding your Freedom of Information Act (FOIA) request of October 31, 2022, for All studies and/or reports in the possession, custody or control of the people who work at/for the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and are responsible for the CDC's claim that parasites cause malaria, that describe controlled experiments using any purified parasite to prove causation of malaria. All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of the alleged "HTLV-I" aka "Human T-Lymphotropic Virus Type I" or the alleged "HTLV-II" aka "Human T-Lymphotropic Virus Type II". All studies and/or reports in the possession, custody or control of the people who work at/for the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and are responsible for the CDC's claim that bacteria can cause pneumonia, that describe controlled experiments using purified Mycoplasma pneumoniae, or any other type of purified bacteria, to prove causation of pneumonia.

Please see the attached letter.

Sincerely,
CDC/ATSDR FOIA Office
770-488-6399

7 attachments

- **23-00174 Final Response .pdf**
  131K
- **23-00174 Part 1 .pdf**
  6474K
- **23-00174 Part 2 (12 5 2022).pdf**
  4726K
- **FOIA request to CDC re_ scientific proof of _HTLV I or II__ or purification.msg**
  103K
- **FOI to CDC re_ proof of any parasite causing malaria.msg**
  201K
- **FOI to CDC re_ proof of any bacteria causing pneumonia.msg**
  202K
- **Revised Acknowledgement- 23-00174 for three requests.pdf**
  167K

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

December 5, 2022

Ms. Christine Massey

[redacted]

Via email: cmssyc@gmail.com

Dear Ms. Massey:

This letter is in response to your 3-part consolidated (attached) Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of October 31, 2022, for:

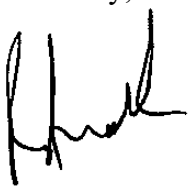
> *1)All studies and/or reports in the possession, custody or control of the people who work at/for the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and are responsible for the CDC's claim that parasites cause malaria, that describe controlled experiments using any purified parasite to prove causation of malaria*
>
> .
>
> *2) All studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of the alleged "HTLV-I" aka "Human T-Lymphotropic Virus Type I" or the alleged "HTLV-II" aka "Human T-Lymphotropic Virus Type II".*
>
> *3) All studies and/or reports in the possession, custody or control of the people who work at/for the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and are responsible for the CDC's claim that bacteria can cause pneumonia, that describe controlled experiments using purified Mycoplasma pneumoniae, or any other type of purified bacteria, to prove causation of pneumonia..*

A search of our records failed to reveal any documents pertaining to your request for Part 3. However, for parts 2, program provides the attached 13 articles and publications. For Part 1, program offers the attached 5 articles/publications.

You may contact our FOIA Public Liaison at 770-488-6246 for any further assistance and to discuss any aspect of your request. Additionally, you may contact the Office of Government Information Services (OGIS) at the National Archives and Records Administration to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services, National Archives and Records Administration, 8601 Adelphi Road-OGIS, College Park, Maryland 20740-6001, e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

If you are not satisfied with the response to this request, you may administratively appeal to the Deputy Agency Chief FOIA Officer, Office of the Assistant Secretary for Public Affairs, U.S. Department of Health and Human Services, via the online portal at https://requests.publiclink.hhs.gov/App/Index.aspx. Please mark both your appeal letter and envelope "FOIA Appeal." Your appeal must be electronically transmitted by March 5, 2022.

Sincerely,



Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
(770) 488-6399
Fax: (404) 235-1852

#23-00174-FOIA

Studies on Human Malaria in Aotus Monkeys. III. Exoerythrocytic Stages of the Salvador II Strain of Plasmodium vivax

Author(s): William E. Collins, Peter G. Contacos, John R. Jumper, Clinton S. Smith and Jimmie C. Skinner

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THE JOURNAL OF PARASITOLOGY
Vol. 59, No. 5, October 1973, p. 859–866

STUDIES ON HUMAN MALARIA IN *AOTUS* MONKEYS. III. EXOERYTHROCYTIC STAGES OF THE SALVADOR II STRAIN OF *PLASMODIUM VIVAX*

William E. Collins, Peter G. Contacos,* John R. Jumper, Clinton S. Smith, and Jimmie C. Skinner
Unit on Primate Malaria, Laboratory of Parasitic Diseases, NIAID, P.O. Box 80190, Chamblee, Georgia 30341

ABSTRACT: Exoerythrocytic stages of the Salvador II strain of *Plasmodium vivax* were demonstrated in sections of liver tissue from *Aotus trivirgatus* monkeys following the intrahepatic inoculation of sporozoites from *Anopheles freeborni* and *A. maculatus* mosquitoes. Six-, 7- and 8-day forms were seen. In general, they appeared to be morphologically similar although smaller than those reported from man.

This report concerns itself with the exoerythrocytic (EE) stages of a strain of human malaria, *Plasmodium vivax*, in a simian host, *Aotus trivirgatus*. The first report of the successful development of *P. vivax* in the *Aotus* monkey was in 1966 (Young et al., 1966; Porter and Young, 1966). Since that time, a number of workers have investigated the use of these animals for many different studies with the human malarias which could previously only be done, if at all, in man. Although exoerythrocytic stages of this parasite have been previously described from man and chimpanzees, very little is known of these stages in this new and potentially widely used host. The only previous report is of a single 8-day body found in biopsy material from an *Aotus* monkey previously inoculated intravenously with the salivary glands from 30 *Anopheles stephensi* mosquitoes infected with a Pakistan strain of *P. vivax* (Draper et al., 1971).

Reported here are 6-, 7-, and 8-day EE bodies of a Salvador strain of *P. vivax* present in biopsy material taken from *Aotus* monkeys.

MATERIALS AND METHODS

The Sal II strain of *P. vivax* was isolated from a natural infection in the area of Las Guarumas, in the state of La Paz, El Salvador (Collins et al., 1973). Blood was inoculated into an *A. trivirgatus* monkey (AO-219) which was then provided by the staff of the Central America Malaria Research Station, CDC, San Salvador. The strain has been maintained by serial blood or sporozoite passage in *A. trivirgatus* monkeys.

The *A. trivirgatus* monkeys were obtained commercially, their origin being Colombia, South America. Prior parasitologic and serologic examinations indicated that they were free of natural malarial infection.

For the inoculations, laboratory-reared *A. freeborni* or *A. maculatus* mosquitoes were fed on monkeys infected with the Sal II strain of *P. vivax*. After 12 to 19 days of extrinsic incubation at 25 C, the salivary glands were removed in 20% monkey serum–saline and lightly crushed under a cover slip. The cover slip was removed and the sporozoites were then drawn up into a syringe in a small quantity of the serum–saline. The monkeys were inoculated intrahepatically according to the technique previously described (Held et al., 1967; Sodeman et al., 1969b). In addition, the bodies of the mosquitoes were lightly crushed in a mortar and pestle with serum–saline. After centrifugation for 30 sec at 1,000 rpm, the supernatant was inoculated intravenously into the monkey. The technique used for the splenectomy of 2 of the monkeys is that described by Sodeman et al. (1970).

Tissues acquired at biopsy were fixed in Carnoy's fluid, embedded in paraffin, and sectioned at 6 μ. These sections were stained with Giemsa by the technique of Shortt and Garnham (1948) as modified by Eyles (1960). Photomicrographs were made with a Zeiss photomicroscope.

RESULTS

A total of six animals was inoculated with sporozoites of the Sal II strain of *P. vivax*. A summary of the number of infected salivary glands inoculated, the days of biopsy, and the resultant infections is presented in Table I.

Monkeys AO-317 and AO-318 were inoculated with 17 and 13 pairs of heavily infected salivary glands, respectively. Examination of approximately 500 sections of biopsy material taken at 5 days failed to reveal the presence of EE bodies. One animal (AO-317) de-

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* Center for Disease Control, Malaria Program, 1600 Clifton Road, Atlanta, Georgia 30333.

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TABLE I. *Summary of observations on six* Aotus trivirgatus *monkeys exposed to infection with the Sal II strain of* Plasmodium vivax *by the intrahepatic inoculation of sporozoites.*

Monkey No.	Mosquito species	No. pairs pos. glands	Days of extrinsci incubation	Biopsy day	EE bodies	Monkey infection	Prepatent period
AO-317	*A. freeborni*	17	12–14	5	–	Yes	27
AO-318*	*A. freeborni*	13	12–14	5	–	No	
AO-259*	*A. maculatus*	32	14–16	6	+	Yes	27
AO-267	*A. freeborni*	29	12–16	7	+	**	
AO-287	*A. freeborni*	15	13–15	8	+	Yes	46
AO-224	*A. freeborni*	55	14–19	10	–	No	

* Splenectomized prior to sporozoite inoculation.
** Died 12 days after sporozoite inoculation.

veloped a patent infection of *P. vivax* after a period of 27 days. The other animal did not develop patent infection.

Monkey AO-224 was inoculated with 55 pairs of heavily infected salivary glands. Examination of approximately 500 sections of biopsy material taken at 10 days failed to reveal the presence of EE bodies. The animal failed to develop a patent infection.

6-day forms

Monkey AO-259 was inoculated with 32 pairs of heavily infected salivary glands. A total of 19 EE bodies were found in approximately 500 sections of biopsy material. The animal developed a patent infection of *P. vivax* after a period of 27 days. The measurements of the midsections of the EE bodies are shown in Table II. The bodies measured from 15.3 to 22.1 μ in width and from 20.4 to 30.6 μ in length. The mean dimensions were 19.1 by 26.0 μ. The mean diameter of the bodies ranged from 19.5 to 26.3 μ with a mean of 22.5 μ.

Photomicrographs of the 6-day forms are presented in Figures 1–9. The bodies were oval in shape and the host cell nucleus was not enlarged. Numerous cytoplasmic aggregates (flocculi) and clefts were apparent.

7-day forms

Monkey AO-267 was inoculated with sporozoites from 29 pairs of heavily infected salivary glands. The EE bodies were relatively abundant in this material. The animal died 12 days after inoculation without developing a patent infection. The measurements of 25 EE bodies are shown in Table II. The bodies measured from 20.4 to 34.0 μ in width and from 25.5 to 39.1 μ in length. The mean dimensions were 25.1 by 31.4 μ. The mean diameter of the bodies ranged from 23.8 to 34.0 μ with a mean of 28.2 μ.

Photomicrographs of the 7-day forms are presented in Figures 10–33. The bodies were oval in shape and demonstrated numerous flocculi.

8-day forms

Monkey AO-287 was inoculated with sporozoites from 15 pairs of heavily infected salivary glands. Only two EE bodies were found in the examination of approximately 500 sections of biopsy material. The animal developed a patent infection of *P. vivax* after a period of 46 days. The measurements of the midsections of the two bodies are shown in Table II. The mean dimensions were 34.8 by 45.0 μ with a mean diameter of 39.9 μ.

Photomicrographs of the 8-day forms are presented in Figures 34–42. The bodies were irregular in shape and contained both small and large flocculi. A prominent vacuole was present in one body (Fig. 40). It was obvious that neither of these forms was mature although there was a considerable enlargement over the 7-day forms.

DISCUSSION

Tissue stages of *P. vivax* have been observed on several occasions. Shortt and Garnham (1948) described EE bodies from a 7-day liver biopsy taken from a human volunteer upon whom approximately 1,700 anopheline mosquitoes, infected with *P. vivax*, were allowed to bite on 2 successive days. In addition, 200 pairs of dissected salivary glands from the same mosquitoes were inoculated

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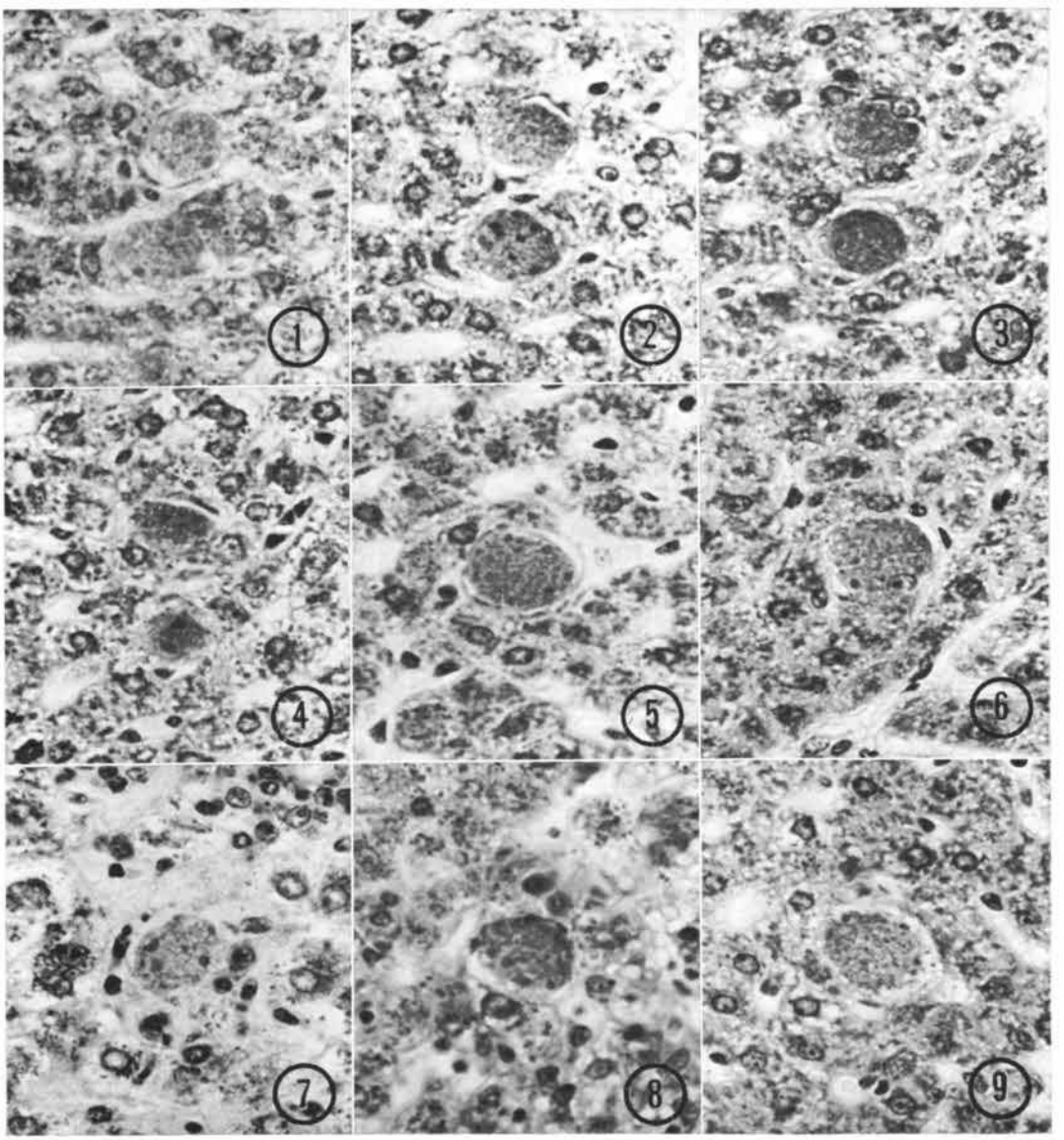

FIGURES 1–42. *Plasmodium vivax* exoerythrocytic bodies in the liver of *Aotus trivirgatus* monkeys. × 580. **1–4**. Serial sections from two adjacent 6-day EE bodies. **5–9**. Midsections from five 6-day EE bodies. **10–16**. Serial sections from a single 7-day EE body. **17–21**. Midsections from five 7-day EE bodies. **22–27**. Serial sections from a single 7-day EE body. **28–32**. Serial sections from a single 7-day EE body. **33**. Midsection from a single 7-day EE body. **34–37**. Serial sections from a single 8-day EE body (3 sections missing). **38–42**. Serial sections from a single 8-day body.

intravenously. The 6-day forms were described as ovoid plasmodial masses measuring about 42 μ in greatest diameter. An estimate of the number of chromatin masses in the parasite at this stage was given as about 800. The 7-day form was a single parasite which appeared to represent the rupture of a fully developed schizont. It would appear that the EE bodies seen in the present study were developing at a much slower rate. At 6 days, the mean diameter was only 22.5 and there was no indication of fully mature schizonts on the 7th day. By the 8th day, however, the mean diameter of 39.9 μ would indicate that

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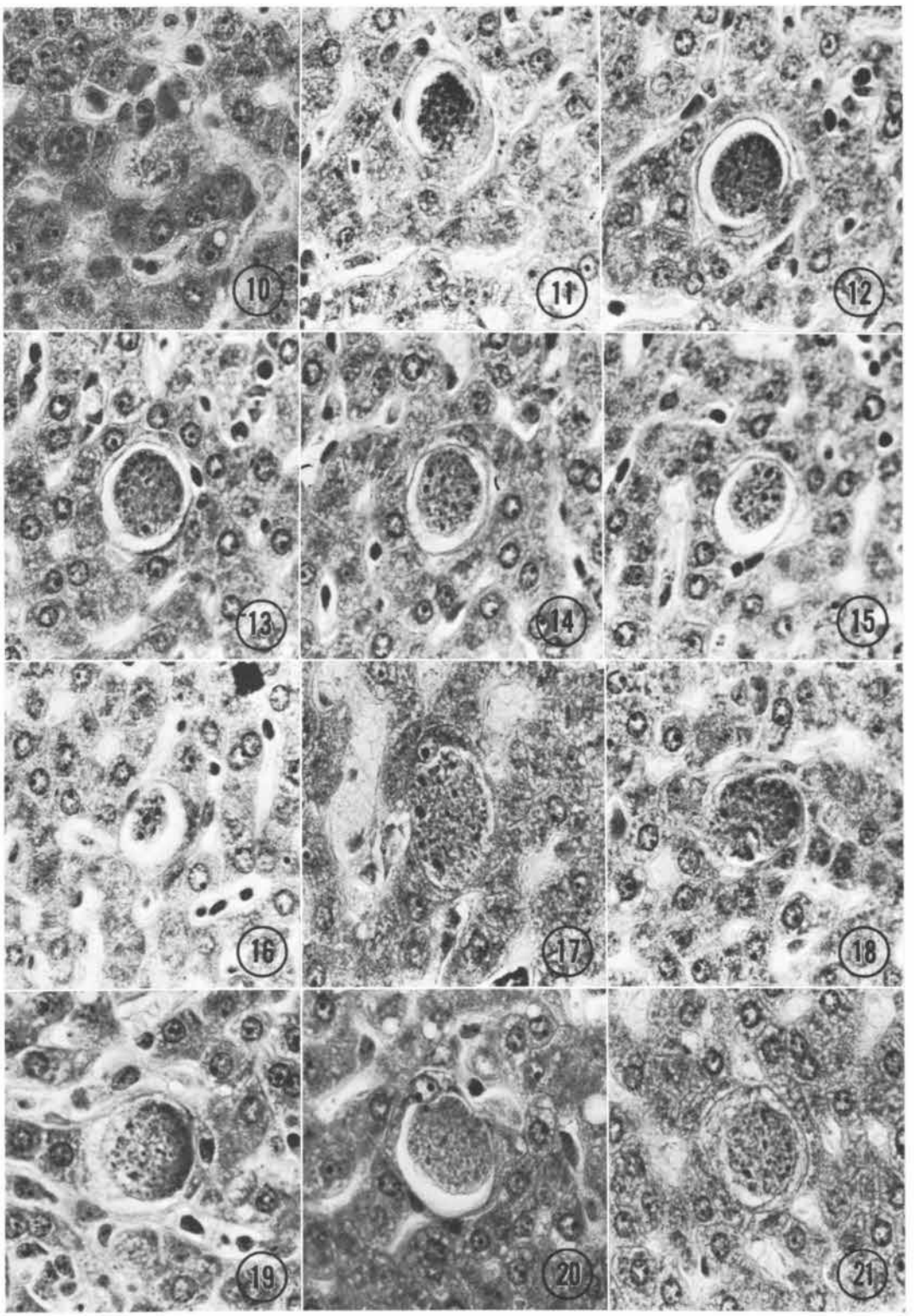

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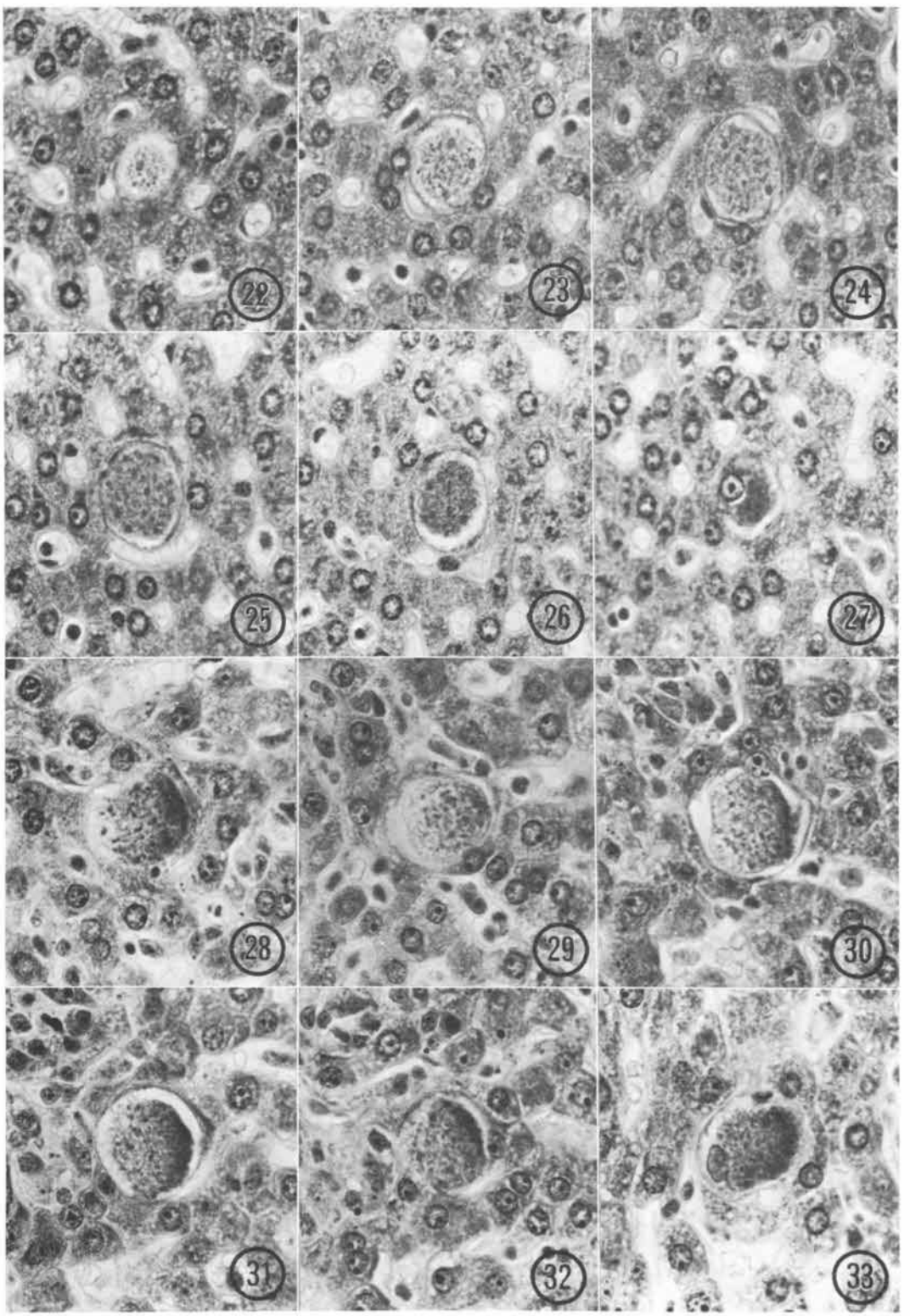

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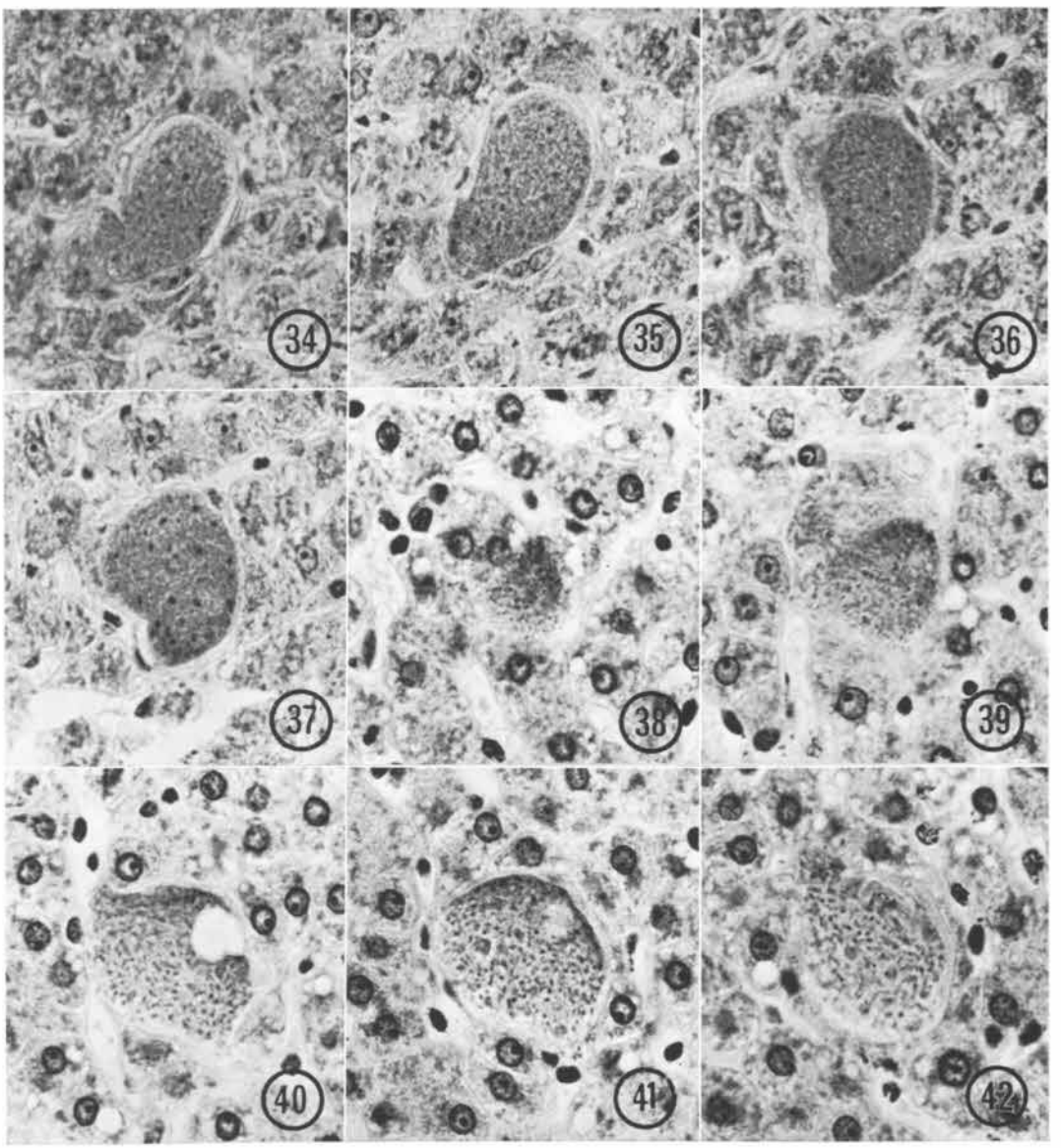

the parasites were approaching the size as seen at 6 days by the previous authors.

Rodhain (1956) described 4-day forms from the chimpanzee (possibly 7-day tissue stages) which ranged in size from 24 μ in diameter up to 47.7 by 35.2 μ. The size would appear to indicate that they were, more likely, 7-day forms. Bray (1957) described 8- and 15-day tissue stages from liver biopsy material taken from a chimpanzee. The EE bodies averaged 52 by 44 μ and were almost always oval in shape. He described immature, premature, and mature schizonts. In the immature stage, one or two larger vacuoles were present, cytoplasm was abundant, and, at times, collected into darker staining aggregates.

There is no information which would indicate whether or not the EE bodies of *P. vivax* take longer to develop in *A. trivirgatus* monkeys than they do in man. Bafort and Kageruka (1972) reported a prepatent period of only 10 days, whereas extended prepatent periods were found here and have been reported elsewhere (Ward et al., 1969; Collins et al., 1973).

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TABLE II. *Measurement of the midsections of 6-, 7-, and 8-day exoerythrocytic (EE) bodies of the Sal II strain of* Plasmodium vivax *in* Aotus trivirgatus *monkeys.*

EE body	6-day		7-day		8-day	
	Dimensions*	Mean diameter**	Dimensions	Mean diameter	Dimensions	Mean diameter
1	15.3 × 23.8	19.5	22.1 × 25.5	23.8	32.3 × 45.9	39.1
2	17.0 × 22.1	19.5	20.4 × 28.9	24.6	37.4 × 44.2	40.8
3	18.7 × 20.4	19.5	22.1 × 27.2	24.6		
4	17.0 × 23.8	20.4	20.4 × 30.6	25.5		
5	17.0 × 25.5	21.2	20.4 × 30.6	25.5		
6	17.0 × 25.5	21.2	22.1 × 28.9	25.5		
7	18.7 × 23.8	21.2	23.8 × 28.9	26.3		
8	18.7 × 23.8	21.2	25.5 × 27.2	26.3		
9	18.7 × 23.8	21.2	25.5 × 27.2	26.3		
10	18.7 × 27.2	22.9	25.5 × 27.2	26.3		
11	18.7 × 27.2	22.9	23.8 × 30.6	27.2		
12	18.7 × 27.2	22.9	23.8 × 32.3	28.0		
13	20.4 × 25.5	22.9	25.5 × 30.6	28.0		
14	20.4 × 28.9	24.6	25.5 × 30.6	28.0		
15	22.1 × 27.1	24.6	23.8 × 34.0	28.9		
16	22.1 × 27.2	24.6	23.8 × 34.0	28.9		
17	20.4 × 30.6	25.5	25.5 × 34.0	29.7		
18	22.1 × 30.6	26.3	28.9 × 30.6	29.7		
19	22.1 × 30.6	26.3	23.8 × 37.4	30.6		
20			25.5 × 35.7	30.6		
21			27.2 × 34.0	30.6		
22			30.6 × 30.6	30.6		
23			25.5 × 39.1	32.3		
24			32.3 × 35.7	34.0		
25			34.0 × 34.0	34.0		
Mean	19.1 × 26.0	22.5	25.1 × 31.4	28.2	34.8 × 45.0	39.9

* Measurements expressed in microns.
** Calculated by adding the length and width and dividing gy 2.

These extended prepatent periods may be due to a very low number of developing EE bodies or to a difficulty on the part of some strains of this parasite in developing in this host.

Nonetheless, it has been shown that EE bodies of both *P. falciparum* (Sodeman et al., 1969a) and *P. vivax* can be demonstrated in the liver tissue of these animals. It is hoped that additional passages, particularly via sporozoites, may increase the adaptation of this parasite to the *Aotus* host to the point where not only will the prepatent periods be similar to those in man, but that the tissue stages will be more readily seen. When, and if, this occurs, the effects of radical curative drugs upon these forms can be studied for their morphological and cytochemical effects.

LITERATURE CITED

BAFORT, J. M., AND P. KAGERUKA. 1972. Mosquito transmission of *Plasmodium vivax* from night monkey to night monkey. Ann. Soc. Belg. Med. Trop. **52:** 235–236.

BRAY, R. S. 1957. Studies on malaria in chimpanzees. II. *Plasmodium vivax*. Am. J. Trop. Med. Hyg. **6:** 514–520.

COLLINS, W. E., P. G. CONTACOS, P. S. STANFILL, AND B. B. RICHARDSON. 1973. Studies on human malaria in *Aotus* monkeys. I. Sporozoite transmission of *Plasmodium vivax* from El Salvador. J. Parasit. In press.

DRAPER, C. C., J. DORREL, P. G. SHUTE, AND J. M. BAFORT. 1971. Exo-erythrocytic schizogony of *Plasmodium vivax* in *Aotus* monkeys. Tr. Roy. Soc. Trop. Med. Hyg. **65:** 417–418.

EYLES, D. E. 1960. The exoerythrocytic cycle of *Plasmodium cynomolgi* and *P. cynomolgi bastianellii* in the rhesus monkey. Am. J. Trop. Med. Hyg. **9:** 543–545.

HELD, J. R., J. R. JUMPER, AND C. S. SMITH. 1967. Direct hepatic inoculation of sporozoites for the study of the exoerythrocytic stages of simian malarias. J. Parasit. **53:** 656–657.

PORTER, J. A., JR., AND M. D. YOUNG. 1966. Susceptibility of Panamanian primates to *Plasmodium vivax*. Mil. Med. **131:** 952–958.

RODHAIN, J. 1956. Les formes preerythrocytaires du *Plasmodium vivax* chez le chimpanzé. Ann. Soc. Belg. Med. Trop. **36:** 99–103.

SHORTT, H. E., AND P. C. C. GARNHAM. 1948. The pre-erythrocytic development of *Plas-*

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modium cynomolgi and *Plasmodium vivax*. Tr. Roy. Soc. Trop. Med. Hyg. **41**: 785–795.

SODEMAN, T. M., P. G. CONTACOS, C. S. SMITH, J. R. JUMPER, AND W. E. COLLINS. 1969a. The exoerythrocytic stages of *Plasmodium falciparum* in *Aotus trivirgatus*. J. Parasit. **55**: 682–683.

———, J. R. HELD, P. G. CONTACOS, J. R. JUMPER, AND C. S. SMITH. 1969b. Studies on the exoerythrocytic stages of simian malaria. IV. *Plasmodium brasilianum*. J. Parasit. **55**: 963–970.

———, J. R. JUMPER, P. G. CONTACOS, AND C. S. SMITH. 1970. A technique for splenectomy of non-human primates. J. Trop. Med. Hyg. **73**: 33–35.

WARD, R. A., L. C. RUTLEDGE, AND R. L. HICKMAN. 1969. Cyclical transmission of Chesson vivax malaria in subhuman primates. Nature **224**: 1126–1127.

YOUNG, M. D., J. A. PORTER, JR., AND C. M. JOHNSON. 1966. *Plasmodium vivax* transmitted from man to monkey to man. Science **153**: 1006–1007.

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countries are greater than those in industrial countries; language barriers, cultural differences, lack of accurate information on age, imperfect understanding about the significance of events &c.[18] Although not absent, the extent of these confounding factors is less in this study because of the controlled nature of the trial and because of the relative cultural and nutritional homogeneity of the two cohorts of children. Evidence which lends support to our findings, though on smaller samples and with less control over other variables, has been reported.[19,20] Both these investigations report deficits in motor competence in children born to mothers on an iodine-deficient diet and thus further support the thesis that the consequence of iodine deficiency is not an all-or-none effect of endemic cretinism but rather gives rise to a range of deficits which may in turn lead to developmental disadvantage.

Endemic cretinism is now a disease of the third world only. Whilst its prevention is a laudable and feasible goal the likelihood that the motor and possibly mental performance of whole populations may be increased by iodine supplementation is of great medical and social importance. The evidence presented here indicates that iodine deficiency may significantly affect general neurological development in the fetal period. Whilst iodine deficiency is not as widespread as protein malnutrition it is more easily remedied.[21]

We thank Miss D. Alberman, Mrs S. Moss, Mrs J. Hartshorne, and the staff of the Anglican Mission at Koinambe for their assistance. We also thank the staff of the Papua New Guinea Departments of Public Health and Education for permission to conduct the study and for their support and assistance. The investigation reported was made possible by a grant from the Wellcome Trust.

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REFERENCES

1. Pharoah POD, Buttfield IH, Hetzel BS. Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. *Lancet* 1971; i: 308–10.
2. Pharoah POD, Buttfield IH, Hetzel BS. The effect of iodine prophylaxis on the incidence of endemic cretinism. *Adv Exp Med Biol* 1972; **30**: 201–21.
3. Pharoah POD, Hornabook RW. Endemic cretinism of recent onset in New Guinea. *Lancet* 1974; ii: 1038–40.
4. Trowbridge FL. Intellectual assessment in primitive societies, with a preliminary report of a study of the effects of iodine supplementation on intelligence. *Adv Exp Med Biol* 1972; **30**: 137–50.
5. Stott DH, Moyes FA, Henderson SE. A test of motor impairment. Slough; National Foundation for Education Research. 1972.
6. Henderson SE, Stott DH. Finding the clumsy child: genesis of a test of motor impairment. *J Hum Mov Stud* 1977; **3**: 38–48.
7. Sloan W. The Lincoln-Oseretsky motor development scale. *Genet Psychol Monog* 1955; **51**: 183–252.
8. Pharoah POD, Ellis SM, Ekins RP, Williams ES. Maternal thyroid function, iodine deficiency and fetal development. *Clin Endocrinol* 1976; **5**: 159–66.
9. Cravioto J, Hambraeus L, Vahlquist B. Early malnutrition and mental development. Stockholm: Almqvist and Wiksell, 1974.
10. Birch HG, Gussow JD. Disadvantaged children: health, nutrition and school failure. New York: Narcourt, Brace and World, and Grune and Stratton, 1970.
11. Tizard J. Early malnutrition, growth and mental development in man. *Br med Bull* 1974; **30**: 169–74.
12. Chase HP, Dabiere CS, Welch NN, O'Brien D. Intra-uterine undernutrition and brain development. *Pediatrics* 1971; **47**: 491–500.
13. Winick M, Rosso P, Waterlow J. Cellular growth of cerebrum, cerebellum and brain stem in normal and marasmic children. *Exp Neurol* 1970; **26**: 393–400.
14. Dobbing J, Sands J. Quantitative growth and development of human brain. *Arch Dis Childh* 1973; **48**: 757–67.
15. Morgane PJ, Miller M, Kemper T. et al. Effects of protein malnutrition on the developing central nervous system in the rat. *Neurosci Neurobehav Rev* 1978; **2**: 137–230.
16. Moncksberg F. Effect of early marasmic malnutrition on subsequent physical and psychological development. In: Scrimshaw NS, Gordon JJ, eds. Malnutrition, learning and behaviour. Cambridge, Mass; M.I.T. Press, 1968.
17. Bartle PR, Griesel RD, Burnett LS. Long-term effects of kwashiorkor on psychomotor ability. Johannesburg SA; Special Report 256 CSIR, 1977.

References continued at foot of next column

CHLOROQUINE-RESISTANT PLASMODIUM FALCIPARUM FROM EAST AFRICA:

Cultivation and Drug Sensitivity of the Tanzanian I/CDC Strain from an American Tourist

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Summary A strain of *Plasmodium falciparum*, designated Tanzanian I/CDC, from an American tourist returning from Tanzania, was isolated in vitro and in the *Aotus* monkey. Clinically, the infection showed a late recrudescent pattern of chloroquine resistance. In 2 inoculated *Aotus* monkeys, the infection recrudesced after a dose of chloroquine (40 mg/kg) curative for sensitive *P. falciparum* strains in the *Aotus* monkey. In 4 additional monkeys two primary infections and one of the recrudescent parasitæmias were cured with a 100 mg/kg dose of chloroquine; the second recrudescent parasitæmia was cured with an additional 40 mg/kg dose of chloroquine. The 48 h in-vitro chloroquine-sensitivity test demonstrated that the Tanzanian I/CDC strain had a pattern of chloroquine resistance similar to a reference resistant strain, the Vietnam-Oak Knoll (FVO). These studies reinforce reports which suggest that chloroquine-resistant malaria is being transmitted in East Africa.

Introduction

RESISTANCE to chloroquine in *Plasmodium falciparum* was first recognised in 1961 by Moore and Lanier[1] in 2 malaria cases from Colombia, and has since been detected in South America, Panama, Southeast Asia, Oceania, and the Indian subcontinent.[2] The drug prophylaxis and therapy of chloroquine-resistant *P. falciparum* has become a major public health problem.

Until recently, in sub-Saharan Africa, the area of the world with the most intense *P. falciparum* transmission, isolated reports of *P. falciparum* resistant to chloroquine have remained open to challenge, on the basis of World Health Organisation (W.H.O.) criteria, of the adequacy of chloroquine therapy or supervision of patient follow-up.[2] In a 1966 report, Jeffery and Gibson[3] were unable to confirm earlier reports of chloroquine resistance from Upper Volta and Liberia. Bruce-Chwatt[4] was unable to find convincing evidence of resistance to chloroquine in Africa up to 1970. Since then, however, additional reports from Africa[5–7] have raised the strong but uncon-

DR CONNOLLY AND OTHERS: REFERENCES—*continued*

18. Tizard J. Ecological studies of malnutrition; problems and methods. In: Cravioto J, Hambraeus L, Vahlqvist B, eds. Early malnutrition and mental development. Stockholm; Almqvist and Wiksell, 1974.
19. Querido A, Bleichrodt N, Djokomoeljanto R. Thyroid hormones and human mental development. *Prog Brain Res* 1978; **48**: 337–44.
20. Roger G, Klees M, Lagasse R, Berguist H, Thilly CH. Psychomotor development of children in regions of severe endemic goitre. *Ann Endocr* 1977; **38**: 50A.
21. Stanbury JB, Ermans A, Hetzel BS, Pretell EA, Querido A. Endemic goitre and cretinism: public health significance and prevention. *WHO Chronicle* 1974; **28**: 220–28.

firmed suspicion that chloroquine-resistant falciparum malaria has emerged there.

Two reports[8,9] of chloroquine-resistant *P. falciparum* in tourists returning from East Africa in 1977 and 1978 appear to satisfy the W.H.O. field-test criteria for chloroquine resistance. 3 patients were treated in hospital with the required doses of chloroquine in countries where reinfection was not possible. In each there was a recrudescence of *P. falciparum* within a month of chloroquine therapy which was cured with drugs effective against chloroquine-resistant strains.

Here we describe the in-vitro cultivation of *P. falciparum* from a patient described in a preliminary note,[10] and the results of in-vitro drug sensitivity testing and studies of the strain in the *Aotus* monkey.

Case-report

A 50-year-old White businessman had been in excellent health before his return from a hunting safari in the Selous area of southern Tanzania. He had not travelled outside the United States or taken antimalarial drugs in the preceding 18 months. From Aug. 15 to Sept. 3, 1978, he was in a mobile camp during the safari and took no malaria chemoprophylaxis. Intermittent fevers to 40·5°C and chills began 4 days after he left Tanzania and returned to Atlanta, Georgia, and he was admitted to hospital. Physical examination was normal except for mild right-upper-quadrant abdominal tenderness; there was no splenomegaly. On the 3rd day of symptoms, *P. falciparum* was identified on thick and thin blood-films. Packed cell volume was 39%, white blood-cell count was 3300/mm^3 with a shift to the left, and a platelet count was 52 000/mm^3. After oral therapy with chloroquine phosphate 1·5 g (base) over 3 days, the patient was discharged.

36 h after treatment was started, the parasite density was reduced, and the patient had no fever. Thick blood-smears at 6 days showed no malaria parasites. 29 days after treatment began, the patient had low-grade fever and malaise. Blood-films showed no malarial parasites. 2 days later he had chills, headache, nausea, and fever to 39·4°C. Physical examination was normal. Blood-smears revealed *P. falciparum* parasites, 5000/mm^3 and the patient was treated with 160 mg trimethoprim and 800 mg sulfamethoxazole twice daily for 7 days.

Parasite density 24 h after treatment began was 1000/mm^3. Thick blood-smears showed no malaria parasites at 48 h. There has been no evidence of a recrudescence of parasitæmia in 3 months of follow-up.

Methods

In-vitro Cultivation

Parasitised blood from the patient, taken before treatment of the recrudescence, was cultivated in vitro by the technique described by Trager and Jensen.[11] An 8% red blood-cell (RBC) suspension was cultivated in RPMI-1640 medium supplement with 10% AB+human serum in candle jars at 37°C. The parasitæmia increased steadily over 3 days, without an interval of adaptation to the culture conditions. By the second week in culture the parasite density was increasing 8-fold to 10-fold every 4 days.

This isolate, designated the Tanzanian I/CDC strain, was maintained with subculture at intervals of 3–4 days by dilution with fresh human RBCs. Daily counts of the percentage of infected RBCs were made from Giemsa-stained thin blood-films.

Studies in the Aotus Monkey

Before treatment of the patient's recrudescent infection, 5.5 ml of whole blood ($4·2\times10^7$ *P. falciparum* parasites) was inoculated intravenously into a splenectomised *Aotus trivirgatus griseimembra* monkey. The infection became patent 21 days later with a peak count of 40 parasites/mm^3, at which time blood was subpassaged to a second splenectomised *A. t. griseimembra* monkey. Subsequently, blood was subpassaged to 4 other splenectomised *Aotus* monkeys in order to adapt the Tanzanian I/CDC strain to *Aotus* and to study the in-vivo response to chloroquine in these monkeys.

Daily thick and thin blood-films were obtained from each monkey; after Giemsa staining, the parasites were counted.[12] Chloroquine phosphate was administered to monkeys under ketamine sedation through an orogastic tube.

Chloroquine-sensitivity Testing

Because, in culture, the Tanzanian I?CDC strain grew asynchronously, as do all *P. falciparum* strains, the standard Rieckmann method of drug-sensitivity testing, which relies on synchronous parasite development, could not be used. To determine the chloroquine sensitivity of the Tanzanian I-CDC strain, we used a modification of the 48 h incubation test originally described by Trager et al.[13].

In our 48 h drug-testing procedure only parasite cultures growing with at least a ten-fold increase in density every 4 days were used. The culture was diluted to a 4% suspension of RBCs (v/v) in RPMI-1640 with 10% human serum. The parasite density was adjusted to 0·25–0·75% by the addition of fresh RBCs.

Chloroquine-sensitivity testing was done in microtitre plates. To 100 µl volumes of culture material was added 5 µl of the appropriate chloroquine concentration. For each chloroquine concentration, 4 replicates were done. After gentle agitation to assure uniform distribution of the drug, the plates were incubated at 37°C in a candle jar. At 24 h, 50 µl of medium was removed and an equal volume of fresh medium with serum was added along with half the original chloroquine dose per well. Thin blood-smears were made from each well at the end of 48 h.

To determine the parasite density, all parasites were counted, irrespective of abnormal parasite morphology produced by chloroquine. The percentage of cells infected with malaria parasites was expressed per 10^4 RBCs, and the results for each strain and chloroquine concentration are the arithmetric mean of four observations.

To characterise the in-vitro chloroquine sensitivity of the Tanzanian I/CDC, two other strains of *P. falciparum* with known sensitivity were tested concurrently:

West African I.—This strain was first isolated in our laboratory from an American tourist returning from West Africa in 1977. Repeated in-vitro testing showed that the strain was highly sensitive to chloroquine (W. Chin, unpublished). In these tests, the West African strain served as a sensitive control.

Vietnam-Oak Knoll (FVO).—This strain was obtained from Dr William Trager, Rockefeller University. Prior testing by Trager[13] and in our laboratory showed that the strain was resistant to chloroquine (50% inhibition of multiplication at 200–300 µg/l). The FVO strain served as a resistant control.

The strain originally cultivated from the patient, Tanzanian I/CDC, was tested for chloroquine sensitivity in the 37th subpassage. The Tanzanian I/CDC strain cultivated from *Aotus* monkey A0–124 (designated Tanzanian I/CDC/A0–124) was tested after 38 subpassages in vitro.

Results

Studies in Aotus Monkeys

The parasitæmia in monkeys A0–106 and A0–112 cleared after chloroquine therapy at 40 mg/kg (base) over 3 days (table). In both monkeys there was a recrudescence of parasitæmia approximately 2 weeks after chloroquine therapy. Parasitæmia was cleared by a second chloroquine treatment at 40 mg/kg (A0–106

INFECTION AND THERAPY OF AOTUS MONKEYS WITH THE TANZANIAN I/CDC STRAIN OF P. FALCIPARUM.

Monkey no.	Source of inoculum	Prepatent period (days)	Therapy			Recrudescence			
			Parasite count (per mm³)	Chloroquine total dose (base)	Days to negative smear	Subpatent period (days)	Chloroquine total dose base	Parasite count (per mm³)	Days to negative smear
A0–68	Patient	21	Peak parasitæmia=40/mm³			..	..	..	..
A0–124	A0–68	1	Peak parasitæmia=48 360/mm³			..	..	..	..
A0–106	A0–124	1	14 136	40 mg/kg	4	16	40 mg/kg	10 416	4*
A0–112	A0–124	1	9672	40 mg/kg	7	14	100 mg/kg	22 692	7*
A0–130	A0–106	9	26 785	100 mg/kg	6*	..	..	..	..
A0–134	A0–106	1	43 388	100 mg/kg	12*	..	..	..	..

* Blood smears remained negative for at least 3 months after therapy.

and 100 mg/kg dose (A0–112), and the infection was cured; blood-smears contained no parasites during a 3-month follow-up.

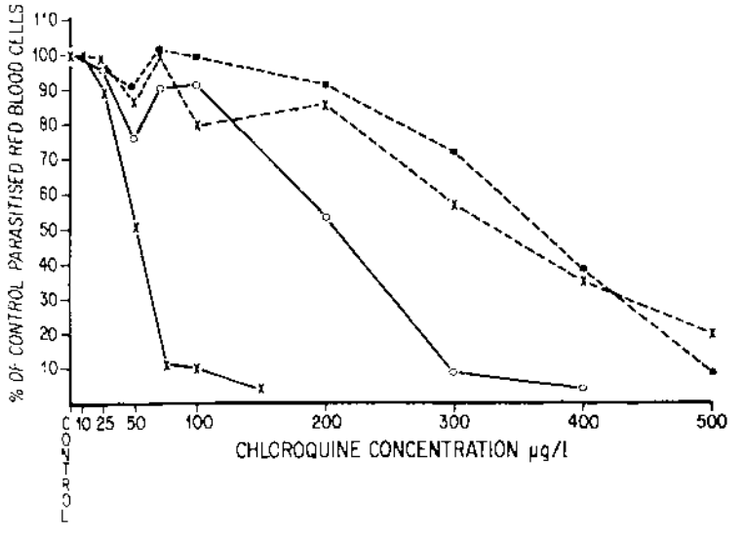
Infection with the Tanzanian I/CDC strain, induced by subpassage of parasitised blood to monkeys A0–130 and A0–134, was cured with chloroquine at a dose of 100 mg/kg (base). There was no recrudescence in over 3 months of follow-up.

Chloroquine-sensitivity Testing

In the West African strain, 50% inhibition of multiplication was induced between 25 and 50 µg/l chloroquine (see figure).

In the reference resistant strain, FVO, 50% inhibition was noted between 200 and 300 µg/l. The profile of drug response in this test system for both reference strains has not changed in our laboratory for the past 12 months.

The multiplication response-curve for the Tanzanian I/CDC strain was similar to the FVO pattern. There was 50% inhibition of multiplication between 300 and 400 µg/l chloroquine. The Tanzanian I/CDC/A0–124 line had a chloroquine-response curve indistinguishable from that of the Tanzanian I/CDC; there was 50% growth inhibition between 300 and 400 µg/l chloroquine.



In-vitro chloroquine sensitivity testing on strains of *P. falciparum*.

x—x West African I; (mean 318 parasites/10⁴ red blood-cells in control);
o—o Vietnam Oak Knoll (207);
x- - -x Tanzanian I/CDC (180);
• - - - • Tanzanian I/CDC/A0124 (260).

Discussion

The infection in the patient from whom the Tanzanian I/CDC strain was derived had a late recrudescent RI pattern of chloroquine resistance, by W.H.O. criteria.[2] The chloroquine dosage in the primary parasitæmia was adequate. Recrudescence of parasitæmia occurred 31 days after initial chloroquine therapy; such a prolonged subpatent interval might not have been detected even in the extended (28 days) W.H.O. field test.

Passage and adaptation of the Tanzanian I/CDC strain in *Aotus* monkeys provided the opportunity for alternative in-vivo observation of chloroquine sensitivity. Schmidt et al.[14] have reported that in *Aotus* monkeys 90% of infections with a chloroquine-sensitive strain of *P. falciparum* (Uganda Palo-Alto) were cured with a total dose of 52·5 mg/kg. In our laboratory the standard chloroquine-sensitive strain in the *Aotus* is the Santa Lucia strain from coastal El Salvador. A chloroquine dose of 40 mg/kg (W. E. Collins, unpublished) cured all 25 infected *Aotus* monkeys, with parasite clearance within 4 days of completion of therapy. The treatment regimen was based upon the equivalent human dosage of 600 mg (or 10 mg/kg in a 60 kg man) over 3 days. Treatment was calcualted as follows: the dosage per kilogram in *Aotus* monkeys (because of the weight/surface area ratio) is four times that of man; the weight of the monkey in kilograms × 10 mg/kg × 4=the total chloroquine dosage for 3 days.

The primary infection with the Tanzanian I/CDC strain in *Aotus* monkeys A0–106 and A0–112 recrudesced after 40 mg/kg chloroquine, demonstrating a degree of resistance. At the 100 mg/kg dose, all infections with the Tanzanian I/CDC strain were cured, indicating that the strain was not in the highly resistant range, as are some Vietnam strains of *P. falciparum* in the *Aotus* monkey.[14]

Of critical importance to the interpretation of chloroquine-sensitivity testing of the Tanzanian I/CDC strain cultivated in vitro was its rapid adaptation to the culture conditions. Strains which require considerable time to adapt to in-vitro culture may attain a degree of drug resistance which was not observed in vivo (W. Chin and W. E. Collins, unpublished). Because there was no adaptation interval, it is less likely that the cultured Tanzanian I/CDC strain was highly selected during cultivation.

Both the line of the Tanzanian I/CDC cultured directly from the patient and the line cultured from the

Aotus monkey had a pattern of multiplication in the presence of chloroquine which clearly suggested resistance. The 48 h test is a more indirect measure of resistance than is the 24 h test of the inhibition of schizogony which can be employed on synchronous infections. Despite this limitation, however, the comparisons of lines of the Tanzanian I/CDC with reference strains, the West African I and FVO, demonstrated that the Tanzanian I/CDC was similar to if not slightly more resistant that the VO strain. Until more experience with other strains and FVO, demonstrated that the Tanzanian I/CDC was similar to if not slightly more resistant than the FVO strain. Until more experience with other strains of *P. falciparum* is vailable, it is not possible precisely to relate the in-vivo chloroquine sensitivity to the 48 h test results.

The ability to induce chloroquine resistance in vitro in an African strain of *P. falciparum*, as recently demonstrated by Nguyen-Dinh and Trager,[15] combined with recent very credible reports of clinical chloroquine resistance from East Africa, have heightened the awareness of this threat. The in-vivo and in-vitro observations on the Tanzanian I/CDC strain reported here provide the most conclusive evidence to date that chloroquine-resistant *P. falciparum* is being transmitted in Africa.

Attention should shift to a concerted effort to map epidemiologically the distribution of chloroquine resistance in Africa, from in-vivo and in-vitro data. The threat of chloroquine resistance in those areas of Africa which are hyperendemic for *P. falciparum* should not be minimised. Strategies for case detection and alternate drug therapies, should they become necessary, need to be anticipated before chloroquine-resistant strains become established and spread to other areas.

We thank Dr Patricia DuBose who cared for the patient from whom the Tanzanian I/CDC strain was isolated.

Requests for reprints should be addressed to C. C. C.

REFERENCES

1. Moore DV, Lanier JE. Observations on two *Plasmodium falciparum* infections with an abnormal response to chloroquine. *Am J Trop Med Hyg* 1961; **10**: 5–9.
2. World Health Organization. Chemotherapy of Malaria and Resistance to Antimalarials. *W.H.O. Tech Rep Ser No. 529*. Geneva, 1973: 39–47.
3. Jeffery GM, Gibson FD. Studies on chloroquine-resistance of *Plasmodium falciparum* in Upper Volta and Liberia, West Africa. *WHO Bull* 1966; **35**: 441–49.
4. Bruce-Chwatt LJ. Resistance of *P. falciparum* to chloroquine in Africa: True or false? *Trans Roy Soc Trop Med Hyg* 1970; **64**: 776–84.
5. Dennis DT, Doberstyn EB, Sissay A, Tesfar GK. Chloroquine tolerance of Ethiopian strains of *P. falciparum*. *Trans Roy Soc Trop Med Hyg* 1974; **68**: 241–45.
6. Omer AH. Response of *Plasmodium falciparum* in Sudan to oral chloroquine. *Am J Trop Med Hyg* 1978; **27**: 853–57.
7. Khan AA, Maguire MJ. Relative chloroquine resistance of *P. falciparum* in Zambia. *Br Med J* 1978; i: 1669–70.
8. Fogh S, Jepsen S, Efferson P. Chloroquine-resistant *Plasmodium falciparum* malaria in Kenya. *Trans Roy Soc Trop Med Hyg* 1979; **73**: 228–29.
9. Kean BH. Chloroquine-resistant falciparum malaria from Africa. *JAMA* 1979; **241**: 395.
10. Center for Disease Control, U.S. Department of Health, Education, and Welfare. *Morb Mortal Wkly Rep* 1978; **27**: 463–64.
11. Träger W, Jensen JB. Human malaria parasites in continuous culture. *Science* 1976; **193**: 673–75.
12. Earle WC, Perez M. Enumeration of parasites in the blood of malaria patients. *J Lab Clin Med* 1932; **17**: 1124–30.
13. Trager W, Robert-Gero M, Lederer E. Antimalarial activity of s-isobutyl adenosine against *Plasmodium falciparum* in culture. *FEBS Letts* 1978; **85**: 826–66.
14. Schmidt LH, Vaughn D, Mueller D, Crosby R, Hamilton R. Activities of various 4-aminoquinolines against infections with chloroquine-resistant strains of *Plasmodium falciparum*. *Antimicrob Ag Chemother* 1977; **11**: 826–43.
15. Nguyen-Dinh P, Trager W. Chloroquine resistance produced in vitro in an African strain of human malaria. *Science* 1978; **200**: 1397–98.

SITE OF EPSTEIN-BARR VIRUS REPLICATION IN THE OROPHARYNX

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Summary Cells and cell-free material in saliva, parotid secretions, and throat washings from patients with acute infectious mononucleosis and patients undergoing tonsillectomy were assayed for the presence of infectious Epstein-Barr (EBV) virus. The agent was invariably present in cell-free form in saliva; neither infectious virus nor viral antigens were found in the cells. Tonsillar lymphocytes from eight patients were also free of EBV. In 10 of 40 patients virus was recovered in secretions from parotid-gland orifices or ducts. These findings suggest that the salivary glands are the site of EBV production in the oropharynx.

Introduction

Epstein-Barr virus (EBV) is shed into the oropharynx by patients with infectious mononucleosis (IM), healthy seropositive individuals,[1,2] and, commonly, by EBV-antibody-positive patients receiving immunosuppressive therapy.[3] The oropharyngeal secretions of patients with EB-virus-associated Burkitt's lymphoma[4] and nasopharyngeal carcinoma,[5] also contain this agent. Infectious virus is detected by its ability to convert lymphocytes into established cell lines, and mature enveloped EBV particles have been seen on rare occasions in concentrated saliva examined by electron microscopy.[6] The presence of infectious EBV in oropharyngeal secretions is clearly of considerable epidemiological importance. However, little is known of the nature and location of cells in the oropharynx in which the cycle leading to release of infectious virus take place.

Although the virus transforms B lymphocytes, the usual lymphocyte relationship in vivo does not lead to the production of infectious virions. However, cultivation of these cells in vitro leads to maturation of virus.[7] Epithelial cells of nasopharyngeal carcinoma also harbour the virus in a covert state; induction of virus can be achieved by treatment of the tumour cells in vitro with bromodeoxyuridine,[8] and induction occurs spontaneously during passage of these cells in the nude mouse.[9]

Transforming activity is more commonly detected in saliva than in samples taken from elsewhere in the oropharynx,[10] and infectious EBV has been recovered during acute IM from specimens taken with a swab applied to the orifice of Stensen's duct.[10] Taken together, these two findings implicate the parotid salivary gland as a potential site of virus production. However, squamous cells in saliva seem to contain EBV DNA as demonstrated by in situ nucleic-acid hybridisation,[11] which suggests that epithelial cells of the buccal mucosa may be a source of EBV.

We sought information on the cellular origin of EBV infection. We assayed cells and cell-free material in saliva and throat washings for infectious virus and

Transmission of Four Central American Strains of Plasmodium vivax from Monkey to Man

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TRANSMISSION OF FOUR CENTRAL AMERICAN STRAINS OF *PLASMODIUM VIVAX* FROM MONKEY TO MAN

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ABSTRACT: Infections of 4 different Central American strains of *Plasmodium vivax* in the *Aotus trivirgatus* monkey were shown to be infectious to *Anopheles freeborni* and *A. maculatus* mosquitoes. The *A. freeborni* were more readily infected than were the *A. maculatus* with each of the strains. A comparison of the infection ratios indicated that each strain of malaria was different with regard to its mosquito infectivity. Transmission of the infection to 17 men was obtained by the bites of infected *A. freeborni* mosquitoes after extrinsic incubation periods of 13 to 18 days. The prepatent periods in the men ranged from 9 to 16 days with a mean of 13.8 days.

The *Aotus trivirgatus* monkey has been shown to be potentially useful as an experimental animal in studies on the human malarias. Young et al. (1966) first reported infection of this monkey with a strain of *Plasmodium vivax* from Panama. Subsequent adaptation of *P. falciparum* and *P. malariae* parasites (Geiman and Meagher, 1967; Geiman and Siddiqui, 1969) has increased interest in the study of different isolates of the malaria parasites in the *Aotus* monkey. One area which has concerned us has been the infection of mosquitoes with malaria by feeding on these animals for subsequent transmission studies to man. Previously, we have reported the results of transmission studies using *P. falciparum* and *P. malariae* from the *Aotus* monkey (Contacos and Collins, 1968, 1969; Collins et al., 1968). Reported here are the results of studies with different isolates of *P. vivax* from Central America.

MATERIALS AND METHODS

The 4 strains of *P. vivax* used in the studies were obtained from naturally acquired infections from El Salvador (Sal—I, Sal—II), Panama (Panama), and Nicaragua (NICA), and provided to us by the staff of the Central America Malaria Research Station, NCDC, San Salvador, El Salvador. Once the strains were established in *A. trivirgatus* monkeys, they were maintained by serial passage of infected blood.

Aotus trivirgatus monkeys, obtained commercially, had their origin in Colombia, South America. Prior serologic and microscopic examination indicated that they were free of natural malarial infection.

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The *Anopheles freeborni* mosquitoes were the F-1 strain originally isolated from Marysville, California, and maintained in the laboratory since then (Hardman, 1947). The *A. maculatus* mosquitoes were obtained from the Institute of Medical Research, Kuala Lumpur, Malaysia (Ow Yang et al., 1963). The techniques used for the feedings on the monkeys and on the human volunteers are those previously reported (Collins et al., 1968).

RESULTS

The results of 285 comparative feedings of *A. freeborni* and *A. maculatus* mosquitoes on 25 infections of *P. vivax* are shown in Table I. Lots of each species were fed simultaneously on a monkey and only in those instances in which at least one of the mosquito species was found to be infected were the results used for comparison. The *A. freeborni* were consistently infected with oocysts at a higher rate than were the *A. maculatus*. With the Panama, Sal—I, and Sal—II strains, the differences in the percentage infection were highly significant ($P < 0.001$) between the two mosquitoes. With the NICA strain, the difference was less apparent ($P = 0.04$). In addition, the intensity of the gut infections was much greater in the *A. freeborni* mosquitoes. With the Panama strain, the mean Gut Infection Index or GII (average number of oocysts per 100 guts) was 179.2 for the *A. freeborni* versus 20.3 for the *A. maculatus* (GII ratio of 100:11.3). A greater difference was found as a result of feeding on the Sal—II strain where the GII ratio was 100:2.5. With all four strains of malaria, the differences between the intensity of the gut infections in the

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TABLE I. *Comparative infectivity of four strains of* Plasmodium vivax *from Central America to* Anopheles freeborni *and* A. maculatus *mosquitoes.*

Strains of parasite	No. lots	*A. freeborni*		*A. maculatus*		Mean GII†		GII* ratio
		Pos./ dissected	Per cent positive	Pos./ dissected	Per cent positive	*A. freeborni*	*A. maculatus*	
Panama	70	360/1,233	29.20	168/1,586	10.59	179.2	20.3	100:11.3
Sal—I	57	236/1,152	20.49	102/1,405	7.26	102.5	10.1	100: 9.9
NICA	22	70/380	19.42	24/473	5.07	270.6	18.4	100: 6.8
Sal—II	136	656/1,068	61.42	433/3,236	13.38	1,002.2	24.8	100: 2.5
Totals	285	1,322/3,833	34.49	727/6,700	10.85	563.6	20.3	100: 3.5

† GII = Gut Infection Index—average number of oocysts per 100 guts.
* GII ratio = Relationship of the GII of the *A. freeborni* mosquitoes to that of the *A. maculatus* where the *A. freeborni* were arbitrarily given a value of 100.

two mosquitoes were highly significant ($P < 0.001$). It was apparent that, for experimental studies, the *A. freeborni* would be the most consistently infected.

The results of dissection of salivary glands of *A. freeborni* mosquitoes infected with the Central American strains of *P. vivax* are shown in Table II. Salivary glands were rated 1+ to 4+ as previously described (Collins et al., 1966). Beginning as early as 12 days after infection, the salivary glands contained large numbers of sporozoites. The percentage infection was higher ($P < 0.004$) in those mosquitoes from lots averaging greater than five oocysts per gut (68.8%) than from those with less than five oocysts per gut (50.9%). However, the intensity of the gland infections was essentially the same in both groups.

The results of the transmission studies to human volunteers are presented in Table III. Seventeen of 18 Caucasian volunteers developed patent infections of *P. vivax* from the bites of infected *A. freeborni* mosquitoes.

TABLE II. *Salivary gland infections in* Anopheles freeborni *mosquitoes infected with Central American strains of* Plasmodium vivax.

Days of incubation	< 5 Oocysts/gut		> 5 Oocysts/gut	
	Pos./ dissected	PGI*	Pos./ dissected	PGI
12–13	4/9	3.8	9/15	2.9
14–15	40/76	3.7	75/112	3.5
16–17	4/10	3.3	19/24	3.9
18–23	9/17	3.7	5/6	3.4
Totals	57/112	3.6	108/157	3.5
Per cent infection	50.9		68.8	

* PGI = Positive Gland Index.

After feeding, the mosquitoes were dissected and the glands rated according to the number of sporozoites present. Thus, volunteer ST received the bites of mosquitoes with a total rating of 3+ whereas volunteer IW received the bites of mosquitoes with a total rating of 68+'s. Each of the four strains of malaria was readily transmitted with prepatent periods which ranged from 9 to 16 days.

TABLE III. *Transmission of Central American strains of* Plasmodium vivax *from* Aotus *monkeys to man by the bites of* Anopheles freeborni *mosquitoes.*

Volunteer	Parasite strain	Days of extrinsic incubation	Gland rating*	Prepatent period
ST	Sal—II	15	3+	15
RA	Sal—II	15	4+	16
LI	Sal—I	14–15	5+	16
TR	NICA	15	6+	12
LO	NICA	15	6+	12
PA	Panama	14	8+	14
PE	Panama	14	8+	14
TO	Panama	14	8+	14
BR	Panama	15	8+	14
WH	Panama	15	8+	15
DI	Sal—I	14–15	8+	NI†
MA	Sal—I	14–15	13+	15
WI	Sal—I	14–15	13+	16
HI	Sal—II	15	24+	13
JO	Sal—II	14–15	25+	15
MA	Sal—II	14–15	25+	14
GR	Sal—II	13–18	50+	9
IW	Sal—II	13–18	68+	10

* Total of salivary gland ratings. Individual mosquitoes are rated 1+ = 1 to 10 sporozoites, 2+ = 11 to 100 sporozoites, 3+ = 101 to 1,000 sporozoites, and 4+ = greater than 1,000 sporozoites in the salivary glands.
† NI = No infection.

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DISCUSSION

Only a relatively few species of anophelines have been tested with regard to their susceptibility to *P. vivax* in the *Aotus* monkey. Young et al. (1966) not only infected *A. albimanus* with the Santa Rosa strain of *P. vivax* from Panama, but were able to transmit the infection by bite to two human volunteers. Later, Baerg and Young (1969) reported that 5.2% of 11,494 *A. albimanus* fed on *A. trivirgatus* monkeys infected with *P. vivax* were infected. In addition, infection was obtained in three of 43 splenectomized *A. trivirgatus* and one of eight splenectomized *Saguinus geoffroyi* monkeys by the bites and/or inoculation of sporozoites from these mosquitoes. In comparative studies between *A. albimanus* and *A. pseudopunctipennis*, the ratio of infected mosquitoes was about 20:1 in favor of the *A. albimanus* (Young, 1970).

Ward et al. (1969) were able to infect *A. balabacensis balabacensis*, *A. stephensi*, and *A. quadrimaculatus* mosquitoes which had fed on *Aotus* monkeys infected with the Chesson strain of *P. vivax*, originally from New Guinea. Transmission was obtained in four splenectomized *Aotus* monkeys and one splenectomized chimpanzee, *Pan troglodytes*, presumably by the bites of all three species of anophelines. The prepatent periods in the *Aotus* monkeys ranged from 12 to 42 days with a mean of 25.8 days. The *A. b. balabacensis* had mean oocyst counts of 61 whereas the other species had 20.

The infection of *A. freeborni* and *A. maculatus* mosquitoes as reported here brings to seven the total number of anophelines which have been reported susceptible to infection with *P. vivax* in the *Aotus* monkey. Although it is not possible to compare in all respects the results from the different laboratories, it would appear that of the seven species, *A. albimanus*, *A. b. balabacensis*, and *A. freeborni* were the better vectors in each laboratory.

By using two or more species of mosquito as standardized test organisms, it is possible to detect differences between different isolates of the malaria parasites. This has been shown previously (Jeffery et al., 1950; Collins et al., 1963) in which *A. albimanus* mosquitoes were readily susceptible to some strains of *P. falciparum* but much less so to others; *A. quadrimaculatus*, the standard mosquito, was susceptible to infection with each of the strains. By using a combination of two standard test mosquitoes, such as *A. freeborni* and *A. maculatus*, differences in infectivity can be determined. The results suggest that the differences between the GII ratio between *A. freeborni* and *A. maculatus* fed on the Panama strain and those fed on the Sal—II strain are such as to separate these two parasites on the basis of mosquito susceptibility.

Studies of *P. vivax* in human volunteers often require sporozoite-induced infections in order to study the effect or action of antimalarial drugs upon relapse. In addition, the passage of infectious blood from one volunteer to another sometimes presents an element of risk due to the passage of secondary infections. Thus, the availability of different strains of *P. vivax* which are infectious to the *Aotus* monkey and which can be readily transmitted by a laboratory-raised mosquito is decidedly advantageous in studies on malarial chemotherapy. In our hands, at least, *A. freeborni* has been shown to be an excellent experimental vector in that it will not only transmit *P. vivax*, but also, as reported previously, *P. falciparum* and *P. malariae* from monkey to man. Based on the present results, *A. maculatus* mosquitoes, although proven experimental vectors of *P. vivax* from man to man, are not readily infected by feeding on these monkeys and thus would appear to be a poor choice as routine experimental vectors in such studies.

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LITERATURE CITED

BAERG, D. C., AND M. D. YOUNG. 1969. Susceptibility of mosquitoes to human malaria induced in Panamanian monkeys. Mil. Med. **134**: 772–779.

COLLINS, W. E., G. M. JEFFERY, AND R. W. BURGESS. 1963. Comparative infectivity of two strains of *Plasmodium falciparum* to *Anopheles quadrimaculatus* Say, *Anopheles freeborni* Aitken, and *Anopheles albimanus* (Wied.). Mosq. News **23**: 102–104.

———, P. G. CONTACOS, ELIZABETH G. GUINN, AND J. R. HELD. 1966. Studies on the transmission of simian malarias. I. Transmission of two strains of *Plasmodium inui* by *Anopheles maculatus* and *A. stephensi*. J. Parasit. **52**: 664–668.

———, ———, ———, M. H. JETER, AND T. M. SODEMAN. 1968. Monkey to man transmission of *Plasmodium falciparum* by *Anopheles freeborni* mosquitoes. J. Parasit. **54**: 1166–1170.

CONTACOS, P. G., AND W. E. COLLINS. 1968. Falciparum malaria transmissible from monkey to man by mosquito bite. Science **161**: 56.

———, AND ———. 1969. *Plasmodium malariae*: transmission from monkey to man by mosquito bite. Science **165**: 918–919.

GEIMAN, Q. M., AND M. J. MEAGHER. 1967. Susceptibility of a New World monkey to *Plasmodium falciparum* from man. Nature **215**: 437–439.

———, AND W. A. SIDDIQUI. 1969. Susceptibility of a New World monkey to *Plasmodium malariae from man*. Am. J. Trop. Med. Hyg. **18**: 351–354.

HARDMAN, N. F. 1947. Studies on imported malarias: 3. Laboratory rearing of western anophelines. J. Natl. Mal. Soc. **6**: 165–172.

JEFFERY, G. M., D. E. EYLES, AND M. D. YOUNG. 1950. The comparative susceptibility of *Anopheles quadrimaculatus* and two strains of *Anopheles albimanus* to a Panama strain of *Plasmodium falciparum*. J. Natl. Mal. Soc. **9**: 349–355.

OW YANG, C. K., F. L. STA MARIA, AND R. H. WHARTON. 1963. Maintenance of a laboratory colony of *Anopheles maculatus* Theobald by artificial mating. Mosq. News **23**: 34–35.

WARD, R. A., L. C. RUTLEDGE, AND R. L. HICKMAN. 1969. Cyclical transmission of Chesson vivax malaria in subhuman primates. Nature **224**: 1126–1127.

YOUNG, M. D. 1970. Natural and induced malarias in western hemisphere monkeys. Lab. Animal Care **20**: 361–367.

———, J. A. PORTER, JR., AND C. M. JOHNSON. 1966. *Plasmodium vivax* transmitted from man to monkey to man. Science **153**: 1006–1007.

BOOK REVIEW . . .

Immunology and the Skin, Advances in Biology of Skin, Vol. XI, ed. by W. Montagna and R. E. Billingham. Appleton-Century-Crofts, New York, 1971, XXVI + 396 p., many figures in bw, 10 in color. $20.00.

This book is comprised of papers given at the 19th Symposium of Skin Biology in September 1969. It is devoted to the underlying mechanisms, tests of sensitivity, theoretical considerations, and practical applications of immunodermatologic reactions. The chapters are written clearly and succinctly. There are numerous pertinent references in each chapter. This volume brings together much detailed material which is scattered in various journals and books. In view of the importance of skin reactions in the pathogenesis and in the diagnosis of many parasitic infections, the subjects discussed should be of interest and use to medical parasitologists. It is unfortunate that there is not a chapter devoted exclusively to the immunodermatologic reactions in parasitic infections. Despite this shortcoming, the principles discussed are certainly applicable. This worthwhile text will be a useful reference.

A. A. Marucci

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A RETROSPECTIVE EXAMINATION OF ANEMIA DURING INFECTION OF HUMANS WITH *PLASMODIUM VIVAX*

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Abstract. A retrospective examination was made of archival data on 98 patient episodes of infection with *Plasmodium vivax* occurring over a period of 4–11 weeks to document changes in hemoglobin (Hb) concentrations associated with continuing parasitemia. The mean percentage change in the Hb concentration for each of the 10 seven-day intervals was −13.4, −10.9, −4.8, 0.12, 0.94, 4.0, 0.69, 11.6, 2.4, and 8.3, respectively. An equilibrium appeared to be established between weeks 4 and 6. Decreases in Hb concentrations were greatest following the first week of parasitemia. Total restoration to preinfection levels did not occur during persistent parasitemia.

INTRODUCTION

The destruction of erythrocytes is a major consequence of infection with *Plasmodium* spp. Infection with *Plasmodium falciparum* can produce acute anemia that can be a major cause of morbidity and mortality if parasitemia is allowed to reach a high density and persist for extended periods of time. Jakeman and others[1] examined parasitemia and anemia data from patients receiving malariatherapy with *P. falciparum* for the treatment of neurosyphilis. Based on a mathematical model, an average of 8.5 erythrocytes were destroyed in addition to each erythrocyte observed to become parasitized. They postulated that this was due to the destruction of uninfected erythrocytes through phagocytosis.

As part of our continuing efforts to search archival records on induced infections in patients for the treatment of neurosyphilis between 1940 and 1963, a similar examination was made on the induction of anemia as a result of infection with *P. vivax*.

Archival data giving daily parasite counts per microliter and weekly determinations of hemoglobin (Hb) concentrations were available. Certain assumptions were necessary to better understand the changes that were recorded.

Plasmodium vivax does not sequester into the deep tissue. It is also well recognized that the parasite has an asexual developmental cycle of approximately 42 hours, depending somewhat on the strain of the parasite.[2] This would indicate that four cycles would be completed during a seven-day period, which was the interval between determinations of Hb concentrations. The mean daily parasite count for the seven days × 4 equals the erythrocytes that were infected and subsequently destroyed during each interval between determinations of Hb concentrations. The hemoglobin concentration at the beginning of the seven-day interval divided by the hemoglobin concentration at the end of the seven-day period would equal the percentage reduction (or increase) in the Hb concentration, the available indicator of anemia. The destruction of erythrocytes (and therefore a reduction in the Hb concentration) by parasitic maturation is balanced by the production of new erythrocytes. Additional erythrocyte destruction could be attributable to a number of different activities, including that proposed by Jakeman and others.[1]

Reported here are the results of a retrospective examination of archival data from 98 induced infections with *P. vivax* for the treatment of paresis and other mental disorders associated with tertiary syphilis. The goal was to document the extent of changes in Hb concentrations in association with continuing parasitemia and to document, as was observed with infections with *P. falciparum*, that anemia associated with infection is markedly greater than can be attributed to parasitic destruction of erythrocytes.

MATERIALS AND METHODS

Patient management. Consent for whatever treatments the hospital staff determined necessary was granted by the families of the patients or the courts when patients were admitted to the hospital. The decision to infect a neurosyphilitic patient with a specific species or strain causing malaria was made as part of standard patient care provided by the medical staff of the South Carolina State Hospital. Patient care and evaluation of clinical endpoints (e.g., fever) were the responsibility of the medical staff. As previously reported,[3] during infection, the temperature, pulse, and respiration were checked every four hours and hourly during paroxysms (fevers) by hospital personnel. During paroxysms, patients were treated symptomatically. Infections were terminated at the direction of the attending physician. Personnel of the U.S. Public Health Service provided the parasites for inoculation, monitored the daily parasite counts to determine the course of infection, and determined Hb concentrations and white blood counts on a weekly basis. All patients undergoing malariatherapy lived in screened wards of the hospital to prevent possible infection of local anophelines.

Treatment. Patients were frequently allowed to maintain parasitemia for relatively short periods of time, mainly from 2 to 11 weeks. Infections were then terminated by treatment with standard antimalarial drugs. Once treated, the data from these patients were excluded from the analysis.

Strains of *P. vivax*. Of the 98 patient episodes for whom weekly hemoglobin concentrations were recorded, 85 were infected with the St. Elizabeth strain of *P. vivax*, 11 with the Chesson strain, and 2 with the Korean strain.

Parasitemia. Patients were infected by the intravenous inoculation of parasitized erythrocytes or via sporozoite inoculation. Thick and thin peripheral blood films were made daily by the method of Earle and Perez,[4] stained with Giemsa, and examined microscopically for the presence of parasites. The threshold of detection was approximately 10 parasites/μL. Asexual and sexual parasites were recorded per microliter of blood. Infections often persisted for many weeks. The number of patients decreased weekly as their infections were terminated; only 14 of the patients had continuing parasitemia

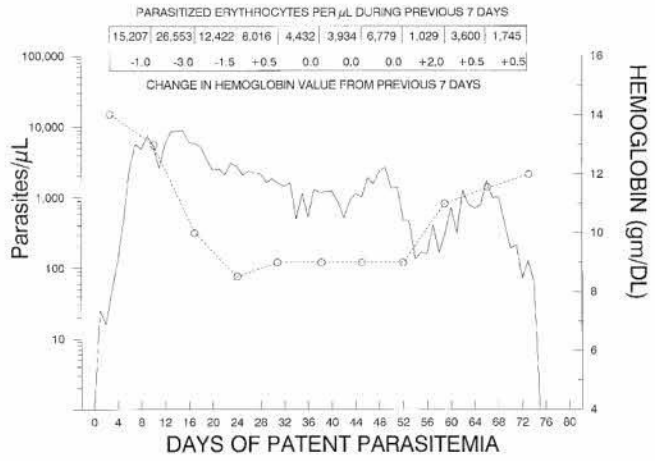

FIGURE 1. Parasitemia, hemoglobin concentrations, and number of parasitized erythrocytes/μL determined at seven-day intervals for patient S-1126 infected with *Plasmodium vivax*. Solid line = parasite counts/μL; dash line = hemoglobin values.

for 11 weeks. The analysis used weekly Hb concentrations and daily parasite counts for those patients still infected through each of 10-weekly intervals.

Data presentation. To monitor the well being of the patients, Hb concentrations were measured weekly, beginning some time during the first seven days of patent parasitemia. This was continued to the week the patient received antimalarial drug treatment. Accumulated parasite counts were calculated up to the time of the first determination of the Hb concentration (week 1); subsequent Hb concentrations were related to the intervening accumulated daily parasite counts through 11 weeks of patent parasitemia. The daily parasite count × 4 = erythrocytes destroyed as attributable to parasite maturation.

Statistical analysis. Piecewise linear regression was used to estimate the decrease in Hb concentrations from week 1 to 4 and the subsequent rate of increase from week 5 to 11 controlling for parasitemia. A paired *t*-test was used to compare mean differences in the initial Hb concentration with the last Hb concentration before treatment. The significance level was set at alpha = 0.05. All statistics were calculated using SAS version 6.0 (SAS Institute, Inc., Cary, NC).

RESULTS

Patient S-1126 (Figure 1) was infected with the St. Elizabeth strain of *P. vivax*. A Hb concentration of 14.0 g/dL was determined on day 3 of patent parasitemia. The accumulated parasite count up to and including day 3 was 89/μL. This was arbitrarily designated as week 1. Following the next seven-day interval (week 1–2), the Hb value was 13 g/dL (a decrease of 1.0 g); the accumulated parasite count for the interval was 26,612/μL (divided by 7 then multiplied by 4 = 15,209 erythrocytes destroyed/μL of blood during this period). This pattern of data collection was continued until the infection was terminated. Each patient infection from initiation to treatment was considered an episode.

The Hb values and the geometric mean number of erythrocytes infected with parasites per microliter during the seven-day interval were then tabulated for each of 98 patient episodes (Table 1 and Figure 2).

All but two episodes had Hb values ≥10 g/dL during the first week of infection. Changes in Hb values were often marked. Between week 1 and week 2, nine patient episodes showed no change in Hb values and four increased. The other 85 patient episodes had decreases in Hb values during this period. Throughout the 11-week period of parasitemia, there were additional reductions in Hb concentrations, coinciding with more frequent instances of increases in value, indicating an apparent replenishment of erythrocytes (Figure 3). During the interval between weeks 10 and 11, only two of 14 patients (14%) had decreases in Hb concentrations.

In the absence of erythrocyte counts per microliter, it is not possible to accurately determine the percentage of erythrocytes destroyed or the percentage of cells replaced. However, during weekly interval 1–2, 19,286 erythrocytes/μL were infected and subsequently destroyed. Assuming an erythrocyte concentration of 5,000,000/μL, this would indicate that only 0.39% of the 13.37% reduction in hemoglobin concentration was due to parasitic erythrocyte destruction. The difference between the Hb value at the end of the seven-day interval and the initial Hb value was used to determine the percentage reduction or increase in the Hb concentration. The mean percentage change in Hb concentration for each of the 10 seven-day intervals beginning with week 2 was −13.4, −10.9, −4.8, 0.12, 0.94, 4.0, 0.69, 11.6, 2.4, and 8.3, respectively. The Hb

TABLE 1
Changes in hemoglobin concentrations (g/dL) during infection of humans with *Plasmodium vivax*

	No. of patients	Parasite count		Hemoglobin concentration (g/dL)				
					Change from		Change from	
		Previous 7 days	Erythrocytes/μL infected*	Mean	Initial	(%)	Previous	(%)
Week 1	98	886	506	12.51				
Week 1–2	98	33,751	19,286	10.80	−1.73	(−13.37)	−1.73	(−13.37)
Week 2–3	97	21,104	12,059	9.61	−2.88	(−22.95)	−1.22	(−10.85)
Week 3–4	91	10,384	5,935	9.03	−3.47	(−27.40)	−0.51	(−4.79)
Week 4–5	85	7,827	4,473	8.98	−3.59	(−28.33)	−0.07	(+0.12)
Week 5–6	61	5,975	3,414	9.26	−3.41	(−26.58)	+0.07	(+0.94)
Week 6–7	41	5,408	3,090	9.55	−3.09	(−24.10)	+0.22	(+3.99)
Week 7–8	28	4,241	2,423	9.68	−3.04	(−23.49)	+0.02	(+0.69)
Week 8–9	23	3,613	2,065	10.52	−2.11	(−16.56)	+1.04	(+11.56)
Week 9–10	17	2,235	1,277	10.50	−2.06	(−16.06)	+0.18	(+2.35)
Week 10–11	14	2,160	1,234	11.00	−1.61	(−12.44)	+0.79	(+8.27)

* Based on a 42-hour developmental cycle for *P. vivax*, there would be 4 generations of parasites each week; mean accumulated parasite count divided by 7 (days) × 4 (generations) = erythrocytes/μL infected by parasites during a 7-day period.

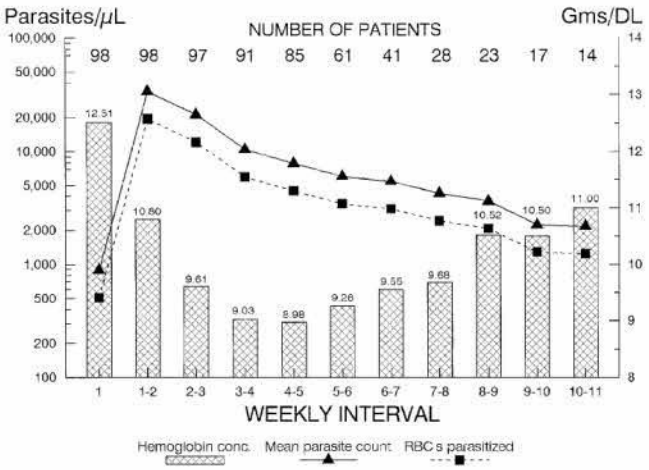

FIGURE 2. Mean hemoglobin values, mean parasite counts, and erythrocytes parasitized during previous seven days for 98 patients infected with *Plasmodium vivax*. Erythrocytes parasitized is based on an estimated four generations during the seven-day interval. conc. = concentration.

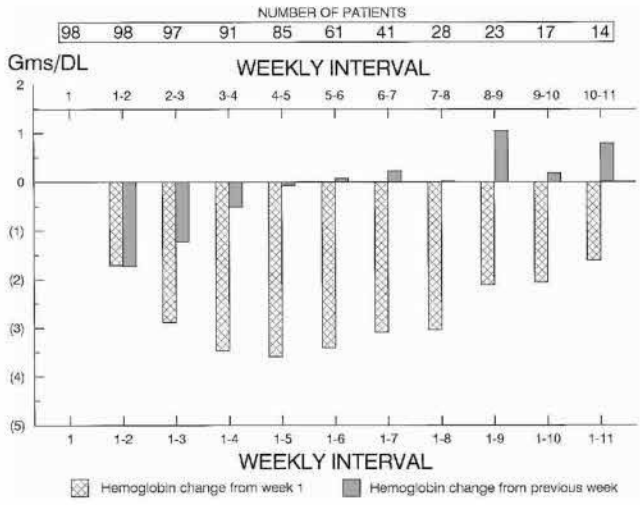

FIGURE 3. Mean changes in hemoglobin concentration (gms/DL) determined at weekly intervals in patients infected with *Plasmodium vivax*. Changes are from initial values and from that of the previous value.

concentration decreased an estimated 0.78 g/dL (95% confidence limit [CL] = –0.86, –0.70) through week 4, then increased an average of 0.34 g/dL (95% CL = 0.13, 0.56) from week 5 to 11. An equilibrium appeared to be established between weeks 4 and 5. Restoration to preinfection Hb levels did not occur at any time during the 11 weeks of persistent parasitemia; rather, the last Hb value before treatment was significantly lower than the initial value ($P < 0.0001$).

DISCUSSION

Early in these infections with *P. vivax*, the decreases in Hb concentrations were far greater than can be accounted for by parasitic activity; later in the infection, Hb values increased somewhat in the continued presence of parasitemia (Table 1). The destruction of erythrocytes (as shown by the reduced concentrations of Hb) was most marked during the first three weeks, and the percentage change from week to week increased at a significant average rate of 0.34 g/dL. It is therefore difficult to explain why the Hb level did not return to near preinfection levels. Certainly, the continued destruction of erythyrocytes by parasitic activity had stabilized, and there was evidence that the percentage change in the value had also stabilized.

Why did the Hb concentrations remain on average greater than 20% below preinfection values? *Plasmodium vivax* primarily invades reticulocytes. It is postulated that the continued parasitemia may have been sufficient to infect and destroy most of the new reticulocytes, thus preventing restoration of the total erythrocyte population.

Normally, one would not consider infection with *P. vivax* as a major contributor to anemia. The marked reduction in Hb concentration could not be attributed to physical destruction of infected erythyrocytes through parasite maturation. The majority of the anemia must be attributed to other activities, such as has been proposed with patients infected with *P. falciparum*. If the continued presence of parasites is sufficient to destroy most of the reticulocytes as they are being produced, and thereby prevent the restoration of Hb levels to preinfection levels, chronic infection with this parasite could have a debilitating effect on the patient.

Generally, one would expect the immune response of the patient to control parasite density and persistence. However, recrudescence, reinfection, and relapse of parasite populations could result in continued parasitemia and anemia in patients infected with *P. vivax*. Studies on the dynamics of infections with *P. vivax* and related species of *Plasmodium*, such as *P. cynomolgi* in nonhuman primates, may provide a better understanding of the role of chronic and persistent infection on anemia caused by this human-infecting parasite.

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REFERENCES

1. Jakeman GN, Saul A, Hogarth WL, Collins WE, 1999. Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. *Parasitology 119:* 127–133.
2. Young MD, 1944. Studies on the periodicity of induced *Plasmodium vivax. J Natl Malaria Soc 3:* 237–240.
3. Collins WE, Jeffery GM, 1999. A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum. Am J Trop Med Hyg 61(Suppl):* 4–48.
4. Earle WE, Perez M, 1932. Enumeration of parasites in the blood of malarial patients. *J Lab Clin Med 17:* 1124–1130.

Parasites & Vectors

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History of the discovery of the malaria parasites and their vectors

Francis EG Cox*

Abstract

Malaria is caused by infection with protozoan parasites belonging to the genus *Plasmodium* transmitted by female *Anopheles* species mosquitoes. Our understanding of the malaria parasites begins in 1880 with the discovery of the parasites in the blood of malaria patients by Alphonse Laveran. The sexual stages in the blood were discovered by William MacCallum in birds infected with a related haematozoan, *Haemoproteus columbae*, in 1897 and the whole of the transmission cycle in culicine mosquitoes and birds infected with *Plasmodium relictum* was elucidated by Ronald Ross in 1897. In 1898 the Italian malariologists, Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava demonstrated conclusively that human malaria was also transmitted by mosquitoes, in this case anophelines. The discovery that malaria parasites developed in the liver before entering the blood stream was made by Henry Shortt and Cyril Garnham in 1948 and the final stage in the life cycle, the presence of dormant stages in the liver, was conclusively demonstrated in 1982 by Wojciech Krotoski. This article traces the main events and stresses the importance of comparative studies in that, apart from the initial discovery of parasites in the blood, every subsequent discovery has been based on studies on non-human malaria parasites and related organisms.

Background

Malaria is an ancient disease and references to what was almost certainly malaria occur in a Chinese document from about 2700 BC, clay tablets from Mesopotamia from 2000 BC, Egyptian papyri from 1570 BC and Hindu texts as far back as the sixth century BC. Such historical records must be regarded with caution but moving into later centuries we are beginning to step onto firmer ground. The early Greeks, including Homer in about 850 BC, Empedocles of Agrigentum in about 550 BC and Hippocrates in about 400 BC, were well aware of the characteristic poor health, malarial fevers and enlarged spleens seen in people living in marshy places. For over 2500 years the idea that malaria fevers were caused by miasmas rising from swamps persisted and it is widely held that the word malaria comes from the Italian mal'aria meaning spoiled air although this has been disputed. With the discovery of bacteria by Antoni van Leeuwenhoek in 1676, and the incrimination of microorganisms as causes of infectious diseases and the development of the germ theory of infection by Louis Pasteur and Robert Koch in 1878-1879, the search for the cause of malaria intensified. Scientific studies only became possible after the discovery of the parasites themselves by Charles Louis Alphonse Laveran in 1880 and the incrimination of mosquitoes as the vectors, first for avian malaria by Ronald Ross in 1897 and then for human malaria by the Italian scientists Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava between 1898 and 1900. Excellent histories of this disease include those by Celli [1], Stephens [2], Scott [3], Russell [4], Foster [5], Garnham [6,7], Harrison [8], Bruce-Chwatt [9], Desowitz [10], McGregor [11], Poser & Bruyn [12] and Schlagenhauf [13].

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The life cycle of the malaria parasites, *Plasmodium* spp

In order to understand the historical events it is necessary to summarise briefly our current state of knowledge. Malaria is caused by infection with five species of *Plasmodium* the life cycles of which are very similar (Figure 1).

Infection begins when (1) sporozoites, the infective stages, are injected by a mosquito and are carried

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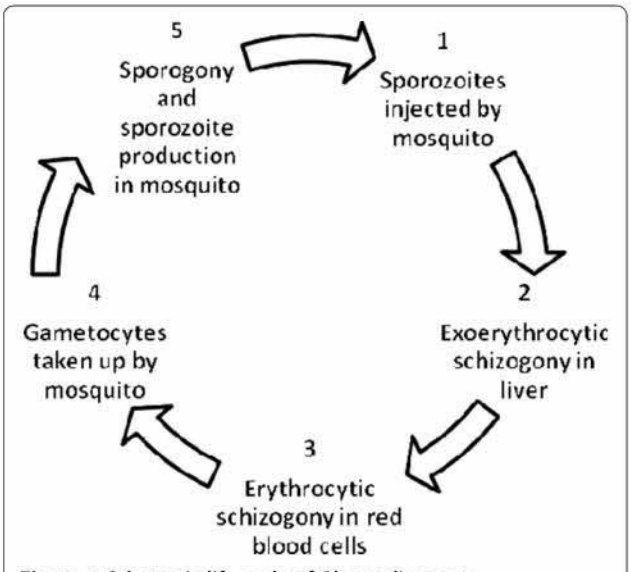

Figure 1 Schematic life cycle of *Plasmodium* spp.

around the body until they invade liver hepatocytes where (2) they undergo a phase of asexual multiplication (exoerythrocytic schizogony) resulting in the production of many uninucleate merozoites. These merozoites flood out into the blood and invade red blood cells where (3) they initiate a second phase of asexual multiplication (erythrocytic schizogony) resulting in the production of about 8-16 merozoites which invade new red blood cells. This process is repeated almost indefinitely and is responsible for the disease, malaria. As the infection progresses, some young merozoites develop into male and female gametocytes that circulate in the peripheral blood until they are (4) taken up by a female anopheline mosquito when it feeds. Within the mosquito (5) the gametocytes mature into male and female gametes, fertilization occurs and a motile zygote (ookinete) is formed within the lumen of the mosquito gut, the beginning of a process known as sporogony. The ookinete penetrates the gut wall and becomes a conspicuous oocyst within which another phase of multiplication occurs resulting in the formation of sporozoites that migrate to the salivary glands of a mosquito and are injected when the mosquito feeds on a new host.

The malaria parasites

Our understanding of the life cycle of the malaria parasites did not proceed in the logical order just outlined but more like a jigsaw in which the various pieces were painstakingly put into place and, like a jigsaw, often involved mistakes and false starts. The story begins with the discovery of the stages in the blood. Many textbooks merely state that 'in 1880 Laveran discovered the malaria parasite' words that do not give this discovery the credit it deserves. In order to understand the background of this discovery it is necessary to go back to the 1870s. The discoveries of Pasteur and Koch had precipitated a search for a bacterial cause for many diseases including malaria. By 1879 the miasma theory was going out of favour and the two theories vying for contention were whether the microorganisms responsible were transmitted (1) by air and inhalation or (2) by water and ingestion. The leading theory was that proposed by the Italian Corrado Tommasi-Crudeli and the German, Theodor Albrecht Edwin Klebs, an eminent microbiologist who had been the first person to see the bacteria responsible for typhoid and diphtheria. Tommasi-Crudeli and Klebs claimed that they had isolated from the waters of the Pontine Marshes, where malaria was prevalent, a bacterium, *Bacillus malariae*, which when isolated in culture and injected into rabbits caused febrile infections accompanied by enlarged spleens reminiscent of malaria [14]. It was against this background that Charles Louis Alphonse Laveran, an unknown French army officer working in Algeria, challenged the perceived wisdom and began in his own words 'to follow the pigment'. Beginning with the known fact that the spleens of malaria patients contained pigment he began to look for pigment in the fresh unstained blood of patients and observed it first in leucocytes and then in or on red blood cells. Looking more carefully, he observed several different forms of erythrocytic organism including crescents, spherical motionless bodies with pigment, spherical moving bodies with pigment and bodies that extruded flagella-like structures all of which he thought were on the outside of the red cells. These observations are particularly interesting because Laveran not only used fresh blood but also a dry objective with a maximum magnification of ×400 diameters. He also suggested a course of events that began with clear spots that grew, acquired pigment and filled the corpuscle which then burst coinciding with the fevers associated with malaria. Laveran meticulously examined the blood of 200 patients and in 148 observed the crescentic bodies in all cases of malaria but never in those without malaria. He also noted that quinine removed these stages from the blood. Laveran quickly realised that he had found a parasitic protozoan which he called *Oscillaria malariae*. He presented his findings to the French Academy of Medical Sciences in December 1880 [15] but failed to persuade any of the eminent microbiologists, zoologists or malariologists of the day that he was seeing anything other than disintegrating red blood cells. Nevertheless he persevered and by 1884 had convinced the leading Italian malariologists including Bignami, Golgi and Marchiafava that malaria was caused by a protozoan and not a bacterium [16]. His biggest triumph came in the same year when he also convinced

the more cynical microbiologists Louis Pasteur, Charles Edouard Chamberland and Pierre Paul Émile Roux. Robert Koch, one of the most influential microbiologists of his time, however, remained sceptical until 1887. Nevertheless in some quarters the miasma theory persisted and as late as 1895 the American R. C. Newton, a supporter of Tommasi-Crudeli, wrote that 'Aerial and aquatic transportation of malaria has been proved' [17]. (This paper is worth reading in full because, although based on what we now know to be false premises, it contains a mass of interesting information about the prevention of malaria such as the use of screens or mosquito nets to exclude insects, closing doors at night and lighting fires out of doors). Laveran was awarded the Nobel Prize for Medicine in 1907 and his discoveries are described in some detail by the Sergent brothers [18] and Bruce-Chwatt [19] as well as in the various histories of malaria listed above.

What was remarkable about Laveran's discovery was that it was without precedent as no protozoan had previously been found inhabiting any kind of human blood cell. Unbeknown to Laveran or the Italian malariologists, however, the Russian physiologist, Vassily Danilewsky had been examining the blood of birds and reptiles in the Ukraine and had discovered a number of parasites including trypanosomes and others that he identified as 'pseudovacules'. Anyone who has studied blood parasites will immediately recognise his description of 'pseudovacuoles' as unstained malaria parasites. By 1885 Danilewsky had recognised the three most common genera of intraerythrocytic blood parasites of birds now known as *Plasmodium*, *Haemoproteus* and *Leucocytozoon* but, as he had published much of his work in Russian, it was not until his three volume book *La Parasitologie Comparée du Sang* had been published in French in 1889 that this information became widely available [20]. Thereafter there began searches for other malaria parasites in reptiles, birds and mammals and this was facilitated by the accidental discovery of a methylene blue-eosin stain by Dimitri Leonidovitch Romanowsky in 1891 [6]. Romanowsky's stains became popular at the beginning of the twentieth century and remain the basis of blood stains such as Leishman's, Giemsa's and Wright's to the present day. These stains colour the nucleus of the parasite red and the cytoplasm blue permitting their easy identification and are used not only for malaria parasites but also for trypanosomes, leishmanias and filarial worms. Romanowsky's discovery is one of the most significant technical advances in the history of parasitology.

Meanwhile the Italian workers, now convinced that malaria was caused by a parasite, took up the challenge with vigour and Marchiafava and Bignami, using a combination of eosin-based blood stains and the oil-immersion microscope objective developed by the Carl Zeiss Company in 1882-4, observed amoeboid movement of the organism. This left them in no doubt that they were dealing with a protozoan parasite that invaded red blood cells, grew within the cells and produced daughter cells that invaded fresh blood cells [21]. Thereafter the Italian views dominated malaria research and, based on observations of the erythrocytic stages of the parasite, Golgi between 1885-6 differentiated between tertian (48 hour periodicity) and quartan (72 hour periodicity) malaria [22] and in 1889-1890 Golgi and Marchiafava further described the differences between mild Spring malaria (benign tertian) and severe Summer-Autumn (malignant tertian) malaria [23].

By this time it had also become clear that the paroxsms characteristic of malaria coincided with the bursting of infected red blood cells and the release of the products of multiplication something that Laveran, who had also realised that in the case of malignant tertian malaria the brain was involved, had proposed [24]. Thus by 1890 it was known that malaria was caused by a protozoan parasite that invaded and multiplied in red blood cells and, after a lot of confusion, that there were three species with specific periodicities and other characteristics responsible for benign tertian (*Haemamoeba vivax*), malignant tertian (*Laverania malariae*) and quartan (*Haemamoeba malariae*) malaria now respectively *Plasmodium vivax*, *P. falciparum* and *P. malariae*. The situation as it existed in 1900 is beautifully summarised by Grassi in his monograph, *Studi di uno Zoologo Sulla Malaria* [25] and, although more details have since been added, this work remains as relevant today as it was 110 years ago. In 1918, John Stephens, working in West Africa, discovered a fourth species which resembled *P. vivax* which he described as *P. ovale* in 1922 [26].

The sexual stages

The action now moves to Canada in 1897 and to the United States a year later where a medical student, William MacCallum, and his colleague, Eugene Opie, while examining the blood of crows infected with *Haemoproteus columbae*, a haematozoan closely related to the malaria parasites, observed flagellated structures which they described in detail and also recorded how the flagellated bodies fused with non-motile bodies to form a vermicule (now called an ookinete) [27]. MacCallum suggested that he was witnessing sexual reproduction that paralleled that in mammals (and, it should be noted, related sporozoans that were already familiar to European zoologists [28,29]) and that the flagellated forms were male gametes, the non-motile forms female gametes and the vermicule the zygote. MacCallum's findings are very significant as he realised that: 'Have we

not here...a sexual process...the result of which is the motile vermiculus? This is a process which we might have expected and which I am confident will be found to occur in the case of the human malaria parasites...' [30]. The significance of this observation initially eluded Ronald Ross (see below) something that remained with him for the rest of his life [31] but was not missed by Patrick Manson who wrote to Ross that 'MacCallum's observation on *Halteridium*; if it is correct, it is of the greatest importance' [31,32]. Here MacCallum reached a dead end because he believed that the vermicule then invaded cells of the vertebrate host but was unable to pursue this line of investigation.

Transmission

Despite all their accumulated knowledge and skills no malariologists could explain how the parasite spread from one human to another. The clues were, however, in place. Over the centuries, circumstantial evidence had accumulated that suggested that mosquitoes might somehow be connected with malaria and by 1883 the American physician, Albert King, had assembled the mass of evidence that was to become known as the mosquito-malaria doctrine [33]. Between 1884 and 1897, Laveran, Manson (who in 1877 had demonstrated that the filarial worms responsible for lymphatic filariasis were transmitted by mosquitoes [34]), and the Italian malariologists, had become increasingly convinced that mosquitoes were involved in the transmission of malaria. Thereafter opinions differed with some observers, including Manson, believing that humans became infected by drinking water contaminated by infected mosquitoes while others thought that the infection was acquired by inhaling dust from dried-up ponds in which infected mosquitoes had died, in other words, variations on the water and ingestion and air and inhalation theories proposed by Tommasi-Crudeli and Klebs in 1879. Manson also toyed with the idea that transmission might be mechanical, i.e. the parasites were passively carried from host to host on the proboscis of a mosquito.

By 1894 Manson, who had spent much of his working life in Taiwan and was then in his 50s and in an established medical practice in London, turned his attention to the possibility of mosquito transmission of malaria but, as he was unable to go to malarious countries himself, he needed someone to carry out the necessary investigations and experiments for him. His colleague-to-be was an unlikely choice, Ronald Ross (Figure 2).

Ronald Ross 1857-1932. In 1897 Ronald Ross working in India discovered that culicine mosquitoes transmitted the avian malaria parasite *Plasmodium relictum* and suggested that human malaria parasites might also be transmitted by mosquitoes. Later, when working in Sierra Leone in 1899, he demonstrated that the human malaria parasites were indeed transmitted by anopheline mosquitoes. In the meantime, however, several Italian scientists had already shown that this was the case.

Figure 2 Ronald Ross 1857-1932. Photograph by courtesy of the Royal Society of Tropical Medicine and Hygiene. Portraits of other scientists who were involved in the elucidation of the life cycle of the malaria parasites can be found elsewhere [9,40].

Ross then aged 37 was an established army surgeon working in India who did not believe that malaria was caused by a blood parasite but thought that it was an intestinal infection. Throughout the second half of 1894, Manson worked on Ross, showed him blood slides containing malaria parasites and convinced him that incriminating a mosquito vector of malaria was a goal worth aiming for. Ross returned to India and over the next four years Manson directed operations at a distance and we are fortunate to have an almost complete collection of the letters that passed between the two men [32]. This was not an easy relationship partly because Ross's first priorities were his military commitments and these inevitably delayed the work he was doing with malaria and partly because, from time to time, Ross seemed more interested in writing poetry and novels. Nevertheless, the cooperation reached a satisfactory conclusion but later ended in acrimony.

Manson, who had access to malaria patients in London, had observed that it was only when blood taken from such patients began to cool that the flagellated forms and subsequent fertilization, as described by MacCallum, appeared and concluded that further development must occur outside the human body in another host, probably a mosquito. Ross, having returned to India, examined several thousand mosquitoes from endemic areas without any success but, remembering Laveran's dictum 'follow the pigment' and Manson's advice to 'follow the flagellum', a reference to the flagella of the male gamete, he eventually found pigmented bodies, which he called spores, on the stomach wall of a mosquito experimentally fed on an infected patient. Ross was no entomologist (in fact the only book he had on entomology was one written for anglers) so he classified the mosquitoes he was studying as grey or barred-back (A), brindled (B), and dappled-winged (C). We now know that the grey mosquitoes were culicines and that the dappled-winged mosquitoes were anophelines. Grey mosquitoes were very common but never contained the pigmented spores. On the other hand the rarer 'dapple-winged' mosquitoes, after being fed on a malaria patient, were found to contain pigmented bodies that ruptured releasing 'rods' that invaded the mosquito's salivary glands. Ross had now made the crucial break-through and had found developmental stages of human malaria parasites in anopheline mosquitoes and, in his letters, he calls August 20th 1897 'Mosquito day' [31,32]. Ross was on the brink of demonstrating that anopheline mosquitoes could transmit human malaria but unfortunately he was not able to complete his studies because at this crucial stage he was posted to Calcutta where there was very little malaria [31]. He did, however, have access to laboratory facilities and, remembering that in 1894 Manson had mentioned the possibility of using malaria parasites of birds in his investigations, he turned his attention to an avian malaria parasite, *Proteosoma relictum* (now called *Plasmodium relictum*), common in many species of birds including crows and sparrows. This parasite, he discovered, was transmitted by his 'grey' (culicine) mosquitoes, probably *Culex fatigans*. Of 242 'grey' mosquitoes fed on infected birds, 178 developed pigmented spores. Ross concluded that mosquitoes fed on infected birds took up male and female gametocytes which fertilized in the mosquito gut and developed into spores on the surface of the mosquito's gut within which rod-like structures were produced that invaded the mosquito's salivary glands and were injected into a new host when the infected mosquito fed. His results were made public in 1898 [35,36]. Ross surmised correctly that human malaria was probably transmitted in the same way and later wrote that 'The triumph of 20 August was now completed and crowned by that of 9 July 1898' [31]. These experiments finally convinced Manson, that malaria was transmitted through the bite of a mosquito contrary to his earlier opinion that the infective stages were discharged into water. He nevertheless still thought that discharge of infective stages into water was the way that filiarial worms were transmitted until it was shown that they too were transmitted via the bite of a mosquito by George Carmichael Low in 1900 [37].

Although Ross had elucidated the whole of life cycle of *Plasmodium relictum* in culicine mosquitoes and had come tantalizingly close to completing the mosquito stages of the human malaria parasites the actual proof of the transmission of human malaria by anopheline mosquitoes still remained unresolved. Ross recorded that one single experiment could bring about the life cycle of human malaria [31] but his military duties took precedence and he was sent to work on an epidemic of plague that was then spreading across India and was not allowed to test his hypothesis because of the plague.

In the meantime several Italian workers were already on the trail. Bignami had suggested in 1896 that mosquitoes might transmit malaria by inoculation but it wasn't until 1898 that he and Grassi, who were fortunate to have access to sites where malaria was present near Rome and in Sicily, produced the final proof when they fed local *Anopheles claviger* mosquitoes on infected patients and subsequently transmitted the infection to uninfected individuals via the bite of these mosquitoes [38]. Over the next two years the Italians proved that only female *Anopheles* mosquitoes could transmit malaria and methodically consolidated their findings and described the whole blood-mosquito life cycles of *P. vivax*, *P. falciparum* and *P. malariae* (see Grassi's classical monograph, *Studi di uno Zoologo Sulla Malaria* [25]). Ross, in the meantime, had been posted to Sierra Leone where within a few weeks after his arrival in 1899 he had demonstrated the development of *P. falciparum, P. vivax* and *P. malariae* in anopheline mosquitoes. Meanwhile, in London, Manson persuaded Bignami and Bastianelli to send him *A. maculipennis* mosquitoes infected with benign tertian malaria which he used to infect his medial student son, Patrick Thurburn, and another volunteer thus completing this part of the story. More detailed accounts of these discoveries can be found in reviews by Ascenzi [39], Dobson [40] and Fantini [41] which were published together in the proceedings of a meeting held in Rome to commemorate one hundred years of malariology.

The discovery of the role of mosquitoes in the transmission of malaria provided malariologists with a new weapon against this ancient disease. In a classical experiment, Grassi dispatched 112 volunteers to the Capaccio Plains, a malarious area in Italy, protected them from

mosquito bites between dusk and dawn and found that only five succumbed to the disease compared with 415 unprotected volunteers who all contracted malaria [25]. Thus the possibility of controlling the disease by reducing contact with infected mosquitoes had been demonstrated. Over the next decades, methods to prevent mosquito biting by avoidance, screening and mosquito proofing dwellings and anti mosquito measures such as by the use of oils and larvivorous fish and draining mosquito habitats had become commonplace [9].

One surprising aspect of this whole story is that some of the clues about arthropod-transmission of blood-inhabiting protozoa were available several years before Ross and the Italian scientists began their investigations. In 1890 the American microbiologists Theobald Smith and Frederick Kilborne had observed that young ticks taken from cattle infected with the piroplasm *Babesia bigemina*, an intraerythrocytic protozoan resembling a malaria parasite, could infect susceptible animals and this was confirmed in a series of meticulously controlled experiments over the next two years [42]. It is strange that none of the participants in the malaria story seemed to be aware of these discoveries, probably because they were published in an American Government Agricultural document. How differently things might have turned out if they had been aware of these discoveries is a matter of speculation.

Exoerythrocytic development

The life cycle in humans, however, remained incompletely understood and nobody knew where the parasites developed during the first 10 days or so after infection during which they could not be seen in the blood. Grassi was the first to suggest that there must be some developmental stage in cells other than red blood cells, possibly white blood cells [25]. This theory was elaborated by Grassi and his colleagues from1893 and 1894 onwards but was later abandoned mainly due to too much reliance on a mistake by the influential German scientist Fritz Schaudinn who, in 1903, described the direct penetration of red blood cells by the infective sporozoites of *P. vivax* [43]. No one else was able to confirm these observations and the phenomenon is now referred to among malariologists unkindly as 'Schaudinn's fallacy'. Nevertheless Schaudinn's ideas were adopted by such authorities as Grassi and dominated scientific opinion for over forty years. Meanwhile evidence that there was a phase of multiplication preceding that in the blood was accumulating from another source, the avian malarias. MacCallum in 1898 had observed developmental stages of *P. relictum* in the liver and spleen of infected birds [30] and thereafter there were numerous somewhat inadequate descriptions of exoerythrocytic development of a number of avian malaria parasites [6,44]. In 1937 Sydney James and Parr Tate conclusively demonstrated that in sporozoite-induced *P. gallinaceum* infections in chickens there was phase of multiplication between the injection of sporozoites and the appearance of parasites in the blood and that this occurred in cells of the reticuloendothelial system [45].

By the late 1930s there was no doubt that in all the avian malaria parasites studied there was a phase of multiplication in various nucleated cells before (and after) parasites appeared in the blood and over the next decade the complete life cycles of a number of avian *Plasmodium* and *Haemoproteus* species, differing only in detail particularly relating to the types of cells involved which varied from species to species, had been worked out. What happened in primates was not so clear and during the 1930s and 1940s there were sporadic reports of parasites in the tissues, particularly in the brain and nervous system, of animals infected with primate and bat malarias. After the end of the Second World War in 1945 malaria research throughout the world intensified and a number of workers became convinced that that there must be an exoerythrocytic stage in the life cycle of the primate malarias but what form this took was not known. This question was not resolved until 1947 when Henry Shortt and Cyril Garnham, working in London, showed that a phase of division in the liver preceded the development of parasites in the blood [46]. The crucial clues came from studies on *Hepatocystis kochi*, another parasite of monkeys first identified by Laveran as *Haemamoeba kochi*. *Hepatocystis* spp. are related to malaria parasites but do not have an erythrocytic stage in their life cycles so these parasites must have only an exoerythrocytic stage which in *H. kochi* is in the parenchyma cells of the liver [47]. This suggested to Shortt, Garnham and their colleagues that the liver might be the place to look for the elusive exoerythrocytic stages of primate malaria parasites and selected *P. cynomolgi* in rhesus monkeys for their investigations. Previous attempts by other workers had failed to find any liver forms so Shortt and Garnham decided to use 500 infected *A. maculipennis atroparvus*, a massive dose of sporozoites, and found exoerythrocytic stages seven days later [48]. Shortly afterwards Shortt, Garnham and their co-workers found exoerythrocytic forms of *P. vivax* in human volunteers [49] and subsequently in volunteers infected with *P. falciparum* in 1949 [50] and *P. ovale* in 1954 [51]. In the meantime the same team had also demonstrated exoerythrocytic stages of *P. inui*, a quartan form of primate malaria. The exoerythrocytic stages of *P. malariae* were more elusive and it was not until 1960 that Robert (Bill) Bray demonstrated their presence in experimentally infected chimpanzees [52]. The story of the discovery of the exoerythrocytic forms of malaria

parasites until 1957 is told in some detail by Bray [44] and updated until 1966 by Garnham [6].

The story of the life cycle of the human malaria parasites was almost complete and had taken nearly 70 years to elucidate. There remained, however, one further question; what caused the long prepatent period between infection and the appearance and reappearance of parasites in the blood seen in some temperate strains of *P. vivax*? This led to the discovery of dormant exoerythrocytic stages, hypnozoites, by Wojciech Krotoski, working with Garnham's team, in 1982 [53].

Other malaria parasites

It has already been noted that malaria-like parasites are commonly found in birds, mammals and reptiles and studies on many of these have contributed to our overall understanding of human malaria. Malaria-like parasites belonging to the genus *Hepatocystis* in non-human primates were first recognised by Laveran in1899 but true malaria parasites, *Plasmodium* spp., were not identified with certainty until 1907 with the independent discoveries of *P. cynomolgi*, *P. inui*, and *P. pitheci* in monkeys imported into Germany from Java [6]. Throughout the 1920s and 1930s there were increasing numbers of reports of new species from wild-caught primates including *P. knowlesi* in 1932 [6,54]. During the 1960s, there were occasional reports of accidental infections with *P cynomolgi*, *P. inui* and *P. knowlesi* in humans suggesting that some primates might act as reservoirs for human malaria but it appeared that the chances of such naturally acquired infections were very remote. However it is now known that humans are at risk from infection with *P. knowlesi*, a malaria parasite with a 24 hour erythrocytic cycle, in Southeast Asia where its natural hosts are macaque and leaf monkeys. Until 1971 there had only been two authenticated cases of naturally acquired human infections with *P. knowlesi* both in peninsular Malaysia. No other cases were recorded until 2004 when a focus of human infections was identified in Sarawak, Malaysian Borneo [55]. Since then there have been several hundred reports of human infections in the region and there is now overwhelming evidence that *P. knowlesi* is a zoonosis involving macaque (*Macaca* spp.) and leaf (*Presbytis* spp.) monkeys as reservoir hosts with mosquitoes belonging to the Leucosphyrus group of *Anopheles* mosquitoes as the vectors in Malaysia and elsewhere in Southeast Asia [56]. Retrospective examination of blood films and the application of the polymerase chain reaction (PCR) and other molecular techniques have revealed that a number of malaria cases previously attributed to *P. malariae* in Malaysia were misidentified and that they were in all probability due to *P. knowlesi* [57].

The first avian malaria parasites were discovered at about the same time as the human species and there are now about 24 species including *P. relictum*, which has contributed most to our understanding of the transmission of human malaria parasites, and *P. gallinaceum* which not only contributed to our understanding of the exoerythrocytic phases of the malaria life cycle but also, because it could easily be maintained and mosquito-transmitted in chickens, served at the main model for chemotherapeutic studies until the discovery of rodent malarias.

The first rodent malaria parasite, *P. berghei*, was identified and isolated from wild rodents in Central Africa by Ignace Vincke and Marcel Lips in 1948 and subsequently adapted to mice, rats, hamsters and gerbils and easily maintained laboratory-bred mosquitoes such as *A. stephensi* [58]. Since then three other species, *P. yoelii, P. vinckei* and *P. chabaudi*, of which there are a number of subspecies and strains, have been identified, isolated and adapted to laboratory rodents and have become the mainstay of studies on chemotherapy and have served as surrogate models of human malaria in the fields of immunology, genetics, molecular biology and biochemistry [59].

In vitro cultivation

One of the most important breakthroughs in malaria research was the development of techniques that enabled scientists to grow the erythrocytic stages of malaria parasites in continuous culture pioneered by William Trager and J.B. Jensen [60] thus freeing investigators from the need to use animals for chemotherapeutic and biochemical studies. The importance of this discovery cannot be overemphasised. For the first time, scientists had access to unlimited quantities of human malaria parasites, particularly *P. falciparum*, thus reducing their dependence on laboratory animals and blood taken from humans. The ease with which the erythrocytic stages could be grown in bulk made it possible not only to test the effects of drugs directly but also to isolate and purify parasite components in order to identify biochemical pathways and molecules of potential use in the development of vaccines and chemotherapy. The cultivation of sexual stages provided insights into the genetics of human malaria parasites and the development of drug resistance. The cultivation of liver stages, although more difficult to achieve, made it possible to develop and test drugs against these stages and provided vital information about the immune responses in the liver. Finally, the cultivation of sporogonic stages has enabled scientists to discover what happens to the parasite in its mosquito vector.

The final [?] step

The final stage in the story of our understanding of the malaria parasites that began when an unknown French

scientist, working by himself in Algeria with a crude microscope, noticed that the blood of patients suffering from malaria contained organisms that he identified as parasitic protozoa culminated 122 years later when a massive team of investigators determined the compete genome of *Plasmodium falciparum* [61] since when the genomes of other malaria parasites have also been published [62].

Conclusions

Over a century later it seems appropriate to attribute the various discoveries concerning the malaria parasites and their transmission as follows. Laveran was the first person to find parasites in the blood of patients infected with malaria in 1880, MacCullum was the first to observe the sexual stages of a malaria-like parasite, *Haemoproteus columbae*, in birds in 1897, Ross was the first to show that any malaria parasite, in this case the avian *Plasmodium relictum*, was transmitted by the bite of infected mosquitoes in 1897 and, by implication, that this would be the case for human malarias and in 1898 Grassi, Bignami and Bastienelli were the first to demonstrate that human malaria parasites were actually transmitted in this way. The most far-reaching discovery made by Ross, and one that is frequently ignored, was that a blood-sucking insect could not only take up infective organisms from an infected individual but could also transmit them some time later when it fed on an uninfected host something that was completely contrary to the received opinion of the time. It took a long time before other investigators realised the universal importance of this discovery and it was not until the first decades of the twentieth century that diseases such as African trypanosomiasis, leishmaniasis, filariasis and loaiasis were discovered to be transmitted by the bites of infected insects. This discovery was not missed by virologists who, after the discovery of viruses, soon established the concept of arthropod-borne or arboviruses or by bacteriologists looking for the mode of transmission of the plague bacillus. The tissues stages in the blood were discovered half a century later, in 1947, by Shortt and Garnham and the final mystery, the persistence of liver stages was established by Krotoski in 1962. The story of the elucidation of the complex life cycle of the malaria parasites was only possible because the various scientists involved were able to transfer knowledge gleaned from non-human malarias in birds and primates to the problem of human malaria thus emphasising the importance of comparative studies in the investigation of human diseases.

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Competing interests

The author declares that they have no competing interests.

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References

1. Celli A: **A History of Malaria in the Italian Campagna from Ancient Times..** *London* 1933.
2. Stephens JWW: *Blackwater Fever, A Historical Survey and Summary made over a Century* London: Hodder and Stoughton 1937.
3. Scott HH: *A History of Tropical Medicine* London: Edward Arnold 1939, **1.**
4. Russell PF: *Man's Mastery of Malaria* London: Oxford University Press 1965.
5. Foster WD: *A History of Parasitology* Edinburgh: Livingstone 1965.
6. Garnham PCC: *Malaria Parasites and other Haemosporidia* Oxford: Blackwell Scientific Publications 1966.
7. Garnham PCC: *Progress in Parasitology* London: Athlone Press 1971.
8. Harrison G: *Mosquitoes and Malaria: A History of the Hostilities since 1880* London: John Murray 1978.
9. Bruce-Chwatt LJ: **History of malaria from prehistory to eradication.** *Malaria: Principles and Practice of Malariology* Edinburgh: Churchill LivingstoneWernsdorfer WH, McGregor I 1988, **1**:1-59.
10. Desowitz R: *The Malaria Capers* New York: WW Norton 1991.
11. McGregor I: **Malaria.** *The Wellcome Trust Illustrated History of Tropical Diseases* London: The Wellcome TrustCox FEG 1996, 230-247.
12. Poser CM, Bruyn GW: *An Illustrated History of Malaria* New York: Parthenon 1999.
13. Schlagenhauf P: **Malaria from pre-history to present.** *Infect Dis Clin N Amer* 2004, **18**:189-205.
14. Klebs E, Tommasi-Crudeli C: *Studi Sulla Natura della Malaria. Rome 1879: (Translated by Drummond E On the Nature of Malaria)* London: Selected Monographs of the New Sydenham Society 1888, **121**:1-56.
15. Laveran A: **Un nouveau parasite trouvé dans le sang de malades atteints de fièvre palustre. Origine parasitaire des accidents de l'impaludisme.** *Bull Mém Soc Méd Hôpitaux Paris* 1881, **17**:158-164.
16. Laveran A: *Traité des Fiévres Palustres avec la Description des Microbes du Paludisme* Paris: Doin 1884.
17. Newton RC: **Some observations which appear to establish the aerial transportation of malaria germs.** *Trans Amer Climatol Assoc* 1895, **11**:91-111.
18. Sergent Et, Sergent Ed: *La Decouverte de Laveran* Paris: Masson 1929.
19. Bruce-Chwatt LJ: **Alphonse Laveran's discovery 100 years ago and today's global fight against malaria.** *J Roy Soc Med* 1981, **74**:531-536.
20. Danilewsky B: **La Parasitologie Comparée du Sang. Nouvelles Recherches sur les Parasites du Sang des Oiseaux: Kharkoff.** 1889, **1.**
21. Marchiafava E, Bignami A: *On Summer-Autumn Malarial Fevers* London: New Sydenham Society 1894.
22. Golgi C: **Sul' infezione malarica.** *Arch Sci Med Torino* 1886, **10**:109-135.
23. Golgi C: **Sul ciclo evolutivo dei parassiti malarici nella febbre terzana: diagnosi differenziale tra i parassiti endoglobulari malarici della terzana e quelli della quartana.** *Arch Sci Med Torino* 1889, **13**:173-196.
24. Laveran A: *Paludism* London: New Sydenham Society 1893.
25. Grassi B: **Studi di uno Zoologo Sulla Malaria. .** *Rome* 1900.
26. Stephens JWW: **A new malaria parasite of man.** *Ann Trop Med Parasitol* 1922, **16**:383-388.
27. MacCallum WG: **On the flagellated form of the malarial parasite.** *Lancet* 1897, **ii**:1240-1241.
28. Wenyon CM: *Protozoology: A Manual for Medical Men, Veterinarians and Zoologists* London: Baillière Tindall Cox 1926.
29. Grassé P-P, (ed.): *Traité de Zoologie. Tome I, Fascicule I. Protozoaires: Rhizopodes, Actinopodes, Sporozoaires, Cnidosporides* Paris: Masson et Cie 1953.
30. MacCallum WG: **On the haematozoan infections of birds.** *J Exp Med* 1898, **3**:117-136.
31. Ross R: *Memoirs with a Full Account of the Great Malaria Problem and its Solution* London: John Murray 1923.
32. Bynum WF, Ovary C: **The Beast in the Mosquito: The Correspondence of Ronald Ross and Patrick Manson.** *Amsterdam* Rodopi BV 1998.
33. King AFA: **Insects and disease. Mosquitoes and malaria.** *Pop Sci* 1883, **23**:644-658.

34. Manson P: **On the development of *Filaria sanguis hominis* and on the mosquito considered as a nurse.** *J Linn Soc (Zool)* 1878, **14**:304-311.
35. Ross R: **The role of the mosquito in the evolution of the malaria parasite.** *Lancet* 1898, **ii**:489.
36. Manson P: **Surgeon-Major Ronald Ross's recent investigations on the mosquito-malaria theory.** *Brit Med J* 1898, **i**:1575-1577.
37. Low GC: **A recent observation on *Filaria nocturna* in *Culex*, probable mode of infection in man.** *Brit Med J* 1900, **i**:1456-7.
38. Grassi B, Bignami A, Bastianelli G: **Ulteriore ricerche sul ciclo dei parassiti malarici umani sul corpo del zanzarone.** *Atti Reale Accad Lincei* 1899, **8**:21-28.
39. Ascenzi A: **Malaria diseases and parasites.** *Parassitologia* 1999, **41**:33-38.
40. Dobson MJ: **The malaria centenary.** *Parassitologia* 1999, **41**:21-32.
41. Fantini B: **The concept of specificity and the Italian contribution to the discovery of the malaria transmission cycle.** *Parassitologia* 1999, **41**:39-47.
42. Smith T, Kilborne FL: **Investigations into the nature, causation and prevention of Texas or southern cattle fever.** *United States Department of Agriculture Bull Bureau Anim Indust* 1893, **1**:1-301.
43. Schaudinn F: **Studien über krankheitserregende Protozoen II. *Plasmodium vivax* (Grassi et Feletti) der Erreger des Tertianfiebers beim Menschen.** *Arbeit Kaiserlich Gesund* 1903, **19**:169-250.
44. Bray RS: *Studies on the Exo-erythrocytic Cycle in the Genus Plasmodium* London: HK Lewis 1957.
45. James SP, Tate P: **Exo-erythrocytic schizogony in *Plasmodium gallinaceum* Brumpt, 1935.** *Parasitology* 1938, **30**:128-139.
46. Shortt HE, Garnham PCC: **Pre-erythrocytic stages in mammalian malaria parasites.** *Nature* 1948, **161**:126.
47. Garnham PCC: **The developmental cycle of *Hepatocystes (Plasmodium) kochi* in the monkey host.** *Trans R Soc Trop Med Hyg* 1948, **41**:601-616.
48. Shortt HE, Garnham PCC: **The preerythrocytic-develoment of *P. cynomolgi* and *P. vivax*.** *Trans R Soc Trop Med Hyg* 1948, **41**:785-795.
49. Shortt HE, Garnham PCC, Covell G, Shute PG: **The pre-erythrocytic stage of human malaria *Plasmodiun vivax*.** *Brit Med J* 1948, **1**:547.
50. Shortt HE, Fairley NH, Covell G, Shute PG, Garnham PCC: **A preliminary note on the pre-erythrocytic stage of *Plasmodium falciparum*.** *Brit Med J* 1949, **2**:1006-8.
51. Garnham PCC, Bray RS, Cooper W, Lainson R, Awad FI, Williamson J: **Pre-erythrocytic stages of human malaria: *Plasmodium ovale*. A preliminary note.** *Brit Med J* 1954, **1**:257.
52. Bray RS: **Studies on malaria in chimpanzees. VIII. The experimental transmission and pre-erythrocytic phase of *Plasmodium malariae* with a note on the host range of the parasite.** *Amer J Trop Med Hyg* 1960, **9**:455-65.
53. Krotoski WA, Collins WE, Bray RS, Garnham PCC, Cogswell FB, Gwadz R, Killick-Kendrick R, Wolf RH, Sinden RL, Koontz C, Stanfill PS: **Demonstration of hypnozoites in sporozoite-transmitted *Plasmodium vivax* infection.** *Amer J Trop Med Hyg* 1982, **31**:1291-1293.
54. Collins WE: **Major animal models in malaria research: simian.** *Malaria: Principles and Practice of Malariology* Edinburgh: Churchill LivingstoneWernsdorfer WH, McGregor I 1988, **2**:1073-1501.
55. Singh B, Lee KS, Matusop A, Radhakrishnan A, Shamsul SSG, Cox-Singh J, Thomas A, Conway DJ: **A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings.** *Lancet* 2004, **363**:1017-24.
56. Galinski MR, Barnwell JW: **Monkey malaria kills four humans.** *Trends Parasitol* 2009, **25**:200-204.
57. Cox-Singh J, Davis TME, Lee-Kim S, Shamsui SSG: ***Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening.** *Clin Infect Dis* 2008, **46**:165-71.
58. Vincke IH, Lips M: **Un nouveau Plasmodium d'un rongeur sauvage du Congo, *Plasmodium berghei* n.sp.** *Ann Soc Belge Méd Trop* 1948, **28**:97-104.
59. Cox FEG: **Major animal models in malaria research: rodent.** *Malaria: Principles and Practice of Malariology* Edinburgh: Churchill LivingstonWernsdorfer WH, McGregor I 1988, **2**:1503-1543.
60. Trager W, Jensen JB: **Human malaria parasites in continuous culture.** *Science* 1976, **193**:673-675.
61. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan M-S, Nene V, Shallom SJ, Suh B, Peterson , Angiuoli JS, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DMA, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B: **Genome sequence of the human malaria parasite *Plasmodium falciparum*.** *Nature* 2002, **419**:489-511.
62. Carlton JM, Escalante AA, Neafsey D, Volkman SK: **Comparative evolutionary genetics of human malaria parasites.** *Trends Parasitol* 2008, **24**:545-550.

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Dear Mr. Harper and Mr. Andoh,

I've finally reviewed the malaria papers that were cited by Roger, and found that none of them match what I required.

It's clear once again that the people running the CDC have no science to back up their claims. And once again, I'm quite certain that going along with such fraud entails various USC violations.

> Below is the related entry in my newsletter which has been disseminated to the public, here https://christinemasseyfois.substack.com/p/germ-fois-del-bigtree-india-project and via my email list and via Facebook here: https://www.facebook.com/photo?fbid=191368700365399&set=a.107538792081724.
>
> *December 5, 2022:*
> *Roger Andoh acting as FOIA Officer in the Office of the Chief Operating Officer,* ***Centers for Disease Control and Prevention*** *failed to provide or cite any record that describes controlled experiments* ***proving that parasites cause malaria****;*
>
> *Instead, Roger cited 5 irrelevant/unscientific papers. Very briefly:*
>
> *1st paper, Studies on Human Malaria....*
> *- small sample size, no controls, invalid independent variable, not even designed to test for causation of malaria*
> *- monkeys were inoculated intrahepatically with crushed mosquito salivary glands and monkey serum-saline, as well as intravenously with supernatant from centrifuged crushed mosquito bodies (minus salivary glands) and monkey serum-saline;*
>
> *2nd paper, Chloroquine Resistant...*
> *- small sample size, no controls, invalid independent variable, not even designed to test for causation of malaria*
> *-infected blood from a human was innoculated intravenously into 1 splenectomized monkey, then blood from that monkey was subpassaged into other splenectomized monkies, etc.*
>
> *Useless animal torture!*
>
> *The remaining papers are equally irrelevant and unhelpful. (I will address the CDC's response to the remaining FOI that is mentioned in this letter, in my next newsletter):*
> *https://www.fluoridefreepeel.ca/wp-content/uploads/2023/03/CDC-parasites-causing-malaria-PACKAGE-redacted.pdf*

by: Christine:
[Quoted text hidden]