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EXPERIMENTAL EFFORTS TO TRANSFER MONKEY MALARIA TO MAN[1]

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A preliminary report on malaria in wild monkeys of Panama was made by the senior author (1) about one year ago but no success attended his efforts to secure a living specimen showing the disease. A later expedition has been made that did secure six juvenile and two infant monkeys with malaria. These animals were captured in the uninhabited portion of Panama known as the Coto and then transferred to our laboratory and placed in screened quarters.

Several experiments in the transfer of monkey malaria to man have been made in the Eastern hemisphere that produced refractory results or were surrounded with circumstances that seemed to leave the question in doubt. In view of the fact that our Western hemisphere monkey malaria is difficult if not impossible to distinguish from human quartan and tertian malaria, it seemed wise to repeat such experiments in our region.

Our experiments have been done in collaboration with the Gorgas Hospital and the Panama Canal Department of the United States Army. The former supplied the necessary hospital requirements and the latter, through the splendid support of the Commanding General of the Department and the Surgeon at the Corozal Post, secured human volunteers and selected from them the class of men desired for the work. We sought for young men who had lived in Northern States where malaria was not endemic and who had never left those regions until assigned to military duty on the Isthmus. During the month of March, 1930, eight volunteers were accepted. Note table 1 for the salient points of interest to us.

[1] Presented at the American College of Surgeons Annual Meeting, October, 1930, at Philadelphia, Pa.

MONKEYS USED IN EXPERIMENT

Three juvenile and one infant red spider monkey (*Ateles geoffroyi* Kuhl) brought from the jungle of the Coto, Republic of Panama and having naturally acquired malarial infections were used as the donors in attempts to infect men and mosquitoes. One juvenile black spider monkey (*Ateles dariensis* Goldman) and one white faced monkey (*Cebus capucinus imitator* Thomas) were used as control animals against the direct blood method used on the men. These two monkeys had spent most of their lives in captivity and were negative daily over a long period of blood film examinations.

TABLE 1

NAME	AGE	TIME ON ISTHMUS	HOME STATE
		days	
R. F. A.	19	14	Michigan
E. C.	21	11	Michigan
A. A. R.	22	14	Michigan
O. A. D.	20	30	Wisconsin
H. J. B.	18	14	Connecticut
A. D.	21	7	Pennsylvania
J. H. K.	21	7	Pennsylvania
C. R. G.	21	7	Iowa

The three juvenile red spider monkeys (Lab. Nos. 3, 4 and 6) were carrying a moderate degree of malarial infection. The infant red spider monkey (Lab. No. 7) was seriously ill with malaria and died before the experiments were concluded.

FIRST STAGE OF EXPERIMENT

The first five men listed in the table were selected for the direct blood transfer from the juvenile red spider monkeys (Nos. 3, 4 and 6) since these animals were large enough to stand the shock of aspirating blood in sufficient amount for use. The infant red spider monkey only weighed 1½ pounds and was too sick to risk as a donor of blood for the men. It was, therefore, saved to act as host for the mosquitoes. The juvenile monkeys carried a moderate but satisfactory degree of malarial infection for use in

the test. These animals were given a general anesthetic (ether) on March 11, 1930, and blood was then aspirated from the heart under aseptic conditions. They recovered from the shock promptly and all of them are still living and showing occasional malarial relapses. The fresh whole blood was immediately administered to the five men without the addition of any saline or citrate solutions. Each man received 1 cc. intravenously and 0.5 cc. subcutaneously except in the case of H. J. B. whose intravenous dose was 0.5 cc. due to trouble caused by coagulation of the specimen. The men were sent to Gorgas Hospital for isolation and observation. There was no apparent reaction to the introduction of this foreign blood. We conducted thin and thick blood film examinations twice a day throughout the entire period of confinement in the hospital in addition to the routine examinations made by Gorgas Hospital.

BLACK SPIDER MONKEY CONTROL (LAB. NO. 11)

At the time these five men were given the direct blood inoculations this control monkey was also given 1 cc. of red spider monkey blood. This was an intraperitoneal and subcutaneous inoculation. The monkey (Lab. No. 11) developed positive blood films for malaria on the eleventh day, later became very ill with the disease and died of it on the forty-eighth day after the inoculation. A solution of quinine (3 grains a day) was given during the last four days of its illness with no apparent effect.

None of the five men developed any noteworthy evidence of illness although a few doubtful intracorpuscular bodies were found in an occasional blood film by some one of the four technicians who searched the daily set of blood films. At the close of two weeks the direct blood method was considered a failure and we resorted to efforts at mosquito transfer with this group as well as the second group of three men.

SECOND STAGE OF EXPERIMENT. TRANSFER EFFORTS WITH MOSQUITOES

We, of course, did not know what, if any, mosquitoes were responsible for malaria in monkeys. This work was done in the

dry season when it is difficult to find mosquito larvae in sufficient abundance to support such an experiment. Since the *Anopheles* mosquitoes play an important rôle in the transfer of human malaria we used all of the available larvae of this genus to rear and feed on our monkeys. We worked with *Anopheles albimanus*, *Anopheles tarsimaculatus*, *Anopheles argyritarsis* and *Anopheles pseudopunctipennis*. These four species happen to include the most important transmitters of malaria in Panama. Darling's (2) experimental work on attempts to infect the *Anopheles* of Panama found that he could infect 70.2 per cent of *A. albimanus*, 60 per cent of *A. tarsimaculatus*, 12.9 per cent of *A. pseudopunctipennis*. He had too few of *A. argyritarsis* to form an opinion but he later found one of this species naturally infected in a house catch that he examined.

Mosquito biting on the malaria monkeys was started with a few lots on March 10, 1930 and was continued until March 25, 1930. Mosquito lots 1, 2, 3, 4, 5, 6, 7 and 8 were fed on the sick infant red spider monkey (Lab. No. 7) and this monkey's blood was richly parasitized. All forms of the parasites could be found but gametes were very abundant, ranging as high on one day as 100 to a field in a thick blood film.

Mosquito lots 9 to 20 were fed on the juvenile red spider (Lab. No. 6). This monkey's blood film contained about 1 gamete to two microscopic fields. See table 2 for mosquitoes used.

On March 19, 1930 the mosquitoes were considered ready to use on the men. These were the last three men on the list. *A. tarsimaculatus* was used on A. D. who received 25 bites from March 20 to April 1, 1930. Lots 7, 8, 10, 12, 13 and C were used. *A. pseudopunctipennis* was fed on J. H. K. who received 30 bites from March 19 to April 1, 1930. Mosquito lots 3, 5 and 11 were used. *A. albimanus* was selected to feed on C. R. G. who received 45 bites from March 19 to 26, 1930. Mosquito lots 1, 2, 4 and 6 were used and all of these were fed on the seriously ill infant monkey No. 7.

These men were observed until April 21 without findings of any consequence to record except in the case of A. D. who ran an elevation of temperature to 100.6 on April second and there-

after had several elevations at or near 100°. On April 17 and May 7 he showed one or two forms that we consider to be malarial parasites but there was nothing satisfactory to report.

TABLE 2

Record of mosquitoes used in biting experiments on the transmission of monkey malaria

LOT NUMBER OF MOSQUITOES	NUMBER OF MOSQUITOES IN LOT	SPECIES OF MOSQUITOES	FED ON INFECTED MONKEY NUMBER	DATE OF INFECTIVE FEEDING
1	17	*A. albimanus*	7	March 10*
2	9	*A. albimanus*	7	March 10–11
3	17	*A. pseudopunctipennis*	7	March 11
4	10	*A. albimanus*	7	March 12–13
5	5	*A. pseudopunctipennis*	7	March 12
6	10	*A. albimanus*	7	March 14–15
7	14	*A. tarsimaculatus*	7	March 14–15
8	7	*A. tarsimaculatus*	7	March 17
9	5	*A. albimanus*	6	March 17
10	5	*A. tarsimaculatus*	6	March 17
11	4	*A. pseudopunctipennis*	6	March 17
12	7	*A. tarsimaculatus*	6	March 19
13	12	*A. tarsimaculatus*	6	March 20
14	4	*A. argyritarsis*	6	March 20
15	19	*A. tarsimaculatus*	6	March 21
16	24	*A. tarsimaculatus*	6	March 21–22
17	8	*A. argyritarsis*	6	March 21–22
18	6	*A. pseudopunctipennis*	6	March 22
19	18	*A. tarsimaculatus*	6	March 24–25
20	14	*A. pseudopunctipennis*	6	March 24–25
21	4	*A. argyritarsis*	6	March 24–25
A	2	*A. argyritarsis*	6	March 17
B	2	*A. albimanus*	6	March 24–25
C	1	*A. tarsimaculatus*	6	March 25

* (This lot nearly all fed on Monkey 7 again on March 14.)

STAGE THREE OF THE EXPERIMENT

It was now decided to use two men from this last group for a direct inoculation subcutaneously of heavily parasitized monkey blood from the black spider monkey (Lab. No. 11). This was the control animal in the first stage of the experimental work. A. D. was permitted to continue since it was thought he might

develop an attack. On April 21, 1930, J. H. K. and C. R. G. were taken to the laboratory where they each received 2 cc. of fresh whole blood from the heart of the sick black spider monkey. This was injected subcutaneously at the lower angle of the left scapula. This monkey's blood was very heavily parasitized at the time. Nothing of note occurred as a result of these injections in the men. The white face monkey (Lab. No. 9) was also injected with blood from the black spider monkey as a control. The intraperitoneal and subcutaneous methods were used. It became positive for malaria on the 11th day and passed a serious illness but now appears to have recovered spontaneously. There are times when its blood films still show a few parasites.

STAGE FOUR OF THE EXPERIMENT

The first group of five men were now subjected to mosquito biting.

A. pseudopunctipennis was used on R. F. A. who received a total of 19 bites, April 1 to 7, 1930. Mosquito lots 3, 5, 11 and 20 were used.

A. tarsimaculatus was used on A. A. R. who received 28 bites from March 30 to April 7, 1930. Mosquito lots 12, 13, 16 and C were used.

A. tarsimaculatus was also used on E. C. C. who received 28 bites from March 30 to April 7, 1930. Mosquito lots 8, 10, 15 and 19 were used.

A. albimanus was used on O. A. D. He received 21 bites from March 27 to April 7, 1930. Mosquito lots 1, 4, 6, 9 and B were used.

A. argyritarsis was used on H. J. B. and he received 15 bites from March 26 to April 7, 1930. Mosquito lots 14, 17, 21 and A were used.

Nothing resulted from these efforts to transfer the parasite.

Mosquitoes dissected three weeks after feeding on monkey malaria revealed glands positive in 2 out of 18 *A. tarsimaculatus*, 1 out of 7 *A. albimanus*. *A. pseudopunctipennis* (11) and *A. argyritarsis* (4) were negative.

The first group of five men were admitted to Gorgas Hospital March 11, 1930, and were discharged April 28, 1930. The case numbers are 313028 to 313032.

The second group of three men were admitted March 19, 1930, and discharged May 12, 1930. The case numbers are 313337 to 313339.

CONCLUSIONS

1. We conclude that red spider monkey malaria and the human benign species of malaria that it so closely resembles are not identical and that the monkey is not, therefore, a reservoir for human malaria, in view of the fact that two control monkeys of different species from the red spider monkey developed acute attacks of malaria on schedule time (eleven days) and that no symptoms and no satisfactory laboratory findings developed in eight men representing the best type of non-immune subjects.

2. It remains to be seen, however, what may happen when an infant monkey (born free of opportunities to acquire monkey malaria) is inoculated with human malaria of the benign species. We await this opportunity, but circumstances indicate it will not acquire the disease.

3. The knowledge gained by our local field and laboratory work on monkeys offers one practical feature. The infant and juvenile monkeys of certain species (*Ateles* and *Cebus*) offer a better animal for research in malaria than the bird which is customarily used.

4. It is interesting to note that two specimens of *A. tarsimaculatus* and one specimen of *A. albimanus* fed on the monkeys showed positive glands on dissection. Further work is indicated in feeding mosquitoes on monkey malaria since it may at least assist in providing teaching material.

REFERENCES

(1) Clark, Herbert C.: A preliminary report on some parasites in the blood of wild monkeys of Panama. Amer. Jour. Trop. Med., January, 1930, x, no. 1, 000.

(2) Darling, S. T.: Studies in Relation to Malaria. Second Edition, page 23. Publication of the Isthmian Canal Commission (N. D.).

DISCUSSION

Colonel Chas. F. Craig (Medical Corps, U. S. Army): The occurrence of malaria in persons travelling through supposedly uninhabited localities has given rise to the query as to whether some animal acts as a reservoir of infection for man. Plasmodia, resembling those causing

malaria, have been found in numerous species of birds, in bats, squirrels, the jumping rat, the flying fox, antelopes, the Indian buffalo, goats, and various species of monkeys. None of the species described, except those occurring in monkeys, have been experimented with as regards the possibility of their transference to man, but the species occurring in monkeys have been studied because of their marked resemblance, in morphology, to those causing malaria in man.

As long ago as 1898, Koch found a plasmodium in the blood of monkeys of the genera Cercopithecus, Cynocephalus and Cercocebus, and to this parasite was given the name of *Plasmodium kochi*. The same parasite was seen later by Ziemann, Bruce and Nabarro, Dutton, Todd and Tobey, Gonder and Berenberg-Gossler, Sergent and others who observed the plasmodium in various species of monkeys belonging to the genus Cercopithecus. It is a common species in these monkeys, and resembles, in its life cycle and morphology, the tertian parasite of man, *Plasmodium vivax*. It is not very pathogenic to monkeys and is affected by quinine. Gonder and Rodenwalt (1908) attempted to infect two human beings with this parasite by the inoculation of blood containing it from infected monkeys, but were unsuccessful in producing the infection.

Another species occurring in monkeys was described by Halberstaedter and Prowazek in 1907, who found a plasmodium in the blood of monkeys in Borneo resembling *Plasmodium vivax*, but accompanied by forms which also resembled *Plasmodium malariae* and *Plasmodium falciparum*. This parasite was named *Plasmodium inui*, and has since been studied by other observers and given different names. This species is common in lower monkeys belonging to several genera, but is not found in the higher apes, as the chimpanzee and the orang-outang. It is very pathogenic to some monkeys, causing death with heavy infections, and quinine has no effect upon this plasmodium.

The same authors described another species of plasmodium occurring in the orang-outang in Borneo in 1907 and named it *Plasmodium pitheci*. In morphology it resembles *Plasmodium vivax*, and is inoculable to other orang-outangs but not to the lower monkeys. This species is considered by Reichenow to be probably identical with one of the plasmodia of man, but no experimental evidence is available which proves this to be true.

In 1917, Reichenow described plamodia that he found in the blood of chimpanzees and gorillas in the Cameroons. In 1920 he more fully described these parasites and stated that in chimpanzees and gorillas there occurred plasmodia indistinguishable in morphology from *Plas-*

modium vivax, *Plasmodium malariae* and *Plasmodium falciparum* of man. As he observed these plasmodia only in apes coming in close contact with man, and thereby liable to bites from mosquitoes infected with the human parasite, he regarded the species in apes as identical with those of man.

Blacklock and Adler (1922–1924) described plasmodia in the blood of chimpanzees in West Africa identical in morphology with the three human species of man, and to the parasite resembling *Plasmodium falciparum*, the name *Plasmodium reichenowi* has been given by Sluiter, Swellengrebel and Ihle (1922). These plasmodia are probably identical with those described by Reichenow. In two experiments upon volunteers, the inoculation of blood, both subcutaneously and intravenously, from chimpanzees infected with these plasmodia, resulted negatively, and they were also unable to infect *Anopheles costalis* from infected chimpanzees. They also endeavored to infect a young chimpanzee by the inoculation of human blood containing *Plasmodium falciparum*, but were unsuccessful.

With the exception of the species described by Doctor Clark, there is a record of but one species of parasite described in monkeys in the Western Hemisphere. This organism, known as *Plasmodium brazilianum*, was found in a monkey belonging to the genus Brachyurus from the Amazon district, and resembling *Plasmodium malariae* of man.

The only instance of the successful transmission of a malaria plasmodium of man to monkeys is the experiment of Mesnil and Roubaud in 1920. These investigators produced an infection in a chimpanzee by the intravenous inoculation of blood from a human case of infection with *Plasmodium vivax*, but they failed in a second attempt with another chimpanzee, and were unable to infect still another chimpanzee by the bites of *Anopheles maculipennis*. The successful result of this experiment may well be questioned, as it may be explained by the lighting up of a pre-existent infection in the chimpanzee with the monkey plasmodium, identical in morphology with *Plasmodium vivax*. The lighting up of the infection may have been due to the injection of a foreign protein, i.e., human blood, and such a latent infection cannot be eliminated. As an instance of such an occurrence, the experiment of Knowles in 1919 may be quoted. This observer inoculated a monkey with 2 cc. of human blood containing crescents of *Plasmodium falciparum*. The monkey became ill about twenty-four hours later and died within forty-eight hours, showing a very intense malarial infection with organisms resembling *Plasmodium falciparum* and *Plasmodium vivax*. As

such intense infection could not have originated from the parasite injected, it was evident that the injection had lighted up a pre-existent, latent infection with the monkey plasmodia. Bass (1922) attempted to produce an infection in four guinea pigs, five rabbits, and one monkey, by the injection of blood containing *Plasmodium falciparum*, but was unsuccessful in all these animals. He stated that horses, mules, dogs, foxes, monkeys, rabbits, screech owls, turtles, frogs and lizards have been inoculated with human blood containing the malaral plasmodium with an infection resulting in no instance.

It is evident that the number of experiments that have been made upon human beings in attempting to transmit plasmodia of monkeys, have been so few that, although they have resulted negatively, it cannot be considered as proven that such transmission is impossible. It is very suggestive that, even among monkeys, it is impossible apparently to transmit the species found in the higher apes to lower monkeys and vice versa, and it is most probable that plasmodia occurring in various animals are so specific that their transmission from one species of animal to another is impossible. However, it should be remembered that in the process of evolution, it is more than probable that parasites of man developed originally from those of some lower animal, and that experiments upon scores of volunteers instead of half a dozen or so, might result in success. For this reason, it is believed that further study should be made of this subject, using a much larger number of volunteers for the purpose, as otherwise it will be impossible to state scientifically that the transmission of monkey malaria to man is impossible.

In his experiments, Doctor Clark was successful for the first time in actually infecting Anopheles mosquitoes with monkey malaria by allowing them to feed upon infected animals. Several observers have experimented along this line, but experiments have been few in number and have been unsuccessful up till now, and in fact until these experiments of Doctor Clark, we did not know whether monkey malaria was transferred by Anopheles or by some other genus of mosquitoes.