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FURTHER OBSERVATIONS ON THE COMPARATIVE SUSCEPTIBILITY OF NEARCTIC AND NEOTROPICAL ANOPHELINES TO COINDIGENOUS STRAINS OF PLASMODIUM FALCIPARUM[1]

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The opportunity afforded by the acquirement of a strain of *Plasmodium falciparum*, evidently derived from the Republic of Panama, suggested the desirability of extending the studies on relative susceptibility already reported (1, 2).

The history of the patient from whom the strain was secured is as follows:

The patient, a white male aged 45, a forester by occupation, had been a resident of Pensacola, Florida, for the year prior to the events described. To his recollection he had never previously experienced any unexplained febrile illness, nor prior to the trip discussed, had ever been in the tropics. He left Pensacola by plane on March 28, 1938, for the Canal Zone, arriving March 30. A few days later he left on a trip to inspect timberlands in the interior of the Republic of Panama, in the course of which he was at the town of David on April 5, but did not spend the night at that place. David was the starting point of an extended camping

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trip to undisclosed points in the region. He returned to the United States by plane before the middle of the month. On April 16, immediately after his arrival in Pensacola, he became ill. experiencing daily chills for three weeks despite quinine therapy, The infection was microscopically diagnosed as falciparum malaria on April 16 or 17. For a few days subsequent to the diagnosis, he was given a total daily dose of 20 grains of quinine, which was soon increased to a minimum of 30 grains, and on a few days as much as 60 grains were given. Although the primary clinical attack was finally checked, parasites continued to be readily detected. Several early relapses were experienced some of which were checked by quinine, while on one occasion a course of atebrin was administered. He went to western North Carolina, with the hope that a change of climate plus further quinine therapy would master the infection. However, on returning to Pensacola in June, the infection again became active and, despite therapy, continued active into July when he returned to North Carolina, remaining until November. On November 25, shortly after returning to Pensacola, a crescent was detected. Through the courtesy of his physician, Dr. Charles A. Born of Pensacola, we were furnished with a quantity of his defibrinated blood, collected on that date, to which sufficient dextrose to make 0.5 per cent, had been added. On its receipt on April 26 at the Florida State Hospital, 10 cc. were intramuscularly inoculated into patient B1878, in whom parasites were detected on December 10, and who experienced a clinical onset on December 14. This strain has been subsequently propagated through several passages by both natural and artificial inoculation. In the subinoculated patients it has shown a tendency to produce more frequent relapses than have other available strains of this species and to require larger amounts of quinine to control.

The protracted clinical course which the original patient experienced, indicates his complete susceptibility. The onset immediately after his return to Pensacola after an absence of less than three weeks suggests that the infection was acquired during the journey. In view of the time of year in which he departed from Pensacola, it is very improbable that the infection could have

been acquired before his departure. Our experience (3) with naturally induced falciparum malaria has presented instances of incubation periods varying from six to twenty-five days, with nearly two thirds terminating the incubation from the eleventh to the thirteenth days after incubation. Obviously counting backward eleven to thirteen days from the patient's onset of illness on April 16, would place him at or near David on his trip to the western part of the republic. From the standpoint of the United States the strain is undoubtedly exotic, while the probabilities favor the idea of its Panamanian origin.

TABLE 1

Results of cross-inoculations with the Long (indigenous to Florida), Mexican and Panamanian strains of P. falciparum

PATIENT	ORIGINAL INFECTION		REINOCULATION	
	Strain	Duration of attack	Strain	Duration of attack
		days		*days*
1175, B1879	Long	11	Mexican	5
1190, B1882	Mexican	9	Long	5
B1863	Long	6	Panamanian	6
B1885	Panamanian	21	Long	10
B1876, 1191	Mexican	10	Panamanian	3
B1883	Panamanian	14	Mexican	6

In order to ascertain whether it was antigenically distinct from the Long and Mexican strains of *P. falciparum* which we have been currently propagating, a series of cross-inoculations of convalescents were performed as outlined in table 1.

The clinical attacks following reinoculation indicate that whatever immunity developed during or subsequent to the primary infection did not afford protection against an attack on reinoculation with parasites of different origin. We thus conclude that these parasites are antigenically distinct and hence represent different strains.

With the permission of the United States Public Health Service, two lots of *Anopheles albimanus* ova, deposited by colonized females maintained in the insectary of the Gorgas Memorial

Institute, Panama, Republic of Panama, were shipped by air express in insulated containers to Tallahassee, Florida. Hatching did not begin until the ova were placed in bowls of water in the Tallahassee insectary. An abundance of imagines were reared from each shipment, the females of which were employed in the comparative infection experiments to be described, with insectary reared female *A. quadrimaculatus* simultaneously applied as controls.

At an appropriate time prior to the expected dates of emergence of the exotic anophelines, susceptible patients were especially

TABLE 2

Comparison of the susceptibility of A. albimanus and A. quadrimaculatus when simultaneously infected with the Long strain of Plasmodium falciparum

SUBJECT	DATE	GAMETOCYTE PER CMM.		EXFLAGELLATION	A. ALBIMANUS							A. QUADRIMACULATUS						
		Male	Female		Lot	Fed	Examined	Per cent +	Cysts			Lot	Fed.	Expelled	Per cent +	Cysts		
									-10	11-30	31-50					-10	11-30	31-50
1213	6/ 7/39	820	570	−	873	7	7	0	−	−	−	874	6	6	33.3	2	−	−
1213	6/ 9/39	510	1,280	−	882	10	9	33.3	3	−	−	883	10	10	80	4	4	−
1213	6/12/39	190	860	−	884	9	8	0	−	−	−	885	10	10	80	7	−	1
1213	6/13/39	0	1,140	−	886	7	7	0	−	−	−	887	9	9	33.3	3	−	−
1221	8/ 8/39	1,660	12,900	+	931	10	9	0	−	−	−	932	11	10	10	1	−	−
All							40	7.5	3					45	48.9	22		
First series							31	9.7	3					35	60.0	21		
Second series							9	0.0	0					10	10.0	1		

inoculated with these different strains of *P. falciparum*, in order that their period of maximum infectiousness would coincide with the availability of the *albimanus* imagines. The various lots enumerated were limited to specimens of each species which actually fed on the patients.

The results of comparative infection experiments in which infection was secured in at least one of the lots on application to patients infected with the Long strain, which is coindigenous with *quadrimaculatus* are presented in table 2.

The results of comparative infection experiments when applied

to a patient infected with the Mexican strain, which is not coindigenous to either anopheline, are presented in table 3.

TABLE 3

Comparative susceptibility of A. albimanus and A. quadrimaculatus when simultaneously infected with the Mexican strain of P. falciparum

SUBJECT	DATE	GAMETOCYTE PER CMM.		EXFLAGELLATION	A. ALBIMANUS								A. QUADRIMACULATUS							
		Male	Female		Lot	Fed	Examined	Per cent +	Cysts				Lot	Fed	Expelled	Per cent +	Cysts			
									-10	11-30	31-50	51-100					-10	11-30	31-50	51-100	101+
B1891	8/3/39	560	380	−	911	12	11	9.2	1	−	−	−	912	11	10	40	2	2	−	−	−
B1891	8/4/39	280	260	+	916	19	9	22.2	2	−	−	−	917	10	10	80	5	3	−	−	−
B1891	8/7/39	230	520	+	924	9	9	11.1	1	−	−	−	925	10	10	80	5	3	−	−	−
B1891	8/9/39	630	0	−	935	9	8	12.5	1	−	−	−	936	5	5	0	−	−	−	−	−
All							37	13.5	5						35	57.1	20			

TABLE 4

Comparison of the susceptibility of A. albimanus and A. quadrimaculatus when simultaneously infected with the Panamanian strain of P. falciparum

SUBJECT	DATE	GAMETOCYTE PER CMM.		EXFLAGELLATION	A. ALBIMANUS							A. QUADRIMACULATUS						
		Male	Female		Lot	Fed	Examined	Per cent +	Cysts			Lot	Fed	Expelled	Per cent +	Cysts		
									-10	11-30	31-50					-10	11-30	31-50
B1890	6/7/39	110 total		−	871	9	9	11.1	1	−	−	872	8	6	0	−	−	−
B1890	6/9/39	60 total		−	880	9	9	11.1	1	−	−	881	9	9	0	−	−	−
B1836	8/2/39	0	340	+	906	12	12	41.7	5	−	−	907	11	10	30	3	−	−
B1836	8/3/39	130	260	−	909	12	12	41.7	4	1	−	910	9	9	55.6	5	−	−
B1836	8/4/39	90	280	−	914	11	10	0	−	−	−	915	8	8	12.5	1	−	−
B1836	8/5/39	60	120	+	918	18	18	0	−	−	−	919	19	19	15.8	3	−	−
B1836	8/6/39	70	220	−	920	22	22	4.5	1	−	−	921	18	18	11.1	2	−	−
B1836	8/7/39	0	210	+	922	11	11	9.2	1	−	−	923	12	12	16.7	2	−	−
All							103	13.6	14					91	17.6	16		
First series							18	11.1	2					15	0.0	0		
Second series							85	14.1	12					76	21.1	16		

The results of comparative infection experiments when applied to patients infected with the Panamanian strain are presented in table 4.

A comparison of the degree of infection after exposure to the Long strain (table 2) indicates that the first patient utilized was probably more infectious than the second, despite the higher gametocyte density in the latter. We believe the poor results with the latter can be attributed to immaturity of the majority of the gametocytes, despite the demonstration of exflagellation. A summation of the results of the experiments on the first patient indicates that *A. albimanus* is significantly less susceptible to infection with the Long strain than *A. quadrimaculatus* ($X^2 = 23.6$; $P = 0.000$).

When the two anophelines are compared in their susceptibility to the Mexican strain (table 3), a parasite presumably exotic to both anophelines, the *quadrimaculatus* exhibits a significantly greater susceptibility ($X^2 = 15.1$; $P = 0.000$).

When both anophelines were applied to the strain presumably coindigenous with the *albimanus*, there is no significant difference in relative susceptibility (Series two: $X^2 = 1.34$; $P = 0.25$). *A. quadrimaculatus* presented a lower frequency of infection than that secured when it was infected with the other strains. The percentage of all *quadrimaculatus* here infected, 17.6 per cent, does not differ materially from the incidence in 113 specimens examined from eight other and independent lots of this anopheline infected with this strain, which was 19.4 per cent. In this connection it is interesting to note that in the case of the first patient employed, with a very low gametocyte count, only the *albimanus* became infected.

Recent infection experiments with *A. albimanus* have been reported from Panama (4) (5) in which presumably indigenous strains of *P. falciparum* were utilized, in which 32.7 and 37.5 per cent of infections were secured. While some of our individual lots have given an incidence slightly in excess of these percentages, our series as a whole gave much lower results.

CONCLUSION

A. albimanus from Panama exhibits a susceptibility to a coindigenous strain of *P. falciparum* similar to that observed in a Floridian strain of *A. quadrimaculatus* when infected with the

same strain. The relative susceptibility of these anophelines to coindigenous and exotic strains is essentially similar to that previously noted (1).

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