at several dilutions, and that dilution giving 35 to 65 plaques per slide was selected to facilitate accurate counting. The plating efficiency for background and immune spleens is approximately twice that observed by us and reported by Jerne using the original procedure.

11. The following calculations for comparing the approximate amounts of antibody synthesized were employed: (Reciprocal of titer × estimated volume) ÷ estimated number of cells; in vivo: [320 × 1 ml (estimated plasma volume)] ÷ (2 × 10^8 cells) = 160 units/10^8 cells; in vitro: (32 × 1 ml) ÷ (2 × 10^7 cells) = 160 units/10^8 cells. The comparison is approximate, since the number of cells in vivo must be estimated and there is no account of half-life.
12. Fetal bovine serum–induced plaques are atypical. Although the size distribution is similar to that observed with cells from immunized animals, their appearance is semiopaque and under microscopic examination they are seen to contain numerous unlysed cells. Plaques from in vitro antigen-stimulated cultures are indistinguishable from those of immunized mice.
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15. This is publication No. 173 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California. This work was supported by PHS grant A1-7007 and by American Cancer Society grant E-395. R.I.M. is supported by PHS Special Fellowship No. 7-F3-CA23,938-02. R.W.D. is supported by a Dernham Fellowship, California Division, American Cancer Society (No. D-100).

20 June 1966

Plasmodium vivax Transmitted from Man to Monkey to Man

Abstract. *Blood forms of human* vivax *malaria infected splenectomized night monkeys (*Aotus trivirgatus*).* Anopheles albimanus *mosquitoes transmitted the infection from a monkey to two human volunteers; parasites and symptoms appeared 11 days later. Blood forms of* vivax *malaria from each of the two humans infected other night monkeys.*

Panama monkeys are being tested to determine whether they can serve as hosts to human-malaria parasites. We now report that *Plasmodium vivax* will grow in night monkeys (*Aotus trivirgatus*).

A patient from Santa Rosa village on the Chagres River, Panama, was admitted to hospital suffering from a *Plasmodium vivax* infection; the parasite count was 6,290/mm³. Blood was drawn, heparinized, and inoculated intraperitoneally into two splenectomized night monkeys; monkey 773 received 56.6 × 10^6 parasites; monkey 776, 62.9 × 10^6 parasites (Fig. 1). At the time of inoculation they received orally an immunosuppressant drug, Imuran (*1*), at the rate of 5 mg per kilogram of body weight.

Before inoculation with human parasites the blood of the monkeys was examined repeatedly to exclude the possibility of a natural infection with malaria, although natural infections have never been reported from night monkeys (*2*); nor were we able to prove natural infections by provocative methods such as splenectomy or drugs.

A patent infection developed in both monkeys on the fourth day. Both showed two peaks of parasitemia: In the blood of monkey 776, at the second and highest peak, parasites reached a maximum of 47,030/mm³ on the 37th day of patency; after patency continued for 54 days the monkey died. At the second (also the highest) peak, parasites in monkey 773 attained a maximum of 24,680/mm³ on the 35th day of patency, at which time chloroquine was given; the parasites disappeared three days later but the monkey died 5 days after receiving the drug.

Anopheles albimanus, which have been in colony at Gorgas Memorial Laboratory for many years, were fed on monkey 773. Fourteen days later, when sporozoites were present in the salivary glands, the mosquitoes bit each of two human volunteers (J.P. and C.J.) and a night monkey (813) by the interrupted-biting method; all three were bitten by each of 181 mosquitoes. Five more mosquitoes also bit both volunteer J.P. and the monkey, so that both suffered a total of 186 bites. Dissection of 100 of the mosquitoes showed a gland-positive rate for sporozoites of 57 percent, so that an estimated 103 infected mosquitoes bit volunteer C.J. and an estimated 106 mosquitoes bit volunteer J.P. and the monkey.

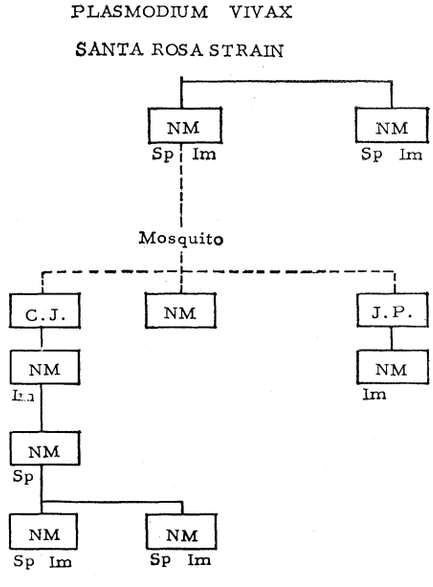

Fig. 1. Transmission of *Plasmodium vivax* from man to the night monkey and then to man. Abbreviations: *NM*, night monkey; *C.J.*, *J.P.*, human volunteers; *Sp.*, splenectomized; *Im.*, received Imuran.

Patent parasitemia and symptoms appeared in the two human volunteers 11 days later, but parasitemia was never patent in monkey 813. A liver biopsy and splenectomy performed on the monkey on the 23rd day disclosed no exoerythrocytic bodies in the biopsied tissue, and patent parasitemia did not develop subsequently.

Blood was drawn from each of the two infected humans and inoculated intraperitoneally into other night monkeys (826 and 825); the monkeys were unaltered but were dosed orally with Imuran at 5 mg/kg. Monkey 826 received blood from J.P.; the parasite count was 2,010/mm³ and the inoculum was 20.1 × 10^6 parasites; 16 days later it was splenectomized. Patent parasitemia appeared on the 41st day. The maximum parasite count was 680/mm³. The monkey died on the 5th day of patency.

Monkey 825 received blood from C.J.; the parasite count was less than 10 per cubic millimeter and the inoculum was less than 0.1 × 10^6 parasites. Patent parasitemia appeared on the 7th day and parasites reached 69,380/mm³ on the 11th day of patency, at which time the parasitemia was terminated by administration of chloroquine.

Vivax malaria has been transmitted by blood from monkey 825 to a splenectomized night monkey, and from the latter to two other splenectomized night monkeys.

Apparently this is the first successful transmission of *P. vivax* to monkeys. The only other animal reported to be susceptible to *P. vivax* is the chimpanzee *Pan satyrus* (*3*). Current work at this laboratory indicates that night monkeys may become useful hosts for the experimental study of human malaria.

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3 July 1966

Antigenic Heterogeneity of Human Immunoglobulin A Proteins

Abstract. *Two types of human IgA-myeloma proteins were distinguished by immunochemical tests. Seven of 51 IgA-myeloma proteins contained an antigenic determinant that was not detected in the other 44 proteins. The distinctive antigenic site was not demonstrated on either the heavy or light polypeptide chains.*

Four classes of immunoglobulin molecules, designated IgG, IgA, IgM, and IgD, are present in normal human serum. Two of these classes, IgG (*1*) and IgM (*2*), have been divided into subclasses on the basis of antigenic differences detected among molecules within each class. This report presents the results of experiments that demonstrate antigenic differences among the IgA molecules and shows that IgA, like IgG and IgM, may be divided into at least two antigenic subclasses.

Antiserums to normal serum IgA and IgA-myeloma proteins were prepared in rabbits and rhesus monkeys. One monkey antiserum (M-90) distinguished two categories of IgA molecules. This antiserum resulted from the immunization of a monkey with a crude preparation of normal human serum IgA, which had been obtained by a combination of zone electrophoresis and anion exchange chromatography (*3*). The monkey was repeatedly injected with this IgA preparation emulsified in complete Freund's adjuvant. Only bleedings obtained during the period 2 to 3 months after primary immunization contained antibodies with the specificity described below. Antiserum M-90 also contained antibodies to many serum proteins other than IgA and was rendered specific for IgA by absorption with human serum that was normal except for a marked deficiency of IgA-globulin.

A number of human serums containing IgA-myeloma proteins were then studied by double diffusion in gel with the absorbed antiserum (Fig. 1, bottom). Some of the precipitin bands spurred over others, indicating that this antiserum detected antigenic differences among the IgA-myeloma proteins.

The antiserum was further absorbed with whole serum from patient Sa until the precipitin reaction between the antiserum and IgA-myeloma protein Sa was abolished. When this absorbed antiserum was retested with the same IgA-myeloma serums, precipitin bands were obtained with some, but not all, of the serums (Fig. 1, top). This indicated that IgA-myeloma proteins could be divided into two groups: those that precipitated with this antiserum and those that did not.

Two IgA-myeloma serums, Fu and Ma, were selected as prototypes for further studies described below. IgA-myeloma proteins that precipitate with the absorbed antiserums will be referred to as Fu type, and the others will be referred to as Ma type.

A survey of 51 serums containing IgA-myeloma proteins showed that seven serums (14 percent) were of the Fu type while 44 serums (86 percent) were of the Ma type (Table 1).

An antiserum specific for Ma type IgA has not been prepared. Since Ma type myelomas are defined by failure to precipitate with the antiserum to Fu, it is not known how many other antigenic types may be included in the Ma group.

Immunoelectrophoretic studies of serum Co (containing Fu type IgA-myeloma protein) indicated that the precipitin band developed by the absorbed monkey antiserum involved only the IgA-protein (Fig. 2). Thus, the absorbed antiserum was reacting with antigenic determinants confined to IgA-myeloma molecules.

IgA molecules consist of heavy and light polypeptide chains which are held together by covalent disulfide bonds and other noncovalent forces. The heavy polypeptide chains carry class-specific antigenic determinants which permit IgA to be distinguished from other immunoglobulin molecules. The light polypeptide chains occur in two antigenic forms, designated κ- and λ-chains. Either κ- or λ-chains may be associated with the heavy chains of an IgA molecule.

Studies were performed to determine whether the Fu type antigenic determinants were on the light or heavy polypeptide chains. IgA-myeloma protein Fu (λ-type) was isolated by zone electrophoresis and anion exchange chromatography (*3*). The protein was reduced with 0.1*M* dithioerythritol and alkylated with 0.22*M* iodoacetamide at *p*H 8.2. The reduced and alkylated protein was acidified with propionic acid and sep-

Table 1. Precipitin reactions of 51 IgA-myeloma containing serums. All serums were tested with the same monkey antiserum which had been absorbed with serum Sa. (+) indicates precipitin reaction in gel; (−) indicates absence of precipitin reaction.

Light chain type	Precipitin reaction	
	Fu-type	Ma-type
	(+)	(−)
κ	0	29
λ	7	15

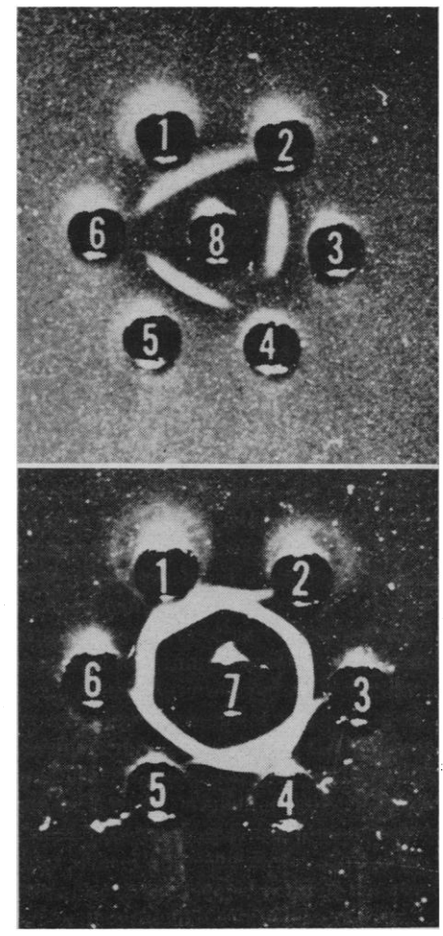

Fig. 1. Ouchterlony reactions of IgA-myeloma serums. All myeloma serums are at a 1/10 dilution. 1, Co; 2, Sa; 3, En; 4, Ra; 5, Hu; 6, Wa; 7, monkey antiserum to IgA absorbed with IgA-deficient serum; 8, the same monkey antiserum additionally absorbed with serum Sa.