other hand, in the rat as in man, the pressor effects of renin or angiotensin are enhanced by sodium retention (*13*).

Although our experiments did not cause chronic hypertension in rats, they may explain how it develops when renin is administered every 8 instead of every 24 hours (*1*). It is likely that a combination of two factors contributes to hypertension: (i) a residual pressor effect at the time of each injection, and (ii) an increase in cardiovascular reactivity to renin which results in a gradual shift of base pressure to hypertensive levels. This view is in accord with the observation that hypertension can be elicited by chronic infusion of subpressor doses of angiotensin (*2, 3*).

Thus, from all available evidence it is clear that renin, like angiotensin, can elicit hypertension and vascular disease. The direct myotropic effect of angiotensin on blood vessels may play a relatively small role except in acute malignant hypertension. The existence of mediated pressor effects that persist in the presence of tachyphylaxis to angiotensin would give more significance to the early observation that renal hypertension is not remitted by experimentally induced renin tachyphylaxis (*14*).

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Immunization of Normal Mouse Spleen Cell Suspensions in vitro

Abstract. *Dissociated cells from the spleens of unimmunized mice were cultured with and without various mammalian erythrocytes. Spleen cell suspensions cultured with heterologous red cells developed levels of hemolytic plaque-forming cells only one $\log_2$ less than those seen in vivo. The reaction is specific for the in vitro immunizing erythrocytes. Antibody was demonstrated in the culture fluids.*

Development of techniques which permit immunocompetent cells to undergo normal immune reactions in vitro has been a major aim in the field of tissue culture. Several investigators have demonstrated such reactions occurring when tissue fragments (*1*) or dissociated cells (*2*) from immunized animals are cultured in the presence of antigen. It has also been reported that tissue fragments from unimmunized animals synthesize small quantities of antibody when cultured with extracts from macrophages exposed to antigen (*3*), when obtained from animals previously injected with either phytohemagglutinin or adjuvant and cultured with antigen (*4*), or when both spleen and thymus fragments are cultured together with antigen (*5*). Very recently it has been reported that tissue fragments from unimmunized animals can be stimulated to synthesize small quantities of antibody to bacteriophage (*6*). In all reports of the in vitro initiation of antibody synthesis in tissues obtained from unimmunized mice, the architecture of the tissue has been preserved. The data to be presented demonstrate that under simple culture conditions, dissociated spleen cells obtained from normal, unimmunized mice can be immunized in vitro and that a response occurs comparable in magnitude to that seen in vivo.

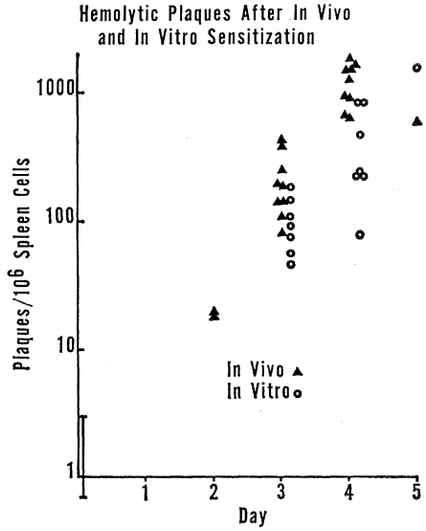

Fig. 1. Hemolytic plaques per 10^6 spleen cells against sheep erythrocytes. In vivo: mice injected intravenously with 0.2 ml 10-percent red cells. In vitro: 1-ml cultures contained 2×10^7 spleen cells and 1 to 2×10^5 sheep red cells. Background plaques (indicated by bar at day zero) = 0.3 to 3.0 per 10^6 spleen cells.

Cells were prepared by gently teasing apart the spleens of normal, unimmunized mice (*7*) in tissue culture medium. One-milliliter cultures containing 2×10^7 spleen cells in Eagle's suspension medium supplemented with "nonessential" amino acids, pyruvate, and 10 percent fetal bovine serum were placed in 35-mm plastic tissue-culture dishes (*8*) and incubated at 37°C in an atmosphere of 7 percent oxygen, 10 percent CO_2, and 83 percent nitrogen. Control cultures and experimental (antigen-containing) cultures were always set up from a single pool of dissociated cells; the experimental cultures contained, in addition to the spleen cells, 1 or 2×10^5 red cells of the appropriate type. The cultures were eccentrically rotated in the horizontal plane at 45 rev/min on a gyrorotary shaker. We added 150 μl of a nutritional cocktail (*9*) to each culture daily. Cultures were harvested and assayed at 3, 4, and sometimes 5 days. Cells were tested for antibody synthesis by the hemolytic plaque technique (*10*), and culture fluids were examined for released antibody. Suspensions of cultured cells were tested in duplicate at several cell concentrations in order to facilitate accurate counting. Forty to 50 percent of the cells planted were recovered; no viability tests were performed. The results are expressed as plaques per 10^6 total recovered cells.

The data presented in Table 1 show that large numbers of plaque-forming cells develop when normal mouse spleen cells are cultured with sheep erythrocytes. In seven consecutive experiments, using four different strains of inbred mice, increases of plaque-forming cells up to 1000 times the background were observed. A marked increase usually occurred between the 3rd and 4th days. It is of considerable interest that significant increases of plaque-forming cells were also observed in spleen cell suspensions cultured in

Table 1. In vitro immunization of normal mouse spleen cells with sheep erythrocytes (seven consecutive experiments).

Mouse strain	Plaques per 10^6 spleen cells				
	Background*	Day 3		Day 4	
		F.B.S.†	S.R.C.‡	F.B.S.†	S.R.C.‡
BL6		23	187	56	853
DBA		27	141	65	879
BDF_1	2.1	25	72	57	210
CAF_1	2.2	12	46	55	240
BDF_1		41	56	7	79
BDF_1	3.0	30	107	31	207
BDF_1	0.5	24	96	112	468
Average	1.2	26	101	55	419
Range	0.3 to 3.0	12 to 41	46 to 187	7 to 112	79 to 879

* Plaques present in spleens of normal, unimmunized mice. Average and range determined on 20 preparations. † 2×10^7 spleen cells cultured in fetal bovine serum (see text). ‡ 2×10^7 spleen cells cultured in fetal bovine serum and 1 or 2×10^5 sheep red cells.

fetal bovine serum but without sheep cells (see below).

The response of cells obtained from animals at various times after intravenous immunization are compared with the responses of cultured cells immunized in vitro in Fig. 1. The data at 3 and 4 days demonstrate that the in vitro response is only one $\log_2$ less than the in vivo response.

A representative experiment demonstrating the specificity of the response is illustrated in Table 2. In this experiment, three types of cultures were made from a single pool of cells obtained from normal, unimmunized CAF_1 mice. One was a fetal bovine serum control, the second contained sheep red cells, and the third contained horse red cells. The three types of cultures were tested on day 3 and day 4 (separately) against sheep and against horse red cells. The results show that in each case the response was specifically directed against the immunizing antigen. Similar experiments showed specificity for sheep and swine red cells.

Culture fluids were tested for hemolytic antibody. Marginal tests were observed with 4-day fluids; titers of 1 : 6 were found with 5-day fluids and titers of 1 : 32 with 6-day fluids. The serums of immunized mice obtained 6 days after intravenous injection of sheep erythrocytes had titers of 1 : 160 and 1 : 320. Thus, the amount of antibody synthesized by 2×10^7 spleen cells immunized in vitro is roughly equivalent to the amount of antibody synthesized by the cells of an immunized mouse (*11*).

Table 2. Specificity of in vitro immunization. Figures are plaques per 10^6 spleen cells.

Red cells in culture	Red cells used in assay			
	Sheep	Horse	Sheep	Horse
	Day 3		Day 4	
None	11	21	55	34
Sheep	46	23	240	21
Horse	15	111	34	144

Inspection of the data presented in Table 1 reveals that mouse spleen cells cultured in fetal bovine serum without sensitizing erythrocytes develop up to a 100-fold increase in the proportion of plaque-forming cells compared to that observed in the original suspension (*9, 12*). Other data (not presented here) show that fetal bovine serum stimulates cultured mouse spleen cells to proliferate as measured by the incorporation of tritiated thymidine. Whether the stimulation of fetal bovine serum is a specific immune reaction and due to foreign antigens (some of which may cross react with erythrocyte antigens), or if it is nonimmune in nature, cannot be decided from these data. The finding of preliminary studies showing that the increase in plaque-forming cells is greater when the assay is performed with bovine cells lends support to the hypothesis that antigenic cross reactivity accounts for part of fetal bovine serum stimulation. However, fetal bovine serum also is thought to contain various growth-promoting factors (*13*). This may account for the increase in plaque-forming cells by a mechanism similar to that suggested by Globerson and Auerbach for the effect they observed with in vivo administered phytohemagglutinin (*4*).

The data presented show that dissociated spleen cells (whose original tissue architecture has been disrupted) obtained from normal mice can be immunized in vitro with relatively simple culture conditions. The response is of only slightly less magnitude than that which occurs in vivo. Salient features of the culture conditions include a relatively high cell concentration, possibly permitting greater cell-to-cell interactions, the use of a nutritional cocktail to promote longevity of the cells, a lower than usual oxygen concentration which approximates closely the physiological level, the use of a rotary shaker, and the inclusion of fetal bovine serum, the complex effects of which are ill understood. Further study is necessary to determine which of the culture conditions are critical for in vitro immunization.

Note added in proof: Globerson and Auerbach (*14*) recently immunized mouse spleen fragments in vitro without the use of phytohemagglutinin, and recovered amounts of antibody similar to those we report.

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7. DBA/2J, C57BL/6J and CAF_1 mice were obtained from the Jackson Laboratories, Bar Harbor, Maine, and $B6D2F_1$ mice were obtained from Simonsen Laboratories, Gilroy, California. Spleen cell suspensions were prepared as described by Jerne and Nordin (*10*), and were found on microscopic examination to consist entirely of single cells. Numerous tests on each of the strains show a range of background hemolytic plaques with sheep red cells of 0.3 to 3 per million spleen cells. The origin of such background plaque-forming cells is unknown and may represent responses to cross reacting environmental antigens. It is of interest that germ-free mice show similar levels of background plaque-forming cells.
8. Falcon Plastics, Los Angeles, California.
9. The nutritional cocktail was prepared by mixing 5 ml of 50 times concentrated essential amino acids (Eagle), 2.5 ml of 100 times concentrated "nonessential" amino acids (Eagle), 500 mg of dextrose in 10 ml Eagle's suspension medium without bicarbonate or supplements, and 25 ml of twice distilled water. The *p*H was adjusted to neutrality with NaOH, 7.5 ml of 7.5-percent $NaHCO_3$ was added, and the mixture was filtered to insure sterility. At the time of feeding, the complete cocktail was prepared by adding one part of fetal bovine serum to two parts of the mixture described.
10. N. K. Jerne and A. A. Nordin, *Science* **140**, 405 (1963). The procedure has been modified and is performed on microscope slides. Eagle's suspension medium prepared without bicarbonate is used and the slides are incubated in a humidifying chamber without a special atmosphere. The modifications simplify the original procedure, facilitating the processing of large numbers of assays. Finished slides are scored unstained without magnification. Cell suspensions were made

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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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.

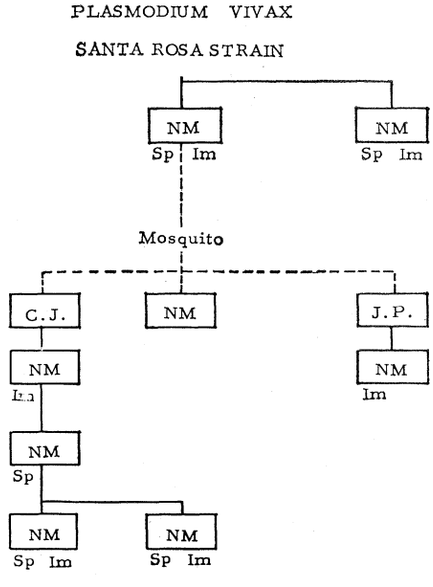

Fig. 1. Transmission of *Plasmodium vivax* from man to the night monkey and then to man. Abbreviations: *NM*, night monkey; *C.J.*, *J.P.*, human volunteers; *Sp.*, splenectomized; *Im.*, received Imuran.

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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