A Naturally Acquired Quotidian-Type Malaria in Man Transferable to Monkeys

Abstract. *A quotidian-type parasite,* Plasmodium knowlesi, *has been found as a natural infection in man. The infection was acquired by a white male during a short visit to peninsular Malaysia. This occurrence constitutes the first proof that simian malaria is a true zoonosis.*

In 1960 the tertian-type simian parasite, *Plasmodium cynomolgi*, B strain (*1*), was accidentally transferred to man by mosquito bite (*2*). Shortly thereafter, other strains of the same species were shown to be infective to man by the same route (*3*). In 1963 the quartan-type parasite of New World monkeys, *P. brasilianum*, was also shown to be infective to man as a result of bites of infected mosquitoes (*4*). Until this present report, none of the simian malarias have been found as natural infections in man.

A 37-year-old white male, B.W., a surveyor for the U.S. Army Map Service, was admitted to the Clinical Center of the National Institutes of Health on 9 April 1965 with complaints of chills, fever, and sweating of several days' duration. His illness began 9 days prior to admission when he was in Bangkok, having returned from 4 weeks in Malaya. At this time he experienced anorexia, mild fatigue, and occasional nausea. Because his symptoms were not severe, he elected to return to the U.S. before seeking medical attention. Three days prior to his admission, the patient had arrived at Travis Air Force Base in California, where he experienced sore throat and shaking chills, associated with high fever and profuse sweating. He was seen by the base physician who treated him for an upper respiratory infection, whereupon he proceeded to his home in Silver Spring, Maryland. There he was seen by his private physician during a chill. Upon finding many ring-form parasites in a smear from the patient's peripheral blood, the physician made a tentative diagnosis of falciparum malaria. The patient was then referred to the Clinical Center at the National Institutes of Health, where the diagnosis of malaria was confirmed, but the infecting parasite was considered to be *Plasmodium malariae* rather than *P. falciparum*. Treatment was started on 10 April 1965 with the conventional regimen of chloroquine followed by primaquine. Prior to treatment, a parasitized blood sample was taken, refrigerated at 4°C, and forwarded to the Laboratory of Parasite Chemotherapy malaria project at the U.S. Penitentiary, Atlanta, Georgia. The blood was inoculated into a healthy Caucasian volunteer on 15 April 1965. Since then it has been serially subinoculated into six additional volunteers (five Caucasians and one Negro), and into rhesus monkeys from each of the first three volunteers. All of the volunteers and each of the monkeys readily became infected.

The salient features of the infection in man include an asexual cycle of approximately 24 hours, a quotidian fever pattern with temperatures as high as 104.8°F, and parasite counts as high as 20,850 per cubic millimeter of blood. The clinical manifestations were moderate to severe, with attacks terminating spontaneously after approximately 2 weeks. In three volunteers in which treatment was required, the parasite yielded readily to conventional antimalarial therapy.

The three rhesus monkeys, *Macaca mulatta*, which received parasitized blood from the volunteers, all died with overwhelming malaria infections within 5 to 7 days after the advent of patency. Virtually 100 percent of the red blood cells were parasitized just before death. No morphologic changes were apparent as a result of subpassage from man to monkey. By inoculating with sporozoites, the infection has been passed from man to monkey and from monkey to monkey each time with fatal results. The earliest prepatent period observed was 5 days. Other simians—the pig-tailed monkey (*M. nemestrina*), the long-tailed monkey (*M. irus*), the squirrel-monkey (*Saimiri sciurea*), and the gibbon (*Hylobates lar lar*)—were inoculated with parasitized blood and all except the gibbon became infected. The parasites were typical and none of the animals died as a result of the infection.

On the basis of its morphology, quotidian periodicity, and pronounced infectiousness to rhesus monkeys, the parasite has been identified as *Plasmodium knowlesi* Sinton and Mulligan, 1933.

This report of a naturally acquired malaria infection in man transferable to monkeys represents the first proof that simian malaria is a *true zoonosis*. The fact that humans can become infected with simian malaria in nature is of special significance at this time because of its possible importance to the program of worldwide malaria eradication.

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Crown-Gall Tumorigenesis: Effect of Temperature on Wound Healing and Conditioning

Abstract. *Temperatures which inhibit crown-gall tumorigenesis in* Kalanchoë daigremontiana *plants also accelerate the rate of wound healing and the rate at which cells in the wound area become competent to react to the tumorigenic stimulus. The concept that the "tumor-inducing principle" is thermolabile and of high molecular weight may be invalid since it is based in part on the lack of effect of temperature on wound healing and conditioning. The rate of division of host cells may determine the success of crown-gall tumorigenesis.*

Crown-gall tumors are formed when a susceptible host plant is wounded, and the wound is infected with a virulent strain of *Agrobacterium tumefaciens* (Conn). Although the agent responsible for the tumorous change in

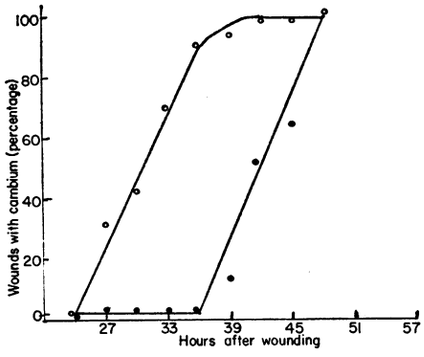

Fig. 1. Time course for the formation of wound cambium at 25° (closed circles) and 32°C (open circles). Each point represents an average of 15 to 20 wounds.

the host cells has not been isolated or identified, it is believed to be thermolabile and of high molecular weight. These properties have been deduced by interpreting calculations of the energy for the inactivation of tumor-formation according to the Arrhenius equation as indicating that either the "tumor-inducing principle" or something intimately associated with its inactivation is a factor of complex structure (*1*). The validity of this technique is predicated on the hypothesis that the thermal arrest of tumorigenesis is due to the inactivation of a tumor-inducing principle and that it is not an effect on host, bacteria, or tumor. This hypothesis is based on negative evidence, that is, on evidence that eliminates other possibilities, which consist, for the most part, of the following observations. (i) *Kalanchoë daigremontiana* (Hamet and Perrier) plants infected with virulent strains of *A. tumefaciens* form tumors at 25° but not at 32°C. (ii) Tumors, once formed, grow at both 25° and 32°C. (iii) The inciting bacteria grow well at 25° and at 32°C. (iv) Conditioning, the process by which cells in the vicinity of a wound become sensitive to tumor-induction, proceeds at both 25° and 32°C. (v) Wound healing in uninfected plants occurs at about the same rate at 25° and 32°C (*2*). These last two points are reexamined in this report.

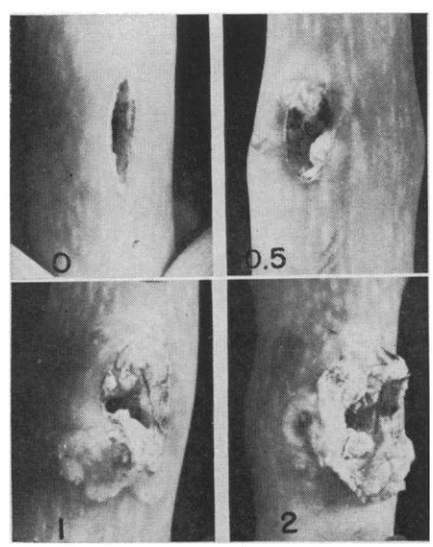

Fig. 2. Standards for scoring tumors of *Kalanchoë*. Scores were obtained by comparing tumors to these photographs. Numbers refer to tumor score.

Kalanchoë daigremontiana plants about 10 cm tall were wounded through two internodes by means of a flat, sterile needle, 2 mm wide and 0.5 mm thick. After the wounding, the plants were placed in growth chambers maintained at either 25° or 32°C. Temperature variations were ± 1.5°C; relative humidity, 70 to 80 percent; and average light intensity was 650 foot candles (7150 lu/m^2) at bench top level. The lights, fluorescent and incandescent, were left on for the duration of the experiments. Plants were removed from the chambers at 3-hour intervals for a period of 48 hours. The wounds were excised, fixed in a mixture of formalin, acetic acid, and alcohol (5:5:90), dehydrated with *t*-butanol, embedded in paraffin, and sectioned at 12 μ. The sections were stained with safranine and counterstained with either orange G or aniline blue.

The large number and small size of *Kalanchoë* chromosomes made it impossible to measure the rate of cell division by comparing mitotic indexes (*3*). It was possible, however, to count the total number of cells and the number of cells with newly formed cell plates in the wound area. From these data the percentage of cells dividing was calculated. This task was somewhat simplified by the observation that the epidermal and pith cells in *Kalanchoë* do not form tumors (*4*). Counts were made of sections of three wounds taken at 3-hour intervals in three separate experiments. No significant difference was observed in the average number of newly formed cell plates in plants maintained at 25° or at 32°C for the first 24 hours after wounding.

This method of measuring the rate of wound healing has two distinct disadvantages: (i) It is not always easy to distinguish newly formed cell walls from older ones, particularly after division has proceeded for some time; and (ii) the cortical area included as part of the wound-healing response can only be arbitrarily delineated. In these experiments an area around the wound that contained between 300 and 350 cells was chosen.

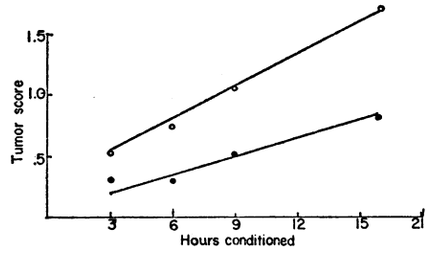

Fig. 3. Comparison of sizes of tumors in plants conditioned at 25° (closed circles) and at 32°C (open circles) for 3, 6, 9, and 16 hours. Each point represents the average of 18 to 20 tumors scored in two experiments. The probability of the null hypothesis according to the Mann-Whitney U test at 3 hours is .086; at 6 hours, .040; at 9 hours, .002; and at 16 hours, .004.

Another measure of the rate of wound healing is the time required for the formation of wound cambium. Since this characteristic structure is easily distinguished, its presence or absence can be scored. Wounds with a definite dividing cambium were scored as 1.0; those with a formed but not dividing cambium, as 0.5; and those without a cambium, as 0. Data obtained by examining a total of 20 to 25 wounds accumulated from three separate experiments are presented in Fig. 1. These data show that plants maintained at 32°C after wounding start to form a wound cambium approximately 12 hours before comparable plants maintained at 25°C. Most of the wounds (90 percent or more) in plants maintained at 32°C formed cambium before any of the plants maintained at 25°C had done so. This indicates that the rate of wound healing at 32°C is faster than at 25°C, and thus contradicts one of the premises upon which the hypothesis on the nature of the tumor-inducing principle is based.

Braun and Mandle (*2*) failed to observe differences in the rate of wound healing at 25° and 32°C. Their histological samples were taken at 24-hour intervals after wounding, and, therefore, they missed points of difference in the wound-healing process at the two temperatures.

To determine the effect of temperature on conditioning during the first 24 hours after wounding, plants were

wounded and kept at 25°C or 32°C for 3, 6, 9, or 16 hours. The plants were then inoculated with *Agrobacterium tumefaciens* strain B6 and placed at 25°C. Twenty-four hours later the plants were placed and left at 32°C to prevent further tumor-induction. Three weeks after inoculation the plants were scored for tumor size by comparison with the photographs shown in Fig. 2. These data are presented in Fig. 3, which shows that plants maintained at 32°C after wounding and before inoculation form larger tumors than comparable plants maintained at 25°C. However, plants conditioned at 32°C for more than 32 hours form significantly smaller tumors than comparable plants conditioned at 25°C. For instance, plants conditioned for 40 hours at 25°C have a mean tumor score of 1.4, whereas those conditioned for 40 hours at 32°C have a mean tumor score of 0.5 after exposure to the bacteria as described above (*5*). It is thus possible to increase or decrease the host response by exposing wounded but uninfected plants to 32°C for various lengths of time.

Although no anatomic differences between plants maintained at 25° or 32°C for the first 24 hours after wounding are detectable by the techniques described, physiological differences are discernable. The significant differences in the conditioning response at 25° and 32°C indicate that the host is affected by even short exposures to 32°C. These findings challenge the tacit assumption that the rate of conditioning is roughly equal at 25° and 32°C.

The conditioning process has been correlated with wound healing; plants infected during the early or late stages of the wound-healing process form smaller tumors than plants infected during the middle of the process, provided the period of exposure to bacteria is limited (*2*). Both wound healing and conditioning have been shown to be accelerated by temperature. Numerous workers have reported that plant cells go through division cycles more rapidly as the temperature increases (*6*). If crown-gall tumorigenesis can occur only during certain parts of the mitotic cycle and requires that this part of the cycle have at least a minimum duration, it is possible that the thermal arrest of tumorigenesis may be due to a decrease of the time available for tumor-induction. This new hypothesis is consonant with the available data and with the reports that progressively smaller tumors are formed in infected plants incubated at temperatures ranging from optimal to the point of thermal cut-off (*7*).

The data presented here do not yield any direct evidence on the actual nature of the tumor-inducing principle. They do, however, challenge two of the premises upon which the concept of a thermolabile tumor-inducing principle with a high molecular weight rests. Although at least part of the thermal arrest of crown-gall tumorigenesis can be explained in terms of host effects, the full elucidation of this phenomenon requires further investigations.

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Osmotic Flow in a Rigid Porous Membrane

Abstract. *The concept of an internal pressure gradient in a rigid porous membrane has been proposed as the basis for osmotic flow. The origin of the pressure in terms of the theory of the chemical potential implies also the existence of states of negative pressure, that is, tension. These states have been observed experimentally by means of a Hepp-type osmometer.*

The classical analysis of swelling in cross-linked charged colloids was based on the idea that the equilibrium state of the interstitial solvent could be regarded as closely related to the Donnan equilibrium. Since in the Donnan equilibrium the crucial role of a pressure difference was well recognized both from theory and experiment, workers in the field of colloids argued convincingly that there was an excess hydrostatic pressure within a colloid phase with respect to its ambient solution. In modern terminology, the excess internal pressure is a consequence of the difference in the mole fraction component of the chemical potential of the solvent between the two phases. The analysis has been applied to equilibrium states of ion-exchange resins. In extending the Teorell-Meyer-Sievers theory of transport processes in

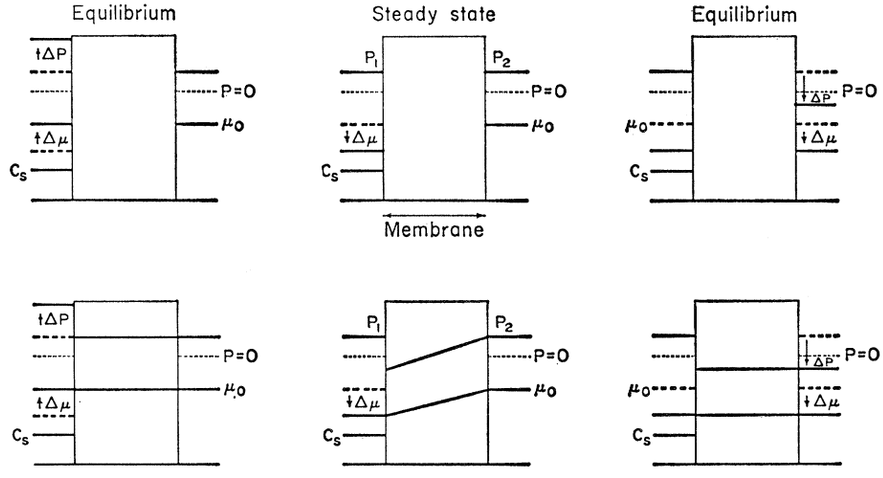

Fig. 1 (top). Thermodynamic variables in the external phases. Fig. 2 (bottom). Profiles of thermodynamic variables throughout the membrane. The zero of the pressure scale is indicated by the fine dotted line and so designated in all diagrams.