3 weeks the flask was practically filled. The contents were trypsinized and transferred to a larger T-flask (75 cm^2) containing 1.8×10^5 rat feeder cells. A week later normal-appearing as well as transformed clones (Fig. 1A) were noted. Specific clones were again marked and, after part of the top of the flask had been removed, the clones were isolated by the cylinder technique (*4*). The cells of each clone were placed in a large T-flask without a feeder layer. Within a week sufficient transformed cells were available for injection into animals; control cells were slower growing, and 3 weeks passed before cells could be harvested for animal testing.

Thus permanent cell lines were obtained from both normal- and transformed-appearing colonies (Fig. 1B). The transformed-derived lines had many of the cell properties known as indices of neoplastic transformation, such as change in morphology and growth behavior, and detachability of cells from the surface of the flask.

To determine whether the transformed cells or the unaltered controls were neoplastic, 10^6 cells were injected intradermally or 5×10^6 cells were injected subcutaneously into weanling hamsters. Cell lines were derived from cells of fetuses of three different hamsters. In no instance did a tumor result from any of the ten control lines. Growing tumors were produced with seven out of eight transformed cell lines, five of which were derived from dense transformed clones and two of which were derived from light transformed clones. Complement fixation tests were negative for antigens of oncogenic viruses known to transform hamster cells in vitro or in vivo. Animals inoculated intradermally with transformed lines took a minimum of 4 months to develop palpable tumors which then grew rapidly and had to be retransplanted before the skin burst. Following subcutaneous inoculation of transformed cells to the dorsum, a period of 6 weeks to 3 months was required to obtain neoplasms. Although these appeared encapsulated, they grew to as much as 20 cm in diameter, and the animals became cachectic. In all cases the primary tumors were fibrosarcomas (Fig. 1C) and tumor metastasis was not observed.

The excised primary tumors were transplantable again, and cell suspensions obtained by treating minced portions of the tumors with trypsin rapidly attached to the plastic surface of the flask. Subsequently, these cells formed cultures resembling the original transformation (Fig. 1D). Particularly apparent were crisscrossing of cellular elements and the piling up of various cells.

Our observations demonstrate that cells derived from cultured fibroblasts are susceptible to neoplastic transformation of benzo[*a*]pyrene and not by pyrene. The quantitation of clonal alterations and the extent of correlation of the morphologically altered clones with tumor production require further investigation.

JOSEPH A. DIPAOLO
RICHARD L. NELSON
PAUL J. DONOVAN
Biology Branch, Etiology, National Cancer Institute, Bethesda, Maryland

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Plasmodium malariae: Transmission from Monkey to Man by Mosquito Bite

Abstract. Anopheles freeborni *mosquitoes were infected by feeding on New World monkeys,* Aotus trivirgatus, *infected with a Nigerian strain of* Plasmodium malariae. *The infection was passed to human volunteers through the bites of these mosquitoes, demonstrating the practicability of using a simian host for infection of mosquitoes with a third species of human malaria parasite and of the use of such mosquitoes to transmit the infection from monkey to man.*

The New World owl monkey, *Aotus trivirgatus,* can now be experimentally infected with three of the four human species of malaria parasites, namely, *Plasmodium vivax* (*1, 2*), *P. falciparum* (*3, 4*), and *P. malariae* (*5*). Young *et al.* (*1*) were able to infect mosquitoes with *P. vivax* which had been established in monkeys and, with the same mosquitoes, transmitted this infection back to man. We reported (*4*) the susceptibility of anopheline mosquitoes to infections with falciparum malaria in owl monkeys and the subsequent transmission of this parasite to man.

In August 1968, we isolated a strain of *P. malariae* from a Nigerian student. On 15 October 1968, blood (2 ml) from an inmate volunteer was inoculated intravenously into a splenectomized owl monkey (AO-74). On 30 December 1968, after a prepatent period of 76 days, parasites were observed in the peripheral blood of this animal. The infection was passed from monkey AO-74 by the intravenous inoculation of parasitized blood to a second splenectomized owl monkey, AO-80, where there was a prepatent period of 7 days. Prior examination indicated the absence of natural malaria infection in these animals. *Anopheles freeborni* mosquitoes fed on both these monkeys became infected and exhibited sporozoites in the salivary glands. The ease with which mosquitoes could be infected with this parasite in the owl monkey was most encouraging, since there are only a few reports of the successful experimental transmission of *P. malariae* to man (*6*).

Six volunteers were exposed to infection with *P. malariae* malaria by the bites of *A. freeborni* mosquitoes infected on monkeys AO-74 or AO-80, or both. Four volunteers were Caucasian, two were Negro. Four of the six volunteers (three Caucasians, one Negro) developed patent infections at 24, 27, 28, and 33 days after exposure. Each of these volunteers had been bitten by two or three mosquitoes with salivary glands heavily infected with sporozoites.

In order to investigate the possible relapse activity in *P. malariae* infections induced by mosquito bites under controlled conditions, all four volunteers were treated therapeutically with either quinine or chloroquine after 3 or 5 days of patent parasitemia and at least one episode of fever. By the time treatment was intiated all four patients had experienced paroxysms with temperature maximums between 39.5° and 39.9°C and parasite counts as high as 50 per cubic millimeter of blood. These volunteers are currently being observed for relapse activity of these infections.

Prior to treatment, parasitized blood was passed by intravenous inoculation from one volunteer to another of compatible blood type; patent infection ob-

tained on day 7. This volunteer experienced multiple paroxysms with maximum fever of 40.8°C and maximum parasitemia of 3450 parasites per cubic millimeter of blood. This infection was terminated with antimalarials because of the occurrence of daily fevers due to multiple asexual cycles of the parasite. However, before treatment, the parasite was blood-passaged to another volunteer, who developed patent parasitemia by day 6. The infection in this volunteer exhibited a quartan fever pattern through four consecutive paroxysms, after which daily fever was observed. Maximum fever of 40.4°C was observed on day 10 and maximum parasitemia of approximately 10,000 parasites per cubic millimeter on day 14. It is considered that the course of parasitemia and the clinical illness observed in the latter two volunteers are typical of *P. malariae* infections in man and would rule out the possibility that a quartan parasite of the owl monkey might have been inadvertently transmitted. The establishment of this strain of *P. malariae* in a small nonhuman primate and its transmissibility through the mosquito further indiate the usefulness of the owl monkey in research in human malaria.

PETER G. CONTACOS
WILLIAM E. COLLINS
National Institute of Allergy and Infectious Diseases, Chamblee, Georgia

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Thermoregulatory Responses to Intra-Abdominal Heating of Sheep

Abstract. *When electrical heat sources were implanted in the abdominal cavities of sheep and heated to dissipate 20 to 22 watts of additional endogenous heat in the animal, a rapid increase in respiratory frequency and respiratory water loss occurred 3 to 5 minutes after the initiation of heating. The response was accompanied by a marked decline of the temperature of the hypothalamus, with an increase of less than 1.0°C in skin temperature over the location of the heaters in the abdomen. When the same skin area was heated externally in the absence of internal heating, no significant response was seen. The results support the concept of the existence of thermoreceptors, located in deep tissues or veins, which play a role in the regulation of body temperature.*

The regulation of the body temperature of homoiothermic animals is thought to be mediated largely by a dual system of neural control involving the hypothalamus and peripheral skin thermoreceptors. Evidence has been accumulating that there is a third major factor in the integrating system responsible for body temperature regulation, namely, deep body thermal receptors (*1*). There have been few direct observations, however, to support the concept of deep temperature receptors. Techniques have been developed in this laboratory to aseptically introduce electrical heat sources at various sites within the animal body and leave them for long periods of time to provide a method for the study of the effects of additional internal heat (*2*) and how they may relate to the effects of heat from an artificial heart. Such stimuli, administered in conjunction with standard procedures for the study of thermoregulatory activity, constitute a means of studying the deep temperature receptor problem.

In recent studies on sheep designed to determine the general physiological effects of additional endogenous heat, we have found that marked thermoregulatory responses occurred upon heating of the viscera and abdominal wall. Additional endogenous heat was introduced over an area of 150 cm^2 between the viscera and abdominal wall of sheep (*3*) while temperatures at different sites, the metabolic rate, and the thermoregulatory responses of respiratory evaporative heat loss and respiratory frequency were monitored.

Figure 1 is a graph of data recorded when a ewe (41 kg, closely sheared) was endogenously heated while being exposed to a constant environmental temperature of 20°C. With intra-abdominal dissipation of 22 watts of heat during a 30-minute period (designated by the arrows labeled *endo* in Fig. 1), there occurred a rapid increase in respiratory evaporative heat loss of 0.5 watt per kilogram of body weight as shown in the curve labeled *HE*. Respiratory frequency, labeled *RF*, increased twofold during the heating period. The most important response at this time was the rapid decline of the temperature measured in the hypothalamus, T_{hypo}, whereas vaginal temperature, T_v, did not change significantly. It has been demonstrated conclusively that cooling the hypothalamus of the mammal causes the animal to respond in a physiological manner that will reduce body heat loss; for example, in the dog, panting will cease, and the blood vessels of the ears will constrict. In our experiments, however, respiratory frequency increased while the hypothalamus was cooling. In the light of our present knowledge regarding the mechanisms of body temperature regulation, the phenomenon could only occur if the respiratory center were driven to increase its activity by signals from heat-sensitive receptors outside the hypothalamus. This is the case when sufficient skin area is heated and thermally sensitive skin receptors initiate appropriate thermoregulatory responses. In our experiments, the temperature of the skin directly over the heaters located beneath the body wall increased approximately 1°C during the endogenous heating period (Fig. 1). When this same skin area was heated 1.5°C by externally applied electric heaters (designated by the arrows labeled *exo* in Fig. 1), no response in the respiratory frequency or deep body temperatures was noted. At 160 minutes in the experiment, the internal heating was repeated for a slightly shorter period, and a similar response of less magnitude occurred as before. Measurement of skin temperatures at selected sites over the rest of the body surface (not shown in Fig. 1) indicated there were no significant changes in temperature at these points during the experiment.

Throughout the time course of these