Reports

Anopheles hackeri, a Vector of Plasmodium knowlesi in Malaya

Abstract. *Anopheles hackeri*, a mosquito commonly found breeding in nipa palm leaf bases along the Malayan coast, was demonstrated to be infected with *Plasmodium knowlesi* by the inoculation of sporozoites into an uninfected rhesus monkey. This was the first demonstration of a natural vector of any monkey malaria.

The discovery that a Malayan strain of *Plasmodium cynomolgi bastianellii*, isolated from the long-tailed macaque, *Macaca irus*, was transmissible to man (*1*) has stimulated inquiries into the vectors of monkey malaria in nature. Although the identity of the main human vectors in Malaya, *Anopheles maculatus, A. letifer, A. umbrosus, A. sundaicus*, and the dark-winged form of *A. barbirostris*, is well established (*2*), virtually nothing is known about transmission of monkey malaria.

There has been some doubt about the origin of oöcyst and sporozoite infections in certain anophelines, and in particular, J. A. Reid of the Institute for Medical Research of the Federation of Malaya suspected that two members of the *A. leucosphyrus* group, *A. hackeri* and *A. pujutensis*, were vectors of monkey malaria. An investigation of their feeding habits showed that neither was attracted to man, but both were caught on monkey bait, and precipitin tests of blood from adults caught by day confirmed that these mosquitoes fed on monkeys in nature (*3*). Efforts have been made to identify the infections in these mosquitoes, and a strain of *Plasmodium knowlesi* has been isolated in a rhesus monkey inoculated with sporozoites from *Anopheles hackeri*.

Until comparatively recently *A. hackeri* was regarded as a rather rare mosquito breeding in split bamboos in inland forest, but, as described by Reid and Weitz (*3*), it is now known to be quite common on the Selangor coast, where the larvae and adults were found in the old leaf bases of the nipa palm (*Nipa fruticans*).

Our studies were made in the vicinity of Kampong Rantau Panjang, north of Klang near the Selangor coast, and this was also the area studied by Reid and Weitz (*3*). In the study area, which bordered on mangrove swamp, nipa palms were extensively cultivated. *Anopheles hackeri* larvae were found in the old leaf bases of the palms, and resting adults could be collected a few inches above the water line at the rate of about five females per man-hour by experienced collectors. During the study period *A. pujutensis* was not found frequently.

Through trapping mosquitoes the conclusion of Reid and Weitz (*3*) that *A. hackeri* is attracted to monkeys was verified. Over a period of 34 nights, 20 *A. hackeri*, including 17 which were blood-fed, were taken in a monkey-baited open net trap on a platform in the canopy 20 ft above ground level. Four mosquitoes were taken in a similar trap on the ground, but none of the four was blood-fed. Nearly all of the mosquitoes were caught on two nights, eight on one night and seven on the other, and each time the majority were caught about 1 hr after sunset. The irregularity in trapping the mosquitoes was puzzling in view of their consistent presence as resting adults in nipa leaf bases and possibly indicated that they are shy feeders. Other trapping methods were unsuccessful.

A man-baited net trap at ground level caught 13 *A. hackeri*. This was surprising, as none had been caught in more extensive efforts by Hodgkin (*2*) or Reid and Weitz (*3*). For our observations the traps were situated close to the breeding grounds, and the catching of *A. hackeri* in the man-baited trap is probably of little significance, since, as Colless (*4*) has shown, mosquitoes flying at random easily enter such traps even when they are unbaited.

Precipitin tests made with blood from freshly fed mosquitoes captured from nipa leaf bases confirmed the finding of Reid and Weitz (*3*) that *A. hackeri* was feeding upon monkeys.

Many mosquitoes from the nipa leaf bases and from the traps were dissected, and only one sporozoite infection was found in over 700 mosquitoes dissected. This is a much lower rate than the 4 percent recorded by Reid and Weitz (*3*). Mosquitoes were dissected in monkey serum-saline (one part serum to four parts physiological saline), and the sporozoites were inoculated intravenously into an uninfected Indian rhesus monkey (*Macaca mulatta*).

Small ring forms were seen in small numbers in the blood 6 days later, and the infection built up with such rapidity that the monkey died in another 3 days. The periodicity was quotidian, and the parasites were morphologically identified as *Plasmodium knowlesi*.

The infection was transferred to a second monkey, and infected blood was shipped to the United States for further study. This strain has been designated the *hackeri* strain of *Plasmodium knowlesi*.

The demonstration that *Anopheles hackeri* is a natural vector of one of the species of monkey malaria is the first such demonstration on record, but it falls far short of explaining the widespread occurrence of monkey malaria. Monkey malarias occur in many areas in which this species of mosquito does not occur commonly. It is possible that other members of the *Anopheles leucosphyrus* complex are involved.

Our laboratories have initiated an extensive investigation of the epidemiology of monkey malarias, of which this study is a part. The transmission of monkey malarias as related to

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

human malarias is being studied in a number of ecologically diverse situations in Malaya, to determine whether malaria is, under any circumstances, a zoonotic disease in nature.

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Suppression of Shoot Formation in Cultured Tobacco Cells by Gibberellic Acid

Abstract. When tobacco pith was cultured on media containing gibberellic acid, shoot formation was observed. The formation of stem structures was strikingly suppressed by concentrations of 0.5 mg/lit. and above.

Application of gibberellins has generally resulted in promotion, rather than suppression, of plant growth processes. One of the few instances of suppression reported is in the rooting of cuttings. Gibberellin treatments not only inhibited rooting of cuttings, but also counteracted the stimulation caused by auxin (*1*). In tests performed with cultured tobacco cells, I found that gibberellic acid indeed prevented formation of roots under otherwise favorable conditions.

The present report considers the effect of gibberellin on the formation of stem structures in cultured cells. A preliminary study showed complete suppression of shoot formation when 5 mg of gibberellic acid per liter was included in the culture medium. Representative cultures can be seen in Fig. 1. The present experiment extends this observation to other concentrations of the growth regulator.

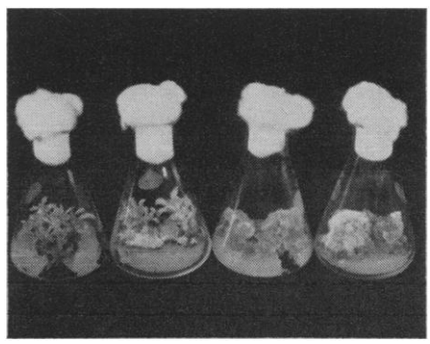

Fig. 1. Formation of shoots in cultured tobacco cells as influenced by gibberellic acid. Pair on left, no gibberellin; pair on right, 5 mg of gibberellic acid per liter.

Tobacco (*Nicotiana tabacum* L. var. Wisconsin 38) pith, which had been in continuous culture for the last several years at the University of Wisconsin, was employed (*2*). The basal medium of Miller *et al.* (*3*), supplemented with three times the level of inorganic phosphate, 0.5 mg of 3-indoleacetic acid, 2 mg of kinetin (*2*), and 150 mg of L-tyrosine per liter, was utilized. The reports of others (*4, 5*) and my experience showed that these levels of the supplements were optimum for shoot formation in cultured tobacco cells. Gibberellic acid (*6*) was included (0, 0.5, 1.0, 5.0 and 10.0 mg/lit.). Solutions of this compound were sterilized by Millipore filtration to avoid any undesirable consequence of heating.

For each level of gibberellin, ten 25- by 150-mm culture tubes, each containing 25 ml of medium, were employed. One piece of callus, roughly 2 mm^3 and weighing about 40 mg, was cultured in each tube. The cultures were maintained at 21° to 27°C under continuous weak light from overhead fluorescent fixtures.

The numbers of cultures with shoot and shoots per culture recorded after 7 wk are shown in Table 1. Controls on basal medium showed profuse shoot development, whereas cultures supplied with gibberellic acid in any concentration exhibited marked suppression. Levels of 5 and 10 mg/lit. of the compound were completely antagonistic. This suppression of shoot formation is most probably not due to toxicity, since no reduction in callus growth was observed in any of the treatments. Furthermore, the concentrations presently employed ranged below that found by Nickell and Tulecke (*7*) to be promotive in growth tests with cell cultures of a large number of species.

These findings, together with earlier reports of inhibition of rooting, show that gibberellin is indeed physiologically distinct from either auxin or kinin. Auxin tends to promote rooting, and kinin enhances shoot formation (*5*). If the amounts of gibberellin presently incorporated into the culture medium can be assumed to have resulted in levels which do not exceed the normal physiological range in the cells, then the data reveal that, whereas gibberellin is known to stimulate organ enlargement, the stem in particular, it antagonizes the initiation of the structure (*8*).

Table 1. Shoot formation in cultured tobacco pith as influenced by gibberellic acid.

Gibberellic acid in medium (mg/lit.)	Cultures with shoot (No.)	Shoots per culture (No.)
0	10	29 ± 4
0.5	3	0.6 ± .4
1.0	4	1.3 ± .6
5.0	0	0
10.0	0	0

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References and Notes

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2. Both tobacco pith and kinetin were obtained through the courtesy of Folke Skoog, University of Wisconsin.
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6. A purified sample containing 90-percent gibberellic acid was kindly furnished by Eli Lilly & Co., Indianapolis, Ind.
7. L. G. Nickell and W. Tulecke, *Botan. Gaz.* **120**, 245 (1959).
8. This report is published with the approval of the director, as technical paper No. 521 of the Hawaii Agricultural Experiment Station.

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Acid-Catalyzed Oxidation of Reduced Pyridine Nucleotides

Abstract. Reduced pyridine nucleotides are oxidatively catalyzed in weakly acidic solutions. The rate is proportional to the acidity, and at constant acidic *p*H, the reaction follows first-order kinetics. The rate of oxidation of reduced triphosphopyridine nucleotide is approximately 3 times that of reduced diphosphopyridine nucleotide. The reaction offers a very plausible explanation for the metabolic efficiency of the malignant tumor cell. It may also play a key role in wound healing and muscle contraction.

When reduced pyridine nucleotides (DPNH and TPNH) (*1*) are dissolved in weakly acidic buffers, they are found to undergo catalytic oxidation which may be followed spectrophotometrically by the decrease in optical density at 340 mμ. If the concentration is expressed in terms of optical density, the reaction follows first-order