the premise that cigarette coal temperatures are about 300°C higher than the coal temperatures in pipes. The results obtained in this laboratory indicate, if anything, that the burning coal in the pipe is at a more elevated temperature than that of the cigarette. We are certainly not qualified to attempt to relate combustion temperatures and carcinogenicity. Furthermore, we are not aware that it has been established that cigarette smoke is carcinogenic. Many reputable investigators have been unable to produce cancer in animals by means of cigarette smoke tars, and no one has yet demonstrated that tobacco smoke is carcinogenic to human beings.

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4 November 1955

E. S. Harlow has criticized the incompleteness of my brief report and points out the fact that the tobacco industry has standardized techniques that simulate human smoking. As previously indicated [*Science* **122**, 514 (1955)], I am a petroleum chemist who was led into this digression by curiosity. In measuring the temperatures that I reported, the individual who donated the particular cigarette, cigar, or pipe was kind enough to smoke it in his usual manner while the temperatures were being measured. This uncontrolled method of determining these temperatures may, in some respects, be responsible for the differences in smoke temperatures found in the apparatus that simulates human smoking and those found in my cursory examination.

I certainly did not intend to perpetuate a misapprehension by suggesting that cigarettes may have a higher coal temperature because of the use of an oxidizing agent, such as potassium chlorate. However, I have observed that certain brands of cigarettes, when placed in an aqueous solution of potassium iodide, will produce a titratable amount of iodine. Furthermore, cigarettes, unlike cigars or pipes, will burn to extinction once they are lit.

The basic point that I would like to reemphasize in the light of Harlow's informative letter concerns the possible chemistry involved in tobacco combustion. As Harlow points out, the temperature a few millimeters from the glowing coal is comparatively quite cold. Therefore, in smoking, tobacco is very rapidly heated to extremely high temperatures. When pure organic compounds are subjected to such treatment in the laboratory, a profusion of very reactive free radicals results. It would, therefore, seem reasonable to postulate that the smoking of tobacco will result in the formation of free radicals. It should be comparatively easy to identify any free radicals in the tobacco smoke by using techniques such as paramagnetic resonance absorption.

Although it is true, as Harlow points out, that recent, well-publicized results of the statistical groups within the American Medical Association do not conclusively prove a relationship between cigarette smoking and lung cancer, one cannot, with true scientific impartiality, ignore these findings. If one accepts the postulate that there may be a possible relationship between cigarette smoking and lung cancer, we must then find a verifiable hypothesis to explain such a relationship. It was from this standpoint that the proposal was made that the differences in coal temperatures between cigarettes and pipes may lead to a difference in the concentration of volatile radicals liberated during the burning of tobacco. As Harlow's results show, the conditions in burning tobacco are those of thermal cracking. The transitory product of thermal cracking is free radicals. There is an overwhelming accumulation of evidence in the scientific literature that free radicals and compounds that readily yield free radicals are carcinogenic. In testing any hypothesis that would relate cigarette smoking to lung cancer, it would, therefore, appear to be of interest to examine cigarette smoke for the presence of free radicals or free-radical precursors.

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Virus of Bats Antigenically Related to St. Louis Encephalitis

A bat survey was begun early in 1954 at the Brooke Army Medical Center, Fort Sam Houston, Tex., because of a malady afflicting the bat colony. An encephalitis was apparent among these animals as manifested by deranged behavior, muscular tremors, urine incontinence, and paretic manifestations. Hundreds of deaths were recorded.

The purpose of this acticle is to report that, on separate occasions, in addition to the isolation of the virus of rabies from bats, a virus antigenically related to St. Louis encephalitis was recovered.

At first, the bat encephalitic manifestations were attributed to the effects of an intensified DDT program in the area. Chemical analyses of 3.62 g of omentum collected from 93 bats revealed a total of 184 μg of DDT, the equivalent of 50 ppm (*1*). Studies conducted by Hayes (*2*) on the relationship between dosage of DDT and its storage in adipose tissue for several species of animals that were fed daily doses of the compound indicate that the storage level we found in bats of 50 ppm of DDT would require a DDT dosage level of about 0.08 mg/kg a day. Rats can withstand daily doses of DDT at more than 100 times this level. It would not be expected, on the basis of results with other species, that any harmful effects would result in bats from this amount of DDT exposure.

Bacteriological studies of brain, liver, and spleen employing differential mediums for the isolation of aerobic and anaerobic bacteria were essentially negative.

From the brain tissue of 335 necropsied bats, nine virus isolates were obtained in white Swiss mice (*3, 4*). These isolates were identified as the virus of rabies by the standard intracerebral neutralization technique. Tests with infected mouse brain tissue against known rabies-immune horse serum demonstrated that the immune serum neutralized both its homologous virus and the newly isolated bat strains.

In addition to confirmed rabies isolations, five other viral agents were recovered from the salivary glands of encephalitic bats in white Swiss mice. The bat salivary gland virus has an incubation period of 5 to 6 days intracerebrally in mice. With the exception of mice and bats, host susceptibility studies including rabbits, hamsters, guinea pigs, and goats were negative.

Reciprocal complement-fixation tests with known neurotropic virus antigens and antiserums and with antigens and antiserums to the bat salivary gland agent suggest that the bat virus shares some antigen common to St. Louis encephalitis virus (Hubbard strain). Thus, specific St. Louis encephalitis guinea pig antiserums consistently fix complement with bat antigens. This fixation is of low order and is not reciprocal, for high-titered bat antiserums will not fix complement with St. Louis encephalitis antigens. No relationship to any other arthropod-borne virus has been demonstrated by complement-fixation tests.

The bat virus is partially neutralized by potent hyperimmune St. Louis encephalitis rabbit serum. There was no evidence of reciprocal activity. Antiserums of western equine encephalomyelitis, eastern equine encephalomyelitis, lymphocytic choriomeningitis, encephalomyocarditis, and rabies exhibited no neutralizing effect against the bat virus.

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25 August 1955

Quantitative Microinjection of Mosquitoes

A method for obtaining oocysts of the malaria parasite free from the mosquito stomach wall was sought as a further step toward the *in vitro* studies of the development of oocysts of *Plasmodium relictum* in *Culex tarsalis* (*1*). Following Weathersby's results (*2*) in obtaining infection of the mosquito (*Aedes aegypti*) with oocysts unattached to the stomach wall by injecting blood infected with the parasite (*Plasmodium gallinaceum*) into the hemocoele of the insect, an attempt was made to use this technique to obtain free oocysts (*3*).

The injection setup consisted of two parts, the injector and the insect holder (Fig. 1). Adapted from the "braking" pipette of Holter (*4*), the injector was made of two components: (i) a needle drawn from glass tubing forming a spindle with a capillary at each end and (ii) a glass tubing jacket, tapered at one end, into which the spindle was sealed and made airtight with Duco cement (Fig. 2, top). For injection, the injector was clamped firmly to a stand in a horizontal position and fastened to a rubber bulb that was provided with a check valve. Solutions to be injected were brought in touch with the point of the needle; the spindle then filled itself through capillary action. Known quantities of solution were obtained by using accurately calibrated spindles of different sizes.

The insect holder was a device similar to that of Wallis (*5*). It was made of glass tubing with a tapering end. The insect was held on the opening of this end by suction (Fig. 2, top). Successful attachment depended on a suitable size of the opening and the shape of the contact area of the insect. An opening with a curved surface could be ground to suit the particular need. The holder was mounted on a clamp that was attached to a mechanical stage by a spring-loaded lever. A screw in control of the lever provided vertical movement, as is shown in Fig. 1. Thus, within a sufficient range, the holder could be fitted to any position demanded.

The injection was carried out by setting the needle point of the injector in the center of the field of the dissecting microscope and then bringing the insect, impelled by the holder, to the needle. When the needle had pierced the body wall of the insect, pressure was maintained on the rubber bulb until the insect was drawn away from the needle in order to avoid backflow of the injected material.

The site selected for injection was the base of the metacoxa (Fig. 2, top); and the age of the mosquitoes used was not less than 3 days, for younger ones did not stand injection well. To immobilize the mosquitoes, a combination of carbon dioxide and cold was found satisfactory. The mosquitoes were first knocked down by CO_2, separated into small lots, and then kept inactive in an ice bath. A continuous CO_2 chamber in which the injection was carried out was found to be very convenient but was abandoned because of the possible effect on the solution to be injected.

Before injecting the parasitized blood of the canary, the technique of injection and the effect of the anticoagulants were tried on both sexes of the mosquitoes. The anticoagulants used were either sodium citrate or heparin in normal saline. At times, glucose was added to the saline-anticoagulant mixture as a possible essential food supplement for the activities of the parasites. Doses of 0.61 mg (comparable to the amount of blood in a fully engorged mosquito) each were injected into the females. Almost 100-percent survival was obtained regularly. Needles varying in diameter from 10 to 90 μ were equally safe, and in one experiment, a needle 300 μ in diameter was used on 13 females, nine of which survived for many days.

Repeated injections were also tried on a total of 21 females in two separate trials. These were fully injected three times on alternate days with a needle 40 μ in diameter, and 19 were still surviving on the third day following the last injection.

However, the males were less hardy,

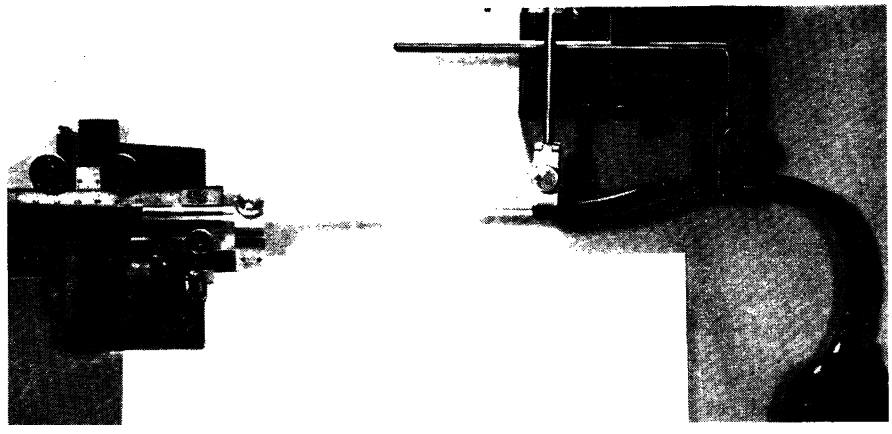

Fig. 1. Insect holder and injector.

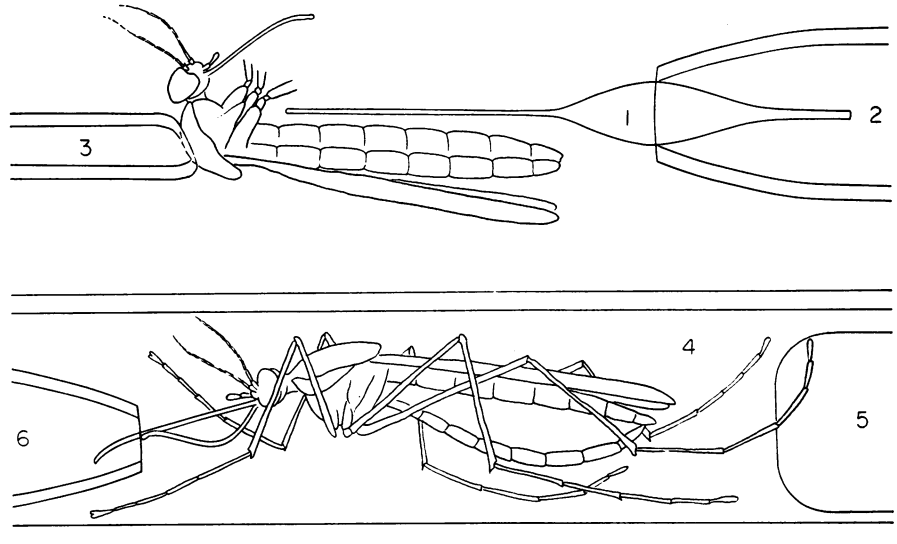

Fig. 2. (Top) Injection of mosquito; (bottom) forced feeding of mosquito. (1) Needle, (2) jacket, (3) insect holder, (4) glass tubing, (5) plastic rod, (6) micropipette.