tion of the aromatase, as might be suggested from studies by Ortiz de Montellano and co-workers (*2, 3*) on rat liver microsomal cytochrome P-450, has not been determined. Identification of the metabolite of NET suicide inhibition reaction of aromatase is in progress by use of ^{3}H-labeled NET.

These findings raise challenging questions on the molecular mechanism of the aromatase reaction and on the possible effects of NET on estrogen in humans taking contraceptive pills.

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Transmission of Oropouche Virus from Man to Hamster by the Midge *Culicoides paraensis*

Abstract. *Oropouche virus (arbovirus family Bunyaviridae, Simbu serological group) was experimentally transmitted from man to hamster by the bite of the midge* Culicoides paraensis. *Infection rates and transmission rates were determined after the midge had engorged on patients with viremia. The threshold titer necessary to enable infection or transmission by the midges was approximately 5.3 $\log_{10}$ of the median lethal dose of the virus in suckling mice per milliliter of blood. Transmission was achieved 6 to 12 days after* C. paraensis *had taken the infective blood meal. This represents conclusive evidence of transmission of an arbovirus of public health importance to man by a member of the Ceratopogonidae family.*

During the past two decades Oropouche (ORO) virus (arbovirus family Bunyaviridae, Simbu serological group) has been recognized as a major cause of human febrile illness in the Amazon region of Brazil. Between 1961 and 1980 numerous outbreaks occurred in urban centers of Pará State, in the eastern part of Amazonia (*1*). At least 165,000 persons were infected, including 130,000 in 1978–1980, when the greatest wave yet recorded affected 16 localities of this state, including the capital (*2, 3*). Outside of the Amazon region, human infection caused by ORO virus has been recognized only in Trinidad, where it was first isolated in 1955, but to date no epidemics have been recorded in that country (*4*).

Three types of clinical syndromes have been associated with ORO virus infection: (i) febrile illness, (ii) febrile illness with rash, and (iii) meningitis or meningismus. Although no fatalities have been attributed to the disease, many patients become severely ill, some to the point of prostration. Acute manifestations usually last one week or less, but many patients experience one or more episodes of recurrence of symptoms for a period of 1 or 2 weeks. Instances of meningitis associated with ORO virus infection were observed during the 1980 outbreak in Pará, and although no sequelae occurred among these patients, it is clear that meningitis is an aggravating component of ORO virus infection (*5*). A rash also is occasionally observed on the trunks, arms, and less commonly on the thighs (*3*).

Oropouche virus probably occurs in nature in two distinct cycles: a sylvatic cycle which is responsible for maintenance of the virus in nature, with primates, sloths, and possibly certain species of wild birds implicated as vertebrate hosts, with the sylvatic vector still unknown; and an urban cycle during which man may be infected and, once infected, probably serves as an amplifying host of the virus among hematophagous insects. Two insect species have been implicated as potential vectors in the urban cycle through epidemiological studies made during outbreaks: the ceratopogonid midge *Culicoides paraensis* and the mosquito *Culex p. quinquefasciatus* (*6, 7*). In laboratory transmission studies of both the suspect vectors the former was found to be the more efficient of the two, but hamsters were used in these experiments as the donor and recipient hosts (*8*). Viremia titers in experimentally infected hamsters are usually much higher than those in man; consequently, the question remained whether *C. paraensis* could become infected when feeding on the lower-titered viremia which is circulated when man is infected. In this report we describe the successful transmission of ORO virus from man to hamsters by the bite of *C. paraensis*. This observation represents the first definitive evidence of transmission of an arbovirus pathogenic to humans by a vector of the family Ceratopogonidae.

Suspected cases of ORO virus infection which occurred during the 1979–1980 Pará outbreak were chosen for the study. Most patients were selected during their first 2 days of illness when viremia titers are usually highest. Blood was collected from febrile patients, diluted 1:10 in phosphate-buffered saline (PBS) with 0.75 percent bovine albumin, and frozen at 70°C for subsequent virus titration. Viremia values were calculated by the method of Reed and Muench (*9*) after inoculation of serial tenfold blood dilutions intracerebrally into suckling mice. Virus identity was confirmed by complement-fixation (CF) tests with the use of mouse brains as antigen and ascitic fluid from hyperimmune mice (HMAF) prepared against the Belém ORO virus prototype strain (BeAn 19991).

All midges used in the transmission experiments were captured as they were attracted to humans at an agriculture research institute near Belém where banana and cacao trees are cultivated (*8*). The *Culicoides* were maintained at 22° to 25°C and 95 percent relative humidity and were provided with a 10 percent sucrose solution which was removed a few hours before they were allowed to feed on the patients.

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Table 1. Transmission of Oropouche virus from man to hamster by *Culicoides paraensis*. Viremia is expressed as $\log_{10}$ of the median lethal dose of the virus per milliliter of blood.

Viremia	Number of patients exposed	Number of patients infecting *Culicoides*	*Culicoides*	
			Number infected/ number engorged (%)	Number transmitted/ number infected (%)
6.3 to 7.3	7	6	12/35 (34)	6/12 (50)
5.3 to 6.2	16	5	15/115 (13)	6/15 (40)
≤ 5.2	4	0	0/31	
Total	27	11	27/181 (15)	12/27 (44)

Shortly after the initial collection of blood from the patients, 20 to 100 *Culicoides* were allowed to engorge for about 1 hour on a patient's hand, usually late in the afternoon. After they had fed the midges were immobilized at 4°C and engorged specimens were removed and placed in a separate holding cage and maintained with 10 percent sucrose solution. Unfed insects were either discarded or held and later tested as a triturated suspension for the presence of virus by intracerebral inoculation into suckling mice. Five or more days after the midges had fed on patients, the midges were given access to laboratory bred, newly weaned Syrian hamsters. The *Culicoides* were placed in small glass tubes, the open ends of which were put in contact with the shaved abdomen of a hamster. One to three insects were allowed to feed on each hamster. After they had fed, the engorged insects were immediately frozen at − 70°C pending virus assay. Engorged midges were later triturated and suspended in PBS containing bovine albumin, and the supernatant was inoculated intracerebrally into suckling mice and Vero cell cultures. Fluids from Vero cells showing cytopathic effect were harvested and ORO virus was identified by a neutralization test with Vero cells and reference HMAF to the prototype ORO virus. Hamsters were observed daily for signs of illness. Brains and livers of morbid hamsters were removed and used as antigens in CF tests with reference HMAF for virus identification. Surviving hamsters were tested for the presence of antibody to ORO virus by a hemagglutination inhibition test 3 weeks after they had been exposed to the bites of the *Culicoides*.

Data obtained in transmission studies are summarized in Table 1. The midges were not infected after feeding on patients circulating less than 5.3 $\log_{10}$ of the median lethal dose of ORO virus in suckling mice ($SMLD_{50}$) per milliliter of blood. Above this apparent threshold both infection and transmission by *Culicoides* was clearly related to the amount of virus in the blood of the human donor. Six of seven patients with a viremia in excess of 6.2 $\log_{10}$ $SMLD_{50}$ per milliliter infected 12 of 35 midges (34 percent). Six of the 12 *Culicoides* (50 percent), which in turn fed on hamsters, transmitted the virus. Infection and transmission rates were lower when midges fed on patients with a viremia of 5.3 to 6.2 $\log_{10}$ $SMLD_{50}$ per milliliter. Virus was not recovered from 514 *Culicoides* which had not taken a visible quantity of blood after exposure to 12 patients.

Although previous studies suggested that *C. paraensis* is the probable urban vector of ORO virus, conclusive evidence had been lacking. Epidemiological evidence was based mainly on the fact that a higher incidence of human infections occurred in areas with high densities of *C. paraensis* (*7*). The rates of isolation of ORO virus from *C. paraensis* collected during outbreaks have been low, however. Virus was isolated from only ten of about 125,000 *C. paraensis* examined, a rate of approximately 1 in 12,500. Our findings suggest that the threshold required to enable *C. paraensis* to transmit ORO virus is close to 5.4 to 5.5 $\log_{10}$ $SMLD_{50}$ per milliliter, a viremia titer not uncommon among ORO patients.

Hamster-to-hamster transmission of ORO virus by the other suspected urban vector, *Cx.p.quinquefasciatus*, has been accomplished (*10*). The threshold of infection for these mosquitoes is high (≥ 9.5 $\log_{10}$ $SMLD_{50}$ per milliliter), well above the usual viremia titers of ORO patients. Efficiency of virus transmission by this mosquito is low. Thus, we conclude that *C. paraensis* is the more important vector of ORO virus.

Culicoides have been recognized as the vectors of certain arboviruses responsible for serious diseases of domestic animals such as bluetongue, African horse sickness, and Akabane fever (*11, 12*). Among arboviruses which cause significant human disease, eastern equine encephalitis and Congo viruses have been sporadically isolated from *Culicoides* (*12*), but these insects are not considered of importance for the maintenance of these agents.

Our findings represent conclusive evidence of an important arbovirus disease of man that is transmitted by *Culicoides*. Methods for the control of *C. paraensis* will have to be developed in order to prevent or interrupt epidemics, particularly in view of the increasing activity of ORO virus in urban centers of eastern Amazonia and the first report of an epidemic in the western part of this region, where the large city of Manaus was extensively affected (*13*). Spread of the virus to other urban centers infested with *C. paraensis* outside of the Amazon region (*14*) must also be considered.

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Transport of Dissolved Amino Acids by the Mussel, *Mytilus edulis*: Demonstration of Net Uptake from Natural Seawater

Abstract. *High-performance liquid chromatography provides direct evidence for substantial removal of naturally occurring specific free amino acids during a single passage of water through the mantle cavity of mussels. This occurs during the few seconds required for passage of the water across the gill, and removal proceeds unabated at ambient substrate concentrations as low as 38 nanomoles per liter.*

The total mass of organic material in solution in the oceans is vast, outweighing the total biomass on the earth (*1*). Marine biologists have speculated for more than a century that this very large potential resource may be available to marine organisms as a nutritional supplement (*2*). Demonstration of rapid influx of specific radiolabeled substrates into marine invertebrates (*3*) has kindled interest in this possibility and stimulated work in the field. Studies in the past two decades strongly support the widespread occurrence of uptake and utilization by marine organisms of elements of the dissolved organic pool (*4*). However, evidence for this conclusion is less direct than is desirable, and considerable controversy still exists about the role of dissolved organic material in invertebrate nutrition (*5*). Since concentrations of specific organic compounds in the natural pool are extremely low, there is no direct analytical evidence for net entry of organic substrates at the submicromolar levels characteristic of natural waters. Furthermore, it has been impossible to exclude completely a possible role of heterotrophic microorganisms, which have been present in the experimental systems studied to date (*6*). We report here that net entry of specific organic solutes from natural waters has been established by direct chemical analysis. A significant contribution to the process by contaminant microorganisms was ruled out by the very rapid changes in concentration, which occurred within seconds.

We prepared fluorescent derivatives of amino acids and other amines in solution with *o*-phthaldialdehyde (OPA). Derivatives were separated by high-performance liquid chromatography (HPLC). Peaks were monitored with a fluorometer, identified by elution time, and quantified by peak area (*7, 8*). The technique has a sensitivity in the picomole range. With a sample of 200 μl of seawater, a quantitative measure of specific amino acids present at concentrations as low as 10 n*M* can be obtained. The linearity of the relation between peak area and concentration was established by chromatography of standards ranging in concentration from 83.5 to 1000 n*M*. Coefficients of determination (r^2) for the three amino acids used were aspartic acid (Asp), .99 (N = 19); serine (Ser), .99 (N = 21); and glycine (Gly), .98 (N = 21).

Specimens of the mussel *Mytilus edulis* were collected locally from Newport Bay in southern California, cleaned with a wire brush, and adapted to room temperature for 24 to 48 hours before use. An animal was placed in 400 ml of medium and allowed to open and begin pumping. The medium was withdrawn and replaced; normally, pumping resumed promptly. Samples were then collected directly from the excurrent siphon of *Mytilus* with a fine plastic cannula positioned with a micromanipulator. Such samples were compared with water samples from the incurrent margin of the animal. Since the animal was unrestrained and pumping normally, differences between incurrent and excurrent samples represented changes that occurred during a single passage of water through the mantle cavity. This procedure has been described in detail (*9*).

The first two tracings in Fig. 1 are chromatograms obtained from 200-μl samples of natural seawater taken respectively from the incurrent margin and the excurrent siphon of an animal as soon as possible after the onset of pumping. All chromatograms are photographs of original records. Fluorescent derivatives of aspartate, serine, and glycine are labeled. The water was collected from the immediate habitat of the animal, brought to the laboratory, and passed through a 0.2-μm Nucleopore filter to remove particulate matter and microorganisms. Analysis of a small sample of water filtered immediately in the field indicated that the brief period between collection of the water sample and filtration in the laboratory did not result in noticeable changes in amino acid composition. In the case presented in Fig. 1, 63, 84, and 72 percent of aspartate, serine, and glycine were removed by the animal during passage of the water through the mantle cavity.

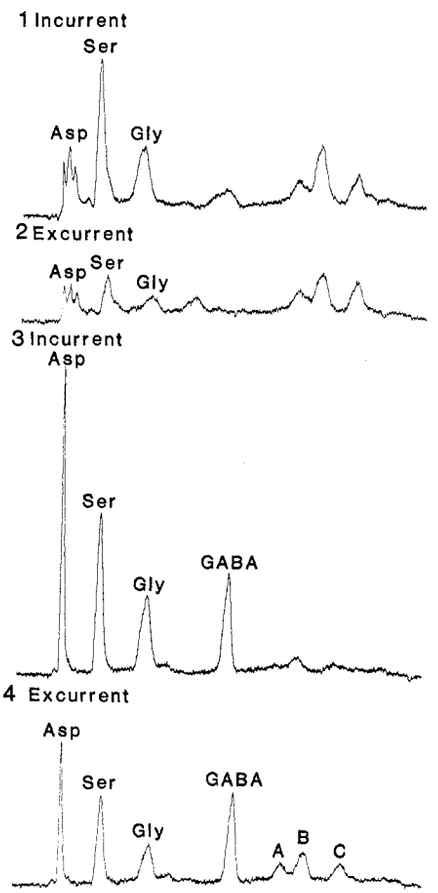

Fig. 1. HPLC chromatograms of natural seawater and an artificial seawater solution. Tracing 1 is natural seawater collected from the immediate habitat of *Mytilus edulis*. Tracing 2 is the same water after passage through the mantle cavity of the animal. Tracing 3 is an artificial seawater solution containing 0.25 μmole per liter of Asp, Ser, Gly, and GABA. Tracing 4 is the artificial solution after passage through the mantle cavity of the animal. Peaks of the four amino acids are labeled. Peaks A, B, and C are material added by the animal during passage of water through the mantle cavity.

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