Table 2. Isolation of Machupo virus from *Calomys callosus* captured in San Joaquin, Bolivia, 20–27 May, 1964. Symbols: +, virus isolated; o, virus not isolated; n, no test or unsatisfactory test.

Block and house number	Virus isolation		
	Kidney	Spleen	Urine
	+	+	o
3–19	+	+	n
8–75	+	o	+
8–75	o	+	n
8–77	o	o	o
9–84	o	o	o
10–94	n	+	+
10–95A	+	+	+
10–95A	o	o	n
10–95A	+	+	n
10–98	+	+	o
10–98	+	+	n
13–117	+	+	n
21–234	+	+	+
28–307	o	o	o
28–308	+	+	o
28–309	+	+	+

virus was found in her urine. The offspring were then transferred to individual boxes, and each was shown to be viremic at 12 and 17 weeks of age. In contrast to adult animals inoculated parenterally, none of these *Calomys* had detectable virus-neutralizing antibodies 20 weeks after potential first exposure to infection.

The demonstration of chronic viruria in experimentally infected rodents stimulated a search for a similar pattern among wild *C. callosus* in San Joaquin, Boliva, where hemorrhagic fever among humans was then active. Seventeen animals were trapped alive from 20 to 27 May 1964, mostly from houses which were probable sources of recent human illness. Samples of voided urine were obtained from 11 of these grossly healthy animals, after which kidneys and spleens were removed. Results of isolation attempts (Table 2) are based on successful reisolation of virus. Machupo virus was recovered with nearly equal frequency from spleen and kidney, and quantitative determinations made in nine cases revealed from $10^{2.8}$ to $10^{5.2}$ $IHLD_{50}$ per gram of tissue. Virus was also detected from 5 of 11 urine specimens, and only from animals whose tissues were positive.

These results suggest certain biological parallels to a limited group of previously described viral agents, including the salivary gland viruses (*8*) and that of lymphocytic choriomeningitis (*9*). In the case of lymphocytic choriomeningitis, mice infected before or shortly after birth become chronically infected; and virus can be found in various tissues, in blood, in urine, and in saliva for long periods, probably for life, in the absence of easily detectable circulating antibodies (*10*). It seems possible that *C. callosus*, when infected prior to or shortly after birth, may also exhibit chronic infection without detectable immune response.

The data also provide a significant new concept that can be evaluated in the search for understanding of the natural history of infection with Machupo and related viruses. Neither Junin nor Tacaribe virus has been shown conclusively to be transmitted by arthropods. Efforts to incriminate such vectors in transmission of Machupo virus have also failed. Continuous contamination of the human environment by infectious urine shed by chronically infected peridomestic rodents could be an important mechanism leading to human disease (*11*).

The presence of viremia in addition to viruria in *C. callosus* preserves the possibility that hematophagous arthropods might play some role in virus transmission. Experimental arthropod transmission studies need to be done. However, if arthropods are not important, some means of rodent to rodent transmission is mandatory. It appears that the events comprising mating, gestation, and neonatal behavior provide the greatest number of potentially reliable mechanisms for maintenance of virus infection. Studies have been initiated to test these possibilities in colonized *C. callosus*.

A classical interpretation of the chronic asymptomatic infection induced by Machupo virus would strongly imply that this agent has had a long and successful history of parasitism in rodents of South America. If this hypothesis is correct, it is possible that human hemorrhagic fever on that continent is not a new disease, but, for a variety of reasons has been recognized as a disease only within the past 15 years.

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Sex Conversion Induced by Hydrostatic Pressure in the Marine Copepod Tigriopus californicus

Abstract. *High hydrostatic pressure applied to the naupliar larval stages of the marine copepod* Tigriopus californicus *converts some individuals that would have become males into females. The copepodid stages are not sensitive to pressure-induced conversion.*

Vacquier (*1*) has presented evidence that high hydrostatic pressures applied for 2 hours to the first naupliar larval stage of the marine copepod *Tigriopus californicus* produced an increased percentage of female survivors. Two hypotheses were advanced to account for this phenomenon: (i) The pressure effect was selective, killing larvae already determined to become males and either not affecting or with a reduced effect upon those destined to become females. (ii) The pressure was converting some potentially male larvae into females.

Norton (*2*) has presented statistical evidence for sex conversion based on Vacquier's (*1*) data. However, we be-

lieve that survival was too low in these earlier experiments to establish conclusively a sex conversion.

We now report new experiments that demonstrate pressure-induced sex conversion during the naupliar stages.

The life cycle of *Tigriopus* consists of six discoidal naupliar stages followed by six copepodid stages in which the gross body form is that of a miniature adult. The sixth copepodid is the adult. Transition to each successive instar is linear with time. Sex cannot be visually distinguished until the fifth copepodid stage. The life cycle requires 14 days at 23°C.

Female copepods were obtained as described by Vacquier (*1*). For each experiment at least 100 egg sacs were dissected from gravid females and pooled in a single dish containing 50 ml of sea water. Nauplii hatch within a few minutes after removal of the egg sac. Usually 20 to 80 nauplii hatch from a single sac as free-swimming, actively feeding larvae. These larval pools were stirred vigorously to insure a random distribution.

For pressurization experiments, 50 larvae of a desired developmental stage were isolated in one 4-ml, diamond-K test tube. A neoprene 000 stopper was inserted so that no air bubbles remained in the tubes. Usually six to ten tubes, each containing 50 larvae, were pressurized at each desired pressure. Pressurization was achieved by use of the apparatus described by Zobell and Oppenheimer (*3*) following the procedure outlined by Vacquier (*1*). All pressure levels were held for 2 hours.

After depressurization each tube of 50 larvae was cultured separately in enriched sea water medium containing the green flagellate *Platymonas* sp. as the food organism. The temperature during pressurization and culturing was 23°C. When the larvae became adults, the cultures were examined and the percent survival and sex ratio were determined.

The data presented in Table 1 are taken from four individual experiments, each representing different naupliar pools. The pressurization was carried out when the great majority of the population were in the stages shown in column 1 of the table. The controls are indicated as 1 atm.

It is apparent from these data that there is not only a drop in the number of males surviving the pressure treatment but that in each of the late naupliar experiments more female *Tigriopus* appeared in the pressurized samples than were present in the controls. The highest number of females obtained in any control group was 183, while in the pertinent experimental group 245 females were found among survivors of 450-atm treatment and 242 among survivors of 550-atm treatment. In the nauplius VI sample, where survival is high, there were 117 females surviving in the control group, 191 in the 450-atm sample, and 159 in the 500-atm sample. These represent approximately 39 percent females in the control and 67 and 62 percent in the two treated groups. Survival in the nauplius V and copepodid I experiments is below normal. This could be caused by injury to the larvae during transfer or contamination of the cultures by detrimental microorganisms. The exact cause is not known.

Table 1. Sex conversion induced by hydrostatic pressure in *Trigiopus*. Conversion can be shown if pressurization occurs during the naupliar stages. Conversion cannot be demonstrated after the onset of the copepodid stages.

Stage	Pressure (atm)	Ratio: No. surviving/ No. treated	Survival (%)	Males (No.)	Females (No.)	Percentage of females among survivors
Nauplius V and VI	1	411/450	91	228	183	45
	450	471/500	94	226	245	52
	500	434/500	87	192	242	56
Nauplius V	1	216/300	72	138	78	36
	450	215/300	72	70	145	68
	500	202/300	67	76	126	62
Nauplius VI	1	297/300	99	180	117	39
	450	286/300	95	95	191	67
	500	256/300	85	97	159	62
Copepodid I	1	157/250	63	105	52	33
	450	229/300	76	145	84	37
	500	281/300	94	197	84	30

These data show that a fraction of each pressurized sample which would normally have become males developed into functional females. The fact that sex conversion does not occur after the onset of the first copepodid stage suggests that sex has been irreversibly determined by this time. These experiments have been repeated with very similar results.

Experiments indicate that at 550 and 650 atm at least 1 hour of pressurization is necessary to suggest conversion in the surviving samples.

Mednikof (*4*) reports that in many species of calanoid copepods only females are described. The percentage of species in which only females are known increases with depth of habitat. These data may represent pressure-induced sex conversion. A mechanism of this type could be of selective value to a bathypelagic copepod inhabiting depths where phytoplankton is scarce, the advantage to the species being increased fecundity.

Since *Tigriopus* is found only in the spray pools of the supralittoral tidal zone, a pressure-induced, sex-converting mechanism cannot be interpreted as having selective advantage. This phenomenon may be a characteristic of the sex-determining mechanism in the Copepoda.

The problem of sex determination in crustacea has been reviewed by Charniaux-Cotton (*5*). In many crustacea thus far examined there exists an androgenic gland associated with the testis or the vas deferens which is necessary for male development. In the amphipod *Orchestia* studied by Charniaux-Cotton (*5*), androgenic gland anlagen are found in all larvae. In those which are genetically male they develop into functional glands which cause the undifferentiated gonads to become testes and produce a functional and morphological male. In larvae genetically determined to become female, the androgenic anlagen do not develop and the undifferentiated gonad becomes an ovary.

It has also been reported by Charniaux-Cotton (*6*) that in the protandrous decapod *Lysmata* the end of the male phase and the beginning of the female phase correlates with the degeneration of the androgenic gland.

Androgenic glands are not known in the Copepoda. However, it is possible that analogous endocrine tissue

may exist in an anatomically nondistinct form, its functional existence made evident only by removal and implantation experiments.

The application of high hydrostatic pressure to *Tigriopus* in the naupliar stages may be damaging a primordial organ or gland which is necessary for male differentiation. Without the influence of a male-determining structure the larvae become females. Evidence that the primary effect of pressure is not upon a biochemical product, such as a gonadotropin, but is on the site of production of a product, appears from the fact that pressurization occurred for only 2 hours. It seems reasonable to assume that any biochemical entity destroyed during this time could be resynthesized after depressurization.

The fact that the copepodid stage is insensitive to conversion may indicate maturation and insensitivity of the hypothetical androgenic gland. An alternative hypothesis is that pressure may be altering the function of genes responsible for male determination. Evidence for pressure-induced gene alteration has been reported in *Neurospora* by McElroy and de la Haba (*7*) and in *Serratia* by Palmer (*8*).

The exact mechanism of pressure-induced sex conversion may not be accessible to further study in *Tigriopus* because the small size of the larvae and adult restrict the precision of endocrinological experiments.

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Oxygen Consumption Rate and Electroencephalographic Stage of Sleep

Abstract. *In five male subjects, and a total of 15 man-nights, oxygen consumption rate ($\dot{V}_{O_2}$) was related to stage of sleep, as defined by electroencephalograms. Gross periodic variations which paralleled change in stage of sleep were discernible in analogue metabolic records. Computations revealed significant differences ($P < .01$) between all stages with $\dot{V}_{O_2}$ highest in stage I REM (dreaming sleep), least in stages III and IV (deep sleep), and intermediate in stage II (light sleep).*

Since the first report that visual dreams were accompanied by a low-voltage, fast electroencephalogram (EEG) and rapid eye movements (stage I REM) (*1*), a number of workers have studied the relationship between physiological indices and EEG stage of sleep. Kamiya noted that pulse and respiration were most rapid and irregular during stage I REM (*2*). Snyder *et al.* reported that blood pressure is higher and fluctuates more than in other stages of sleep (*3*). Evarts found cortical discharge rates to be highest during stage I REM sleep, almost approaching those of wakefulness (*4*).

Nocturnal patterns of gross energy expenditure have also been described, but thus far without reference to EEG recordings (*5–7*). The present study seeks to clarify the relationship between the rate of oxygen consumption ($\dot{V}_{O_2}$) during the night and the level of sleep as reflected by the EEG.

Determinations were made on five subjects for a total of 15 man-nights. The group comprised five males, ranging in age from 19 to 35, with a mean of 26 years. All subjects were caucasian and in good general health. Each reported to the laboratory at his usual bedtime for three consecutive nights, during which measurements were obtained under conditions of natural sleep. The subjects slept on a comfortable bed in a temperature-controlled metabolic chamber adjacent to the instrument laboratory. The EEG patterns were recorded from the right frontal and parietal areas by means of monopolar, silver-disc surface electrodes and a six-channel Grass Polygraph, model P7. Electrooculograms were similarly measured with electrodes placed at the outer canthus of each eye. Body movements were recorded by a transducer placed on the bedspring approximately at the level of the lumbar region of the body.

Respiratory exchange was measured continuously during sleep by means of an automatic gas analysis system similar to that described by Buskirk *et al.* (*7*). In this system the subject's head was enclosed in a specially constructed plastic hood, through which ambient air was drawn at a constant rate of 80 liter/min (approximately eight times ventilation volume). A sample of the mixture of ambient air and expired gases was diverted through paramagnetic and infrared gas analyzers for simultaneous analysis of oxygen and carbon dioxide content. The results of these analyses were converted into electrical outputs and written out to provide continuous and permanent analogue records. Values for rates of oxygen consumption were computed from the data by means of standard respiratory equations, after conversion

Table 1. Comparison of $\dot{V}_{O_2}$ in stages of sleep throughout the night (pooled data for 15 man-nights). Means are given with standard deviations. Only continuous stages lasting 15 minutes or more were used in this analysis. The fact that t is positive signifies that x is greater than y.

Stage of sleep			Mean $\dot{V}_{O_2}$ (cm³/min)		t value*	Level of significance
x	vs.	y	x	y		
I REM	vs.	II	228 ± 25	220 ± 27	3.82	$P < .01$
I REM	vs.	III + IV	228 ± 25	214 ± 26	6.64	$P < .001$
II	vs.	III + IV	220 ± 27	214 ± 26	3.09	$P < .01$

* The t-test was computed as follows (*13*): $t = (\bar{x}_1 - \bar{x}_2)/Sp[(1/N_1) + (1/N_2)]^{\frac{1}{2}}$, where: $Sp = [(N_1 - 1)S_1^2] + [(N_2 - 1)S_2^2]/(N_1 + N_2 - 2)$; $S_1^2 = \Sigma (x_{t_1} - \bar{x})^2/(N - 1)$; $\bar{x}_1$, $\bar{x}_2$ are means of sleep stages being compared; and N_1, N_2, are number of 5-minute observations taken in each stage. The significance levels quoted are for a two-tailed test.