Table I. Effect of different concentrations of actinomycin *D* on the replication of infectious pancreatic necrosis (IPN) virus[a]

Concentration of actinomycin D (μg/ml)	Virus infectivity titer[b]
0.0	7.8
0.2	5.0
0.5	4.7
1.0	4.5
2.0	5.0

[a] Duplicate cultures of RTG-2 cells were pretreated with maintenance medium containing the designated concentrations of actinomycin D, 2 h prior to infection. Immediately before infection, the cultures were washed free of residual actinomycin with 3 changes of fresh maintenance medium. All cultures were then infected with approximately 100 $TCID_{50}$'s of virus per cell. Following a 1 h adsorption period at 22 °C, unadsorbed virus was removed by washing the cell sheet 3 times with Earles balanced salts solution. All cultures were then reincubated at 22 °C in maintenance medium containing the appropriate concentration of actinomycin D. The experiment was terminated at 24 h after infection. [b] Log_{10} of the number of $TCID_{50}$'s per 1.0 ml.

Table II. Effect of the time of addition of actinomycin *D* on the replication of infectious pancreatic necrosis (IPN) virus[a]

Actinomycin addition[b] (h)		Virus infectivity titer[c]
–[d]		7.8
1	Pre-infection	5.3
0		5.1
1	Post-infection	6.7
2	Post-infection	7.9
3	Post-infection	7.3
4	Post-infection	7.3
5	Post-infection	7.5
6	Post-infection	7.9
9	Post-infection	7.8
11	Post-infection	7.3

[a] All cultures were infected at time 0 with approximately 100 $TCID_{50}$'s of virus per cell as in Table I. Following virus adsorption the cultures were incubated in normal maintenance medium. The experiment was terminated 24 h after infection. [b] At the designated intervals, the normal maintenance medium on all cultures was replaced with maintenance medium containing actinomycin D (2.0 μg/ml). In the case of cultures treated with actinomycin prior to infection, maintenance medium containing the antibiotic was added immediately following the virus adsorption. [c] Log_{10} of the number of $TCID_{50}$'s per 1.0 ml. [d] Actinomycin D omitted.

of cells with actinomycin or the addition of the antibiotic at the time of infection resulted in a greater than 99% inhibition in the production of infective virus. A significant, but not as great, inhibition of IPN virus production was also observed when actinomycin was added within 1 h after infection. However, exposure to the antibiotic at 2 h after infection or later had no effect on the replication of IPN virus.

Microscopic examination of uninfected RTG-2 cultures treated with actinomycin D (2.0 μg/ml) revealed cells noticeably more rounded and granular than those of untreated controls. This cytotoxicity of actinomycin for RTG-2 cells was first apparent approximately 10–12 h after exposure to the antibiotic. The extent of cytological damage increased with time until 24 h after exposure, when cell damage and cell loss was extensive. The degree of cytotoxicity observed was less severe when concentrations of 0.2–0.5 μg actinomycin/ml were employed. The results presented in Table II indicate that the inhibition of IPN virus by actinomycin D is not an indirect result of the cytotoxicity of the antibiotic for RTG-2 cells. In cells exposed to actinomycin C from 2 h after infection until the experiment was terminated, IPN virus replicated to titers equivalent to those found in similarly infected untreated cells.

Although IPN virus resembles members of the reovirus group in size and morphology[15,16] and its effect on macromolecule synthesis in the infected cell (unpublished data), the results reported here indicate a sensitivity of the virus for actinomycin D which differs from that reported for the reoviruses. Instead, the pattern of inhibition of IPN virus by actinomycin D appears to parallel in many ways that described for the myxoviruses and poliovirus[1–6].

The mechanism by which actinomycin D inhibits the multiplication of IPN virus is not known. Assuming that IPN replicates in a manner similar to other single-stranded RNA viruses, an effect of actinomycin on the structure or activity of the replicative form (RF) is a possible mechanism. Alternatively, actinomycin may interfere with a constitutive host function required for virus multiplication. In any event, the actinomycin sensitive step in the replication of IPN virus occurs early in the infection process.

Résumé. La croissance du virus de la nécrose infectieuse pancréatique dans les cultures des cellules de la truite est arrêtée dans une proportion de plus de 99% par l'actinomycine D, à toutes les concentrations essayées. La réaction sensible à l'actinomycine a lieu dans les premières phases du processus infectieux.

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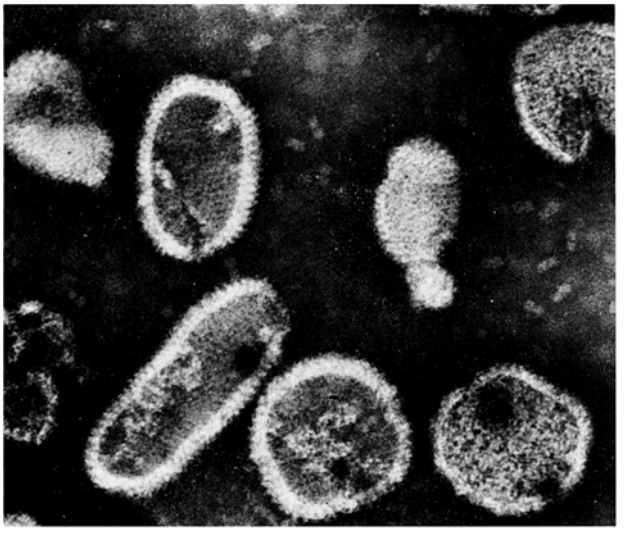
A Virus of a New Type Pathogenic to Crustacea

Very few virus diseases have been observed in marine invertebrates. In crustacea the only virus described is that discovered by Vago[1] in *Macropipus depurator* L. We have recently observed a new virus disease in *M. depurator* populations of the French Mediterranean coast. The diease is characterized by a weakening of the animals and a rapid mortality without obvious symptom.

From the haemolymph and from suspensions of different tissues of diseased crabs, an ovoid, often elongated, virus has been isolated. The virion, 150–300 nm in length, is surrounded by an envelope of composedsub-units arrang-

[1] C. Vago, *Nature*, Lond. *209*, 1290 (1966).



Infectious viral particles isolated from the haemolymph and the haemocytes of diseased *Macropipus depurator* ×. 126,000

The cytoplasm of heart cells observed in electron microscopy on ultrathin sections shows an arrangement in groups of ovoid virions.

These virions have been purified by differential and density gradient centrifugations. Injection of purified viruses to healthy crabs provoked the disease.

Further studies on the nucleic acid and the ultrastructure will enable us to define the position of this virus which shows some characteristics common to rhabdoviruses. Nevertheless, it seems that this virus cannot be included in one of the types of viruses already known in comparative virology.

The disease seems frequent in crab populations of the Mediterranean Coast, for many samplings enabled us to observe lesions and isolate the virus. It is sometimes associated with another virus, 50–60 nm in diameter, probably the one found by VAGO[1] in the same Mediterranean area.

Résumé. Une maladie virale a été mise en évidence chez le Décapode *Macropipus depurator*. Le virus isolé et purifié est de 150 à 300 nm de long, ovoïde et enveloppé. Il est expérimentalement très pathogène. Les groupements de virions ont été montrés dans le cytoplasme des cellules cardiaques.

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ed in regular lines forming a helical structure on the surface. The virion is homogenous inside but contains several elements denser to electrons (Figure).

The Spatial Orientation of Desert Ants, *Cataglyphis bicolor*, Before Sunrise and After Sunset

In two early papers SANTSCHI[1,2] suggested that desert ants (*Formicinae: Cataglyphis bicolor*; *Myrmicinae: Monomorium salomonis*) would be able to use the moon and even some brighter stars for nocturnal orientation. Although these conclusions are often cited in handbooks and review articles about ant behavior[3,4], they have not been scrutinized until now. In recent years, however, the problem of celestial orientation during the night has become more interesting, since 1. the orientation toward the moon's azimuth has been tested in several arthropods[5–8], and 2. the ability to recognize patterns of bright circumpolar stars could be proved in vertebrates[9–11]. In contrast to the overwhelming evidence for a sun-compass orientation in both arthropods and vertebrates, there are no unambiguous data about a compass orientation towards the azimuth of the moon or even of some brighter stars. Studying the visual orientation of desert ants, *Cataglyphis bicolor*[12–15], we therefore investigated the orientation performances of these desert ants during different time intervals between sunset and sunrise.

Since *Cataglyphis bicolor* is a predatory and solitary hunter, never performing mass foraging along scent trails, single ants can be successfully trained to go to feeding places. In the experiments dealt with here, the individually marked ants were trained from the nest entrance to a special azimuth α_f along a distance of 10–20 m. As in *Cataglyphis bicolor* foraging is usually restricted to day-time, the ants had to be trained under day-light conditions and were afterwards tested during the night. For each foraging run R_f the coordinates of the feeding place were determined by means of a grid of thin threads (training grid, mesh width 1.0 m), which extended over the whole experimental area. During the night, 2–100 h after having been captured at the feeding place (azimuth α_f), the ants were placed in a testing grid, far away from the training grid and completely unknown to the ants. There they were released at point P_1. Using a red light beam (Filtraflex-DT edge filter 580 nm) the return runs R_r could be exactly recorded until the ants started to search around at random (point P_2). For each return run P_1P_2 the mean direction α_r was determined graphically. Data about light intensity LI (Lux), wind direction w_f and w_r, wind velocity WV (m/sec), temperature, relative humidity and atmospheric pressure were automatically recorded during R_f and R_r.

For direction-finding during the time between sunset and sunrise, *Cataglyphis bicolor* would be able theoreti-

[1] F. SANTHSCHI, Rev. Suisse Zool. *19*, 303 (1911).
[2] F. SANTSCHI, Rev. zool. Afric. *11*, 111 (1923).
[3] F. BERNARD, *Les fourmis (Hymenoptera, Formicidae) d'Europe occidentale et septentrionale* (Masson et Cie, Paris 1918).
[4] J. H. SUDD, *An introduction to the Behaviour of Ants* (Edward Arnold Ltd., London 1967).
[5] R. JANDER, Z. vergl. Physiol. *40*, 162 (1957).
[6] F. PAPI and L. PARDI, Z. vergl. Physiol. *41*, 583 (1959).
[7] F. PAPI, Cold Spring Harbor Symp. quant. Biol. *25*, 475 (1960).
[8] J. T. ENRIGHT, Proc. AIBS Symp. Animal Orientation and Navigation (Wallops Station, Virginia, 1971), in press.
[9] H. G. WALLRAFF, Zool. Anz. Suppl. *32*, 348 (1969).
[10] S. T. EMLEN, Auk *84*, 309, 463 (1967).
[11] S. T. EMLEN, Science *165*, 716 (1969).
[12] R. WEHNER, Rev. Suisse Zool. *75*, 1076 (1968).
[13] R. WEHNER and R. MENZEL, Science *164*, 192 (1969).
[14] R. WEHNER, Verh. dt. zool. Ges. *64*, 238 (1970).
[15] R. WEHNER, Proc. AIBS Symp. Animal Orientation and Navigation (Wallops Station, Virginia, 1971), in press.