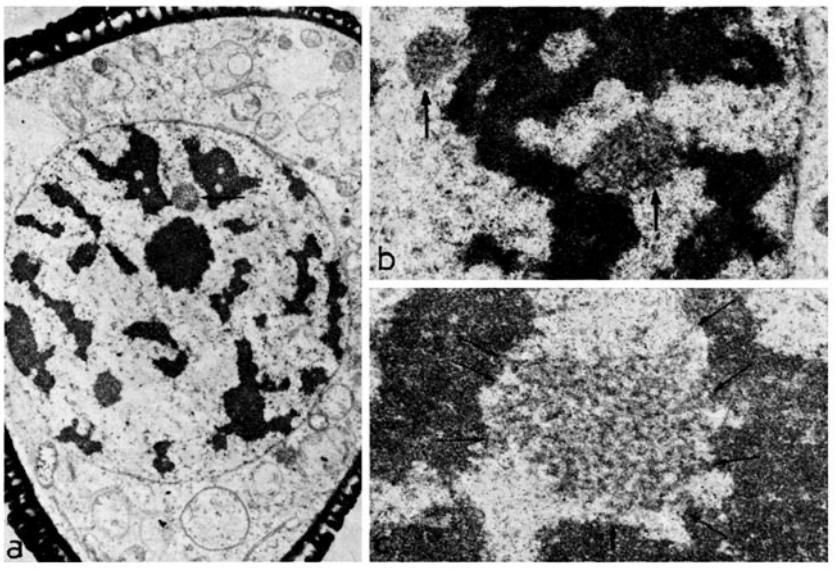

Fig. 1. a) A nucleus with the typical chromatin masses and 'micropuff' (arrow). ×4,663.
b) Part of a nucleus. 2 micropuffs (arrows), closely linked to the chromatin, can be observed. ×17,485.
c) The relationship between the micropuff and the chromatin masses can be observed (arrows). ×32,500.

mations with a diameter of 1 μm which appeared closely associated, at the same time, with a number of chromatin masses (Figure 1, a and b). The morphology of these formations appears to correspond exactly to the 'micropuffs' described by LAFONTAINE[1] and LAFONTAINE and LORD[2]. They consist, structurally, of dense elements forming fine cords of strings approximately 100 Å thick, less dense than the chromatin and enveloped in a formation of even lower density (Figure 1c). The connexions between these formations and the adjacent chromatin masses are apparently established by means of cords coming out from the chromatin masses (Figure 1c), and we might deduce from this that the chromatin mass gives rise to differentiations similar to the puffs in polytene chromosomes[3,4]. On the basis of this similarity we may connect the activities of both types of chromosome during the interphase, resulting in these closely similar formations.

Resumen. En núcleos de microsporas de *Allium cepa* se describen estructuras de 1 μm de diámetro, de baja densidad y estrechamente relacionadas con varias masas de cromatina, las que se equiparan con los denominados «micropuff» recientemente descritos.

P. ESPONDA[5] and G. GIMÉNEZ-MARTÍN

Department of Cytology,
Institute of Cellular Biology, C.S.I.C.,
Velázquez 144, Madrid (Spain), 23 November 1970.

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[5] Member of the Biology Department Staff of the University of Chile (Valparaíso).

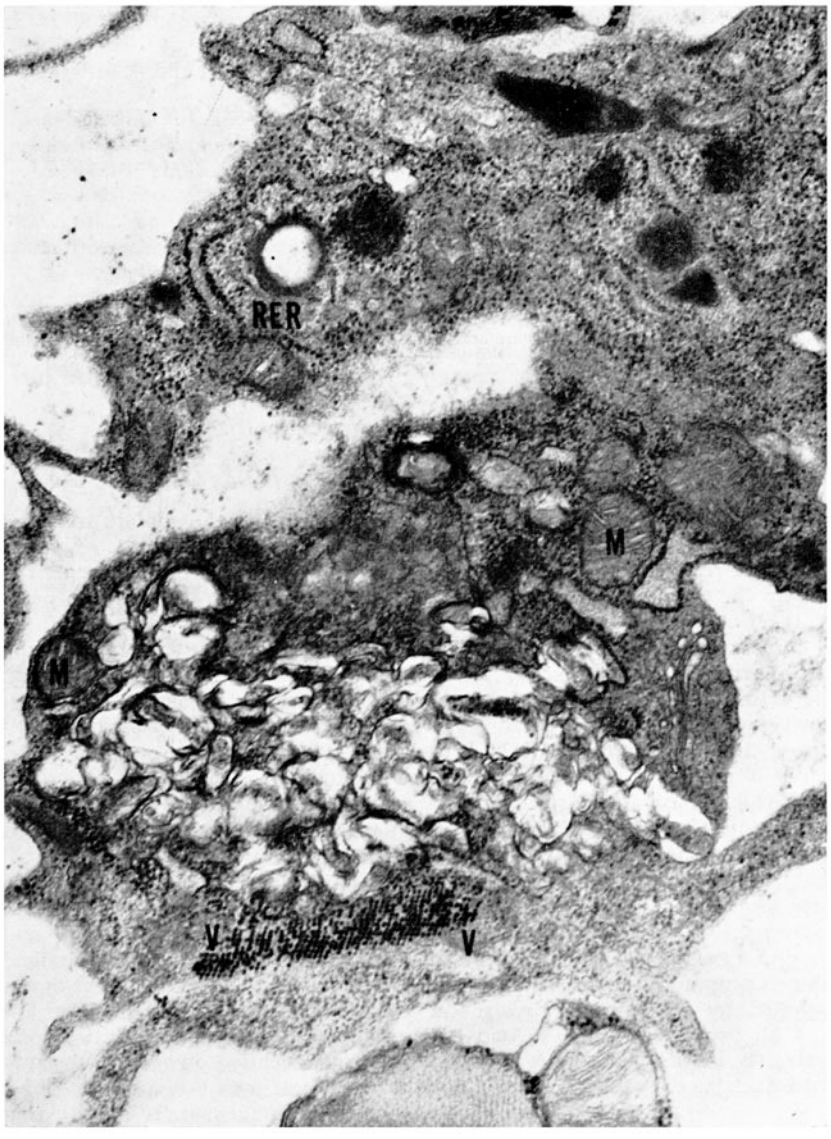
Encephalomyocarditis Viral Valvulitis in New-Born Mouse[1]

Infection with the encephalomyocarditis (EMC) virus has not been demonstrated in man, although KOCH[2] has isolated the virus from cerebrospinal fluid, earwashings, blood and feces of 3 patients. Myocarditis[3,4] and mural and valvular endocarditis[5] have been produced in mice by EMC virus in this laboratory. KILHAM, MASON and DAVIES[6] showed that the EMC virus can produce valvulitis in the mongoose.

However, EMC virus has never been demonstrated previously to lodge directly in the heart valves of infected animals. The purpose of this report is to present the finding of an EMC virus crystal in an infected fibroblast of the mitral valve of a mouse with the use of the electron microscope.

EMC virus stock was obtained from GLASGOW[7]. After 3 passages in mouse cardiac cells and 3 passages in L-cells, the cultured virus fluid was determined to have a titer of 10^{-6} $TCID_{50}$. Control fluid was prepared from non-infected L-cells. 14 new-born HaM/ICR mice were injected i.p. with 0.1 ml viral culture fluid and 6 new-born mice were injected with virus-free tissue culture fluid. At the end of 24 h, the tissues were excised and fixed in 5% glutaraldehyde in phosphate buffer for 3 h. After a buffer rinse of 2–3 h, the tissues were postfixed in 1% osmium tetroxide in phosphate buffer for 1.5 h. Tissues were then dehydrated in a graded series of methanol and embedded in epoxy resin. Thin sections were examined with a Siemens Elmiskop 1 electron microscope after they were stained with uranyl acetate and lead citrate.

A viral crystal was found in the valve of 1 of the 14 animals studied, and lesions in valvular fibroblasts were found in 5 animals. However, viral crystals and myocardial lesions were found in the myocardium of all 14 mice. Because the sections studied represent only a small portion of



Valvular tissue of a suckling mouse infected with EMC virus and sacrificed 24 h after i.p. inoculation. Note the vesiculation and vacuolation in the lower half of the fibroblast. The damage is intimately associated with the viral crystal (V). The top portion of the cell appears normal, with well preserved rough endoplasmic reticulum (RER). The mitochondria (M) show only slight swelling of the cristae. ×27,500.

the valves, it is quite likely that viral crystals were present but missed in the valves of more of the infected animals. Nevertheless, demonstration of the virus in one such animal proves that the virus can invade heart valves and produce valvular disease. The valves and myocardium of the control mice were normal.

The ultrastructural lesions consisted of the formation of vacuoles and membrane-bound vesicles within the cytoplasm of the valvular fibroblast. In the animal in which the viral crystal was located (Figure 1), the lesion consisted of vesiculation and vacuolation in intimate association with the viral crystal. In that portion of the cell in which no viral crystal was found, the cytoplasm appeared essentially normal. In the region in which the viral crystal was located, the rough endoplasmic reticulum (RER) was generally lacking, although it was abundant in the unaffected area. The mitochondria were well preserved, with only slight swelling of the cristae.

Based on the fact that a viral crystal has been located within the valvular fibroblast of a mouse infected with EMC virus, we conclude that there is a direct infection of cardiac valvular tissue by the EMC virus. This crystal is similar in appearance to that found in the myocardium[3] of the mouse. We further believe that the lesions found in the valvular tissue were produced by infection with EMC virus. The adenovirus has been reported to produce valvulitis in mice[8,9]. In adenoviral infection, intranuclear virus

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crystals were found in the fibroblasts of the valves. Definite virus-like particles and valvular cell damage have been previously demonstrated by electron microscopy in mice infected with Coxsackie B_4 virus[10]. These studies show viruses to be pathogenic for the valves of the mouse and indicate further the possible role of viruses in the production of valvular heart disease in man.

Résumé. Un cristal du virus EMC fut trouvé dans le fibroblaste valvulaire d'une souris atteinte de la maladie provoquée par ce virus. La description de ce cristal et des changements pathologiques corrélatifs survenus dans le tissu valvulaire ont établis que ce virus est au moins capable d'envahir des valvules du cœur et de produire la maladie valvulaire.

G. E. BURCH and J. M. HARB[11]

Department of Medicine of the Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans (Louisiana 70112, USA), 29 January 1971

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[11] From the Department of Medicine of the Tulane University School of Medicine and the Charity Hospital of Louisiana, New Orleans

Enzymatic and Biological Studies of Cholera (*Vibrio cholerae*) Toxin

It has been well recognized that death due to cholera (*Vibrio cholerae* or *V. comma*) infection is due to the loss of enormous amounts of fluid and electrolytes. A recent study indicates, that pathogenesis is caused by the toxin produced by the bacterium and not by the organism itself[1–5]. Choleragen was isolated from cholera toxin and found to be responsible for choleraic diarrhea in infant rabbits[1], and in humans[6]. Cholera toxin is a mixture of different proteins of which identity and function have not been well investigated. It is, therefore, important to know not only the choleragen present in the toxin but also other components which may contribute to the pathogenic effect in cholera infection.

In this study the investigation of cholera toxin other than choleragen was made. This report will describe the presence or absence of certain enzymes, hemolytic activity, and capillary tube permeability activity.

Cholera toxin was prepared by the method of FINKELSTEIN et al.[7] at the Merck, Sharp and Dohme Company. The lyophilized toxin was generously supplied by Dr. JOHN R. SEAL, of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Before the investigation was made, the absence of intact bacterium was confirmed by inoculating the cholera toxin on agar plates.

Hyaluronidase activity was followed by measuring the decrease in turbidity of a hyaluronic acid-protein complex[8]. Acetylocholine esterase activity was assayed by following the hydrolysis of indophenyl acetate as described by KRAMER and GRAMSON[9]. Phosphodiesterase activity was spectrophotometrically measured by following the hydrolysis of the substrate Ca-*bis*-(*p*-nitrophenyl)-phosphate[10]. Proteolytic activity using casein as the substrate was carried out following the method of KUNITZ[11].

For the coagulation test, 90% clottable fibrinogen was dissolved in 10 ml of 0.001 *M* phosphate, 0.8% NaCl at pH 6.2, 0.5 ml of clear supernatant solution after centrifugation was incubated with 0.5 ml toxin solution (10 mg/ml). Hydrolyses of N-benzoyl-L-arginine ethyl ester, *p*-toluene-sulfonyl-L-arginine methyl ester, and acetyl-L-tyrosine ethyl ester were carried out as described previously[12]. Leucine aminopeptidase activity was determined by a modified method of GOLDBARG and RUTTENBURG[13]. Acid and alkaline phosphatase activities were determined spectrophotometrically on the substrate *p*-nitrophenyl phosphate and *o*-carboxyphenyl phosphate[14]. Phospholipase A was measured by titrating released fatty acids from lecithin[15].

For the hemolytic assay, rabbit erythrocytes were washed first with physiological saline solution then followed by 2, 4, 6-trimethyl pyridine containing 0.005 *M*

Enzyme or biological activities	Substrate	Presence or absence of activities
Hyaluronidase	Hyaluronic acid	+
DNase	DNA	+
Acetylcholinesterase	Indophenyl acetate	+
Phosphodiesterase	Ca-*bis*-(*p*-nitrophenyl)-phosphate	+
Proteolytic enzyme	Casein	+
Coagulation	Fibrinogen	+
Amino acid esterase	N-benzoyl-L-arginine ethyl ester	—
	p-toluenesulfonyl-L-arginine methyl ester	—
	Acetyl-L-tyrosine ethyl ester	—
Leucine amino peptidase	L-luecyl-β-naphthylamide	—
Acid phosphatase	*o*-carboxyphenyl phosphate	—
Alkaline phosphatase	*p*-nitrophenyl phosphate *o*-carboxyphenyl phosphate	—
RNase	RNA	—
Phospholipase A	Lecithin	—
Hemolysis		—
Capillary permeability		+

+, presence of enzyme or biological activity; —, absence of activities.

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