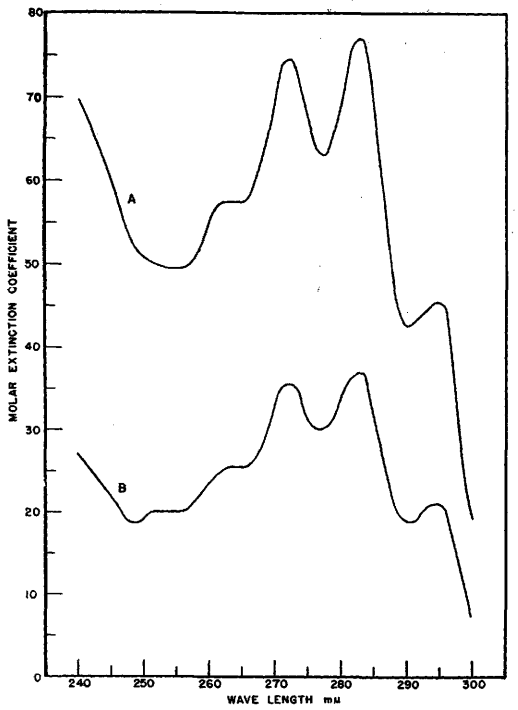

FIG. 1.

Ultraviolet adsorption of the high melting compound (curve A) and its acetate (curve B).

The acetate was hydrogenated in the same manner. The product of the hydrogenation was recovered from the solution and recrystallized once from 95% ethanol. The compound melted at 130.5-132.0° and had the following composition:

Found:		C: 80.52	H: 12.02
		80.71	12.03
	Avg	80.62	12.02
Calculated for:	$C_{29}H_{50}O_2$	80.87	11.70
	$C_{30}H_{52}O_2$	81.05	11.80
	$C_{32}H_{56}O_2$	81.29	11.94

Summary. A compound of steroid nature, having the probable formula of $C_{28}H_{46}O$, has been isolated from cane juice. This compound has therapeutic activity in alleviating an induced stiffness in guinea pigs. It has not been possible to further elucidate the structure of this compound, since not enough material was available at the time the project had to be discontinued, due to circumstances beyond control of the two senior authors. The isolations, however, tend to confirm the findings by Oleson *et al.* and Petering *et al.*, in that the antistiffness factor is steroid in nature.

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17165. Electron Microscope Studies of the Vesicle and Spinal Fluids from a Case of Herpes Zoster.*

ALFRED S. EVANS† AND JOSEPH L. MELNICK. (Introduced by F. G. Blake.)
(With the technical assistance of Ruth Peabody.)
From Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

The pathogenesis of herpes zoster has not been resolved. Even though recent work has suggested that the virus may multiply in the skin, the part played by the central nervous system in this infection is not clear. Like the virus of varicella, with which it is either closely related or identical, the virus of herpes zoster cannot readily be passed to laboratory animals, making work difficult in this disease. However, elementary bodies of varicella and herpes zoster have been described as occurring in vesicular fluids when such fluids have been examined in the optical microscope.[1-3] These bodies have been characterized more fully in the electron microscope by Ruska,[4] by Rake

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† National Institutes of Health Postdoctorate Research Fellow.

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[1] Paschen, E., *Hyg. Rundschaw*, 1919, **29**, 269, 313.

[2] Aimes, C. R., *Lancet*, 1933, **224**, 1015.

[3] van Rooyen, C. E., and Illingsworth, R. S., *Brit. Med. J.*, 1944, **2**, 526.

[4] Ruska, H., *Scientia*, 1943, **37**, 16.

and his coworkers,[5,6] and by Farrant and O'Connor.[7] We have been able to confirm these findings in regard to finding elementary bodies in the vesicular fluids of varicella and of herpes zoster, and in addition have demonstrated particles of similar size in the cerebrospinal fluid of a patient with herpes zoster. The purpose of this paper is to present these findings and to compare the particles with those found in the vesicular fluid from a case of herpes simplex.

Case report. The patient studied was a 28-year-old post-graduate student who was admitted to the New Haven Hospital on December 14, 1948 with complaints of frontal headache of 10 days duration becoming more severe in the last 5 days, and of fever of 3 days duration. He had not noted chest pain or a skin rash. Previous hospital records disclosed that he had been under investigation for an unexplained splenomegaly and hyperglobulinemia since 1944. Physical examination, including a neurological evaluation, was not remarkable except for a group of early vesicles on an erythematous base which extended from the 9th and 10th thoracic vertebra posterially nearly to the mid-line anteriorally on the left side over the 6th and 7th ribs, and a spleen that was palpable 2 cm below the costal margin. There was no hyperaesthesia over the affected skin areas. Laboratory work on the day of admission revealed a normal white, red, and differential count. A lumbar puncture done on the following day yielded clear fluid with the following characteristics: leucocyte count 145 per cmm, all of which were mononuclear, protein 156 mg%, Wassermann negative and bacterial culture negative. Initial pressure was 170 mm of water, final pressure, 120. A second spinal puncture on the next day revealed an increase in cell count to 225, which were predominantly lymphocytes. The patient remained asymptomatic except for headache, but had daily fever up to 102°F for 4 days. Convalescence was uneventful.

Materials and methods. For the electron microscope preparations, vesicle fluid from the patient was obtained with a tuberculin syringe on the day following admission. It was collected in 0.5 ml of saline, and after a clearing centrifugation at 2000 r.p.m. for 20 minutes was spun at 18,000 r.p.m. for 30 minutes in a cooled rotor, 6 inches in diameter. The sediment was resuspended in 0.2 ml of distilled water. Two samples of cerebrospinal fluid of this patient in amounts of 2 and 8 ml were handled in a similar fashion as were the samples of vesicular fluid from cases of varicella. The specimen of herpes simplex fluid shown was obtained from a lesion on the upper lip of a patient with a respiratory infection. It was collected in 0.2 ml of saline and examined without centrifugation. Spinal fluid from 20 patients with various ailments (tuberculous meningitis, poliomyelitis, and noninfectious neurological disorders) were examined, 15 without centrifugation and 5 after centrifugation as above.

Small drops of the samples were placed on collodion covered 200-mesh stainless steel grids and allowed to dry. They were then shadow-cast with chromium at an angle of 1 to 7, and examined in a RCA electron microscope, type EMU. The magnification was calibrated by photographing a silica replica of a diffraction grating (15,000 lines per inch) and by the

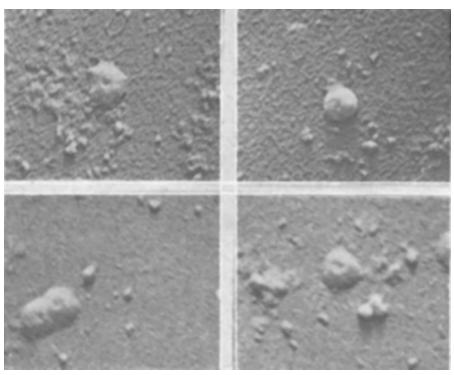

FIG. 1.
Electron micrographs of vesicle fluid from a patient with herpes zoster. × 20,700.

[5] Nagler, F. P. O., and Rake, G., *J. Bact.*, 1948, **55**, 45.

[6] Rake, G., Blank, H., Coriell, L. L., Nagler, F. P. O., and Scott, T. F. M., *J. Bact.*, 1948, **56**, 293.

[7] Farrant, J. L., and O'Connor, J. L., *Nature*, 1949, **163**, 260.

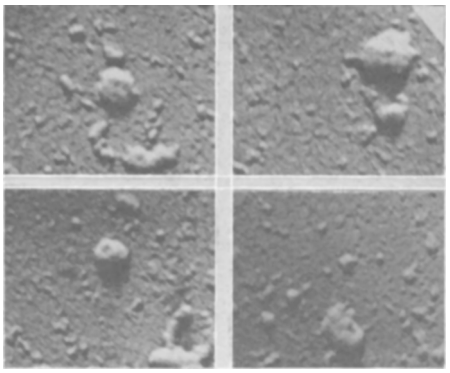

FIG. 2.
Electron micrographs of spinal fluid from a patient with herpes zoster. × 20,700.

use of latex balls of 259 mu diameter as described by Williams and Backus.[8]

Results. Fig. 1 illustrates typical bodies found in the vesicular fluid of the patient with herpes zoster and Fig. 2 those found in his spinal fluid. As seen from the frequency distribution of particles of varying diameters in Fig. 3, there is no significant difference between the average diameter of the elementary bodies found in either fluid, being 230 mμ for those in the vesicular fluid and 224 mμ for those in the spinal fluid.

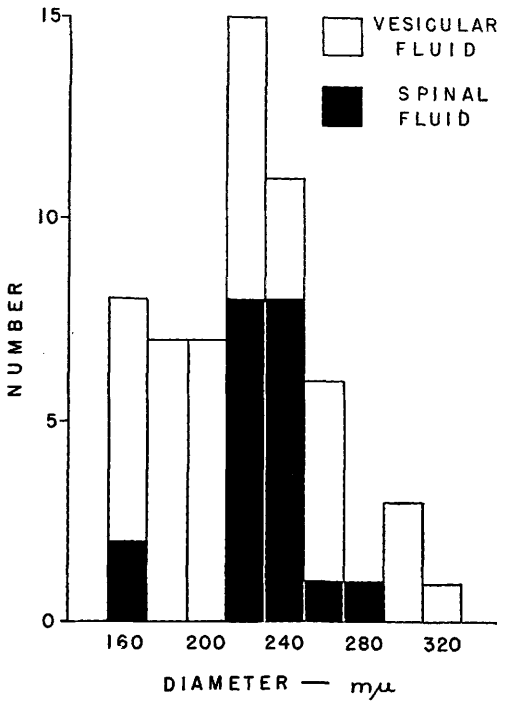

FIG. 3.
Frequency distribution of particles seen in the vesicular and spinal fluids of a case of herpes zoster.

No bodies of this sort were seen in any of the spinal fluids examined from patients with other diseases.

Fig. 4 illustrates elementary bodies obtained from vesicles of a patient with chicken pox, the average diameters of the particles being 244 mμ (range 200-280). The diameters of particles from 2 other patients with varicella averaged 250 mμ (range 190-320) and 225 mμ (range 180-250). The overall average of the particles from these 3 patients was 245 mμ.

Fig. 5 is a micrograph of elementary bodies from vesicle fluid of a patient with herpes simplex showing elementary bodies having an average diameter of 213 mμ (range 200-220).

It is difficult from any of our photographs to speak of brick-shaped elementary bodies such as discussed by Rake *et al.*[5] However some of the particles appear to have corners and in the measurements the average diameter of each particle was used. The preparations seem to be characterized by surface irregularities and particularly a central area of depression in the middle of which often appears a small elevated round structure. The latter was particularly marked in the bodies from herpes simplex (Fig. 5).

Discussion. These observations tend to confirm those recently presented by Rake and his colleagues[6] as well as those of Farrant and O'Connor[7] bearing on the similarities in elementary bodies observed in vesicular fluids from patients with herpes zoster and with varicella. Moreover, in addition to finding elementary bodies in the vesicular fluid of a patient with herpes zoster we have found particles of similar size in the spinal fluid of this patient. It is noteworthy that in the absence of clinical evidence of central nervous system involvement other than headache this patient showed a pleocytosis of 145 and 225 cells (chiefly lymphocytes) per cmm on 2 successive days during his disease. In a series

[8] Williams, R. C., and Backus, R. C., *J. App. Phys.*, 1948, **19**, 119.

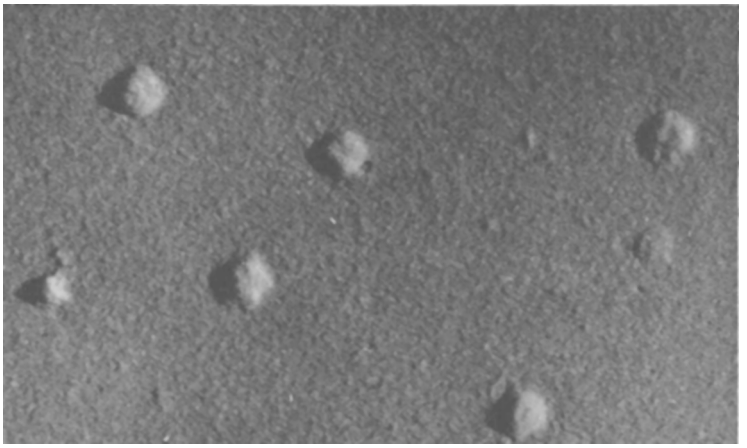

FIG. 4.
Electron micrographs of vesicle fluid from a patient with chicken pox. ×36,200.

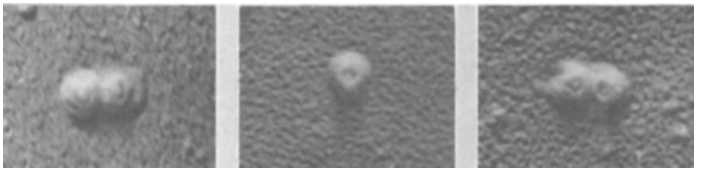

FIG. 5.
Electron micrographs of vesicle fluid from a patient with herpes simplex. × 27,500.

of 137 cases of herpes zoster reported by Gais and Abrahamson[9] lumbar punctures were done in 11: in 6 the count was normal while 5 showed a pleocytosis of 40 to 110 cells per cmm, chiefly lymphocytes.

Whereas herpes simplex appears to be unrelated to herpes zoster, similar appearing bodies have been detected in vesicular fluid here also. These bodies are 200-220 mμ in diameter which is considerably larger than that found by filtration studies of herpes virus contained in rabbit testicle and brain tissue by Elford *et al.*[10] However to our knowledge, filtration studies of the virus in vesicular fluid have not been done.

Summary. 1. Electron microscope studies of vesicular and spinal fluids from a patient with herpes zoster have demonstrated in both particles of similar size averaging 227 mμ in diameter.

2. These particles are compared to those in vesicular fluids from patients with herpes simplex and chicken pox. In herpes simplex particles averaging 213 mμ in diameter were found, and in chicken pox, particles of 245 mμ.

[9] Gais, E. S., and Abrahamson, R. H., *Am. J. Med. Sci.*, 1939, **197**, 817.

[10] Elford, W. J., Perdrau, J. R., and Smith, W., *J. Path. and Bact.*, 1933, **36**, 49.

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