using a variety of techniques. Glucose was identified by paper chromatography[6], and as the phenylosatriazole derivative[7]. Qualitative and quantitative[8] application of the Seliwanoff reaction showed the presence of a ketose; the optical rotation of the solution identified this as fructose. Rhamnose, which is a component of asiaticoside, was absent. Estimations of total reducing power, and of formaldehyde produced on oxidation with periodate[9], combined with the previous data, gave for centelloside the composition: centellic acid : glucose : fructose = 1 : 10 : 2 (mol.). Since strict proof of the homogeneity of the preparation is lacking, these figures must be regarded as provisional, but they show that centelloside carries a much larger sugar system than asiaticoside, and to this, and to the fructose content, the hydrophilic nature is probably due.

A property of centelloside which calls for special comment is the behaviour with warm dilute alkali, which rapidly sets free the salt of the aglycone, while destruction of the sugar colours the solution yellow. This high lability to alkali makes it unlikely that any part of the sugar is bound glycosidically to triterpene hydroxyl groups, and is sufficient to account for the action on Fehling's solution without assuming a free reducing centre in the sugar system. The aglycone, centellic acid, has no reducing properties.

The neutral nature of centelloside suggests at once that the linkage between sugar and triterpene is of the ester type. It must be borne in mind, however, that the carboxyl of centellic acid is sterically hindered, like that of oleanolic acid, and the behaviour of two synthetic glucose derivatives of the latter has been found to accord with theoretical expectation, and to diverge from that of centelloside. 1-β-Oleanolyl glucose, m.p. 216–218° (decomp.), $[\alpha]_D = +46{\cdot}5°$ (alcohol), is hydrolysed by acids, but stable to alkalis. 6-(Acetyloleanolyl) glucose, m.p. 235° (decomp.), $[\alpha]_D = +81°$ (alcohol), although destroyed by alkalis, is stable to acids. The alkalilability of centelloside remains unexplained, and clarification is being sought, *inter alia*, by the preparation of centellic esters of different sugars.

It is of interest that the Ceylonese variety of *Centella asiatica* apparently produces no asiaticoside but this closely related compound. Whether this reflects a morphological difference, or merely one of habitat or season, we are not competent to decide.

We are indebted to Dr. J. L. Simonsen, director of the Colonial Products Research Council, and to Dr. R. Child, director of the Coconut Research Scheme, Lunuwila, Ceylon, for making the fresh plant material available to us; the plants were identified botanically at the Department of Agriculture, Peredeniya, Ceylon.

B. Lythgoe
S. Trippett

University Chemical Laboratory,
Cambridge.
Aug. 24.

[1] Bontems, J. E., *Bull. Sci. pharmacol.*, **49**, 186 (1941).
[2] *Nature*, **155**, 601 (1945).
[3] Boiteau, P., Buzas, A., Lederer, E., and Polonsky, J. (see preceding communication).
[4] Grimes, Ch., and Boiteau, P., 8th Annual Report of the Botanical and Zoological Park of Tananarive, Madagascar, 1947.
[5] Bhattacharyya, S. C., and Lythgoe, B. (see preceding communication).
[6] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).
[7] Hann, R. M., and Hudson, C. S., *J. Amer. Chem. Soc.*, **66**, 735 (1944)
[8] Bacon, J. S. D., and Bell, D. J., *Biochem. J.*, **42**, 397 (1948).
[9] Reeves, R. E., *J. Amer. Chem. Soc.*, **63**, 1476 (1941).

ELEMENTARY BODIES OF VARICELLA AND HERPES ZOSTER

By J. L. FARRANT
Division of Industrial Chemistry,
Council for Scientific and Industrial Research, Melbourne
AND
J. L. O'CONNOR
Commonwealth Serum Laboratories,
Commonwealth Department of Health, Melbourne

DESPITE the prevalence of varicella and herpes zoster, little information is available concerning the infective agents themselves, since susceptible experimental animals providing adequate quantities of the viruses have not been found. Optical microscopical observations on the morphology and size of stained varicella virus have been made by Aragão[1], Paschen[2], Amies[3] and, more recently, by van Rooyen and Illingworth[4]. The last-named workers describe the infective agent as having a diameter of 1,250–1,750 A., and as being appreciably smaller than the elementary bodies of vaccinia and variola. Particles of the size stated are at the limit of resolution of the optical microscope, so little information is available concerning the elementary body itself.

Ruska[5] has reported an electron microscopic study of these viruses in a journal not available to us. Ruska's work was published before the 'shadow-casting' technique of Williams and Wyckoff[6] was devised, but we have used this method in a study of these viruses. The electron microscope used was an R.C.A. Model *EMU*.

The material used was fluid withdrawn from early vesicles in cases of chickenpox and shingles and variously diluted with normal saline to one-fifth to one-twentieth of the original concentration. Approximately 0·01 c.c. of the diluted fluid was allowed to evaporate to dryness on a collodion film stretched across a wire-mesh specimen screen, washed with distilled water to remove soluble salts and then 'shadow-cast' with platinum as recommended by Williams and Backus[7]. A thickness of *c.* 7 A. of platinum was deposited at such an angle that the shadow-lengths were 3·9 times the heights of the corresponding particles.

In fluid direct from the vesicles of four of the five cases of chickenpox examined, a number of bodies of regular size and shape were seen. The identity of these bodies with the causative agent of varicella was established by making two sets of preparations. The first (*a*) consisted of equal volumes of vesicle fluid diluted one-in-five with normal saline and of serum similarly diluted, taken from a patient 24 hours after the onset of an attack of chickenpox. Set (*b*) was similarly prepared except that the serum used was taken from the same patient on the eighth day of the disease. Both mixtures were allowed to stand for one hour at 22° C. before a portion was transferred to specimen screens and dried. Preparation (*a*) showed a uniform dispersion of particles which occurred singly; by contrast, preparation (*b*) showed considerable aggregation of the particles (Fig. 1).

Only two cases of herpes zoster were available for examination, and the vesicle fluid of one of these was found to contain particles indistinguishable in size

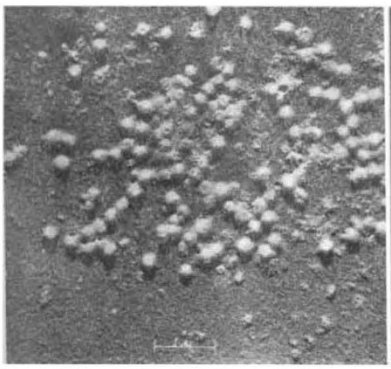

Fig. 1

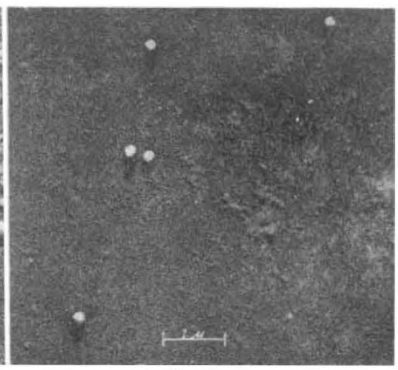

Fig. 2

Fig. 1. Varicella virus agglutinated with convalescent serum. Shadow-cast with platinum. Shadow ratio, 3·9

Fig. 2. Varicella virus fixed with 0·5 per cent osmic acid. Shadow-cast with platinum. Shadow ratio, 3·9

and shape from those observed in varicella. In view of the well-known fact that an attack of either herpes zoster or of varicella affords immunity against the other, attempts to observe agglutination of herpes zoster virus by convalescent serum from a case of varicella were made. Once again the convalescent serum caused much more marked aggregation of the virus than did the onset serum.

The profile of the virus particles shown in the micrographs is roughly circular; but measurements of shadow-lengths (see table) indicate that they are not truly spherical but oblate. In fact, the appearance of the particles is that of caps of ellipsoids, suggesting that the particles have collapsed to various extents on drying at room temperature. Evidence in support of this view was obtained by fixing the particles by the addition of osmic acid or formalin to the virus suspensions in saline. When so fixed, the virus particles approach more closely to the spherical form when dried (Fig. 2). As the micrographs yielded insufficient information for the calculation of the volumes of the unfixed virus particles as caps of ellipsoids, they were treated as caps of spheres and their volumes were found to agree with those of the fixed particles treated as ellipsoids (see table).

	No. of particles measured	Mean diameter (A.)	Mean height (A.)	Mean volume (10^6 A.^{3})
Varicella virus	77	2420±30	730±15	1880±60
Varicella virus and onset serum	30	2420±30	750±30	1950±90
Varicella virus and convalescent serum	45	2450±40	800±20	2150±80
Varicella virus and 5 per cent formalin	14	1860±70	1030±40	1870±160
Varicella virus and 0·5 per cent osmic acid	14	1750±35	1180±50	1890±110
Herpes zoster virus	19	2530±80	790±30	2240±160

Hodge[8], of this Laboratory, has observed a similar effect on fixing influenza virus particles with phosphotungstic acid. Similar observations have been made by Dawson and McFarlane[9] on the brick-shaped particles of vaccinia virus. They found particles dried at room temperature had the same lateral dimensions as freeze-dried particles but were only half the height. Particles fixed with osmic acid had the same volume as the particles dried at room temperature; but all dimensions were 20 per cent less than those of freeze-dried particles. It appears that fixed particles retain their true shape to a greater extent than those dried at room temperature, but freeze-drying is required if the true shape and size are sought.

Statistical treatment shows that the untreated varicella virus particles are significantly smaller than those treated with convalescent serum and are also smaller than herpes zoster virus particles. However, our preparations contained too much extraneous protein material to allow any real significance to be attached to these differences. The mean particle dimensions and their standard errors given in the table depend on magnification calibration obtained by means of interferometrically measured glass fibres as described by Farrant and Hodge[10].

Virus particles treated with phosphotungstic acid and osmic acid show areas of differing densities suggesting internal structure, but no evidence of this could be seen in the unstained particles.

The identity of these bodies with the causative agent of varicella is not conclusively established, as it has not been possible to attempt the reproduction of the disease by purified suspensions of them. It is not, therefore, possible to satisfy Koch's postulates; but the occurrence of the particles in the vesicle fluid of four out of five patients suffering from chickenpox, and the development of serum agglutinins for the bodies observed, during the course of the disease, give a high degree of probability that they are the cause of chickenpox.

Since this work was completed, we have received a paper by Rake and Nagler[11] showing electron micrographs of varicella virus. The particle dimensions in the horizontal plane, given by them, differ from those obtained by us; but it is evident from our own observations that size depends, to a great extent, upon the mode of preparation of the specimen for examination.

We desire to thank Dr. W. Stephenson and Dr. A. Ferris, of the Infectious Diseases Hospital, Fairfield, Melbourne, who kindly supplied the specimens for investigation. We also thank Dr. A. L. G. Rees, of the Division of Industrial Chemistry, for his interest in this work.

[1] Aragão, H. (1911), quoted in Van Rooyen and Rhodes, "Virus Diseases of Man", 225 (Oxf. Univ. Press, 1940).
[2] Paschen, E., *Deutsch. Med. Wschr.*, **43**, 746 (1917).
[3] Amies. C. R., *Lancet*, i, 1015 (1933).
[4] Van Rooyen, C. E., and Illingworth, R. S., *Brit. Med. J.*, ii, 526 (1944).
[5] Ruska, H., *Klin. Wochenschr.*, **22**, 703 (1943).
[6] Williams, R. C., and Wyckoff, R. W. G., *J. App. Phys.*, **15**, 712 (1944).
[7] Williams, R. C., and Backus, R. C., *J. App. Phys.*, **19**, 119 (1948).
[8] Hodge, A. J., unpublished work.
[9] Dawson, I. M., and McFarlane, A. S., *Nature*, **161**, 464 (1948).
[10] Farrant, J. L., and Hodge, A. J., *J. App. Phys.*, **19**, Sept. (1948).
[11] Rake, G., and Nagler, F. P. O., *J. Bact.*, **55**, 45 (1948).