TABLE III.
Sporicidal Activity of Penicillin for Heated and Non-heated Spores.

Culture	Penicillin 5u/ml added to milk—viable spores per ml					
	Non-heated spores			Heated spores[1]		
	0 hr × 1000	5 hr × 1000	27 hr × 1000	0 hr × 1000	5 hr × 1000	27 hr × 1000
B. subtilis (LB)	14.7	29.7	4.2	293	2.4	0.03
	(293)[2]	(148)	(61)	(290)	(0.5)	(0.04)
" " (6)	205	28.5	2.1	241	0.8	0.06
	(241)	(19)	(2.7)	(241)	(0.4)	(0.1)
B. megatherium (696)	231	86	23	116	25	6.2
B. cereus (232)	267	2,500[3]	Millions	164	1,200[3]	Millions
B. stearothermophilus (1518)	292	96	18.5	196	108	16.6

[1] 95°-15 min for LB, 6 and 1518; 85°-15 min for *B. megatherium* and *B. cereus*.
[2] Figures in parentheses show plate count after 95°-15 min.
[3] Both spores and vegetative cells.
Incubation temperature: *B. subtilis*, 45° C; *B. megatherium* and *B. cereus*, 30° C; *B. stearothermophilus*, 55° C.

clearly unable to prevent vegetation of germinated spores. Whether this is due to penicillinase production in the intermediate stages of germination or to other factors is not known. Increasing the concentration of penicillin or incubation of the suspensions containing it for longer periods, only slightly increased its sporicidal activity, indicating that even in susceptible species there exists a very small fraction of spores which are relatively resistant to penicillin.

The extraordinary effectiveness of penicillin in low concentration against certain types of spores, together with its non-toxic nature, suggests potential usefulness in non-medical fields, including that of food preservation. The possibility of employing penicillin in conjunction with mild heating merits consideration. Within a very limited sphere of observation, we have observed that a high degree of resistance to penicillin is correlated with a low degree of thermal resistance. Our observations upon the sporicidal activity of penicillin are being extended and will be reported later.

Summary. Under suitable conditions of incubation, penicillin in relatively low concentration is actively sporicidal against many aerobic species of bacteria. In sensitive species the sporicidal activity of penicillin is much greater in milk than in water and is usually enhanced by sublethal heating of the spores before treatment with penicillin.

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Electron Shadow-Micrography of Virus Particles.*

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The wave length of the light used with an optical microscope fixes the size of the smallest detail that it will resolve. For the shortest ultraviolet light it is practical to employ, this is in the neighborhood of 0.15 micron. The electron microscope, however, has such a great ultimate resolving power that, at least for the present, the smallest object it will reveal has a size determined by other factors. Photomicrographs produced with electrons are essentially shadow-graphs in which regions that are relatively light on the original negative correspond to regions in the sample which deflect (scatter) a larger percentage of the incident electrons,

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and as long as the problem is not complicated by the crystallinity of the object this ability to scatter depends on the amount of matter in the way of the electron beam. It is found in practice that particles of biological origin often cannot be observed simply because they do not contain enough matter to deflect a detectable percentage of the incident electrons. The problem of extending and improving electron microscopic vision of such small objects can therefore be said to be one of increasing the contrast between different parts of a preparation and between the preparation and the substrate that supports it. One way to do this is by staining with some heavy metal or radical which is selectively adsorbed. Staining with phosphotungstic acid has already been effectively used in the electron microscopy of tendon and muscle.[1] We have approached the problem from a different point of view and have found that "shadow-casting" with a thin film of metal deposited *in vacuo* is a very effective way of revealing the shape as well as the presence of many minute biological objects. The present note illustrates the application of this method to the examination of suspensions of purified viruses, as represented by influenza and the tobacco mosaic virus protein.

Shadow-casting was originally developed[2] as a way of measuring the heights of objects under the electron microscope. It consists in depositing an exceedingly thin (ca 7 mμ) layer of metal obliquely on to the preparation to be examined in the microscope. High points on the surface intercept more than an average number of the condensing atoms and at the same time shield areas immediately behind them. These shielded areas, devoid of deposited metal, appear as "shadows" of the elevated regions responsible for them. Just as an observer on a mountain-top or in a plane sees many details of a landscape at sun-rise,

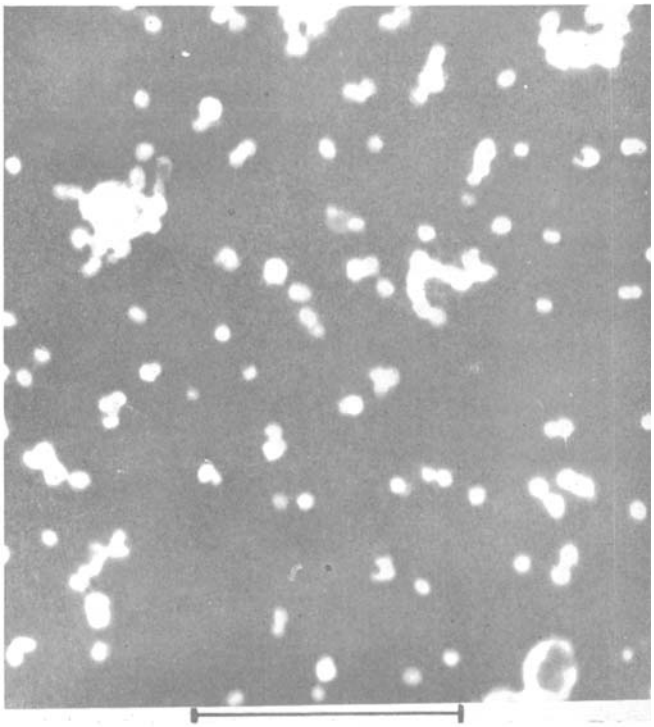

FIG. 1.
An electron micrograph of a suspension of purified PR-8 influenza virus. The numerous round bodies, and their aggregates, are what are now generally considered to be the elementary virus units. In this and each of the other photographs the inscribed line has a length on the photograph corresponding to one micron on the sample.

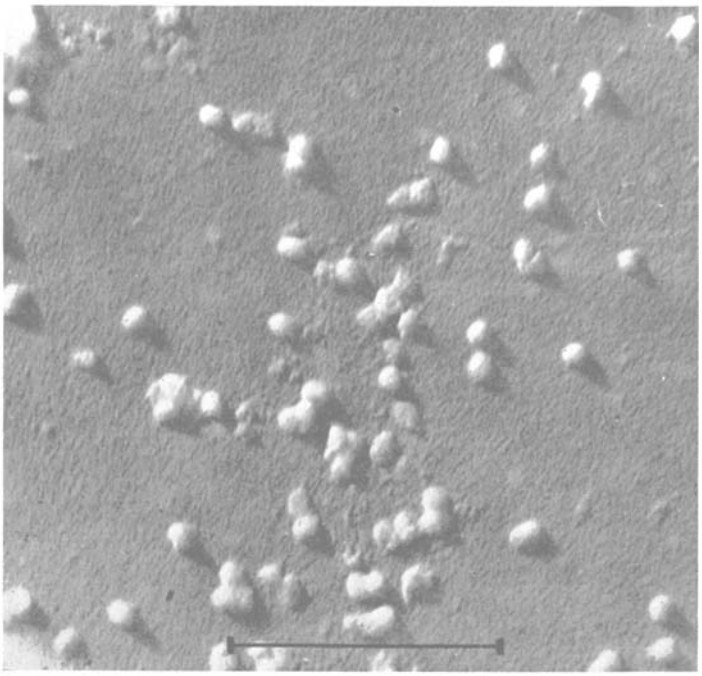

FIG. 2.
A micrograph of a similar preparation after it has been shadowed by the oblique deposition upon it of a thin layer of chromium.

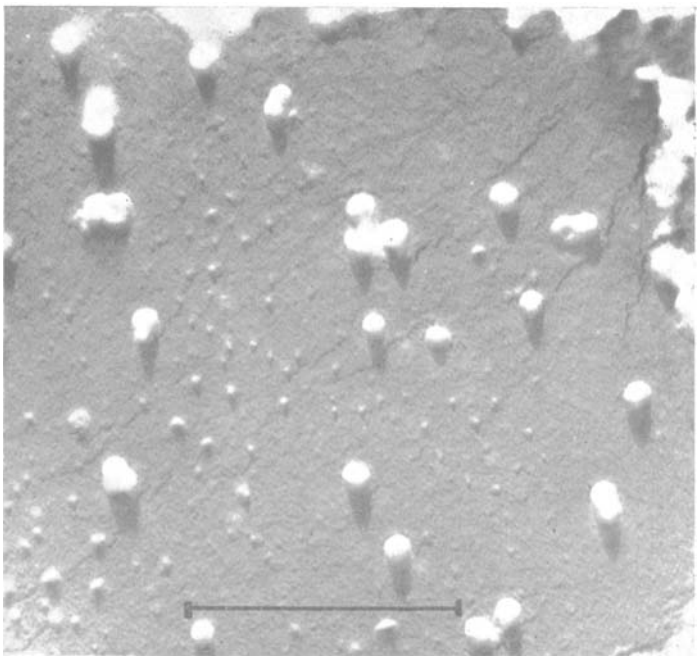

FIG. 3.
A field in another similarly shadowed influenza preparation.

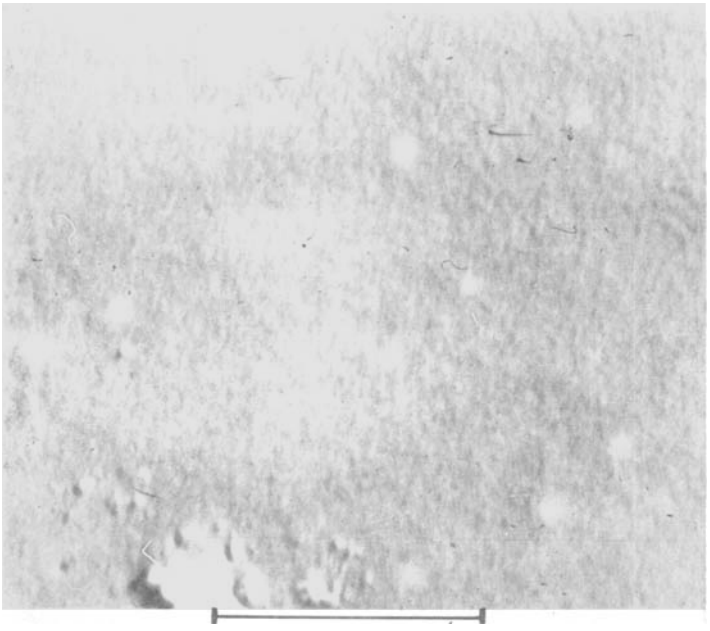

FIG. 4.
A micrograph of a shadowed blank screen to show the texture of the substrate. This field was chosen to include some dirt which appears at the bottom of the picture.

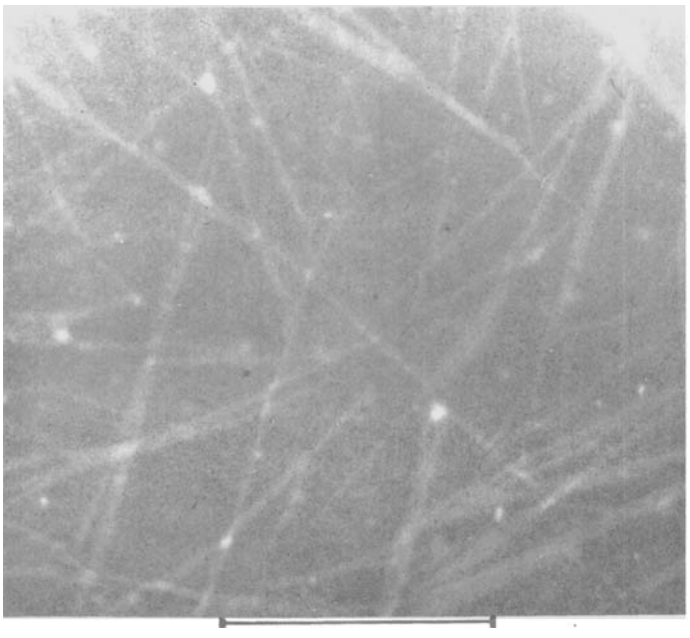

FIG. 5.
A micrograph of a dilute suspension of the tobacco mosaic virus protein.

FIG. 6.
A field of a similar but more dilute tobacco mosaic protein suspension after shadowing.

or sunset, which would be invisible to him at noon, so metal shadow-casting brings out much detail that is not to be seen in an unshadowed (or in a vertically coated) preparation.

The PR-8 influenza virus used in this work has been purified by the differential high speed centrifugation of allantoic fluid of diseased chick embryos. Our centrifugal cycle did not differ in any significant detail from that employed by others[3] for the purification of this virus; following their suggestions it has been found advantageous to suspend the sedimented virus pellets in $CaCl_2$-Ringer solution and to make further necessary dilutions with 0.02M $CaCl_2$ solution. The tobacco mosaic virus was an ultracentrifugally purified preparation kindly furnished by W. M. Stanley. Dilutions of this virus have been made in distilled water. Both viruses have been prepared for microscopy by placing suitably diluted micro-drops on the usual collodion-covered screens and withdrawing as much as possible of the droplets after they have stood in contact with the screens for from 2 to 5 minutes. When a photograph of the same electron microscopic field was wanted before and after metal-shadowing the evaporated metal film was laid down without removing the preparation from its holder.

The electron microscope was a Type EMB instrument manufactured by RCA. The originals of the photographs of this paper were taken at 10000x on fine grained plates. Magnification was established with a thin metallic replica of a 15000 line-per-inch grating.

The shadowing metallic film was evaporated on to the specimens following the technique customary in this work. It is of course essential to use a metal that exhibits little structure of its own. Very few metals fulfill this requirement. Of those that do, chromium is satisfactory in a calculated thickness of ca 7 mμ

[1] Jakus, M. A., Hall, C. E., and Schmitt, F. O., *J.A.C.S.*, 1944, **66**, 313.

[2] Williams, R. C., and Wyckoff, R. W. G., *J. Applied Physics*, 1944, **15**, 712.

[3] Taylor, A. R., Sharp, D. G., Beard, D., Beard, J. W., Dingle, J. H., and Feller, A. E., *J. Immunology*, 1943, **47**, 261.

and as such has been used throughout the present work. The best angle at which to deposit the metal film will depend on the preparation, being greater for the smaller objects. For our studies this angle has been such as to make the lengths of the shadows from 5 to 10 times the heights of the shadowed objects.

Fig. 1 is a micrograph of a typical PR-8 influenza virus preparation while Figs. 2 and 3 are similar fields after the deposition of chromium shadows. They and the other pictures are negatives; that is, areas that are black, including the shadows, are those that are relatively transparent to the electron beam. The numerous circular bodies in Fig. 1 show up in the shadowed figures as little globules; they are the same as the electron microscopic objects of ca 75 mμ diameter that have previously been identified with the elementary bodies of influenza. Shadowing makes it clear that many of the apparently elongated and giant forms seen in direct electron micrographs are clusters of these globular bodies. In the shadow pictures these chains and aggregates notably resemble microcolonies of streptococci and staphylococci[4] but as far as the pictures reproduced here are concerned no conclusions can be drawn from these resemblances.

Many particles much smaller than the influenza bodies are present in some fields of the shadowed preparations (Fig. 3). They probably are the same as the small particles previously described.[5] In addition to this particulate matter, shadowing also outlines any soluble material that may still be accompanying or contaminating the preparation. A small portion of such a mass with embedded influenza bodies is just on the edge at the top of Fig. 3. Similar masses in a variety of forms are common on other photographs we have taken.

To interpret these micrographs it is necessary to know how much of their fine detail is contributed by the structure of the collodion substrate and the metal film. Experience shows that the substrate is never entirely smooth and that its inequalities become more apparent as the shadowing is more oblique. The surface appearance of collodion films made in the same way and shadowed at the same angle is remarkably constant, but it is well to prepare blanks from each lot of collodionized screens. A field of such a control shadowed at a 5 to 1 angle and chosen to contain some dirt particles to aid in focusing is given in Fig. 4. The seemingly rougher texture of the collodion under more oblique shadowing is evident in the tobacco mosaic picture of Fig. 6.

As was shown several years ago[6] a purified preparation of the tobacco mosaic protein contains fine fibrils that differ widely in length but have a more or less constant diameter of ca 15 mμ. A direct electron micrograph of the protein used in the present experiments is reproduced in Fig. 5. After being shadowed by a layer of chromium deposited to give ten-to-one shadows, a field of this preparation containing only a few fibrils looks like Fig. 6.

The technique of shadow-micrography is of such evident help in determining the shape and size of these small objects that we are applying it to a more thorough study of influenza and other viruses, and of some of the larger macromolecules.

Summary. A new procedure is described for the electron microscopy of small objects. This technique, illustrated by photographs of purified influenza and tobacco mosaic viruses, involves the oblique evaporation of a thin film of metal over the preparation before micrography. The three-dimensional effect it produces gives new information about the heights and shapes of objects seen in the preparation.

[4] Wyckoff, R. W. G., *J. Exp. Med.*, 1934, **59**, 381.

[5] Chambers, L. A., Henle, W., Lauffer, M. A., and Anderson, T. F., *J. Exp. Med.*, 1943, **77**, 265; Taylor *et al.* op. cit.

[6] Stanley, W. M., and Anderson, T. F., *J. Biol. Chem.*, 1941, **139**, 325.