TABLE II.
Plasma Bicarbonate in mM per Liter of Water, pH of Plasma, Dry Residue of Plasma, and Hematocrit Ratio. Definition of 0, Δ_1, Δ_2 in Legend of Table I.

Dog No.	Plasma bicarbonate			Plasma pH			Dry residue of plasma, g/kg H_2O			Hematocrit, % erythrocytes		
	0	Δ_1	Δ_2	0	Δ_1	Δ_2	0	Δ_1	Δ_2	0	Δ_1	Δ_2
1	24.6	—0.6					75.5	+2.0		39.7	+ 5.7	
2	22.0	—2.2		7.38	0.00		79.6	—2.2		34.7	+ 0.3	
3	23.2	—2.5	—1.0	7.39	+0.11	+0.01	86.8	—1.6	—1.0	38.1	+11.9	+3.2
4	21.8	—7.2		7.40			82.5	+6.7		51.2	+12.2	
5	23.9	—5.4	—0.4	7.41	+0.09	+0.15	93.0	—1.3	—0.4	34.6	+ 6.2	+1.5
6	21.1	—0.6	—1.1	7.51	—0.03	—0.02	87.8	—4.0	—0.5	38.7	+ 4.0	+5.8
7	19.0	—4.5	—1.7	7.47	—0.01	—0.03	85.7	+6.4	—1.0	40.6	+ 8.1	+3.6

and 7, Δ_1 fell only 3.0 and 2.3 mM respectively. Since corpuscular water did not change we estimate that the chloride lost from the erythrocytes of these dogs could have raised the extracellular chloride 0.19 mM.

The sharp fall Δ_1 in the chloride of whole blood (Table I) is referable mainly to the increased proportion of corpuscles, which contain less chloride than plasma.

The plasma pH, measured in order to calculate bicarbonate and complicated by artificial respiration in dogs 5, 6, 7, is listed in Table II. The bicarbonate fell faster than chloride throughout, probably in relation to accumulating metabolic acids. However during the first 2 hours its value declined so much more than chloride in proportion to the normal concentrations of each as to suggest a special relation to the potassium administered. Whether the effect was related to a release of lactic acid by the potassium tetany or to some other factor remains unknown.

Summary. In nephrectomy a sudden experimental elevation of the extracellular potassium did not lower the plasma chloride as much as predicted from the Boyle-Conway concept. The chloride fell 3.17 mM while the potassium rose 6.03 mM and the correlation 0.465 between these is insignificant. The proportional fall in plasma sodium slightly exceeded that for chloride. The bicarbonate of plasma fell so rapidly initially as to suggest a special relation to the administered potassium.

15053

Shadowed Electron Micrographs of Bacteria.*

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In a recent note[1] we have described a procedure, based on metallic shadow-casting,[2] for enhancing the visibility of very small particles under the electron microscope and making apparent many details of their shapes which are not seen in other ways. This technic consists in obliquely depositing in a vacuum a thin layer of a relatively structureless metal such as chromium onto the electron microscopic preparation. The distribution of thickness of this layer, which should be of such an average thickness as to be partially transparent to the electron microscope beam, is determined by the contours of the preparation.

* Supported in part by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265.

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

Relatively tall objects collect a thicker deposit in the direction of the oncoming metal atoms and at the same time shield other areas to produce what might be called permanent "shadows" of themselves. As the previously published micrographs of influenza and tobacco mosaic virus particles[1] have shown, this results in a three-dimensional effect which brings out many details invisible by direct electron micrography. When this technic is applied to the photography of bacteria it proves to be equally useful for these larger objects. The present note describes this application and illustrates it with electron micrographs of several typical organisms.

Photographs of the following bacteria† are reproduced in the figures of this paper: (1) *Bacillus subtilis,* a recently isolated smooth strain; (2) *Bacillus mesentericus vulgatus,* a strain of the "Michigan" type cultivated for many years in the laboratory; (3) *Salmonella typhimurium,* a strain cultivated for 3 years in the laboratory; (4) *Bacterium typhosum,* an old laboratory strain; (5) *Staphylococcus aureus,* a strain isolated 3 years ago from human material; (6) *Streptococcus hemolyticus* (beta), scarlet fever strain IV (Dick); (7) *Streptococcus pneumoniæ,* Type II, an old laboratory strain.

Bacteria to be photographed were grown in broth or on agar slants, the cultures varying in age from 24 hours to 3 months. Organisms were washed from the slants; the resulting suspensions were made 0.5% with respect to formalin and then sedimented by centrifugation at 2000 r.p.m. Broth cultures were similarly formalinized and sedimented. In either case the sedimented cells were resuspended in distilled water and washed twice by alternate centrifugation and resuspension in water. Preparations for electron microscopy were made from such purified suspensions by adding micro-drops of appropriate dilutions to the surface of the usual collodion-covered metal screens. Films of metallic chromium of *ca.* 7 millimicron calculated average thickness were then evaporated obliquely onto the top surfaces of these preparations after they had thoroughly dried in the air. Metal evaporation was carried out as in the work with viruses except that for these large objects the angle of deposition was chosen so that shadow-lengths would be only twice the heights of the objects casting them.

Organisms‡ in an old culture of *B. subtilis* appear as in Fig. 1 after being shadowed. The central protoplasm in the 3 bacilli lying side by side stand out as opaque masses. The neighboring, more transparent, convoluted mass from which a single flagellum seems to issue is perhaps the extruded protoplasm from a fourth disintegrated cell. An isolated *subtilis* bacillus is shown in Fig. 2a. This cell, like those of the preceding figure, has a relatively flat shelf of sharply bounded substance beyond its opaque central protoplasm; this probably outlines the cell wall from which the central mass has retracted on aging and drying. When this bacillus is photographed with

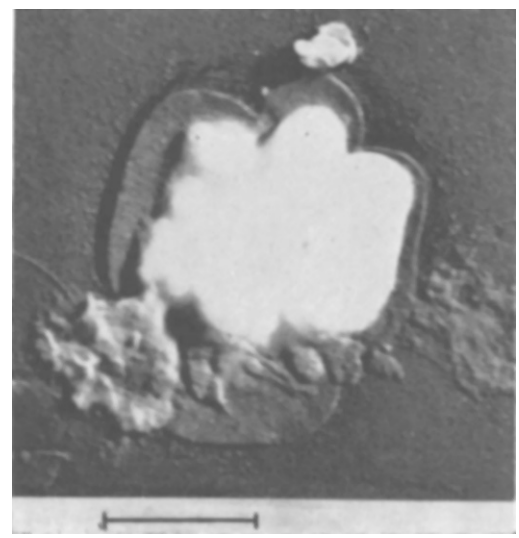

FIG. 1.

A shadowed electron micrograph of a group of *B. subtilis* organisms. The scale of magnification of this and the following photographs is given by the one micron scale appended to each. It shows that the magnification in these reproductions, except Fig. 4, is about 20,000 times.

† We are indebted to Dr. J. M. Bourn of the Michigan Department of Health Laboratories for bacterial cultures.

‡ For excellent electron micrographs of subtilis and subtilis-like bacilli see for example Mudd, S., Polevitzky, K., Anderson, T. F., and Chambers, L. A., *J. Bact.*, 1942, **42**, 251; Dubin, I. N., and Sharp, D. G., *J. Bact.*, 1944, **48**, 313.

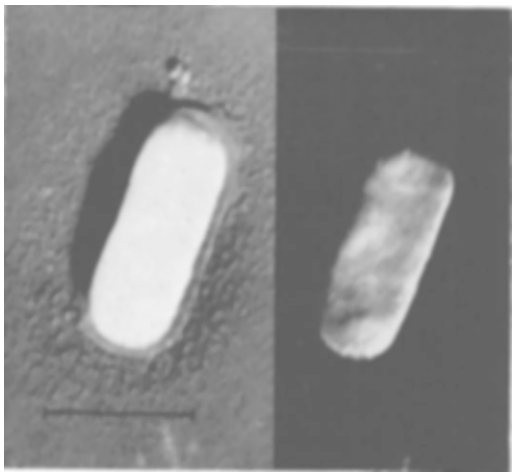

FIG. 2a (left).
A light exposure of a younger single cell of *B. subtilis* after metal-shadowing, showing the cell outline and wall and, by its "shadow," the general shape of the bacillus.

FIG. 2b (right).
A prolonged exposure of the same shadowed preparation showing the surface contours of the bacillus.

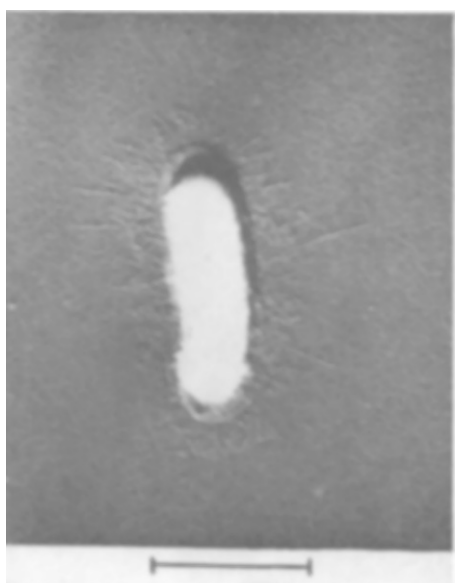

FIG. 3.
A shadowed micrograph of a single organism from a culture of *Bact. typhimurium*. The exceedingly fine peritrichate flagella of the cell are evident.

such a prolonged exposure that it appears partially transparent, Fig. 2b is obtained. This is primarily a record of the contours of its surface since the electron scattering of the deposited metal so greatly exceeds that of the bacterial protoplasm that it concentrates the observed scattering in this superficial layer. Other examples of the delineation of surface structure that can be made through such prolonged exposures are given in subsequent photographs. As would be expected, the related *B. mesentericus vulgatus* gives photographs much like those shown above for *B. subtilis*. Several old cells, all but one of which were undoubtedly dead before the preparation was made, are reproduced in Fig. 5. Fragments of extruded protoplasm and many of the very thin flagella possessed by these big rods are strewn over the field. The large spore at the bottom of this picture has structure which is reflected in its partial transparency to the electron beam.

Photographs of 2 flagellated members of the genus Bacterium are reproduced in Fig. 3 and 4. The single cell of *Bact. typhimurium* (Fig. 3) is almost entirely filled with its protoplasm but a narrow surrounding shelf is visible and beyond this the very fine peritrichate flagella are readily seen. The much

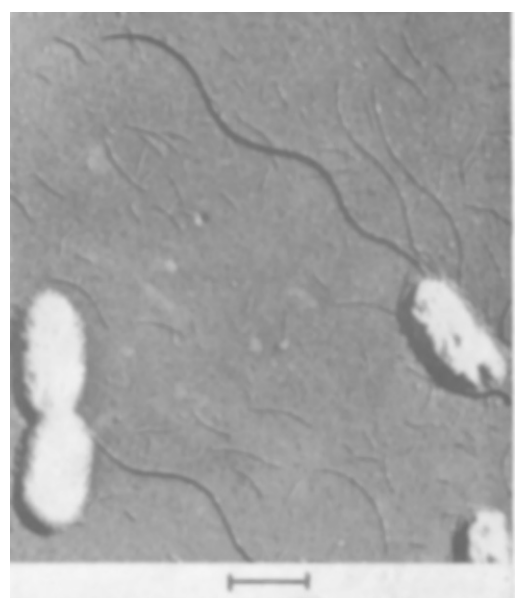

FIG. 4.
A shadowed micrograph of 3 cells of *Bact. typhosum* showing flagella. The magnification has been reduced to about half that of the other figures in order to include an entire flagellum. Note also the many fragments of finer flagella.

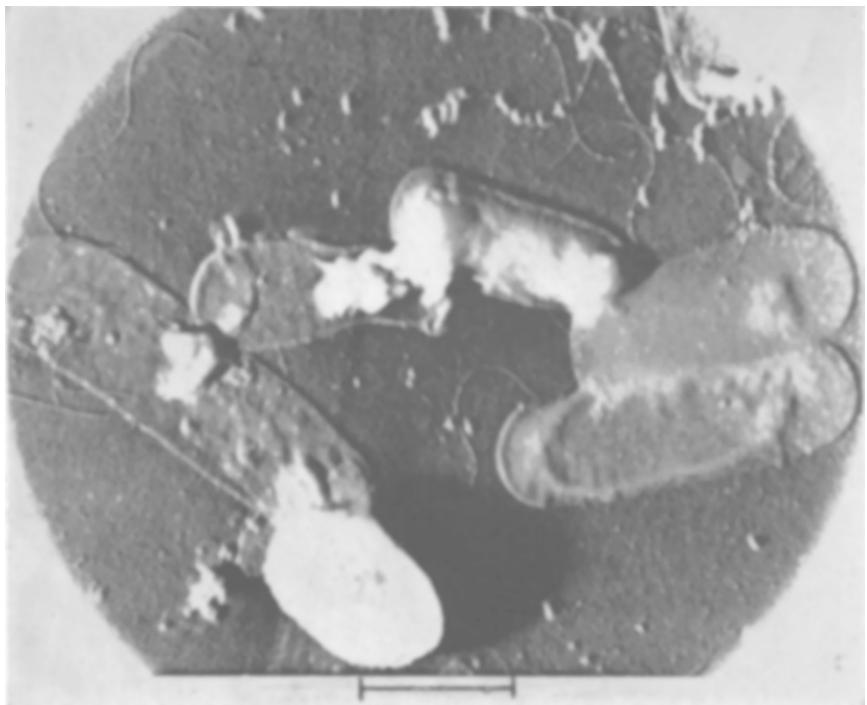

FIG. 5. A shadowed micrograph of several old cells and a spore from a culture of *B. mesentericus vulgatus*.

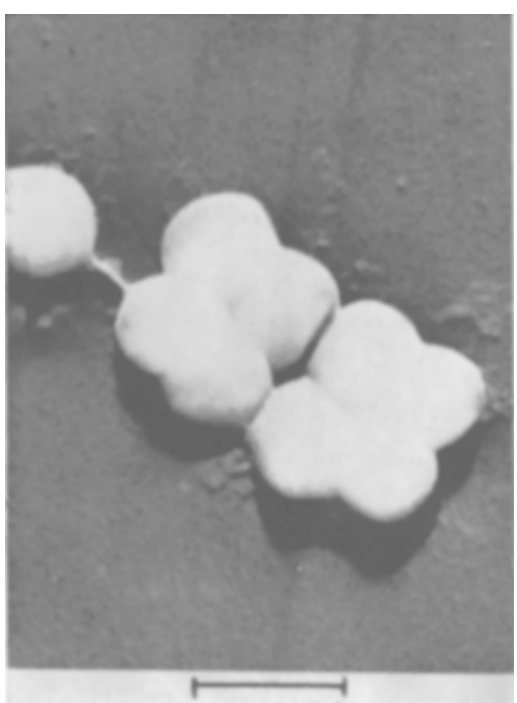

FIG. 6.
A shadowed micrograph of several cells from a culture of *Str. hemolyticus* IV (Dick).

thicker flagella of some typhoid bacilli[3] are evident in Fig. 4. They cast shadows of very different lengths and must therefore vary greatly in thickness; often they appear more as ribbons than as threads.

The photographs that follow are of various cocci. The spherical streptococci of Fig. 6 are bound together by connections whose details can be clearly seen. Details of their surface as well as of the double cup-like structure, faintly apparent in the single coccus on the edge of this figure can be brought out by longer exposure. The wrinkled surfaces of the diplococci of a 24-hour culture of Type II pneumococci are evident in the prolonged exposure of Fig. 7. While it is possible with care to bring out slight indications of capsular substance surrounding such pneumococci, these indications are so slight as to defy satisfactory reproduction. Fig. 8 and 9 are examples of the pleomorphism seen in cultures of *S. aureus*. The surfaces of the cocci of both figures are at least as rough as those of the pneumococci. The white, especially opaque

[3] Mudd, S., and Anderson, T. F., *J. Exp. Med.*, 1942, **76**, 103.

FIG. 7.
A prolonged exposure of a shadowed preparation of diplococci from a culture of *Str. pneumoniæ* II, showing the surface structure of these cells.

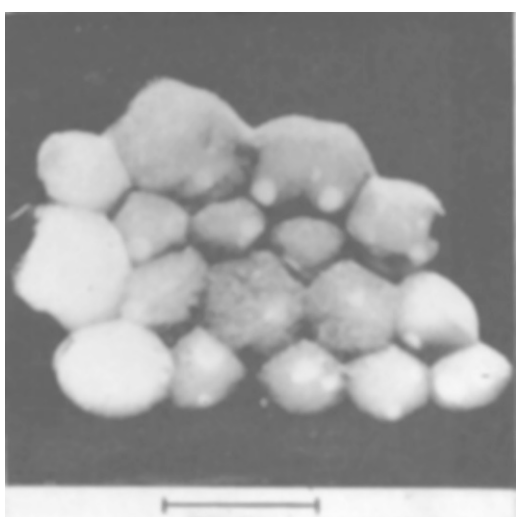

FIG. 8.
A prolonged exposure of a shadowed preparation of a group of organisms from a culture of *Staph. aureus*.

regions in every cell of Fig. 8 are also present in unshadowed photographs.[4] Another struc-

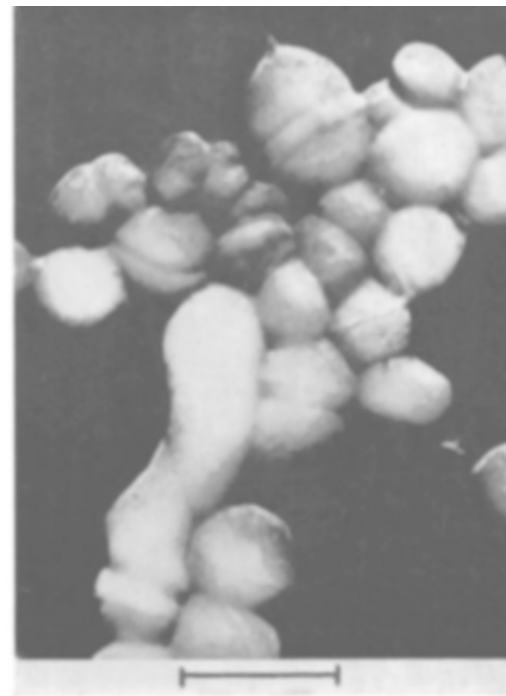

FIG. 9.
Another field from an old culture of *Staph. cereus* showing cells of various shapes.

ture often observed in these staphylococci is the broad equatorial band that is especially evident in the large form at the top of Fig. 9. Possibly it is an early stage of the division process that appears more developed in cells such as that at the center of this figure.

The foregoing micrographs illustrate the electron micrographic appearance of metal-shadowed bacteria. Our experience indicates that most information can be obtained by making two exposures of a shadowed preparation: (1) a light one to show the general shape of the organism, its flagellar structure, and any evidence there may be of a capsule or other substance surrounding the cell proper, and (2) a prolonged exposure which will bring out details of its surface.

Summary. Metal shadow-cast preparations reveal under electron microscopy many details of bacterial morphology. The three-dimensional effect achieved with this technic brings out with especial clarity the shapes of organ-

[4] Knaysi, G., and Mudd, S., *J. Bact.*, 1943, **45**, 348.

isms and the contours of their surfaces; shadowing also makes evident their flagellar and other extra-cellular processes. Illustrative shadowed electron micrographs are given of subtilis-like and typhoid-like organisms and of several forms of cocci.

15054 P

Cultivation of Viruses of the Psittacosis Group in the Allantoic Cavity of Chick Embryos.*

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Many of the viruses of the psittacosis group have been cultivated successfully in fertile hens' eggs, the yolk sac being most satisfactory for this purpose. Using this particularly susceptible tissue, preparation of large amounts of virus has been possible.[1] Such preparations cannot be regarded as ideal for some purposes, however, such as attempts at purification of the virus[2] or for production of antigens, because of the large amount of admixed tissue debris and yolk material, removable only with difficulty. It occurred to us that the allantois, being embryologically similar to the yolk sac, might also show special susceptibility to the viruses of this group. The allantoic cavity has been extremely useful in cultivation of influenza virus,[3] and the advantages in the case of the latter virus might be expected to hold for others as well.

After our first attempts to adapt mouse pneumonitis virus, one of the agents of this group, to the allantoic cavity, the publication of Williams[4] appeared, in which he reports successful cultivation of psittacosis virus in the allantoic cavity and in greater amounts than obtained in the yolk sac. We have been able to confirm his finding and have extended the study to other viruses of the group. The present preliminary report records the successful cultivation of a strain of psittacosis, human pneumonitis (SF), and meningopneumonitis (F-97) virus in the allantoic cavity. The few attempts made so far to adapt mouse pneumonitis virus to the allantoic cavity have not been successful. Williams reported failure to cultivate lymphogranuloma venereum virus in this tissue.

The strains of virus used in the present experiments have been employed in previous work in this laboratory and their sources have been recorded.[2] The first inoculations into allantoic cavities were made with emulsions of infected mouse tissue or yolk sac, and subsequent passages were made with 0.25 cc quantities of undiluted allantoic fluid. The preparations of human pneumonitis and feline pneumonitis virus, designed for the first allantoic injections, proved to be contaminated with bacteria, a circumstance overcome by adding sulfadiazine to the contaminated emulsions in the amounts of 68 to 75 mg per egg. Before use the eggs were incubated at 38 to 39°C for 7 or 8 days and at 37°C for 2 days. After the inoculations were made, incubation was continued at 35° C. When movements of the embryo became feeble, indicating impending death, usually on the 3rd, 4th or 5th day, the eggs were chilled at 4° C for a few hours and the allantoic fluids then withdrawn. Five to 6 cc per

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1 Grace, A. W., Rake, G., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 259.

2 Hilleman, M. R., *J. Infect. Dis.*, 1945, **76**, 96.

3 Burnet, F. M., *Aust. J. Exp. Biol. and Med. Sci.*, 1941, **19**, 291.

4 Williams, S. E., *Aust. J. Exp. Biol. and Med. Sci.*, 1944, **22**, 205.