Termination of the masking noise was followed by a "rebound" increase in the frequency and duration of PS episodes (Fig. 2). This was invariably true in each of the ten 3-day records involving 24 hours of noise. In the subsequent quiet day the rabbit appeared to "recover" all of the PS it had missed during the noisy period. The results are reminiscent of Dement's findings relative to the deprivation of PS and dreaming in man(4). It is impossible to determine whether the rabbits were dreaming, but the apparent need to recover the missing episodes of PS suggests that PS may play a fundamental and essential role in the normal physiology of sleep in the lower mammals as well as man.

Summary. Spontaneous episodes of "paradoxical" sleep are plentiful in the rabbit adapted to its surroundings. The phenomenon is almost completely inhibited during 24 hours of noise which does not prevent ordinary sleep with spindles and slow waves. Following the masking noise period the animal recovers the missing paradoxical sleep episodes, indicating that the latter may be of fundamental physiological importance.

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Electron Microscopic Structure of Chicken Embryo Lethal Orphan Virus.*† (28726)

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Horne *et al*(3) reported on the electron microscopic structure of adenovirus type 5. The virus showed the cubic symmetry of an icosahedron possessing 252 capsomeres. The diameter of the virus was 70 mμ and the distance between the centers of adjacent capsomeres was 70 Å. Davies *et al*(1) reported that the virus of infectious canine hepatitis also was an icosahedron possessing 252 capsomeres. The diameter of this virus was 82 mμ and the distance between the centers of adjacent capsomeres was 90 Å.

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The chicken embryo lethal orphan (CELO) virus is widely spread as an inapparent infection in chickens and turkeys. Yates and Fry(8) isolated several strains of CELO virus from embryonating chicken eggs that were inoculated with various tissues or exudates. In characterizing the CELO virus, Yates(7) reported that this virus did not pass through a 50 mμ membrane filter, and by electron microscopic study he indicated the virus to be 80 mμ in size. Petek *et al*(6) studied sections of cell cultures infected with CELO virus under the electron microscope and reported the virus to be approximately 70 mμ in size. This paper presents the structure and size of the CELO virus as studied with negative stain techniques under the electron microscope.

Materials and methods. Preparation of virus. The CELO virus was obtained through the courtesy of Dr. V. J. Yates. The virus was propagated in the allantoic cavity of 10- to 11-day-old embryonating chicken eggs obtained from a U. S. Pullorum-Typhoid clean hatchery. The eggs were inoculated with 0.1 ml of a 10^{-2} dilution of the virus by the allantoic cavity route and incubated at 37°C. Death of the embryo within 24 hours was considered non-specific. After incubation for 48 hours, followed by 4 to 6 hours refrigeration, the allantoic fluid was harvested and stored at —40°C.

Purification of virus. When used, the virus was centrifuged in a refrigerated centrifuge at 3000 rpm for 10 minutes. The titer (ELD_{50} per ml) of the virus was 10^9 to 10^{10}. The purification of the virus was done by passing the virus preparation through a Sephadex (G-75, Medium—Pharmacia, Uppsala, Sweden) column as described by Per Flodin(5). Sodium chloride (0.1 M) was used as an eluent. The portion of the eluate containing the maximum virus concentration, as determined by a modified hemagglutination test for CELO virus, Dutta and Pomeroy(2), was dialyzed against 0.05 M ammonium acetate for 4 to 6 hours at 4°C. This dialyzed preparation was centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge and the supernate was used for electron microscopic studies.

Phosphotungstic acid. A 2% suspension of phosphotungstic acid in distilled water, the pH adjusted to 7 with 1 M KOH solution, was used for negative staining.

Grids. Copper grids (200 mesh) with a formvar membrane prepared from a 0.15% solution of formvar in chloroform and shadowed with carbon were used.

Preparation of specimen for examination in electron microscope. The purified virus preparation and the phosphotungstic acid were mixed in equal parts. The membrane side of the grid was touched to the mixture and excess fluid was removed by touching the sides of the grid with a filter paper. The grids were allowed to dry and then examined under the electron microscope (RCA EMU 3F).

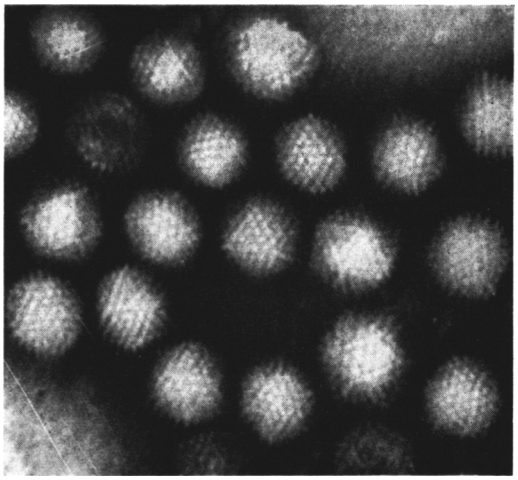

FIG. 1. Electron micrograph of chicken embryo lethal orphan (CELO) virus negatively stained with phosphotungstic acid. × 168,000.

Results. The CELO virus has an average diameter of 73 mμ (69-76 mμ). This virus is a regular icosahedron (20 faces) having a cubic symmetry in which each face is an equilateral triangle. Each virus particle has subunit structures, the capsomeres, which are arranged in a definite pattern. It can be noted from the electron micrograph that the capsomeres which occupy the apices of the faces lie in 5-fold symmetry axes and are surrounded by 5 other capsomeres, whereas those on the edges or faces are surrounded by 6 other capsomeres. Along each edge of the equilateral triangular face there are 6 capsomeres resulting in a total of 252 capsomeres in each virus particle. The distance between the centers of adjacent capsomeres is about 76 Å. The virus is without an envelope.

Discussion. Horne(4) reported that viruses fall into 3 main symmetry groups: those with cubic symmetry (*e.g.* Adenovirus, Infectious Canine Hepatitis, Herpes Simplex, Polyoma), those with helical symmetry (*e.g.* Newcastle Disease, Mumps, Influenza) and those with complex symmetry or combined symmetries (*e.g.* Vaccinia). The class of polyhedrons that have cubic symmetry includes the regular tetrahedron (4 faces), dodecahedron (12 faces) and icosahedron (20 faces). In a regular icosahedron, each face is an equilateral triangle. In such a regular icosahedral virus

the total number of capsomeres can be determined with the formula $10(n-1)^2+2$, where n is the number of capsomeres present along one edge of a triangular face.

There are structural similarities regarding the shape, number of faces and number of capsomeres among the adenovirus, infectious canine hepatitis virus and CELO virus. All 3 viruses have cubic symmetry, are icosahedron and have 252 capsomeres in each virus particle. The diameter of the adenovirus is 70 mμ, infectious canine hepatitis is 82 mμ and CELO virus is 73 mμ. Further, Petek *et al* (6) reported the similarities in physio-chemical and biological properties of the adenovirus and CELO virus. Because of the similarities in morphological, physio-chemical and biological properties of adenovirus and CELO virus, it seems justified to classify the CELO virus in the adenovirus group.

Summary. The chicken embryo lethal orphan (CELO) virus is 73 mμ in diameter and the distance between the centers of adjacent capsomeres is 76 Å. The virus is a regular icosahedron (cubic symmetry) having 252 capsomeres and without an envelope. The CELO virus could be classified in the adenovirus group.

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Effect of Sodium on Juxtaglomerular Index and Zona Glomerulosa in Experimental Nephrosis.* (28727)

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It has been established that the degree of granulation of renal juxtaglomerular cells (JGI) may be affected by the sodium state of the animal. Reduction in dietary sodium or adrenalectomy results in hypergranulation whereas the converse is obtained by salt overload and some situations characterized by sodium retention. These effects have been equated with accompanying alterations of blood volume and the juxtaglomerular apparatus has been visualized as a stretch receptor (1). Recent studies in our laboratory, although not subverting this latter concept, have indicated the significance of electrolyte composition in accounting for the decreased JGI consistently observed in untouched kidneys of rats with renal hypertension induced by the application of a constricting clip about the renal artery of the contralateral member (2). Investigation of the effect of sodium depletion and salt overload on the nephrotic syndrome, which is characterized by sodium retention and decreased blood volume, would also appear to represent a suitable experimental model in which to delineate electrolyte and pressor or hemodynamic factors which may be responsible for alterations in JGI. Tobian *et al*(3) noted statistically significant greater JGI in ascitic than non-ascitic rats with the nephrotic syndrome induced by administration of aminonucleoside, although comparison with the normal was not made. Since JGI represents a reliable index of renin activity and aldosterone secretion may be reflected by the appearance of the zona glomerulosa of adrenal cortex, studies of these parameters should provide pertinent infor-

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