with crude extracts of Salmonella.

1. Neter, E., *Bact. Rev.*, 1956, v20, 166.
2. Cavelti, P., *J. Immunol.*, 1947, v57, 141.
3. Singer, J. M., Plotz, G. M., *Am. J. Med.*, 1956, v21, 888.
4. Gilboa-Garber, N., Nelken, D., *Nature*, 1963, v197, 158.
5. Pick, E., Nelken, D., *ibid.*, 1963, v197, 157.
6. Bozicevich, J., Tobie, J. E., Thomas, E. H., Hoyem, H. M., Ward, S. B., *Public Health Reps.*, 1951, v66, 806.
7. Bozicevich, J., Bunim, J. J., Freund, J., Ward, S. B., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 180.
8. Bozicevich, J., Nasou, J. P., Kayhoe, D. E., *ibid.*, 1960, v103, 636.
9. Goodman, H. C., Bozicevich, J., *Immunological Methods*, J. Ackroyd, Ed., Blackwell & Co., England, in press.
10. Ribi, E., Haskins, W. T., Landy, M., Milner, K. C., *J. Exp. Med.*, 1961, v114, 647.
11. Ribi, E., Haskins, W. T., Milner, K. C., Anacker, R. L., Ritter, D. B., Goode, G., Trapani, R. J., Landy, M., *J. Bact.*, 1962, v84, 803.
12. Digeon, M., Raynaud, M., Turpin, A., *Ann. Inst. Pasteur*, 1952, v82, 206.
13. Ribi, E., Haskins, W. T., Landy, M., Milner, K. C., *Bact. Rev.*, 1961, v25, 427.
14. Shear, M. J., Turner, F. C., *J. Nat. Cancer Inst.*, 1943, v4, 81.
15. Davies, D. A. L., Morgan, W. T. J., Mosimann, W., *Biochem. J.*, 1954, v56, 572.
16. Wright, G. G., Feinberg, R. J., *J. Immunol.*, 1951, v68, 65.
17. Heidelberger, M., Kendall, F. E., Scherp, H. W., *J. Exp. Med.*, 1936, v64, 559.
18. Webster, M. E., Sagin, J. F., Anderson, P. R., Breese, S. S., Freeman, M. E., Landy, M., *J. Immunol.*, 1954, v73, 16.
19. Coulson, E. J., Stevens, H., *ibid.*, 1961, v86, 241.
20. Baer, H., Naylor, I., Bibbel, N., Rosenfield, R. E., *ibid.*, 1959, v82, 183.
21. Landy, M., Johnson, A. G., Webster, M. E., *Am. J. Hyg.*, 1961, v73, 55.
22. Landy, M., Trapani, R. J., Clark, W. R., *ibid.*, 1955, v62, 54.
23. Landy, M., Johnson, A. G., Webster, M. E., Sagin, J. F., *J. Immunol.*, 1955, v74, 466.

Received September 3, 1963. P.S.E.B.M., 1963, v114.

"Rebound" Recovery from Deprivation of Paradoxical Sleep in the Rabbit.* (28725)

N. KHAZAN† AND CHARLES H. SAWYER

Department of Anatomy and Brain Research Institute, University of California at Los Angeles, California

"Paradoxical sleep" (PS) in mammals has been described as a distinct phase of sleep, apparently profound, characterized by a total loss of postural tone and by rapid eye movements. It has an abrupt onset in the course of ordinary "spindle sleep," and its electroencephalographic (EEG) correlate consists of low voltage fast cortical activity similar to that of arousal(1-3). In man the PS phase has been associated with dreaming, and deprivation of PS by awakening the subject at the onset of each PS episode led to psychological disturbances and an increased incidence of PS when sleep was again undisturbed(4).

In rabbits a pattern now recognized as PS was described(5) as a phase in the "EEG afterreaction" to coitus in the female with "chronic" electrodes implanted in cortical and subcortical regions of the brain. The EEG record was characterized by a fast (8c/sec) high amplitude theta rhythm in the hippocampus and related regions (called the phase of "hippocampal hyperactivity") and a depression of intrinsic activity in the olfactory bulb. During this phase the rabbit's head was on the floor with her ears depressed and the only notable motor activity was a twitching of the eyelids and jerky movements of the jaws. The reaction could be induced by

* Supported by U.S.P.H.S., N.I.H. grant.

† Postdoctoral NIH Research Fellow from Dept. of Applied Pharmacology, Hebrew Univ.-Hadassah Med. School, Jerusalem, Israel.

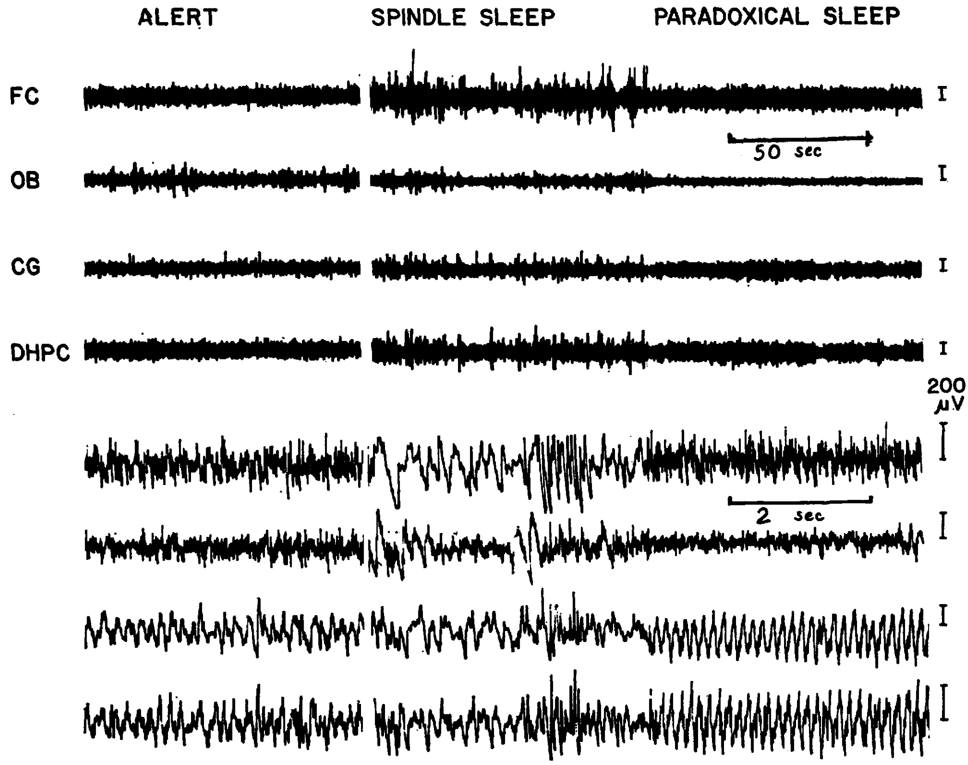

FIG. 1. Comparative EEG recordings at low speed (0.6 mm/sec) and moderate speed (15 mm/sec). Note the easily diagnosed changes in the record during the 3 states, alert, spindle sleep, and paradoxical sleep. Abbreviations: FC, frontal cortex; OB, olfactory bulb; CG, central gray; DHPC, dorsal hippocampus.

low frequency stimulation of the hypothalamus or regions of the limbic system(6). During these experiments which were conducted in a noisy room with a "white" masking noise, PS seldom if ever occurred spontaneously. Under those conditions the stimulation threshold for the induction of PS varied with the hormonal condition of the animal(7). More recently it has been noted that the induction of paradoxical sleep can be conditioned to a neutral tone(8). Paradoxical sleep in the rabbit has also been described by Faure(9).

In studying EEG correlates of sleep patterns in rabbits in a sound attenuated observation room we observed several spontaneous episodes of paradoxical sleep after the rabbit had become adapted to the room(10). This observation suggested a more extensive investigation of the frequency and duration of these spontaneous episodes under a variety of experimental conditions. The present report describes the relative incidence of sleep with spindles and slow waves, paradoxical sleep and wakefulness throughout the day and night periods and the effect of a masking noise on these patterns.

Materials and methods. Observations were made on 10 mature ovariectomized New Zealand White rabbits with electrodes implanted chronically in such regions as frontal cortex, olfactory bulb, septum, dorsal hippocampus, amygdala, central gray and midbrain reticular formation by methods described earlier (5). The rabbits were caged in a light controlled room with 12 hours of darkness per day, but the observation room during recording sessions was constantly illuminated. Continuous EEG recordings were made with a Grass EEG apparatus for periods up to several days during which the rabbit was disturbed minimally. This excluded the acute

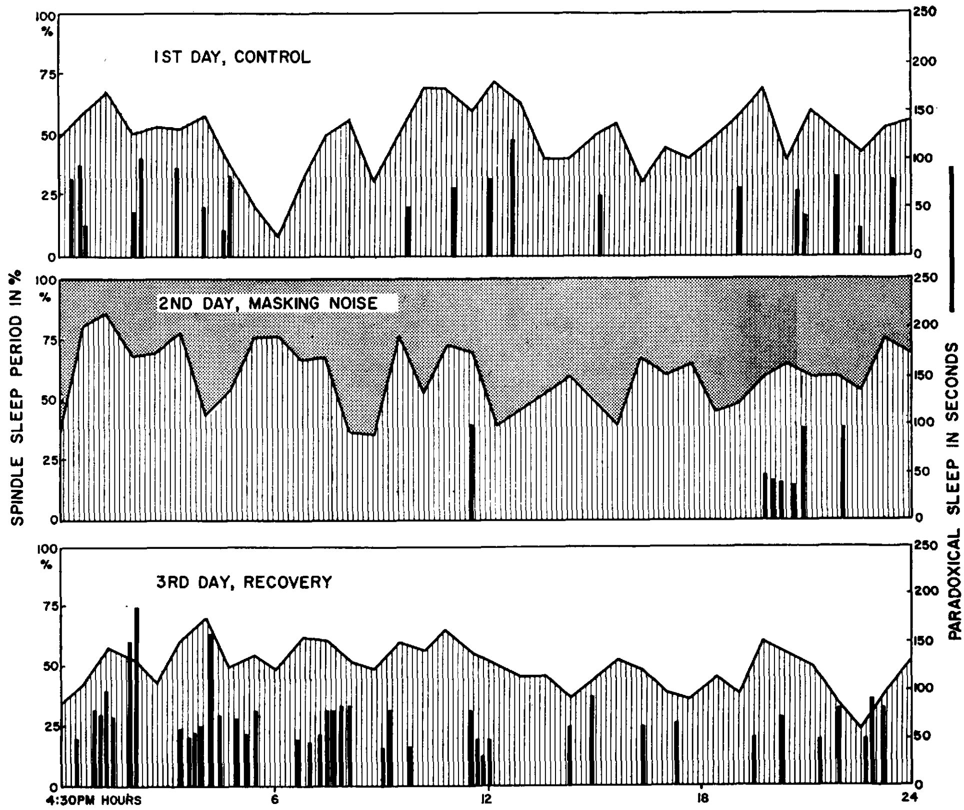

FIG. 2. Incidence of episodes of paradoxical sleep recorded continuously for 3 days. A white masking noise was presented during the second 24 hours. Note the highest incidence of PS (rebound) during the third day.

stress factors commonly encountered in short term experiments. The frequency and duration of PS and ordinary sleep were then analyzed for successive 24-hour periods under control and experimental conditions. The results include 10 sets of continuous recordings over 3-day periods each consisting of 24 hours of control record, 24 hours of masking noise and 24 hours of recovery. The white masking noise level registered 78 decibels (db) as compared with an ambient background level of 52 db measured with a General Radio Co., Type 1551-A sound-level meter.

Results and discussion. Even at a paper speed as slow as 0.6 mm/sec, which was the usual speed used, the characteristic patterns of alertness, ordinary sleep and paradoxical sleep are easily distinguishable (Fig. 1).

The 24-hour sleep record of the well adapted rabbit in the sound-attenuated chamber shows 10-12 hours of spindle sleep almost evenly spaced and 20-30 episodes of PS ranging from 30 seconds to 6 minutes (Fig. 2). No clear diurnal rhythm was noted. Different rabbits revealed sleep patterns sufficiently individualized in their frequency, duration and distribution of episodes as to permit identification of the subject. PS periods were infrequent until the animal became thoroughly adapted to the observation room.

White masking noise almost obliterated the PS episodes for 20 hours or more after which the rabbit gradually became habituated to it. In the course of a week the animal became dishabituated, for a second session with masking noise a week later was equally inhibitory to PS. Surprisingly, spindle sleep was not inhibited by the white noise; if anything, there was a slight increase in the amount of ordinary sleep during the 24 hours of noise.

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.

The authors thank Mrs. Enid Busser for technical help and Mrs. Suzanne Arlen for preparing the figures.

1. Dement, W., *Electroencephalog. Clin. Neurophysiol.*, 1958, v10, 291.
2. Jouvet, M., Michel, F., Courjon, J., *C. R. Soc. Biol.*, Paris, 1959, v153, 1024.
3. Jouvet, M., *Arch. ital. Biol.*, 1962, v100, 125.
4. Dement, W., *Science*, 1960, v131, 1705.
5. Sawyer, C. H., Kawakami, M., *Endocrinology*, 1959, v65, 622.
6. Kawakami, M., Sawyer, C. H., *ibid.*, 1959, v65, 631.
7. ———, *ibid.*, 1959, v65, 652.
8. ———, *The Physiologist*, 1962, v5, 165.
9. Faure, J., *Rev. Neurol.*, 1962, v106, 190.
10. Khazan, N., Kawakami, M., Sawyer, C. H., *The Pharmacologist*, 1963, v5, 266.

Received September 3, 1963. P.S.E.B.M., 1963, v114.

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

S. K. DUTTA AND B. S. POMEROY
Department of Veterinary Bacteriology and Public Health, College of Veterinary Medicine, University of Minnesota, St. Paul

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.

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.

* This study was supported in part by grants from Dr. Salsbury's Laboratories, Charles City, Iowa, and Animal Disease and Parasite Research Division, U.S.D.A.

† Published as paper no. 5202, Scientific Journal Series of Minn. Agri. Exp. Station.