represent cases of chromosomal differentiation (*3*, *7*).

The virus-infected larvae frequently show clear symptoms of disease, and, although surviving for several weeks, they are unable to grow at normal rates. The virus seems to induce a higher metabolic activity, resulting mainly in an increase in the size of the chromosomes. As revealed by autoradiography, synthesis of DNA in the chromosomes is greatly enhanced after the infection. The presence of easily breakable points in some of the chromosomes is an indication that the multiplication of virus inside the cell interferes with synthesis of some chromosomal constituent. This is a point of interest, since in *R. angelae* the size and structure of chromosomes permit us to do some detailed work on the relation of the virus to the chromosome. This perhaps will help us to understand the deleterious effects of many viruses on mitotic chromosomes described in several papers dealing mainly with mammalian chromosomes (*8*). Paton *et al.*, and others as well (*9*), have described interesting cases of changes in morphology and behavior of chromosomes induced by mycoplasma in tissue cultures of human cells. Some of these results may be related to the virus-induced changes observed in the chromosomes of *R. angelae*.

Rhynchiosciara angelae was previously one of the most favorable organisms for study of the activity of chromosomes and specific chromosomal regions in differentiation (*2*, *3*). The present findings demonstrate that *R. angelae* is also uniquely suitable for studies on the response of chromosomes and genes to infective agents. It is the only known organism in which gene activities can be studied morphologically in at least eight different tissues. Probably many of the reactions of the chromosomes and genes of *R. angelae* to those two microorganisms are good indications of what occurs in many infections in other organisms.

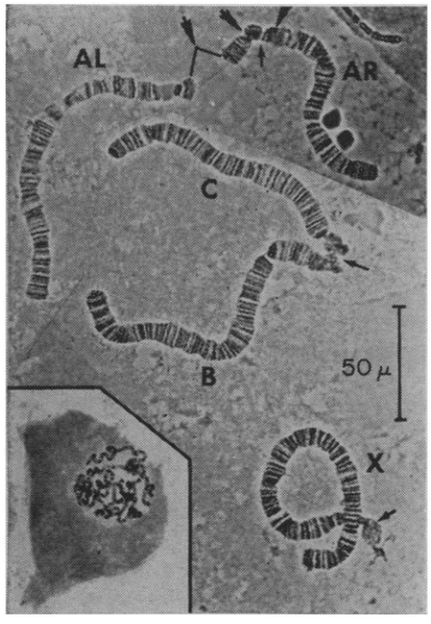

Fig. 3. The polytene chromosomes (A, B, C, and X) of a virus-infected cell from the gastric caecum (composite photomicrograph). *AR* and *AL* represent respectively the right and the left arms of chromosome A. The short arrows indicate the heterochromatic portions at the centromeric region of the chromosomes. At the base of the X chromosome, the nucleolus-organizing region is dispersed, a branched structure inside a dispersed nucleolus not visible in this picture being formed. The long arrows near the centromeric region of chromosome A indicate three easily breakable points frequently seen in chromosomes from cells infected by the virus. Inset: a normal cell of the same gastric caecum, at the same scale to show the increase in size of the chromosomes of infected cells.

CRODOWALDO PAVAN
RENATO BASILE

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

References and Notes

1. M. Diaz and C. Pavan, *Cienc. Cult. (São Paulo)* **16**, 247 (1964); M. Diaz and C. Pavan, *Proc. Nat. Acad. Sci. U.S.* **54**, 1321 (1965).
2. C. Pavan, *Brookhaven Symp. Biol.* **18**, 222 (1965).
3. M. E. Breuer and C. Pavan, *Chromosoma* **7**, 371 (1955); C. Pavan, *Proc. X Intern. Congr. Genet. 10th (Montreal)* **1**, 321 (1959).
4. M. J. D. White, *J. Morphol.* **78**, 201 (1946); *ibid.* **80**, 1 (1947).
5. C. Pavan, M. Diaz, R. Basile, unpublished results.
6. M. Lyon, *Nature* **190**, 372 (1961); L. B. Russell, *Trans. N.Y. Acad. Sci.* **26**, 726 (1964); H. J. Muller, *Harvey Lectures* (1947–48) **43**, 165 (1950).
7. C. Pavan, *Monogr. Natl. Cancer Inst.* **18**, 309 (1965).
8. B. Hampar and S. A. Ellison, *Nature* **192**, 145 (1961); P. S. Moorhead and E. Saksela, *J. Cell. Comp. Physiol.* **62**, 57 (1963); N. Wald, A. C. Upton, V. K. Jenkins, W. H. Borges, *Science* **143**, 810 (1964).
9. G. R. Paton, J. P. Jacobs, Dr. F. T. Perkins, *Nature* **207**, 43 (1965); J. Fogh and H. Fogh, *Proc. Soc. Expt. Biol. Med.* **119**, 233 (1965); C. C. Randall, L. G. Gafford, G. A. Gentry, L. A. Lawson, *Science* **149**, 1098 (1965); R. M. Nardone, J. Todd, P. Gonzalez, E. V. Gaffney, *ibid.*, p. 1100 (1965).

10. We thank Dr. Ellen Mattingly, Dr. R. B. Cumming, and Dr. J. E. Trosko for criticism and suggestions; C. J. Whitmire, Jr., and J. D. Amundson for editorial assistance; L. C. Gomes Simões for the supply of some strains of flies from Brazil; and Miss Shirley A. Preston for the technical assistance. Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

* Visiting investigators from the University of São Paulo, São Paulo, Brazil.

2 February 1966

Dreaming Sleep in Man: Changes in Urine Volume and Osmolality

Abstract. *Epochs of dreaming sleep, as measured by rapid eye movements, consistently correlated with biphasic change in urine volume and osmolality in catheterized human subjects. Marked decrease in volume and increase in osmolality were followed by a hypotonic diuresis.*

Recent studies (*1*) demonstrating autonomic and respiratory changes associated with the rapid eye movement (REM) state during sleep prompted us to attempt to elucidate neuroendocrine correlates associated with this state, because subcortical systems influencing peripheral physiological events have been shown to act through the pituitary-adrenal axis (*2*); we studied urinine volume, osmolality, creatinine concentration, and steroid and catechol amine metabolites serially collected during all-night sleep in man. We now report a consistent urinary-volume correlate of the REM state and some findings that suggest the mechanism involved.

Seven male urology patients aged 45 to 74, whose renal function was normal and who were habituated to urinary catheters for several days, were studied for a total of 11 nights by means of conventional electroencephalographic, electromyographic, and electrooculographic recordings in a sound-attenuated, darkened room (*3*). The urinary catheters were led from the room into a volume-regulated fraction collector which permitted continuous collection of urine samples of uniform volume, the volumes being measured accurately after collection. The electrical recordings were scored for REM state without knowledge of the data from the urine studies, by use of Dement's criteria (*4*). The time of onset and duration of the REM periods were carefully determined. The filling of each tube in the fraction collector was time-locked to the electrophysiologic data by an event recorder fed into an amplifier of one channel of the electroencephalograph. Urinary creatinine was determined by the method of Jaffe (*5*). The osmolality of the urine samples was measured by freezing-point depression, by use of an Advanced osmometer.

All 41 REM-state epochs recorded during the 11 nights were associated with urine-volume responses of varying magnitude (Figs. 1 and 2). With some

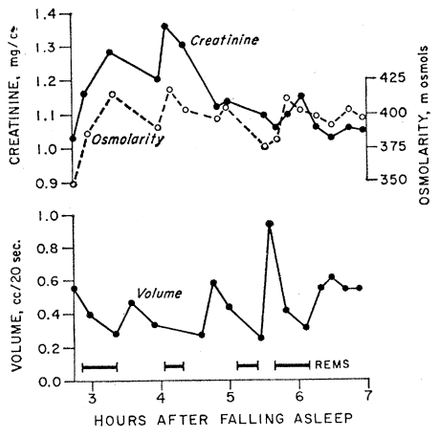

Fig. 1. Simultaneous plot of urine volume, osmolarity (osmolality), creatinine concentration, and REM-state epochs during a typical night's sleep. The hypotonic diuresis associated with the first hour or so of recumbency and sleep has been omitted.

variability in latency, but usually within 2 minutes of the onset of the REM state, there was a marked decrease in the drop rate of urine from the catheter into the fraction collector. This decrease was followed by an increase of even greater magnitude after the end of the REM state. These changes are smoothed-out somewhat in Figs. 1 and 2 because of the overlap of antidiuretic and diuretic effects in the serial, constant-volume samples.

One possible explanation of this change was a mechanical factor, such as alteration of bladder tone or compression of the catheter lumen by penile erections that have been shown to accompany the REM state (*6*). However, reciprocal changes in urine osmolality and creatinine were associated with the volumetric shifts (Figs. 1 and 2); mechanical storage or blockage effects in the urinary collecting system would not have altered the composition of the urine in such a fashion. This association suggested that the change in urine flow associated with the REM state was a change in renal function. Pitts (*7*) concluded that, except for renal disease states or extremely marked volemic changes leading to circulatory collapse, changes in urine flow in man are for the most part effected by changes in water reabsorption, not by changes in filtration rate. Two neuroendocrine mechanisms affecting water and solute reabsorption by the kidney tubule, which could be triggered by shifts in central nervous system events, are the releases of aldosterone from the adrenal (with a resulting increase in sodium reabsorption and an isosmotic decrease in volume) and of antidiuretic hormone–like substances from the posterior pituitary, with a hypertonic volume reduction. Our data show a consistent inverse change in urine osmolality with change in volume, which indicates an alteration primarily in the water rather than in the sodium reabsorptive mechanism. However, before concluding that the urine-volume changes were secondary to release of antidiuretic hormone, some indication of the constancy of glomerular filtration rate is necessary. Since these studies were done during sleep and were of repeated measurements, the more accurate reflection of glomerular filtration rate, inulin clearance, could not be used. Although endogenous creatinine clearance in man has great individual variability and, in addition to being filtered, creatinine is partially secreted by the tubule (wherefore its clearance exaggerates the glomerular filtration rate), clearance is used by some groups (*8*) as a rough measure of glomerular filtration rate. Since the creatinine concentrations in plasma did not change significantly between morning and evening in our subjects, the expression for the change in creatinine clearance

$$(Uv'\ Uc'/Pc') - (Uv\ Uc/Pc)$$

becomes

$$Uv'\ Uc' - Uv\ Uc$$

Uv is urine volume per minute, *Uc* is creatinine concentration in urine in milligrams per milliliter, and *Pc* is creatinine concentration in plasma in milligrams per milliliter. Since *Uv* varies inversely with *Uc* (see Figs. 1 and 2), $Uv\ Uc \cong k$ and $Uv'\ Uc' - Uv\ Uc \cong 0$. Therefore, although this is a rough approximation because of brief collection periods that included both low and high output of urine, it appears that the changes in volume and osmolality were independent of changes in glomerular filtration; this could be expected in view of the reported stability of the glomerular filtration rate in healthy man (*9*).

The antidiuretic hormone-like initial effect possibly may have been secondary to cardiorespiratory irregularities (*1*) associated with the REM state and consequent release of antidiuretic hormone triggered by brain-stem vasomotor reflexes (*10*). Changes of pressure in both the left atrium and the carotid baroreceptors have been reported to influence release of antidiuretic hormone as part of the blood-volume regulatory system. However, the quite-short latency and regularity of appearance of the hypertonic decrease in urine flow during the first 2

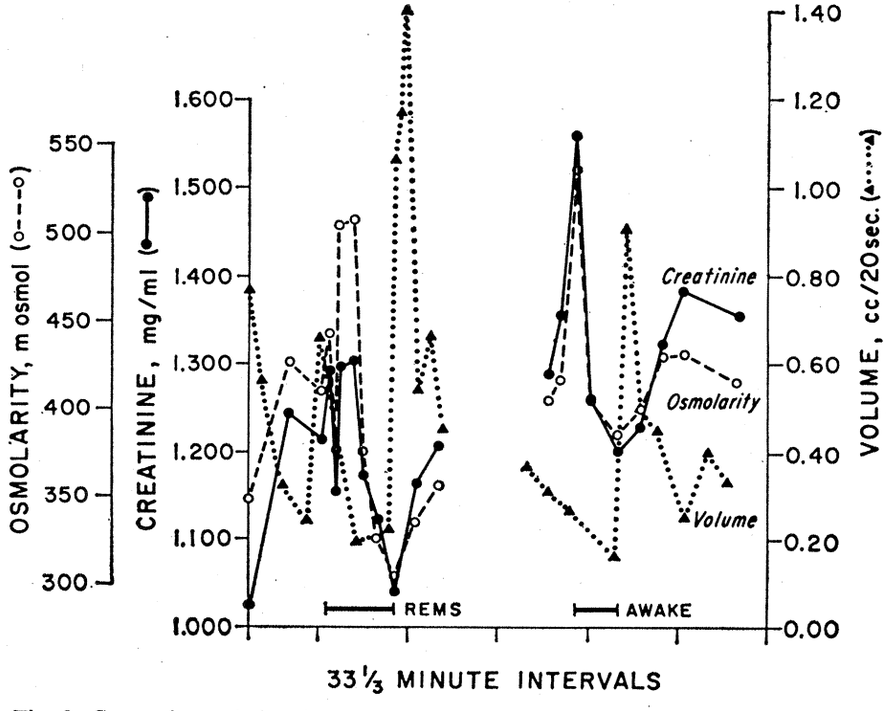

Fig. 2. Comparison of the changes in some urine variables during a REM state and a middle-of-the-night arousal period; note the similarity of the directions of change.

minutes of the REM state, combined with the irregular time of appearance of cardiovascular irregularities (*11*), make this explanation unlikely.

The volume and osmolality changes were more likely concomitants of central nervous system changes associated with the REM state. The evidence is (*12*) that the REM state may be a special kind of "arousal," with many of its central and peripheral manifestations resembling more an awake than a sleeping state. We have recently reviewed several studies indicating that various kinds of emotional-arousal states in man are associated with decreases in urine volume (*13*), probably secondary to release of antidiuretic hormone. Our results demonstrate that short periods of lying awake in the middle of the night (not the early diuretic phenomena associated with lying down and going to sleep) were associated with the same biphasic response of urine volume and osmolality that is seen in the REM state (Fig. 2). Brainstem and limbic "activation' associated with the REM state (*1*) may have been transmitted to the hypothalamic nuclei, concerned with control of antidiuretic hormone, by way of established pathways (*2*).

A. J. MANDELL, B. CHAFFEY, P. BRILL
M. P. MANDELL, J. RODNICK
R. T. RUBIN, R. SHEFF
Departments of Psychiatry and *Urology, University of California, Center for the Health Sciences, Los Angeles*, and *Wadsworth Veterans Administration Hospital, Los Angeles*

References and Notes

1. F. Snyder, *Amer. J. Psychiat.* **122**, 377 (1965).
2. J. D. Green, *Physiol. Rev.* **44**, 461 (1964); W. J. W. Nauta, in *Advances in Neuroendocrinology*, A. V. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963); A. J. Mandell, L. F. Chapman, R. W. Rand, R. D. Walter, *Science* **139**, 1212 (1963).
3. A. Jacobson, A. Kales, J. R. Zweizig, J. Kales, *Amer. J. EEG Technol.* **5**, 5 (1965).
4. W. Dement, "The physiology of dreaming," thesis, Univ. of Chicago, Chicago (1958).
5. J. Peters, *J. Biol. Chem.* **146**, 179 (1942).
6. C. Fisher, J. Gross, J. Zuch, *Arch. Gen. Psychiat.* **12**, 29 (1965).
7. R. F. Pitts, *Physiology of the Kidney and Body Fluids* (Year Book, Chicago, 1963), p. 66.
8. G. Mattar, H. L. Barnett, H. McNamera, H. D. Lauson, *J. Clin. Invest.* **31**, 938 (1952).
9. M. B. Strauss, *Arch. Internal Med.* **103**, 489 (1959).
10. G. Farrell and A. N. Taylor, *Ann. Rev. Physiol.* **24**, 471 (1962).
11. F. Snyder, *Science* **142**, 1313 (1964).
12. W. C. Winters, *EEG Clin. Neurophysiol.* **17**, 234, (1964).
13. A. J. Mandell, *Amer. Heart J.* **65**, 572 (1963).
14. Assisted by NASA grant NsG 237-62 under the aegis of the Space Biology Laboratory; and by the Brain Research Institute, U.C.L.A. Health Science Center.

13 January 1966

Parturient Mice: Effect of Environment on Labor

Abstract. *More first-born pups and greater numbers of pups were born in a familiar cage having a covered nesting box than in a glass bowl, when pregnant mice at term were alternated between contrasting environments. Current theories emphasize the influence on labor of mechanical and hormonal factors; these data suggest that environmental factors also may play a role.*

Current physiologic theories and medical and veterinary therapeutic practices concentrate almost exclusively on the endocrine and mechanical factors involved in the onset and progress of labor (*1*). Ecologic and clinical observations (*2*) suggest, however, that environmental factors also may influence labor to some extent.

This experiment was designed to test the hypothesis that environmental factors play a role in labor. White mice of the CF1 strain were used because previous work had shown them to be placid (*3*) and otherwise suited to the design of the experiment. Dated pregnancies were produced by adding male mice to a colony of nulliparous female mice for approximately 13 hours from 8 p.m. to 9 a.m.; the lighting was normal. Eighteen days later, females judged to be probably pregnant were placed individually in cages with opaque sides and nesting boxes—replicas of the cages that had housed the mice before removal from the colony. Each cage contained a wooden nesting box (22.5 by 15 by 9.4 cm) having a hole in the side to permit entry of the mouse and containing soft rayon bedding; a wooden top, completely covering the box and sheltering the mouse from view, was removable to permit complete inspection of the box during the experiment.

The preference of nulliparous pregnant mice at term for the covered nesting box is statistically indicated by the fact that they were found inside the box in 47 of 60 observations of 21 individuals at 2- and 4-hour intervals. There is no record of the sites of delivery; observation was not continuous, but delivery regularly seemed to occur within the covered nesting box.

All pregnant mice were left overnight in their individual cages containing nesting boxes. Next day, between 1 and 2 p.m., all cages were moved to a large observation room, and another type of cage that was to serve as an unfamiliar, unsheltered environment was placed beside each cage.

This second type of cage consisted of a standard glass fish bowl about 17.5 cm high; the bottom contained two cups of coarse gravel, with a quarter cup of uriniferous bedding from cat cages to imbue the bowl with a strange odor. Wire mesh atop the bowl retained the mouse. Both types of cage contained food pellets. Since water is difficult to administer in a glass bowl, both experimental environments contained only apples instead of water for moisture; apples had proved sufficient to maintain a parturient mouse of this strain during both labor and the first week of lactation.

Mice were randomly divided into

Table 1. Birth sequence distribution: numbers of pups born in the two environments. F, Familiar cage with covered nesting box; U, unfamiliar glass bowl.

Mothers moved every 2 hours (2-hour intervals)					Mothers moved hourly (1-hour intervals)							
F	U	F	U	F	F	U	F	U	F	U	F	U
2		4			1				2		5	3*
2		1	4		4							
1	6			1	3	4						
1		6	4		3	1	1	1	1			
10					1	8	1					
1	1	6			1		6	5				
7	5				7							
6	3				1	2	2					
1	6	5			3	3						
5	8					3	3		8			
	3	8				1	4	9				
	1	5	1			1	9					
						4						

* Killed at end of experiment, 26 hours after these births; two pups found at autopsy.