by a process of breakage and rejoining. Furthermore, since normal recombination frequencies can be attained in the absence of DNA synthesis (Fig. 1), breakage and rejoining is probably the major mechanism for recombination in this virus. The possibility that a small amount of DNA synthesis may be involved in the recombination process is not excluded. However, the basic process is clearly one of physical exchange between molecules rather than a copying of genetic information from two molecules in the process of making a third (*17*).

E. SIMON

Department of Biological Sciences, Purdue University, Lafayette, Indiana

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23 July 1965

Cestode in North Dakota: Echinococcus in Field Mice

Abstract. *Field mice* (Microtus pennsylvanicus *and* Peromyscus maniculatus) *are naturally infected with* Echinococcus multilocularis. *Thus, the sylvatic cycle (fox to field mice to fox) has been established in North Dakota. This cestode is expected to extend its range to other agricultural regions of the continental United States where similar conditions favorable for the completion of its life cycle exist.*

Echinococcus multilocularis (Leuckart, 1863) was reported first from the continental United States by Leiby and Olsen (*1*) who found the adult cestodes in red foxes (*Vulpes vulpes*) from Ward County, North Dakota. Biologic and morphologic studies (*2*) on the larval stages which developed in experimentally infected cotton rats (*Sigmodon hispidus*) have confirmed that the North Dakota cestode is *E. multilocularis* and is indistinguishable from the species found in Alaska (*3*).

Preliminary investigations in North Dakota, during the late spring of 1965, have revealed a high prevalence of sylvatic multilocular echinococcosis. Of 47 field mice examined for natural infections, 3 of 32 *Microtus pennsylvanicus* and 3 of 15 *Peromyscus maniculatus* harbored *Echinococcus multilocularis*. With the exception of a single *Peromyscus maniculatus*, in which the cystic stages occurred in both the liver and spleen, the infections were confined to the liver. In all cases, the larvae seemed normally developed with large numbers of scolices. To the best of my knowledge, this constitutes the first report of *E. multilocularis* occurring naturally in the above rodents; however, experimental infections with the Alaskan strain have been established in both *Microtus pennsylvanicus* (*4*) and *Peromyscus maniculatus* (*5*).

The work by Leiby and Olsen (*1*) and my study confirm that the sylvatic cycle of *Echinococcus multilocularis* (fox to field mice to fox) is well established in North Dakota. In view of present knowledge, it should be expected that its range will eventually extend throughout the agricultural regions of the United States where conditions for completion of its life cycle are favorable.

Vogel (*6*) ascertained that dogs, cats, and foxes served equally well as definitive hosts for the Eurasian strain of *E. multilocularis*. Therefore, it is reasonable to assume that dogs and cats in rural areas will become infected when they feed upon field mice harboring the larval cestode, and occasional infection of man in the United States could occur as a result of his association with these domestic animals. Also, as indicated by Rausch (*3*), there is some risk in the handling of foxes by trappers, hunters, and fur handlers.

PAUL D. LEIBY

Department of Biology, Minot State College, Minot, North Dakota

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14 July 1965

Periodic Respiratory Pattern Occurring in Conjunction with Eye Movements during Sleep

Abstract. *With each flurry of rapid eye movements during the sleep of human subjects there is a decreased amplitude of respiration and a slight increase in rate. Occasionally the rhythmic breathing pattern may even resemble Cheyne-Stokes respiration. The consistency of this breathing pattern suggests that respiration in this stage of sleep is not a direct function of dream content.*

The initial report (*1*) regarding the occurrence of a rapid eye movement (REM) stage of sleep indicated that the cardiac and respiratory rates were slightly elevated during that stage as compared with either the preceding or following stages. In the decade that ensued, these results were essentially confirmed, although great stress (*2, 3*) was placed on the purported irregularity of the respiratory pattern in the REM stage of sleep. Inasmuch as respiration (sighing, hyperventilation, compulsive breath-holding, and so forth) reflects

the emotional state of the awake individual, the erratic breathing in the REM period gave some credence to the view that such breathing simply mirrors the emotional content of a coexisting dream.

The present report shows that respiration in the REM, or dreaming, stage of sleep has a distinctly regular pattern which is similar for all the individuals observed. Furthermore, the pattern at the beginning of a REM period is practically identical with the one observed at the end of such a period.

The alteration of respiration during short bursts of eye movements and at the initiation of a long train of eye movements was systematically in the same direction—apnea. (A REM period consists of bursts of eye movements which last from a fraction of a second to several minutes and which are separated from each other by varying periods of ocular quiescence.) Since apnea or hypopnea was noted to occur during the first 45 seconds of rapid eye movements, it is reasonable to assume that the resulting alteration of the gas tensions of the blood would induce respiratory changes in a direction opposite to that of the initial effect. Consequently, it is suggested that the reports (*2, 3*) affirming an irregularity of respiration during the REM period were based on the masking effect imposed by secondary respiratory responses.

Eleven normal adult subjects were permitted to sleep through the night while polygraphic recordings were made in the conventional manner (*1*) of frontal and occipital electroencephalograms, of electro-oculograms of horizontal and vertical vectors of eye movements, and of the electrocardiogram. Arterial oxygen saturation was monitored with a Waters pediatric earpiece. In addition, respiration was recorded through a thoracic pneumograph and by means of an intranasal catheter which led a sample of expired air to a Beckman capnograph for detection of CO_2.

Owing to the location of the catheter, a marked reduction in ventilatory volume allowed the expired air sample to be diluted by atmospheric air, thereby leading to CO_2 values lower than the true values for expired air. However, in the period of ocular inactivity preceding a burst of eye movements (hereafter called "eye burst"), the tidal respiratory exchange was normal, and therefore the CO_2 values for the expired air at this time were accurate. The expired CO_2 reflects alterations of the alveolar CO_2. Consequently, the method employed permitted recognition of any trend in CO_2 level prior to an eye burst. When the thoracic pneumograph tracing revealed apnea, the spuriously low CO_2 values did subserve the function of indicating that the reduced ventilatory volume was not due simply to a shift from thoracic to abdominal breathing.

Table 1. Percentage change in respiratory rate and amplitude during the beginning of an eye burst (equivalent to the duration of four breaths) as compared to the period of ocular quiescence immediately preceding the eye burst. REM period II immediately follows REM period I, but these are not necessarily the second and first REM periods of the night.

Respiratory parameter	Percentage change			
	REM period I		REM period II	
	1st half	2nd half	1st half	2nd half
Amplitude	−16.6	−15.3	−15.4	−18.9
Rate	+11.1	+10.8	+ 9.8	+10.3

Scoring of the data was accomplished by first establishing groups of rapid eye movements which constituted discrete eye bursts. Those eye movements which were separated by a duration of less than 1.5 respiratory cycles were considered to belong to the same eye burst; eye movements separated by 1.5 or more respiratory cycles were classified as separate eye bursts. Thus the entire REM stage of sleep was marked off into segments of ocular quiescence interspersed among eye bursts. (There are usually several eye bursts during a REM period, and usually several REM periods during a night's sleep.)

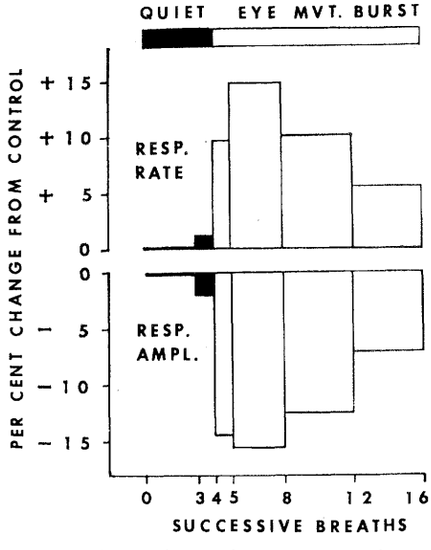

Fig. 1. Change in respiratory rate and amplitude during a burst of rapid eye movements. The first three breaths, represented by heavy horizontal lines, are the control values occurring in the period of ocular quiescence just before the eye burst. The vertical black bar represents the fourth, or last, breath of the quiescent segment. White bars indicate breaths in the eye burst period.

A total of 391 pairs of segments of ocular quiescence and motility were analyzed breath-by-breath; the range of number of pairs per subject was 12 to 70. A value for each respiratory parameter was obtained for each of four breaths immediately preceding an eye burst, and for each of a maximum of 12 consecutive breaths coincident with the beginning of an eye burst. Average values were computed for each of the breaths; the value of a respiratory parameter (for example, amplitude) for a given respiratory cycle was compared with each of the measurements computed for the preceding cycles. Heart rates were determined for eight of the subjects by obtaining average values for segments of records corresponding to the last four breaths of periods of ocular quiescence as well as for the immediately succeeding groups of breaths. Statistical significance was established through the use of Fisher's *t*-test for paired data; a two-tailed test was employed in all instances.

The results (Fig. 1) revealed no significant difference in either the respiratory rate or amplitude between any of the four breaths constituting the segment of ocular quiescence. The fourth breath (the one just before the eye burst) suggested a slight change in rate and amplitude, but in all likelihood the change was merely an indication of the small error resulting from the inexactitude of correlating fractional respiratory cycles with the precise beginning of the eye burst. The first breath coincident with the eye burst showed a precipitous decrease in amplitude as well as a rise in rate as compared with the first three of the four preceding breaths, which are represented in Fig. 1 as the quiescent control period; these changes, 15.6 and 9.4 percent, respectively, were significant at the .001 level of probability. Peak changes in respiratory rate and amplitude occurred during the second, third, and fourth breaths of the eye burst. With longer eye bursts, the changes in respiration began to diminish, so that the amplitude at the end of the measured segment was only 8.1 percent ($p < .05$) below the quiescent

control, while the rate was only 5.5 percent above the control (statistically insignificant).

The respiratory changes associated with eye bursts were quite consistent within the REM period when the data for the first halves of the REM periods were compared with the results for the second halves of the same REM periods (Table 1). The amplitude was lower and the rate was higher by almost the same amount in both halves of the REM periods. Similarly, a comparison of the respiratory changes within one REM period with those occurring in a succeeding REM period revealed no statistically significant differences (Table 1).

It would appear that although respiration during the REM period is not uniform, the pattern is nevertheless far from irregular or erratic. In some individuals, when the eye bursts are not especially long, the inhibition of the inspiratory phase of respiration can be correlated with the eye bursts without the need for a statistical analysis. Figure 2 is a sample of such an instance and shows, furthermore, that a Cheyne-Stokes-like pattern of respiration, which is occasionally observed at the onset of sleep in normal persons (*4*), can be observed during the REM or dreaming stage of sleep. The same illustration also reveals that minor fluctuations in arterial O_2 saturation are concomitant with the eye bursts.

Alteration of the respiratory gas tensions of the blood would not appear to be a likely cause of the onset of either the rapid eye movements or of the changes in chest movements. Actually, the arterial O_2 did not fall significantly until the third breath after the eye burst had already begun (Fig. 3), and then, as the eye movements continued for nine more breaths, the arterial O_2 saturation fell to an average of 95.8 percent ($p < .001$), a level which is still too high on the oxygen-hemoglobin dissociation curve to be an effective respiratory stimulant. The fall of O_2 saturation was undoubtedly the aftermath of the reduction of respiratory amplitude, and it reflected an inadequacy of ventilation which was not entirely compensated by the increase in rate.

As reported in a preliminary communication (*5*), the decrease of arterial O_2 saturation coincident with an eye burst could be extreme, so that single values of 92 percent were occasionally measured. Subjects attaining such low

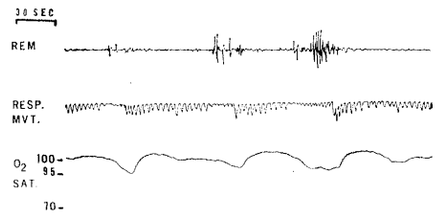

Fig. 2. Sample record showing Cheyne-Stokes-like pattern of breathing during a rapid eye movement stage of sleep in a normal subject. Note that fluctuations of arterial O_2 saturation and respiratory movements are in phase with the three bursts of eye movements indicated by the uppermost tracing.

O_2 values in their sleep were unable to achieve the same drop in arterial O_2 saturation by voluntarily holding the breath to the breaking point in the waking state. In the absence of data for alveolar CO_2 tension during the occurrence of an eye burst, it would be speculative to suggest that the respiratory center's responsiveness to CO_2 during the eye burst phase of the REM period has been reduced compared to the waking state.

The concentration of CO_2 in the expired air during ocular quiescence (Fig. 3) was stable at 4.6 and 4.7 percent for five successive breaths preceding the eye burst and showed no significant trend which might implicate the gas as a causative factor for the REM phenomena.

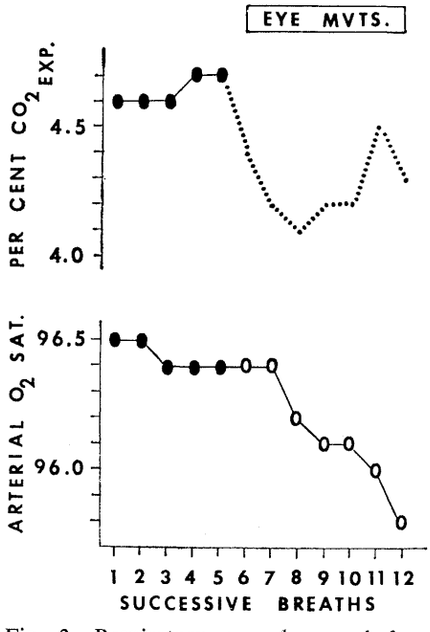

Fig. 3. Respiratory gas changes before (filled ovals) and during (open ovals) a burst of rapid eye movements. Note that there was no significant alteration in the gas tensions from the first to fifth successive breaths. The dotted line represents CO_2 values during an eye burst and merely indicates reduction of tidal volume (see text).

Heart function, like respiratory activity, has been reported previously to be highly irregular (*2*) during the REM period, and also to exhibit an increased rate (*1*) as compared with the stages of sleep either before or after the REM period. Again, as with respiration, the present results provide a different view. On the basis of 246 paired measurements, the mean heart rate for the duration equal to the four breaths preceding an eye burst was 55.9 beats per minute, as compared with 55.8 per minute for a similar duration coincident with the start of an eye burst. During eye bursts that lasted longer than four breaths, the heart rate averaged 3.3 percent lower than during the quiescent control period. Although no statistical significance is attached to the alteration of the heart rate during an eye burst, it is apparent that the heart rate certainly did not increase during the first 12 breaths concurrent with the eye burst. Incidentally, there is a striking resemblance between the cardiorespiratory function during the REM stage of sleep and the description (*6*) of a concurrent apnea and bradycardia occurring during drowsiness.

The present results suggest that physiological activity within the REM period is dichotomized. First, when the eyes are inactive, there is a stage of sleep characterized by regular cardiorespiratory function, albeit the rate of activity is increased compared with other sleep stages. Second, when the eyes are in motion, there is an increase in the occipital alpha rhythm (*7*) together with a depression of ventilation and a possible slowing of the heart. The alternation of periods of ocular quiescence and ocular motility leads to a consistent switching from one physiological pattern to the other. However, with the prolongation of the flurries of eye movements, compensatory cardiorespiratory reactions obliterate the identity of the two patterns.

Unless all dreams have the same emotional content, it does not appear likely that they would affect respiration in so consistent a manner. Even the eye movements which had been postulated (*1*) to represent the motor manifestation of visual imagery during the course of a dream are most likely not directly linked to dream content. Since visual

imagery in the waking state usually, although not always, leads to a reduction of the alpha rhythm, it is difficult to reconcile the increased occipital alpha rhythm at the time of the eye bursts. The periodicity of respiration points to a trigger in the central nervous system more prosaic than that of dream content.

EUGENE ASERINSKY
Department of Physiology,
Jefferson Medical College,
Philadelphia, Pennsylvania

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Trans-Aconitic Acid in Range Grasses in Early Spring

Abstract. trans-*Aconitate ion, an inhibitor of the tricarboxylic acid cycle, was identified in range grasses as* trans-*aconitic acid, which was isolated in crystalline form. It occurs in surprisingly high concentrations in early-season forage grasses. Dry-weight concentrations of* trans-*aconitate vary with season and species; concentrations of between 1 and 2.5 percent are common in mixed pasture grasses, but are higher in certain species such as* Hordeum leporinum *(3.5 percent) and* Phalaris tuberosa *var.* stenoptera *(4.2 percent). Leaves of western larkspur* (Delphinium hesperium) *contain 12.2 percent* trans-*aconitate.* trans-*Aconitate may be partially responsible for nutritional disorders, such as grass tetany (hypomagnesemia), that occur in grazing cattle in early spring.*

During the early spring of 1964 and again in 1965, samples of range grass were collected for analysis in connection with especially severe seasonal outbreaks of grass tetany (hypomagnesemia) in range cattle of the lower Sierran foothills of central California. When applying a quantitative polarographic method to determine nitrate concentrations (*1*) in dried samples, we noted a surprisingly large amount of a soluble, polarographically reducing substance in charcoal-clarified aqueous extracts from the samples. The polarographic wave caused by this substance thoroughly masked the nitrate-diffusion current. Supporting electrolytes were devised to give well-defined, reproducible, polarographic waves from the unknown so that relative concentrations could be determined accurately. The polarograph was then used to follow the material through fractionation procedures that led to isolation and identification as *trans*-aconitic acid. At this point the polarographic method automatically became a quantitative means of determining *trans*-aconitate in plant materials.

The water-soluble fraction of the grasses was subjected to paper chromatography, a mixture of mesityl oxide, formic acid, and water being used for elution (*2*). The material moved with an R_F value essentially the same as that of fumaric acid, but unlike authentic fumaric acid it did not react in the presence of fumarase either directly or when extracted from the chromatographic paper. Consequently, 10 g of dried plant material was extracted with water, and the extract was clarified with charcoal, acidified, and further extracted with ether. The unknown reducible substance passed from the aqueous into the ether phase and crystallized when the ether was evaporated. Refined polarographic examinations of half-wave potentials of the crystalline material, in both acid and neutral supporting electrolytes, suggested that it was probably *trans*-aconitic acid. Spacings from x-ray powder-diffraction patterns and titrimetric curves of the crystalline material were identical with those of authentic *trans*-aconitic acid (Table 1). Melting points were not sharply defined, indicating slight impurity.

Powder-diffraction patterns (Cu-Kα) showed fewer distinguishable lines and greater background scattering than in the reference sample of *trans*-aconitic acid because of the lower degree of crystallinity of the isolated material; relative intensities of the principal lines were not identical but were in keeping with the lower degree of crystallinity (Table 1). Thus, polarographic reduction potentials, x-ray diffraction data, coincidence of titrimetric curves made with NaOH, melting-point data, and consideration of the chemistry of extraction determined that the material isolated from samples of dry grass was slightly impure *trans*-aconitic acid.

Polarographic analyses were made of other dried samples of mixed forage grasses from ranges where animals were prone to grass tetany. These samples, which had been collected in early spring from new growth and dried for 48 hours at 70°C, revealed a consistently high content of 1.5 to 2.5 percent *trans*-aconitate. Lawn clippings from outside our laboratory yielded much less (0.05 percent). Certain dried samples of individual wild and cultivated species have yielded as much as 3.5 and 4.2 percent *trans*-aconitic acid equivalent (*Hordeum leporinum* and *Phalaris tuberosa* var. *stenoptera,* respectively) in early spring, whereas dried mature grass left from the preceding fall contained no detectable aconitate. Dried leaves of *Delphinium hesperium* (western larkspur), a member of the same family as the aconites, contained 12.2 percent aconitate, mostly as *trans*-aconitate.

There are relatively few references to the occurrence of high concentrations of aconitic acid in plant materials. Roberts and Martin (*3*) report concentrations of 2 percent aconitate in solids from dried sugar cane juice, with an additional 1 percent distributed among fumaric, mesaconic, succinic, glycolic, citric, malic, syringic, and oxalic acids. Our drying procedure for samples (48 hours at 70°C) was found to change aconitate from the *cis* to the *trans* isomer; conversion rates increase with temperature and with increasing acidity or alkalinity (*4*).

The high concentration of *trans*-aconitate in these samples of grass suggested interesting biochemical questions relative to the *trans*-aconitate content of the growing plants, as well as to possible consequences for grazing animals. Because *trans*-aconitate is a competitive inhibitor of aconitase in the tricarboxylic acid cycle (*5*), it was important to ascertain the relative distributions of *cis*- and *trans*-aconitate in fresh, green plants.

MacLennan and Beevers (*6*) showed that corn plants synthesize *trans*-aconitate when they are fed acetate labeled with C^{14} at position 1; of the aconitate-C^{14} generated from the acetate, 95 percent was found as *trans*-aconitate and