show. In view of the composition of the shell of oysters, composed mainly of calcite (rhombohedral $CaCO_3$) and minor amounts of aragonite (orthorhombic $CaCO_3$), it is an interesting question which mineral is in the resilium.

To test its composition, a very small hammer and punch were used to break off small pieces of the resilium of *Crassostrea virginica* (Gmelin) until they made up several cubic centimeters. These pieces were checked under the binocular microscope for adhering fragments of the shell. All such contaminated material was discarded. The pure resilium material was ground to a powder. The x-ray powder diagram made by Dr. J. F. Burst of Shell Development Company shows the curve of the mineral aragonite.

H. B. STENZEL
Shell Development Company, Exploration and Production Research Division, Houston, Texas

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22 January 1962

Sleep Deprivation, Age, and Exhaustion Time in the Rat

Abstract. Male rats were maintained on a constantly moving wheel in a study of prolonged sleep deprivation. The results obtained revealed a striking negative relationship between age and resistance to exhaustion.

In an earlier study of the effects of prolonged sleep deprivation, six young male hooded rats, 60 days old, were placed on a constantly moving wheel for 27 days. One animal was lost after 17 days. When older animals were tested in a similar manner, it was noted that exhaustion occurred much earlier. This study reports on the relationship of this age variable to exhaustion time.

Six male animals from the following six age groups were obtained from the University of Florida colony: group I, 63 days old; group II, 89 days old; group III, 147 days old; group IV, 170 days old; and group V, 220 days old. At least two litters were represented in each group. The rats were placed, in individual 4½- by 7½-inch cubicles, on wheels, two-thirds submerged in water, which rotated at a constant speed of approximately 2 rev/min. Food trays were placed in each cubicle so that the animals could feed at any time. The rats remained on these wheels continuously except when they were removed for weighing at 24-hour intervals. The total distance covered by an animal during a day was 0.7 mile. The rats, when exhausted, fell from the wheel into the water and were unable to remount the wheel. Animals were removed from the experiment when they fell into the water after being replaced on the wheel three times during a 15-minute period.

Figure 1 shows for each age group the time at which the criterion of exhaustion was reached. The group I (63 day) animals were removed after 9 days although none had reached the exhaustion criterion. It may be recalled, at this point, that 60-day-old rats were run 27 days with the loss of one animal after 17 days. The only difference in the treatment of the 60-day-old rats was that they were kept off the wheel for a longer time (approximately 20 minutes) because several additional measures were taken.

The rats' weight loss and their intake of food while on the wheel were measured. The average percentages of weight retention for each of the five groups after 48 hours were as follows: group I, 95.8; group II, 86.2; group III, 89.2; group IV, 90.6; and group V, 93.3. These averages do not include values for one animal from group V and one from group III that did not last 48 hours. These weight losses, however, give a distorted picture in the case of group I, and to some extent in the case of group II, as these animals are still in a growth gain period; thus these figures map represent a considerable suppression of weight gain. There is a .20 correlation (rank order) between amount of weight lost in 48 hours and terminal exhaustion time in groups II through V. Finally, the overall weight loss for all animals at the weighing before exhaustion was 15.81 percent.

The average change in food intake from the first day on the wheel to the second day on the wheel was as follows for the five groups (in percent): group I, 248.7; group II, 53.4; group III, 99.1; group IV, 88.8; and group V, 45.3. These figures represent the total food intake during the second day divided by the total food intake during the first day in percentage terms. The correlation between the change in food intake and the change in exhaustion time was not significant.

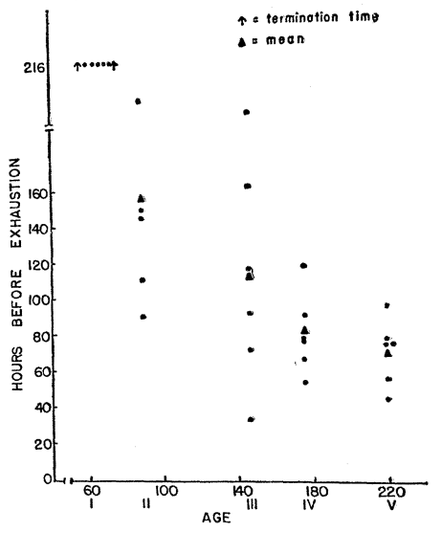

Fig. 1. Exhaustion times of each rat and group means as a function of age. Note that, in group I (age 63 days), runs were terminated after 216 hours.

In conclusion, the data show a clear-cut relationship between exhaustion time and age. Because the amount of activity involved is far below the normal free-run activity of a rat and because the weight loss before exhaustion is certainly within survival limits, it is at least plausible to hypothesize that this exhaustion is related to sleep deprivation (*1*).

WILSE B. WEBB
HARMAN W. AGNEW, JR.
Department of Psychology, University of Florida, Gainesville

Note

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Arrangement of DNA in Living Sperm: A Biophysical Analysis

Abstract. The submicroscopic arrangement of DNA molecules in living sperm is analyzed by new, highly sensitive polarization optical techniques. It is concluded that the molecules are arranged as a coil of a coil in sperm chromosomes, which in turn appear to be arranged in single file with a definite sequence.

Despite major advances in our knowledge of the molecular structure of DNA (deoxyribonucleic acid) (*1*, *2*) and its genetic significance, little is yet known of the exact arrangement of this molecule in chromosomes of living cells. We have inquired into this problem by developing a new method of fine structure analysis which takes advantage of

the crystalline optical property of DNA in living sperm heads and the changes induced in it by irradiation with polarized ultraviolet light. The general approach to the problem and our conclusions will be outlined below.

The needle-shaped sperm heads found in a wide variety of organisms show a strong negative birefringence *(3)*. The birefringence is intrinsic and has been attributed to a regular orientation of the DNA(-protein) molecules aligned parallel to the length of the sperm head. We have recently found that the negative birefringence of various insect sperm heads can be abolished locally when a small spot of ultraviolet of wavelengths between 2500 and 3100 A is shone on the sperm (*4*). This loss of birefringence must reflect an *in situ* alteration and disorientation of the DNA molecules since no loss of refractility accompanies the change of birefringence.

When the irradiating ultraviolet beam is plane polarized, the birefringence is reduced three to four times more effectively with the ultraviolet *E*(electric)-vector polarized perpendicular to the sperm axis than with it polarized parallel. This agrees with the negative ultraviolet dichroism (see *5*, *6*; the ultraviolet absorption is greater when light is polarized perpendicular to the molecular axis since the purine and pyrimidine bases lie in this plane) and the dichroic ratio found in oriented gels of pure DNA (*6*), and strongly suggests that the ultraviolet energy is absorbed directly by the purine or pyrimidine bases of the DNA molecules in the irradiated area. In fact, we find that fibers made of pure DNA gels respond to polarized ultraviolet irradiation and loose their birefringence in much the same way as do the insect sperm heads.

In the mature sperm of the cave cricket, *Ceuthophilus nigricans*, the axis of birefringence is only on the average parallel to the geometric axis of the sperm. With a high-resolution rectified polarizing microscope *(7)* the optic axis is found to zigzag regularly within the smooth head as though the "crystalline" material were ordered into a microscopic helix (Fig. 1). When this sperm is exposed to ultraviolet polarized at 45° to the head, the zigs and zags ("microdomains") each respond independently to the irradiation and loose their birefringence at different rates depending on their azimuth orientations (Fig. 1). Apparently the microdomains containing molecules whose bases lie parallel to the *E*-vector absorb more ultraviolet and loose their birefringence faster than those whose bases are crossed. This should induce, as was actually observed, a rotation of the average optic axis of the sperm head away from the sperm axis and towards the direction of the *E*-vector of the irradiating ultraviolet ray. Thus by observing the rotation of the average optic axis of the sperm, the approximate azimuthal inclinations of the zigzag domains which existed before the irradiation could be estimated.

This same effect can be used to demonstrate the presence of submicroscopic zigzags or coils in pure DNA fibers and also in the fruit-fly sperm head even though its structure is too small to be resolved with the light microscope.

These experiments suggest that the observed changes take place at a truly molecular level or, what is more likely, at a level involving only local segments of the long DNA molecules. Short segments of the molecules must act as independent dichroic absorbers which loose their birefringence upon ultraviolet irradiation. If this reasoning is correct, one should be able to determine the exact submicroscopic arrangement

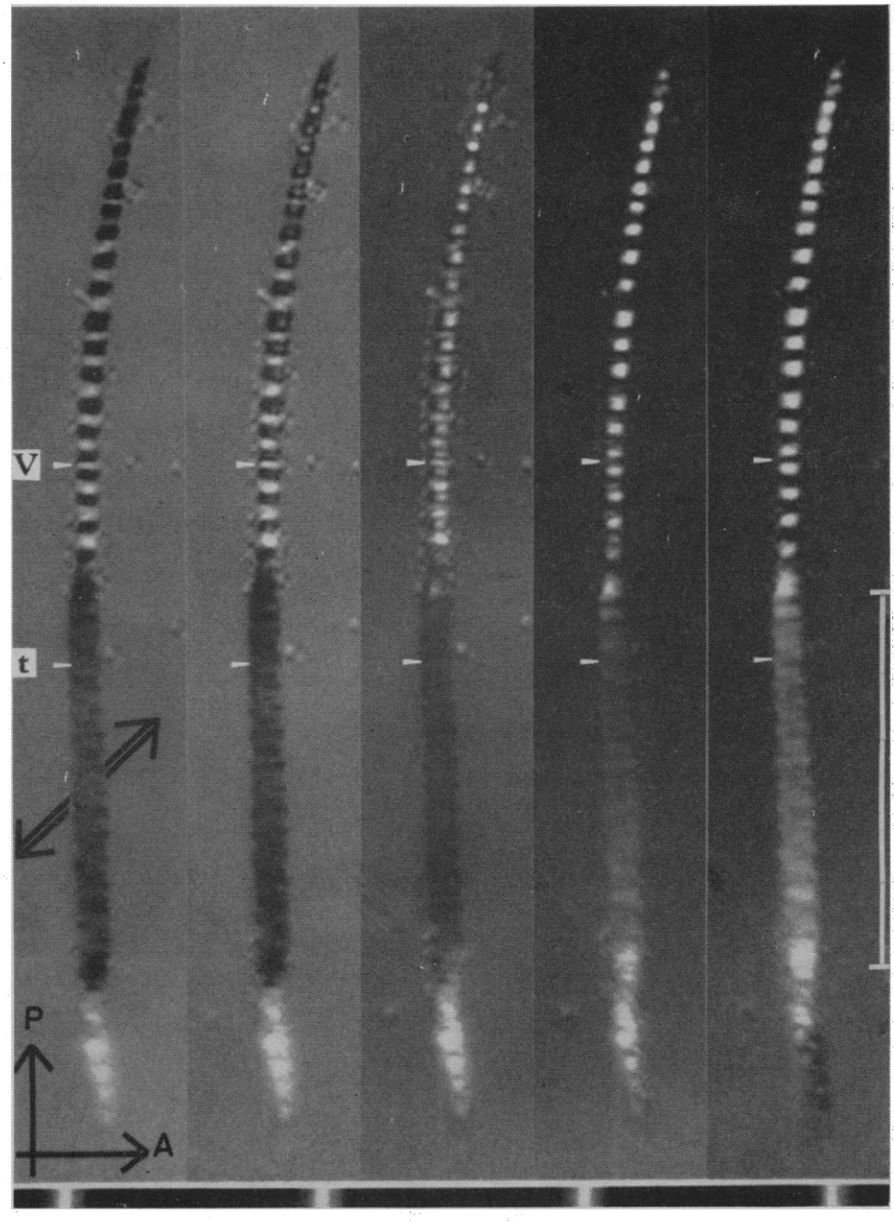

Fig. 1. Sperm head of the cave cricket (*Ceuthophilus nigricans*) at various compensations as viewed through the rectified polarizing microscope. The bracket to the right of the figure shows the region of the sperm exposed to polarized ultraviolet whose *E*-vector lies in the direction indicated by the double dark arrow. Scale interval, 10 μ.

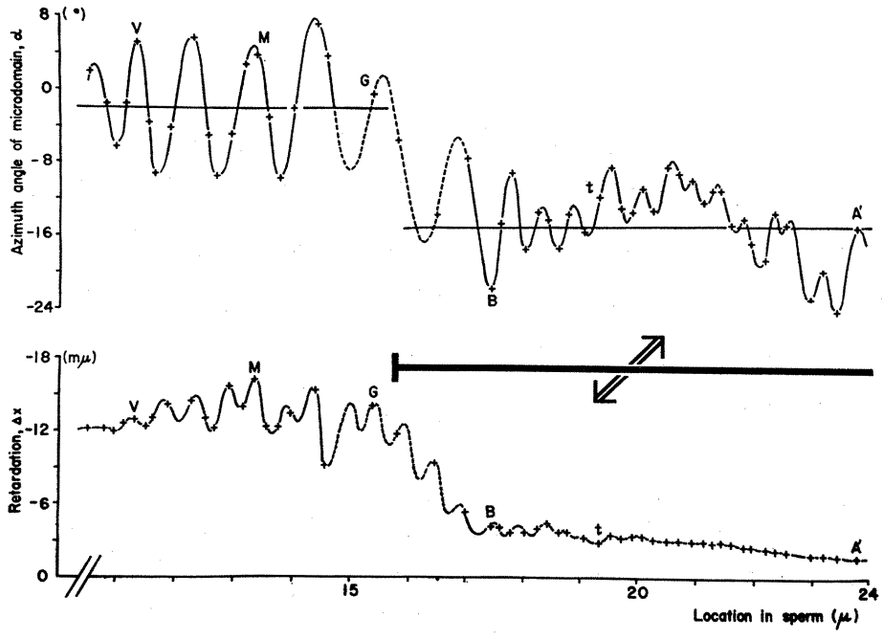

Fig. 2. The distribution of azimuth angles and retardations in microdomains measured along a 14-μ length of the cave cricket sperm head. The dark bar and the arrow define the area and the direction of the *E*-vector of the irradiating ultraviolet beam. Compare with Fig. 1 in which points *V* and *t* are indicated.

of the DNA molecules in the sperm of the cave cricket by measuring the change of optical property in each microdomain before and after irradiation with polarized ultraviolet light.

We therefore developed a method for measuring the retardation and azimuth orientation of each microdomain to a precision of 0.1° at each 0.3-μ^2 area in microscopic objects including intact living cells. Photographs of the specimen are first taken through the rectified polarizing microscope with the elliptical compensator set at various orientations. With the rectified microscope the diffraction pattern of weakly retarding objects is properly corrected (*8*). The densities of the specimen image and of the background are then measured with an automatic scanning microdensitometer. These measurements are used to determine the compensator angle giving extinction at each relevant point of the specimen. This process is repeated with the specimen oriented in several directions and from the total data the azimuth orientation and retardation of each point of the specimen are calculated.

Measurements by this method along the length of partially irradiated cave cricket sperm showed unexpected changes in the azimuth angles in the irradiated area (Fig. 2). The changes cannot be explained simply by assuming that the birefringence of each whole zigzag domain is differentially removed by polarized ultraviolet, but could be explained if the bases within the microdomains were again zigzagged or crisscrossed at a submicroscopic level. This indicates that there exist at least two orders of coiling or zigzagging of DNA (or their bases) in the sperm head, with the average molecular axis oriented parallel to the long axis of the head.

From the structure of DNA molecules derived from x-ray analysis (*2*) and the measurements provided by our experiments, we were led to postulate the following model for the packing arrangement of chromosomes and DNA in the cave cricket sperm head. The 20- to 30-A-thick DNA(-protein) molecules, presumably as a bundle several hundred angstroms in diameter, are wound to form a very long helix approximately 2000 A thick. This in turn is wound into another elongated helix approximately 8000 A in diameter, two such helices intertwined together with some matrix making up the sperm chromosomes. The microscopic zigzag domains would then reflect the gyres of the intertwined chromonemata while the overall optical property of the sperm head reflects the alignment of those portions of the DNA molecules which within the coiled coil run roughly parallel to the sperm head.

The coiled chromosomes are disposed lengthwise in the sperm head, arranged one after another. Work in progress strongly indicates that the chromosomes are actually arranged in a definite sequence.

The coiled coil model is consistent with certain classical cytological views of chromosome structure (*9*), but does not necessarily fit with current interpretations of sperm head structure based on electron microscopy (see, for example, *10*). Models consistent with the latter do not fit our observations on living sperm well, and it is possible that the electron microscope image of the sperm head suffers from artifacts of fixation. In any event, it now appears probable that a rather definitive choice of models can be made by further optical studies on living sperm heads (*11*).

SHINYA INOUÉ
HIDEMI SATO

Department of Cytology,
Dartmouth Medical School,
Hanover, New Hampshire

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11. This research was supported in part by NSF grant No. G19487 and USPHS grant No. CA 04552-04 awarded to one of us (S.I.). The procedure of our experiments, details of the observations, and the arguments are in preparation for presentation elsewhere.

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