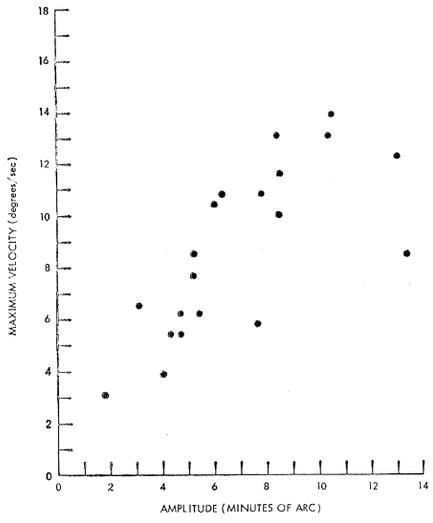

Fig. 2. Maximum velocity versus amplitude for microsaccades.

ment; variation of starting position and direction of movement; for movements between visible fixation points or in total darkness with conditioned eye movements. He further found that average velocities of secondary saccadic corrections fell on the same curve obtained for the types of movements described above. The range of amplitudes used in his study was from 1 to 35 degrees.

The data of Gurevich indicate that a single physiological system is responsible for a wide variety of saccadic eye movements. In an attempt to determine whether microsaccades (flicks), the small (1 to 30 minutes of arc) involuntary saccades observed during fixation, are the output of this same system, we have studied the maximum velocity of such movements.

The subject viewed a grid composed of three vertical wires and one horizontal wire (0.13 mm in diameter) superimposed on a circular, 4-degree, transilluminated field at optical infinity. The three vertical wires were 1 degree apart and were used for position calibration. The intersection of the central vertical wire and the horizontal wire served as a fixation cross. Viewing was monocular with the left eye, the right eye being occluded. Eye position was monitored by the previously described method (*5*) of differential reflection of infrared light from the iris and sclera. Only horizontal movements were recorded. The signal proportional to eye position was recorded on one channel of a recorder (Sanborn, model 320). This signal was also electronically differentiated, and the derivative was recorded on the other channel of the recorder. Further amplification (by a factor of about 5) was provided by recording signals proportional to the pen positions on the eye-position and velocity channels on a second recorder (Visicorder, model 1508). Thus, two recorders were used, the first being used primarily to keep both signals on scale and to provide an immediate check on the linearity of calibrations. Records from the second instrument were used in all analyses.

Calibration of the velocity channel was accomplished by recording a triangular wave on the eye-position channel and its derivative on the velocity channel. All recorder gains and calibrations were unchanged for this procedure. Thus, given the amplitude of the triangular wave on the eye-position channel and the frequency of the wave, a velocity in degrees per second could be related to a given deflection on the velocity channel. Such calibrations were made for at least three frequencies within the range of velocities observed in the experiment.

Figure 1 shows some typical microsaccades and their velocity traces. These are two movements of roughly the same size, one with a great deal more overshoot than the other. Note that the overshoot is proportionately much greater than that normally seen with larger saccades. Figure 2 is a plot of maximum velocity in degrees per second (ordinate) as a function of amplitudes in minutes of arc (abscissa). It is clear that velocity is an increasing function of amplitude for these movements.

In Fig. 3 the data from Fig. 2 are replotted, and data points from larger voluntary saccades and secondary corrective saccades are added. The latter data, which are in agreement with those of Westheimer (*1*) and Hyde (*2*), were obtained in the same manner as those for the microsaccades, except that, of course, the stimulus conditions were different. The points are plotted on logarithmic scales because of the large ranges involved. A smooth, continuous curve through all data points is clearly justified, indicating, indeed, that microsaccades, voluntary saccades, and involuntary corrective saccades are produced by the same physiological system, or that a single motor element serves to limit the dynamics of all three types of saccades. Since the data in Fig. 3 are plotted on logarithmic coordinates, the curvilinearity of the velocity-amplitude relationship is deemphasized, because of scale compression. It is still clear, however, that saturation of peak velocity sets in at high amplitudes.

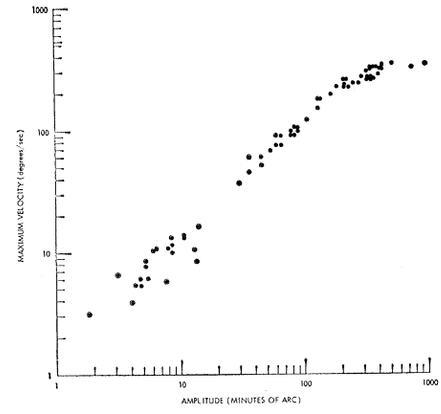

Fig. 3. Maximum velocity versus amplitude for microsaccades, involuntary corrective saccades, and voluntary saccades.

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5 August 1965

Mitosis: Induction by Cultures of Human Peripheral Lymphocytes

Abstract. *Ribosomal RNA extracted from peripheral lymphocytes, which had been recently stimulated by specific antigens to which the donor was sensitized, is capable of promoting transformation and mitosis when added to cultures of autologous unstimulated lymphocytes.*

In cultures of peripheral lymphocytes containing a specific antigen to which the donor has been sensitized, a variable proportion of the cells undergoes transformation to large lymphocytes and blast cells, some of

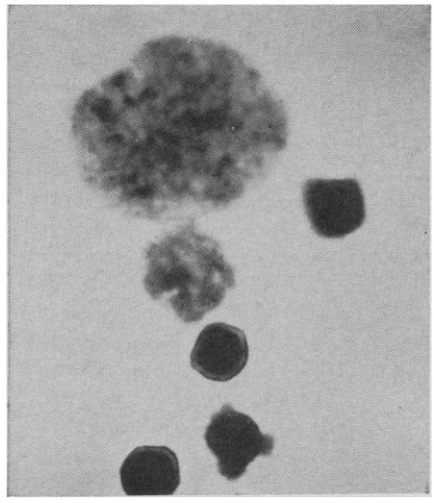

Fig. 1. Transforming cell and satellites.

which then undergo mitosis (see *1*).

Such cell transformation and mitosis have been induced by bacterial antigens (*2*), by viral antigens (*3*), by drugs (*4*), by tissues such as skin (*5*), and by cells and cell fractions of homologous lymphocytes (*6, 7*). It is believed that a specific antigen-antibody reaction within the growing cells is responsible for these changes, which differ from the nonspecific stimulation brought about by phytohemagglutinin because clumping of the transforming cells, which occurs before mitosis with phytohemagglutinin, is not observed; occasionally a cytoplasmic bridge of morphologic continuity can be discerned between a transforming cell and one or two satellite small lymphoctyes (Fig. 1).

We now report on an attempt to identify the cell component that gives rise to this phenomenon. We have obtained evidence that the phenomenon is probably mediated by the ribosomes. It may be produced by ribonucleic acid extracted from the cells, and it can be abolished by ribonuclease.

Peripheral lymphocytes from four normal individuals of known immune status were cultured and studied for mitosis and cell transformation by the technique described previously (*5*). Tetanus toxoid (0.05 ml), tuberculin (0.005 mg of purified protein derivative), poliovirus (0.05 ml of Sabin triple vaccine) and typhoid endotoxin (10 μg) were added to separate portions of cultures each containing 6 to 8 million lymphocytes in 8 ml of a culture medium consisting of 15 percent fetal calf serum in Eagle's medium 199 to which penicillin and streptomycin had been added. Cultures were incubated for 4 to 24 hours, then centrifuged at 800 rev/min for 10 minutes. The supernatant was discarded, and the cells were washed twice in medium 199 and centrifuged again. The cellular sediment was divided into two equal parts. One part was added to a fresh culture of lymphocytes from the same individual, comprising 4 million lymphocytes suspended in 8 ml of medium 199. This was incubated for 5 days, the result being positive for mitosis and cell transformation.

The second part of the sediment—consisting of washed, presumably sensitized lymphocytes—was used for the extraction of RNA with 3 ml of Perry's acetate buffer (*8*) at *p*H 5.0 containing 0.5 percent sodium dodecyl sulfate and magnesium chloride. The resulting suspension was extracted three times with water-saturated phenol; the phenol layer was discarded. The aqueous phase and the redissolved precipitate at the water-phenol interface were tested separately for ability to induce mitosis and cell transformation in a culture of fresh autologous lymphocytes; only the material from the interface layer stimulated mitosis. The material from the interface layer was dialyzed against Perry's solution, without sodium dodecyl sulfate but containing 5 percent sucrose. After dialysis for 22 hours it was layered on 4 ml of a sucrose gradient (5 to 20 percent) in lusteroid tubes and centrifuged in a model L Spino ultracentrifuge for 10 hours at 35,000 rev/min. Five fractions were collected by puncturing the bottom of the gradient tubes. These were dialyzed against medium 199 and added to separate portions of autologous lymphocytes (4 million cells in 8 ml); they were then cultured and examined for mitosis and cell transformation.

The bottom fractions I and II of lymphocytic RNA persistently stimulated mitosis. These two fractions were treated with ribonuclease (0.04 μg/ml RNA extract) for 1.5 hours at 37°C. They were then dialyzed, in a stretched cellophane membrane, against saline

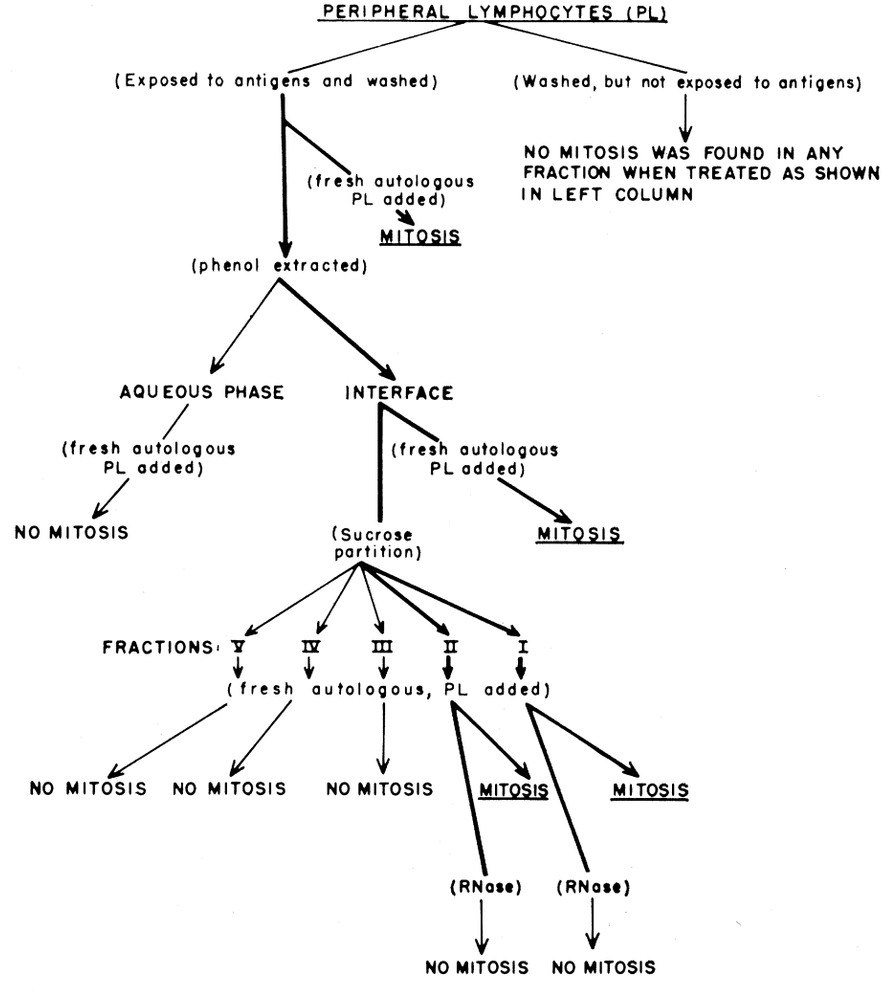

Fig. 2. Mitosis (and cell transformation) induced by RNA extracted from sensitized cells.

with constant mixing and changing of the saline. On incubation no mitosis-stimulating activity was found.

In a control study RNA was extracted in the same manner from lymphocytes (from the same donor) which had not been incubated with the various specific antigens. When this was added to fresh autologous lymphocyte cultures no fraction showed mitotic activity.

Further controls consisted of lymphocyte cultures from the same donor; to one set nothing was added, and these cultures gave uniformly negative results. On the other hand, controls to which phytohemagglutinin was added were uniformly positive.

Each culture was scored for cell transformation and mitosis from counts of 1000 cells. The result was considered positive when cell counts indicated more than 3 percent transformation and over 0.1 percent mitosis (Fig. 2). Only the lymphocytes, which had been challenged with specific antigens to which the donor was presumably sensitized, were capable of stimulating autologous unchallenged lymphocytes to transform and divide. The same mitosis-stimulating and transforming activity was obtained with the total RNA extracted from the stimulated lymphocytes, the interface RNA, and fractions I and II of the sucrose gradient. The activity thus appeared to reside in the heavy, ribosomal RNA fractions. The fact that the same RNA fractions from unstimulated lymphocytes did not stimulate mitosis suggests the possibility that the active ribosomal fractions may have formed complexes with, or somehow have been altered by, the challenging antigen.

It remains to be demonstrated, however, whether the ribosomal RNA which is responsible for the transforming and mitosis-stimulating activity resides in that portion of the ribosomes which are rapidly labeled as opposed to the heavier ribosomes which are not rapidly labeled in growing cultures.

It is not known yet whether cytoplasmic connections occasionally seen (Fig. 1) between transforming cells and small lymphocytes form a bridge of functional continuity caused by cytochemotaxis to allow for transport of particular material between an immunologically competent small lymphocyte and a newly informed transforming cell.

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Diapause Induction in Daphnia Requires Two Stimuli

Abstract. *Short-day photoperiods in conjunction with a second stimulus were required to induce sexual reproduction (and diapause) in an autumnally diapausing strain of* Daphnia pulex. *The second stimulus was associated with the unalkalized portion of a small lake and with density of the population in laboratory cultures. Diapause was induced at 12° and 19°C.*

The cladoceran *Daphnia* alternates parthenogenetic with sexual reproduction in a variety of seasonal cycles. Development of the sexually derived embryo is arrested in an early stage, and the arrest is considered a true diapause (*1*). The appearance of sexual reproduction has been explained on the basis of either environmental or intrinsic regulation. In the latter explanation, which arose apparently from studies at a time that coincided with the season of sexual reproduction (*2*), it was assumed that only certain generations and broods were capable of sexual reproduction. The temperature-independent and synchronous initiation of sexual reproduction (and diapause) at different altitudes (*3*) and the simultaneous appearance in lake population and laboratory culture (*4*) were offered in support of intrinsic regulation. Those cases for environmental control were accounted for by the proposal that a period of lability precedes obligatory sexual reproduction. There are ample arguments opposing intrinsic control in the early discussions of reproduction in *Daphnia* (*5*).

Environmental stimuli associated with the reversal from parthenogenesis include density of culture, evaporation of habitat, starvation, low temperature, diet, and metabolic depressants (*6*, *7*). The possibility of photoperiod influence was overlooked, and, without such a possibility, many studies seem not to distinguish regulation in populations which reproduce sexually (initiate diapause) only in autumn from regulation in those which repeat in spring and autumn.

Fortuitous circumstances permit a new hypothesis consistent with the results of earlier studies. In the unlimed (Paul Lake) part of a recently separated lake (Fig. 1), the population of *D. pulex* (and *D. rosea*) initiate sexual reproduction (diapause) in autumn only with considerable precision. No evidence for other than parthenogenesis (nondiapause) was discovered in the other portion (Peter Lake), to which hydrated lime had been added. To account for the precision of onset and the restriction of sexual reproduction to unlimed Paul Lake, the hypothesis proposes a need for two stimuli, one of which could be photoperiod.

The hypothesis was tested with cultures brought into the laboratory and maintained on cell suspensions of *Chlamydomonas reinhardi* Dangeard, Indiana University strain 89, in lake water or synthetic substitute. In the tests of photocontrol, groups of five young *Daphnia* were reared throughout life in glass vials (25 by 95 mm) containing 20 ml of medium. At 2-day intervals the number of released parthenogenetic or sexual (ephippial) broods was counted, and the adults were transferred to fresh medium in which was suspended 1.5×10^5 cells per milliliter. Cycles of light and dark (LD) were regulated by clock-controlled switches; light intensity of approximately 1350 lux was generated by "daylight" fluorescent tubes.

Both populations responded to photoperiod in the laboratory. In the Paul Lake stock, which was studied in sufficient detail, the transitional reversal from parthenogenesis to sexuality was promoted by LD 13.5:10.5 when cultures were maintained at 19°C (Fig.