senger RNA, sensitive techniques (*14*) have failed to demonstrate increased ribonuclease activity in metaphase cells.

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Blood Flow in the Microvasculature of the Conjunctiva of Man

Abstract. *Cinephotomicrography of capillary blood flow in conjunctival vessels of man were carried out at 116 pictures per second. Red cells did not move in streamlines but rather in a heterogenous mixing type of flow. Flow in vessels of patients whose red cells were grossly aggregated was very slow and often stagnant.*

Ever since the observations of blood flow in the capillary vessels of small animals by Poiseuille and Krogh, attempts have been made to examine these same phenomena in man. An external, transparent vascular membrane is not available in man without surgical procedure, hence the microscopy of capillary blood flow must be studied by reflected light from normally exposed surface vessels such as those in the conjunctiva of the eye, or through translucent tissues such as finger webs or nail beds. In addition to problems of resolution in these areas, observations are also difficult because of the serious artifacts of heat stress or injury when the necessarily intense light is focused on the vascular tissue under examination. Cinephotomicrography is also complicated by the fact that filming rates must be increased by a factor equal to the image magnification by the lens system in order to retain time resolution.

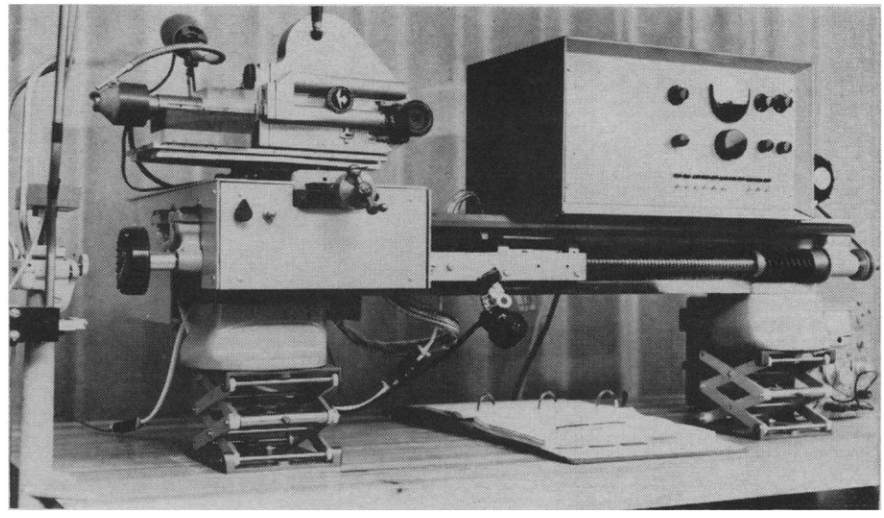

Fig. 1. Camera, lens system, and power source mounted on lathe bed. Headrest at left.

Improved techniques of pulsed high-intensity (stroboscopic) lighting, light manipulation by fiber optic bundles, and increased film speed have been utilized to overcome some of these difficulties in order that observations of blood flow in the microvasculature of man might be recorded without alteration or influence of the subject. With a modified 16-mm camera and a long-working-distance (14 mm) objective lens, mounted 180 mm from the film plane, a magnification of 20 times the true image size was obtained on film with resolution of 2 μ. Stroboscopic light synchronized with the camera was conducted from a xenon flash tube to the tissue by way of fiber optic bundles (diameter, 9.5 cm; length, 0.6 m) attached to the lens mounting. The entire unit was constructed on a heavy, 1.5-m lathe bed (Fig. 1). A precision loaded lead screw was used as the focusing mechanism. The subject was comfortably seated in front of the apparatus with his head resting in a head-and-chin rest and his gaze fixed on a small point of light off to the side at 45° angle. The mass of the instrument and the table precluded its use in the prostrate patient, but the machine could be brought to the bedside for photography of a patient in the sitting position. Filming runs lasted 4 to 5 seconds at rates of 116 pictures per second. Film type was high-speed fine-grain reversal, ASA 160 daylight. The procedure was innocuous and no untoward reactions to the brief stimulus of light were noted.

Film studies of blood flow in vessels of diameters from 10 μ (capillaries) up to 200 μ (arterioles and venules) in normal man (20 studies of seven subjects) revealed a continuous heterogenous mixing type flow rather than streamline flow (Figs. 2 and 3). Erythrocyte aggregation was commonly observed in vessels of 50-μ diameter and less, while flow within capillaries often

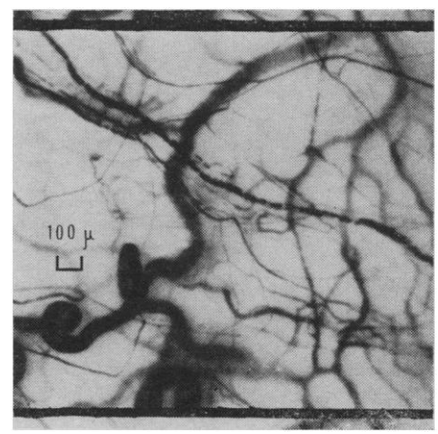

Fig. 2. Bulbar conjunctival vessels of man. Magnification on original 16-mm film was × 4.

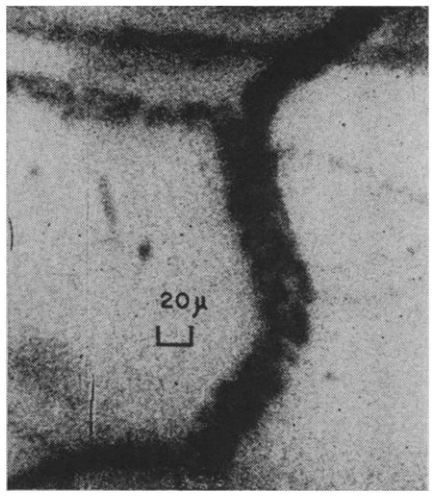

Fig. 3. Venule of bulbar conjunctiva of man. Magnification on original 16-mm film was × 20.

revealed large gaps of plasma between orderly files of erythrocytes. Similar studies in 13 patients, chosen because of profound erythrocyte aggregation of their blood in vitro (five patients with myeloma, five patients with severe coronary artery disease, and three patients with macroglobulinemia) revealed marked cell aggregation (clumps of 5 to 30 cells) in all vessels. Flow was considerably slower than in the normal, and in many vessels stasis was apparent. Vessel patterns demonstrated marked tortuosity and dilatation. These observations support the earlier recordings of conjunctival blood flow observed at lower magnifications (*1*). The observations of a cell-free layer of plasma at the wall, as noted in studies in vitro (*2*), were not confirmed by these studies in vivo. Red cells moved in a very heterogenous fashion without maintaining any uniform streamlines in both arterioles and venules.

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Culture of a Planktonic Calanoid Copepod through Multiple Generations

Abstract. Acartia tonsa *has been propagated through 12 filial generations during 1 year in small laboratory cultures. The mean generation time was 25 days at 17°C on a slightly suboptimum, mixed algal diet. Body size, reproductive capacity, and generation time were unchanged during the culture period.*

Copepods, particularly calanoid copepods, are the principal link between primary (photosynthetic) producers and carnivores in the sea. Their biology has been studied in the laboratory for several decades with the use of animals taken directly from nature. However, many aspects of their biology need to be studied under a degree of experimental control possible only with material of known uniform age and physiological status. For such studies substantial numbers of copepods must be raised in the laboratory under defined conditions.

The few instances where marine copepods have been bred and reared in the laboratory have involved noncalanoid species (*1*) and, in one instance, a nonplanktonic calanoid (*2*). Holoplanktonic marine calanoids seem to have been completely refractory to laboratory propagation for over 50 years, although several species, when brought into the laboratory after having mated in nature, will produce eggs that can be raised to the adult stage (*3*). Subsequent generations apparently have not been obtained in such cases.

From October 1963 to March 1964, starting with adults taken from the Chesapeake Bay, we successfully raised limited numbers of five consecutive generations of the holoplanktonic calanoid *Acartia tonsa* Dana (*4*). The animals were lost after an air-conditioning failure in our culture room, but we have now reestablished a culture of the same species from adults taken off the Virginia coast in late 1964. This new line has thus far been propagated into the 12th filial generation and continues to exhibit a vigorous and normal-appearing reproductive potential. Descriptions of our technique and the growth characteristics of the first ten generations are given in this report.

Stock animals are maintained and most experiments are carried out unaerated in various-sized pyrex crystallizing dishes covered with a flat piece of glass. Dishes that are 190 mm in diameter and contain 1500 ml of culture allow better survival than smaller dishes do, even when each size contains the same number of copepods per unit volume (*5*). Membrane-filtered sea water (salinity about 31 per mille) is used unsupplemented and usually unbuffered. We have buffered certain experiments with tris [tris (hydroxymethyl) amino methane] at 500 mg per liter brought to the *p*H of sea water with HCl. This system has no effect on the survival of nauplii or adults, and continued exposure likewise has no effect on hatching and maturation. Other systems and higher tris concentrations were not tested. All stocks and those experiments in which temperature and illumination are not variables are kept in a constant-temperature room at 17° ± 1°C under constant illumination in the range of 650 to 1300 lux (*6*).

Food organisms are added from pure cultures of various flagellates and diatoms at the time of each transfer to fresh medium and thereafter at 3-day intervals. Since the F_2 generation we have used a mixture of approximately equal parts of *Isochrysis galbana, Rhodomonas* sp., and an unidentified small diatom (5 to 6 μ). About 10,000 (total) cells per milliliter of copepod culture are added at the initial feeding and about 4000 cells per milliliter at each subsequent feeding. This allows a concentration of about 10,000 to 38,000 cells per milliliter to be maintained in the culture dishes at all times. The same feeding regimen is used for nauplii, copepodites, and adults.

Stock nauplii are not ordinarily removed from the parental dish until nauplius stage III or IV is reached. Offspring are then transferred into fresh medium to a final concentration of about 40 nauplii per liter. After producing-females are obtained, the concentration is further reduced to no more than five such animals in a 1500-ml culture in order to restrict production in any given dish and thereby prevent excessive concentrations of nauplii (*7*). Transfers are made with pipettes that have apertures of 1 to 3 mm in diameter, the size depending on the developmental stage of the animals involved. The animals are released very slowly beneath the surface of the medium. Great care is necessary in handling these animals, and significant differences in survival have been found when identical cultures were transferred