

leukocytes, while in our own work with the lymph we have studied the alternations in permeability. In the present series of observations we have studied the changes in the blood sugar during malarial fever (artificial inoculation) with particular reference to the crisis.

In the fasting patient we have determined that the period of the chill and rise in temperature is associated with a decrease in the blood sugar level. This corresponds to the time of a peripheral leukopenia and a diminished concentration of blood protein. With the crisis and fall in temperature, blood sugar increases, leukocytes increase in the peripheral blood stream and the blood proteins increase. These pictures can be regarded as a result of a reversal of the autonomic balance. In the first stage we have a peripheral sympathetic tonus with a splanchnic parasympathetic orientation; in the period of defervescence, a peripheral parasympathetic and splanchnic sympathetic tonus. We cannot, of course, exclude the effect of metabolic utilization of the sugar. We believe, however, that the magnitude of the sugar mobilization during the period of defervescence would indicate a splanchnic sympathetic effect.

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The culture of planarian tissues *in vitro*.

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This preliminary paper is devoted to a description of the technique and some suggestive results of the culture *in vitro* of the tissues of the flatworm, *Planaria dorotocephala*. Practical sterilization of the material has been accomplished by exposing the worms, in a small amount of water, to ultra-violet radiation from a quartz mercury arc lamp at a distance of 35 cm. during 4 minutes. After such an exposure the bacteria associated with the worms are practically all killed or prevented from reproducing, without injury to the worms. The hanging-drop, as well as the petri-dish technique were used for the tissue cultures.

Buffer solutions composed of the common physiological salts,

in order to be tolerated by the Planarian explants, must be between $1/8$ and $1/25$ the concentration of chick serum. The optimum concentration of Planaria is $1/10$ to $1/12$ the chicken concentration. Intact worms, however, tolerate indefinitely all concentrations between well-water and buffer solutions containing from $1/4$ to $1/5$ the salt-content of vertebrate-isotonic solutions. The organ of osmotic regulation seems to be the external epithelium of the worm.

In hanging-drop cultures the cells survive in fluid media for as long as 10 to 15 days; they are pseudopodially active, and show some cell division, but not active, sheet-like proliferation. When support and tension are afforded to the explant by the addition of a drop of agar, a conspicuous sheet-like outgrowth of parenchyma may be seen within 12 hours. The cells will migrate along fine silk strands, but do not spread in sheets. Under certain conditions, cells in petri dish cultures may survive as long as 64 days.

Serum or tissue extracts of the following animals have been introduced into the culture media: tapeworm, clam, snail, isopod, frog, sheep, without injurious effect upon the explant. Sheep serum is stimulating. The artificial media used are buffer solutions to which are added a dextrose, dextrose and peptone, glycogen, leucine, arginine and tyrosine, respectively. Dextrose or glycogen prolongs the life of the cells considerably beyond their time in a buffer solution alone. The mixture of dextrose and peptone perceptibly stimulates them to migration and division. The amino-acids used are unfavorable, leucine least so.

The various types of cells have been identified in culture, and their behavior studied.

The size of a typical explant is about $1/120$, or less, the volume of a whole worm. When such pieces are introduced into hanging drop conditions, a variable percentage of them may fail to spread out amorphously in the form of tissue cultures, and may round up and form individuals, some of them obviously polar, and some of them such that no polarity is discernible. The polar individuals are the more viable. One apolar individual is recorded to have changed its behavior to that of the polar type; many times, the polar type has been observed to disintegrate rather suddenly, and one or more of the apolar type to form subsequently from the debris. Apolar individuals may form originally from fragments of the explant.