Direct Measurement of Leukocyte Motility: Effects of pH and Temperature[1] (35894)

G. G. NAHAS, M. L. TANNIERES, AND J. F. LENNON

Columbia University College of Physicians and Surgeons, New York, New York 10032; and Institut de Pathologie Cellulaire, Hôpital Bicêtre, 94-Le Kremlin-Bicêtre, France

Because of their random nature, rates of locomotion of polymorphonuclear neutrophils (PMN) can only be measured by direct, visual techniques that will take into account the multidirectional pathway which they follow. Few such studies have been performed, and data relating the effects of temperature and pH to the rate of locomotion of PMN observed by direct, visual techniques are incomplete and were performed several decades ago with elementary methods (1–3). As a result, Q_{10} values available for this biological process are fragmentary (4).

The purpose of the present study was to obtain more extensive data on the subject with up-to-date techniques to determine to what extent temperature and pH affect, over a wide range, the rate of locomotion of PMN. Such measurements could also be compared with those performed by the indirect technique using the migration of leukocytes through Millipore filters (5).

Methods. A special system (Fig. 1) was designed to study PMN under conditions of a constant microenvironment. This system comprises two parts: a series of equilibrating tonometers (Eschweiler) and a microobservation chamber in communication with the latter. The equilibrating tonometers (volume: 15 ml) are immersed in a constant temperature bath. They are connected through humidifiers to gas mixtures of known concentrations of O_2, N_2 and CO_2. The observation chamber is made of a glass slide and a cover slip sealed with resin and connected with two polyethylene tubes (0.5-mm i.d.). One of the tubes is connected to the equilibrating tonometer, the other to a syringe. The system allows for an anaerobic filling of the observation chamber, after the blood cells have been equilibrated in the tonometer. The observation chamber is placed under a phase contrast microscope. The objective of the microscope is fitted with a device (6) which maintains constant the temperature of the preparation under microscopic observation. This device consists of a metal coil placed around the objective of the microscope. Temperature can be rapidly modified from 0 to 50° by circulating through the coil warm water or supercooled alcohol. The objective is isolated from the microscope by an insulating ring; changes in temperature are only transmitted to the center of the glass observation chamber. Temperatures could be continuously recorded by means of a thermocouple placed next to the cover slip. The image of the preparation under the microscope was transmitted to a television screen, according to the technique described by Bessis (7).

PMN from healthy human donors were sampled according to conventional techniques and equilibrated in the tonometers. The pO_2 of the equilibrating gases was maintained at 150 mm Hg. The pCO_2 was changed from 15 to 150 mm Hg. The pH of the plasma in which the PMN were suspended was varied over the range of 5.0 to 8.1 either by changing pCO_2 or by addition of 0.15 *M* lactic acid or 0.3 *M* tris(hydroxymethyl)aminomethane (THAM).

After equilibration, the PMN suspension was transferred into the observation chamber. The outline of the PMN was drawn on the television screen with a soft lead pencil every 10 sec for 90 sec. Subsequently, a

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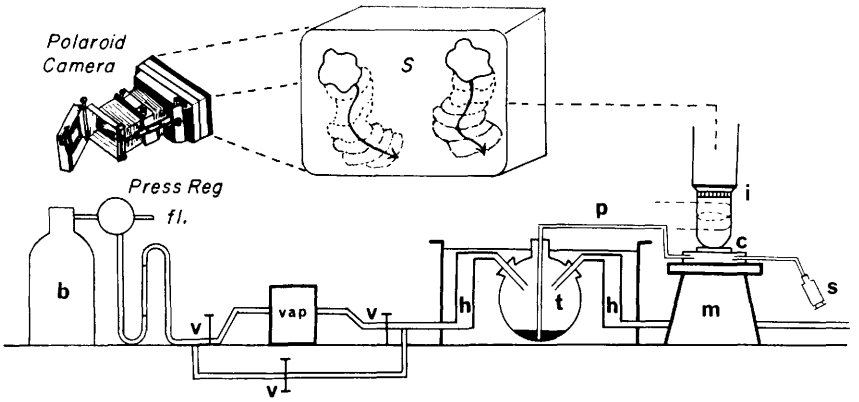

FIG. 1. System for the direct study of blood cells under controlled microenvironment. This system comprises: a tank (b) containing known concentration of equilibrating gases with pressure reducer (Press Reg) and flowmeter (fl); a calibrated vaporizer for each of the anesthetic vapors utilized; a series of valves (v) for bypassing or using the vaporizer; a thermostated bath with tonometer (t) and humidifiers (h); a microscopic air-sealed observation chamber connected to the tonometer by means of a polyethylene tube (p) and to a syringe (s) for filling; a phase contrast microscope (m) and an objective which is thermally controlled and isolated from the microscope by means of an insulating ring; a television screen (S) for observing the blood cells; a Polaroid camera which can be triggered at regular intervals.

camera was placed in front of the TV screen and successive pictures were taken at 10-sec intervals on the same film. The exact pathway followed by the PMN could be accurately measured (μ/sec). Eight to 20 measurements were made at each temperature or pH studied.

Results. The rate of PMN locomotion in a medium of pH 7.4 as a function of temperature is represented in Fig. 2. At 6° the PMN were immobile; the maximum velocity was observed at 42°. At higher temperature a fragmentation of the PMN occurred with rapid loss of motility.

The rate of PMN locomotion as a function of pH presents a bimodal distribution which is observed when temperature increases above 23°. The inhibitory effect of alkaline pH beyond 7.6 contrasts with the lack of effect of acid pH until 6.5 (Fig. 3).

Discussion. Rate of locomotion of PMN is markedly affected by temperature, especially between 23 and 42°. This rise in temperature is associated with a ninefold increase in velocity. The maximum velocity observed for human PMN (0.94 μ/sec $\pm$ 0.006) was at pH 7.43 and 42°. This value is at variance with that reported by McCutcheon (2), who observed a maximum velocity of 0.65 μ/sec $\pm$ 0.002 at 40°, with a sharp drop thereafter. But McCutcheon did not control pH. These velocities are also three times greater than those which can be calculated from the data of Bryant *et al.* (5), who measured PMN migration over 1 to 4 hr. These authors used an indirect method which did not allow for

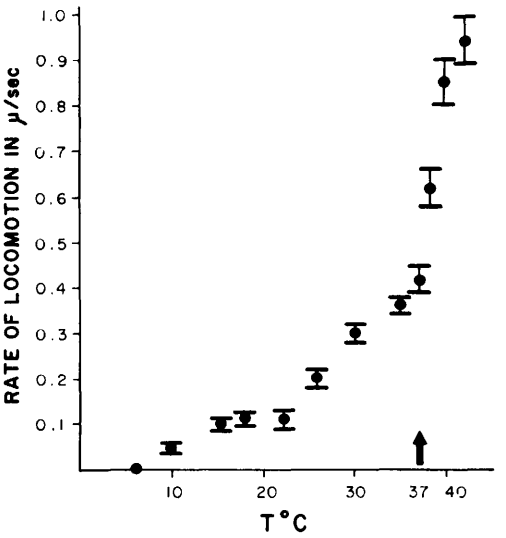

FIG. 2. Rate of locomotion of human polymorphonuclear neutrophils in function of temperature (pH 7.43).

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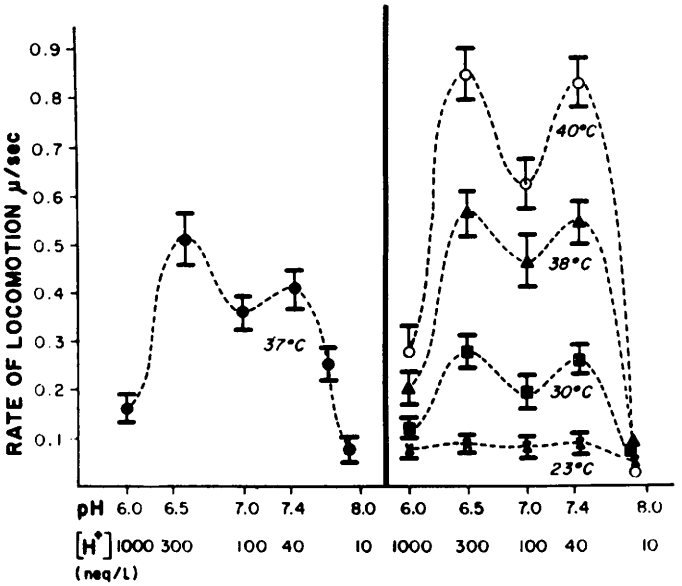

FIG. 3. Rate of locomotion of human polymorphonuclear neutrophils in function of pH and temperature.

the measurement of the total pathway travelled by the PMN and which was not sensitive enough to indicate the interactions of temperature and pH on the rate of locomotion of PMN. The Q_{10} calculated from the present data was 3.1 between 12 and 22°, 4.0 between 22 and 32°, 5.2 between 32 and 42°. These are higher than those calculated from data reported by early workers (4).

The rate of PMN locomotion as a function of pH (Fig. 3) presents a bimodal distribution similar to that reported for amoeba Proteus (8) with peak velocities at pH 6.5 and 7.4. Such a bimodal distribution was observed at four different temperatures. Changes of pH towards acidity over a significant range from pH 7.4 to 6.5 does not alter or even increase rate of locomotion. By contrast, alkaline pH beyond 7.6 produces a significant decrease in rate of locomotion which is completely and irreversibly inhibited at pH 7.9 at all temperatures. This contrast is more apparent when $[H^+]$ concentration is used in nEq/liter, instead of pH. Rate of PMN locomotion is not greatly affected when $[H^+]$ changes from 40 nEq/liter (pH 7.4) to 320 nEq/liter (pH 6.5). However, it is totally inhibited when $[H^+]$ decreases from 32 nEq/liter (pH 7.5) to 10 nEq/liter (pH 8.0). When blood is collected in open tubes with heparin, CO_2 escapes and pH of the plasma tends to become alkaline (pH 7.5–7.7), a factor which will decrease rate of PMN motility.

Summary. Rate of locomotion of human PMN is markedly enhanced by temperature between 32 and 42° ($Q_{10} = 5.2$). This rate is not significantly altered between pH 6.5 and 7.5. Beyond pH 7.6 there is a significant and rapid inhibition of motility.

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