(acido acetico = p. eboll. 118,1°C). Ciò può costituire un inconveniente notevole quando si lavori con sostanze stabili solo in ristretti limiti di acidità.

Allo scopo di eliminare questo inconveniente abbiamo studiato un nuovo tampone volatile capace di mantenere inalterato il pH durante la concentrazione. Risponde a tale scopo l'acetato di etilendiamina. L'etilendiamina bolle a 118°C, ha cioè un punto di ebollizione molto prossimo a quello dell'acido acetico (118,1°C), il tampone può pertanto venir concentrato sotto vuoto a varie temperature senza variare sensibilmente il suo pH. Questo tampone è ormai da più di un anno in uso con buoni risultati nel nostro laboratorio.

Oltre a questa favorevole caratteristica l'acetato di etilendiamina: 1) può essere eliminato per liofilizzazione secondo STEIN e MOORE[2], 2) a differenza dei sali di piridina non interferisce nelle determinazioni della densità ottica allo ultravioletto, 3) permette lo svolgersi di numerose reazioni chimiche senza interferirvi, 4) a differenza dei sali di collidina che subiscono l'ossidazione anodica è relativamente stabile alla ossidazione, 5) può essere applicato alla cromatografia degli aminoacidi in quanto non interferisce nella reazione alla ninidrina secondo MOORE e STEIN[7] quando si raccolgano dalla colonna aliquote di 0,1–0,5 ml nelle quali la concentrazione della amina sia pari o inferiore al 0,05 *M*.

Il diagramma indica come si debbano miscelare soluzioni 0,05 *M* di etilendiamina ed acido acetico per ottenere liquidi aventi una determinata acidità (pH a 20°C). La molarità dei tamponi così ottenuti è particolarmente adatta per l'elettroforesi su colonna di cellulosa.

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Summary

A volatile ethilendiamine-acetate buffer is described which is capable of keeping the pH unaltered during volatilization. The advantages of this buffer in electrophoretic and chromatographic procedures, in comparison with other volatile buffers, are discussed.

[7] S. MOORE e W. H. STEIN, J. biol. Chem. *176*, 367 (1948).

Motility of Leukocytes in Slide Cells

MARTIN *et al.*[1] found that the speed of guinea pig leukocytes migrating in closed slide cells is constant during the first 6 h of incubation and moves in a linear fashion with time. Similar curves of the migration of leukocytes were obtained by ELBERG and SCHNEIDER[2]. In the curves obtained by KETCHEL and FAVOUR[3] during the study of the motility of human leukocytes in sealed capillary tubes, leukocytes also migrated at a constant rate during the first 6–8 h of incubation.

In studying the motility of human leukocytes (POLÁKOVÁ[4], POLÁK and POLÁKOVÁ[5], POLÁK, POLÁKOVÁ, and ŠKVARIL[6]), our results were not in full agreement with those obtained by the above-mentioned authors. A more detailed analysis of leukocyte motility was therefore carried out.

Methods.—Leukocyte motility was studied in slide cells by a slightly modified method (MARTIN *et al.*[1]). The cells were prepared from silicone-coated standard microscope slides. The experiments were carried out on the blood of blood donors. The concentration of heparin (Novo) used in our experiments was 0·001 mg/cm³ of blood. After filling, the slide cells were sealed hermetically and centrifuged at 2200 rpm for 10 min, thereby separating the blood into erythrocytes, leukocytes and plasma layers. The slide cells were then examined directly under the microscope and those in which in isolated cases, leukocytes were found in the plasma layer, were excluded. The slide cells were then placed in incubators at an angle of 70°. During this procedure the blood and slide cells were kept at a temperature of +1 – +36°C.

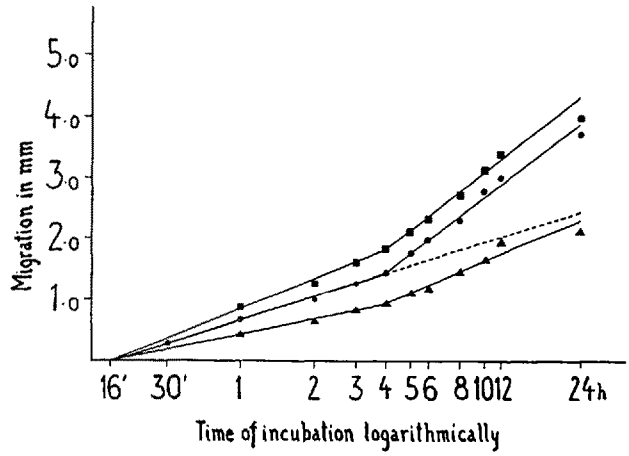

Fig. 1.—Migration curves at different temperatures. *x* time plotted logarithmically, *y* migration in mm. Upper curve —40°C, middle curve —37°C, lower curve 30°C. The points on the curve are arithmetic means.

The rate of the leukocyte migration was read under a microscope in the same slide cells at definite time intervals inside the thermostabile chamber.

Results.—The blood of 37 blood donors was examined. In parallel slide cells, filled with the blood of the same blood donor, the measurements of the rate of migration were carried out at intervals of 1, 2, 3, 4, 6, 8, 12, and 24 h at a temperature of 30, 37, and 40°C. For technical reasons, it was not possible to carry out the readings at all time intervals. In slide cells incubated at 37°C, migration was also read off after 30 min.

The statistical evaluation of the influence of both increased (40°C) and decreased (30°C) temperature on leukocyte motility was carried out in such a way that migration values at 37°C was taken as a basis, the differences were calculated and evaluated using *t*-test (CRAMÉR, 1946). The results are shown in the Table.

In Figure 1, where the migration was plotted semilogarithmically in time, the migration curves at different temperatures are shown. From these it follows that leukocyte motility increases at 40°C and decreases at 30°C as compared with the leukocyte motilities at 37°C.

[1] S. P. MARTIN, C. H. PIERCE, G. MIDDLEBROOK, and J. DUBOS, J. exp. Med. *91*, 381 (1950).

[2] S. S. ELBERG and P. SCHNEIDER, J. infect. Dis. *93*, 36 (1953).

[3] M. M. KETCHEL and C. B. FAVOUR, J. exp. Med. *101*, 647 (1955).

[4] K. POLÁKOVÁ, Cs. pediatrie *10*, 101 (1955).

[5] H. POLÁK and K. POLÁKOVÁ, Acta haemat. *16*, 385 (1956).

[6] H. POLÁK, K. POLÁKOVÁ, and ŠKVARIL, Cs. pediatrie *11*, 464 (1956).

Time in hours		½	1	2	3	4	5	6	8	10	12	24
37°	n	11	23	33	25	34	24	35	18	12	13	37
	M	314·1	698·0	1016·9	1305·3	1455·7	1740·8	1980·7	2348·0	2791·2	3073·8	3716·9
	σ	54·6	209·8	326·4	450·4	537·8	525·7	550·6	654·9	694·0	711·9	1010·8
Δ 30 – 37°	n		8	8	9	10	8	11	8	6	6	11
	Δ_1		267·5	390·0	417·0	548·5	595·2	759·3	841·1	1066·6	1107·0	1488·5
	$\sigma\Delta_1$		167·5	347·5	288·1	452·7	369·8	546·0	622·9	425·8	472·5	864·0
	t		4·2276	2·971	4·0932	3·6304	4·2577	4·3980	3·5725	5·6014	5·2381	5·4471
	P		0·01	0·05	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01
Δ 37–40°	n		22	32	24	32	23	33	17	11	12	34
	Δ_2		202·4	278·6	356·5	419·4	429·6	440·7	457·9	349·0	340·0	301·4
	$\sigma\Delta_2$		102·4	141·3	185·5	190·0	211·6	236·3	248·9	236·2	297·9	534·7
	t		4·5826	10·94	9·2136	12·296	9·5250	10·7947	7·3578	4·6724	3·7841	1·8600
	P		0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·1

It can be seen from Figure 1 that the migration proceeds in two straight lines intersecting at the 4th hour.

Both parts of the experimental curve can be expressed by exponential equations of the type $t = b \cdot e^{c \cdot \mu}$ expressing the law of growth. In the equation: t = time; μ = migration in μ; c, b = constants.

In our experiments migration starts between the 15th to 16th min (as seen by direct observation) which corresponds to the time necessary for the formation of a fibrin network on which the leukocytes move inside the slide cells. Up to the 4th hour the curve follows a straight line. For the calculation of this part of the curve (at a temperature of 37° C) values of 30 min and 4 h were used. The equation may be expressed:

$$\mu = \frac{\log t - \log b}{c \cdot \log e}.$$

The constants were calculated:

$b = 0{\cdot}256$ dependent on the time at which migration starts;

$c = 0{\cdot}00189$ dependent on the rate of migration.

Therefore $\mu = 1220 \cdot \log t - 721$.

At the 4th hour, the slope of the curve abruptly changes and becomes steeper. This second phase can be calculated similarly from the values obtained at 4 and 10 h with the resulting equation $\mu = 3250 \cdot \log t - 502{\cdot}5$.

The rate of migration (r) at individual time intervals was calculated as the function of time. For the first phase:

$$\frac{\Delta \mu}{\Delta t} = 1220 \cdot \frac{1}{t} \cdot \frac{1}{\ln 10}$$

which becomes after simplification:

$$r_1 = \frac{530}{t}$$

for the first phase,

$$r_2 = \frac{1412}{t}$$

for the second phase, which are hyperbolic equations.

The course of the migration rate in the different time intervals is shown in Figure 2. A striking feature of the curve is the sharp increase in the rate of migration at about the 4th hour of incubation, the nature of which we have tried to elucidate. We found that the sudden increase in the migration rate is not caused by a sudden change in pH, nor is it caused by a change in rH, in which the chief changes occur during the first 30 min.

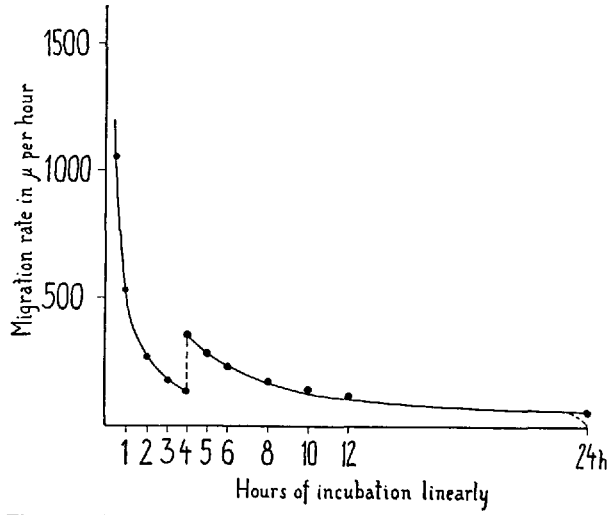

Fig. 2. – Migration rate at different time intervals. x time in hours, y rate of migration in μ/h.

It may be asked whether the increase in the migration rate at about the 4th hour is not due to metabolic or degeneration products of the leukocytes themselves and whether a similar process does not occur in tissues during inflammation.

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3rd Clinic for Internal Diseases, Charles University, Prague, April 11, 1957.

Zusammenfassung

Die Leukozytenbewegung wurde in Glaskammern während 24 h Inkubation beobachtet: die Kurve der Leukozytenbewegung besteht aus 2 Phasen, welche mittels 2 Exponentialgleichungen (durch das Wachstumsgesetz bestimmt) ausgedrückt werden können.

In der vierten Stunde der Inkubation tritt eine Steigerung der Leukozytenbewegung auf.

Die Leukozytenbewegung bei einer Temperatur von 30 und 40° C wurde mit der bei 37° C verglichen: Die Leukozytenbewegung ist bei 30° C langsamer und bei 40° C schneller als bei 37° C.