or 20 weeks of age, but other possibilities exist such as an effect of CY on the emergence of tumor cells. Present data are insufficient to allow for a conclusion.

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Heterogeneity in filterability of erythrocytes from malaria *(Plasmodium berghei)*-infected blood

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Summary. Erythrocytes from *Plasmodium berghei*-infected blood show a decrease in deformability with increasing parasitaemia, as measured by filterability through polycarbonate sieves. A major fraction of cells carrying mature parasites and a smaller fraction carrying ring-stage parasites account for the obstruction of filtration, while the remaining infected cells do not contribute to the decrease in filterability. The relation of filterability to metabolic status in infected cells is discussed.

The deformability of erythrocytes plays an important role in their passage through capillaries and associated processes. Erythrocytes of reduced deformability may not be capable of passage through restricted areas of circulation such as those in the spleen, and are thereby removed from the circulation[2,3]. In some malaria infections[4-6], such as those resulting from *Plasmodium knowlesi, Plasmodium coatneyi* and *Plasmodium yoelii* YM, it is believed that reduced deformability could lead to capillary obstruction in the brain contributing to cerebral complications. Apart from cerebral complications, other lesions in malaria which involve altered rheologic properties of the erythrocytes include renal failure and liver necrosis[4,5]. Filterability of erythrocytes through micropores having similar dimensions to those of capillaries has been widely used[7-9] as an indicator of deformability. Studies with infected simian erythrocytes[4] have indicated that alterations in the microcirculation are correlated with their filterability. It is unclear, however, whether a similar decrease in filterability occurs generally with all malaria infections, or only in those with cerebral complications or prominent microcirculatory disturbances. Although *P. berghei* infection of the mouse is normally not associated with cerebral involvement, many of the pathological lesions could be related to alterations of the rheological properties of the erythrocytes. This paper reports a decrease in the filterability of *P. berghei*-infected mouse erythrocytes, and the heterogeneity of this decrease in erythrocytes carrying parasites at the same stage of maturation.

Materials and methods. *P. berghei*-infected blood was obtained by cardiac puncture of infected Swiss mice, 5 days after i.p. inoculation with 10^7–10^8 parasitized erythrocytes. No reticulocytosis ensued during this period, as observed by Brilliant Cresyl Blue staining. Cells from normal and infected blood were collected in ACD solution and washed 3 times with either a buffer containing 25 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 86 mM glucose and 50 mM Na_2HPO_4, pH 7.4 or (in ATP depletion experiments) with a buffer

Changes in parasitaemia, filtration time and ATP content of erythrocytes from *P. berghei*-infected blood on repeated filtration

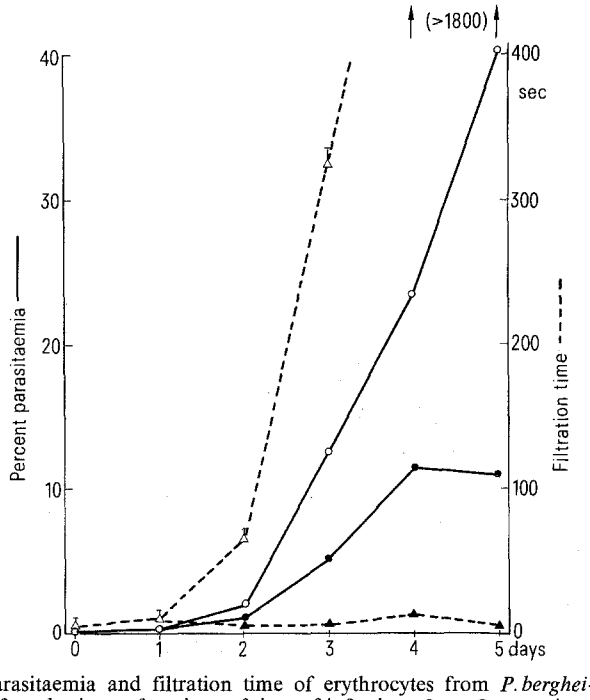
Source	Number of filtration	Parasitaemia (%) Total	Ring	Mature	Filtration time (sec)	ATP content (nmole/10^9 cells)
Normal	1–4	–	–	–	5.3± 0.7	64.9±14.0
Infected, day 3	1	12.6	5.7	6.9	326 ±10	61
	2	6.5	3.9	2.6	12.8	66
	3	3.4	2.2	1.2	7.1± 1.6	52
Infected, day 5	1	40.3	6.4	33.9	>1800	58
	2	22.0	4.4	17.6	>1800	76
	3	20.0	4.4	15.6	77.4	72
	4	13.0	3.5	9.5	6.0± 2.3	68
Normal, ATP-depleted	1	–	–	–	139	2
Infected, day 3, ATP-depleted	1	12.0	–	–	463	2

Erythrocyte suspensions (1.0×10^9 cells/ml) were repeatedly applied on 3 μm polycarbonate filter under 10 cm water pressure, using a new filter each time. Before each filtration, the parasitaemia and ATP content was determined and the erythrocyte concentration appropriately adjusted.

containing 75 mM NaCl, 75 mM KCl, 10 mM Tris-HCl and 0.1 mM EGTA, pH 7.5, and then 3 times more with a similar buffer without EGTA. Leucocytes were removed by passage of a 50% cell suspension through a column of cellulose CF-11 after the 1st wash[10]. Cells were stained with Giemsa for parasite counts.

The filtration apparatus consisted of a modified 10 ml pipette connected with a holder for a 2.5 cm filter. Polycarbonate membrane filters, 3 µm sieves (Nucleopore Corp.) were used, and filterability was measured by a method similar to that of Gregersen *et al.*[7], using the time required for passage of 1 ml suspension containing 1.0×10^9 cells through the filter under a positive pressure of 10 cm H_2O. ATP content was measured by a luciferin-luciferase method[11]. ATP was depleted from cells according to Lew[12].

Results and discussion. As parasitaemia develops, filterability of the erythrocytes from infected mice through 3 µm polycarbonate sieves is markedly decreased (fig.). This is seen clearly starting from day 2 after infection, where filtration time is increased 10-fold while the parasitaemia is only 2%. The decreased filterability is not related to the proportion of reticulocytes, which remained at 1.22 ± 0.15% throughout the course of infection, or mean cell volume (60.5 ± 1.3 µm^3 for normal, 59.7 ± 0.6 µm^3 for infected erythrocytes at 32% parasitaemia). The fact that flow decrease occurs at very low parasitaemia indicates that only a small proportion of cells with reduced deformability, presumably the infected cells, can obstruct the pores effectively, and/or that all cells from infected blood, not only infected cells, have reduced deformability. Our results may be compared with those of Miller et al.[4,5] which showed obstruction of flow through 5 µm sieves for rhesus erythrocytes parasitized at more than 10%, indicating that interaction between infected cells may be necessary for the obstruction. The smaller pore size of the filter in our experiment may account for differences in sensitivity of filterability to increase in parasitaemia.



Parasitaemia and filtration time of erythrocytes from *P. berghei*-infected mice as functions of time of infection. ○——○, parasitaemia; and △——△, filtration time of erythrocytes from infected blood; ●——●, parasitaemia; and ▲——▲, filtration time of the erythrocytes which had been previously subjected to 4 filtrations.

Apart from dependence on parasitaemia, Miller et al.[4,5] showed that mature trophozoites and schizonts caused more obstruction than young trophozoites (ring). In *P. berghei* infection, which is asynchronous, such a difference in obstruction should result in a lower parasitaemia of cells in the filtrate, mainly due to removal of cells containing mature parasites. Furthermore, if the erythrocytes from infected blood are subjected to repeated filtration, the filtration time should become increasingly smaller. Results of repeated filtration experiments presented in the table shows that erythrocytes carrying mature parasites are indeed preferentially, but not exclusively, removed and the filtration time is gradually decreased. After the 4th filtration, the filtration time is not significantly different from that of normal erythrocytes (table and fig.). However, erythrocytes from infected blood still have significant amounts of both ring-stage and mature parasites, the proportion of the latter being smaller than before filtration. These results clearly show a heterogeneity in the deformability of the infected cell population at all stages of development.

Many factors could contribute to reduced deformability of erythrocytes, including cellular ATP, Ca^{2+} and content of other cations[13,14]. Although some plasmodial infections have been shown to lead to ATP depletion[15], which could result in decreased deformability, *P. berghei*-infected mouse erythrocytes have an ATP content comparable to that of normal cells (table and Areekul. et al.[16]). Furthermore, depletion of ATP from normal cells, achieved through metabolic inhibition[12], resulted in an increase in filtration time which, although large, is still much lower than the filtration time of erythrocytes from infected blood, while ATP depletion of the latter led to only a small further increase in filtration time (table). It is therefore unlikely that the differences in filterability are explained by differences in ATP content. High intracellular Ca^{2+} also resulted in cell rigidity[13,14]. The Ca^{2+} content of human erythrocytes has been found to increase with *P. falciparum* infection, reaching a value of approximately 10 times the normal content at 60% parasitaemia[17], while the content of monkey erythrocytes has been reported not to increase with *P. knowlesi* infection[18]. It is possible that changes in Ca^{2+} content and its cellular distribution contribute to the deformability change observed.

The observed decrease in filterability of erythrocytes from *P. berghei*-infected mice may have some pathophysiological implications. Although there are few cerebral complications in this malaria model, other pathological changes[19] could be linked with decreased deformability, such as increased destruction of the erythrocytes during passage through the spleen, and other liver, renal and pulmonary changes. Other lesions such as antibody-coating of the erythrocytes could also lead to their removal and destruction[20]; however, clearance of *P. berghei*-infected erythrocytes in rats appears to depend on rheologic properties more than on an antibody-dependent process[3].

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Peroxidase and phospholipid deficiency in human eosinophilic granulocytes – A marker in population genetics

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Summary. A large scale investigation was carried out in order to establish the frequency of peroxidase and phospholipid deficiency of eosinophilic granulocytes among various ethnic groups in Israel. The simplicity of the method for staining eosinophilic peroxidase renders it a useful marker for the study of population genetics.

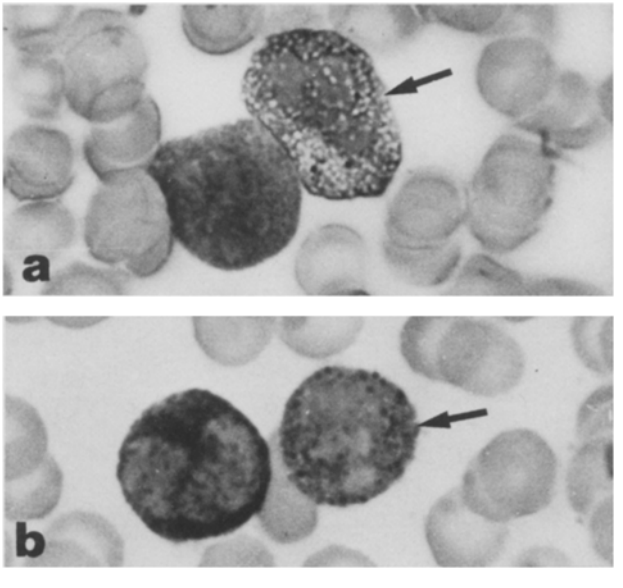
A 'new' hereditary defect of human eosinophilic granulocytes, consisting of complete absence of phospholipid and peroxidase staining was described in 1968 by Presentey[1]. The anomaly did not seem to be associated with particular clinical symptoms. The first 2 cases were encountered in families of Yemenite Jews[2] and an additional case was found in an Iranian Jew[3]. Family studies suggested an autosomal recessive mode of inheritance. The 1st population survey showed that the trait was not rare among Yemenite Jews; there were decreasing frequencies in Iraqi, Iranian and North-African Jews[4], but no cases were found among Ashkenazi of Central and East European origin, nor among Sepharadi Jews of the Balkans. A further study[5] disclosed the occurrence of the eosinophilic defect among Israeli Arabs of the Galilee, with a frequency close to that found among Iraqi, Iranian and North African Jews[4]. Since then, the study has been continued on a large scale, including a total of 69,133 people. The distribution of the affected individuals according to ethnic groups and the respective gene frequencies are presented in the table.

The anomaly was detected by screening the blood smears of all patients sent to a regional laboratory for routine hematological examinations, using specific peroxidase staining of the eosinophils according to Undritz[6] and staining for phospholipids according to Lison[7] (fig.a and b). Smears showing negative staining with these methods were subsequently subjected to nonspecific peroxidase staining according to Graham-Knoll[8] in order to ascertain that the anomaly did not affect the neutrophils. Special care was taken to identify family links between positive cases for further genetic studies.

The overall results of the study carried out during the period 1970–1976 are summarized in the table. They enclose the findings out of a total Jewish population of 63,465 individuals living in the Rehovot district and grouped according to ethnic origin as well as those obtained from the study of 1182 from the Galilee region. As shown in the table, the highest frequency of affected individuals as well as that of the mutant gene was found among Yemenite Jews, followed in decreasing order by Jews of North-African and Iraqi-Persian extraction and by the Arab population studied. So far, the eosinophilic anomaly has not been detected among Sepharadi Jews of the Balkans. Its rare occurrence among Ashkenazi Jews (L:11,325) let us presume that sporadic cases would be found also among non-Jewish European populations, whose genetic make-up is close to that of Ashkenazi Jews.

One such case has been already reported in the literature[9] and an additional case was incidentally detected by us in a Swedish gril residing temporarily in Israel.

Out of the 88 cases carrying the anomaly, 14 had family links and 74 were unrelated. The mode of inheritance was compatible with an autosomal recessive character of the mutant gene as previously suggested. Moreover, 14 individuals exhibited a partial loss of enzyme activity, manifested by a weak peroxidase staining of the eosinophils. Their



a) Positive peroxidase staining of neutrophilic and eosinophilic (arrow) granulocytes of a normal patient. b) Positive peroxidase staining of neutrophilic granulocytes and negative staining of eosinophilic granulocyte (arrow) of a deficient patient.