When lymphoid cell suspension was obtained from rabbits treated with Freund's adjuvant only, no specific phagocytosis of erythrocytes could be detected. On common incubation of killed staphylococci with hypersensitive lymphoid cells and erythrocytes no distinct influence of the degree of bacterial phagocytosis by macrophages was observed.

The incubation of erythrocytes with fresh sera of sensitized rabbits led to their partial destruction or damage. These erythrocytes, after washing, were not phagocytized. We suppose, therefore, that the factor in question is probably not a conventional opsonin.

Further experiments are in progress to analyse the distinct nature of the above-mentioned factor and its role in the mechanism of the reaction.

It was shown that on simultaneous incubation, a factor is released from sensitized lymphoid cells which is capable of inducing specific phagocytosis or adherence of erythrocytes to macrophages from normal animals. We think that these results are related to similar findings described in the studies of cell migration quoted in the introduction of our paper as well as to the problem of the so-called cytophilic antibodies[6-8] and possibly also of the so-called cellular immunity[9].

Zusammenfassung. Mit In-vitro-Experimenten wird gezeigt, dass in Anwesenheit von sensibilisierten Kaninchen-Lymphozyten Schaferythrozyten spezifisch an normale Kaninchen-Makrophagen adsorbieren und von diesen dann phagozytiert werden.

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Alterations of the Erythrocyte Membrane Caused by Fluorinated Dinitrobenzenes

Acetylcholinesterase (ACHE) appears to be located at the outer surface of the human erythrocyte membrane[1,2]. We have recently shown that this enzyme was inactivated when red cells were treated with 1-fluoro-2,4-dinitrobenzene (FDNB) and 1,5-difluoro-2,4-dinitrobenzene (DFDNB)[3]. The effect was irreversible and characteristically distinct for each reagent. While ACHE inactivation was greater with DFDNB than with FDNB, exposure to the former rendered the residual activity more resistant to heat and urea than treatment with the latter. FDNB reacts with free amino, histidyl, tyrosyl or sulfhydryl groups to yield dinitrophenyl derivatives, and DFDNB reacts with 2 such groups, provided they are 5 Å apart, to give dinitrophenylene cross-links[4]. The increased stability of the residual ACHE activity following DFDNB treatment was ascribed to the formation of cross-linked derivatives[3]. Since ACHE activity is reduced in new-borns affected with ABO hemolytic disease[5] and inasmuch as blood-group specific antigenic receptors also are located at the red cell surface[6], we have investigated the influence of ABO antibodies on ACHE inactivation by FDNB and DFDNB and the possible changes in the agglutinability of cells thus treated.

Blood obtained from normal adult individuals of blood group A or B was centrifuged and the plasma and buffy coat were removed by suction. The erythrocytes were washed twice with 20 vol. of cold 0.15 *M* NaCl and 5 times with 20 vol. of chilled 0.1 *M* sodium-potassium phosphate buffer, pH 8.0. 5% stock solutions of FDNB and DFDNB in methanol were prepared daily and stored at 4 °C. A 0.5% suspension of A erythrocytes was incubated for 60 min at 25 °C and pH 8.0 with $5 \times 10^{-4} M$ FDNB and DFDNB. Methanol was added to the controls. Following incubation the cells were washed 5 times with 20 vol. of buffer and adjusted to a 50% suspension after the last centrifugation. ACHE activity was measured at 25 °C on replicate 0.1% cell suspensions in 0.1 *M* phosphate buffer, pH 8.0 using acetylthiocholine iodide as substrate and 5:5′-dithiobis-(2-nitrobenzoic acid) as color reagent[5]. Residual enzyme activity was related to controls. Agglutinability was ascertained with human anti-A serum (Ortho Diagnostics). In some experiments, A or B erythrocytes were agglutinated with an excess of homologous human antiserum. The agglutinated cells were dispersed and washed 5 times with buffer prior to the exposure to FDNB and DFDNB. Finally, A or B erythrocytes were incubated with homologous human antiserum in amounts insufficient to cause direct agglutination, but sufficient to produce agglutination with rabbit anti-human globulin serum. Cells thus treated were washed with 0.1 *M* phosphate buffer, pH 8.0 and then incubated with the reagents. Following this treatment and multiple washings with buffer, ACHE activity and agglutinability by rabbit anti-human globulin serum were determined. Agglutination was judged on a 1 to 4 scale.

In Table I it can be seen that while treatment of erythrocytes with $5 \times 10^{-4} M$ FDNB caused only a moderate reduction of ACHE activity, exposure to the same concentration of DFDNB and under identical conditions resulted in a much greater loss of activity. It can also be observed that whereas cells treated with FDNB were agglutinated by homologous antiserum, those incubated with DFDNB did not agglutinate. However, agglutination of FDNB-treated erythrocytes was invariably followed by hemolysis. When red cells were agglutinated with an excess of homologous antibody and then exposed to FDNB or DFDNB, reduction of ACHE

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activity was not affected. The coating of erythrocytes with homologous antiserum in amounts which did not cause direct agglutination, also failed to enhance the reduction of enzyme activity when the cells were subsequently exposed to the reagents (Table II). In testing the agglutinability of these cells with anti-human globulin serum, immediate lysis was noted with the antibody-coated, FDNB-treated erythrocytes, whereas the sensitized cells incubated with DFDNB had become non-agglutinable. Essentially the same results were also obtained when group B erythrocytes were similarly treated.

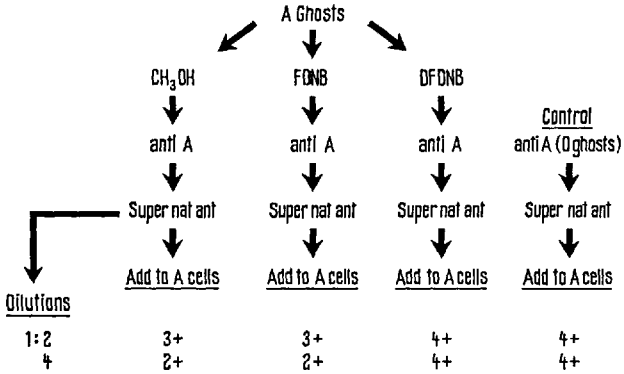
The markedly distinct behaviour of FDNB- and of DFDNB-treated cells, prompted a study of their antibody-binding capacity. Since erythrocytes exposed to FDNB became fragile, cell-free membrane preparations (or red cell ghosts), obtained by osmotic lysis, were used in these experiments. A 0.5% ghost suspension, prepared from blood-group A red cells, was treated at 25 °C and pH 8.0 with $5 \times 10^{-4} M$ of the reagents, washed 5 times with buffer and then incubated for 30 min at 22 °C with anti-A serum. Methanol-treated A ghosts and blood group O ghosts served as controls. Following incubation, the membranes were sedimented by centrifugation and the supernatant was diluted and tested for the presence of residual anti-A antibodies, using a standard 6% suspension of A erythrocytes (Affirmagen, Ortho Diagnostics). In the Figure it can be seen that whereas the ghosts treated with FDNB bound almost as much antibody as the control preparations, those exposed to DFDNB took up a relatively small amount as evidenced by comparison with the O ghosts.

Table I. Effect of FDNB and DFDNB on ACHE activity and agglutinability of group A erythrocytes

Treatment	% ACHE activity	Agglutination with anti-A serum
None	100	4+
Methanol	98	4+
FDNB	71	3+ (lysis)
DFDNB	7	0

Table II. Effect of FDNB and DFDNB on ACHE activity and agglutinability of antibody-coated group A erythrocytes

Treatments	% ACHE activity	Agglutination with anti-human globulin serum
None	100	0
Anti-A + buffer	100	4+
Anti-A + methanol	100	4+
Anti-A + FDNB	73	lysis
Anti-A + DFDNB	7	0



Antibody uptake by erythrocyte membranes treated with FDNB and DFDNB. Agglutination was judged as indicated in text.

The indifference of ACHE to blood-group specific antibodies is reminiscent of our previous studies on the effects of proteolytic enzymes[1], and tannic acid[7] on this stromal enzyme. As in the present work, in no case could we observe a protection of ACHE or an enhancement of inactivation by agglutinating the cells with their ABO specific antisera before the exposure to the inactivating agents. Our results indicate that treatment with FDNB does not interfere with agglutination by homologous antisera, but cells thus treated become extremely fragile. BERG et al.[8] have correlated the fragility of FDNB-treated erythrocytes with the osmotic imbalance created by their increased permeability to small cations. The persistent agglutinability of these cells was further illustrated by the almost normal binding capacity of anti-A serum by A ghosts pretreated with FDNB. In contrast, the loss of agglutinability and the striking reduction in antibody uptake caused by DFDNB indicate that the formed dinitro-phenylene cross-links prevented the attachment of antibody to its specific receptor site. These observations differ from the suppression of ABO blood-group agglutinability noted when red cells were treated with formalin[9] or with tannic acid[10,11], since the uptake of antibody was not significantly affected by these agents. Our findings with FDNB- and DFDNB-treated erythrocytes and ghosts are in keeping with the dissimilarity with which these reagents modify the surface of the human red cell[8,12].

Zusammenfassung. Inaktivierung der Acetylcholinesterase in der Erythrozytenmembran mit 1-Fluor-2,4-dinitrobenzol und 1,5-Difluor-2,4-dinitrobenzol konnte durch blutgruppenspezifische Antikörper nicht beeinflusst werden. Nach Difluordinitrobenzol-Behandlung zeigten die Blutkörperchen Verlust der Agglutinabilität und die Membranen eine Herabsetzung ihrer Absorptionsfähigkeit für homologe Antikörper.

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