1.3±0.4; pHSA, 1.7±0.6; pMSA, 1.8±0.6 and pHRP 27.0±7.9. The mean weight ratios of heterologous polymeric albumins to homologous pHSA, determined for a 50% inhibition (0.47 for pBSA, 0.53 for pRSA, 0.76 for pGSA and 1.06 for pMSA) as well as the complete radioimmunoassay inhibition curves (fig.) proved that pBSA, pRSA and pGSA are better inhibitors than pHSA, while pMSA inhibitory capacity is close to that of pHSA. The inhibitory capacity of pHRP was 15–34 times weaker than that of polymeric albumins, as can be seen from the mean weight ratios of pHRP to polymeric albumins for 50% inhibition. Similar results were obtained for AAA found in normal human sera. Thus the means ±SD of inhibitors in µg required for 50% inhibition in 3 normal human sera were: pBSA, 1.4±0.5; pRSA, 1.6±0.5; pGSA, 1.4±0.3; pHSA, 2.3±1.3, pMSA, 2.4±1.4 and pHRP, 34.0±4.3.

In the table are given the results of the inhibition experiments performed with normal rabbit and mouse AAA. The weight ratios of heterologous polymeric albumins to homologous pRSA at maximum inhibition of passive hemagglutination showed that rabbit AAA react better with pBSA and pHSA than pRSA. The reacting capacity of rabbit AAA with pMSA was comparable with that shown with pRSA. In the mouse system heterologous pHSA showed a stronger inhibitory capacity than homologous pMSA on the reaction between mouse AAA and ^{125}I-pHSA.

The similar, or even stronger, AAA binding activity shown by heterologous vs homologous polymeric albumins demonstrate the lack of species specificity for AAA in both pathologic and normal human and animal sera. Thus our results extend the findings of Thung and Gerber[13] who showed that AAA in pathologic sera react with both polymeric homologous human albumin and heterologous bovine and rat albumin. The lack of species specificity has also been demonstrated for AAA elicited in rabbits by immunization with GA-polymerized rabbit albumin[17] and not for rabbit and mouse antibodies elicited against GA-polymerized immunoglobulin[18]. The capacity of GA-treated heterologous albumins to react with AAA shows that GA treatment produces similar antigenic determinants in albumins of different species. GA-treated peroxidase, used as a control, inhibited, to some extent, the reaction between AAA and pHSA, thus suggesting that pHSA and pHRP have some common antigenic determinants. It is reasonable to assume that after GA treatment albumin and peroxidase share mainly the chemical groups which appear following the reaction of GA with the ε-amino groups of the lysine residues[19], and not the antigenic determinants dependent on the polymerization process, since the latter are probably related to the particular amino acid environment of each protein[7]. This supposition could explain the high capacity of GA-polymerized albumins to react with AAA from different species and the weak reacting capacity of the GA-polymerized peroxidase not related to serum albumin.

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Phagocytosis in diabetic subjects: increase in hydrophobicity of granulocyte cytoplasmic membrane

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Summary. Granulocytes from diabetic subjects have impaired ability to engulf bacteria; the data obtained suggest that the alterations are correlated with an increase in surface hydrophobicity, as measured by contact angle.

Subjects with diabetes mellitus have altered lipid-metabolism, which is reflected in the cellular membrane. An increase in microviscosity, that is to say, a decrease in fluidity, of the erythrocyte membrane was revealed by analyses using fluorescence-depolarization[1]. The reduced membrane fluidity is apparently related to an increase in the molar ratio of cholesterol-to-phospholipids (C/PL)[1,2]. Alterations in the C/PL ratio must give rise to changes in

the surface tension of the cellular membrane, as revealed by alterations in surface hydrophobicity. On the other hand, investigations of the physicochemical surface properties of bacteria, in relation to their liability to phagocytosis, showed that hydrophobic interaction is important for host-parasite interactions[3]. The interfacial properties of monolayers of the phagocytic cells and the various bacteria are best studied by contact-angle measurements[4].

It is well known that diabetic subjects show impaired resistance to infections. Nolan et al.[5] reported abnormalities in migration, ingestion and killing of bacteria by granulocytes, and Bagdade et al.[6] demonstrated that granulocyte-adherence may also be impaired in patients with controlled diabetes.

The present investigation was devoted to the study of the surface-hydrophobicity and the ability to phagocytize in granulocytes from diabetics. Diabetic subjects (type II) of both sexes aged between 40 and 60, who had been suffering from fasting hyperglycemia for over 3 years, were selected. Patients who suffered from vascular complications but did not suffer from acidosis and ketonuria were orally treated with anti-diabetic drugs. Pheripheral blood granulocytes were isolated using heparin (10 U/ml). Leukocyte enrichement was achieved by centrifugation at $350\times g$ for 30 min. The leukocyte rich buffy-coat layers thus obtained were treated with NH_4Cl (0.17 M) for 5 min in ice to lyse the residual erythrocytes. The suspension was subsequently washed 3 times for 10 min by centrifuging in Hanks solution at $250\times g$. The pellet was then resuspended in Hanks solution at 5×10^6 cells/ml; 0.4 ml of each suspension was put into Costar plates (Nunclan, Delta, Denmark) and incubated for 30 min at 37 °C to promote the adherence of polynucleates to the plate surface. After this incubation, the supernatant with non-adhering cells was removed, and the wells were then washed 3 times with normal saline solution. The monocellular film formed at the bottom of the wells was then dried at room temperature for 60 min, before proceeding to contact-angle measurements. These were carried out according to the method described by Dahlgren and Sunquist[4]. Briefly, a 10-µl normal saline drop was deposited on the dry monocellular film, and the drop diameter was then measured with a Bausch and Lomb stereomicrometer: from its diameter we calculated the contact-angle using the equation of Dahlgren and Sunquist[4]; these authors have already calculated and tabulated solutions of this equation for different diameters of drops.

Cholesterolemia, glycemia, triglyceridemia and phospholipidemia determinations were performed by conventional methods.

For phagocytosis tests, the leukocyte-rich layer was stratified on MSL (Milieu de separation des lymphocytes-Eurobio, Paris) and then centrifugated at $300\times g$ for 30 min. The granulocyte pellet was washed by centrifuging 3 times for 10 min at $250\times g$ in Hanks solution, and then resuspended in Hanks solution at 2×10^6 cells/ml. Phagocytic index was determined following the technique of Van Zwet and al.[7]. Briefly, 2×10^6 cells/ml suspended in RPMI 1640 and 2% fetal serum were incubated with *Staphylococcus epidermidis* (10^6 cells/ml). After 30 min a 0.5-ml sample was taken, immediately added to 1.5 ml of ice-cold RPMI 1640 to stop phagocytosis, and centrifuged at $110\times g$ for 4 min. 2 0.1-ml aliquots of 3 consecutive supernatant dilutions, with an expected number of 10^2-10^4/ml, were plated. Plates with 10-500 colonies were counted and the number of bacteria in the supernatant was calculated.

All diabetic subjects from whom granulocytes had been taken exhibited mean basal glycemia of 240 ± 20 mg% and mean triglyceridemia of 455 ± 40 mg% after a 15-h fast. Normal subjects from the control group exhibited basal glycemia of 80 ± 10 mg% and triglyceridemia of 140 ± 20 mg%. The surface hydrophobicity of polynucleates taken from diabetic subjects' peripheral blood, determined by contact-angle measurements, is shown in the table. A contact-angle increase demonstrates an increase in surface hydrophobicity, which in turn alters the surface tension and plays a fundamental role in the engulfing of bacteria by the phagocytic cells. In fact, the change in the phagocytic index of granulocytes from diabetic subjects is inversely proportional to the increase in surface-hydrophobicity. The cholesterol/phospholipids ratio is the same in plasma and in erythrocyte cytoplasmic membrane, as shown by Otaji et al.[1]. In our experiments C/PL ratio is related to the contact-angle and to the phagocytic index (table). None of the cases studied has shown a wider contact-angle with an increase in the phagocytic index. Thus, hydrophobicity increment, phagocytic index, and C/PL ratio in granulocytes are closely related.

As more and more has been learned about the complex structure of the cell membrane, it has become evident that the composition and microviscosity of membrane lipids must be a critical controlling factor for cell functions. Interfacial tension is a major physicochemical cell-surface parameter, and probably plays a crucial role in the decision that a phagocyte makes between ingestion and non-ingestion. The decreased phagocytic ability observed in our diabetic subjects is probably caused by altered surface hydrophobicity of the granulocytes, which in turn is due to observable metabolic alterations.

Correlation of various parameters in normal and diabetic subjects

	Calculated contact-angle[a]	Phagocytic index[b]	Plasma C/PL mole ratio
Normal subjects[c]	16.5° ±0.08	0.30±0.05	0.4±0.05
Diabetic subjects[c]	20.30°±2.0	0.10±0.03	0.6±0.05

[a] The contact-angle is calculated by solving the equation: $V=\frac{\pi a^3}{6}[3\,\mathrm{tg}\nu/2+(\mathrm{tg}\nu/2)^3]$ where the diameter is $2a$[4]. [b] Phagocytic index $=\log N_o-\log N_t$ (N_o and N_t indicate the number of bacterial cells in the suspension at time 0 and after 30 min in presence of granulocytes[7].). [c] Mean±SEM of 10 experiments.

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