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Fluidity of rat liver Golgi membranes in streptozotocin diabetes. A spin label study

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The mobility of 5-doxylstearic acid spin label (5-SASL) in the intact rat liver Golgi membranes of streptozotocin diabetes was studied as a function of free blood sugar level and temperature. During development of diabetes, indicated by the increase of the free blood sugar level, the membrane fluidity measured in the physiological temperature range (1) does not change in comparison with control in light diabetes, (2) decreases significantly in advanced diabetes and (3) again increases to the control level in heavy diabetes (the free blood sugar levels being 200–250 mg/100 ml, 250–350 mg/100 ml and greater than 350 mg/100 ml, respectively). The development of streptozotocin diabetes is accompanied by significant changes in lipid composition of liver Golgi membranes as also shown in our previous observations. The measurements of motion of 5-SASL in Golgi membranes as well as in vesicles, made from commercially available lipids of composition close to the liver Golgi membranes, show that a decrease of cholesterol contents is the main factor which induces the increase membrane fluidity. We suggest that in the heavy diabetes the hemeostatic regulation in the lipid composition leads to minimization of alterations in membrane fluidity to obtain comparatively normal activity of certain membrane enzymes.

Introduction

Significant changes in enzyme activity, morphology and lipid composition of the rat liver Golgi apparatus in streptozotocin-diabetic rats have been previously reported [1–3]. Many studies have also been carried out on erythrocyte membranes in both insulin-dependent diabetes in humans and streptozotocin-induced diabetes in rats [4–8] showing different phospholipid and cholesterol contents and alteration in theirs osmotic resistance. These works suggest changes in cell membranes fluidity during development of diabetes, which can in turn contribute to the change in activities of membrane-associated proteins, transport across membranes, shape of organelles, etc.

The physiological properties of membrane lipids can influence the activity of membrane proteins in several ways: (a) the fluidity of membrane lipids may contribute to rotational or translational diffusion of proteins in fluid lipid regions, (b) lateral phase separation in the membrane may include separation of proteins, (c) changes of lipid composition can expose different parts of integral proteins. It was shown, for example, that increase of the proportion of unsaturated fatty acids in the plasma membrane [9] as well as in liposomes with reconstituted insulin receptors [10] leads to an increase in insulin-receptor sites available for insulin. Such changes are associated with an increase in cell responsiveness to insulin.

Spin label electron spin resonance (ESR) was one of the first physical techniques used for examination of human dystrophic tissue [11,12] showing the complex effect of cholesterol and unsaturation of alkyl chains on membrane fluidity. In this paper we employed the spin-label method to examine the fluidity of rat liver Golgi membranes in streptozotocin diabetes. The 5-doxylstearic acid spin label (5-SASL) was used because its ESR spectra reflect an alkyl chain mobility of phsopholipids. Also, nitroxide moiety of 5-SASL is located in the hydrocarbon region of the membrane which is strongly influenced by the rigid core of cholesterol [13]. It was expected, that changes in cholesterol contents observed in Golgi membranes [2,3,8]

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Sph, sphingomyelin; Chol, cholesterol; 5-SASL, 5-doxylstearic acid spin label; ESR, electron spin resonance.

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would affect the movement of 5-SASL. To separate effects of cholesterol and unsaturation of fatty acids, ESR measurements were made within wide range of temperatures (10 °C to 42 °C) for Golgi membranes and liposomes with lipid contents close to that of the liver Golgi membranes. Principal results of alterations in fluidity of Golgi membranes are presented as a function of blood sugar level in streptozotocin-diabetic rats.

Materials and Methods

Animals. Two groups of female Wistar rats, aged about 6 months, 170–230 g in weight, and fed ad libitum with commercial pelleted food and tap water were used. The rats were not starved before killing. To estimate free blood sugar level, the blood was taken from the liver vein with a syringe with one drop of heparin from the rats anaesthetised with ether. Next, their livers were removed and immediately used for Golgi membrane preparations. The experimental groups were as follows: (i) control rats, untreated (9 animals), (ii) streptozotocin-diabetic rats (13 animals) in which diabetes was induced by intraperitoneal injection of streptozotocin in doses of 60–70 mg per kg of body weight in 50 mM sodium citrate (pH 4.5). The rats were starved for about 10 h before streptozotocin administration. The animals were considered diabetic, when on the eleventh day they revealed of free blood sugar levels more than 200 mg/100 ml. They were divided into three subgroups: (1) with a free blood sugar level of 200–250 mg/100 ml, arbitrarily called 'light diabetes'; (2) with a free blood sugar level of 250–350 mg/100 ml, 'advanced diabetes'; (3) with a free blood sugar level of more than 350 mg/100 ml, 'heavy diabetes'.

Reagents. All phospholipids and streptozotocin were purchased from Sigma (St. Louis, MO), crystallized cholesterol cames from Boehringer Mannheim (Indianapolis, IN), 5-SASL was purchased from Molecular Probes (Junction City, OR). Tris and serum bovine albumin came from Koch-Light Labs Ltd (Colnbrook, Bucks, U.K.). All other reagents were analytical grade and cam from POChem (Gliwice, Poland).

Preparation of Golgi membranes. Golgi-rich membrane fractions were isolated by the one-step gradient procedure of Fleischer [14] from whole liver of indidivudal rat, immediately after killing. The purity of the membrane fractions was previously checked by morphological [15] and enzymatic [16] methods. Specific activity of Golgi membranes marker enzyme, the galactosyl transferase was about 30–50-times higher in fraction than in respective homogenate. Immediately after isolation, the fractions were used for ESR measurements. Total lipids from Golgi-rich membrane fractions of individual rats were extracted according to Kates [17] and chloroform solutions of lipids were stored at −30 °C before labeling and ESR measurements.

Preparation of lipid vesicles. Isoladed lipids from individual rats (about 1 to 4 mg in chloroform) were mixed with chloroform solution of spin label at molar ratio of probe to lipid of about 1/100 and dried with a stream of nitrogen and further dried under reduced pressure (about 0.1 mmHg) for at least 12 h. The 0.9% NaCl in 0.05 M Tris-HCl (at pH 8.5) 0.1–0.2 ml were added to dried lipid at about 45 °C and vortexed vigorously. Samples were also sonicated for 10 min in a 125 W-water bath. Liposomes (multilamellar dispersion of lipids) from commercially available lipids were prepared in a similar way. Proper mixtures of lipids ($1.0 \cdot 10^{-5}$ mol) and spin label ($1.0 \cdot 10^{-7}$ mol) were used. Samples were ready for ESR measurement after vortexing, no sonication was applied.

Spin labeling of Golgi membranes. The suspension (0.5 ml) of Golgi membranes was added to a glass tube with the film of 5-SASL on the bottom obtained after chloroform evaporation ($1.5 \cdot 10^{-8}$ mol of 5-SASL per mg of protein). The tube was vigorously vortexed for 1 min at 25 °C, filled up with the same buffer and centrifuged 30 min at $80000 \times g$. Pelleted membranes were used for ESR measurements. To avoid spin–spin interaction 'magnetically diluted' spin label concentration was used (similar or lower than used for labeling other biological membranes [18,19]). To ensure that all probe carboxyl groups were ionized in the membrane a rather high pH had been chosen [20,21]. The apparent pK of 5-SASL in phosphatidylcholine (PC) membranes is 7.25.

Analytical estimations. The blood sugar level was estimated by the method of Somogyi and Nelson [22]. The proteins were determined by method of Lowry et al. [23] with crystalline bovine serum albumin used as a standard.

ESR spectral measurements. ESR measurements were carried out on a Varian E-3 spectrometer equipped with a variable temperature-controlling unit. The sample for measurements was taken in Pasteur pipette and sealed on the end with Miniseal wax. The temperatures were monitored using copper-constantan thermocouple that was placed in the sample just above the active volume of the cavity. The conditions of the measurements were: microwave power 5 mW and modulation amplitude 1 G. The recorder scan range was checked using 10^{-4} M solution of 2,2,6,6-tetramethylpiperidine-*N*-oxyl-4-one.

Results

The 5-SASL spin label used in this work is thought to orient in the lipid bilayer part of cell membranes with its ionized carboxyl end anchored near the polar heads of the lipid molecules and its long alkyl chain in the average parallel to the alkyl chains of the membrane lipids [24]. In biological and model membranes 5-SASL undergoes rapid anisotropic motion about the long axis of the spin label and wobbling of the long axis within

the confines of a cone imposed by the membrane environment. The anisotropic rotational motion of the spin label gives rise to the new feature of the ESR spectra, that can be used to calculate the order parameter of 5-SASL. Because the maximum splitting value is directly related to the order parameter of 5-SASL [25], it has been used as a convenient parameter to evaluate the membrane fluidity. Howeve, at lower temperatures (10 °C, 20 °C) the ESR spectra are in the slow motion region and therefore maximum splitting reflects not only the order parameter of 5-SASL but also the rate of alkyl chain motion. These two values cannot be determined without spectral simulation. The maximum splitting value - an empirical parameter used in this work increases with increase of alkyl chain order and with decrease of rate of alkyl cain motion. For brevity, we describe this observation as the decrerase of 5-SASL mobility. ESR spectra of 5-SASL in Golgi membranes are given in Figs. 1A and B. The spectra are similar to those obtained for other 5-SASL-labeled model [13,24] and biological membranes [18,19,26] (see also ESR spectra in Fig. 1C). Fig. 1 indicates, that the label undergoes rapid anisotropic motion in the membrane. ESR spectra were registered at five different temperatures: 10 °C, 20 °C, 30 °C, 37 °C and 42 °C for all samples. The same results were obtained during heating and cooling of the sample. Results did not change after storing the sample for 48 h at 4 °C, and during that time no detectable reduction of nitroxide free radical was observed.

Fig. 2 shows the mean values of maximum splittings and standard deviations calculated by Student's *t*-test for nine control rats. All values of maximum splittings for streptozotocin-diabetic rats were shown as a function of free sugar level in the blood. There are four remarkable features in this figure: (1) At physiological range of temperature membrane fluidity does not change in the light diabetes, (2) it decreases significantly in the advanced diabetes, (3) and again returns to the control value in the heavy diabetes, (4) At lower temperatures

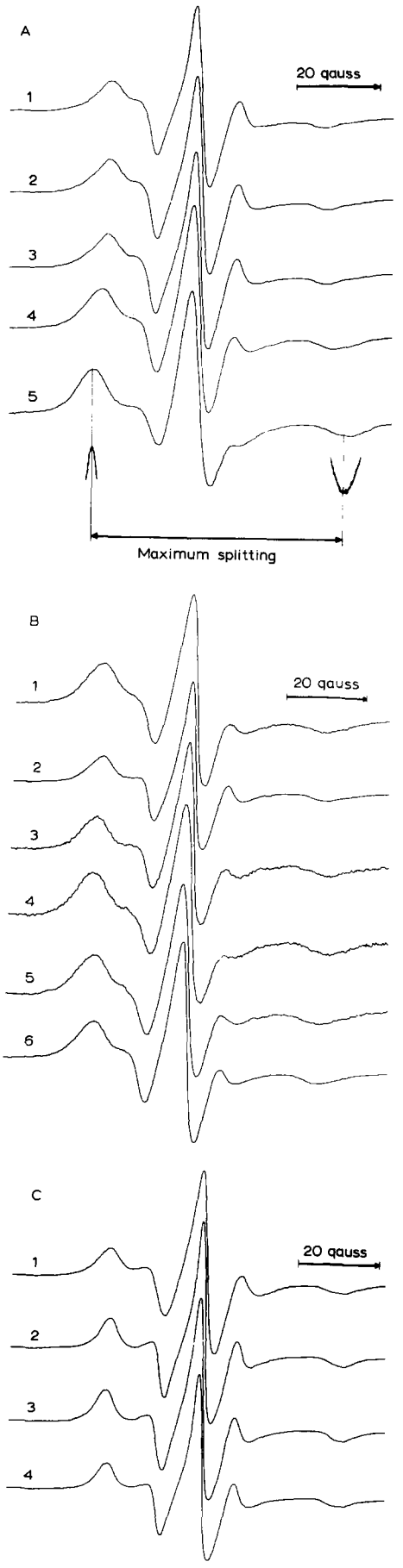

Fig. 1. (A) ESR spectra of 5-SASL in liver Golgi membranes of streptozotocin-diabetic rat (free blood sugar level 800 mg/100 ml) measured at: (1) 42 °C, (2) 37 °C, (3) 30 °C, (4) 20 °C and (5) 10 °C. The measured value of maximum splitting is indicated. The outer wings were also magnified by recoreding at 5–8-times higher receiver gain and with slower scan tim. Note the very good signal to noise ratio. (B) ESR spectra of 5-SASL in liver Golgi membranes of control (1) and streptozotocin-diabetic rats (2–6) measured at 20 °C. The free blood sugar level was: (1) 150 mg/100 ml; (2) 250 mg/100 ml; (3) 280 mg/100 ml; (4) 300 mg/100 ml; (5) 350 mg/100 ml and (6) 800 mg/100 ml. (C) ESR spectra of 5-SASL measured at 20 °C in: dispersion of lipids extracted from liver Golgi membranes of streptozotocin-diabetic rat with a free blood sugar level of 450 mg/100 ml (1), model membranes from commercially available lipids with lipid composition similar to Golgi membranes of diabetic (2) and control (3) rats according to Kordowiak [3] and control rat (4) according to Tartakoff [28]. See Results for details.

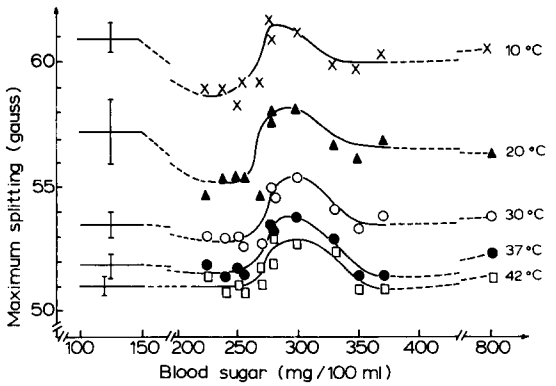

Fig. 2. Maximum splitting of 5-SASL in Golgi membranes plotted against free blood sugar level at various temperatures. For control group both the mean maximum splitting values and the standard deviations are given. The free blood sugar levels were: 100–150 mg/100 ml in control, 200–250 mg/100 ml in light diabetes, 250–350 mg/100 ml in advanced diabetes and greater than 350 mg/100 ml in heavy diabetes. Student's t-test analysis of the results at 37 °C indicates that the changes in membrane fluidity between the three investigated groups of rats are statistically significant ($t = 7.05$, $\alpha < 0.001$ and $t = 4.69$, $0.001 < \alpha < 0.01$ between light and advanced and between advanced and heavy diabetes, respectively).

(10 °C, 20 °C) this relationship is shown by a similar curve but the curve is shifted towards higher fluidity giving values lower than the control maximum splitting values in light and heavy diabetes (higher fluidity) and similar to the control values in advanced diabetes. To understand better the influence of temperature on the Golgi membrane fluidity in control and diabetic animals the plot of maximum splitting as a function of temperature was produced from the mean values of maximum splitting for control, light diabetes and advanced diabetes (Fig. 3). These curves show no critical breaking points in the range of 10 °C to 42 °C, so it is valid to compare the fluidity at either temperature [27]. It can be seen that changes in the membrane composition occurring in the light diabetes cause a marked increase in membrane fluidity only at low temperatures (10 °C) but influences only a little the fluidity at high temperatures (37 °C–42 °C). In advanced diabetes the membrane fluidity decreases again within the full range of temperatures resulting in a small increase in fluidity at 10 °C and a marked decrease of fluidity at 37 °C in comparison to control values. During further aggravation into heavy diabetes membrane fluidity increases again to reach the values observed at light diabetes (see Fig. 2, data not shown in Fig. 3).

We tried to obtain liposomes from lipids isolated from Golgi membranes. Both vortexing and sonication always produce a dense paste like pellet on a bottom of the test tube. It is likely, that large amount of phosphatidylethanolamine (PE) present in Golgi membranes [3,28] produces vessicles aggregation. For comparison ESR spectrum of 5-SASL incorporated into the suspension of lipids extracted from Golgi membranes of diabetic rat with free blood sugar level of 400 mg/100 ml is shown in Fig. 1C. Maximum splitting of this spectrum (56 G) and its shape are similar to spectra of 5-SASL in Golgi membranes shown in Fig. 1B (spectra 2, 3 and 6). Because of the poorly defined structure of the lipid-water dispersion these results were not considered.

The results of measurement of maximum splitting values of 5-SASL from liposomes made of commercially availabel lipids are shown in Fig. 4. We tried to keep the lipid composition of liposomes as near as possible to the values given by Kordowiak [3] (PC = 25, PE = 9, phosphatidylserine (PS) = 9, sphingomyelin (Sph) = 7, cholesterol (CHol) = 50 all expressed as mol%) for Golgi membrane of control rats and (PC = 36, PE = 15, PS = 7, Sph = 9, Chol = 33) for Golgi membranes from streptozotocin-diabetic rats. The liposomes with lipid composition given by Tartakoff [28] for control rat liver Golgi membranes (PC = 37, PE = 16, PS = 10, Sph = 12 and Chol = 25) were also prepared. ESR spectra of

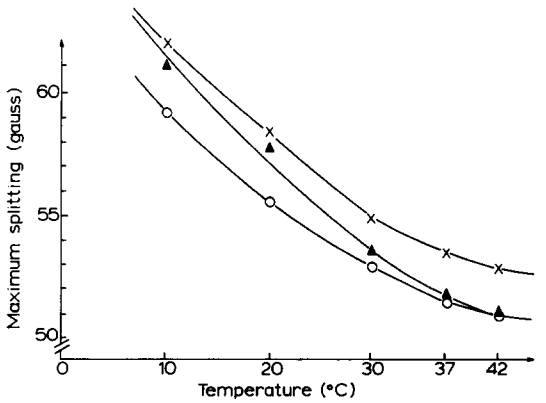

Fig. 3. Maximum splitting of 5-SASL in Golgi membranes plotted against temperature for: ▲, control; ○, light diabetes (240 mg/100 ml); and ×, advanced diabetes (300 mg/100 ml free blood sugar). Values of the free blood sugar level given in parentheses.

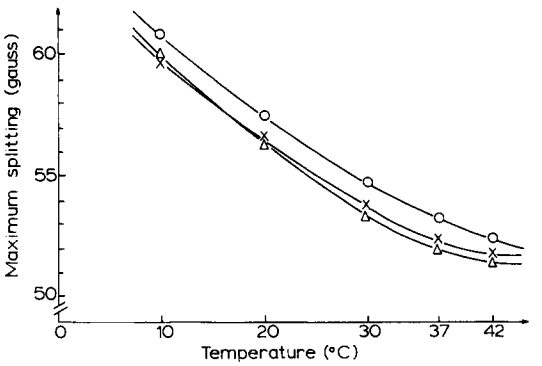

Fig. 4. Maximum splitting of 5-SASL in model membranes plotted against temperature. Lipid composition of model membranes was similar to Golgi membranes of: control (○) and diabetic rats (×) according to Kordowiak [3] and to control rats (△) according to Tartakoff [28]. See Results for details.

5-SASL in these model systems are shown in Fig. 1C. It can be seen that cholesterol to lipid ratio is the main factor which influences the membrane fluidity. It has to be pointed out, that we did not change the unsaturation of fatty acids in lipids for liposomes preparation. All lipids with certain degree of unsaturation purchased from Sigma were isolated from natural sources: PC from egg yolk and PE, PS and Sph from bovine brain. Additionally we have not data on the changing of degree of unsaturation in rat liver Golgi membranes during development of diabetes.

ESR spectrum of 5-SASL labeled biological membranes and suspension of lipids mixture is a time and spatial average arising from spin labels in all environments in the membrane. It can be seen from Fig. 1, that the linewidth of ESR spectrum of 5-SASL in Golgi membrane > linewidth of lipid dispersion of lipids extracted from Golgi membranes > linewidth of liposomes made of commercially available lipids. This increase can arise from the distribution of local viscosities in the sample (5-SASL is located in domains with different lipid composition) and/or from slower rotational motion about the long axis of the spin label. However, ESR spectra were analysed as one-component spectra because at all temperatures no clear evidence of a second component exists. Very little peacks from free 5-SASL in aqueous phase can be neglected, because they do not affect the maximum splitting. It has to be pointed out, that for Golgi membranes with lowest fluidity (from diabetic rat with a free blood sugar level of 300 mg/100 ml), the maximum splitting and shape of ESR spectrum of 5-SASL recorded at 20 °C (Fig. 2B, spectrum 4) is similar to the ESR spectrum for Golgi membranes with higher fluidity from diabetic rats with free blood sugar levels of 800 mg/100 ml and 250 mg/100 ml but recorded at 10 °C (Fig. 1A spectrum 5). This indicates, that lipid composition as well as temperature contribute in the same way to the motion of 5-SASL. It is also evident, that the ESR spectrum is a one-component only spectrum.

Discussion

In streptozotocin diabetes the composition of cell membranes changes significantly. The cholesterol content in Golgi membranes, smooth microsomal membranes as well as in smooth endoplasmic reticulum, is lower than in control [1–3]. In Golgi membranes, the ratio of phospholipids to protein also increases by a factor of two [3], however, the total lipid to protein ratio does not change significantly. As reported by Faas and Carter [1] fatty acid unsaturation was also decreased in microsomal membranes as a result of lower desaturase activity (mainly $\Delta 6$ and $\Delta 9$ desaturase activities). All these composition changes influenced the membrane fluidity in the two opposite ways: the decrease of cholesterol contents resulted in an increase of membrane fluidity [29]; the decrease of fatty acid unsaturation in the presence of a high cholesterol level caused the membrane fluidity to decrease [13] and a increase of the phospholipid protein ratio increased the membrane fluidity [30]. All these changes in Golgi membranes composition are summarized in Table I.

We would like to explain the changes in Golgi membrane fluidity (measured as changes in maximum splitting of 5-SASL), during development of diabetes as a result of alterations in membrane composition reported above. It is likely, that in light diabetes the increase in membrane fluidity is mainly caused by a decrease of cholesterol content. According to Kusumi et al. [13], decrease of cholesterol content in liposomes made of unsaturated lipids (egg yolk PC, dioleoyl PC) greater decrease the maximum splitting values of 5-SASL at lower (10 °C) than at higher temperature (35 °C–40 °C). Similarly, the decrease of Golgi membrane fluidity in light diabetes, in comparison with control, is bigger at low temperatures than at the physiological temperature range. In advanced diabetes, the decrease in unsaturation of fatty acids is probably responsible for the decrease in membrane fluidity within the whole temperature range, compared with light diabetes. We can also

TABLE I

The total lipid, phospholipid and cholesterol to protein ratios in rat liver Golgi-rich membrane fractions from control and streptozotocin diabetes, as well as the rat liver microsomal desaturase activity

Group of rats	Total lipid- [a] protein ratio (mg/mg)	Phospholipid- [b] protein ratio (μmol P/mg)	Cholesterol- [b] protein ratio (μmol chol/mg)	Activity of microsomal [c] fatty acid desaturase	
Control	0.76 (0.65–0.90)	0.22 ± 0.18	0.26 ± 0.15	18:0 → 18:1 18:2 → 18:3	100% 100%
Streptozotocin-diabetic	0.82 (0.64–1.00)	0.54 ± 0.31	0.26 ± 0.06	18:0 → 18:1 18:2 → 18:3	11% 52%

[a] The mean values and minimal and maximal values (in parentheses) are given (present study).
[b] The values according to Kordowiak [3]. The mean values ± standard deviations are given.
[c] Data obtained by Fass and Carter [1].

claim, that in heavy diabetes the increase in lipid/protein ratio causes increased membrane fluidity. Gaffney [31] has provided evidence that 10% change in unsaturation of lipid hydrocarbon chains or 5% variation in the mole fraction of cholesterol or 20–30% change in the fraction of integral proteins in the membrane would change the maximum splitting in the ESR spectrum of 5-SASL by 1–3%. Our explanation would be more valid if all changes of membrane composition were measured as a function of the free sugar level in the blood or as a function of time after streptozotocin administration. Some support for our hypothesis came from the fact, that changes in the cholesterol level in Golgi membranes appeared on the 10th or 11th day after streptozotocin injection, for free blood sugar levels higher than 200 mg/100 ml, while the decrease in fatty acid desaturase activity occurred only between the 14th and 21st days after streptozotocin injection [1] when the free blood sugar level was greater than 300 mg/100 ml.

Model membrane measurements show that decreasing of cholesterol content causes an increase of membrane fluidity. No difference in cholesterol effects at low and high temperature was detected (Fig. 4) probably as a result in parallel alterations in phospholipid composition. This model disregards changes in unsaturation and length of fatty acids as well as the presence of cholesterol esters.

It is commonly accepted that membrane fluidity influences the activity of membrane-associated enzymes [32]. Also in streptozotocin diabetes the activity of certain enzymes is altered [16,33]. For example Holloway and Garfield [2] postulated that an increase in fluidity of the smooth endoplasmic reticulum membranes measured by lipid-soluble fluorescent probe contributes to the increase in glucose-6-phosphatase activity. The control mechanism of the membrane fluidity is multifactorial. Effects of some factors on membrane fluidity can accumulate or may neutralize each other. The physiological consequences of change in membrane fluidity are manifested by changes in activity of enzymes and have to be minimized by homeostatic mechanisms of the organism. It is likely, that these mechanisms are working during development of diabetes: after a marked decrease in Golgi membranes fluidity in advanced diabetes, the fluidity returns again to the control level in heavy diabetes. It should be pointed out, that the final effect is obtained at an membrane composition different from that in control while the fluidity remains the same only at the physiological temperature. In the same model of diabetes, the level of corticosterone and/or ketone bodies in the blood has shown a similar pattern of changes [34], however, in that case the alterations have been measured a function of time and not considered in relation to the free sugar level in the blood.

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