THE USE OF FLUORESCENT PROBES TO DETECT STRUCTURAL CHANGES IN MEMBRANES OF THE ENDOPLASMIC RETICULUM OF THE LIVER IN RATS KEPT ON AN ATHEROGENIC DIET

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It has become evident in recent years that the physical state of the lipid phase of biomembranes, which depends in particular on their cholesterol content, influences the function of membranes and may perhaps play an important role in the origin of atherosclerosis [1-4]. Nevertheless, the possibility of changes in the physical structure of membranes in vivo during adaptation of the body to external factors has been inadequately studied.

It was accordingly decided to study whether adaptive changes take place in the physical structure of membranes in vivo during exposure to physiological conditions: a change in the standard diet to a balanced, semisynthetic diet or to a diet rich in cholesterol.

EXPERIMENTAL METHOD

Male noninbred albino rats, after weaning, were fed on No. 1 granulated food with the necessary additives. When the body weight reached 120-150 g the animals of group 1 were transferred to a balanced semisynthetic diet (diet I) [5]: 60.7% potato flour, 22% casein, 10% plum oil, 4% mixed salts [6], 3% cellulose, 0.2% choline chloride, and 0.1% of a mixture of water-soluble vitamins in glucose [7]. Simultaneously the animals of group 2 were transferred to a similar diet with the addition of 0.5% cholesterol and 0.5% deoxycholic acid (diet II) [8]. At definite time intervals the rats were decapitated (five animals at each experimental point) and microsomes were isolated [9]. The content of protein, phospholipids, and cholesterol was determined as in the previous investigation [9], lipids being extracted by Folch's method [10]. Glucose-6-phosphatase activity was determined by the method in [11]. Liposomes were obtained by the ethanol method [12]. The properties of the fluorescent probes – pyrene, 1-anilinonaphthalene-8-sulfonate (ANS), and 4-dimethylamino-chalcone (DMC) – were described in [13-15]. Fluorescence of the probes was excited at 334, 365, and 404 nm respectively and measured as described previously [15].

EXPERIMENTAL RESULTS

As Fig. 1 (curves 1 and 3) shows, replacement of the ordinary diet by a balanced semisynthetic diet led to changes in the lipid composition of the microsomal membranes and in activity of the marker enzyme of the microsomes. Atherogenic diet II also led to changes in these parameters, but the effect was more marked in this case and occurred sooner (curves 2 and 4). Meanwhile, changes took place in the physical structure of the membranes, for the fluorescence of the membrane probes was altered (Fig. 1c-e). Atherogenic diet II also had a more marked effect than diet I on the probes (Fig. 1, Table 1).

It might be supposed that changes in the physical structure of the microsomal membranes recorded by fluorescent probes would be the direct result of enrichment of the membranes with cholesterol. To test this hypothesis, the behavior of these same probes was investigated in model lipid membranes (liposomes), differing in their cholesterol content. As Fig. 2 shows, cholesterol affected the intensity of fluorescence of all probes, and also changed the shape of the fluorescence spectrum of DMC and pyrene (this change can be characterized by parameters F_{497}/F_{546} and F_{470}/F_{400} respectively).

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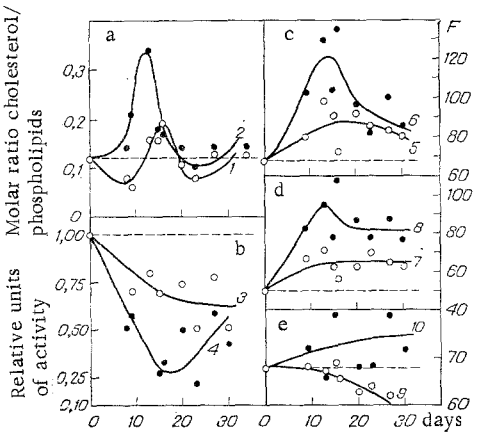

Fig. 1

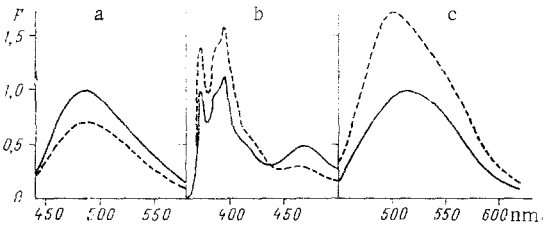

Fig. 2

Fig. 1. Effect of synthetic diet I (empty circles) and cholesterol-enriched diet II (filled circles) on molar ratio cholesterol/phospholipids (a), activity of glucose-6-phosphatase relative to its activity at t = 0 (b), on the intensity of fluorescence of ANS (c), pyrene (d), and DMC (e) probes in a suspension of microsomes. Abscissa, time (in days) after switching rats to diet I or II. F) Intensity at maximum of fluorescence spectrum (in relative units) measured at concentrations of probes of 15 μM and of microsomal protein of 0.5 mg/ml.

Fig. 2. Fluorescence spectra of ANS (a), pyrene (b), and DMC (c) in suspension of liposomes composed of egg phosphatidylcholine (0.5 mg phospholipid/ml), not containing (continuous lines) and containing (broken lines) 0.33 M cholesterol. Concentration of probes 15 μM.

TABLE 1. Changes in Parameters of Fluorescence of Probes in Suspension of Liver Microsomes of Rats Kept on Qualitatively Different Diets (M ± m)*

Fluorescent probe	Parameter of fluorescence	Diet I	Diet II	P‡
ANS	F_{470}	100† ±4	124±9	<0,02
DMC	F_{497}	100† ±1	107±3	<0,1
	F_{497}/F_{546}	0,75±0,01	0,76±0,01	>0,1
Pyrene	F_{400}	100† ±8	134±17	<0,01
	F_{470}/F_{400}	0,65±0,08	0,60±0,11	>0,1

*Mean results of eight independent measurements made in the course of 30 days after switching the rats to diet I or II are given.
†Mean value of this parameter in the case of diet I was taken as 100.
‡Significance of differences between effects of diet I and diet II.

The reaction of the probes in model membranes and microsomes can now be compared. The increase in the intensity of fluorescence of DMC and pyrene in microsomes, particularly marked in the case of the atherogenic diet (Fig. 1d, e; Table 1), can be the direct result of the increase in the cholesterol content, for it took place in liposomes also (Fig. 2b, c). Meanwhile, the increase in fluorescence of ANS in the microsomes (see Fig. 1c and Table 1) did not correlate with the decrease in fluorescence in the liposomes (Fig. 2a); probably the change of diet not only caused an increase in the cholesterol content in the microsomes, but also possibly led to a decrease in the negative charge on their surface, to which ANS is highly sensitive [14]. Analysis of the shape of the spectra of DMC and pyrene also indicates that changes in the physical structure of the microsomal membranes were connected both with an increase in the cholesterol content and with certain other structural changes (possibly changes in the protein/ lipid ratio, modification of the lipids during their peroxidation, and so on).

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