Studies on serum lipoprotein in the neonatal period

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With 4 figures and 2 tables

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Introduction

The serum content of lipids in newborns is determined partly by the maternal-fetal transport (5, 6), and partly by the lipid synthesis by the newborn (30). Cholesterol seems to be formed in the newborn (30) but it may also pass the placental barrier (7, 40). On the other hand, the low content of unesterified fatty acids in serum of newborns and the high content of these acids in pregnant women supports the theory that free fatty acids cannot pass the placenta. This may be explained by the binding of fatty acids to albumin (2, 31). This theory is furthermore supported by data indicating that the human fetus covers its energy requirement by carbohydrate (41). Corresponding to this the newborn have low levels of serum lipoproteins (33) and cholesterol (23, 39).

The determination of lipids and lipoproteins in the newborn period by means of the uptake of lipophilic dyes applied to serum on filter paper, has given contradicting results with regard to the time dependent changes in the serum lipoproteins (1, 12, 22, 33, 34). Furthermore, older paper electrophoretic methods (32, 33) do not make it possible clearly to separate and identify the pre-β-lipoprotein from the main fractions consisting of α-1- and β-lipoprotein, as well as the chylomicrons. We have therefore found it relevant to elucidate the neonatal changes in serum lipoproteins in more detail, both by means of recent methods enabling an optimal separation of serum lipoproteins (15) and also by the recently developed quantitative radial immunodiffusion technique, which in the present paper has been used for quantitative estimates of β-lipoprotein (10). This method seems more direct and accurate than the indirect assay based upon the determination of serum cholesterol (23, 39).

Materials and methods

Patient material

The sera from newborns and their mothers all originate from the neonatal and obstetric departments, Rigshospitalet, Copenhagen. The blood samples were taken 1–2 hours after the morning meal. At birth, blood was taken from the mothers by puncture of a cubital vein and from the newborn as cord blood. After birth the blood was taken from the infants as heel blood. The blood was centrifuged after coagulation and the serum samples were analysed within three hours.

Data from 18 children with a birth weight above 2500 g were compared with data from 7 children with birth weight below 2500g. All children were healthy and thriving well, they
were fed mothers milk supplemented with "half skimmed milk" Eledon (E). The data of these two groups were finally compared with those from 3 children with birth weight above 2500g fed exclusively, (E).

**Chemicals**

Unless stated otherwise, the chemicals used were of the highest obtainable purity form British Drug Houses, Dorset, England.

**Methods**

Separation and semi-quantitative estimation of serum lipoproteins was made by electrophoresing the serum proteins in albumin containing agar-agarose-gel. (35) (0.26 g agarose + 0.30 g DIFCO-special Noble agar (DIFCO, Ltd., Chicago, USA) + 0.25 g bovine albumin (Berlingwerke, Marburg an der Lahn, Germany) / 100 ml buffer). The agar and agarose were suspended in 0.05 M Na-barbital buffer (pH 8.6) and slowly heated in a water bath to 95°. After complete solubilization of the agar the mixture was cooled down to 45° and albumin was added during agitation. The homogeneous mixture was cast on microscope slides (1 x 7 cm), 1 ml per slide. In order to avoid migration of the proteins between the agar-gel and the glass surface, the slides were coated with aqueous agar prior to application of the electrophoretic gel. This was done by pouring a 95° warm aqueous agar solution (0.50 g DIFCO special noble agar / 100 ml distilled water) on the slides (about 1 ml per slide). After drying off the slides at room temperature (22°) the slides were coated with a thin, firmly attached layer of agar and they were now ready for casting the electrophoretic medium (vide supra). The electrophoretic slides were stored at 4° until used.

A systematic study of the optimal conditions for separation of serum lipoproteins in the electrophoretic equipment recommended by Wieme (45), which allows fast separation below light petrol ether, revealed that:

1. the light petrol ether did not disturb the electrophoretic separation and distribution of lipoproteins if the transverse through (1.5 cm x 1 mm) for the applied serum sample (20 μl) was sealed prior to electrophoresis with a 50° warm agar-buffer.

2. the maximal separation of alpha-, pre-beta, beta- and chylomicrons was obtained by application of the sample 1 cm from the cathodic edge, i.e. 2 mm from the cathodic agar block of Wieme's apparatus (43).

3. the electrophoretic time was 32 min. at 7.5 volt per cm in a 1.0 mm thick agar layer.

After an electrophoretic separation for 32 min. at 28°, the slides were fixed for 5 min. in a mixture of 70 vols. ethanol + 25 vols. distilled water + 5 vols. glacial acetic acid. Hereafter, the slides were dried below filter paper at room temperature (22°). The lipoproteins were stained in a solution containing: 200 mg Sudan Black suspended in 100 ml 60% aqueous ethanol heated to 90°, cooled to 22° and filtered prior to use. (The staining solution was stable for one week). The background on the slides was finally destained (10 min.) in 50% aqueous ethanol.

The amount of staining material bound to the lipoprotein fractions was estimated by scanning the electrophoretic slides in a Vitatron scanning equipment consisting of photometer (UFD 100, Vitatron Ltd., Dieren, Holland), a scanning unit equipped with photomultiplier no 200/21, and slit-with 0.25 mm and finally recorder UR 100, equipped with an integration unit. The scanning was performed at 604 mm. By application of increasing dilutions of serum in 0.9% (w/v) NaCl and of increasing dilutions of purified beta-lipoprotein (vide infra), it was possible to demonstrate a linear relationship between peak-area and the reciprocal value of the dilution. However, this linear relationship showed a different slope for the different lipoprotein fractions (cf. fig. 1.) and furthermore, the chylomicron curve showed a typical asymptotic feature at higher concentrations of this fraction. The percentage distribution of the lipoprotein patterns visualized below, therefore give only approximate relative percentages but they can, in agreement with fig. 1. be corrected for difference in staining capability.