From the Neurochemical Institute, the Institute of Hygiene, University of Copenhagen and the Department of Pediatrics, University Hospital (Rigshospitalet) Copenhagen (Denmark)

Tracing and Treatment of Children with Essential Familial Hypercholesterolemia

By G. E. Andersen and J. Clausen

With 7 figures and 2 tables

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Essential familial hypercholesterolemia is characterized by hypercholesterolemia, xanthelasmas, tendinous and cutaneous xanthomas and early development of coronary disease (11, 18, 19). In Scandinavia this syndrome is also called *Harbitz-Müller's* disease. It is normally regarded as being transmitted as a simple dominant trait (14, 22). The heterozygous patients will develop ischemic heart disease during their forties (males) or during their fifties (females) (25). The rare homozygous individuals will develop ischemic heart disease already before the age of 20 (5, 11, 13). After the introduction of lipoprotein electrophoresis as a diagnostic tool, the disease is now normally called type-II hyperbetalipoproteinemia in the classification of *Fredrickson* et al. (8). However, quantitative assay of serum-cholesterol and serum-triglyceride is sufficient to diagnose this entity.

As it is theoretically possible that lowering of serum-cholesterol can delay the development of atherosclerosis in the coronary arteries, we have found it relevant to trace and treat the disease already during childhood. Hereby, it is our hope to demonstrate that the prophylactic dietary approach is easier to handle than a secondary dietary approach after the first heart-infarct during the forties or fifties, as it is well-known that it is easier to change the diet early in life than later.

Material and clinical methods

Patient material

A total number of 144 individuals have been examined. They fall into four groups:

1. 59 individuals from Pedigree III, VI, IX, XI and XIII of *Kornerup's* original 14 Danish families having essential hypercholesterolemia and xanthomatosis (14) (cf. fig. 1, 2, 3, 4, 5 and 6).
2. 40 relatives of seven patients (Pedigree XIV–XXI) who from 1960 to 1970 were admitted to one of the medical departments in Copenhagen under the diagnosis: essential hypercholesterolemia and heart-infarct before the age of 45. (Essential hypercholesterolemia is here used synonymous with primary hypercholesterolemia as no explanatory factors such as diabetes, liver-, thyroid- or renal disease could be demonstrated. The diagnosis of heart-infarct was based on enzymatic, cardiographic or autopsy findings.) (Fig. 1, 2, 3, 4 and 5.)
3. 38 relatives of seven patients (Pedigree No. XXIII–XXV) (Fig. 1, 2, 3, 4, 5 and 6) from 1960 to 1970 were admitted to one of the medical departments in

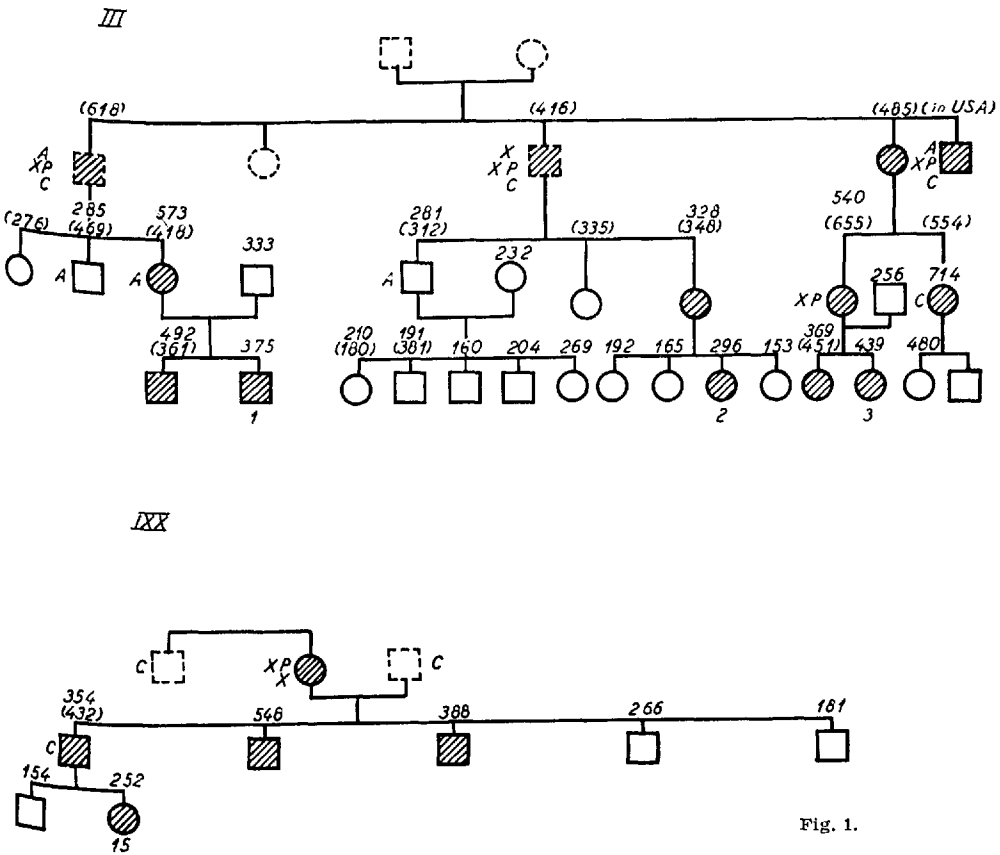

Fig. 1.

= normal man/woman
= hypercholesterolemic man/woman
= diseased man/woman
= sibship
= married couple

XP = palpebral xanthelasmas
X = tendinous or cutaneous xanthomas (palpebral xanthelasmas excepted)
C = angina pectoris or sudden death from coronary disease
A = arcus senilis

Interpretation of Legends in Fig. 1 to 6.
The symbols above on the left-hand side are used in the pedigrees. Figures in parentheses above the symbol indicate serum-cholesterol in milligrams per 100 ml in 1941–43 or 1954. Figures without brackets above the symbol indicate serum-cholesterol in milligrams per 100 ml in 1971. The figures 1–16 of the lower row refer to children who are considered to have familial hypercholesterolemia.

Copenhagen under the diagnosis: essential hypercholesterolemia and angina pectoris and/or heart-infarct after the age of 50. (The diagnosis angina pectoris was based on symptoms plus cardiographic changes.)

4. Three children of one patient who, from 1960 to 1970, was admitted to one of the medical departments in Copenhagen under the diagonsis: essential familial hypercholesterolemia and xanthomatosis (Pedigree XXII) (Fig. 5).

As many relatives of the patients were traced and examined as possible. All causes of death were verified from hospital records or death certificates. All the blood samples were taken after 13 hours fasting and analysed within four hours.

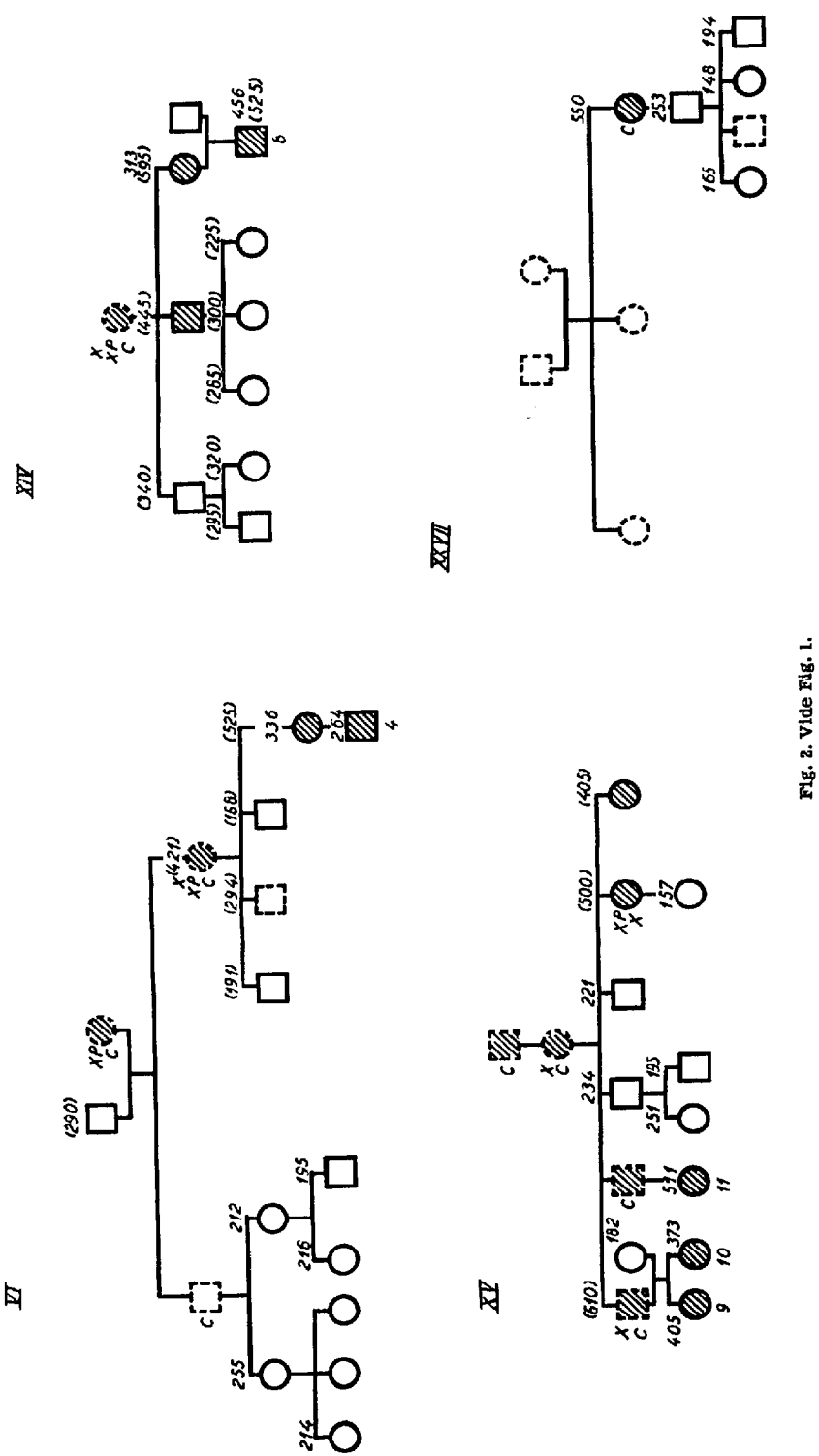

Fig. 2. Vide Fig. 1.

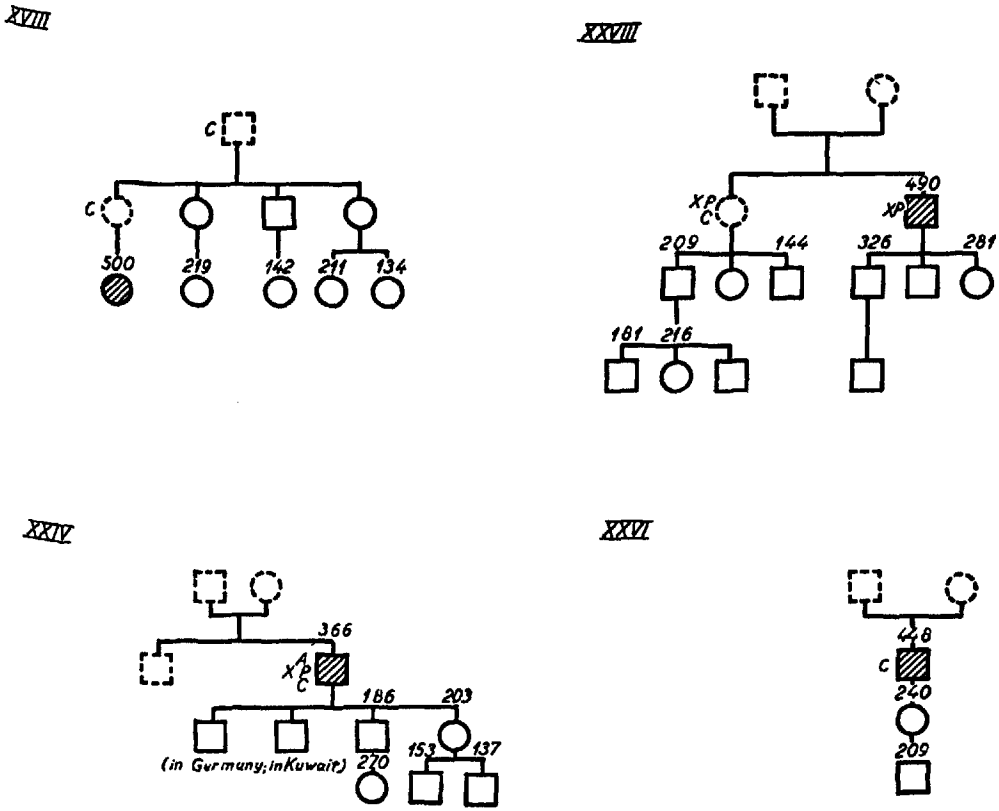

Fig. 3. Vide Fig. 1.

Normal material

13 healthy boys and 13 healthy girls were studied for serum levels of cholesterol, triglyceride and β-lipoprotein after 13 hours fasting.

Chemicals

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

Methods

Separation and semi-quantitative estimation of serum-lipoproteins were made by electrophorezing the serum proteins in an 0.5 % (w/v) agarose-gel (DIFCO, Ltd., Chicago, U.S.A.). The agarose was suspended in 0.05 M Na-barbital buffer (pH 8.6) and slowly heated in a water bath to 95°. The homogenous mixture was cast on a microscope slide (1 × 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.

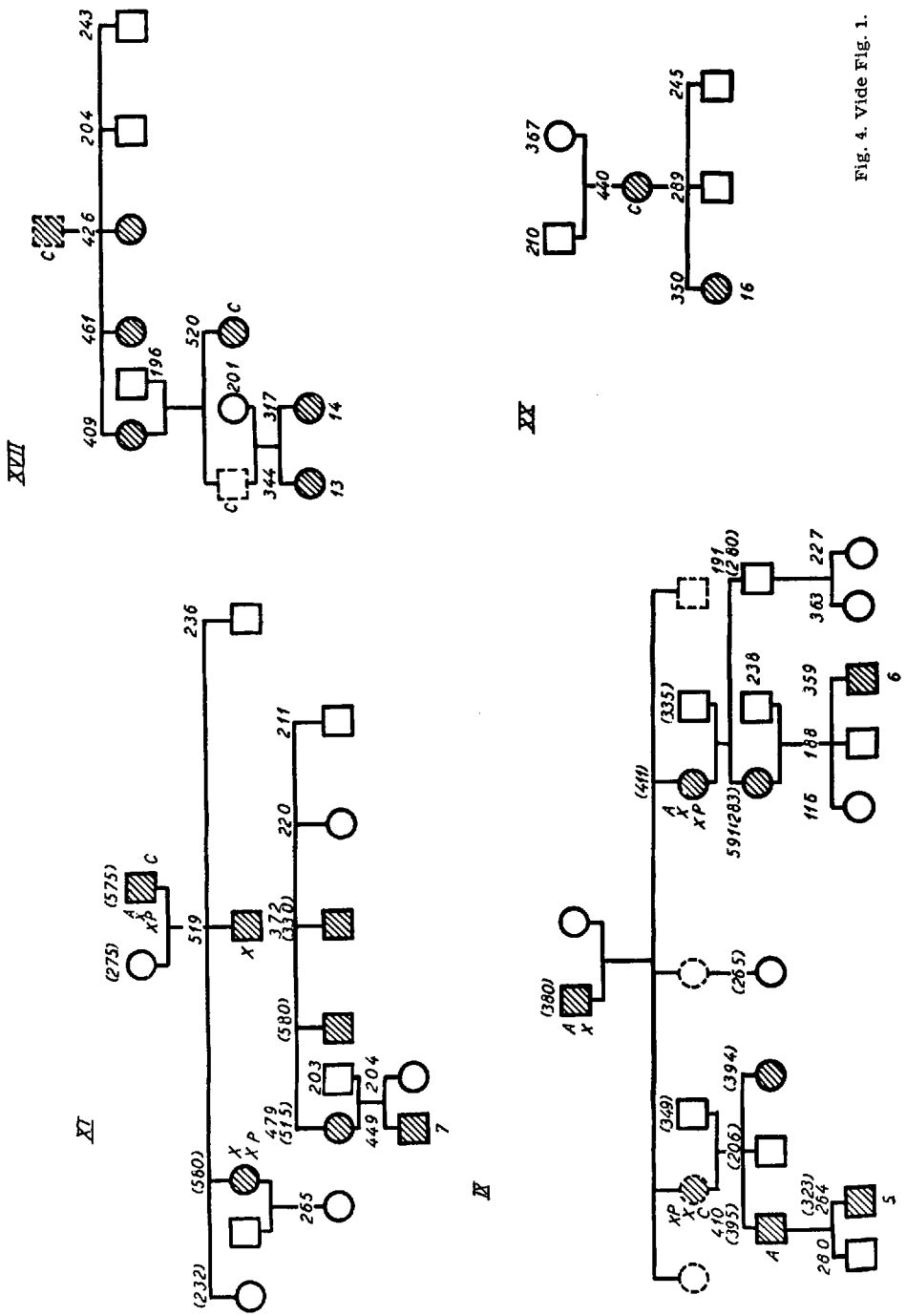

Fig. 4. Vide Fig. 1.

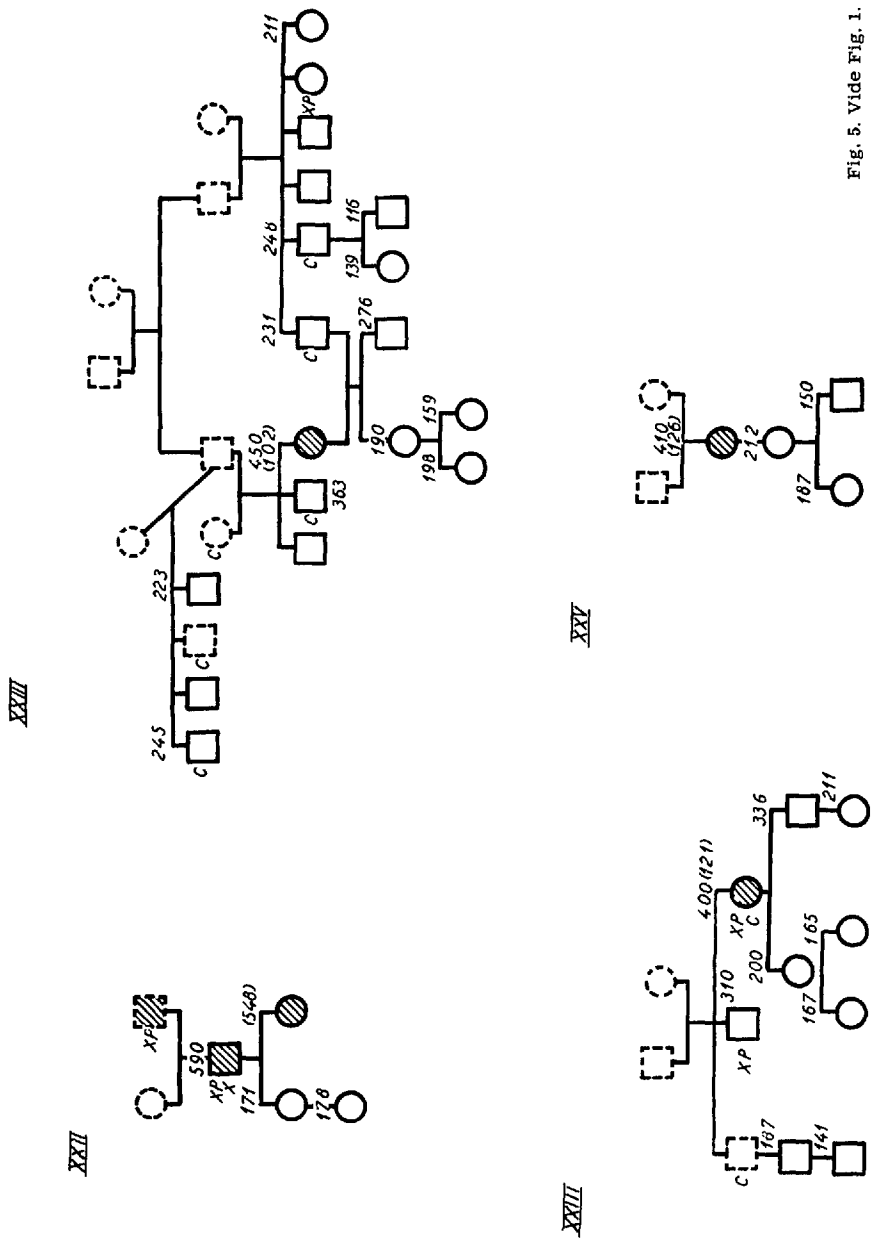

Fig. 5. Vide Fig. 1.

A systematic study of the optimal conditions for separation of serum proteins in the electrophoretic equipment recommended by *Wieme* (29), 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 trough (1.5 cm × 1 mm) for the

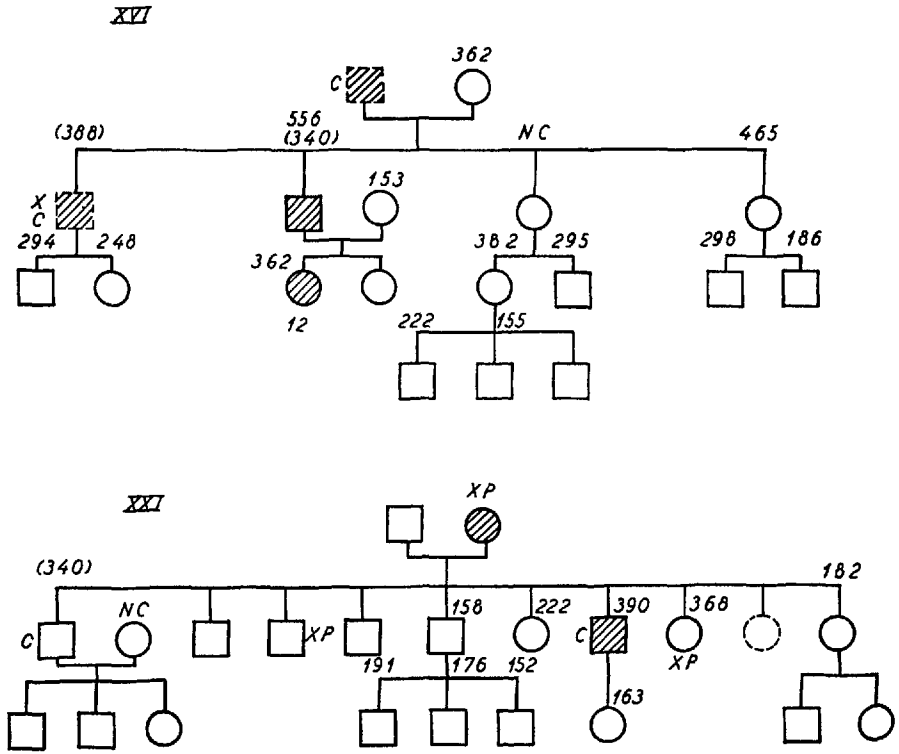

Fig. 6. Vide Fig. 1.

applied serum sample (20 μl) was sealed prior to electrophoresis with a 50° warm agar-buffer.

2. The maximal separation of alpha-, pre-β- and β-lipoprotein 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 (29).

3. The electrophoresis was made at 7.5 volt per cm in a 1.0 mm thick agar layer. In order to obtain an identical migration rate lateral to the sample trough, 2 μl of aqueous bovine albumin (Behringwerke, Marburg, W. Germany) 5 % (w/v) containing 1 % bilirubin was applied and electrophorezed together with the sample. Hereby, the yellow albumin zone of the lipoprotein was used as an electrophoretic marker. The electrophoresis was continued for around 32 min., until the albumin zone was 2.5 cm from the application trough. Hereby a clear separation of the lipoproteins was obtained. 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 Oil Red (or 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

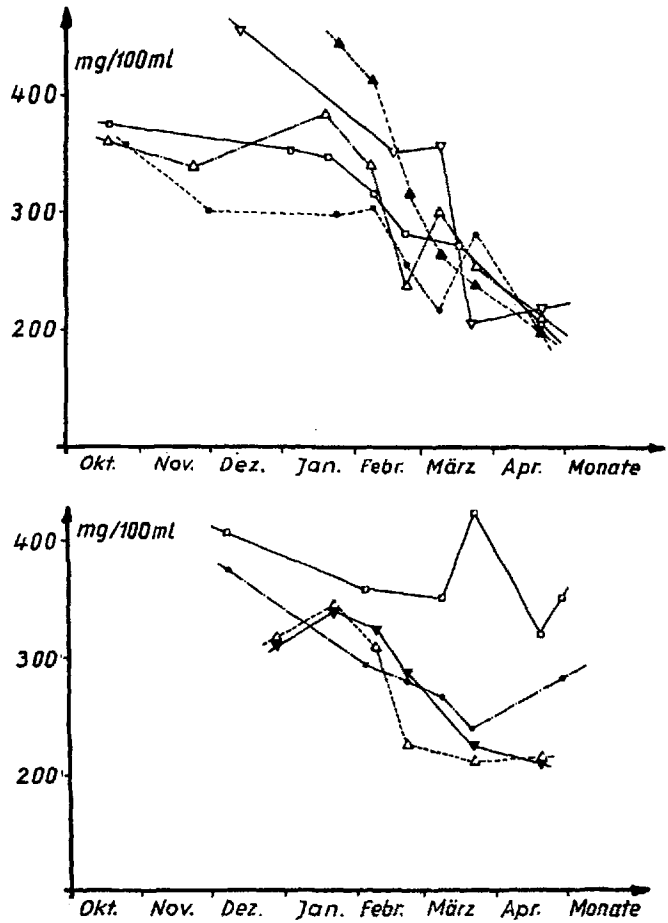

Fig. 7. Dietary induced changes in serum-cholesterol values of nine patients treated with a diet low in saturated fat, enriched with corn oil (for detail, see the text).
Ordinate: serum cholesterol mg/100 ml
Abscissa: time in month

Upper part:
patient K. D. no. 270366 diet from 25th January
patient K. A. no. 140465 diet from 6th January
patient C. T. no. 071061 diet from 25th January
patient J. F. no 160226 diet from 20th January
patient L. G. no. 070252 diet from 15th December

Lower part:
patient M. O. no. 070951 diet from 8th January
patient B. O. no. 220654 diet from 8th January
patient T. F. no. 100757 diet from 22nd January
patient G. F. no. 040361 diet from 22nd January

equipment consisting of photometer (UFD 100, Vitatron Ltd., Dieren, Holland), a scanning unit equipped with photomultipler 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 nm. By application of increasing dilutions of serum in 0.9 % (w/v) NaCl and of increasing dilutions of purified β-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 (9).

The percentage distribution of the lipoprotein patterns visualized below, therefore gives only approximate relative percentages.

Quantitative immuno-chemical estimation of β-lipoprotein

Was performed by means of the quantitative radial immuno-diffusion (4) under the experimental conditions described by *Clausen* (4). A standard solution of immuno-chemically pure β-lipoprotein was prepared from 100 ml pooled normal human serum by precipitation with dextran sulphate in the presence of $CaCl_2$ (3). The precipitation, mainly consisting of β-lipoprotein, was dissolved in 3.02 % (w/v) Na-citrate and finally dialyzed against 0.15 M Na-phosphate buffer (pH 6.5). The lipoprotein was re-precipitated, dialyzed once more and finally purified by filtration on Sephadex G-200. 10 ml dialyzed fraction (about 0.5 g protein/100 ml) was applied on a Sephadex G-200 column (40 × 2 cm). The column was made by heating the Sephadex G-200 powder for 2 min., to 90° in 0.15 M Na-phosphate buffer (pH 6.5) and then cooled to room temperature (room temperature, 22°). During the elution procedure the buffer remained 10 cm above the Sephadex layer. With the LKB fraction collector (LKB Ltd., Stockholm, Sweden) type 7000 Ultro Rac, equipped with a 10 ml Siphon stand, the β-lipoprotein was collected in the first 40 ml. The total protein content on every step of the isolation procedure was estimated by means of the *Lowry* method (15).

The purity of the β-lipoprotein was collected by micro-immuno-electrophoresis of 1.5 µl 5 % (w/v) liproprotein solution using either 100 µl polyvalent horse antiserum against normal human serum or 100 µl mono-specific anti-β-lipoprotein from rabbits. The experimental conditions used were those given by *Clausen* (4). The first mentioned antiserum was obtained from the central laboratory of the Dutch Red Cross, Amsterdam (batch No. PHO 13 P 10). The last mentioned anti-serum was made in the laboratory by protracted immunization of rabbits with 250 µl 5 % (w/v) lipoprotein solution (4). The anti-serum obtained by immunization for three months was mono-specific against β-lipoprotein when tested against normal human serum in micro-immuno-electrophoresis (4).

Initial studies revealed a linear relationship between the amount of β-lipoprotein applied (1.5 µl solution) in the radial immuno-diffusion plate and the diameter of the circular diffusion area. The recovery was estimated to 95 % when purified β-lipoprotein was added to normal human serum. The standard deviation (SD) was found to be 5 %.

Due to the common subunits (8) between pre-β- and β-lipoprotein, the immunological assay determines the total content of both lipoprotein fractions, hereafter designated "β-lipoprotein".

Assay of serum-cholesterol

Was made by the method of *Abell* et al. (1) using Sigma cholesterol standard (Ch-S), standard for chromatography (99.0 % pure), lot No. 400–7260). Initial studies revealed a recovery of cholesterol from human serum of 98 % and SD is 2 %.

Assay of glycerides in serum was made by the enzymic method of *Eggstein* (7). This method is based upon estimation of glycerol after alkaline hydrolysis of the serum glycerides. The recovery of triglycerides from human serum was assayed to be 96 % and SD to 5 %.

Statistical evaluation of data of determination of the standard deviation (SD). Also, the normal distribution of the normal data was controlled by estimation of the degree of "Skewness" (σ). The statistical evaluation was made on Olivetti's computer: Programma No. 101, using the programs Nos. 1.50 and 1.52 as made by *Williams* (30).

Results

A total number of 16 relatives under the age of 20 were found (9 between 5–10 years, 2 between 10–15 years and 5 between 15–20 years), all having the biochemical features typical of familial hypercholesterolemia, i.e. increased β-lipoprotein concentrations with increased serum-cholesterol and normal serum-triglyceride levels, and these abnormalities were also demonstrated in at least one of the parents. All children were heterozygous.

The great difficulty in judging the biochemical data obtained is the lack of normal values of cholesterol, β-lipoprotein and triglycerides in children. For Scandinavian children normal values of cholesterol have earlier been reported by *Kornerup* (14), *Rafstedt* (23), *Wamberg* (27) and *Sterky* et al. (26). As 9 of the 16 children were between 5–10 years, 13 healthy boys and 13 healthy girls between 5–10 years old were assayed for serum-cholesterol, -triglyceride and-β-lipoprotein (cf. table 1). Of the sixteen children with familial hypercholesterolemia, nine were selected for dietary studies, their parents being cooperative and interested in trying a treatment. In group 3 no children were found to have familial hypercholesterolemia. It is possible, that their parents with essential hypercholesterolemia are a minus variant in a normal distribution. In all cases mentioned below, the agarose-electrophoresis revealed a predominant β-lipoprotein fraction and no distinct pre-β-lipoprotein fraction. The nine individuals of group 2 are characterized by the following clinical and laboratory data (cf. fig. 1, 2, 3, 4, 5, 6).

Table 1. Serum lipid values of 26 normal children (age 5 to 10 years)

	serum-cholesterol mg/100 ml	serum-β-lipoprotein*) mg/100 ml
Normal boys	174.7 ± 42.0 r: 116 – 262	405.5 ± 64.8 r: 315 – 525
Normal girls	182.7 ± 40.1 r: 134 – 252	472.1 ± 117.4 r: 315– 655

	serum-lipoprotein distribution in %			serum-triglyceride mg/100 ml
	α-lipoprotein (2 fractions)	pre-β-lipoprotein**)	β-lipoprotein	
Normal boys	20.2 ± 7.0	29.7 ± 9.4	50.1 ± 8.7	108.8 ± 28.2 r: 66 – 160
Normal girls	17.5 ± 4.1	24.5 ± 8.8	58.1 ± 11.2	112.0 ± 28.0 r: 77 – 159

r: range of values

*) immuno-chemically determined therefore including the pre-β-lipoprotein.

**) Total stain in area between α- and β-Uroprotein fractions.

Pedigree III no. 1. C. T. (No. 071 061), 9 years, weight 33.5 kg.

Physical examination:	normal. No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	375 mg/100 ml
Serum-triglyceride:	127 mg/100 ml
Serum-β-lipoprotein:	905 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 5.8 % pre-β-lipoprotein: 13.2 % β-lipoprotein: 81.0 %

Serum-GPT: 10 U/l (normal 5–25 U/l), serum-LDH: 291 U/l (normal 104–370 U/l) serum-alkaline-phosphatase: 111 U/l (normal $<$ 100 U/l), serum-thymol: 1 MacLagan-units, plasma-fibrinogen: 0.25 g/100 ml (normal 0.20 to 0.40 g/100 ml), serum-creatinin: 0.8 mg/100 ml, urine-microscopy: normal, serum-PBJ: 6.5 μg/100 ml, serum-T_3-test: normal, serum-calcium: 9.8 mg/100 ml (normal 9.1–10.7 mg/100 ml), serum-phosphorous (inorganic): 4.0 mg/100 ml (normal: 2.5–4.6 mg/100 ml).

Fasting serum-glucose level:	102 mg/100 ml (method: Hexokinase).
Peroral glucose-load:	33.5 g of glucose anhydr. Increase to maximum 260 mg/100 ml, decrease prolonged and not below starting value. Abnormal glucose-load.

Pedigree IX no. 6. K. D. (No. 270 366), 5 years, weight 19.0 kg.

Physical examination:	normal: No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	359 mg/100 ml
Serum-triglyceride:	123 mg/100 ml
Serum-β-lipoprotein:	850 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 14.8 % pre-β-lipoprotein: 21.1 % β-lipoprotein: 64.0 %

Serum-GPT: 11 U/l, serum-LDH: 360 U/l, serum-alkaline-phosphatase: 82 U/l, serum-thymol: 0 MacLagan-units, plasma-fibrinogen: 0.25 g/100 ml, serum-creatinin: 0.8 mg/100 ml, urine-microscopy: normal, serum-PBJ: 6.3 μg/100 ml, serum-T_3-test: normal, serum-calcium: 10.1 mg/100 ml, serum-phosphorous (inorganic): 4.1 mg/100 ml.

Fasting serum-glucose level:	91 mg/100 ml
Peroral glucose load:	(19.0 g of glucose anhydr.) Normal.

Pedigree XI no. 7. K. A. (No. 140 465), 6 years, weight 19.5 kg.

Physical examination:	normal. No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	449 mg/100 ml
Serum-triglyceride:	87 mg/100 ml
Serum-β-lipoprotein:	900 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 15.2 % pre-β-lipoprotein: 3.8 % β-lipoprotein: 81.0 %

Serum-GPT: 10 U/l, serum-LDH: 290 U/l, serum-alkaline-phosphatase: 98 U/l, serum-thymol: 0 MacLagan-units, plasma-fibrinogen: 0.53 g/100 ml, serum-creatinin: 0.8 mg/100 ml, urine-microscopy: normal, serum-PBJ: 9.5 μg/100 ml, serum-T_3-test: normal, serum-calcium: 9.8 mg/100 ml, serum-phosphorous (inorganic): 4.6 mg/100 ml.

Fasting serum glucose level:	73 mg/100 ml
Peroral glucose load:	(After 19.5 g of glucose anhydr.) Normal.

Pedigree XIV no. 8. L. G. (No. 070252), 19 years. Weight: 67.5 kg.

Physical examination:	normal. No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	612 mg/100 ml
Serum-triglyceride:	152 mg/100 ml
Serum-β-lipoprotein:	905 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 8.4 %
	pre-β-lipoprotein: 15.8 %
	β-lipoprotein: 75.8 %

Serum-GPT: 10 U/l, serum-LDH: 160 U/l, serum-alkaline-phosphatase: 31 U/l, serum-thymol: 2 MacLagan-units, plasma-fibrinogen: 0.34 g/100 ml, serum-creatinin: 1.0 mg/100 ml, urine-microscopy: normal, serum-calcium: 9.2 mg/100 ml, serum-phosphorous (inorganic): 3.9 mg/100 ml.

Fasting serum-glucose level:	80 mg/100 ml
Peroral glucose load:	(After 67.5 g and glucose anhydr.): normal.

Pedigree XV no. 9. M. O. (No. 070951), 19 years. Father had xanthomas and died suddenly 34 years old of heart-infarct. His mother had xanthomas and died suddenly 50 years old of heart-infarct. Her father had xanthomas and died suddenly 51 years old of heart-infarct.
Weight: 53.0 kg.

Physical examination:	normal. No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	405 mg/100 ml
Serum-triglyceride:	215 mg/100 ml
Serum-β-lipoprotein:	714 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 2.9 %
	pre-β-lipoprotein: 9.6 %
	β-lipoprotein: 87.5 %

Serum-GPT: 10 U/l, serum-LDH: 219 U/l, serum-alkaline-phosphatase: 21 U/l, serum-thymol: 0 MacLagan-units, plasma-fibrinogen: 0.35 g/100 ml, serum-creatinin: 1.0 mg/100 ml, urine-microscopy: normal, serum-PBJ: 9.4 μg/100 ml, serum-thyroxine: 98.0 $\pm$ 3.5 nmol/l (slightly increased), serum-calcium: 9.1 mg/100 ml, serum-phosphorous (inorganic): 3.4 mg/100 ml.

Fasting serum-glucose level:	99 mg/100 ml
Peroral glucose load:	(After 53.0 g of glucose anhydr.): normal.

Pedigree XV no. 10. B. O. (No. 220 654), 16 years. Weight: 54.0 kg.

Physical examination:	normal. No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	373 mg/100 ml
Serum-triglyceride:	136 mg/100 ml
Serum-β-lipoprotein:	651 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 6.9 %
	pre-β-lipoprotein: 5.7 %
	β-lipoprotein: 87.4 %

Serum-GPT: 7 U/l, serum-LDH: 196 U/l, serum-alkaline-phosphatase: 19 U/l, serum-thymol: 1 MacLagan-units, plasma-fibrinogen: 0.28 g/100 ml, serum-creatinin: 1.0 mg/100 ml, urine-microscopy: normal, serum-thyroxine: 168 ± 3.5 nmol/l (slightly increased), serum-calcium: 9.9 mg/100 ml, serum-phosphorous (inorganic): 3.6 mg/100 ml.

Fasting serum-glucose level:	77 mg/100 ml
Peroral glucose load:	(After 54 g of glucose anhydr.): normal.

Pedigree XVI no. 12. J. F. (No. 160 226), 9 years. Father 38 years old, no arcus, xanthelasmas nor xanthomas, no heart symptoms, normal ECG. Father's data:

Serum-cholesterol:	340 mg/100 ml
Serum-triglyceride:	126 mg/100 ml

One brother who had xanthomas died suddenly 39 years old of heart-infarct.
Weight: 27.2 kg.

Physical examination:	normal. No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	362 mg/100 ml
Serum-triglyceride:	126 mg/100 ml
Serum-β-lipoprotein:	860 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 15.5 %
	pre-β-lipoprotein: 9.5 %
	β-lipoprotein: 75.0 %

Serum-GPT: 10 U/l, serum-LDH: 286 U/l, serum-alkaline-phosphatase: 108 U/l, serum-thymol: 2 MacLagan-units, plasma-fibrinogen: 0.28 g/100 ml, serum-creatinin: 0.7 mg/100 ml, urine-microscopy: normal, serum-PBJ: 7.2 μg/100 ml, serum-T_3-test: normal, serum-calcium: 9.8 mg/100 ml, serum-phosphorous (inorganic): 4.5 mg/100 ml.

Fasting serum-glucose level:	89 mg/100 ml
Peroral glucose load:	(After 27.2 g of glucose anhydr.): normal.

Pedigree XVII no. 13. T. F. (No. 100 757), 13 years old. Father died suddenly 34 years old of heart-infarct. No xanthelasmas nor xanthomas were described, no serum-cholesterol was taken. His sister has had two heart-infarcts 39 and 40 years old. No arcus, xanthelasmas nor xanthomas. Her serum-cholesterol: 520 mg/100 ml, serum-triglyceride: 1.93 mm/l. Her mother is 70 years old, never cardiac symptoms, no arcus, xanthelasmas

nor xanthomas. Serum-cholesterol: 409 mg/100 ml, serum-triglyceride: 286 mg/100 ml.
Weight: 40.0 kg.

Serum-cholesterol:	312 mg/100 ml
Serum-triglyceride:	92 mg/100 ml
Serum-β-lipoprotein:	835 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 23.3 %
	pre-β-lipoprotein: 0.0 %
	β-lipoprotein: 76.7 %

Serum-GPT: 7 U/l, serum-LDH: 290 U/l, serum-alkaline-phosphatase: 100 U/l, serum-thymol: 1 MacLagan-unit, plasma-fibrinogen: 0.29 g/100 ml, serum-creatinin: 0.8 mg/100 ml, urine-microscopy: normal, serum-PBJ: 6.6 μg/100 ml, serum-T_3-test: normal, serum-calcium: 9.6 mg/100 ml, serum-phosphorous (inorganic): 4.8 mg/100 ml.

Fasting serum-glucose level:	97 mg/100 ml
Peroral glucose load:	(After 40.0 g of glucose anhydr.): normal.

Pedigree XVII no. 14. G. F. (No. 040361), 10 years old. Weight: 26.0 kg.

Physical examination:	normal. No arcus, xanthelasmas nor xanthomas.
Serum-cholesterol:	317 mg/100 ml
Serum-triglyceride:	72 mg/100 ml
Serum-β-lipoprotein:	835 mg/100 ml
Serum-lipoprotein distribution:	α-lipoprotein: 26.4 %
	pre-β-lipoprotein: 0.0 %
	β-lipoprotein: 73.6 %

Serum-GPT: 13 U/l, serum-LDH: 330 U/l, serum-alkaline-phosphatase: 64 U/l, serum-thymol: 1 MacLagan-units, plasma-fibrinogen: 0.26 g/100 ml, serum-creatinin: 0.8 mg/100 ml, urine-microscopy: normal, serum-PBJ: 7.7 μg/100 ml, serum-T_3-test: normal, serum-calcium: 9.9 mg/100 ml, serum-phosphorous (inorganic): 4.4 mg/100 ml.

Fasting serum-glucose level:	79 mg/ml
Peroral glucose load:	(After 26.0 g of glucose anhydr.): normal.

Treatment

Little has been published about treatment of familial hypercholesterolemia in children. Following the principles of *Segall* et al. (24) the above-mentioned 9 patients were put on a diet containing less than 15 g of saturated fat daily. In order to avoid the necessity of adding too much carbohydrate and in order to maintain an adequate caloric intake, also to improve the palatability of an otherwise low fat diet, a free amount (on an average 20–30 g per day) of corn-oil (63 % linoleic acid) and dietary vegetable-oil margarine (45 % linoleic acid) were given (on an average 16 g linoleic acid per day).

The calories were supplied as 65 % carbohydrate, 25 % protein, 4 % saturated fat and 6 % polyunsaturated fat. The diet was introduced at home, as the parents of these 9 selected cases were keenly interested in

Table 2

Serum values in mg/100 ml

Pedigree III – 1 C.T.								
Cholesterol	375 (27/10)	354 (5/ 1)	347 (21/ 1)	316 (9/2)	285 (23/2)	272 (17/3)	300 (20/4)	258 (22/6)
Triglyceride	127	248	233	95	391	186		
β-lipoprotein*)	905	775	835	715	750	750	725	750
Pedigree IX – 6 K.D.								
Cholesterol	359 (27/10)	303 (1/12)	300 (25/1)	304 (9/2)	257 (23/2)	218 (9/3)	281 (24/3)	205 (20/4)
Triglyceride	123	112	73	100	107	83	112	
β-lipoprotein	850	900	755	835	750		825	800
Pedigree XI – 7 K.A.								
Cholesterol	449 (8/12)		412 (12/2)	332 (24/2)	227 (9/3)	240 (24/3)	200 (20/4)	
Triglyceride	87		103	90	106	73		
β-lipoprotein	900		975	880	880	850	820	
Pedigree XIV – 8. L.G.								
Cholesterol	612 (13/10)	456 (15/12)	352 (18/2)	356 (9/3)	207 (23/3)	216 (20/4)		
Triglyceride	152	107	149	179	127			
β-lipoprotein	905	835	880		850	870		
Pedigree XV – 9 M.O.								
Cholesterol		405 (8/12)	297 (5/2)	359 (23/2)	350 (9/3)	421 (22/3)	318 (20/4)	340 (22/6)
Triglyceride		215	209	176	175	190		
β-lipoprotein		875	950	975	975	950	900	
Pedigree XV – 10 B.O.								
Cholesterol	373 (14/9)	373 (8/12)	292 (5/2)	279 (23/2)	266 (9/3)	239 (22/3)	281 (29/4)	287 (22/6)
Triglyceride	136	170	160	213	150	203		
β-lipoprotein	651	700	735	700	750	765	750	725
Pedigree XVI – 12 J.F.								
Cholesterol	362 (20/10)	342 (24/11)	384 (20/1)	341 (8/2)	262 (23/2)	302 (9/3)	254 (24/3)	209 (20/4)
Triglyceride	126	97	112	105	149	120	99	
β-lipoprotein	860		975	835	975	950	900	900
Pedigree XVII – 13 T.F.								
Cholesterol	312 (29/12)	344 (22/1)	323 (9/2)	287 (23/2)	224 (23/3)	209 (20/4)		
Triglyceride	92	132	113	99	97			
β-lipoprotein	835	975	925	880	800	850		
Pedigree XVII – 14 G.F.								
Cholesterol	317 (29/12)	340 (22/1)	308 (9/2)	224 (23/2)	210 (23/3)	212 (20/4)		
Triglyceride	72	89	76	127	87			
β-lipoprotein	835	835	660	800	810	825		

*) immuno-chemically determined therefore including the pre-β-lipoprotein

the project. It was only difficult to keep this diet, low in saturated fat, during the first week. At the end of the second week, most of the problems were forgotten.

A mean reduction in serum-cholesterol of 139 mg/100 ml has been achieved so far (table 2). The fasting triglyceride changes are also given in table 2. The changes in cholesterol are shown in fig. 7. No constant change occurred in serum-triglycerides but the serum-cholesterol values continue, in all cases, to decline for more than three months (maximally 54 % in three months, case no. 070 252). This change in serum-cholesterol was not associated with corresponding changes in serum-β-lipoprotein. Minor intermittent rises in the serum-cholesterol values were found, explainable as deduced from interrogative information given by the patients, that they did not strictly follow the diet advised in these periods, but supplemented it with sweet things such as chocolate. Body-weight has remained constant within 1 kg under the treatment.

Discussion

The need to prevent cardio-vascular diseases is increasing in industrialised countries. Also pediatricians should take part in this prevention, being the first to eliminate atherogenetic factors. Several reports have compared serum-cholesterol and atherosclerosis and related atherosclerosis to dietary factors, such as high caloric intake, high consumption of saturated fat and suboptimal intake of essential fatty acids (2, 12, 13, 16, 20, 28). Thus, on the basis of these data, dietary experiments designed to prevent coronary thrombosis have been successfully carried out in adults. However, since atheromas may be formed prior to birth and in childhood (17), dietary experiments should be brought forward for groups of children with high risks of coronary thrombosis.

We have chosen a disease, known for many years to have a high rate of early heart-infarct, where it is possible to make the diagnosis in childhood. The present study revealed 16 children with biochemical abnormalities, having hypercholesterolemia and increased β-lipoprotein, in families of which one parent had developed ischemic heart disease before the age of 45. There was no case of pronounced secondary hypertriglyceridemia in these families.

In another group of families with the same biochemical lipid-patterns and ischemic heart disease developed clinically after the age of 50, no children were found having abnormal lipids.

These data support the idea, that all children of parents with ischemic heart disease before the age of 45 should have their serum-lipids determined and in the case of essential hypercholesterolemia, be put on a diet low in saturated fat, lowering their serum-cholesterol.

It was not difficult to make the children accept the diet, and it was particularly easy the younger the children were. In pedigree XV no. 9 the serum-cholesterol has risen in spite of a strict diet. We plan to put her on diet plus Cholestyramine. During the dietary treatment, serum-cholesterol decreased (maximally 54 %) for more than three months. This decline was not associated with a corresponding decline in serum-β-lipoprotein

(assayed together with the pre-β-lipoprotein). These findings may indicate that the basic abnormality in *Fredrickson* Type-II hypercholesterolemia is not in the cholesterol-metabolism, but linked to the turn-over of the apoprotein of the lipoprotein. This seems to be supported by the data of *Grundy* et al. (10) showing hypercholesterolemia to be associated with changes in pool size of cholesterol.

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Summary

144 individuals with personal or familial occurrence of coronary heart disease were studied in order to trace *Fredrickson's* type-II hyper-β-lipoproteinemia (familial hypercholesterolemia, *Harbitz-Müller's* disease).

18 families with familial hypercholesterolemia were traced among 99 individuals. These families belonged to patients having coronary attacks before the age of 45. 30 relatives of seven patients admitted to medical treatment of coronary heart disease after the age of 50 did not reveal occurrence of familial hypercholesterolemia.

Among the first group of families 16 children were found with hypercholesterolemia (9 aged 5–10 years, 2 aged 10–15 years and 5 aged 15–20 years) nine of these whose parents were cooperative concerning dietary treatment were selected for a detailed study of the disease (assay of serum-cholesterol, -triglyceride, -β-lipoprotein and -lipoprotein distribution) and hereafter treated for more than three months with a diet low in saturated fat. During this period the serum-cholesterol was nearly normalized in all patients (a mean decline in serum cholesterol of 139 mg/100 ml serum was estimated). No child did not respond on the treatment. This argues in favour of a tracing and treatment of this disease entity as soon as possible after birth in order to avoid vascular deposition of cholesterol. No change in total serum-β-lipoprotein was induced by the diet. This finding argues in favour of the disease being caused by a decrease in degradation of the apo-β-lipoprotein rather than by an abnormality of the cholesterol metabolism.

Zusammenfassung

144 Personen mit familiärem Vorkommen koronarer Herzattacken wurden untersucht, um *Fredricksons* Typ-II-Hyper-β-Lipoproteinämie (familiäre Hypercholesterinämie, *Harbitz-Müllers* Krankheitsbild) zu überprüfen. Unter 18 Familien mit hereditärer Hypercholesterinämie wurden 99 Personen beobachtet. Diese Familien gehörten zu Patienten, die vor dem 45. Jahr eine Koronarattacke gehabt hatten.

30 Angehörige von 7 Patienten wurden zur medizinischen Behandlung der koronaren Herzattacken angenommen. Nach dem 50. Jahr wiesen sie keine Vorkommen von familiärer Hypercholesterinämie auf.

In der ersten Gruppe der Familien wurden 16 Kinder mit Hypercholesterinämie gefunden (9 im Alter von 5–10 Jahren, 2 im Alter von 10–15 Jahren und

5 im Alter von 15–20 Jahren). Neun derselben, deren Eltern an der diätetischen Behandlung teilgenommen hatten, wurden für eine detaillierte Untersuchung des Krankheitsbildes (Versuche des Serum-Cholesterins, der Triglyzeride, der β-Lipoproteine und der Verteilung von Lipoproteinen) ausgewählt. Sie wurden danach, mehr als 3 Monate lang, mit einer Diät mit geringem Gehalt an gesättigtem Fett behandelt. Durch diese Periode hindurch wurde das Serum-Cholesterin fast normal bei allen Patienten (Mittelwert vom Serum-Cholesterin von 139 mg/100 ml Serum). Alle Kinder wiesen dieses Resultat auf. Dieses Resultat spricht stark dafür, die der Krankheit ausgesetzten Personen so schnell wie möglich nach der Geburt dieser Behandlung zu unterziehen, um die vaskuläre Anhäufung von Cholesterin zu vermeiden. Bei dieser Diät wurde keine Änderung im Total-Serum-β-Lipoproteingehalt bewirkt.

Die Befunde sprechen dafür, daß die Krankheit ihre Ursache in einem herabgesetzten Abbau des Apo-Lipoproteins hat.

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Authors' addresses:

Dr. *Jørgen Clausen,* Neurochemical Institute, Rådmandsgade 58
Dr. *Gunnar Eg Andersen,* Department of Pediatrics, Copenhagen (Denmark)