IMMUNO DIAGNOSTICS INFORMATIONS

LIPIDOPHOR

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Following a lecture given at the HDL Methodology Workshop
from March 12th to 14th, 1979 in San Francisco,
organized by the National Heart, Lung, and Blood Institute (NHLBI),
Bethesda, USA.

Electrophoresis of Plasmalipoproteins

Lecturer: Dietrich Seidel, M.D., Göttingen, FRG

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Electrophoresis of Plasmalipoproteins

Our present knowledge about the role and possible antagonistic function of the major lipoprotein fractions in atherogenesis, justifies great emphasis on the improvement of our analytical methodology in this field of clinical research.

The aim of this study was to optimize and standardize lipoprotein-electrophoresis for quantification of lipoprotein fractions and to determine the ratio of β-lipoproteins to α-lipoproteins.

This ratio we feel, is probably the most important parameter to determine in the future. The method developed and used is primarily based on agarose gel electrophoresis followed by polyanionprecipitation and densitometric measurement of lipoprotein bands. We originally published the method with dextrane sulphate and $CaCl_2$, as precipitating solution. But the precision of the method in particular for measurement of α-lipoproteins has markedly improved by using a two-step-procedure with precipitation solutions instead, which are part of the LIPIDOPHOR ALL IN 12-test-kit.

The conditions for the electrophoresis are specified in the leaflet of the test-kit.

The conditions for the polyanionprecipitation were as follows:

> Immediately after the electrophoretic separation, the gel plates are placed into a bath of developer solution 1 in order to get a fast fixation of the lipoproteins. After 1 hour this solution is replaced by developer solution 2 in which the plates are stored for two more hours. The difference between solution 1 and 2 is only in sodium chloride concentration. This is required to make sure that no globulines are present after the incubation period.

For standardization isolated and purified intact lipoprotein fractions were used. For the preparation of these standard fractions we started out with approximately 600 ml of plasma from 6 different subjects (two normals, two type II and two type IV hyperlipoproteinemics).

The isolated fractions were checked for purity by lipoprotein electrophoresis, electronmicroscopy, immunological techniques, ultracentrifugation and protein-lipid determination. Of each sample, 3 aliquots were lyophilized for 48 hours and their dry weight determined. Also 6 aliquots of each sample were electrophoresed at different concentrations (Fig. 1).

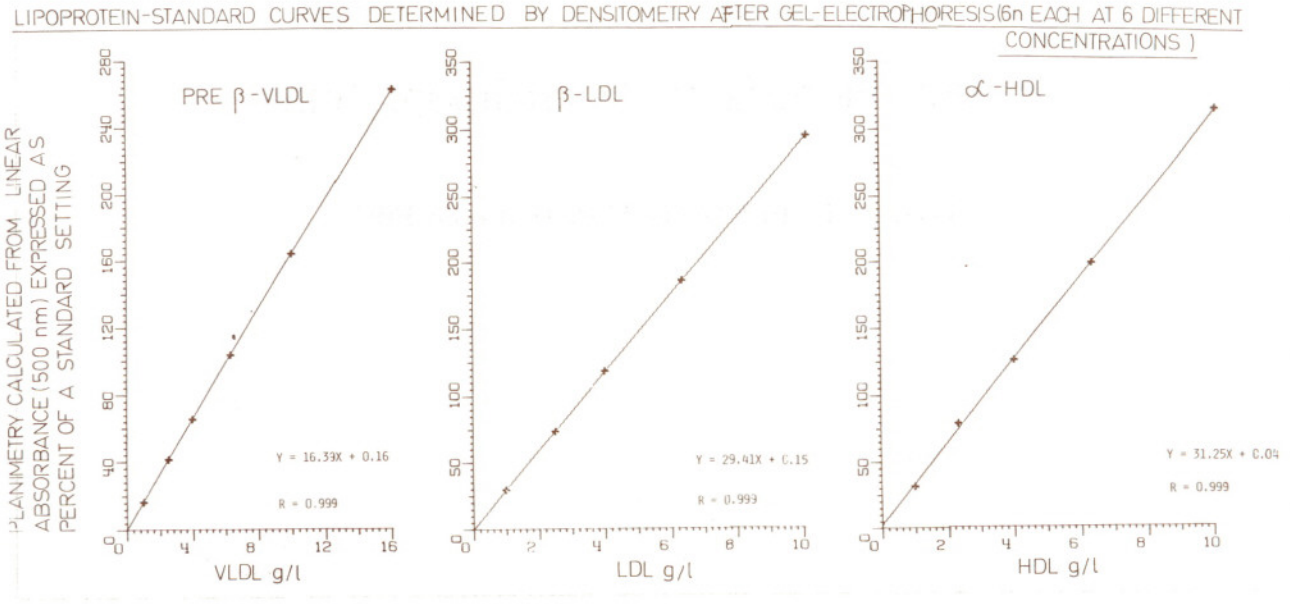

Fig. 1

Each experimental point of the standard curves represents the mean of samples from the 6 different subjects. The area integral is determined densitometrically at 500 nm. With these experiments we established the lipoprotein mass: area integral relationship for the 3 major lipoprotein fractions. From these correlation-curves exact values can now easily be calculated for each fraction.

In absolute terms these values are in part dependent on the experimental conditions and densitometer used.

Not so, however, and this is important to understand, their relative relationship, which was determined to be as:

α-lipoprotein :	β-lipoprotein :	pre-β-lipoprotein
1	0.94	0.52

The precision of the method for lipoprotein mass quantification which includes exact pipetting of the sample to the plate and densitometric scanning is satisfactory with a coefficient of variance between 3 and 4% for the three fractions. The low CV of 1.3% for determination of the β-lipoprotein : α-lipoprotein ratio is interesting to note. It indicates, that separation and densitometric scanning as such, is extremely precise.

We now tested, whether the relative cholesterol content of a lipoprotein fraction, separated by gel-electrophoresis, is sufficiently constant to allow calculation of lipoprotein cholesterol from lipoprotein mass and vice versa.

For this we used two sets of experiments. In a first series we calculated on the basis of the relative cholesterol content of the various lipoproteins (45% for β-lipoproteins, 15% for pre-β-lipoproteins and 18% for α-lipoproteins) the β-cholesterol, the pre-β-cholesterol and the α-cholesterol in different serum samples, on which the lipoprotein mass concentration had been determined as discussed. In addition the concentration of the total cholesterol was measured in these serum samples. Comparision of both, revealed a highly significant correlation (Fig. 2).

In the second set of experiments the lipoprotein-bands were cut off the plates after electrophoretic separation and densitometry. Thereafter, the lipoproteins were eluted from the slices and their cholesterol content directly measured enzymatically (Merck, Darmstadt). Again comparision of α-cholesterol measured directly from the bands versus α-cholesterol determined on the basis of densitometric scanning revealed a correlation coefficient of 0.9913, which is unexpectedly high and very satisfactory (Fig. 3).

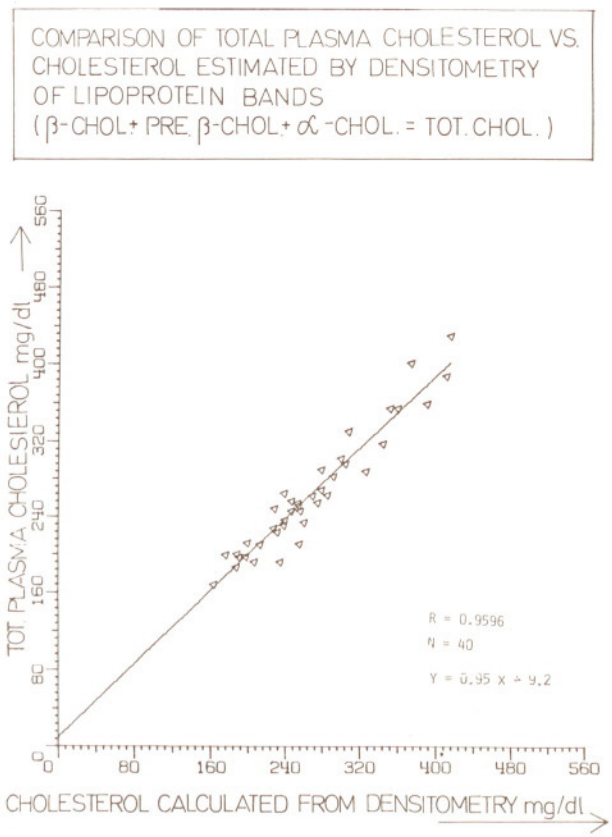

Fig. 2

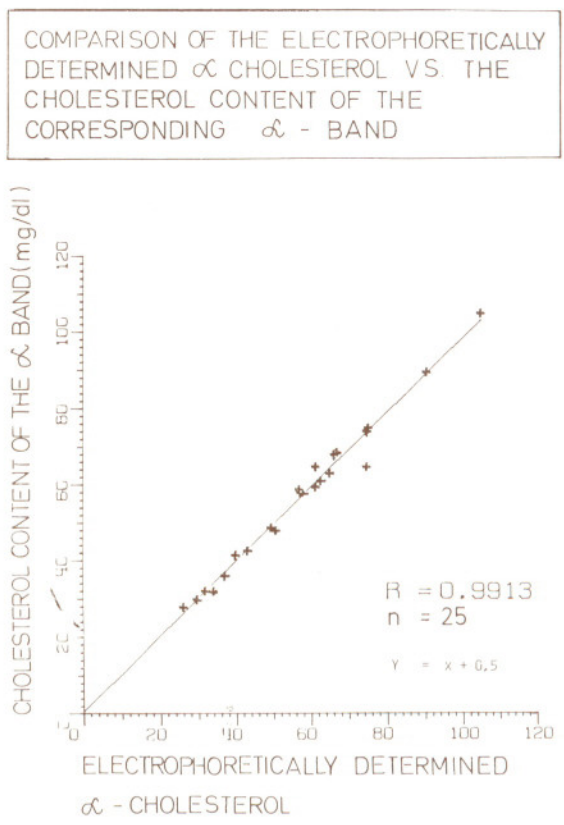

Fig. 3

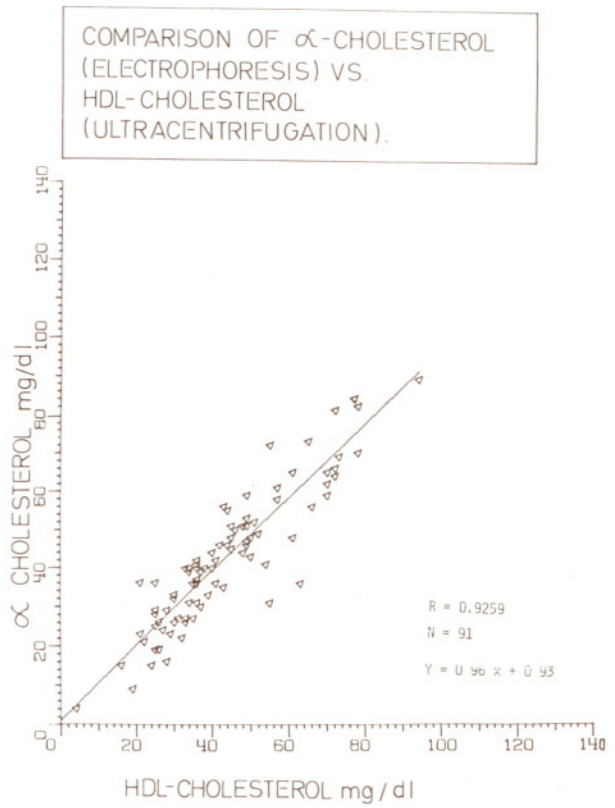

Fig. 4

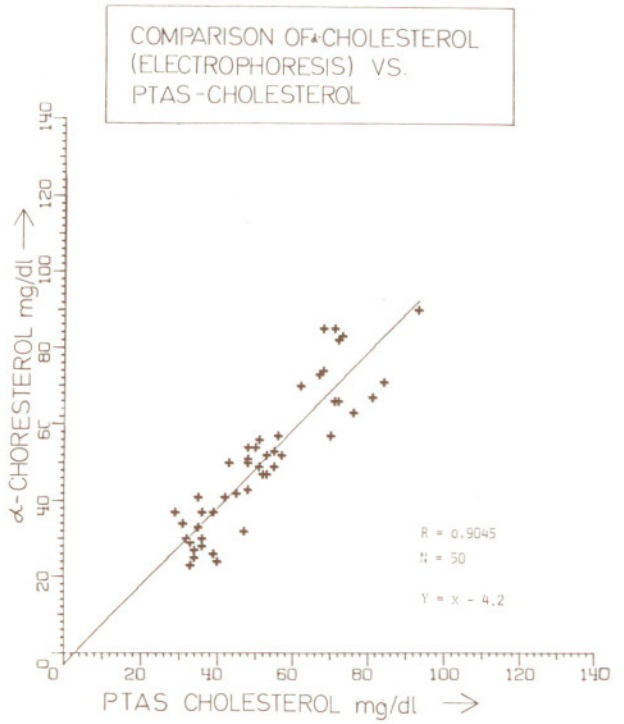

Fig. 5

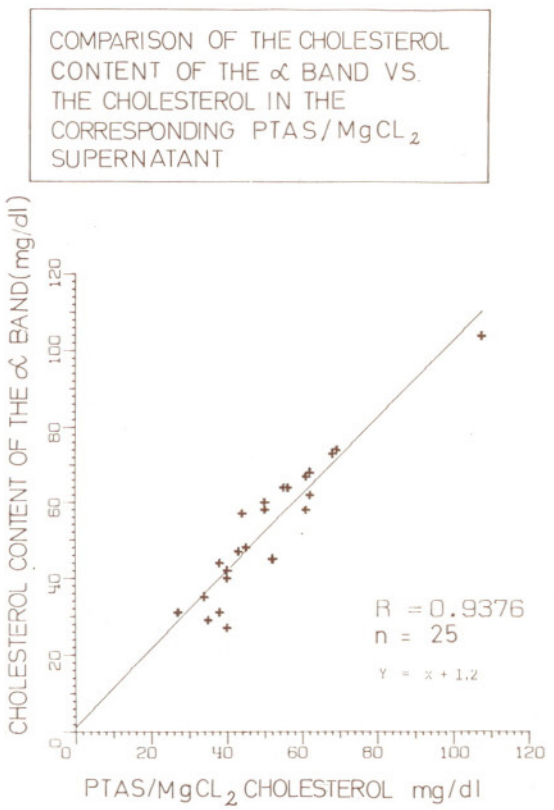

Fig. 6a

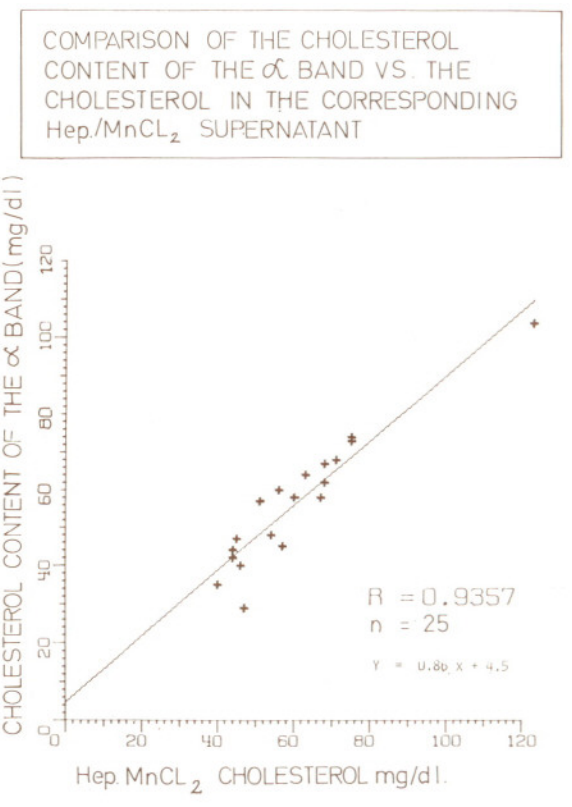

Fig. 6b

This correlation holds true also for the β- and pre-β-lipoprotein fractions quantitated in the two ways.

We feel, that the outcome of these experiments allows calculation of lipoprotein mass to lipoprotein cholesterol, at least for general use and as long as no abnormally composed lipoprotein fraction is present. It is therefore also possible to apply densitometry based on relative area measurement for lipoprotein quantification. The advantage of this type of densitometry, which is generally used for serum protein quantification, is its independence of exact pipetting and therefore provides higher precision.

A rather simple computer program allows calculation of lipoprotein concentration from determination of the relative area integrals of the fractions and from total plasma cholesterol measurement. The method is extremely precise and precision is an important requirement of a method for general use in clinical chemistry.

This holds, not only for measurement of the individual lipoprotein fractions, but also and in particular for the biologically important ratio of β-lipoprotein : α-lipoprotein.

I now like to present some data comparing the α-cholesterol determination described here, with data obtained from lipoprotein fractionation and subsequent cholesterol measurement by ultracentrifugation and polyanionprecipitation (Fig. 4, Fig. 5, Fig. 6 a and b). It can be seen, that the correlation between the methods tested, is always satisfactory.

We were now interested to see, whether the electrophoretically determined β/α lipoprotein ratio is rather stabil in a person or if it shows significant fluctuation during the day or between days.

It turned out, that the ratio is more stabil than the concentration of one of the two usually determined plasma lipids, triglycerides and cholesterol (Fig. 7 a, b and c).

Presently, we have two joint clinical trials running, which are designed to evaluate the possible diagnostic power of quantification of lipoproteins in the way presented here and in particular to relate the β/α-lipoprotein ratio to the likelihood of coronary heart disease. We have some belief, that in particular this parameter is not only more predictive than quantification of plasma lipids alone, but also more than measurement of the fractions.

COMPARISON OF THE β-LP: α-LP RATIOS DETERMINED IN SAMPLES TAKEN IN 1 HOUR INTERVALS FROM DIFFERENT SUBJECTS

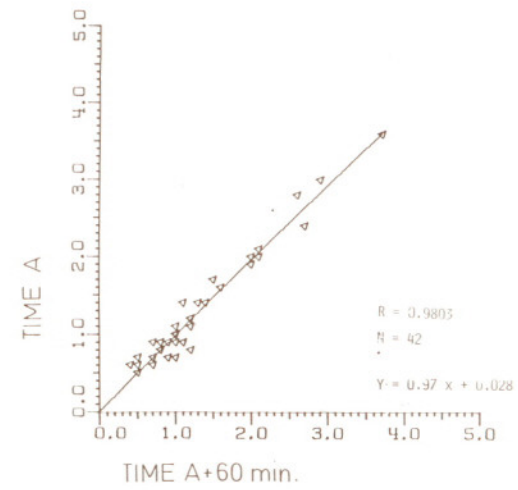

Fig. 7a

COMPARISON OF THE β-LP: α-LP RATIOS DETERMINED IN SAMPLES TAKEN IN 28 DAYS INTERVALS FROM DIFFERENT SUBJECTS.

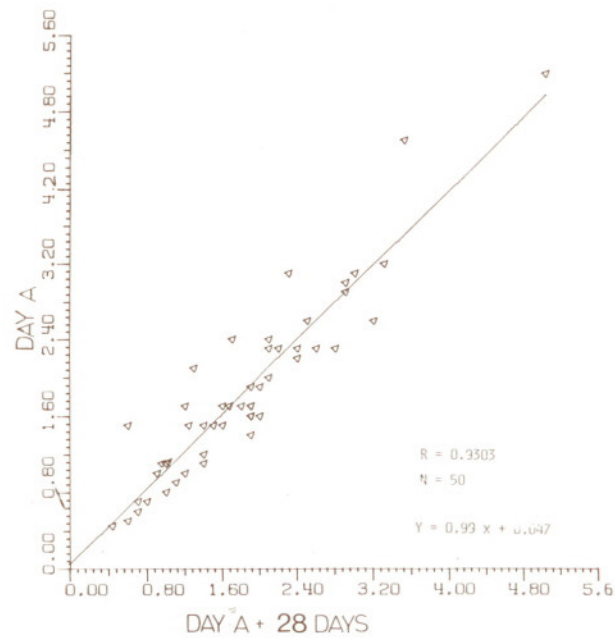

Fig. 7b

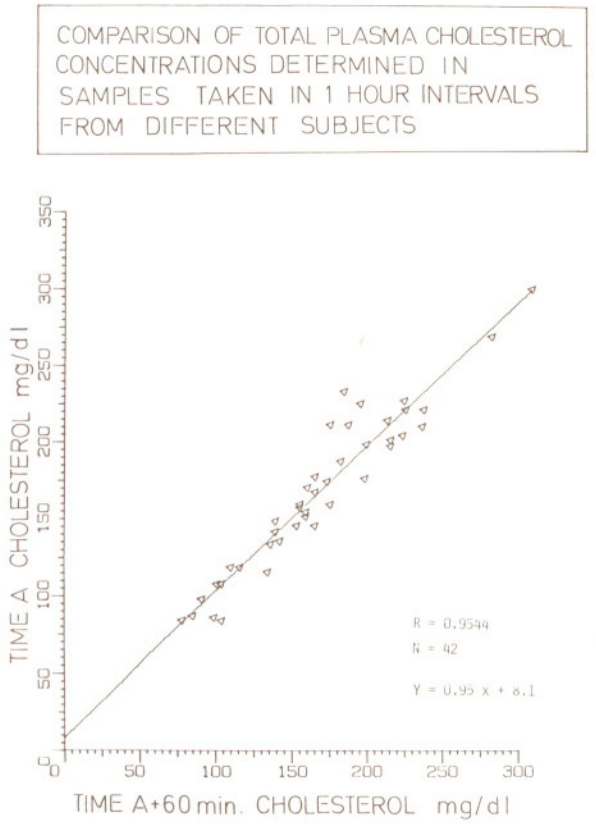

Fig. 7c

A meaningful application of methods used in clinical chemistry and even more so in epidemiology, should not only provide a high diagnostic power, but should also be sufficiently precise to allow conclusive information of the different lipoproteins in atherogenesis. I think, this holds in particular true for measurement of the β/α-lipoprotein ratio.

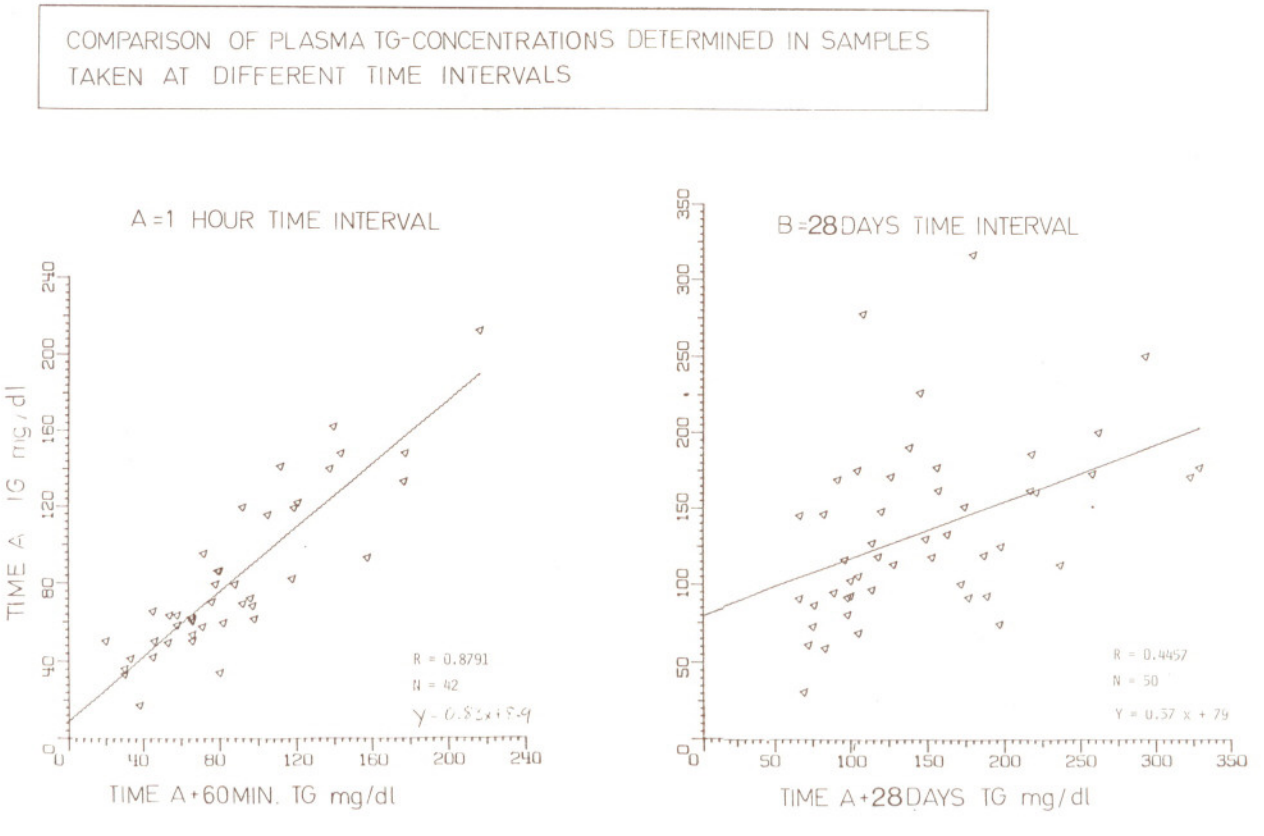

Fig. 7d

In summary:

1. Lipoprotein-gel-electrophoresis followed by polyanionprecipitation allows mass quantification of electrophoretically separated lipoprotein fractions by densitometry.

2. Measurement of cholesterol eluted from separated lipoprotein bands highly correlates with cholesterol calculated from the corresponding area integrals.

3. Total cholesterol correlates well with the sum of electrophoretically determined β- + pre-β- + α-cholesterol.

4. Correlation between α-cholesterol and HDL-cholesterol or precipitation supernatant cholesterol is satisfactory.

5. The β-lipoprotein : α-lipoprotein ratio remains rather constant in vivo and is more constant than plasma lipids.

6. Clinical data indicate that quantification of electrophoretically separated lipoprotein fractions and in particular determination of the β-lipoprotein : α-lipoprotein ratio may prove to be a valid clinical chemical tool.

7. The described method is simple and easy to perform and highly precise. It therefore fulfills an important requirement of a clinical chemical method.

References: On request available.