Heterogeneity of Human Plasma High Density Lipoprotein (36886)

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While it has been well established that the protein moiety of high density lipoprotein (HDL) of human and animal origin is heterogeneous physically, chemically and immunochemically (1–8), the total HDL as isolated in the preparative ultracentrifuge has been considered to be a homogeneous entity (9, 10) representing a family of species continuously varying in molecular weight and hydrated density (11, 12). HDL has been shown to separate into several components by starch gel electrophoresis (13) or polyacrylamide gel electrophoresis (14) or analytical gel electrofocusing (15); however, the analytical evidence does not distinguish between polymorphic forms and components of different chemical composition. Our studies using preparative isoelectric focusing suggest that total HDL consists of several discrete lipoprotein species.

Materials and Methods. The HDL was prepared and purified from fresh batches of plasma as described by Sodhi, Bhatnager and MacKenzie (16). It was labeled with ^{3}H-cholesterol (Amersham/Searle) by incubating a mixture of HDL and a suspension of ^{3}H-cholesterol in human serum albumin (17) at 4° overnight. The mixture was ultracentrifuged again to float the labeled HDL to remove the albumin. It was dialyzed against 0.15 *M* NaCl (pH 7.0) with several changes at 4° and against distilled water immediately prior to electrofocusing. Labeled HDL (50 to 200 mg) was electrofocused at 10° by the method of Vesterberg and Svensson (18) using an LKB, Model 8101, electrofocusing column (LKB Produktor AB, Stockholm Bromma 1, Sweden) and 2% carrier ampholytes (Ampholines) according to LKB Instruction Manual (19). Although a pH range of 3–10 was used initially, the 3–6 range was used in subsequent experiments. The applied power was maintained at 2.5 W during the 48 to 72 hr necessary to achieve equilibrium. Fractions of 1.5 to 2 ml were collected. The pH of each fraction was determined at 25° using a Sargent-Welch, Model NX, pH meter. Protein in each fraction was determined by measuring its optical density at 280 nm in a Beckman Model DB-G spectrophotometer or by the method of Lowry *et al.* (20) using bovine serum albumin (Mann Research Laboratories, Inc.) as standard, after 100-fold dilution to minimize the interference due to Ampholine (21). The ^{3}H activity was measured in a Packard Model 3003 TriCarb scintillation spectrometer. Aliquots from fractions were pooled to reconstitute HDL and this was compared with the aliquot from the HDL used for electrofocusing. The two preparations were subjected to electrophoresis on Agarose gel (22).

Results and Discussion. Figure 1A shows a typical separation pattern of HDL on preparative isoelectric focusing. The optical density profile (solid line) indicated the presence of discrete components with peaks or shoulders having specific isoelectric points. The protein distribution in individual components determined by the Lowry method closely paralleled the optical density curve. Results of several such experiments (Table I) show

TABLE I. Isoelectric Points of Major HDL Fractions.

Expt no.	p*I* values				
1[a]	3.85	4.32	4.64	4.97	5.23
2	3.95	4.30	4.75	4.95	5.20
3	4.00	4.25	4.65	4.95	5.15
4[b]	3.99	4.40	4.78	5.03	5.22

[a] A 3–10 pH range was used for this experiment only.

[b] HDL used for this experiment was tritiated.

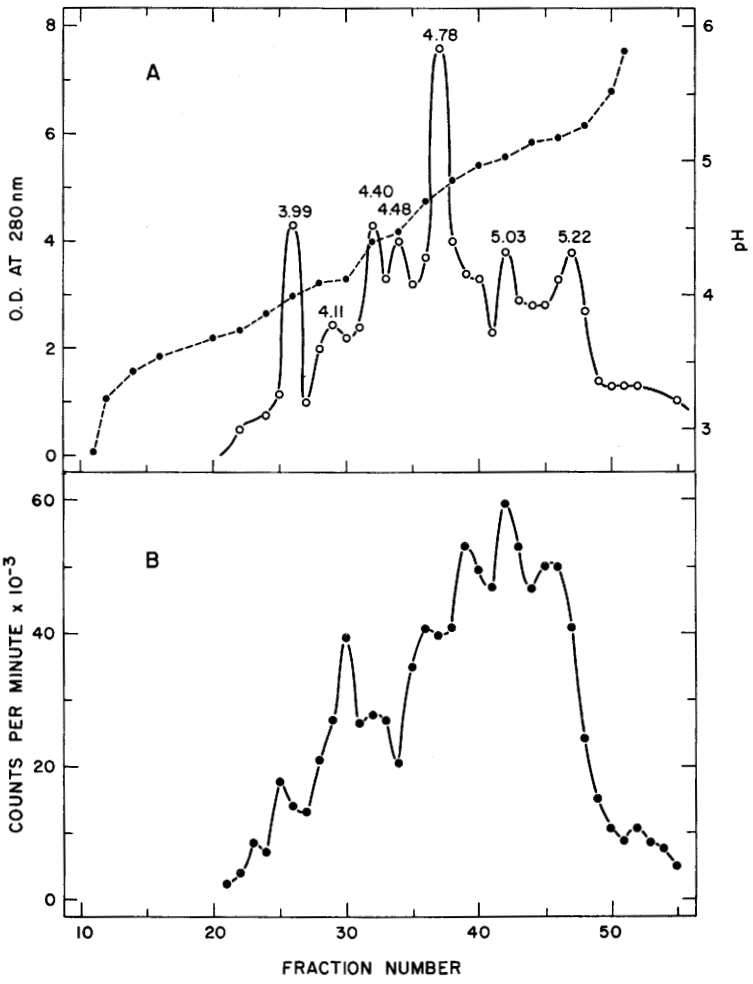

FIG. 1A. Optical density profile (—) and pH gradient (- -) obtained by preparative isoelectric focusing of plasma HDL. The optical densities were determined after appropriate dilution of the fractions. (B) ^{3}H-Cholesterol activity in counts per minute per 0.1 ml.

that the major components consistently focused in a narrow pH range and the separation pattern was reproducible. The minor differences in the isoelectric points of the components could be due to inherent differences in the sera (15). Samples applied to the column were recovered quantitatively in all experiments.

The results of electrofocusing HDL labeled with ^{3}H-cholesterol (Fig. 1B) indicated that (i) ^{3}H-cholesterol was distributed over the same pH range as protein, and (ii) the tritium:protein ratios were different in different components. It may also be observed from the figure that all protein peaks and tritium activity peaks do not coincide. This may be because the observed protein peaks may represent more than one component possibly containing different amounts of ^{3}H-cholesterol. Nevertheless these results suggest that the lipids were associated with protein.

Figure 2 shows the results on Agarose electrophoresis. The mobility of HDL reconstituted by pooling all fractions eluted after electrofocusing was identical to that of the starting material. We also determined that the presence of Ampholine, in the same concentration as that used for electrofocusing

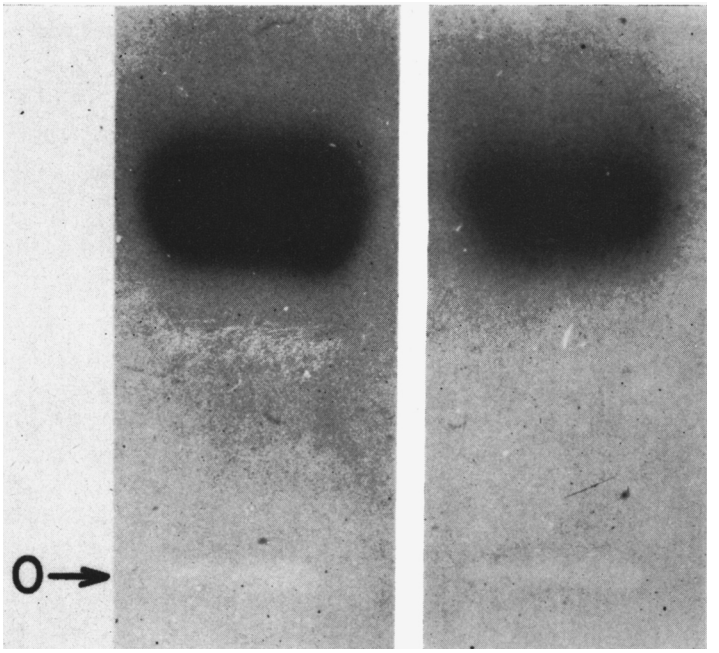

FIG. 2. Agarose gel electrophoregram of HDL (left) and 'reconstituted' HDL (right).

had no effect on the electrophoretic mobility of HDL. These results suggest that the method of separation did not denature the lipoprotein.

It is unlikely that different protein peaks represented artifacts arising either from a chemical reaction between the Ampholines and the proteins or from differential binding of proteins to ampholytes. It has been shown that basic Ampholines may interact with acidic proteins to produce artifacts (23) but we were dealing with acidic Ampholines and acidic proteins thus minimizing the possibility of such a chemical interaction. Moreover, similar results were obtained (Table I) whether the Ampholines used were entirely acidic (pH 3–6) or they covered both acidic and basic range (pH 3–10). Ampholines have also been shown not to bind proteins isolated by isoelectric focusing (24). Furthermore the process of labeling HDL with ^{3}H-cholesterol could not account for the heterogeneity observed since the results with labeled and nonlabeled samples were similar.

Based on the above observations, it is suggested that high density lipoprotein as isolated in the preparative ultracentrifuge is a heterogeneous mixture of several lipoprotein subspecies.

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1. Shore, B., and Shore, V., Biochemistry **8**, 4510 (1969).

2. Scanu, A. M., Toth, J., Edelstein, C., Koga, S., and Stilleer, E., Biochemistry **8**, 3309 (1969).

3. Albers, J. J., Albers, L. V., and Aladjem, F., Biochem. Med. **5**, 48 (1971).

4. Rudman, D., Garcia, L. A., and Howard, C. H., J. Clin. Invest. **49**, 365 (1970).

5. Koga, S., Bolis, L., and Scanu, A. M., Biochim. Biophys. Acta **236**, 416 (1971).

6. Scanu, A. M., Reader, W., and Edelstein, C., Biochim. Biophys. Acta. **160**, 32 (1968).

7. Shore, B., and Shore, V., Biochemistry **7**, 2773 (1968).

8. Shore, V., and Shore, B., Biochemistry **7**, 3396 (1968).

9. Shore, B., and Shore, V., Biochem. Biophys. Res. Commun. **1**, 228 (1959).

10. Irwin, E. C., PhD thesis, University of Alberta, Edmonton, Alberta, 1969.

11. Oncley, J. C., Biopolymers **7**, 119 (1969).

12. Scanu, A. M., *in* "Structural and Functional

Aspects of Lipoproteins in Living Systems" (E. Tria and A. M. Scanu, eds.), Chap. C3. Academic Press, New York (1969).

13. Scanu, A. M., and Granda, J. L., Progr. Clin. Pathol. **1**, 398 (1966).

14. Narayan, K. A., Narayan, S., and Kummerow, F. A., Nature (London) **205**, 246 (1965).

15. Kostner, G., Albert, W., and Holasok, A., Hoppe-Seyler's Z. Physiol. Chem. **350**, 1347 (1969).

16. Sodhi, H. S., Bhatnagar, R. K., and MacKenzie, S. L., Can. J. Biochem. **49**, 1076 (1971).

17. Porte, D., Jr., and Havel, R. J., J. Lipid. Res. **2**, 357 (1961)

18. Vesterberg, O., and Svensson, H., Acta Chem. Scand. **20**, 820 (1966).

19. Instruction Manual, LKB-Produkter AB, Stockholm-Bromma 1, Sweden.

20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. T., J. Biol. Chem. **193**, 265 (1951).

21. Page, M., Biochim. Biophys. Acta **236**, 571 (1971).

22. Noble, R. P., J. Lipid Res. **9**, 693 (1968).

23. Frater, R., J. Chromatogr. **50**, 469 (1970).

24. Hayes, M. G., and Wellner, D., J. Biol. Chem. **244**, 6636 (1969).

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