UNIVERSITY OF ILLINOIS

May 13 1987

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Guy Riccitelli

ENTITLED An In Vitro Study of Factors which May Alter the Production of High Density Lipoproteins in the Human Body

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science in Biochemistry

Ana Jonas
Instructor in Charge

APPROVED: Howard Hayes

HEAD OF DEPARTMENT OF Biochemistry

O-1364

AN *IN VITRO* STUDY OF FACTORS WHICH MAY ALTER THE PRODUCTION OF HIGH DENSITY LIPOPROTEINS IN THE HUMAN BODY

BY

GUY RICCITELLI

THESIS

for the

DEGREE OF BACHELOR OF SCIENCE

IN

BIOCHEMISTRY

College of Liberal Arts and Sciences

University of Illinois

Urbana, Illinois

1987

TABLE OF CONTENTS

INTRODUCTION

Lipids found in human plasma are transported as components of lipoproteins. In this form, lipids such as triglycerides, cholesterol and cholesterol esters, and phospholipids are associated with a number of proteins known as apolipoproteins. These lipoproteins are composed of a nutral lipid core surrounded by a layer of cholesterol, phospholipid, and apolipoprotein, oriented so that the polar head groups and amino acids are external.[1] The various lipoproteins are classified according to ultracentrifugal densities, and consist of Chylomicrons, Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), and High Density Lipoproteins (HDL).

There are dynamic interactions between all of these lipoproteins. Chylomicrons, the least dense of all the lipoproteins, are formed in the intestine, and function as a transport system for dietary triglycerides. VLDL are synthesized in the liver and small intestine and carry endogenous triglycerides before degrading into LDL. These LDL contain approximately 60% of total serum cholesterol for the most part in the form of a cholesterol ester rich core. A high level of circulating plasma LDL concentration has been associated with an increased risk of coronary heart disease[2].

While much research has been done on LDL, growing evidence of HDL's role in the prevention of atherosclerotic disease has increased interest into the study of this lipoprotein[3,4]. Nascent High Density

Lipoproteins are formed in several ways *in vivo*. They are formed, in part, in the circulation from surface components of Chylomicrons and VLDL, as well as in the liver and intestines. These nascent HDLs are less than 200 angstroms in diameter and approximately 45 angstroms thick. In some manner not yet fully understood, these nascent HDL particles are able to acquire cholesterol from cellular membranes.[5] This cholesterol is then acted upon by the enzyme Lecithin Cholesterol Acyl Transferase (LCAT). LCAT acts by transforming the cholesterol into cholesterol esters, which are then incorporated into the lipid core. This fills the core of the nascent particle, transforming it into a mature, spherical HDL particle. This uptake and conversion of cholesterol occurs so rapidly that, in normal plasma, only mature HDL may be detected. This mature particle continues to circulate, and transfers these cholesterol esters to LDL and VLDL which transport them to the liver, adrenal cortex, and gonads, where they may be metabolized or excreted[1,5].

The mechanism by which nascent High Density Lipoproteins are formed is, as yet, not fully understood. Discoidal, HDL-like micelles have proven to be an accurate model of nascent HDL particles and have been used in many experiments. Apolipoproteins A-I and C's may be isolated from human HDL by delipidation and separated by gel-filtration by passage through two Sephacryl 200 columns (90 x 2.5 cm each) in series. Apolipoprotein C's may also be prepared in a slightly different manner using plasma VLDL as a source as cited by Jonas[6,7]. In the following experiments, discoidal, micellar complexes will be used in a study of factors which may have an impact upon HDL production *in vivo* Two

series of *in vitro* experiments were performed in this study.

The first series of experiments was a study of product formation. A series of vesicle preparations were made up in order to illustrate what effect apolipoprotein C's might have when added to an Apo A-I / lipid vesicle system. Two controls were used in this experiment, a system into which only Apo A-I was added and a system containing only Apo C's. In addition, three other systems were set up containing both apolipoproteins in varying orders of addition. By comparison with the standards, the effects of the Apo C's upon the system may be seen. Lipid vesicles in this series of experiments were prepared by utilizing an ultrasonication technique similar to that used by Huang[8].

The second series of experiment used a different approach to the problem. In this series, rather than be concerned with a study of products, the main focus was a study of the kinetics of particle formation in the presence of various lipids modifying the lipid bilayer. Similar work has been done by Pownall *et al.*[9,10] and others[11,12]. This series of experiments will expand upon this work by studying the effects of palmitic acid, myristic acid, di-myristoyl phosphatidyl ethanolamine (DMPE), di-palmitoyl phosphatidyl ethanolamine (DPPE), di-palmitoyl phosphatidyl serine (DPPS), and cholesterol upon the spontaneous degradation by Apo A-I of multi-lamellar vesicles (MLV's) of di-myristoyl phosphatidyl choline (DMPC) into discoidal particles as noted by Jonas[7].

It should be noted that DMPC vesicles are not an entirely physiologically accurate model. Furthermore, they are somewhat

unstable in the presence of apolipoproteins.[13,14] They are used for several important reasons, however. First, the T_m of DMPC is only 23.9°C. Since the rate of degradation of particles is fastest at the gel-liquid crystalline transition temperature due to the increased permeability of the matrix[9], this temperature, near room temperature is very efficient to work with. Secondly, since only one type of lipid is present in DMPC vesicles, any change in the kinetics of reaction must have occured due to the addition of the alternate lipid. A number of different concentrations of the additional lipid were studied to analyze what trends were being generated.

EXPERIMENTAL ANALYSIS

I. Materials--

The phospholipids used in both series of experiments, egg-Phosphatidyl Choline (egg-PC), DMPC, DMPE, DPPE, and DPPS, were purchased from the Sigma Chemical Co. in the L-configuration at ≥98% purity. The cholesterol, as well as the long chain fatty acids, myristic acid (C_{14}), and palmitic acid (C_{16}) were also purchased from Sigma Chemical Co.. The ^{14}C-labelled cholesterol was obtained from New England Nuclear Co.. The Spectropore #3 and #4 tubing was obtained from Spectrum Medical Industries.

Apolipoprotein A-I and the mixture of apolipoprotein C's were prepared via the Jonas and Matz method[12], with an original reference to Edelstein *et al*[13]. Apo A-I and Apo C's were stored, lyophilized, at -20^{0}C. They were then resuspended in separate preparations in 3M guanidine hydrochloride (GuHCl) and dialized individually against a standard salt buffer consisting of : 0.01M Tris HCl, 0.15M NaCl, 1mM NaN_3, and 0.005% EDTA, at a pH of 8.0, and temperature of 4^{0}C. The sonications carried out in the first series of experiments were done on a Heat Systems Ultrasonic Sonicator. The ^{14}C-cholesterol was counted on a Beckman LS-3801 Liquid Scintillation Counter. The bovine serum albumin (BSA) and ferritin used in column calibration were obtained from Sigma Chemical Co. The column used in separating complexes was

packed with BioGel Agarose A-5m beads purchased from BioRad Laboratories. The reagents used in the Lowry and Chen assays[17,18,20] were obtained from suitable suppliers and were of reagent grade. The absorbance at 280nm of the samples was measured on a Beckman Model 35 Spectrophotometer. Standard equipment for both Gradient Gel Electrophoresis and SDS- Polyacrylamide Gel Electrophoresis were used for these analyses. In the second series of experiments, the decrease in light scattering intensity of the MLV's after the addition of Apo A-I was measured on a Perkin-Elmer MPF-3 Fluorescence Spectrophotometer.

II. Methods--

A. Preparation- The vesicles in the first series of experiments were formed by using an ultrasonication method similar to that pioneered by Huang[8] with an original reference to Saunders[19]. A series of five mixtures of egg-phosphatidyl choline (egg-PC) and cholesterol were made so that the final egg-PC concentration would be 8 mg/ml and the egg-PC/cholesterol molar ratio would be 100/1. Into each mixture of lipid, approximately 2.0×10^5 cpm's of ^{14}C-cholesterol were added. These lipids were mixed in a scintillation vial in a solution of (2:1) $CHCl_3$:MeOH and were dried down completely under pure nitrogen. Standard salt buffer was then added and the mixture vortexed for three minutes, until all traces of lipid had been removed from the sides of the

vial. This left a dispersion of cloudy, milky-white Multi-Lamellar Vesicles (MLVs).

This dispersion was then ultrasonicated under nitrogen at setting 6 for 3 minute bursts with a minute break between each burst. This procedure was continued for 20-30 minutes until the dispersion cleared and took on the pale blue color of suspended titanium. The dispersion was then centrifuged in a Beckman Model J21B Centrifuge at 15,000 rpms for one hour at 5^{0}C. The supernatant was removed from the titanium pellet and stored at 4^{0}C.

Prior to use in these experiments, separate solutions of apolipoproteins were made from lyophilized Apo A-I and a mixture of Apo-C's. These apolipoproteins were resuspended in 3M GuHCl and dialyzed extensively in a standard salt buffer. The concentrations of these proteins were checked by a modified Lowry assay [17,18] (for both apolipoproteins separately) and by a measurement of A_{280} (for Apo A-I. using an extinction coefficient of 11.5 cm^2/gm [21]).

The apolipoproteins were then incubated for three hours with the lipid vesicles. Molar ratios of 100:1 lipid : protein were used, with 2mg of Apo A-I being the amount from which lipid concentration was calculated. In cases where both Apo A-I and the mixture of Apo C's were added to the same reaction, equal 2mg amounts of each were added.

Five different solutions were prepared. In tube 1, a control, only Apo C's were added. In tube 2, another control, only Apo A-I was added. In tube 3, both Apo A-I and Apo C's were added simultaneously. In tube 4, Apo A-I was first added and the tube incubated for three hours; then

Apo C's were added and the tube incubated again for three hours. Tube 5 was treated similarly, only Apo C's were added and incubated prior to the subsequent addition of Apo A-1 and second incubation. All incubations were carried out at 37°C.

These five samples were then individually run down a BioGel A-5m column (2 x 60 cm), and a total of eighty 2ml fractions were collected from each. For each fraction, both the A_{280} and counts of ^{14}C-cholesterol were measured and several fractions were pooled around points of absorbance or cpm maxima. (See figures 1-5).

Since the main focus of the second series of experiments was the kinetics of protein:lipid interaction rather than the product formation, a different method of preparation was necessary. This method of preparation was begun similarly to that used in the first series, but the Multi-Lamellar Vesicles were not subsequently ultrasonicated. DMPC was used in this experiment rather than egg-PC for reasons noted above, and was mixed with a single additional lipid. The amount of this additional lipid was varied between 2-10%, and several lipids were introduced into DMPC MLV's in this way. Apolipoprotein A-1 stock solutions were prepared as noted before.

B. Absorbance- For each pooled fraction, a measurement of absorbtion at 280nm was made. The various aromatic amino acid residues present in the apolipoproteins will absorb at this wavelength. As a result, only

those fractions containing free or lipid-bound protein, as well as very large particles, should give a significant absorbtion. Further, a Lowry test, as modified by Markwell, was performed [17,18]. The results of these two tests should agree.

C. Measurement of Radioactivity- An aliquot of each pooled fraction was mixed into liquid scintillation fluid and placed into a Beckman Liquid Scintillation Counter as noted above. Each escaping electron has the potential to excite a molecule of fluorophore in the scintillation fluid. When this molecule relaxes it emits a brief flash of light which may be picked up and counted. For each fraction, a measurement of cpm's (counts per minute) of ^{14}C-cholesterol was made. Only those fractions containing lipid should give significant counts.

D. Phosphate Analysis- Each pooled fraction was analyzed by the Chen *et al*. [20] assay for organic phosphate. A standard curve for each day of measurement was set up by using inorganic phosphate standards. From this data, the fractions containing phospholipids may be identified and the phospholipid concentration calculated.

E. Column Calibration- Samples of BSA, ferritin, and phospholipid complexes of a known size were run through the BioGel A-5m column. From the positions at which they elute (as measured by A_{280} for the proteins and cpm's for the complexes), an idea of the column's separating ability can be learned. This will give a rough estimate of the size of the sample complexes eluted during the experimental runs.

F. Gradient Gel Electrophoresis- For each of the pooled fractions a sample was run through a 4-30% gradient gel. These gels were then analyzed on an LKB Bromma Ultroscan Laser Densitometer. A cocktail mixture of standards containing albumin, lactate dehyrogenase, catalase, ferritin, and thyroglobin was placed in two lanes on each gel. This method of analysis allows a great deal of sensitivity in measuring the size of major species present in each pooled fraction. It should be noted, however, that only a comparitively rough estimate may be made of any species larger than thyroglobin, the largest standard.

G. Sodium Deodecyl Sulfate- Polyacrylamide Gel Electrophoresis- The sodium deodecyl sulfate present in this gel acts as a detergent, breaking up the lipid complexes and freeing bound apolipoprotein. Each pooled fraction was run through a highly cross-linked (10%) gel against a number of small protein standards. This type of analysis allows a differentiation between vesicles containing bound Apo A-I and bound

Apo C's. This will allow an insight into what is occuring in the mixed Apolipoprotein systems.

G. Fluorescence Analysis- In the second series of experiments, a dispersion of multi-lamellar vesicles was formed from DMPC and additional incorporated lipid as noted above. Each of the samples of MLV's was incubated at a specific temperature in a Perkin- Elmer Fluorescence Spectrophotometer. The large heterogeneous multi-lamellar vesicles scatter incident light easily. The scattered light can be read at 90^{o} to the incident light using a fluorescence spectrophotometer. Discoidal particles about 100 angstroms in diameter, on the other hand, scatter much less light. When Apolipoprotein A-I is introduced to the MLV system, it has been shown by Pownall *et al.*[9,10] and others [6,11] that the Apo A-I spontaneously breaks down the multi-lamellar vesicles into small, discoidal particles similar to nascent HDL. This breakdown has been studied by Pownall using a spectrophotometer. In this analysis the breakdown will be kinetically studied over time by observing the decrease in light scattering by the MLV dispersion after the inclusion of Apo A-I. The fluorescence spectrophotometer was set to both excite and detect scattered light at 350nm. No filter was used in this analysis. Both the excitation window and the emission window were set equally between two and three nanometers, in order to obtain an initial light scattering intensity of between 90 and 95 fluorescence units. After complete temperature equilibration of the dispersion, Apo A-I was added

and mixed by inversion, and the decrease in light scattering measured as a function of time. It should be noted that a control reaction mixture containing no Apo A-I was also analyzed to test for settling of the MLV dispersion.

RESULTS

A. Gel Filtration Chromatograpy- In the first series of experiments, the reaction mixtures were run through a column of BioGel A-5m in order to separate the product particles. The results have been summed up in Figures 1-5. Gel Filtration Chromatography is an important analytical tool used to separate various molecules based upon their molecular size. The column in this experiment was filled with a number of beads made of Agarose, an inert substance. Large macromolecules are able to flow around these beads and elute in an earlier fraction. Apolipoproteins in their unbound state are smaller than the apolipoprotein: lipid complexes and, thus, are eluted in a later position. The relative sizes of these protein-lipid complexes can also be indicated by their position of elution. This data has been presented in Section D.

B. Protein Measurement and CPM Data- In the first series of experiments, both the absorbance at 280 nm and the counts of ^{14}C-cholesterol were measured for each pooled fraction. This data has been summed up in Table 1. It was noted that in one of the pooled fractions (D4) the A_{280} was extremely high while the number of counts was virtually at background level. Other fractions (A1, C3, C4, E3) show similar profiles. It was inferred from this data and from the position of

elution of these fractions that the majority of this fraction, if not the entirety, in fraction D4, consisted of unbound protein. The Lowry test proved somewhat inconclusive due to low protein amounts, however there did exist cases where a measureable amount of protein was present. These conformed with the pooled fractions containing high absorbance and low counts.

C. Phosphate Analysis- The results of these analyses have been recorded in Table 2. As can be seen, the pooled fractions which contained a high level of ^{14}C-cholesterol also contained a higher level of phosphate. This is to be expected since the phosphate is in the fraction that originates from the phospholipid bilayer of the complexes. This is where the cholesterol should also be incorporated.

D. Column Calibration-- A minor problem in resolution between the standards somewhat marred the effectiveness of this analysis. As a result of the standards eluting in fractions of such close proximity (ferritin- #56, lipid complexes- #58, and BSA- #60), an accurate standard curve could not be drawn. This was believed to have resulted from packing changes in the column by the time this analysis was performed. It should be noted that the unbound proteins eluted in a later fraction than that occupied by the suspected apolipoprotein lipid complexes. It was also of interest that the pooled fractions containing the suspected

complexes eluted from the column in a range from two to twenty fractions earlier than the largest of the protein standards. This would seem to indicate a heterogeneous collection of complexes, ranging in size from slightly larger than ferritin (79 angstroms in diameter) to significantly larger than ferritin. The exact size of the complexes can not be calculated due to the lack of an accurate standard curve.

E. Gradient Gel Electrophoresis- By utilizing the method of gradient gel electrophoresis, it is possible to calculate fairly accurately the size of the complexes. The stain used in this analysis stains for proteins either in the lipid-bound or unbound state. Due to the length of time that the samples were electrophoresed (24 hours), however, any unbound Apo A-I or Apo C's are able to pass completely through the gel. Only those proteins which are bound in complex form will be sufficiently retarded to show up on the gels. By comparing the positions of the complexes on the gel, as measured by the laser densitomiter, to those of the standards, the size of the complexes may be calculated. This information can be seen in Table 4. It was noted, however, that the range of sizes of the complexes went from a diameter of approximately 67 angstroms to greater than 250 angstroms. (It should be remembered that there are complications associated with the calculation of sizes in the upper regions of this gel.) This is interesting, since it agrees roughly with the results collected from the column calibration. Also a point of major interest was that the smaller sized complexes (<100 angstroms in diameter) were only formed

when both Apo A-I and Apo C s were present. It did not seem to make much of a difference in which order the apolipoproteins were added, only the presence of both in the reaction.

F. Sodium Deodecyl Sulfate- Polyacrylamide Gel Electrophoresis- Any Apo C's which might be present would be travelling with the dye front and would be difficult to see. Furthermore, since a mixture of different Apo C's was used, this band would be diluted and spread out, and thus, also difficult to locate. As expected, this proved to be the case. No definite bands can be made out corresponding to the mixture of Apo C's. Bands corresponding to Apo A-I are present, however. The fractions which produced a band corresponding to Apo A-I are : B2 (faint), C3 (strong), C4 (faint), D3 (strong), and E3 (very faint).

G. Kinetic Study of Light Scattering Decrease- The results of this analysis have been summed up in Tables 4-9. It was noted that the addition of between 2-10 % of certain lipids (palmitic acid, myristic acid, DMPE, and DPPE) had a negative effect upon the rate of MLV degradation corresponding to the level of added lipid as compared to a system without such added lipid. The addition of low level amounts of cholesterol seemed to have a positive effect upon the rate[6], however,

increasing the amount of cholesterol past the 6 molar % point had a negative effect. As shown in this study as well as in similar studies done by Epand *et al.*[11], the addition of DPPS to a similar system had a positive effect upon the rate of MLV degradation. It was noted in this study that the rate of degradation is not greatly dependent upon the temperature until the T_m is reached (see Table 6). At this temperature there is a large increase in rate (see Table 10). Similar results have been found by Pownall *et al.*[9].

DISCUSSION

In the first series of experiments, it appeared as if the addition of both Apo A-I and the mixture of Apo C's were necessary for the formation of smaller size complexes (<100 angstroms). It did not appear important which of the two apolipoproteins was added first. In the two control experiments, where only one of the two types of apolipoproteins was added, a group of large particles were formed in the range from 250-300 angstroms in radius. Moreover, the cpm peak for Trial A was narrower than that for any other trial. It would seem that the addition of Apo C's alone gives a large, but more homogeneous population of particles. Trial B seems to indicate that Apo A-I is capable of spontaneously forming a population of large heterogeneous particles containing differing amounts of lipids and protein as has been shown before[6].

In each of the three experimental trials (see Figures 3-5), a shoulder area appears in the cpm data in the range from fraction #50 to #54. This shoulder is absent from the controls. This is an indication that smaller sized complexes are being formed. This conclusion is borne out by the gradient gel data. In all but one of these shoulder region fractions, (C2), all the complexes are under 100 angstroms in diameter. In pooled fraction C2, two distinct species of particle are evident. This could have been caused by the range of fractions which were chosen.

SDS-Polyacrylamide analysis of the four pooled fractions which gave smaller sized complexes indicates that Apo A-I is definitely present in at least three of these fractions. It seems entirely plausible that at least a portion of the Apo A-I present is bound to lipid complexes. It has been shown by Jonas and others [6,22,23] that small complex discs contain two Apo A-I's whereas larger discs contain three Apo A-I's. It can not be said for certain that the larger particles formed in this study are large discs of this type. This is due to the fact that it is difficult to distinguish between discoidal micelles and spherical vesicles.

From the data obtained, it appears as if there is some sort of protein-protein interaction between the Apo A-I and the Apo C's. The exact nature of this interaction can only be hypothesized at this point. It is possible that one of the apolipoproteins has a sort of catalytic effect on the system. One possible mechanism is that, for a time, both apolipoproteins may be attached to the same complex. This dual binding may somehow induce smaller particles to be formed. A second possible mechanism is that one of the apolipoproteins may bind to the lipid dispersion first, while the second apolipoprotein binds slower, but more preferentially to the large complexes which have formed. When this second apolipoprotein binds, it binds to a smaller amount of lipid bilayer. This means that the lipid bilayer is more highly curved, and thus, more likely to exhibit defects in the bilayer lattice. Since it did not matter which of the apolipoproteins was added first, it appears as if three hours of incubation may be enough to allow the system to completely equilibrate. Further experimentation with systems incubated for shorter

lengths of time may allow a distinction to be made or suggest a more concrete mechanism.

The second series of experiments gave some interesting results. In Henry J. Pownall's opinion[9], the kinetics of the incorporation of Apo A-I into lipid vesicles is controlled less by any changes in the stucture of Apo A-I than it is by the "permeability" of the lipid bilayer. This is due to the fact that the protein:lipid bilayer interaction appears to be hydrophobic in nature. In the bilayer, the polar heads are ideally spaced a certain distance apart. Each phospholipid is in constant movement, however, and when enough space is present, Apo A-I is able to find a place to bind. This is why bilayers at a temperature equal to the T_m react more quickly with Apo A-I. Lattice defects are more numerous in the vicinity of a coexisting gel-liquid crystalline state. The liquid crystalline state is much more permeable than the gel state, but it appears that when the system is energetic enough (ie. when the experimental temperature is much greater than the T_m) the Apo A-I can not act quickly enough to attack the lattice defect.

This idea of permeability may also give an explanation for the effects of incorporated lipids on the kinetics of bilayer breakdown. When thinking of a phospholipid it is easy to picture a cylinder where the hydrophobic effect exerted by the fatty acyl chains is compensated by the hydrophilic effect of the large polar head group. Fatty acids, however, have a large hydrophilic head, relative to one fatty acyl chain. Thus, fatty acids may be pictured as a cone. Phosphatidyl ethanolamine is the

opposite. It has a small polar head group with a strongly hydrophobic end formed by two fatty acyl chains. Its appearance may be pictured as an inverted cone. Somehow, these two molecules with their opposite shapes, seem to cause the same effect, a "stiffening" of the membrane, which reduces membrane permeability. It is easy to imagine the inverted cone shape of the phophatidyl ethanolamine allowing the polar head groups to move closer together in that region due to polar head group interaction. It is harder to picture what physical effect the fatty acids might create that would "stiffen" the bilayer.

Cholesterol has been shown in the past to increase permeability when introduced in low concentration[6]. It is supposed that the small amount of cholesterol provides the defect sought for by the Apo A-I. In higher concentrations, the cholesterol stays between the phospholipids and prevents the free movement of the fatty acyl chains. This hydrophobic bonding "stiffens" the bi-layer, thus making it more crystalline-like. According to Epand's hypothesis[11], anionic phospholipids act by spreading the T_m of a phospholipid complex so that it occurs over a much broader range. Thus, with increasing amounts of DPPS, the bi-layer acts more like it is at the critical transition state, where it is much more permeable to Apo A-I. Epand also suggests that the negative charge present at the polar head groups may make the polar head groups spread apart due to electrostatic repulsion. This, however, appears to be somewhat contraindicated by the results of the kinetic studies of palmitic and myristic acid effects in this study.

In conclusion, it appears as if several of the factors examined in this

study may have an effect upon HDL production *in vivo*. Apo C's appear to, in some way, facilitate the formation of smaller size particles *in vitro*. It is possible that they may have a similar function in the body. Certain lipids present within the body have an effect upon the rate of breakdown of large lipid bi-layers *in vitro*. It seems possible that a similar effect may occur within the body and adjust the *in vivo* rate of HDL formation.

REFERENCES

1. Zubay, G., Biochemistry (1984) Addison Wesley Publishing Co., Inc., Reading, Mass., 555-560

2. Gotto,A.M., Jr., Robertson,A.L., Jr., Epstein, S.E., DeBakey, M.E., and McCollum, C.M., III, (1985) Atherosclerosis, Upjohn Co., Kalamazoo, Mich.

3. Taylor, K.B., and Anthony, L.E. (1983) Clinical Nutrition, McGraw-Hill Inc., St. Louis

4. Tall, Alan R., and Small, D.M., (1980) Advances in Lipid Research, Vol. 17, Academic Press Inc.,

5. Werb,Z., and Cohn, A.Z., (1971) Journal of Experimental Medicine 134, 1545

6. Jonas, Ana, (1986) Methods of Enzymology 128, 553-582

7. Herbert,P.N., Shulman, R.S., Levy, R.I., and Frederickson, D.S., (1973) J. Biol. Chem. 248, 4941

8. Huang, C., (1969) Biochemistry 8, 344

9. Pownall, H.J., Massey, J.B., Kusserow, S.K., and Gotto, A.M., Jr., (1978) Biochemistry 17 1183-1187

10. Pownall,H.J., Pao, Q., Hickson, D., Sparrow, J.T., Kusserow, S.K., and Massey, J.B., (1981) Biochemistry 20, 6630-6635

11. Surewicz, W.K., Epand, R.M., Pownall, H.J., and Hui, S.W., (1986) J. Biol. Chem. 261, 16191-16197

12. Tony, M.F., and Kuksis, A., (1986) Biochem. Cell Biol. Vol.64, 264-272

13. Hui, S.W., Epand, R.M., Dell, K.R., Epand, R.F., and Orlowski, R.C., (1984) Biochim. Biophys. Acta 772, 264-272

14. Epand,R.M., (1982) Biochim. Biophys. Acta 712, 146-151

15. Jonas, A., and Matz, C.E., (1982) Biochemistry 21, 6867-6872

16. Edelstein, C., Lim, C.T., and Scanu, A.M., , (1972) J. Biol. Chem. 247, 5842-5849

17. Lowry,N.H., Rosebrough,N.J., Farr, A.L., and Randall,R.J., ,(1953) J. Biol. Chem. 193, 265-275

18. Markwell, M.K., Hass,S.M., Bieber,L.L., and Tolbert, N.E., (1978) Anal. Biochem. 87, 206-210

19. Saunders, L.,Perrin, J., and Gammick, D., (1962) J. Pharm. Pharmacol. 14

20. Chen, P.S., Toribaro,T.Y., and Warner,H., (1956) Anal. Chem. 28, 1756-1758

21. Gwynn, J., Brewer, H.B., Jr., Osborne, J.C., Jr., and Edelhoch, H., (1974) J. Biol. Chem. 249, 2411-2416

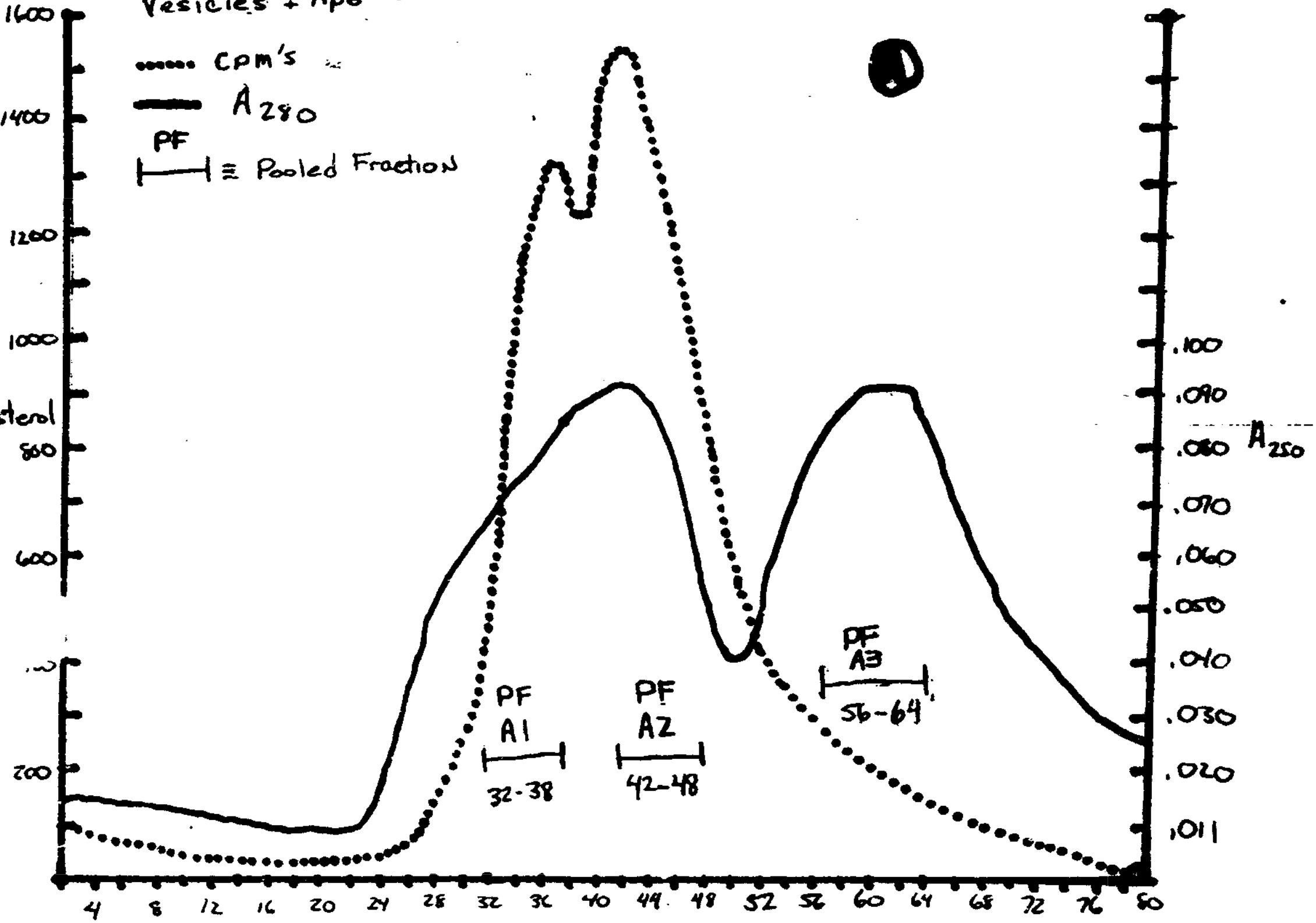
22. Swaney, J.B., (1980) J. Biol. Chem. 255, 8791

23 Wetterau, J.R., and A. Jonas, (1983) J. Biol. Chem. 258, 10961

LIST OF ABBREVIATIONS

VLDL	Very low density lipoproteins
LDL	Low density lipoproteins
HDL	High density lipoproteins
LCAT	Lecithin cholesterol acyl transferase
Apo A-I	Apolipoprotein A-I
Apo C's	Apolipoprotein C's
DMPC	Dimyristoyl phosphatidyl choline
egg- PC	Egg- phosphatidyl choline
DMPE	Dimyristoyl phosphatidyl ethanolamine
DPPE	Dipalmitoyl phosphatidyl ethanolamine
DPPS	Dipalmitoyl phosphatidyl serine
MLV	Multi-lamellar vesicle
GuHCl	Guanidine Hydrochloride
BSA	Bovine serum albumin
SDS	Sodium deodecyl sulfate
PF	Pooled fraction

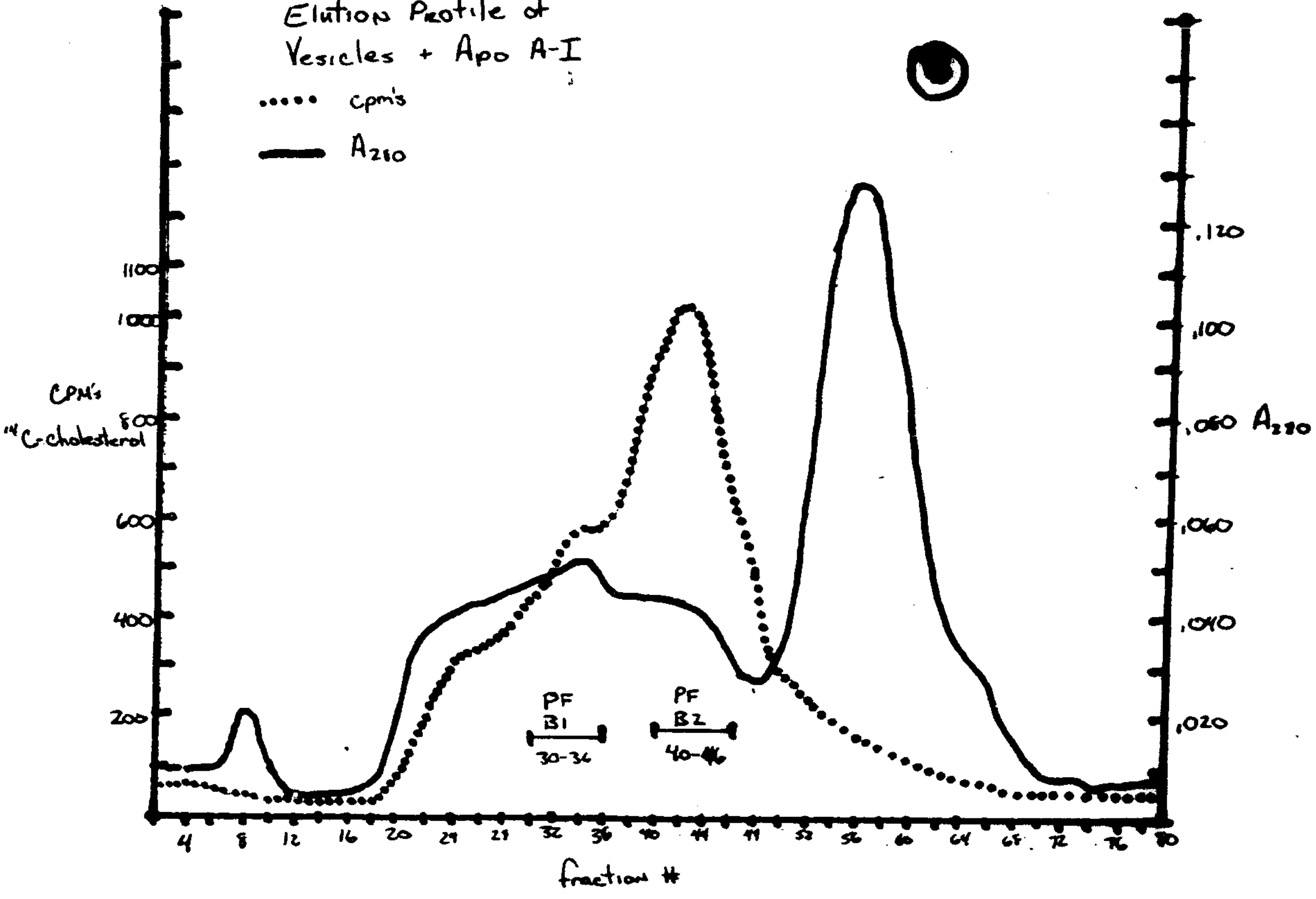
Elution Profile of Vesicles + Apo C's



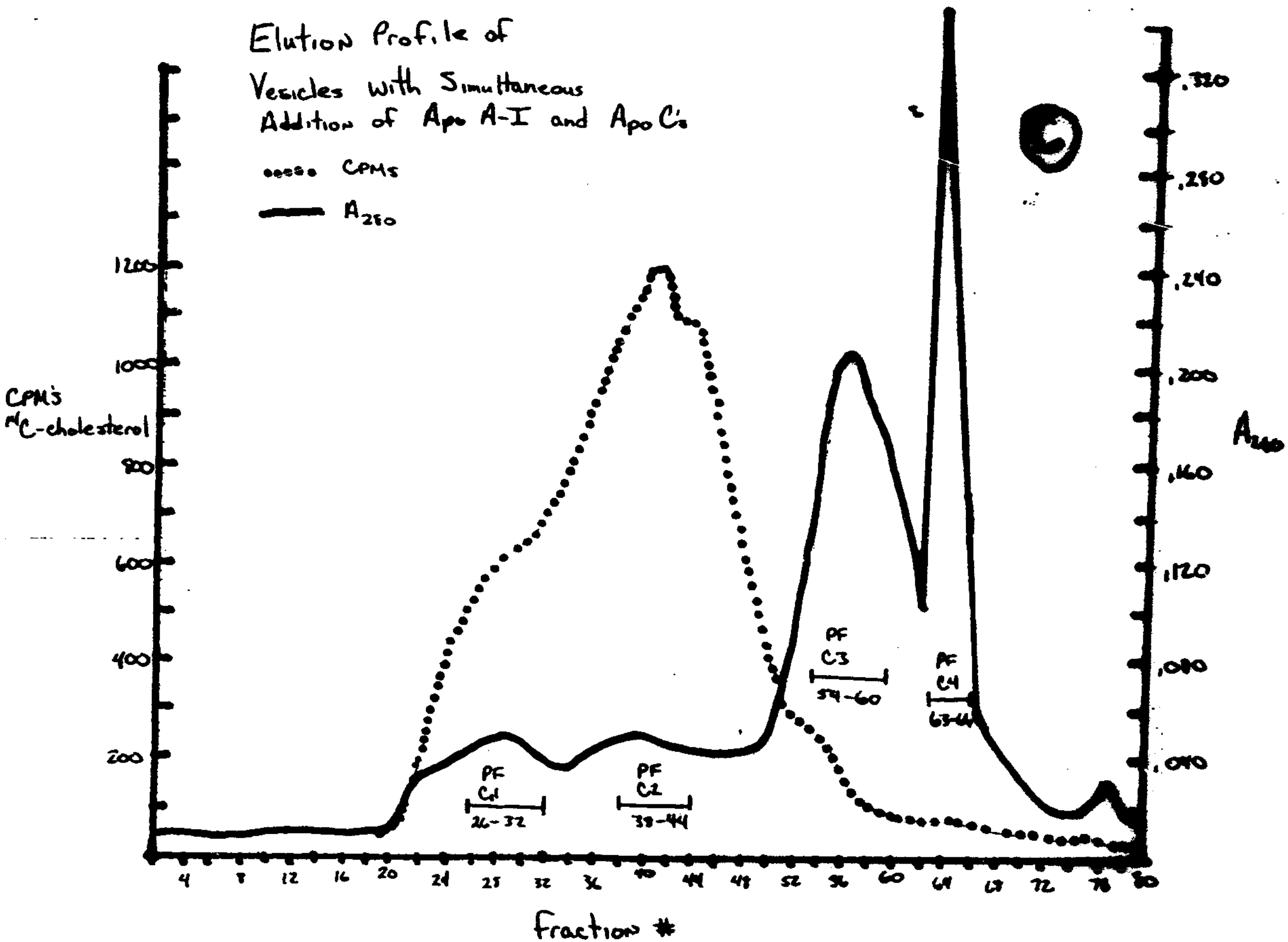
Elution Profile of
Vesicles + Apo A-I

..... cpm's

——— A_{210}



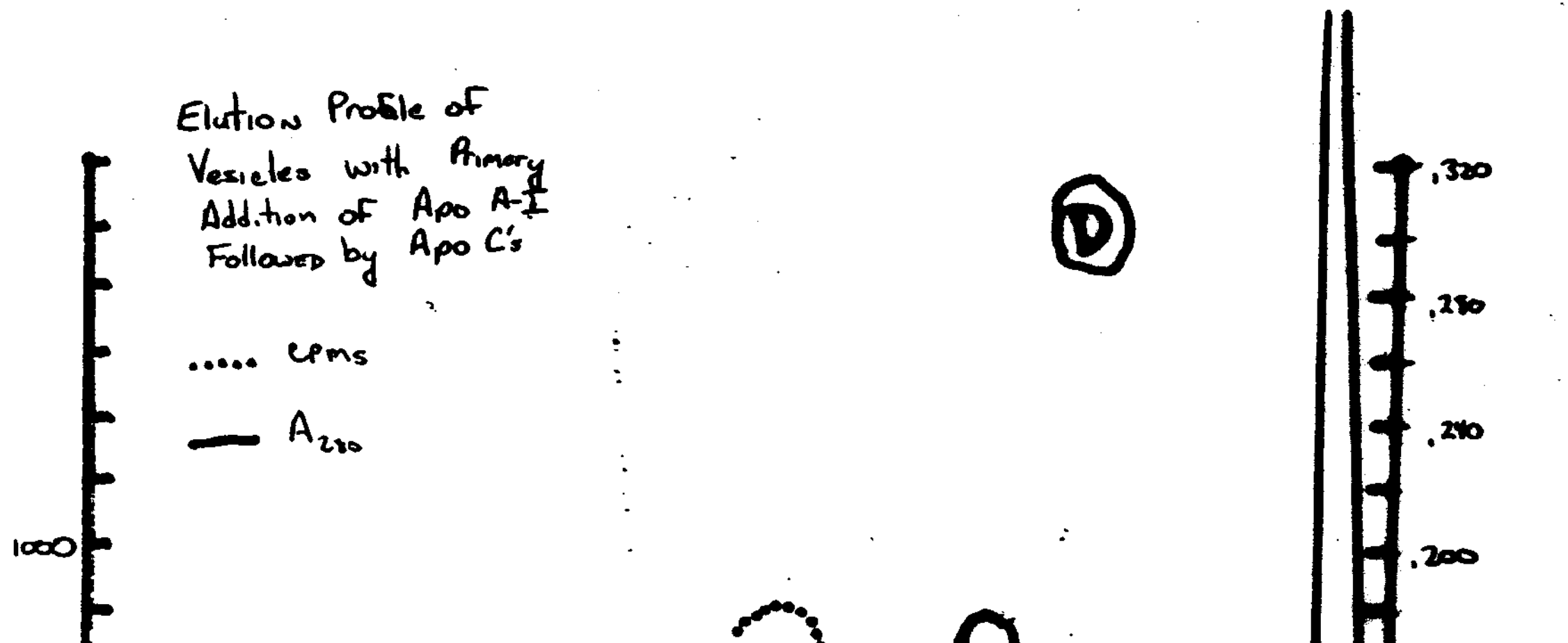
Elution Profile of
Vesicles with Simultaneous
Addition of Apo A-I and Apo C's



Elution Profile of Vesicles with Primary Addition of Apo A-I Followed by Apo C's

..... cpms

— A_{280}



3C

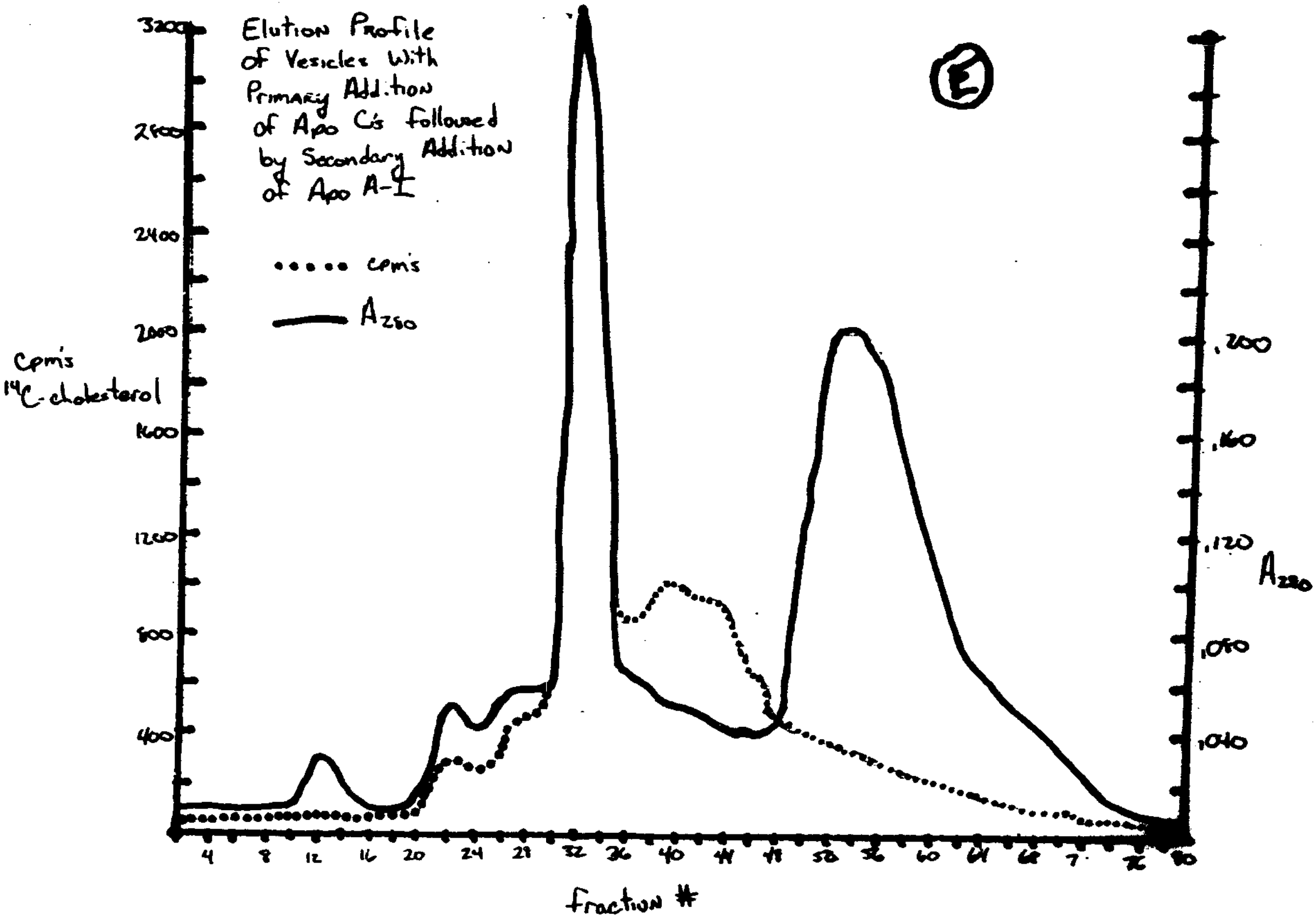

Elution Profile of Vesicles With Primary Addition of Apo Cs followed by Secondary Addition of Apo A-I
..... cpm's
—— A280
(E)
cpm's 14C-cholesterol
3200
2800
2400
2000
1600
1200
800
400
.200
.160
.120
.080
.040
A280
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80
Fraction #

Table 1.
Absorbance and Counts of Eluted Pooled Fractions

Sample (PF)	cpms	A_{280}
A1	771.5	0.055
A2	1203.0	0.068
A3	114.5	0.079
B1	513.5	0.040
B2	877.5	0.030
C1	553.0	0.032
C2	1073.5	0.037
C3	257.5	0.163
C4	61.0	0.183
D1	473.5	0.042
D2	830.0	0.033
D3	443.0	0.120
D4	32.0	0.431
E1	663.0	0.070
E2	813.5	0.027
E3	299.0	0.166

Table 2.
Phosphate concentration of pooled fractions

Sample (PF)	Average phosphate concentration x 10^{-4}M
A1	6.13
A2	8.06
A3	1.97
B1	2.69
B2	6.02
C1	6.18
C2	7.53
C3	4.18
C4	2.20
D1	3.59
D2	5.97
D3	5.70
E1	4.69
E2	6.08
E3	3.39

Table 3
Measurement of Size of Particle Contained in Pooled Fractions
As Calculated by Gradient Gel Electrophoresis.

(PF)	Diameter of Major Species Present (in angstroms)	
A1	>250	
A2	>250	
A3	No Particles Present	
B1	>250	
B2	247	>250
C1	>250	
C2	67.3	>250
C3	69.54	.16
C4	>250	
D1	>250	>250
D2	245.1	>250
D3	78.3	92.8
D4	No Particles Present	
E1	>250	
E2	>250	>250
E3	78.14	

Table 4
Kinetic Analysis of Rate of Degradation of MLV's Containing Palmitic Acid After 3 and 10 minutes (n-2)
T-21.5°C

% Palmitic Acid	R_3 (min^{-1})	R_{10} (min^{-1})
0	10.3	4.7
2	10.3	5.2
4	6.7	3.6
6	4.0	1.8
8	1.3	1.15
10	2.2	2.2

Table 5
Kinetic Analysis of Rate of Degradation of MLV's Containing Myristic Acid After 3 and 10 minutes (n-2)
T-21.5°C

% Myristic Acid	R_3 (min^{-1})	R_{10} (min^{-1})
0	10.1	4.7
2	5.0	2.5
4	x	x
6	4.2	2.15
8	2.2	1.5
10	2.0	1.7

Table 6
Kinetic Analysis of Rate of Degradation of MLV's Containing DPPE After 3 and 10 minutes (n-2)
T-21.5°C

% DPPE	R_3 (min^{-1})	R_{10} (min^{-1})
0	8.0	3.0
2	5.3	2.1
4	6.3	3.6
6	4.0	1.65
8	2.15	2.2
10	3.65	1.95

Table 7
Kinetic Analysis of Rate of Degradation of MLV's Containing DMPE After 3 and 10 minutes (n-2)
T-21.5°C

% DMPE	R_3 (min^{-1})	R_{10} (min^{-1})
0	9.5	3.8
2	x	x
4	2.85	1.55
6	2.15	1.85
8	2.5	2.15
10	1.6	1.65

Table 8
Kinetic Analysis of Rate of Degradation of MLV's Containing DPPS After 3 and 10 minutes (n-2)
T-21.5°C

% DPPS	R_3 (min^{-1})	R_{10} (min^{-1})
0	10.5	6.15
2	8.0	5.1
4	9.7	5.3
6	14.7	6.9
8	16.8	8.15
10	19.65	7.8

Table 9
Kinetic Analysis of Rate of Degradation of MLV's Containing Cholesterol After 3 and 10 minutes (n-2)
T-21.5°C

% Cholesterol	R_3 (min^{-1})	R_{10} (min^{-1})
0	9.15	3.6
2	10.5	4.1
4	x	3.2
6	11.3	5.8
8	8.0	5.05
10	6.85	4.95

Table 10
Temperature Effects on Average Rate of MLV Degradation

Temperature °C	R_3 (min^{-1})
21.5	9.4
22.0	6.65
23.0	8.7
23.5	8.0
23.8	7.7
* 24.1	14.0
** 25.1	13.3

* Note: R_1 ~35 min^{-1} and reaction is approximately 75% complete after 1 min.
**Similar to above.