[CONTRIBUTION FROM THE DIVISION OF MEDICAL PHYSICS, UNIVERSITY OF CALIFORNIA]

Degradation of S_f20-400 and High Density Lipoproteins of Human Sera by Ethyl Ether[1]

BY SHUKI HAYASHI,[2] FRANK LINDGREN AND ALEX NICHOLS

RECEIVED MAY 24, 1958

The S_f20-400 and the high density lipoproteins of human sera have been degraded by partially extracting their lipid content with ethyl ether. The resultant degradation products have been analyzed both chemically and ultracentrifugally. In the case of the S_f20-400 class lipoproteins, the ether extracted more than 60% of the total lipids. The composition of the extracted lipids (mostly glycerides) was approximately constant for each of four successive extractions. On the other hand, the main lipid constituent of the lipoprotein fragments was phospholipid. In the case of the high density lipoproteins, the ether extracted only a very small amount of lipids. In spite of this resistance to ether degradation an essentially lipid-free protein fragment was produced, the molecular weight of which was calculated to be approximately 40,000.

Introduction

Lipoproteins of human sera exhibit considerable differences in such properties as density, molecular size and chemical composition.[3,4] Generally, these lipoproteins have been fractionated on the basis of their densities by ultracentrifugal flotation.[5] In these procedures the lipoproteins have been fractionated into three broad density classes: (1) lipoproteins with densities less than 1.007 g./ml., (2) lipoproteins with densities between 1.007 and 1.063 g./ml., and (3) lipoproteins with densities between 1.063 and 1.20 g./ml. These lipoprotein classes have been characterized and designated S_f-20-400, S_f0-20 and the high density lipoproteins, respectively. Ultracentrifugal studies indicate that each of these lipoprotein classes exhibits a distribution in density and molecular size. Further, chemical analysis of the lipid composition of each of these broad lipoprotein classes indicates gross composition differences between the classes as well as variations in composition within each class.[6,7]

These data have suggested possible structural relationships in the various lipoprotein groups.[8] However, formulation of the actual structure and molecular associations present in each of the lipoprotein classes requires additional information. This report describes efforts directed toward obtaining such information by studying the degradation of lipoprotein molecules and the products formed during such degradation.

The early work of Macheboeuf on horse blood lipoproteins was one of the first studies bearing on the structure of lipoproteins.[9] He found these lipoproteins to be chemically stable with regard to repeated precipitation by ammonium sulfate and solubilization in an aqueous medium. When shaken with ether, these lipoproteins were found to liberate partially their constituent lipids. A more complete extraction of lipids was found to require a critical concentration of alcohol in the ether. Similar observations with the chemically fractionated human sera lipoproteins were made by Oncley, *et al.*,[10] which indicated that partial extraction of lipids with ether produced water-soluble degraded lipoproteins. More recently similar findings have been reported by Avigan.[11]

In the experiment presently described, ethyl ether was used in the degradation of lipoproteins of both the S_f20-400 class and the high density class. The degradation products arising from each of these classes of lipoproteins have been isolated into the following density groups: those less dense than 1.007 g./ml., (2) those between 1.007 g./ml. and 1.065 g./ml., (3) those between 1.065 g./ml. and 1.20 g./ml. and (4) those more dense than 1.20 g./ml. These degradation products so isolated were analyzed ultracentrifugally and chemically.

Experimental

Preparation of the S_f20-400 Class and the High Density Lipoproteins.—The source of the lipoproteins was blood of a 45 year old healthy male, drawn 5 hr. after a meal consisting of 85 g. of corn oil blended in skim milk. The serum lipoproteins were fractionated into the S_f20-400 class, the S_f0-20 class and the high density lipoproteins by preparative ultracentrifugation[6] except for one modification. In this experiment NaBr was used to raise all solution densities.[12] This modification was made since $NaNO_3$ would interfere with the Kjeldahl nitrogen determinations.

The flotation patterns of the isolated S_f20-400 class and the high density lipoprotein preparations are shown in Figs. 1 and 2, respectively.

One-ml. fractions of the solution immediately below the top 1-ml. fraction following each preparative ultracentrifugation were collected for the purpose of determining the non-protein nitrogen values of each lipoprotein preparation. This NPN nitrogen was assumed to be in the form of such small molecular substances as urea, uric acid and free amino acids. The possibility that discrepancies might arise from using the second milliliter NPN value due to sedimentation of these small molecular substances was tested. Following 24 hr. ultracentrifugation (as used for the lipoprotein isolation), 2% urea and 2% leucine solutions showed average decreases in concentration in the 1st milliliter of 1.4 and 2.9%, respectively. Correspondingly, the decreases observed in the 2nd milliliter fractions were 0.7 and 2.6%, respectively. Thus, if one uses the NPN value of the second milliliter fraction as an estimate of the NPN concentration in the top milliliter fraction, only an error of approximately 1% is involved.

(1) Supported in part by the U. S. Public Health Service, Fellowship HF-6585-C and the U. S. Atomic Energy Commission.

(2) Physics Department, University of California, Davis, California.

(3) F. T. Lindgren, H. A. Elliott and J. W. Gofman, *J. Phys. Colloid Chem.*, **55**, 80 (1951).

(4) J. L. Oncley, K. W. Walton and D. G. Cornwell, THIS JOURNAL, **79**, 4666 (1956).

(5) O. F. deLalla and J. W. Gofman, "Methods of Biochemical Analysis," Vol. I, ed. D. Glick, Interscience Publishers Inc., New York, N. Y., 1954, pp. 459–478.

(6) F. T. Lindgren, A. V. Nichols and N. K. Freeman, *J. Phys. Chem.*, **59**, 930 (1955).

(7) L. A. Hillyard, G. Entenman, H. Feinberg and I. L. Chaikoff, *J. Biol. Chem.*, **214**, 79 (1955).

(8) F. T. Lindgren, N. K. Freeman, A. V. Nichols and J. W. Gofman, "The Blood Lipids and the Clearing Factor," IIIrd International Conference on Biochemical Problems of Lipids, Brussels, July 1956, pp. 224.

(9) M. A. Macheboeuf, *Bull. chim. biol.*, **11**, 268 (1929);

(10) J. L. Oncley, F. R. N. Gurd and M. Melin, THIS JOURNAL, **72**, 458 (1950).

(11) J. Avigan, *J. Biol. Chem.*, **226**, 957 (1957).

(12) All density values indicated for the preparative procedures are for 20° and do not include the contributions to solution density of proteins and lipoproteins.

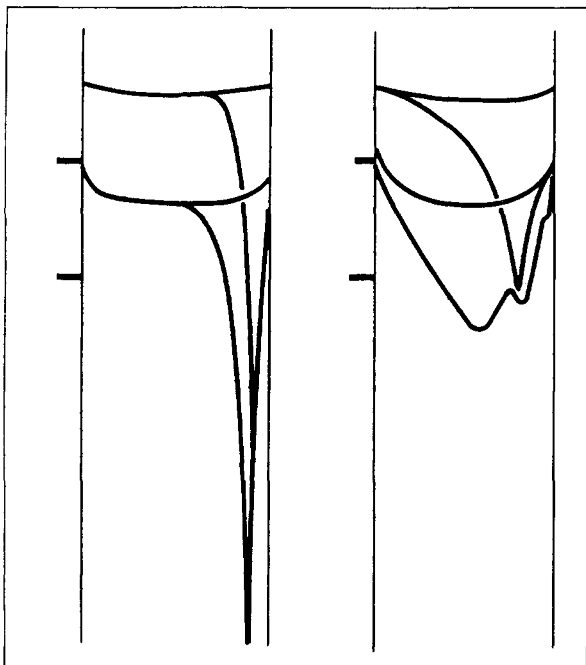


Fig. 1.—Ultracentrifugal flotation patterns of the initial S_f20-400 class lipoproteins. The first frame was taken when rotor reached full speed (52,640 r.p.m.) and the second frame was 6 minutes later. The solution density, at 20°, for the upper patterns was 1.0072 g./ml. and 1.0638 g./ml., for the lower patterns. Flotation proceeds toward the left. For all analytic runs double sectored analytical cells were used allowing accurate positioning of the base line. A wire angle of 45° was used.

Ether Degradation of the S_f20-400 Class Lipoproteins.—The isolated preparation of the S_f20-400 class lipoproteins was diluted with a 1.007 g./ml. solution of NaBr such that the final solution density remained 1.007 g./ml. and the lipoprotein concentration was reduced to 2.02%. Four 6.0-ml. aliquots of this solution were placed into 25 × 150 mm. screw-cap culture tubes provided with Teflon gaskets. Thirty ml. of peroxide free ethyl ether[13] was layered over each lipoprotein aliquot. The aliquot tubes, after capping, at room temperature, were placed in an approximately horizontal position on a rocking platform which provided a gentle stirring motion, without turbulent mixing of the ether and the aqueous phases. After 30 minutes of rocking, the aliquot tubes were centrifuged at low speed in a Size 2 International Centrifuge, and the ether phases were removed. The tubes were refilled with fresh ether and the liquid extractions were repeated three times. The extraction time of the last three steps was 15 minutes for each step. Finally, the extracting solvent was evaporated under a stream of nitrogen and the extracted lipids were further dried *in vacuo* over P_2O_5.

After the extraction, nitrogen was streamed over the aqueous aliquot phases for 12 hr. to remove the solubilized ether. To minimize the evaporation of these aqueous phases, the nitrogen was first bubbled through a column of water. A slight loss in volume occurred, but this was corrected by addition of distilled water.

The degraded lipoproteins procured by the extraction process were isolated in three density groups by preparative ultracentrifugation (Spinco, Model L, Centrifuge in a type 40.3 rotor at 40,000 r.p.m.) in three successive 24 hr. runs. In the first run the aliquot solutions were centrifuged which yielded top ml. fractions containing those degradation products of densities less than 1.007 g./ml. The bottom 3-ml. contents of the preparative tubes of this run were mixed with an equal volume of NaBr solution which increased the solvent density to 1.065 g./ml. This solution was centrifuged in the second preparative run yielding top ml. fractions containing those degraded lipoproteins of densities between 1.007 and 1.065 g./ml. The bottom 3-ml. contents of these preparative tubes of the second run were mixed with an equal volume of solution of NaBr which increased the solvent density to 1.20 g./ml., and this mixture was centrifuged in the final run which yielded top ml. fractions containing those degradation products of densities between 1.065 and 1.20 g./ml.

(13) The ether was equilibrated prior to use with aqueous ferrous sulfate to remove peroxides.

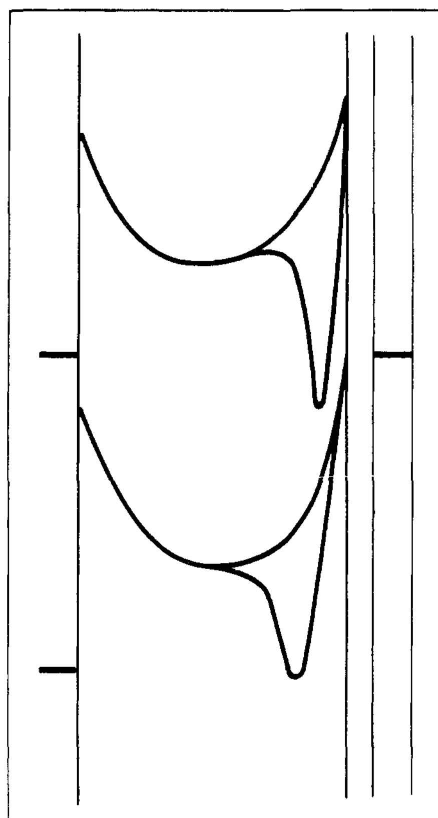


Fig. 2.—Ultracentrifugal flotation patterns of the initial high density lipoproteins (HDL-2 and HDL-3). Patterns were taken 32 minutes after rotor reached full speed (52,640 r.p.m.). The solution densities, at 20°, for the upper and lower patterns are 1.1964 and 1.3061 g./ml., respectively. Flotation proceeds toward the left.

Ether Degradation of the High Density Lipoproteins.—The isolated preparation of high density lipoproteins was diluted with distilled water to give a final solvent density of 1.065 g./ml. The lipoprotein concentration was reduced to 1.37%. The preparation of aliquots, the partial extraction of lipids by ether and the subsequent removal of the dissolved ether from the aliquot solutions were identical with the steps used in the treatment of the S_f20-400 class lipoproteins (see previous section).

The degraded lipoproteins arising from the high density lipoproteins were ultracentrifugally isolated into the following three density groups: those with densities (1) less than 1.065 g./ml., (2) between 1.065 g./ml. and 1.20 g./ml. and (3) greater than 1.20 g./ml.

Extraction and Analysis of the Total Lipids.—The extractions of the total lipids from the lipoproteins were made according to the method described by Freeman, *et al.*[14]

The total lipid extracts were analyzed by silicic acid chromatography combined with infrared absorption spectrophotometry.[14] The infrared absorption measurements were made using a Baird Associates, Model 5, Recording Spectrophotometer.

Determination of Protein.—The Kjeldahl nitrogen values were determined for the delipidized portion of the lipopro-

(14) N. K. Freeman, F. T. Lindgren, Y. C. Ng and A. V. Nichols, *J. Biol. Chem.*, **227**, 449 (1957).

teins according to the method of Ma and Zuazaga.[15] After ultracentrifugation of each lipoprotein solution, the 2nd ml. fractions directly below the lipoprotein containing fractions were used to determine the non-protein nitrogen values. Any lipoproteins contaminating the non-protein nitrogen background solutions were removed by precipitation with 10% trichloroacetic acid.

The Kjeldahl nitrogen values were converted to total protein by the factor 6.67 mg. protein per mg. nitrogen.[16]

Ultracentrifugal Analyses.—The ultracentrifugal analyses were made with a Spinco, Model E, Analytical Ultracentrifuge. Flotation coefficients were measured from the slope of the ln x *vs.* $\omega^2 t$ plots, where x indicates the radial distance of the maximum ordinates of the flotation pattern.

The estimates of the hydrated densities of the lipoproteins were made from the extrapolated values of the solution densities at which zero sedimentation velocity would occur on the ηS *vs.* ρ plots (η is the viscosity of the lipoprotein free solvent relative to water as calculated from data in the International Critical Tables, S is the measured flotation rate and ρ is the solution density). Since it was not known whether or not S was dependent on concentration, the ηS *vs.* ρ measurements were made with lipoprotein concentration kept constant.

Results and Discussion

Degradation Products of the S_f20-400 Class Lipoproteins.—The S_f20-400 lipoprotein solution initially contained 440.8 mg. of lipids. Following the partial extraction with ether, 167 mg. of lipids (40% of the total lipids) remained in soluble form within the aqueous phase. A total of 273.9 mg. of lipids was extracted. Table I presents the weights

TABLE I

WEIGHTS AND COMPOSITIONS OF LIPIDS EXTRACTED BY ETHYL ETHER FROM THE AQUEOUS SOLUTION OF S_f20-400 CLASS LIPOPROTEINS INITIALLY CONTAINING 440.8 MG. OF LIPID

Extraction step	Wt. of lipids extracted, mg.	Composition of lipid, wt. %[a] CSE	CS	PL	TG	FA
1st	47.8	13	9	13	64	2
2nd	78.8	15	9	13	62	2
3rd	92.2	15	8	13	63	1
4th	55.3	15	9	13	62	1

[a] CSE, CS, PL, TG and FA denote, respectively, cholesteryl esters (as oleate), cholesterol, phospholipids (as lecithin), triglycerides (as triolein) and unesterified fatty acids (as oleic acid). The values are averages of two determinations.

and compositions of these extracted lipids. Within the limits of experimental errors, the composition of each of the successively extracted lipids is indistinguishable from each other. However, these lipid compositions are significantly different from the total lipid composition of the initial S_f20-400 class lipoproteins (see Table II).

The soluble degraded lipoproteins or lipoprotein fragments resulting from the ether extraction of the S_f20-400 lipoproteins were also found to exhibit a wide distribution of density and molecular size.

After fractionation these degraded lipoproteins were studied by analytical ultracentrifugation. Figure 3 shows a comparison of the ultracentrifugal distribution of the initial S_f20-400 class lipoprotein with that of the fractionated degradation products. To facilitate comparison, this figure was drawn by replotting the ultracentrifugal flotation patterns with reference to a straight baseline.

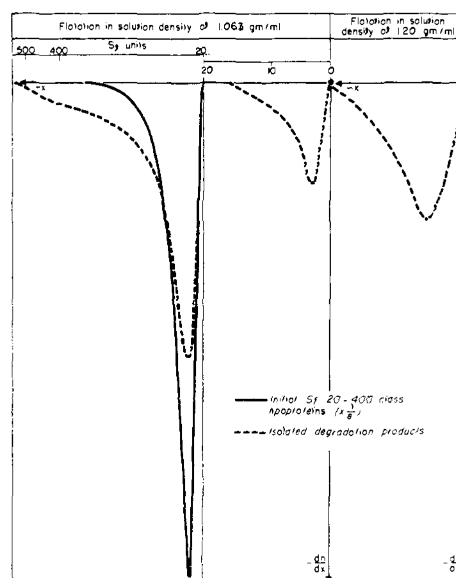


Fig. 3.—Comparison of the ultracentrifuge flotation diagrams of the initial S_f20-400 class lipoproteins and their degradation products. The areas designated by the solid line and dotted lines are proportional to the weight of the initial material used and weights of the fractionated degradation products, respectively.

The flotation pattern for the product S_f20-400 lipoproteins of densities less than 1.007 g./ml. has a peak with approximately the same flotation rate as that of the initial S_f20–400 class lipoproteins. However, the increased skewness of the product pattern suggests the production of some lipoproteins of higher S_f rates not initially present. Alternatively, of course, this increase in S_f rate might also result from a lowered molecular density.

TABLE II

COMPOSITION OF THE INITIAL AND THE DEGRADED S_f20-400 CLASS LIPOPROTEINS

	Lipoprotein[a,b] compn., mg. Protein	Lipid	Lipid composition, wt. %[b] CSE	CS	PL	TG	FA
Initial S_f20-400 lipoproteins	40.6	440.8	11	8	23	58	1
Degraded S_f20-400 d^{20}_4 <1.007 g./ml.	4.3	56.9	15	7	18	57	2
Degraded S_f20-400 1.007 < d^{20}_4 < 1.065 g./ml.	2.6	14.6	5	13	61	18	4
Degraded S_f20-400 1.065 < d^{20}_4 < 1.20 g./ml.	18.2	21.6	5	9	63	17	6

[a] In amounts actually isolated for each density range. [b] Averages of at least two determinations.

Except for this additional skewness which represents a small portion of the total distribution, these low density degraded lipoproteins appear to be ultracentrifugally equivalent to the initial S_f20-400 class lipoproteins. Comparison of the lipid composition of this low density fraction of the degraded lipoproteins with the initial S_f20-400 class lipoproteins also shows very little difference. Since the extraction of lipids from the initial lipoproteins had

(15) T. S. Ma and G. Zuazaga, *Ind. Eng. Chem., Anal. Ed.*, **14**, 280 (1942).

(16) B. Shore, *Arch. Biochem. Biophys.*, **71**, 1 (1957).

not been carried to completion,[17] it is reasonable to expect that some lipoproteins of the S_f20-400 class might remain relatively unchanged.

The degraded lipoproteins fractionated into the two density ranges of from 1.007 to 1.065 g./ml. and from 1.065 to 1.20 g./ml. have ultracentrifugal distributions which are presented in Fig. 3. Table II presents the chemical compositions found for these fractions. Table IV shows the estimates of some of the physical properties for these distributions of lipoproteins. Molecular weights were calculated from Svedberg's equation for sedimentation velocity assuming spherical particles.

The naturally occurring serum lipoproteins fractionated in the density ranges from 1.007 to 1.063 g./ml. and from 1.063 to 1.20 g./ml. have been previously characterized.[5] Comparison of the chemical and physical properties of each of the degraded lipoprotein fractions with the natural serum lipoprotein fractions of the corresponding density ranges reveals similarities in hydrated density and lipid to protein ratio. However, the calculated molecular weights are comparable only in order of magnitude and the lipid chemical compositions are grossly different. In contrast to the naturally occurring lipoproteins, the degraded lipoproteins have a high content of phospholipids and a low content of cholesteryl esters.

Inspection of Table II shows only a slight variation in the lipid composition for the two fractions of the degraded lipoproteins whose density ranges are from 1.007 to 1.065 g./ml. and from 1.065 to 1.20 g./ml. On the other hand, the lipid to protein ratios differ by a factor of five.

A closer inspection of the differences between these two groups of degraded lipoproteins can be made by comparing the weights of the lipid and protein per mole of lipoproteins. From Table IV, it is calculated that a mole of degraded lipoproteins in the density range from 1.007 to 1.065 g./ml. has approximately 1.4×10^6 g. of lipid and 2.4×10^5 g. of protein and a mole of degraded lipoproteins of densities betwen 1.065 and 1.20 g./ ml. has approximately 4.4×10^5 g. of lipid and 3.8×10^5 g. of protein. Between these two groups of degraded lipoproteins of closely similar lipid composition the weights of lipid per mole lipoprotein differ by a factor of three while the weights of protein per mole lipoprotein differ only by a factor of one and a half.

It is significant that in each of the successive ether extractions performed on the S_f20-400 class lipoproteins the composition of the lipid was essentially constant. Also, the lipid content of the degraded lipoproteins varies from particles of one density to another though the composition of these lipids remains relatively constant. These observations suggest that ether extraction of lipids from lipoproteins may be a selective process which involves particular combinations of lipid constituents. Since they are displaced from the lipoproteins in these particular combinations, they may also exist in this same or similar combination in the intact lipoprotein. This may be indicative of some structural features of the lipid moiety of lipoproteins. Similarly, the lipids of the degraded lipoproteins may also exist as particular combinations of lipids, mutually bound together, associated with the protein moiety.

The foregoing discussion may be extended by considering the mole ratios of the lipid constituents. The mole ratios with respect to cholesteryl ester (as oleate) : cholesterol : phospholipid (as lecithin) : triglyceride (as oleate) of the lipids extracted by ether are approximately 1.3:1.4:1:4.3. For the degraded lipoprotein fractions of the density ranges from 1.007 to 1.065 g./ml. and from 1.065 to 1.20 g./ml. the ratios are approximately: 0.10: 0.43:1:0.26 and 0.10:0.29:1:0.23, respectively. The ratios among cholesteryl esters, phospholipid and triglyceride are approximately the same for the two fractions of the degraded lipoproteins. The variable component is cholesterol which is higher for the fraction with the higher lipid to protein ratio.

It would be of interest to know what mole ratios can be obtained by extraction with solvents other than ethyl ether. In the present study it is interesting to note that the number of hydroxyl, acidic and basic groups in the combination of lipid constituents extracted by the ether are fewer than in the combination of lipid constituents extracted by the addition of a more polar solvent such as methyl alcohol to the ether. If these mole ratios could be obtained with extracting solvents other than ether, then such mole ratios may suggest some of the lipid–lipid molecular associations involved in the structure of lipoproteins.

Degradation Products of the High Density Lipoprotein.—The ether in contact with the aqueous solution of the initial high density lipoproteins extracted only 3.05 mg. from 152.2 mg. of lipids originally present. The weights (in mg.) of the lipids extracted in each of the four extraction steps are as follows: 1.08, 0.69, 0.48 and 0.80. Although a negligible amount of lipid was extracted, a considerable alteration of the physical properties of the lipoproteins was effected by the ether as can be seen in Fig. 4.

Inspection of Table III shows no distinguishable differences in the lipid compositions between the initial material and the isolated degradation products. However, the average lipid-to-protein ratios

TABLE III

COMPOSITION OF THE INITIAL AND THE DEGRADED HIGH DENSITY LIPOPROTEINS

	Lipoprotein compn.,[a] mg.		Lipid composition, wt. %[b]				
	Protein	Lipid	CSE	CS	PL	TG	FA
Initial HDL	162	152	32	6	44	16	3
Degraded HDL d^{20}_4 m 1.065 g./ml.	13.3	51.5	34	7	41	16	3
Degraded HDL $1.065 < d^{20}_4 <$ 1.20 g./ml.	37.6	59.8	31	6	46	13	4
Degraded HDL d^{20}_4 1.20 g./ml.	48.8	0.6	..	..	..	..	..

[a] In amounts actually isolated for each density range. [b] Averages of two determinations.

(17) In a preliminary experiment with a similar system, it was found that ether extracted approximately 80% of the total lipids of the S_f20-400 class lipoproteins in about 90 minutes. Further extraction for several hours yielded only a negligible amount of additional lipid.

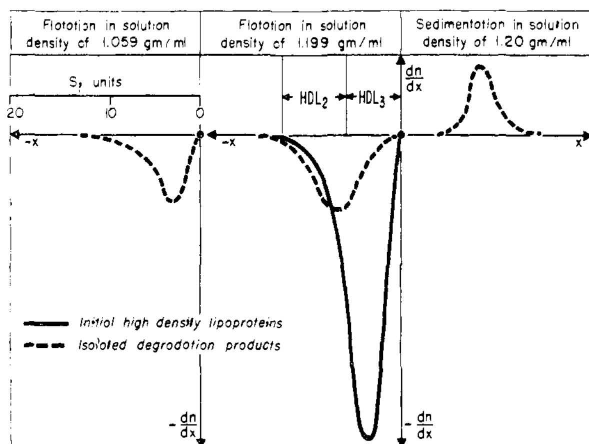


Fig. 4.—Comparison of the ultracentrifugal diagrams of the initial high density lipoproteins (HDL-2 and HDL-3) and their degradation products. The initial distribution contains predominantly HDL-3 class lipoproteins. After extraction there is a pronounced reduction of the HDL-3 class resulting in a lipoprotein distribution principally within the HDL-2 class.

show significant changes for each of the isolated degradation products from that of the initial material.

TABLE IV

SOME PHYSICAL PROPERTIES OF THE DEGRADATION PRODUCTS FROM THE S_f20-400 AND THE HIGH DENSITY LIPOPROTEINS

	Density range, g./ml.	Hydrated density	Mol. wt.	Wt. % lipid
Degraded S_f20-400 lipoproteins	1.007–1.065	1.05	1.6×10^6	85
	1.065–1.20	1.11	8.2×10^5	54
Degraded HDL	<1.065	1.04	1.1×10^6	79
	1.065–1.20	1.09	5.2×10^5	61
	>1.20	1.32	4.3×10^4	1

The degradation product which sedimented in the solution density of 1.199 g./ml. was found to contain only 1.2% lipid by weight. The sedimentation patterns of this degradation product are shown in Fig. 5. The hydrated density was estimated (by ηS *vs.* ρ study) to be 1.32 g./ml. and the calculated molecular weight assuming spherical particles was 40,000. It is of interest that in a similar study on centrifugally isolated high density lipoproteins, Scanu, *et al.*,[18] obtained by prolonged alcohol–ether and ether extraction at $-20°$ a soluble protein containing only 0.5% lipid. The molecular weight of this protein, calculated by sedimentation and diffusion, assuming a partial specific volume of 0.729, was 75,000. However, it is not possible directly to compare their results with ours since the conditions of extraction as well as the manner of characterizing the soluble protein degradation product were different.

(18) A. Scanu, L. A. Lewis and F. M. Bumpus, *Arch. Biochem. and Biophys.*, **74**, 390 (1958).

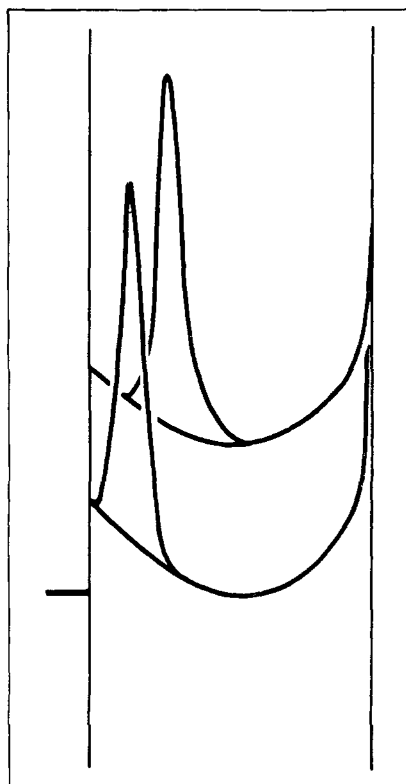


Fig. 5.—Ultracentrifugal sedimentation patterns of the essentially lipid-free degradation product from the high density lipoproteins taken 108 minutes after rotor reached full speed (52,640 r.p.m.). The solution density, at 20°, for the upper pattern was 1.098 g./ml. and 1.199 g./ml. for the lower pattern. Sedimentation proceeds toward the right.

Ultracentrifugal analyses show that the appearance of the degradation products was accompanied by a significant reduction in HDL-3[19] lipoprotein concentration. This observation together with the fact that a negligible amount of lipid was extracted by the ether suggests the dissociation of the HDL-3 lipoproteins into an essentially lipid-free protein product and a lipid or lipoprotein product. The latter apparently is involved in the formation of the lower density, higher molecular weight products. Table IV shows that the amount of protein per molecule of product (expressed as grams protein per mole lipoprotein) is comparable to that calculated for the HDL-2 lipoprotein. A reasonable mechanism for the above degradation process might be the dissociation of HDL-3 lipoproteins into at least two products, (1) a lipid-free protein unit and (2) a lipid-containing unit. In the degradation process one or more of the latter units might then associate with a HDL-2 lipoprotein to form a larger low-density macromolecular complex.

Avigan[11] found that the high density lipoproteins were stable against ethyl ether extraction at 4° and that their delipidization with an alcohol–ether mixture yielded soluble protein molecules. However, in our investigation extraction with ether at about 25° yielded an appreciable amount of soluble, essentially lipid-free, protein molecules. These

(19) R. N. Hazelwood, THIS JOURNAL, **80**, 2152 (1958).

similar experiments at different temperatures suggest that stability of the high density lipoprotein against ether extraction is strongly temperature dependent.

Acknowledgments.—The authors wish to acknowledge helpful discussions with Drs. John W. Gofman and N. K. Freeman.

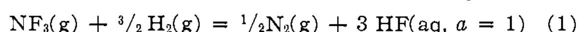
BERKELEY, CALIFORNIA

COMMUNICATIONS TO THE EDITOR

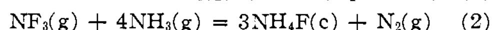
HEAT OF FORMATION OF NITROGEN TRIFLUORIDE AND THE N-F BOND ENERGY

Sir:

The heats of reaction (1) and (2) have been determined using calorimetric procedures customary in our laboratory, and reasonably concordant values for the heat of formation of NF_3 have been



$$NF_3(g) + {}^3/_2 H_2(g) = {}^1/_2 N_2(g) + 3\ HF(aq, a = 1) \quad (1)$$



$$NF_3(g) + 4NH_3(g) = 3NH_4F(c) + N_2(g) \quad (2)$$

derived from the measurements.

For reaction (1) measured quantities of NF_3 and excess hydrogen over water were ignited in a bomb. No residual NF_3 was found in the products. ΔH^0_{25} for reaction (1) was determined by four experiments for which averages are shown in Table I as calculated on the basis of (a) NF_3 dosage and (b) HF titration. Uncertainties listed are standard deviations of the means.

Reaction (2), carried out by igniting a measured quantity of NF_3 mixed with excess ammonia, with no water, resulted in the formation of a crystalline deposit of NH_4F on the bomb walls. Eight experiments were performed. In Table I are shown values for ΔH^0_{25} calculated from (c) volume of NF_3, (d) weight of NF_3, (e) weight of NH_4F, (f) moles NH_3 determined by analysis of the NH_4F, and the mean of these weighted inversely as the squares of the standard deviations.

TABLE I

RESULTS OF CALORIMETRIC MEASUREMENTS

	Reaction	Basis of calculation	ΔH^0_{25} kj./mole	$\Delta Hf^0_{25}(NF_3)$ kcal./mole
(a)	(1)	NF_3	-845.6 ± 1.7	
(b)	(1)	HF	-871.9 ± 0.6	
	(1)	Mean	-859.0 ± 13.4 (= -205.3 ± 3.2 kcal./mole)	-30.7 ± 3.4
(c)	(2)	NF_3 volume	-1084.1 ± 7.1	
(d)	(2)	NF_3 weight	-1081.1 ± 3.3	
(e)	(2)	NH_4F weight	-1087.0 ± 2.5	
(f)	(2)	NH_3 titration	-1098.7 ± 8.4	
	(2)	Mean	-1085.7 ± 4.2 (= -259.5 ± 1.0 kcal./mole)	-29.4 ± 2.1
	(1 and 2)	Mean		-29.7 ± 1.8

The heat of formation of NF_3 is calculated from the measurements on reaction (1) using ΔH^0-[HF(aq., $a = 1$)] = -78.66 kcal./mole.[1] It is calculated from the measurements on reaction (2) using $\Delta H_f[NH_3(g)] = -11.04$ kcal./mole[1] and $\Delta H_f[NH_4F(c)] = -111.0$ kcal./mole.[2] In the latter calculation, the principal uncertainty is in the heat of formation of NH_4F, which we estimate to be ± 0.6 kcal./mole. The weighted average of the results gives $\Delta H_{f25}[NF_3] = -29.7 \pm 1.8$ kcal./mole, and indicates that NF_3 is slightly more stable than was found by Ruff and Wallauer.[3,1]

With E(F–F) = 37.7 ± 1 kcal./mole[4] and E-(N≡N) = 225.92 ± 0.1 kcal./mole,[5,1] the mean N–F bond energy E(N–F) in the NF_3 molecule is 66.4 ± 0.8 kcal./mole. Following Reese and Dibeler[6] the dissociation energies of the individual bonds are estimated to be at 25°: $D(NF_2–F)$ = 74.0; D(NF–F) = 62.6 D(N–F) = 62.6 kcal./mole.

(1) National Bureau of Standards Circular 500, 1952.

(2) T. L. Higgins, "Dissertation Abstracts," **17**, 1231 (1957).

(3) O. Ruff and H. Wallauer, *Z. anorg. allgem. Chem.*, **196**, 421 (1931).

(4) W. H. Evans, T. R. Munson and D. D. Wagman, *J. Research, Nat. Bur. Standards*, **55**, 147 (1955).

(5) L. Brewer and A. W. Searcy, *Ann. Rev. Phys. Chem.*, **7**, 221 (1956).

(6) R. M. Reese and V. H. Dibeler, *J. Chem. Phys.*, **24**, 1175 (1956).

NATIONAL BUREAU OF STANDARDS
WASHINGTON 25, D. C.

GEORGE T. ARMSTRONG
SIDNEY MARANTZ
CHARLES F. COYLE

RECEIVED MAY 27, 1959

A MODEL FOR CELLULAR CATION DISCRIMINATION

Sir:

The preferential uptake of potassium (or rubidium) ions from solutions containing 20–30 times more sodium than potassium by cells is a fundamental fact of biology. However, no cationic discrimination system has been isolated from cells nor does any known chelating agent or ion exchange resin exhibit this degree of preference for potassium over sodium.[1] This note describes results with orthoclase, a silicate feldspar ($KAlSi_3O_8$) which shows properties of cationic selection, similar to that exhibited by living cells.

In the orthoclase lattice there is insufficient free space surrounding the potassium to accommodate a single water molecule in the structure.[2] When orthoclase particles[3] (20–80 mesh) are incubated with Rb^{86} or Na^{22} (added as the chloride) these cations are taken up from solution (esti-

(1) *Cf.* I. M. Glynn, *Progress in Biophysics*, **8**, 241 (1957).

(2) C. E. Marshall, "Colloid Chemistry of the Silicate Minerals," Academic Press, New York, N. Y., 1949, pp. 23–25.

(3) Silicate minerals, obtained from Wards Natural Science Establishment, N. Y., included orthoclase *var.* Adularia (Brimfield, Mass.), microcline (Ontario), and leucite (Rome); samples were crushed, sieved, and washed with water, ethanol, and ether.