

Serum Lipoproteins and Apolipoproteins in Rats with Streptozotocin-Induced Diabetes

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ABSTRACT The lipoproteins of rats fed a high sucrose diet and made diabetic by administration of 45 mg/kg of streptozotocin were studied. All lipoprotein classes were found to be present in increased concentrations. The apolipoprotein composition of the various lipoprotein fractions was studied by polyacrylamide-gel electrophoresis in the presence of 8 M urea, isoelectric focusing in the presence of 8 M urea, and sodium dodecyl sulfate gel electrophoresis in polyacrylamide gels. In the very low density lipoproteins (VLDL) of diabetic rats, there was a marked alteration in the relative amounts of C proteins by polyacrylamide-gel electrophoresis, and this was found by isoelectric focusing to be primarily a relative increase in C-III-3 apoprotein and a decrease in C-III-0. In addition, in the diabetic rats, the VLDL contained a protein of mol wt 46,000, the A-IV protein, which normally is only present in the high density lipoproteins. In the high density lipoproteins, (HDL) the same alterations in pattern of the C proteins seen in the VLDL were present. Furthermore, the arginine-rich and A-IV protein normally present in HDL could not be detected in the HDL, although the other apolipoproteins are present.

Apolipoprotein concentrations were determined by quantitative immunoelectrophoresis. It was found that in the diabetic rats there was an increase in the total amount of apo-B in the plasma, with the increment divided proportionately between the VLDL and the low density lipoprotein (LDL). The total apo-C concentration of plasma increased minimally. The A-IV concentration of plasma increased by 27%; it decreased markedly in the HDL, but appeared in increased amounts in both VLDL and in the $d > 1.21$ fraction. The arginine-rich protein decreased

by 63% in the plasma and decreased significantly in the HDL, but increased in VLDL, LDL, and in the $d > 1.21$ fraction.

These alterations in apolipoprotein patterns in diabetic animals suggest that the apolipoproteins may play an important role in determining the concentration of the various lipoprotein fractions, or may be the result of altered metabolism of the lipoproteins. These lipoproteins with altered apolipoprotein composition may have important biologic differences from normal lipoproteins. Nevertheless, the HDL, despite the fact that it is deficient in some of its major constituents, was unchanged in its cholesterol content.

INTRODUCTION

We have shown that marked hyperlipoproteinemia can be produced by the intravenous injection of 45 mg per kg of streptozotocin to rats fed a diet high in sucrose (1). Serum triglyceride concentration increased markedly and cholesterol and phospholipid concentrations also increased.

It is now well established, both in man and in the rat, that the protein portion of the various lipoproteins contain heterogeneous subunit proteins, the apolipoproteins (2-7). In a previous publication Swaney et al. (8) have described the apolipoproteins of rat high density lipoprotein (HDL):¹ they contain in addition to its major component, A-I, the C apolipoproteins, an arginine-rich protein of mol wt 35,000, and an additional protein of mol wt 46,000, that has been designated A-IV. It has been demonstrated that alterations of apolipoprotein composition take place after cholesterol or sucrose feeding to rats (9, 10), monkeys (11), and rabbits (12). Alterations of composition and concentration of apolipoproteins have also been shown to occur in human disease, as in

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¹Abbreviations used in this paper: HDL, high density lipoproteins; LDL, low density lipoproteins; SDS, sodium dodecyl sulfate; VLDL, very low density lipoproteins.

hypothyroidism (13), type III hyperlipoproteinemia (14), and in obstructive jaundice (15).

In this paper, we present data on the serum lipoprotein concentrations and on the apolipoprotein composition of the major plasma lipoproteins in diabetes mellitus induced in sucrose-fed rats. The distribution of certain of the apolipoproteins among the various lipoprotein classes was also measured in these animals.

METHODS

Induction of diabetes

Male Sprague-Dawley rats (Holtzman Co., Madison, Wis.), weighing 200–300 g were used. The animals were fed a semi-purified sucrose-rich diet which contained (wt/wt): 20% vitamin-free casein, 60% sucrose, 5% lard, 1% vitamin mixture, 4% salt mixture, and 10% cellulose (obtained from General Biochemical Div., Chagrin Falls, Ohio) for a 3-wk period, after which time they were divided into two groups: sucrose-fed control rats and sucrose-fed diabetic rats. Diabetes mellitus was produced by i.v. injection into the tail vein of 45 mg/kg body weight of streptozotocin (supplied by Dr. W. Dulin of The Upjohn Co., Kalamazoo, Mich.).

The streptozotocin was freshly dissolved in 0.05 M citric acid pH 4.5 and was injected within 5 min of its preparation. The volume of administered streptozotocin in solution did not exceed 0.5 ml for each rat. Control rats were injected with 0.5 ml 0.05 M citric acid containing no streptozotocin. During the first 24 h after injection, the rats were supplied with 5% glucose in their drinking water, as it is known that hypoglycemia occurs 7–12 h after administration of streptozotocin (16). The rats were observed for glycosuria and ketonuria throughout the study.

With this dose of streptozotocin, plasma glucose was markedly increased; plasma immunoreactive insulin markedly decreased; and ketonuria did not occur. The animals were sacrificed 12 days after the administration of streptozotocin by exsanguination from the abdominal aorta. In each instance, the bloods were obtained without prior fasting.

Separation of lipoproteins

Lipoprotein separation was carried out by the method of Havel et al. (17), using the SW 41 Rotor in the Beckman model L-2 65B ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) at 15°C. Chylomicrons were removed after ultracentrifugation at 10,000 *g* for 30 min. Very low density lipoproteins (VLDL) of $d < 1.006$ and low density lipoproteins (LDL), $d 1.006$ – 1.063 , were separated by 20 h ultracentrifugation at 200,000 *g*, while HDL of $d 1.063$ – 1.21 were separated by 40 h of ultracentrifugation at 200,000 *g*. Densities above 1.006 were adjusted by addition of KBr solutions.

The isolated fractions were separated using a Spinco tube cutter (Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.); they were washed once by resuspending them in their respective density solutions and then repeating the ultracentrifugal separations. The washed lipoprotein fractions were exhaustively dialyzed against water with 0.005% EDTA pH 7.0 at 4°C.

To obtain apolipoproteins free of lipid, the isolated fractions were lyophilized and delipidated at 4°C with a mixture of 3:1 absolute ethanol:anhydrous diethyl-ether, as

described by Brown et al. (3). As suggested by Scanu and Edelstein (18), cold ether was then added to the mixture to adjust it to 1:1 ethanol (vol/vol). The precipitate was centrifuged at 4°C at 2,000 rpm for 45 min. The delipidated apolipoproteins were dissolved in 0.2 M Tris-HCl buffer pH 8.2 containing 0.06 M sodium decyl sulfate and 8 M urea.

Chemical methods

Proteins were determined by the method of Lowry et al. (19), as modified by Sata et al. (20). Cholesterol was determined by the method of Abell et al. (21).

Electrophoresis

Polyacrylamide disk gel electrophoresis was carried out using 10% gels in 8 M urea (22). The stacking gel and separating gel contained Tris-HCl 0.7 M, pH 6.6–6.8, and 0.12 M, pH 8.8–8.9, respectively. Gels were polymerized in glass tubes, 6 × 150 mm OD. A discontinuous buffer system was used consisting of Tris-glycine buffer, pH 9.05 at the cathode and Tris-HCl buffer, pH 8.1 at the anode; 75–125 μ g of protein was applied to each of the tubes. Electrophoresis was carried out for a 3-h period at 15°C with a constant current of 3.5 mA per tube. The gels were fixed and stained for protein with 1% Coomassie Blue in 7% acetic acid and 15% trichloroacetic acid (TCA) for 18 h. Destaining was carried out in 7% acetic acid for 48 h. Densitometric scanning of gels was performed in a Gilford Spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) with a linear transport unit at a wave length of 527 nm. The area under the peaks was integrated during scanning. Since purified apolipoproteins were not available, the staining response of the various apolipoproteins was determined indirectly by applying varying amounts of delipidated VLDL or HDL to the gels and comparing the percent distribution of the various apolipoproteins, as was done previously by Glickman and Kirsch (23). When varying amounts of protein were applied, the relative distribution of the apolipoproteins was found to be constant, suggesting that for each apolipoprotein its optical density is directly proportional to the amount of protein present.

Isoelectric focusing was performed on the apoproteins of VLDL and HDL (24). 75–125 μ g of protein was applied to each gel containing 7.5% acrylamide in 8 M urea. The catholyte and anolyte consisted of 0.02 N NaOH and 0.01 M H_3PO_4 , respectively. The pH gradient was established with ampholytes in the pH 4.0–6.0 range (LKB Instruments, Inc., Rockville, Md.) diluted to 2% (wt/vol) and the gels were prefocused with a current of 1 mA per tube. After a potential of 400 V was attained, the electrofocusing of the protein was allowed to proceed for 5–6 h at a temperature of 15°C. The gels were fixed in 6% TCA and stained with Coomassie Blue, as described by Malik and Berrie (25). Densitometry of gels was performed as described above.

Sodium dodecyl sulfate (SDS) polyacrylamide-gel electrophoresis was performed according to the method of Shapiro et al. (26), as modified by Maizel (27). Acrylamide gels of 12.5% were polymerized in tubes of 6 × 100 mm OD, and a continuous buffer system, consisting of 0.1 M sodium phosphate pH 7.0 containing 0.1% SDS was used. Electrophoresis was carried out for a 20-h period at 30 V; the temperature was maintained at 15°C; and 10–20 μ g of protein was applied to each gel. Before electrophoresis, the proteins were reduced by heating with 1% 2-mercaptoethanol for 1 min at 100°C. The gels were stained with

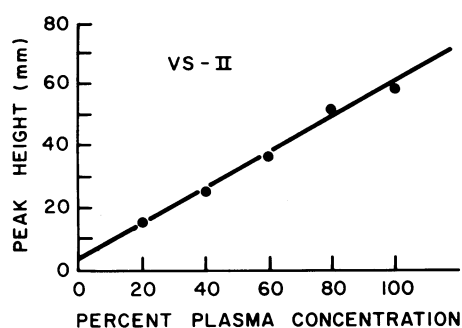


FIGURE 1 Relationship between concentrations of plasma and height of peak as determined by quantitative immunoelectrophoresis against antiserum to VS-II (arginine-rich protein). Whole rat serum was diluted in goat serum to give the various concentrations.

0.2% Coomassie Blue in 50% methanol and 9% acetic acid for a period of 20 h. They were then destained in 7% acetic acid and 5% methanol for 24 h.

Quantitative immunoelectrophoresis was used for determination of apolipoprotein concentration. The rocket method of Laurell (28) was used. The plates for the quantitative immunoelectrophoresis were 200 × 100 × 2 mm in size. The agarose (Seakem, Marine Colloids, Inc., Rockland, Maine) was made up in a 1% solution in 0.025 M barbital buffer pH 8.6. The antiserum was mixed with the agarose solution at 50°C. The concentration of a specific antiserum ranged from 0.2 to 7%, depending on the apolipoprotein being determined. During electrophoresis, precipitation occurs at the point of antigen-antibody equivalence forming a rocket-shaped peak, the height of which is a function of the amount of antigen applied. By this method, it is possible to determine the relative concentration of specific proteins in the various lipoprotein classes.

Apolipoprotein concentration was determined on whole plasma or on lipoprotein fractions. We had found (29) as did Laurell, that isolated proteins behave differently from proteins present in solution with other proteins. We have, therefore, determined the apolipoprotein content of the various samples by measuring the apolipoprotein content of the various infranatants, i.e. the $d > 1.006$ fraction, the $d > 1.063$ fraction, and the $d > 1.21$ fraction. These fractions were prepared by ultracentrifugation as previously de-

TABLE I
Plasma Lipoprotein-Protein Concentrations in Sucrose-Fed Control and Diabetic Rats

| | No. of pools | VLDL | LDL | HDL |
|------------------------------------|--------------|-------------|------------|------------|
| <i>mg protein/100 ml of plasma</i> | | | | |
| Control | 4* | 10.0 ± 1.0† | 2.5 ± 0.5 | 37.6 ± 7.5 |
| Diabetic | 7§ | 69.9 ± 12.0 | 14.4 ± 2.4 | 63.9 ± 6.5 |

* Each pool was prepared by combining equal volumes of plasma from six rats.

† Mean ± SEM.

§ Each pool was prepared by combining equal volumes of plasma obtained from three rats, 12 days after streptozotocin administration.

TABLE II
Plasma Lipoprotein Lipid Concentrations in Sucrose-Fed Control and Diabetic Rats

| | VLDL | | LDL | HDL |
|----------------------------|--------|-------|-------|-------|
| | TG* | Chol* | Chol | Chol |
| <i>mg/100 ml of plasma</i> | | | | |
| Control | 188† | 7.6† | 2.6§ | 32.0§ |
| Diabetic | 1,055† | 56.7† | 32.9† | 54.4† |

* TG, triglyceride; chol, cholesterol.

† Values are the mean of duplicate analyses of five pools of plasma; each pool contains the plasma obtained from three rats.

§ Values are the mean of duplicate analyses of three pools of plasma; each pool contains the plasma obtained from four to six rats.

scribed; dialyzed against 0.85% NaCl solution containing 1 mg/ml of EDTA, pH 7.4; and were then adjusted to their original volume of 5 ml with 0.85% sodium chloride solution. The amount of apoprotein in a given density fraction is, thus, the difference between the peak heights of the successive infranatants, e.g., the amount of apo-B in VLDL is obtained by subtracting the amount of apo-B in the $d > 1.006$ fraction from that found in the whole plasma. The amounts of each apoprotein are expressed in terms of the percent of that present in whole plasma. When various dilutions of plasma were subjected to quantitative immunoelectrophoresis, peak heights found were proportional to the amount of protein present (Fig. 1).

Preparation of antisera

Antisera was prepared in goats by intramuscular and intradermal injection of mixtures of equal volumes of antigen (0.5–5 mg of protein) and complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) (30). In addition, 2.5 ml of pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.) was administered subcutaneously. Antigens were injected either two or three times at 3–4-wk intervals.

The following antigens were used: LDL (d 1.030–1.050) prepared by ultracentrifugation from normal rat serum; the antiserum obtained was absorbed with HDL. A mixture of C proteins (VS-III) was also used as an antigen. This is the fraction obtained when apo-VLDL is eluted from Sephadex G-200 columns (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) by the method of Bersot et al. (6). By polyacrylamide-gel electrophoresis, the various C proteins are found in this fraction. Therefore, it is heterogeneous and results in the production of a polyvalent antiserum. The VS-II fraction of rat apo-VLDL was used as an antigen. In this fraction, only the arginine-rich protein could be detected by SDS-gel electrophoresis. A-1 was obtained by gel filtration on Sephadex G-200. The fraction at the HS-2 peak contained only A-I and was used as the antigen. A-IV antigen was prepared by preparative SDS-gel electrophoresis, cutting an appropriate band and triturating it in phosphate buffer. The various antisera were tested against whole serum and column fractions by the double-diffusion method, and they did not cross react but did react with the appropriate antigen.

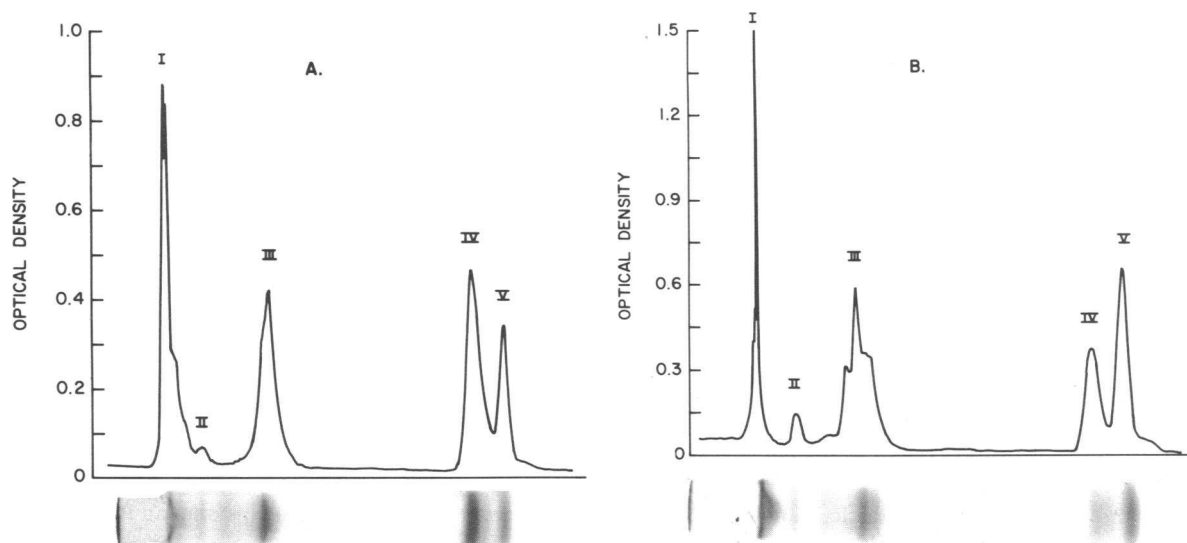


FIGURE 2 Polyacrylamide-gel electrophoresis in 8 M urea of apo-VLDL from (A) sucrose-fed control rats and (B) diabetic rats. Shown in the figure is a photograph of the gel and superimposed above the photograph is its densitometric scan.

RESULTS

Serum lipoprotein concentrations. The administration of streptozotocin resulted in a sevenfold increase in VLDL protein and marked increase in LDL and HDL protein, Table I. The triglyceride present in VLDL (Table II) increased sixfold, whereas the cholesterol increased eightfold. The cholesterol content of LDL increased to a similar extent, while that in HDL almost doubled.

The apolipoproteins of VLDL. The electrophoretic patterns of apo-VLDL obtained in 10% polyacrylamide gels in 8 M urea are shown in Fig. 2, where the patterns from sucrose-fed rats (A) are compared with the patterns from diabetic rats (B). Five major bands are present: band I is located between the stacking and the running gels, and has been identified previously as the apo-B protein; band II has been identified as C-I by Herbert et al. (31); band III corresponds to the arginine-rich protein in control rats. In the diabetic rat, band III is asymmetrical, suggesting the presence of other proteins.

Bands IV and V are the C proteins other than C-I. It is apparent that there is relatively more band IV than band V protein in VLDL of the sucrose-fed control rats. In contrast, however, the gels from the diabetic rats contain relatively more band V than band IV protein. The predominance of band V in the diabetic VLDL is apparent in the scans. The peaks were integrated, and the ratio of area under peak IV to that under peak V measured. This was found to be 1.92 in the controls, whereas in the diabetic rats it was 0.59. Herbert et al. (31) found

the less acid peak (band IV) to contain C-III-0, as well as C-II, and the more acidic band (band V) to contain C-III-3. Further resolution of the C proteins was obtained by isoelectric focusing. In Fig. 3 are shown the patterns of apo-VLDL from sucrose-fed control and diabetic rats. The distribution of the various C proteins is altered in the diabetic rat. Band 2 (C-III-0) is predomi-

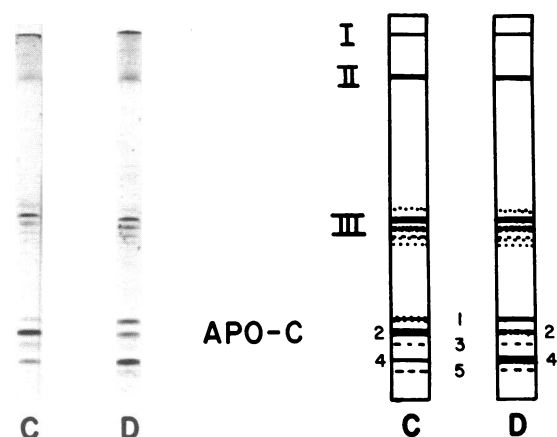


FIGURE 3 Isoelectric focusing on polyacrylamide gels over the pH range 4.0-6.0 of apo-VLDL from (C) sucrose-fed control rats and (D) diabetic rats. On the left, are shown the photographs of the original gels; on the right, schematic diagram. Areas I and III represent the apo-B and arginine-rich proteins, respectively. The apo-C proteins are numbered 1-5 in the order of their isoelectric points. These have been identified by Swaney and Gidez (personal communication) as follows: band 1 is C-II, band 2 is C-III-0, band 3 may be C-III-1, band 4 is C-III-3, and band 5 may be C-III-4.

TABLE III
Distribution of C-Apolipoproteins in APO-VLDL of Sucrose-Fed Control and Diabetic Rats Separated by Isoelectric Focusing

| Band no.*..... | 1 | 2 | 3 | 4 | 5 |
|----------------------|-----------|----------|------------|----------|----------|
| Identification†..... | C-II | C-III-0 | C-III-1 | C-III-3 | C-III-4 |
| | | | % of total | | |
| Control | 10.8±0.5§ | 40.4±0.5 | 18.3±1.2 | 23.4±1.9 | 7.1±0.2 |
| Diabetic | 18.7±0.7 | 22.9±1.1 | 7.3±0.3 | 40.1±1.4 | 11.0±0.3 |

* Refer to fig. 3.

† See legend to fig. 3.

§ Values represent the means of duplicate scans of VLDL preparations from four pools of control plasma and seven pools of diabetic plasma. Each pool contained plasma from at least three rats. The total area of the C proteins was taken as 100%.

nant in the control; in the diabetic, band 4 (C-III-3) predominates. These bands of the apo-C proteins were scanned and the peaks integrated. The results are shown in Table III, where alterations in the relative distribution of the C protein bands in diabetes are shown quantitatively.

Swaney et al. (8) have previously shown that SDS-gel electrophoresis is useful for resolution of apolipoproteins of mol wt above 20,000. Fig. 4 shows the SDS-gel electrophoresis of apo-VLDL from sucrose-fed control and diabetic rats. In the procedure used, the C proteins are not well resolved and there are a number of unidentified minor bands. The apo-B proteins do not enter the gel. In the control rats (C), there is only one other high mol wt apolipoprotein. It has a mol wt of 35,000 and corresponds to the arginine-rich protein found in HDL (8). The apo-VLDL from the diabetic rats contains in addition, a band of higher mol wt (46,000) which corresponds to the A-IV component of apo-HDL of control rats.

The apolipoproteins of HDL. Separation of the apo-HDL proteins using 10% polyacrylamide gels in 8 M urea and also by isoelectric focusing revealed changes in

the distribution of the C proteins similar to those found in apo-VLDL.

By SDS-gel electrophoresis (Fig. 5), marked differences between the sucrose-fed control and diabetic high molecular weight apoproteins of HDL were found. In the control, three proteins are clearly seen in apo-HDL. They are A-I of mol wt 26,000, the arginine-rich with mol wt 35,000, and A-IV with mol wt 46,000. In contrast, in the apo-HDL from the diabetic, only the A-I was seen, while the arginine-rich protein and A-IV were not detected. These findings were confirmed by isoelectric focusing of apo-HDL. Since the relative amounts of the C proteins are low in HDL, they are poorly visualized in these gels.

Quantitation of the apolipoproteins by quantitative immunoelectrophoresis. The results are summarized in Table IV. Apo-B is increased in whole plasma of diabetic rats by 86% and the increment is distributed between VLDL and LDL in the same proportion as in serum from sucrose-fed control rats. The apo-C in whole plasma increased by 15% in the diabetic rats. However, its distribution was altered with the percentage in VLDL increasing and that in HDL decreasing. A-I was in-

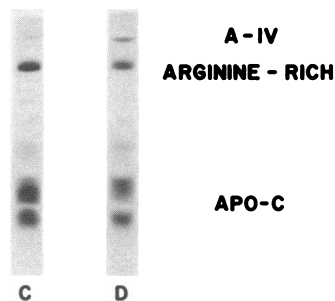


FIGURE 4 SDS electrophoresis in 12.5% polyacrylamide gels of apo-VLDL from (C) sucrose-fed control and (D) diabetic rats.

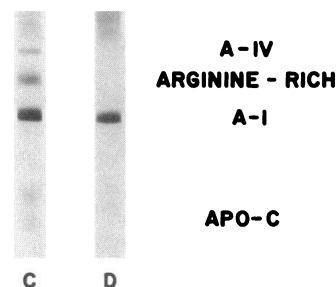


FIGURE 5 SDS gel electrophoresis in 12.5% polyacrylamide gels of apo-HDL from (C) sucrose-fed control and (D) diabetic rats.

TABLE IV
*The Relative Plasma Concentration and Distribution of Apolipoproteins in the Lipoproteins in Sucrose-Fed Control and Diabetic Rats**

| Apo-lipoprotein | | Plasma† (% of control) | Distribution between lipoprotein classes, % | | | |
|-----------------|----------|---------------------------|---|-----|-----|------------|
| | | | VLDL | LDL | HDL | $d > 1.21$ |
| Apo-B | Control | 100 | 71 | 29 | ND§ | ND |
| | Diabetic | 186 | 69 | 30 | <5% | ND |
| Apo-C | Control | 100 | 14 | 8 | 78 | ND |
| | Diabetic | 115 | 23 | 12 | 65 | ND |
| A-1 | Control | 100 | 8 | 10 | 82 | <5% |
| | Diabetic | 146 | 13 | 7 | 65 | 15 |
| A-IV | Control | 100 | 18 | 13 | 67 | <5% |
| | Diabetic | 127 | 28 | 5 | 13 | 54 |
| ARP | Control | 100 | 15 | 10 | 63 | 12 |
| | Diabetic | 63 | 38 | 27 | 18 | 17 |

* Average of duplicate determinations on two pools of plasma obtained from three to six rats.

† The amount present in plasma from nondiabetic rats was assigned a value of 100%.

§ ND, Nondetectable (<2%).

creased by 46% in the plasma of diabetic rats with the proportion in HDL reduced and a significant amount was present in the $d > 1.21$ fraction. The A-IV protein in plasma increased by 27% and shows a markedly altered distribution between the diabetic and nondiabetic rats, with the proportion in HDL decreasing from 67% of the total to 13%, while that in VLDL increased, but most striking was the increased amount present in the $d > 1.21$ fraction. The arginine-rich protein concentration decreased in the diabetic plasma to 63% of the control value. However, its distribution was also greatly altered:

the percent in HDL decreased markedly and the remainder appeared in VLDL, LDL, and in the $d > 1.21$ fraction.

One example of this technique is shown in Fig. 6, in which plasma from diabetic rats and sucrose-fed control rats and their respective $d > 1.006$, $d > 1.063$, and $d > 1.21$ infranatants were quantitated using antiserum to the VS-II fraction (high arginine). The decreased concentration of this apoprotein in the whole plasma of the diabetic is apparent. In the control, most of this protein is in the 1.063-1.21 fraction (HDL), whereas in the diabetic the amount present in that fraction is markedly reduced. As previously noted, the arginine-rich and A-IV proteins could not be detected in the HDL of diabetic rats using SDS-gel electrophoresis, whereas it was detected by the Laurell method. This difference is due to the greater sensitivity of the immunologic technique.

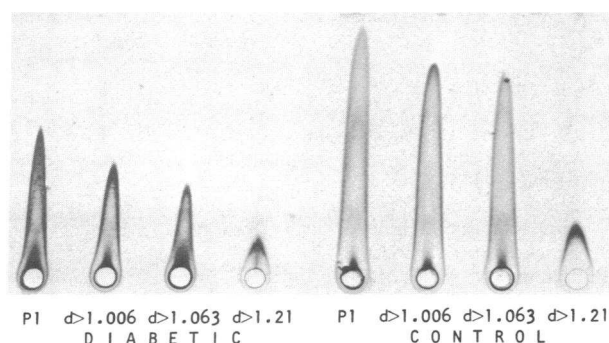


FIGURE 6 Quantitative immunoelectrophoresis of plasma ultracentrifugal fractions. The plates contained 1% agarose with 5% vol/vol of antiserum to the VS-II (arginine-rich fraction). P1 is whole plasma; the remaining peaks are the infranatant fractions obtained by ultracentrifugation of plasma at the density indicated.

DISCUSSION

In this study, lipoprotein classes were isolated by ultracentrifugation at the densities conventionally used for separation of lipoproteins. We have previously shown that the rat VLDL can be separated at $d < 1.006$; LDL in the rat fed a normal diet separates in the density range of 1.03-1.05; and the HDL separates at a density of 1.063-1.21 (29). Preliminary removal of chylomicrons was carried out in these studies because blood samples were taken from nonfasted animals. Since an intermedi-

ate fraction (d 1.006-1.03) is not present in the sucrose-fed control or diabetic rats, the conditions used in this study adequately separate the major lipoprotein classes. The degree of the separations can be judged from the data in Table IV, which show that VLDL and LDL contain small amounts of the HDL proteins, A-I and A-IV. Similarly, HDL contained no significant amounts of apo-B. There is an apparent discrepancy between the results in Table IV and Figs. 2A and 4, in which A-I and A-IV could not be detected in the VLDL from the sucrose-fed nondiabetic rats. These differences may be the result of differences in sensitivity of the methods, but also may reflect differences in the preparation of the samples for the different methods, e.g., for polyacrylamide-gel electrophoresis the lipoproteins were washed by repeated ultracentrifugation and then subjected to treatment with organic solvents to remove the lipid.

The increased concentration of VLDL can be the result of either increased production, decreased removal, or both. Recent studies by Reaven and Reaven (32) in rats studied 7 days after administration of streptozotocin found no evidence of increased synthesis of VLDL-triglyceride, and it was concluded that the hypertriglyceridemia is due to a defect in VLDL removal. It is possible that the LDL concentration increased because of increased formation of LDL from VLDL. This might occur as the result of an absolute increase in the rate of degradation of VLDL resulting from the large increase in its plasma concentration (33).

Of considerable interest is the rise in HDL protein concentration, especially since HDL found in the diabetic rats is deficient in certain of the major apolipoproteins of HDL prepared from the serum of sucrose-fed rats. Whether the alteration in the apolipoprotein pattern of HDL is related to the alterations in the rate of production or removal of this has not been established. However, it should be noted that despite these alterations in apolipoprotein composition, the ratio between cholesterol and protein are similar in the diabetic and nondiabetic rats (Table I and II). The marked decrease in amount of arginine-rich protein in HDL, despite the increase in concentration of HDL protein and cholesterol, suggests that this protein is not an obligatory constituent of HDL, nor is it essential for cholesterol transport in HDL. It is unlikely that the decreased amount of arginine-rich protein in HDL is due to transfer to other lipoprotein classes, since the total amount in the serum is decreased. The HDL of the diabetic is also deficient in the A-IV protein, but its level in the plasma is increased, and the protein is found in increased amounts in VLDL. This probably accounts for the asymmetry of peak III in Fig. 1.

In earlier studies (34), we had postulated the presence of apolipoprotein in the $d > 1.21$ fraction, and the

findings of A-IV, arginine-rich apolipoprotein, and A-I in increased amounts in that fraction from diabetic rats suggests the desirability of further study of apolipoproteins in the $d > 1.21$ fraction and their metabolic significance.

The alterations in C protein distribution found by isoelectric focusing indicated that in the diabetic rat, as compared to the sucrose-fed controls, a C-III component containing more sialic acid predominates. It is of interest that in diabetes other serum glycoproteins are also present in increased concentrations (35). Since C-II has been shown to be an activator of lipoprotein lipase (36, 37), whereas C-III has been shown to be an inhibitor in man (38), it is tempting to speculate that the altered ratios of the C proteins, including the ratio of C-III-0 to C-III-3 might influence lipoprotein lipase activity.

From these studies it cannot be determined how the changes in apoprotein patterns are related to the hyperlipoproteinemia: whether the alterations in pattern cause hyperlipoproteinemia, or whether these alterations are the result of the metabolic changes that produce hyperlipoproteinemia.

These alterations in apolipoprotein composition of the lipoproteins in diabetic rats suggest that similar changes may occur in human diabetes. Schonfeld et al. (39) have recently found increased concentrations of apo-B in hyperlipemic diabetic patients as we have found in the diabetic rat. Based on our findings of alterations in the composition and distribution of other apolipoproteins, it is apparent that similar studies should be undertaken in man to obtain better understanding of the pathophysiology of the hyperlipoproteinemia of diabetes.

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