Serum Lipoproteins in Experimental Diabetes. I. Serum Lipoprotein Pattern of Normal and Depancreatized Dogs.* (20968)

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Studies of the past few years on serum lipoproteins suggest that the nature of the protein carrier and the type of linkage between protein and lipid are of fundamental importance in determining the metabolic fate of the lipid moiety. Experimental and clinical evidence further suggests that the *in vivo* transformation of serum lipoproteins usually proceeds from the larger molecules to the smaller ones, the former being the immediate result of fat absorption or mobilization, the latter being most probably related to lipid utilization in cell metabolism(1-6). Gofman and co-workers(7) reported that a strong statistical correlation exists between an increased concentration of certain lipoproteins (Sf_{10-20} and Sf_{20-100}) and clinical and experimental atherosclerosis suggesting an etiologic relationship. In view of the fact that atherosclerosis is more frequent in diabetic than in non-diabetic subjects, the lipoprotein pattern of diabetic patients has been analyzed in several laboratories and, although some results suggest an increase in the Sf_{10-20} and Sf_{20-100} fractions(8), others do not(9).

A study of serum lipoproteins in various types of experimental diabetes, under different conditions of treatment, seemed therefore of particular interest. In this paper, data will be presented on the lipoprotein patterns of normal and depancreatized dogs, with and without insulin treatment, as determined by zone-electrophoresis on filter paper.

Material and methods. Twenty normal dogs, kept on a constant diet of commercial dog food, were fasted about 18 hours. Blood samples were obtained, the serum separated by centrifugation and analyzed to establish normal lipoprotein pattern of the animal. Nine dogs were depancreatized and kept on a constant diet of raw horse meat, sucrose and raw pancreas in quantities sufficient to prevent fatty infiltration of the liver(10). After recovery from the operation, insulin requirement for complete control of glycosuria in 24-hour urine specimen was determined for each animal and varied between 10 and 40 units. Dose of insulin was then diminished or insulin withdrawn completely for several days, daily measurements of the 24-hour output of urine and glucose continued, and the serum lipoprotein pattern studied under various degrees of diabetic control. Forty lipoprotein determinations were made. Qualitative tests for ketone bodies in urine and blood were performed daily. Quantitative determinations of blood glucose and ketone bodies were made periodically and repeated every time lipoprotein analysis was performed. Fractionation of lipoproteins was performed as previously described(1,2) with an apparatus designed according to Durrum(11) and Flynn and de Mayo(12) with minor modifications. Strips of filter paper (Schleicher and Schull 598) 11 × 45 cm were used as support for electromigration and placed in the tank with the ends immersed in barbital buffer at pH 8.6 and μ 0.05. When the front of the "buffer" had climbed to within a few centimeters from the middle of the strip, serum (0.4 ml) was applied to the middle of the strip with a graduated pipette, as a transverse band as uniform as possible. After a few minutes to allow equilibration between serum and buffer, the circuit was closed, and a current of 140-160 volts and about 2 mA/strip, was allowed to flow from a rectifier. Electrophoresis was continued for 16 hours at room temperature. The strips were then dried in oven at 90°C for one half hour and cut longitudinally in 3 parts, 2, 2 and 5 cm wide respectively. The 2 narrow strips were used to identify the various lipoprotein fractions: one was treated with bromphenol blue to stain the proteins and the other with Sudan III to stain the lipids. The intensity of color was measured with the Beckman spectrophotometer by means of a

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special attachment. The largest strip was then cut into sections corresponding to the position of the bands stained by Sudan III. Each section was extracted 3 times with boiling alcohol-ether mixture (3:1), extracts were pooled into a 10 ml glass-stoppered cylinder and, after cooling, the volume was brought to 10 ml with alcohol-ether mixture. A portion of each extract was analyzed for total cholesterol according to the methods of Bloor, Pelkan and Allen(13) adapted to spectrophotometry. Another portion was analyzed for lipid phosphorus according to the method of Youngburg and Youngburg as modified by Horwitt(14), after digestion with 10% perchloric acid. Portions of the serum itself were analyzed similarly. Total cholesterol and lipid phosphorus content of the various fractions were calculated as per cent of total amount recovered from the strip, and as per cent of total amount found in the serum. Recovery of the lipids from the strip was greater than 90%.

Results. Most of the lipids of dog serum are in complexes with high electrophoretic mobility. These constitute a group of fractions whose mobility ranges from that of α_2-globulins to albumin: boundaries between these fractions are often difficult to recognize and a broad band covering albumin-α-globulin area appears after staining with Sudan III. In a few normal sera there appears a distinct band between the α_1 and α_2-globulin. In addition, in some normal sera, lipids can be extracted from a zone immediately ahead of the albumin fraction, where, under certain conditions, significant amounts of lipoprotein migrate(15). The slow-migrating fractions, *i.e.*, the β-lipoproteins, and the fraction which does not migrate at all in filter paper electrophoresis and which will be referred to as the $\beta_2\gamma$-fraction, are present in low concentration in normal post-absorptive dog serum and account only for 15-20% of the total serum lipids (Fig. 1).

Fig. 2 reproduces protein and lipid pattern of normal dog serum as obtained through photometric evaluation of stained protein (bromphenol blue) and lipid (Sudan III) moieties. Lipid composition varies markedly from fraction to fraction. Thus the fast-moving fractions (albumin-α-globulin) are relatively richer in lipid phosphorus than the β-globulin fraction. The average $\frac{\text{cholesterol}}{\text{lipid P}}$ ratio is 18.3 for the albumin-α-globulin and 31.4 for the β-globulin group (Table I). This difference is statistically significant ($P = .03$). Comparison of the intensity of Sudan III stain of various fractions with cholesterol and lipid phosphorus distribution suggests that β fractions are richer in neutral fat than the fast-moving lipoproteins. This tendency of neutral fat to attach itself to the β lipoproteins was observed also after a fatty meal

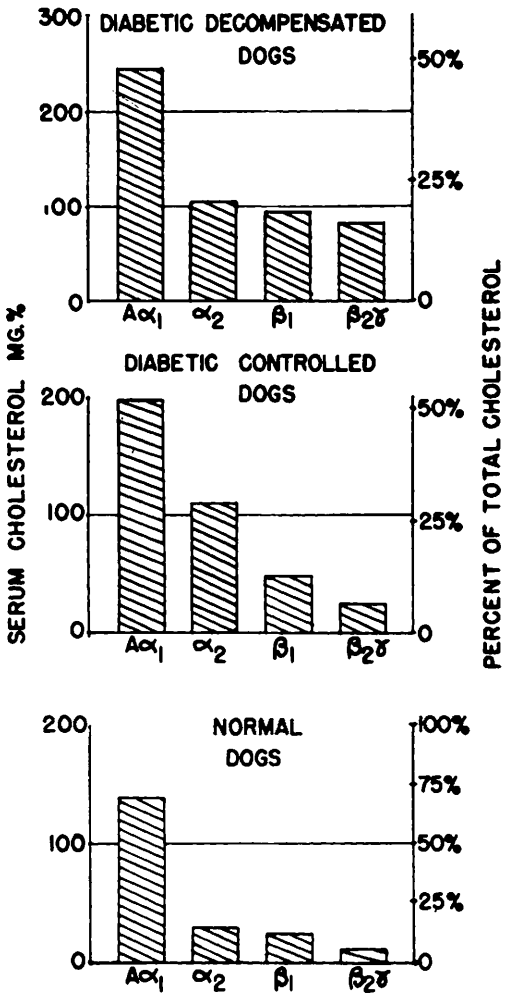

FIG. 1. Distribution of cholesterol in the main serum lipoprotein fractions of normal and diabetic dogs.

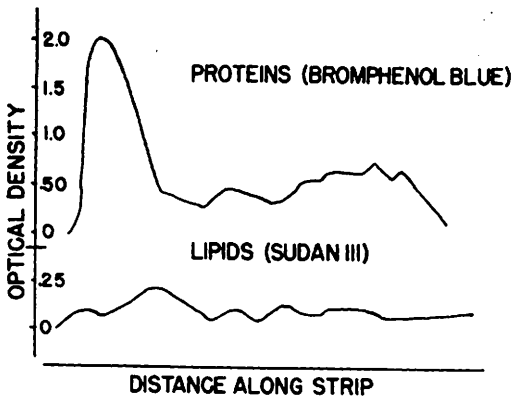

FIG. 2. Protein and lipid patterns of normal dog serum. Galvanometer readings. Instrument set at zero with unstained portion of strip.

when extra neutral fat appeared mostly in the $\beta_2\gamma$ fraction(15).

Depancreatized dogs under insulin treatment. In this group of animals, the level of serum cholesterol was consistently higher than in the control group. Total lipid phosphorus was also increased, but less than cholesterol. Relative distribution of lipids between the two main lipoprotein groups did not differ from normal serum, but often there was a change in the subfractions of the albumin-α-globulin group with a rise in the α_2-components, apparently at the expense of the faster migrating ones (Fig. 1). In this group of animals, the $\frac{\text{cholesterol}}{\text{lipid P}}$ ratio in whole serum and in albumin-α-globulin fraction was higher than in the normal animal. As in the normal animal, the value of the ratio was lower in the albumin-α-globulin than in the β-globulin fraction.

TABLE I. $\frac{\text{Cholesterol}}{\text{Lipid phosphorus}}$ Ratio in Alpha and Beta Lipoproteins of 15 Normal Dogs.

	Albumin-α-lipoprotein	β-lipoprotein
	14.9	27.8
	18.3	16.0
	17.8	26.6
	18.0	25.7
	17.5	101.0
	21.4	37.8
	15.3	27.5
	15.7	24.5
	15.7	21.1
	15.7	23.1
	28.6	38.0
	18.7	21.5
	20.1	21.9
	19.1	28.2
	18.0	26.1
Avg	18.3 S.D. 3.4	31.1 S.D. 20.1

Depancreatized dogs without insulin treatment. Three to 5 days after withdrawal of insulin, with increasing glycosuria and ketosis, there developed a further steep rise of serum cholesterol and a moderate increase of lipid phosphorus, usually associated with visible lipemia. The degree of change seemed to depend upon degree of decompensation; with minor decompensation, *i.e.*, shortly after withdrawal of insulin in dogs with high insulin requirement or later in mildly diabetic dogs, the lipoprotein pattern maintained the same general features observed during the period of insulin control showing only a rather uniform rise of lipid content of all lipoprotein fractions. On the other hand, when decompensation was severe and ketosis marked, β-globulin fractions increased markedly, while albumin-α-globulin fractions either remained at the level reached at the beginning of decompensation or decreased. As severe decompensation set in, the average $\frac{\text{cholesterol}}{\text{lipid P}}$ ratio showed a further rise in all fractions, but more in β-globulin than in albumin-α-globulin fraction. In some decompensated dogs, an increase in the fraction faster than albumin was observed; in these cases, the fraction accounted for 5-20% of total serum cholesterol and lipid phosphorus.

Fig. 3 represents the correlation between the cholesterol content of the serum and cholesterol content of 2 main lipoprotein groups in normal and depancreatized dogs. Although the correlation between serum and albumin-α-globulin cholesterol is fairly good both in normal and controlled diabetic dogs, in decompensation, the rise in serum cholesterol is due mostly to a rise in the β-globulin fraction while the albumin-α-globulin fraction shows the irregularity of behavior noted above. When insulin treatment is restored, in a few days the picture returns to that of the control period. Typical course of events in a depancreatized animal with diabetes of moderate severity is represented in Fig. 4, in which the cholesterol content of the whole

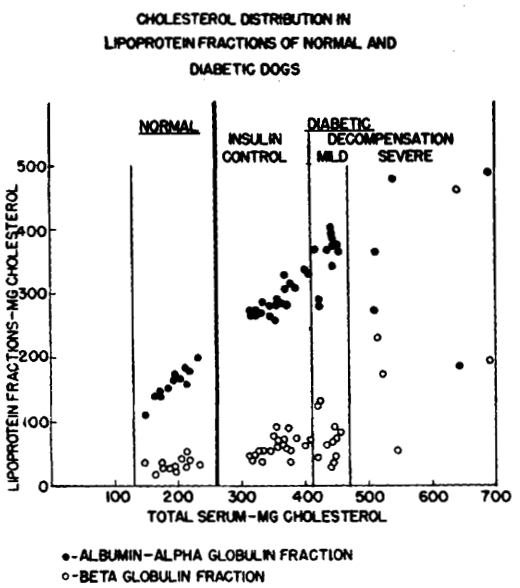

FIG. 3. Correlation between total serum cholesterol and lipoprotein cholesterol in normal and diabetic dogs.

serum and of the various fractions is being used to indicate the behavior of the lipoproteins.

Discussion. The results appear to indicate the following: 1. From the electrophoretic point of view, lipoprotein pattern of the normal dog consists of 3 major groups containing 5 fractions. The first group contains albumin and α-globulin and carries the largest proportion of serum lipids. The second group is that of β-globulins. This group normally has a low lipid content, but this increases after fat absorption. The third group is that of the β_2-γ-globulins which do not migrate at all. It is interesting to note that contrary to what happens in the normal dog, most lipids present in normal human serum are carried by the β-lipoprotein fraction and only a small amount by albumin-α-globulin fractions. 2. Moderate impairment of carbohydrate utilization, such as occurs in depancreatized dogs under insulin treatment, is associated with a rise of all lipoprotein fractions. This might indicate a "loading" of the fat transportation system, probably due to an increased fat mobilization. 3. When severe decompensation of diabetes sets in, the additional fat mobilized no longer can be handled normally and this is associated with a sharp rise of the β-lipoproteins. Perhaps this is due to an absolute or relative inadequacy of the factors involved in the conversion of the large molecule lipoproteins into smaller ones in the liver. 4. In the serum of diabetic dogs, there is a rise of the $\frac{\text{cholesterol}}{\text{lipid P}}$ ratio, the physiological implications of which are not clear at present.

The marked differences in the serum lipoprotein patterns of man and dog, demonstrated also by ultracentrifugal analysis(16), cautions against direct transfer of these results to human pathology. However, there are indications that an analogy of functions might exist between corresponding lipoprotein fractions in these two species: a) both fat absorption and heparin administration modify the same fractions in the serum of man and dog(15), and b) at high levels of serum lipids, under conditions suggesting an impairment of the normal conversion of lipoproteins, the β fractions become carriers of the bulk of serum lipids in both animals. The difference in relative proportions of various fractions, therefore, seems to be the result of

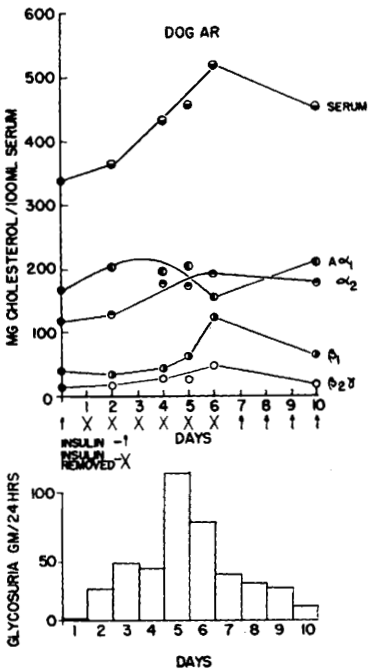

FIG. 4. Effect of insulin on serum lipoproteins in the depancreatized dog.

a different equilibrium and, probably, does not indicate a basic difference in the mechanism of lipid transport. For these reasons, it does not appear unreasonable to apply some of the findings obtained in the diabetic dog to diabetic man and suggest, tentatively, that a strict control of diabetes must be adopted to prevent those lipemic abnormalities suspected of having a pathogenetic relationship to atherosclerosis.

Summary. 1) Serum lipoproteins of normal and depancreatized dogs have been studied by means of zone electrophoresis on filter paper. 2) Dog serum contains 3 main groups of lipoprotein fractions: a fast-migrating albumin-α-globulin group, a slow-migrating β-globulin group and a β_2-γ-globulin group which does not migrate at all. 3) The bulk of serum lipids is carried by the albumin-α-globulin fractions and only a small amount by the β-globulins. This is the opposite of what happens in normal man. 4) Serum of depancreatized dogs under insulin control contains more total cholesterol and total lipid P than normal serum. The $\frac{\text{cholesterol}}{\text{lipid P}}$ ratio is increased. There is a tendency for an increase in amount of the slower migrating components of albumin-α-globulin fraction at the expense of the faster ones. 5) Withdrawal of insulin is followed by an immediate uniform increase of all lipoprotein fractions. As decompensation sets in, the β-globulin fraction increases out of proportion with other fractions. Thus, in decompensated diabetic dogs, as in man, the β-globulin fraction becomes the principal carrier of serum lipids. 6) The possible significance of these findings is discussed.

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Particle Size of Yellow Fever Virus. (20969)

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Since the original ultrafiltration experiments on yellow fever virus by Bauer and Hughes (1) and the ultracentrifugation studies on the virus by Pickles and Bauer(2) no work on the measurement of this virus has been reported.

Recently the technic of particle size determination using the Spinco preparative ultracentrifuge became available Polson and Linder(3). This method which allows sedimentation constants to be determined with fair accuracy and without laborious preliminary purification was used to determine the particle size of the yellow fever virus.