mal level even at an age having a highest incidence of serious development of glomerulosclerosis. The observation suggests that biosynthesis of basement membrane reflected by its glucosyltransferase activity does not accelerate in genetically transmitted microangiopathy.

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[10] This work was supported by grant No. 3T01 AM 05617-05S1 from National Institutes of Health, Institute of Anthritis, Metabolism, and Digestive Diseases.

## Changes in the Apoprotein Composition of Very Low Density Lipoproteins in Man Following Eating

The serum concentrations of very low density lipoproteins (VLDL) and of triglycerides are increased following a carbohydrate and/or fat rich meal[1–4]. It has been well established in recent years that the protein moiety of VLDL consists of numerous different apoproteins, several of which have been clearly characterized, others which have not[5–15]. The purpose of the present investigation was to ascertain whether, following ingestion of a meal, there is a general increase in all the apoproteins of VLDL, or whether the relative concentrations of only certain of the apoproteins increases, while the concentrations of others might not change, or might even decrease.

*Materials and methods.* 4 normal individuals, 3 males and 1 female participated in an experiment of fasting, eating, and fasting. A light meal in the evening was followed by no food during the next 15 h; blood sample No. 1 was then drawn. Between 11.00 h and noon of that day the 4 participants ate a 2,000 Cal meal consisting of 40% animal fat, 20% protein and 40% carbohydrate. Following completion of the meal, blood samples No. 2–5 were drawn after 2, 4, 7, and 21 h, respectively. Serum was obtained. To each serum sample EDTA was added to $10^{-3}$ *M*. Lipoproteins were isolated as follows: chylomicrons were removed by 2 sequential ultracentrifugations at 12,000 rpm for 25 min in a Beckman 30.2 rotor. VLDL was then isolated as previously described[14]. VLDL was delipidated by dropwise addition of cold ethanol-ether (3:1) in the ratio of 3 volumes solvent to 1 volume VLDL. Each sample was rotated for 16 h at 7 °C and then centrifuged at 2,000 rpm for 30 min. The supernatant was decanted and cold ethanol-ether (3:2 v/v) was added in the same approximate volume as the first solvent. The tubes were then rotated for another 16 h. After centrifugation the precipitate was washed 3 times with cold ether, and then dried. The isoelectric focusing gels were prepared as follows: the gel solution contained 3 g acrylamide (3%), 0.2 g N,N′-methylene-*bis*-acrylamide (0.002%), 48 g urea (8 *M*), and 4 ml 40% ampholine (pH $^{3.5-10}$) in a volume of 90 ml. To this gel solution were added 10 ml of 0.004% riboflavin containing 50 mg ammo-

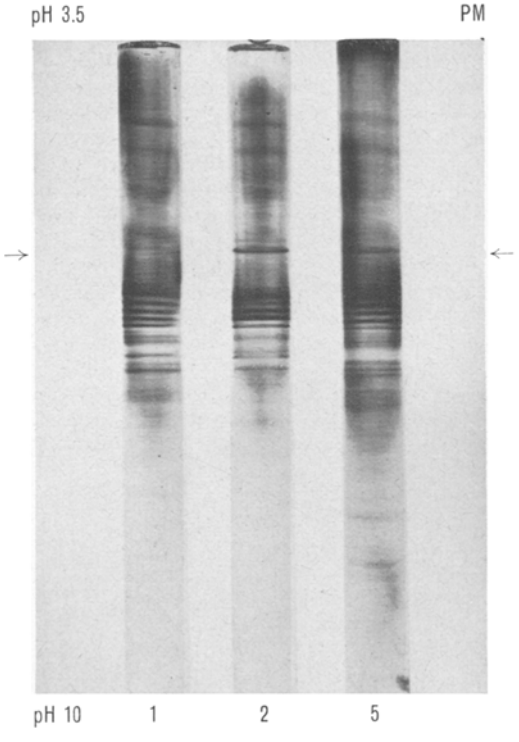


Fig. 1. Isoelectric focusing results of the apoproteins of serum very low density lipoproteins obtained from individual P.M. (1) before meal, and (2) 2 h, and (5) 21 h ,respectively, after the meal.

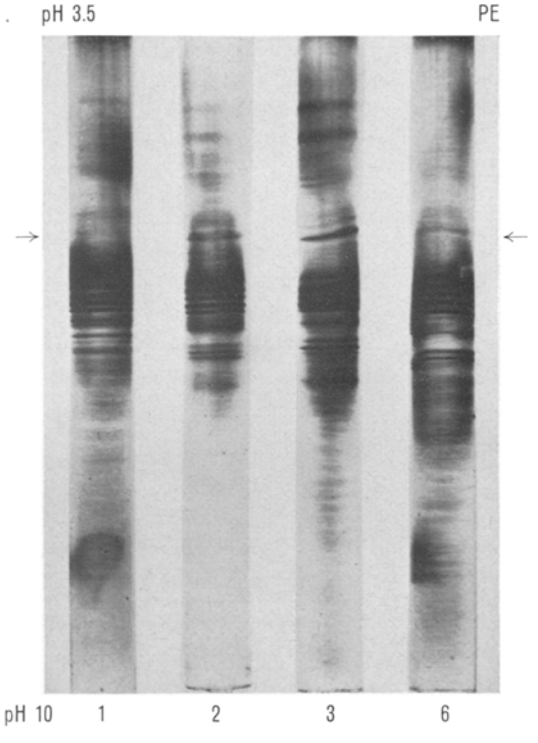


Fig. 2. Isoelectric focusing results of the apoproteins of serum very low density lipoproteins obtained from individual P.F. (1) before meal, and (2) 2 h, (3) 4 h, and (5) 21 h, respectively, after the meal.

nium persulfate. After thorough mixing, the solution was poured into 24 acid cleaned columns, 7 mm i.d. Each column was overlayed with water and allowed to polymerize for 1 h under fluorescent light.

The apoVLDL samples were prepared by adding 0.2 ml of 5% Triton X-100 to 0.5 mg apoVLDL powder. The samples were sonicated for about 3 min until the powder was evenly dispersed in the Triton. Then 0.2 ml of 5% Triton, containing 0.1 *M* $K_2CO_3$ and 8 *M* urea, was added to each sample along with an additional 0.1 g of urea. The samples were again sonicated for about 3 min. A volume of 0.2 ml of each sample preparation was then added to the top of every gel column. The samples were overlayed with a solution containing 0.05 ml ampholine, 100 mg sucrose and methyl green dye in a volume of 2 ml to a height of 5 mm above the sample. Every column was then filled with the top electrode (anode) solution, 0.24 *N* $H_2SO_4$. The cathode solution was 0.48 *N* NaOH.

The starting current was 1 mA/gel, about 50 volts. After 1 h the voltage was increased to about 100 volts. Thereafter the voltage was increased every half hour, not exceeding 1 mA/gel until 400 volts was reached. The gels were allowed to run an additional hour after the current had decreased to 4 mA or less. The entire run took about $4^1/_2$ h.

The gels were removed from the columns and washed in 10% trichloracetic acid (TCA) overnight. TCA was changed the next morning and washing was continued for 1–2 h. The gels were then rinsed in distilled water and washed in destaining solution (5:5:1 methanol-water-acetic) acid for at least $^1/_2$ h to hasten staining. The gels were then stained in 0.1% Coomassie Blue in 5:5:1 methanol-water-acetic acid with stirring in a 37 °C water bath for $^1/_2$ h. The gels were destained in 3–4 h. They were removed from the destainer and stored in 3% acetic acid.

*Results.* The results of isoelectric focusing runs of the VLDL apoproteins of the 4 individuals are shown in Figures 1–4. It can be seen that there are some 20 or more bands in each of the gels. The significant finding is that most of the bands with certain significant exceptions are of similar intensity when particular bands from different bleedings are compared. The two notable exceptions: 1. In the region of pH 4.7, marked by an arrow, there is no, or at most only a very weak apoprotein band in sera of sample No. 1 of each of the individuals; there is an intensely staining band in sample No. 2 of each


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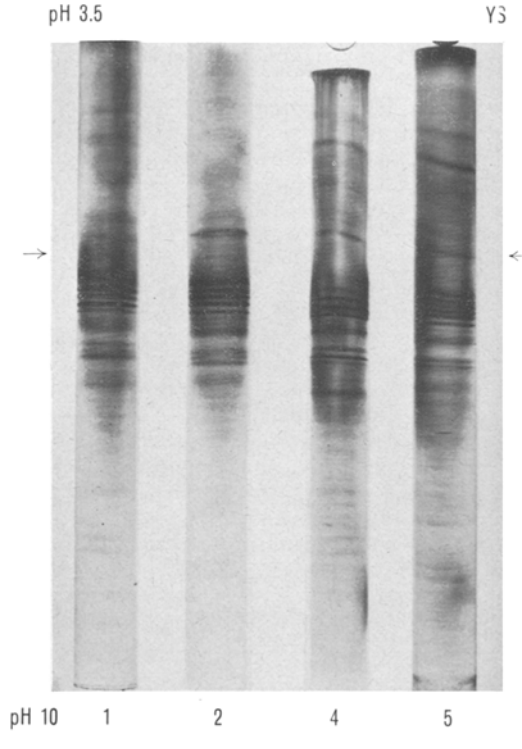


Fig. 3. Isoelectric focusing results of the apoproteins of serum very low density lipoproteins obtained from individual Y.S. (1) before meal, and (2) 2 h after, (3) 4 h, and (5) 21 h, respectively, after the meal.

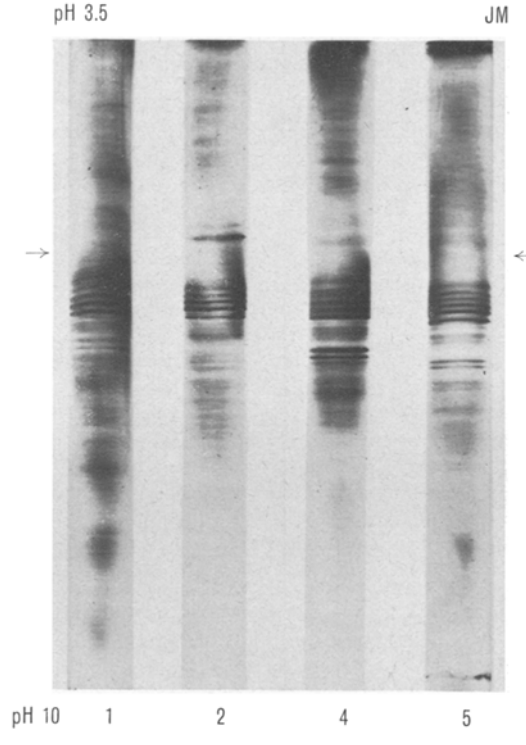


Fig. 4. Isoelectric focusing results of the apoproteins of serum very low density lipoproteins obtained from individual J. M. (1) before meal, and (2) 2 h, (4) 7 h, and (5) 21 h, respectively, after the meal.

individual, i.e., 2 h after the meal; in the later samples the intensity of that band then decreases progressively with time following eating such that it has or has nearly returned to base line level in sample No. 5. 2. There are differences in the apoprotein bands in the region between pH 6.5 and 7.5 between the pre- and post eating samples. More intensely staining bands occur in samples 2 and 3 than in 1 and 5. These differences are not as clear as those in (1). They may, none the less, be similarly significant. No attempt was made in this experiment to quantitate the staining intensity of the bands or to further identify the apoproteins which give rise to these bands.

*Discussion.* We believe that the finding that only a small number of VLDL apoproteins increases significantly following food intake and that the concentrations of the majority of the apoproteins remains unaffected is interesting and significant. Qualitative as the present findings are, they are quite adequate to exclude what might appear to be the most plausible assumption about apoprotein function in lipid transport, namely that in response to food intake, simply more of the same VLDL is produced as that which is circulating in the fasting state. Newly formed VLDL following food intake obviously has a different apoprotein composition than VLDL present in the fasting state.

Some of the questions which arise are: What is the origin of these new apoprotein? Are they newly synthesized proteins, perhaps synthesized in the gut, or are they some of the minor high density apoproteins? What kinds of lipoprotein molecules carry these apoproteins? Are these apoproteins carried as special subpopulations[14,16,17]? Are each of the apoproteins or subpopulations under independent metabolic control or is the control of certain of them linked? These questions will remain open until the apoproteins and subpopulations are isolated and characterized so that quantitative metabolic studies can be carried out.

*Summary.* The concentrations of certain of the very low density human serum lipoprotein apoproteins were found to increase following a meal, while the concentrations of the majority of the apoproteins were found to be unaffected.

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## Effect of Sex Hormones on Uric Acid Metabolism in Rats

It has been known for many years that the average serum urate concentration of pre-menopausal females is approximately 1.0 mg per 100 ml lower than that of males[1]. The existence of this sex difference in serum urate concentration has been attributed to the hormonal alteration found between both sexes. The purpose of this study is to evaluate the effect of some sex hormones on urate metabolism in animal experiments.

*Methods and materials.* Wistar rats weighing 150–200 g were used in the present study. Plasma and urinary uric acid concentration were estimated, using an enzymic spectrophotometric method of LIDDLE[2]. Allantoin was measured by the method of LARSON[3]. Blood samples were taken before and after the completion of the daily administration of sex hormones. 24-hour urine samples were collected in every group during successive 7 days before and after the administration of hormones. Estrogen or progesterone were administered to female rats and androgen to male rats. The daily doses of estrogen, progesterone and androgen i.m. were 10 mg, 100 mg and 100 mg/kg body weight, respectively. These hormones were daily administered for 7 days. Blood samples were analyzed for concentration of uric acid. Urinary excretion of uric acid and allantoin were measured and calculated in 24-hour urine samples.

*Results.* The results of the present study are summarized in the Table. When 100 mg/kg body weight estrogen was given daily for 7 days in female rats, the mean plasma urate level (1.85 $\pm$ 0.76 mg/100 ml) and the mean excretion of uric acid plus allantoin (72.89 $\pm$ 11.54 mg/day) did not change (1.87 $\pm$ 0.93 mg/100 ml, 75.17 $\pm$ 11.72 mg/day).

On injection of androgen, 10 mg/kg body weight in male rats, the mean plasma urate level was slightly elevated from 2.10 $\pm$ 0.81 mg/100 ml to 2.24 $\pm$ 0.84 mg/100 ml and the mean urinary excretion of uric acid plus allantoin was slightly increased from 73.73 $\pm$ 14.33 mg/day to 78.02 $\pm$ 14.65 mg/day. But these differences were not significant.

The mean plasma urate concentration was significantly reduced from 2.43 $\pm$ 1.04 mg/100 ml to 1.53 $\pm$ 0.57 mg/100 ml and the mean urinary excretion of uric acid plus allantoin decreased from 60.15 $\pm$ 19.81 mg/day to 54.71 $\pm$ 16.56 mg/day by the injection of progesterone 100 mg/kg body weight in female rats.

*Discussion.* It is well recognized that plasma urate levels reflect both de novo purine synthesis and renal excretion of uric acid. It is also generally accepted that plasma urate levels in children do not differ between both sexes[1]. In pre-menopausal adult women, plasma urate levels are about 1.0 mg/100 ml lower than those of men, and they are gradually increased in menopause. This age and sex difference in urate metabolism suggests endocrine alterations in men.

MIKKELSEN et al.[1] showed in population studies in Tecumseh that the plasma urate levels in pregnant women were significantly lower than those of age-matched non-pregnant women. From these findings they suggested that estrogen and/or progesterone may have urate-depressing ability. Similar observations were reported by BOYLE et al.[4]. They studied the influence of pregnancy on urate metabolism and confirmed that the serum urate levels are significantly lower during early and middle pregnancy than those of age-matched female controls. They showed that the urate excretion was increased in middle and late pregnancy. They consider

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