Adenosine monophosphate did not affect the TSH-sensitive adenylate cyclase. Neither 8-Br–cyclic AMP by itself nor AMP by itself affected the basal level of cyclic AMP (see Fig. 2).

Hence, elevated intracellular cyclic AMP induces receptor patching and reduces the concentration of cyclic AMP normally induced by TSH.

Receptor internalization induced by insulin and epidermal growth factor (EGF) (*15*, *17*) seems to be related to the well-characterized process of insulin and EGF-induced receptor loss or down regulation (*18*, *19*). However, cyclic AMP does not affect the down regulation of insulin or EGF but it does induce down regulation of TSH receptors. This raises the interesting possibility that part of the regulation of TSH activity occurs at the level of receptor clustering when cyclic AMP production is stimulated and that cyclic AMP acts both as the second messenger of TSH and also as the regulator of the level of its membrane receptor.

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16. The $FRTL_5$ cells (10^6 cells per dish) were derived from TSH for 18 hours. Then the cells were washed with Krebs-Ringer Hepes buffer and exposed for 2 hours to [^{3}H]adenine (1.5 μCi) at room temperature. After being washed, half of the samples were exposed to 1 m*M* 8-Br–cyclic AMP for 30 minutes at 37°C and the second half were incubated with medium for the same period. After several washes the cells were treated with solutions containing TSH and isobutylmethylxanthine. The accumulation of cyclic AMP was determined according to Y. Solomon, *Cyclic Nucleotide Res.* **10**, 35 (1979). The ^{3}H-labeled cyclic AMP was separated from other tritiated intermediates by passing the reaction mixture through a Dowex alumina column. The separated ^{3}H-labeled cyclic AMP was counted in a β-scintillation counter.
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Type III Hyperlipoproteinemia Associated with Apolipoprotein E Deficiency

Abstract. *Subjects with type III hyperlipoproteinemia develop premature atherosclerosis and have hyperlipidemia due to an increase in cholesterol-rich very low density lipoproteins (VLDL) of abnormal electrophoretic mobility. Apolipoprotein E is a major protein constituent of VLDL and appears to be important for the hepatic uptake of triglyceride-rich lipoproteins. A new kindred of patients with type III hyperlipoproteinemia is described in which no plasma apolipoprotein E could be detected, consistent with the concept that type III hyperlipoproteinemia may be due to an absence or striking deficiency of apolipoprotein E.*

Patients with type III hyperlipoproteinemia (HLP) often develop premature coronary artery and peripheral vascular disease and may have palmar, tubo-eruptive, and tendinous xanthomas (*1*). The hyperlipidemia in these subjects generally is not detectable prior to the third decade of life and is exacerbated by obesity, increased caloric intake, and hypothyroidism (*1*). Type III HLP patients have increased concentrations of plasma cholesterol and triglyceride (*1*). The concentrations of low density lipoprotein (LDL) (density 1.019 to 1.063) and high density lipoprotein (HDL) may be normal or decreased in these subjects (*2*); chylomicrons are often present in the fasting state, and very low density lipoprotein (VLDL) (density < 1.006 g/ml) and intermediate density lipoprotein (IDL) (density 1.006 to 1.019 g/ml) are increased (*1*). The VLDL isolated from type III HLP plasma has an increased ratio of cholesterol to triglyceride, and

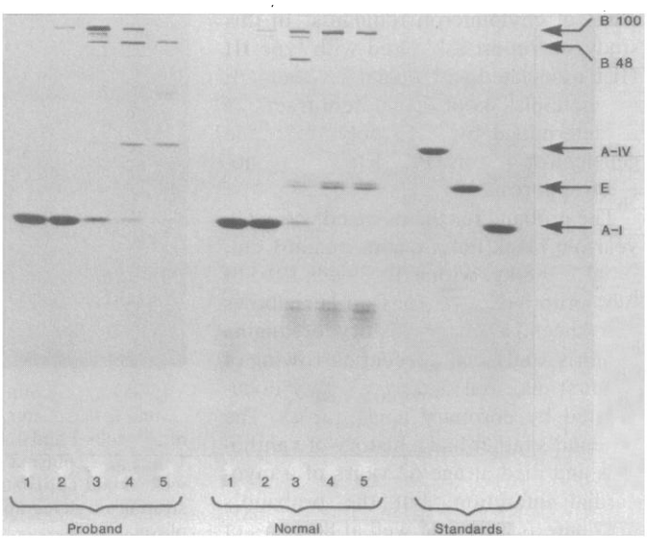

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of plasma lipoproteins from a normal subject and the proband showing no detectable apoE, but the presence of apoB-48 and apoA-IV in the proband's lipoproteins. Identical 15-μg protein loads of 1, HDL_{2a+3}; 2, HDL_{2b}; 3, LDL; 4, IDL, and 5, VLDL were run on gels. Apolipoprotein A-IV, apoE, and apoA-I standards are shown.

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Table 1. Plasma lipid and lipoprotein cholesterol values in normal subjects (N = 1088), subjects with type III HLP (N = 66), the proband, and siblings and offspring of the proband. The proband and siblings 1 and 2 had no detectable apoE. Values in individual subjects represent the mean of three determinations, and means ± standard deviation are shown for study groups.

Subject	Plasma		Cholesterol			Ratio of VLDL cholesterol to plasma triglyceride
	Cholesterol (mg/dl)	Triglyceride (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	
Normal	189 ± 40	87 ± 43	16 ± 11	123 ± 35	50 ± 14	0.18 ± 0.05
Type III HLP	441 ± 153	694 ± 486	292 ± 158	111 ± 53	38 ± 19	0.42 ± 0.14
Proband	442	171	160	193	69	0.94
Sibling 1	614	294	381	200	33	1.30
Sibling 2	499	167	196	244	59	1.17
Mean	512 ± 97	211 ± 72	246 ± 119	212 ± 28	54 ± 19	1.14 ± 0.18
Offspring 1	240	169	24	171	45	0.14
Offspring 2	181	111	19	123	39	0.17

migrates in the β as well as the pre-β position on lipoprotein electrophoresis, in contrast to normal VLDL which usually migrates in the pre-β region only (*1*). The ratio of VLDL cholesterol to plasma triglyceride in type III HLP subjects is generally in excess of 0.30, in contrast to normal individuals in which this ratio is approximately 0.18 (*1*).

The concentration of apolipoprotein (apo) E, a protein constituent (molecular weight approximately 38,000) of plasma lipoproteins, is increased in the plasma of most type III HLP subjects (*3*), and most of it is found within VLDL (*4*). Apolipoprotein E appears to be associated with chylomicron remnants in plasma, and may be important for the receptor-mediated uptake of these particles by the liver (*5*). Apolipoprotein E has several isoforms on isoelectric focusing (IEF), and type III HLP patients have been reported to have an abnormal apoE pattern on IEF (*6*). Apolipoprotein E from type III HLP plasma is catabolized at a slower fractional catabolic rate on lipoproteins than is normal apoE in both normal and type III HLP subjects (*7*), consistent with the concept that some patients with type III HLP have an abnormal apoE resulting in impaired catabolism of chylomicron remnants. In this study we report a kindred with type III HLP associated with no detectable apoE in whole plasma or lipoprotein fractions as determined by IEF, polyacrylamide gel electrophoresis (PAGE), or immunoelectrophoresis.

The proband for this kindred was a 60-year-old black female of height 161 cm, weight 69.8 kg, with a 10-year history of tubo-eruptive xanthomas on her elbows and knees, a 3-year history of angina pectoris, and an 80 percent narrowing of the first diagonal coronary artery documented by coronary angiography. The proband's father had a history of xanthomas and died at age 62 years of a myocardial infarction, but the proband's mother was alive and well at 86 years of age. The patient had seven siblings, three of whom had xanthomas, and she had two offspring with no xanthomas.

Blood was obtained in 0.1 percent EDTA after an overnight fast (12 to 14 hours) from the proband, her two offspring, and two of the three siblings with a history of xanthomas and elevated plasma lipid concentrations. One sibling was a 45-year-old male and the other a 48-year-old female. The offspring of the proband were a 33-year-old male and a 38-year-old female. The subjects were on an unrestricted diet, were not receiving medication known to affect plasma lipid concentrations, and had normal serum thyroid, liver, and kidney function tests and normal immunoglobulin. Plasma cholesterol and triglyceride and lipoprotein cholesterol concentrations were quantitated by standard Lipid Research Clinics methodology (*8*), and lipoprotein electrophoresis was performed on paper (*8*) as well as agarose (*9*). In addition, plasma lipoproteins (VLDL, IDL, and LDL and HDL_{2b}, density 1.063 to 1.10 g/ml, and HDL_{2a+3}, density 1.10 to 1.21 g/ml) were isolated by sequential ultracentrifugation in Beckman 40.3 rotors and Beckman L2-65B ultracentrifuges (*10*). Lipoprotein fractions were delipidated with a mixture of methanol and chloroform (1:3 by volume) and analyzed by IEF (*p*H 4 to 6) (*6*) and sodium dodecyl sulfate (SDS)–PAGE, with 15 percent polyacrylamide gel, as described (*11*). We also examined the various forms of apolipoprotein B, using 3.5 percent acrylamide PAGE (*12*). Apolipoprotein E was isolated from the supernatant (1.019 g/ml) of normal plasma by heparin-Sepharose affinity chromatography (*13*), and monospecific antiserums were prepared in rabbits. Apolipoprotein A-IV was isolated from the supernatant of human thoracic duct lymph (1.019 g/ml) by gel permeation chromatography (*14*). Plasma and lipoprotein fractions were subjected to immunodiffusion and immunoelectrophoresis at varying ratios of sample and antiserum (1:1 to 1:512) for the detection of apoE.

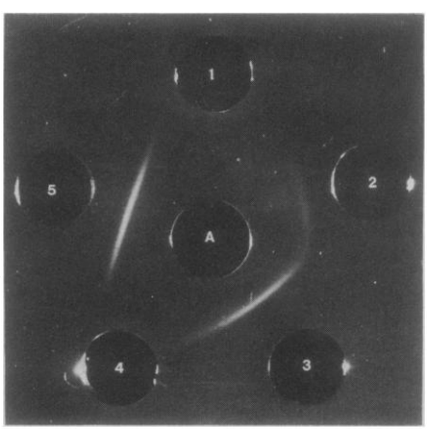

Fig. 2. Immunodiffusion plate with apoE antiserum in the center well, the proband's plasma in wells 1 and 4, the plasma from offspring 1 in well 2, plasma from a normal subject in well 3, and apoE standard in well 5, demonstrating no detectable apoE in the proband's plasma.

As shown in Table 1 (*2*), the proband and two of her siblings had type III HLP on the basis of abnormal lipoprotein electrophoretic patterns (with VLDL of broad β mobility), increased plasma lipid concentrations, and increased ratios of VLDL cholesterol to plasma triglyceride. Only trace amounts of chylomicrons were present in the fasting plasma of these patients. Affected members of this kindred are somewhat distinct from other type III HLP subjects in that they have lower plasma triglyceride concentrations, higher LDL cholesterol values, and significantly higher ratios of VLDL cholesterol to plasma triglyceride. The offspring of the proband had normal plasma lipid and lipoprotein cholesterol values (see Table 1).

Analysis of lipoprotein apolipoproteins by SDS-PAGE (Fig. 1) revealed an absence of apoE in the proband and two of her siblings and the presence of apoE in her offspring. In addition, the proband's VLDL, IDL, and LDL contained

significant amounts of lower molecular weight apoB and apoA-IV in contrast to corresponding normal lipoprotein fractions. The results of immunodiffusion studies (Fig. 2) indicate the absence of apoE in the proband's plasma, and its presence in normal plasma and in the plasma of the proband's offspring. The proband's plasma contained apolipoproteins A-I, A-II, B, C-I, C-II, and C-III as tested by radial immunodiffusion with a mean apoC-II plasma concentration of 6.0 mg/dl (normal, 2.5 ± 1.5 mg/dl).

Chylomicrons released by intestinal epithelial cells contain apolipoproteins B, A-I, A-II, and A-IV (*12, 14, 15*). Most lymph chylomicron apoB is comprised of lower molecular weight apoB or "B-48" (*12*). Chylomicrons in lymph appear to acquire significant quantities of the C apolipoproteins, presumably as a result of transfer from HDL which has filtered from plasma into lymph (*16*). After entry into plasma, lymph chylomicrons are acted on by lipoprotein lipase, resulting in the hydrolysis of triglyceride and the transfer of apoA-I, apoA-II, the C apolipoproteins, and lipid to HDL (*15*), and the formation of chylomicron remnants within the VLDL and IDL density region (*17*). Chylomicron remnants contain both apoB and apoE (*5*). On the basis of studies with radioactively labeled chylomicrons in man, only a very small fraction of chylomicron apoB is transferred to LDL (*15*). The chylomicron remnants are rapidly removed from the circulation by the liver, and this catabolic process appears to be mediated in part by an apoE receptor in the rat. The importance of this receptor-mediated process in man is unknown (*5*). This uptake phenomenon is enhanced by estrogen administration (*18*). Estrogens have been reported to ameliorate the hyperlipidemia in type III HLP female subjects in contrast to other forms of hyperlipidemia (*19*). Most patients with type III HLP have increased plasma concentrations of an abnormal apoE as demonstrated by IEF (*6*), and this protein abnormality results in a delayed catabolism of triglyceride-rich lipoproteins because of a decreased hepatic uptake (*6, 7*).

Members of the kindred with type III HLP described herein, because of their lack of detectable plasma apoE, differ from other known type III HLP kindreds, all of which have shown increased amounts of an abnormal apoE. In addition, these subjects had only mild hypertriglyceridemia, increased LDL cholesterol, and a much higher ratio of VLDL cholesterol to plasma triglyceride than reported in other type III HLP subjects. Apolipoprotein A-IV and lower molecular weight apoB (B-48), two lymph chylomicron apolipoprotein constituents generally not found in normal plasma IDL and LDL, were present in significant quantities in the IDL and LDL of these patients with apoE deficiency. These data are consistent with the following concepts: (i) apoE is important for the catabolism of chylomicron remnants; (ii) apoE deficiency results in the accumulation of chylomicron remnants in plasma, type III HLP, tubo-eruptive xanthomas, and premature coronary artery disease; and (iii) apoE deficiency represents a new disease entity.

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Intestinal Diffusion Barrier: Unstirred Water Layer or Membrane Surface Mucous Coat?

Abstract. *The dimensions of the small intestinal diffusion barrier interposed between luminal nutrients and their membrane receptors were determined from kinetic analysis of substrate hydrolysis by integral surface membrane enzymes. The calculated equivalent thickness of the unstirred water layer was too large to be compatible with the known dimensions of rat intestine. The discrepancy could be reconciled by consideration of the mucous coat overlying the intestinal surface membrane. Integral surface membrane proteins could not be labeled by an iodine-125 probe unless the surface coat was first removed. The mucoprotein surface coat appears to constitute an important diffusion barrier for nutrients seeking their digestive and transport sites on the outer intestinal membrane.*

Before a solute in the small intestinal lumen can interact with receptors for hydrolysis or transport, it must pass through a diffusion barrier, which modifies the kinetics of nutrient assimilation (*1*). It has been suggested that this barrier is an unstirred water layer located at the intestinal lumen–membrane interface (*1*).

We have examined the intestinal diffusion barrier by means of a kinetic analysis of the surface membrane hydrolases, sucrase, lactase, and aminooligopeptidase. These enzymes are known to operate at the luminal-cell interface of the intestinal mucosal cell (*2, 3*); when the kinetics of rat jejunum enzymes in vivo are compared to those of the isolated, pure enzymes (*4–7*), the effective thickness of the overlying water diffusion barrier can be calculated (*8*).

Carbohydrates were made up to 0.3*M* in deionized water and diluted with buffer (0.14*M* NaCl and 0.01*M* sodium, po-

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