Table III. Effect of cordycepin on *o*-diphenolase activity

Additions	*o*-Diphenolase activity	
	Enzyme units	Control (%)
Control	600	100
Cordycepin a) 10^{-5} *M*	670	112
b) 10^{-4} *M*	580	97
c) 3×10^{-4} *M*	620	103

The enzyme activity was measured in crude extract prepared from excized embryos germinated (48 h) in continuous presence of cordycepin.

Table IV. Effect of cycloheximide (CHI) on the *o*-diphenolase activity and ^{3}H-leucine incorporation in the eluted fraction

Additions	*o*-Diphenolase activity in eluted fraction		^{3}H-leucine incorporation in eluted fraction	
	Enzyme units	Inhibition (%)	cpm/mg protein	Inhibition (%)
Control	30	—	1,120	—
CHI, 2 µg/ml	18	40	486	57
CHI, 4 µg/ml	0	100	68	94

The dialyzed crude extract was fractionated on acrylamide gels and the region of 2 fast moving isoenzymes of *o*-diphenolase was eluted with 0.05 *M* phosphate buffer (pH 6.6) and designated as eluted fraction.

Pisum[13] and soybean[14]. In sea urchin eggs, Act-D failed to inhibit the activation of protein synthesis, although it retarded mRNA formation[10]. Also, in germinating cotton cotyledons[11], the induction of protease and isocitratase is supported by pre-existing mRNA and is not inhibited by Act-D.

Fractionation of crude extract on acrylamide gels revealed 4 *o*-diphenolase isoenzymes at zero h germination (D_1–D_4 in Figure 2). After 48 h germination, 6 new isoenzymes (D_5–D_{10}) were formed (Figure 2). Embryos cultured in presence of CHI (5 µg/ml) showed a virtual disappearance of 6 newly formed isoenzymes (D_5–D_{10}). Furthermore, CHI (2 and 4 µg/ml) caused a concomitant decrease in enzyme activity and ^{3}H-leucine incorporation in the eluted fraction obtained from the fast moving isoenzyme bands (D_9–D_{10}) of *o*-diphenolase (Table IV). This indicated that the stimulation of *o*-diphenolase represents de novo enzyme synthesis. Unlike CHI, Act-D and cordycepin failed to abolish the activity of newly formed *o*-diphenolase isoenzymes (Figure 2). Briefly then, the stimulation of *o*-diphenolase activity and its isoenzyme formation during early germination of wheat embryos is supported by conserved message already present in ungerminated embryo. Thus the stimulation of *o*-diphenolase enzyme seems to be regulated at the translational level.

Zusammenfassung. Nachweis, dass die Stimulation der *o*-Diphenolase-Aktivität und der Isoenzymbildung während des frühen Keimungsstadiums von Weizenembryonen von einem konservierten und bereits im ungekeimten Samen vorgebildeten Informationsträger abhängig ist.

SHANT R. TANEJA and R. C. SACHAR[15]

Division of Biochemistry
Indian Agricultural Research Institute
New Delhi-110012 (India), 10 March 1975.

[13] G. R. BARKER and M. RIEBER, Biochem. J. *105*, 1195 (1967).
[14] T. MORI, F. IBUKI, S. MATSUSHITA and T. HATA, Arch. Biochem. Biophys. *124*, 607 (1968).
[15] Acknowledgments. Actinomycin D was a generous gift from Merck Sharp and Dohme. One of us (SRT) is grateful to Indian Council of Agricultural Research for the award of Senior Research Fellowship.

Renal Glucose Utilization in Genetically Diabetic Microangiopathy

The common feature of diabetic microangiopathy is associated with thickening of the capillary basement membrane[1]. A detailed chemical analysis of human diabetic basement membrane reveals the fact that the content of hexose and hydroxylysine increased[2,3]. In alloxan diabetic rat kidney, an elevation of post-ribosomal glucosyltransferase activity[3] is found which may be responsible for the increased amount of glomerular basement membrane[4]. However, it is yet unknown whether glucosyltransferase or/and other factors are involved in the genetically transmitted diabetic microangiopathy.

A human type glomerulosclerosis (diffuse, exudative and nodular) associated with protein urea and increased blood urea nitrogen levels was described in the non-obese genetically diabetic KK mice[5]. Thus KK mice are the ideal model for studying the biochemical change during the development of genetically transmitted diabetic microangiopathy.

Since glucose content was increased in the hydroxylysine-linked disaccharide unit[2], present investigation was to study glucose utilization by renal tissue of KK mice during the process of microangiopathy development. Moreover, the glucosyltransferase which incorporated glucose from UDPG (uridine diphosphoglucose) to β-D-galactose-hydroxylysine basement membrane was also examined in KK mice.

Materials and methods. The KK mice were maintained under constant laboratory temperature and regular mouse chow diet, containing 11% fat was given.

D-glucose-^{14}C (U), and UDPG-^{14}C (U) were purchased from New England Nuclear, Company. Calf skin collagen was the product of Sigma Company. NCS Tissue solubilizer was purchased from Amersham/Searle. All other chemicals were of reagent grade.

Non-fasted KK mice and Swiss albino mice were sacrificed by stunning. Kidneys were removed and dryed by Kimex. The tissue was weighed, cut and placed in 1.5 ml of Krebs-Ringer-bicarbonate solution (KRB) for 15 min. The preincubated tissue was then transferred to a flask

[1] J. M. B. BLOODWORTH, jr., Diabetes *12*, 99 (1963).
[2] P. J. BEISSWENGER and R. G. SPIRO, Science *168*, 596 (1970).
[3] R. G. SPIRO and M. J. SPIRO, Diabetes *20*, 641 (1971).
[4] D. J. PETERSON, W. C. GREENE and G. M. REAVEN, Diabetes *20*, 649 (1971).
[5] R. A. CAMERINI-DAVALOS, W. OPPERMANN, R. MITTL and T. EHRENRLICH, Diabetologia *6*, 324 (1970).

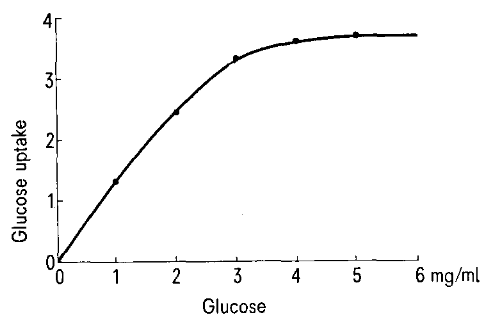
containing 3 mg/ml of glucose plus 10^6 cpm of glucose-^{14}C (U). After 4.5 h, tissue was washed with KRB buffer to remove any excessive counts and homogenized in 70% ethanol. The ethanol-soluble fraction was separated from the ethanol insoluble fraction by centrifugation at 2,000 *g* for 2 min. The ethanol insoluble material was dissolved in NCS. Both fractions were counted in BRAY's solution[6]. All countings were done in a nuclear Chicago model liquid scintillation counter.

Glucosyltransferase assay. Kidney after isolation from animals was homogenized in 0.3 ml of 0.15 *M Tris* acetate buffer containing 2 m*M* 2-mercaptoethanol at pH 6.9. The homogenate and its washing were transferred into a microcentrifuge tube and the volume of homogenate was brought to 0.6 ml. The homogenate was centrifuged at 10,000 *g* in a Beckman microfuge B for 10 min. 50µl of 10,000 *g* supernatant was incubated in 0.1 ml of medium consisting of 0.24 mmole of *tris*-acetate, pH 6.9; 5 µmole $MnCl_2$, 0.3 µmole 2-mercaptoethanol, UDPG-^{14}C (0.34 mCi), 3 mg of collagen. After 2 h incubation at 37 °C, 4 ml of phosphotungstic acid was added into the tube. The precipitated protein was centrifuged at 2,000 *g* for 10 min. The supernatant was decanted and the pellet was further washed 3 times with 2 ml of cold 11% trichloroacetic acid (TCA) and then 1 time with 2 ml of the mixture of ethanol and ether (1:1, v/v). The pellet was suspended in 1 ml of 0.1 *M* NaOH and heated in a water bath at 85 °C. The NaOH solubilized material was counted in BRAY's solution.

Total protein of 10,000 *g* supernatant was determined by Biuret method[7], with bovine serum albumin used as standard. The enzyme activity is expressed as cpm of UDPG-^{14}C incorporated into the collagen per mg of protein in supernatant.

Results and discussion. The glucose utilization during the development of glomerusclerosis in KK mice was examined in kidney. The total glucose uptake by renal tissue from one-half-month-old Swiss albino mice is linear with exogenous glucose concentration up to 3 mg/ml (Figure). This fact was used to study glucose utilization in 15 day to 6-month-old KK mice and age-matched normal controls. Both 45-day-old KK and Swiss albino mice show the maximum glucose utilization. A slight increase in glucose uptake was observed from KK mice of all ages compared with normals (Table I). The incidence of significant glomerular lession in 2-6-month-old KK mice was 77%, but only 23% was observed in normals[5]. If glucose utilization is disturbed in KK mice, one expects to see a larger change in glucose utilization, the present study suggests that the development of renal glomerulsclerosis in KK mice does not influence the renal glucose uptake.

Glucose utilization was also examined at the level of incorporation of glucose by glucosyltransferase from UDPG to collagen. The glucosyltransferase activity in 4-month- and 6-month-old KK and controls does not have significant statistical differences (Table II). It is now clear that the mechanism of genetic transmitted microangiopathy is unrelated to the elevation of glucosyltransferase activity. Instead of increased biosynthesis of basement membrane in KK mice; possibly, the basement membrane breakdown may be slower, which will also lead to the accumulation of glomerular basement membrane in renal cortical tissue. Sinc e microangiopathy might be the pathogenetic factor for diabetes mellitus[8], further study on degradation of glomerular basement membrane is clearly indicated.



Effect of Glucose concentration on renal glucose uptake. Uptake is expressed as mg of glucose uptake/4.5 h/100 mg of dry tissue.

Table I. Renal glucose utilization in the KK and normal mice

Mice used	Age (days)	mg of glucose/100 mg tissue/4.5 h		
		EtOH-soluble fraction	EtOH-insoluble fraction	Total
Norm	16	2.2	1.3	3.5 ± 0.1
KK	15	2.7	1.2	3.9 ± 0.1
Norm	30	3.3	1.7	5.0 ± 0.1
KK	30	3.6	1.9	5.5 ± 0.1
Norm	45	4.6	1.8	6.4 ± 0.2
KK	45	5.6	1.9	7.5 ± 0.1
Norm	60	3.1	0.2	3.3 ± 0.2
KK	60	3.3	0.2	3.5 ± 0.2
Norm	159	3.5	0.3	3.8 ± 0.1
KK	170	3.7	0.35	4.1 ± 0.1

The data are expressed as mean of 4 mice followed with standard deviation.

Table II. Glucosyltransferase activity in renal cortex of KK and control mice

Age group	Activity (cpm/mg of protein)	
	SA (control)	KK
4 months	759 ± 47	857 ± 63
6 months	527 ± 28.1	519 ± 24.6

The data are expressed as mean of 10 animals followed with SEM. SA, Swiss Albino.

Summary. Glucose uptake into kidney tissue is not influenced by the development of glomerulosclerosis in KK mice. Glucosyltransferase activity remains at a nor-

[6] G. BRAY, Analyt. Biochem. *1*, 279 (1960).
[7] A. G. GORNALL, C. S. BARDAWILL and M. M. DAVID, J. biol. Chem. *177*, 751 (1949).
[8] M. D. SIPERSTEIN, in *Pathogenesis of Diabetes Mellitus* (Eds. E. CERASI and R. LUFT; John Wiley and Sons, New York 1970), p. 81.

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.

M. CHEN[9], C. VELASCO and
R. A. CAMERINI-DAVALOS[10]

Department of Biochemistry, Purdue University, West Lafayette (Indiana 47907, USA), and Diabetes Center, Department of Medicine, New York Medical College, New York (N.Y., USA), 10 June 1975.

[9] M. C. was a NIH trainee in Diabetes Center, Department of Medicine, New York Medical College, New York, New York, USA.

[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-

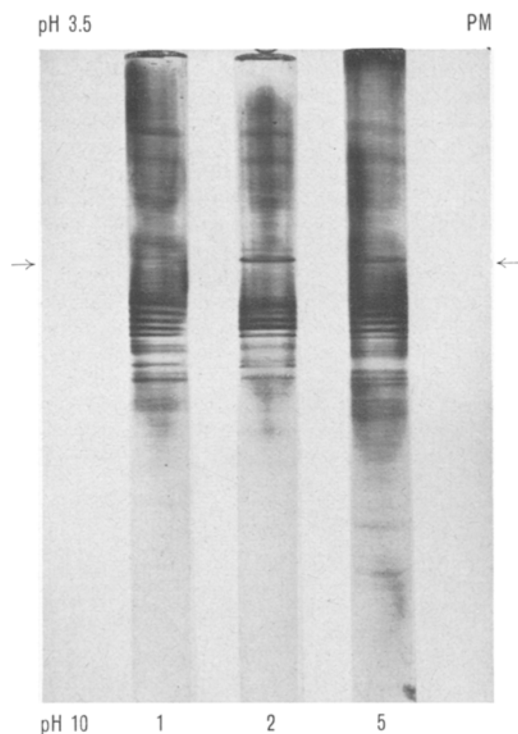


Fig. 1. Isoelectric focusing results of the apoproteins of serum very low density lipoproteins obtained from individual P.M. (1) before meal, and (2) 2 h, and (5) 21 h ,respectively, after the meal.

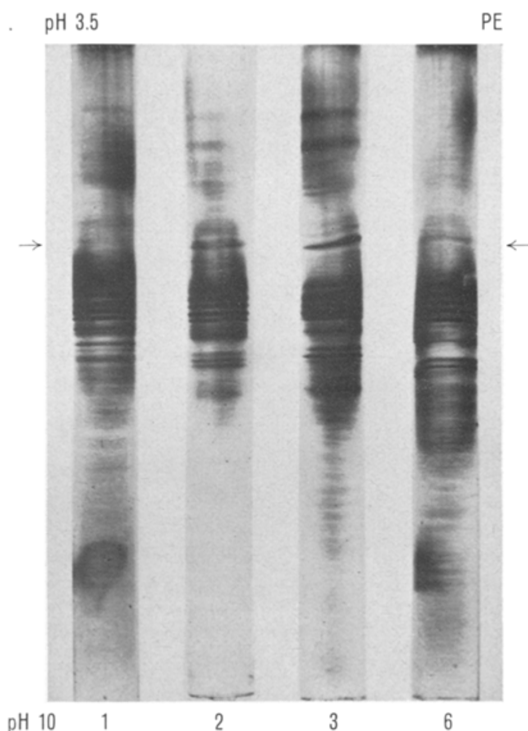


Fig. 2. Isoelectric focusing results of the apoproteins of serum very low density lipoproteins obtained from individual P.F. (1) before meal, and (2) 2 h, (3) 4 h, and (5) 21 h, respectively, after the meal.