MEDICAL GRAND ROUNDS

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THE MANAGEMENT OF TYPE I DIABETES IN THE 1980s

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ERRATA AND OMISSIONS

Page 1, line 4 from bottom: Reference is Drury, T., M. Harris: The changing prevalence of diabetes in the U.S. Diabetes 29(Suppl. 2):43A, 1980.

Page 13: Last paragraph should read: "A diabetic kidney mistakenly transplanted into a nondiabetic..."

Page 51: Line 5 should read "...systems to prevent hyperglycemia and over-glycosylation..."

Page 51: Lines 7 and 8 should read "Development of specialized private and public hyperglycemia control centers..."

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THE DILEMMA OF DIABETES

Throughout the professional lifetime of all currently active physicians one of the most intractable dilemmas of medicine has been the controversy concerning the relationship between hyperglycemic control and diabetic complications. In the half century since the introduction of insulin the controversy has evolved from an often bitter contest of opposing faiths (Joslin vs. Tolstoi), unsupported by any scientific evidence, to its present status, in which a recent accumulation of scientific data has for some participants in the controversy been decisive (26). However, until very recently, the controversy was an academic one, because there seemed to be no feasible alternative to the accepted methods of diabetic management with their attendant high risk of diabetic complications. Recently, however, there have been three major developments which make feasible alternatives to current therapeutic practice an immediate possibility: 1) the availability of home monitoring methods for daily profiles of glycemia; 2) methods for an integrated assessment of antecedent control of hyperglycemia and of glycosylation of proteins; and 3) new technics for delivery of insulin which maintain near-normal blood glucose profiles and levels of hemoglobin A_{1C} for extended periods of time.

Given these advances in metabolic monitoring capability and in glycemic control, the clinical community is now compelled seriously to reappraise its present technics of glucose monitoring and control of diabetics and to reach a decision as to how to treat diabetes in the coming decade.

The stakes in this decision are enormous both in terms of human suffering and in economic costs. There are 20-30 million diabetics in the world, of which 8 million live in the United States. Of the latter, 1.5 million, including 100,000 children, take daily injections of insulin. The prevalence of the disease may be increasing; a six-fold increase in the prevalence of diabetes has apparently occurred between 1936 and 1978 (reference abstract 169 in ADA program). The incidence of Type I diabetes, which is approximately the same in Europe, in New Zealand and in Rochester, Minnesota, ranges from 7-19.6 new cases per 100,000 inhabitants or 14-40,000 new cases per year in the U.S.A. (36).

THE RESULTS OF CURRENT MANAGEMENT OF DIABETES IN THE UNITED STATES

A. Morbidity with current treatment:

1. Blindness is 25 times as common in diabetics as in nondiabetics, 8.5 persons per 100,000 or about 20,000 Americans, are registered as legally blind from diabetic retinopathy (90); several times more are visually impaired.

2. Renal disease is 17 times more common (more than 50% of Type I diabetics treated by conventional therapy will develop end-stage renal failure between 10 and 30 years after the onset of their diabetes).

3. Gangrene is 5 times more common.

4. Heart disease is twice as common.

B. Mortality with Current Treatment: Average life expectancy is one-third less than that of the general population. The death rate when diabetes is listed as the primary diagnosis is 17.7 per 100,000 people; the overall death rate is 42.7 per 100,000 among diabetics. Death directly attributable to diabetes plus its complications is 30.3 per 100,000.

C. Economic costs of diabetes with current treatment:

1. Overall costs: The 8.5 million diabetics in the U.S.A. generated a total cost of $15.4 billion in 1979. The "average" diabetic generated costs of $1,845.32 during 1979, 65.8% of which was direct and 48.3% was the result of lost work days. The 1.9 million insulin-requiring diabetics generated $4.5 billion in costs or approximately $2,394 per patient, the costs being approximately 21% higher per patient for an insulin-requiring diabetic (158).

2. Medical costs: Direct medical costs attributable to diabetes in the state of Kentucky were estimated between $88-134 million per year. Diabetes was the leading cause of hospital admission by disease in Kentucky and accounted for 5.23% of all hospital admissions.

   The average cost of a diabetic hospitalization ranged from $1,037 to $1,248, significantly more than nondiabetic hospitalizations.

3. Cost of diabetic retinopathy: $75,000,000 per year in lost income and welfare alone (90,108).

4. Cost of diabetic nephropathy: The cost of diabetic nephropathy alone is expected to reach $750 million per year by 1984 for the treatment of diabetic uremic patients alone, who will, according to some estimates, constitute ~60% of the patients undergoing chronic dialysis. Diabetics have been estimated to account for 25% of all patients currently undergoing dialysis by the National Conference on Diabetes Advisory Board, although National Medical Care estimates it to be only about 15% at the present time.

Conclusion: The current methods of treating diabetes in the U.S.A. are associated with a very high morbidity and mortality and cost due to microangiopathic and other complications of the disease.

A CASE IN POINT

I.P. is a 46-year-old white male automobile mechanic admitted to the VA Medical Center for renal dialysis. He has had Type I diabetes since the age of 19. While serving in Korea he experienced polyuria, polydypsia, polyphagia and weight loss, and was discharged from the military service on 35 U of NPH/day. Except for occasional hypoglycemia while working, he was virtually free from problems in diabetic management until age 40, when he noticed failing vision in his left eye. Opthalmologic examination revealed severe retinopathy with microaneurysms, soft exhudates, blot hemorrhages and neovascularization. Other findings included absent ankle jerks, diminished sensation in the stocking area, postural hypotension and EKG evidence of an old posterior wall myocardial infarction. Urinalysis revealed 3 grams of protein/24 hours and 0 to 1+ glycosuria. Fasting plasma glucose levels ranged between 130 and 180. Creatinine was 3.9 mg/dl.

Renal function deteriorated following discharge and for the past year he has required chronic renal dialysis.

Conclusion: By current standards he had been a well-controlled diabetic. When seen by his physician three to four times per year his fasting blood glucose levels were almost always under 150 mg%. Urine tests done faithfully at home were generally 1+ or negative. He supplemented his basic dose of intermediate insulin with regular insulin whenever urine tests revealed glycosuria.

Yet, he developed serious complications of the disease, despite the fact that by today's standard of medical practice he was considered to be a "well-controlled" juvenile-type diabetic who enjoyed 21 years of relatively complication-free life. The development of microvascular complications in a "well-controlled" patient can mean only one of two things:

1. Either the complications of diabetes can develop despite good control, and are therefore independent of the magnitude and duration of the metabolic abnormalities in which case there is no reason to modify current therapy;

2. Or, metabolic abnormalities are directly or indirectly responsible for these complications and current treatment is to blame and should be modified; the insulin regimes now generally employed, i.e., 1 or 2 injections of an intermediate-acting insulin, usually cannot achieve "adequate" glycemic control, and the methods of monitoring (occasional fasting glucose levels and home urine determinations) cannot differentiate clearly between adequate and inadequate metabolic control.

The purpose of these Grand Rounds will be to review the available evidence for and against each of these possibilities.

SIX CRITICAL QUESTIONS

Question 1: Is clinical microangiopathy dependent or independent of hyperglycemia (and/or its associated abnormalities)?

Question 2: Are the current methods of management of diabetes satisfactory?

Question 3: If not, why is current therapy not satisfactory?

Question 4: How should diabetes be treated in the 1980s?

Question 5: Will elimination of hyperglycemia prevent clinical microangiopathy or provide other benefits; will it impose any risks?

Question 6: If there is no clearcut proof that normalization of glycemia can prevent clinical microangiopathy, is it acceptable to continue in the 1980s the current methods of therapy pending scientific proof of the superiority of the new therapy?

EVIDENCE AGAINST THE HYPERGLYCEMIA-MICROANGIOPATHY CONNECTION

A. Results of the Dallas Study of muscle capillary basement membranes:

1. Muscle capillary basement membranes are thickened in diabetics and prediabetics (179)

TABLE I

QUADRICEP CAPILLARY BASEMENT MEMBRANE WIDTH
IN NORMAL, DIABETIC AND PREDIABETIC SUBJECTS

Subjects	Average Basement Membrane Width (A)	Prevalence of Basement Membrane Thickening (%)
Normal (N=50)	1080 ± 27	8
Diabetic (N=51)	2403 ± 119	98
Prediabetic (N=30)	1373 ± 44	53

This study, published in 1968 by Siperstein, Unger and Madison, reported that almost all overtly diabetic patients had thickened quadriceps muscle capillary basement membranes (MCBM), and that half of normoglycemic prediabetics (offspring of two diabetic parents) also had significant, if slight, thickening.

However, Williamson, using different fixation and measurement technics, obtained different results and a long controversy ensued (218,222). It is now generally conceded that, while the observation of >two-fold MCBM thickening in overt diabetes is correct, the small difference in the prediabetics was probably due to an anomolously low value in the control subjects, most of whom were under 40 years in age. [Age has since been shown by Kilo et al. (99) to be a positive correlate with MCBM thickness, and Siperstein himself found a MCBM value 60% greater in a second group of normal controls (7).]

2. Duration of diabetes does not influence MCBM thickening: Siperstein et al. (179) failed to find a relationship between duration of diabetes and the MCBM thickening.

However, all other studies, (37,145,218,223) including a subsequent study by Siperstein's lab, have observed a relationship between duration of diabetes and MCBM thickness (7). Indeed, MCBM may be normal at the time of onset of Type I diabetes (217) and the kidney CBM is clearly normal at the start (79,111,141). Diabetic children do not exhibit consistent MCBM thickening (37,163).

There is an unpublished report that with excellent control Type I diabetics have normal MCBM values 5-20 years after onset, while poorly controlled patients develop thickened MCBMs in less than 5 years (81).

3. Patients with secondary diabetes (due to pancreatic insufficiency, hyperlipemia, Cushing's syndrome) do not exhibit MCBM thickening (179): According to Siperstein et al. (179), only one of eight patients with diabetes secondary to chronic pancreatitis exhibited MCBM thickening.

   However, Yodaiker (223) studied 20 such patients, all without a family history of diabetes, and found them to have thickened MCBM (223). Raskin (164) reported that rebiopsy of eight such patients 1.5-9 years after their initial biopsy showed a significant increase in MCBM in all but one patient; although only one of eight had been abnormal originally, five of eight were abnormal on rebiopsy. Verdonk et al. also studied clinical microangiopathy (retinopathy and nephropathy) in diabetes secondary to acute and chronic pancreatitis but with inconclusive results (210).

B. Studies of the role of hereditary factors in diabetic vascular disease: Marks et al. report (119) that MCBM thickening occurs in HLA-DR_4 nondiabetic parents of Type I diabetic children without MCBM thickening. DR_4 negative parents of such children were normal. If confirmed, these findings would support independence of MCBM thickening of hyperglycemia in relatives of diabetics.

   However, the difference in MCBM width was only 400 A, about 1 S.D. from the mean, and the ability of this method to distinguish small differences has previously been challenged (28). Moreover, Deckert et al. (40) found no relationship between HLA-antigens and the presence of retinopathy in long-standing Type I diabetics and Pyke and Tattersall suggest that non-HLA genes are involved. Retinopathy is common in concordant twins with Type 1 diabetes, but in discordant twins, only the diabetic twin has the retinopathy (160).

   Others, including Siperstein, have reported normal MCBM in nondiabetic monozygotic twins of diabetics (65,92). Jervell and Solheim (84), like Deckert, found no relationship between HLA types and nephropathy or retinopathy. Finally, MCBM thickening may not be correlated with clinical microangiopathy, at least not with retinopathy (49).

C. Studies of nodular glomerulosclerosis and diabetic retinopathy in nondiabetics: Careful scrutiny of the world literature reveals only two bona fide case reports in which apparent diabetic microangiopathy occurred in a "nondiabetic" (75,193). Other reports are questionable. Considering that the pathologic manifestations of diabetic vascular disease are not entirely specific, the rarity of such reports opposes, rather than supports, the premise of independence of microangiopathy and hyperglycemia.

Conclusions: MCBMs clearly are thickened in established diabetes, but the evidence of independence from hyperglycemia is soft. The subtle differences observed in certain genetically predisposed but nondiabetic groups must be interpreted with caution in view of the technical problems of reproducibility of MCBM measurements in the near-normal range, the sparse distribution of muscle capillaries and their indistinct outer border. The evidence of an inherent and independent vascular abnormality is outweighed by the positive relationship between duration of hyperglycemia and MCBM thickening, the absence of MCBM thickening at the onset of Type I diabetes, the development of MCBM thickening in secondary pancreatic diabetes, and the discordance of MCBM thickening in monozygotic twins. Even if there is a subtle morphologically detectable microvascular abnormality in normoglycemic patients with a diabetic inheritance, evidence that it can progress to clinical microvascular disease independent of hyperglycemia is nonexistent.

EVIDENCE FAVORING A RELATIONSHIP BETWEEN HYPERGLYCEMIA (OR AN UNIDENTIFIED CONCOMITANT OF HYPERGLYCEMIA) AND DIABETIC MICROANGIOPATHY

A. Clinicopathologic evidence of a relationship between hyperglycemia and microangiopathy

1. Nature's own study: It has been pointed out (207) that nature may have provided compelling evidence of a relationship between hyperglycemia and microangiopathy (Table II).

TABLE II

Blood Glucose	Number of Patients	Development of Clinical Diabetic Microangiopathy
Normal	>96% of the human race	Virtually never
Overtly Diabetic	<4% of the human race	Common

This, obviously, is an anecdote - not a real study. It consists of unequal, unmatched groups that were never actually surveyed and it is retrospective. Yet, a well-controlled survey of renal tissue tends to bear it out; Kamenetzky et al. found no diabetic nephropathy in 62 nondiabetic Pima Indians, while 55% of 43 diabetic Indians had nodular glomerulosclerosis and 44% had exhudative glomerular lesions (91).

Conclusion: Even if there is a genetic predisposition for diabetic microangiopathy, there is no evidence that it is expressed clinically in the absence of hyperglycemia (or some unidentified concomitant of hyperglycemia).

2. Retrospective clinical studies: The many attempts to determine the relationship between hyperglycemia and diabetic microangiopathy through retrospective clinical analysis is scientifically unsound because of selection bias and inability to quantify either the degree of hyperglycemia or the magnitude of the complications. According to Knowles (101) 51 of 85 such studies concluded that good control is beneficial. Only a few are cited herein (27,34, 103,109,159,192). Several concluded that bad control is associated with more frequent and more severe complications (35,56,82, 87). In the Joslin Clinic study of Type I diabetics of 10-36

years duration (94) only one of 101 nephropathics came from the "well-controlled" group. No patients with grade 4 retinopathy and only 3 with grade 3 retinopathy came from this group. Nerve conduction velocity was also related to better control. Of greatest interest in the retrospective category is the famous Malmo Study (87). Between 1922-1935 (before the advent of longer-acting insulins) all patients received multiple injections of regular insulin. From 1936-1945 longer-acting insulins were used. Even though the duration of the diabetes was 8.6 years longer in the 1922-1935 group, they had less retinopathy and nephropathy than the 1936-1945 group! Retrospective analyses in the United Kingdom and in Boston (136,170) found that most 20-40 year survivors had received multiple injections.

Conclusion: While certain of these studies, particularly the Malmo Study, are extremely provocative, none are scientifically conclusive because of inherent flaws in retrospective studies of diabetes.

3. Prospective clinical studies:

   a. Study of mortality: Goodkin reported the mortality in poorly controlled diabetics to be 2.5 times that of better controlled patients in a 20-year prospective study (70).

   b. The Phoenix study of retinopathy: In 590 Pima Indians over 24 years old, the 6-year incidence of retinopathy (microaneurysms, hemorrhages and neovascularization) increased in relation to the 2-hour plasma glucose level of a 75 g oral glucose tolerance test 6 years earlier (Figure 1) (114). All Pima diabetics are Type II.

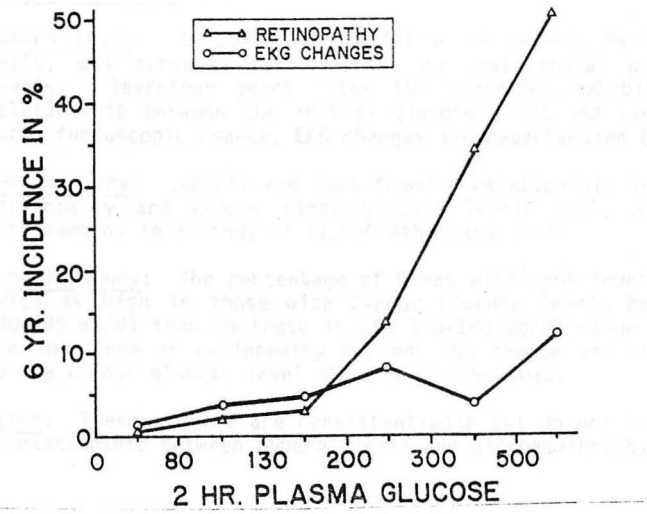

Figure 1: EKG changes and retinopathy as a function of plasma glucose (114).

c. Brussels study of diabetic triopathy: In the most ambitious prospective study of diabetes in medical history, 4398 cases were followed for 25 years (109,110,154-156). A relationship between retinopathy and duration and severity of hyperglycemia was noted after 12 years of Type I diabetes. Other prospective studies also report that only very good control prevented worsening of retinopathy (104,130) and proteinuria (131,194).

d. The Paris study of microaneurysms: In 1968 randomly assigned insulin-dependent patients were placed on a regime of long-acting insulin given once a day or 2-3 injections per day (200-202), and followed by fluorescein angiography. After 3 (85) and 4 years (53) both the mean yearly number of microaneurysms and mean fasting blood glucose levels were lower in the multiple injection group. Although the study was criticized because of a shifting of about 25% of patients from one regime to another, in fact this reduced rather than increased the significance of their findings. It has been extended with an additional year of observation which discloses an annual increase in microaneurysms of 9 ± 1 in the single dose group vs. 3 ± 1 in the multiple dose group ($p<0.001$)(53). This fits well with the Malmo study (87).

Conclusion: These studies, despite certain imperfections in design, provide impressive clinical support for the value of good control but fall short of proof.

4. Epidemiologic studies:

a. Oxford Study: In 1946 the population of Oxford, Massachusetts, was screened for diabetes by postprandial glucose levels. Seventeen years later the diabetics exhibited a relationship between the initial glucose level and survival rate, funduscopic change, EKG changes and hypertension (142).

b. London study: Jarrett and Keen found a relationship between retinopathy and 2-hour blood glucose levels (83), as did Katsilambros in a study of 21,000 Athenians (93).

c. Phoenix study: The percentage of Pimas with proteinuria was twice as high in those with 2-hour glucose levels between 200-299 mg/dl than in those in the 140-160 mg/dl range (91). The incidence of retinopathy but not EKG change was related to the 2-hour glucose level after a glucose load.

Conclusion: These studies are consistent with but do not prove a causal relationship between hyperglycemia and microangiopathy.

5. Pathologic studies of the human kidney in hyperglycemic environment:

   a. Danish Study: Osterby found no difference in glomerular basement membranes between nondiabetics and new Type I diabetics. One to 2 years later slight but significant thickening was present in the diabetics; after 3.5-5 years still greater thickening had occurred and mesangial changes were present (139,140).

   b. Scottish Study: Ireland et al. observed glomerular basement membrane thickening in patients with secondary nongenetic diabetes (80).

   c. The Minnesota Study: Serial annual biopsies of normal kidneys transplanted into diabetics revealed the development within 2-3 years of vascular lesions indistinguishable from those of diabetic nephropathy, including a 2- to 3-fold increase in GMB thickness (24,121), and immunofluorescence for IgG and albumin in tubular and glomerular GBM (122)! This never occurs in kidneys transplanted into nondiabetics!

   Conclusion: These studies, particularly the Minnesota study, would seem to provide the most decisive evidence of relationship between hyperglycemia and diabetic nephropathy. Assuming that the recipient's blood vessels did not invade the donor's kidney (most improbable), the Minnesota study is the ultimate proof of a relationship between nephropathy and the metabolic environment.

6. Pathologic studies of the hyperglycemic environment in animals:

   a. Retina:

      1. Alloxan diabetic dogs (also growth hormone induced and spontaneously diabetic dogs) develop after 2-4 years microaneurysms, hemorrhages and exhudates identical to human diabetic retinopathy save for the absence of proliferative disease (51,137). This can be inhibited by good control with insulin (16,46).

      2. Experimentally diabetic rats develop neovascularization which is prevented by islet transplantation (137).

   b. Kidneys:

      1. Alloxan diabetic dogs develop in several years diffuse glomerulosclerosis and Kimmelsteil-Wilson lesions which are inhibited by good control of hyperglycemia with insulin (16,52).

Conclusion: These and other studies not cited herein favor a pathogenic relationship between hyperglycemia (or its concomitant abnormalities) and diabetic microangiopathic disease of animals, but because extrapolation from animals to man can always be questioned, this evidence may be regarded as inconclusive.

7. Studies of the ability of near-normalization of glycemia to induce regression of diabetic complications:

   a. London Study: After 3 months of a mean blood glucose of 108 mg/dl, a patient with preproliferative retinopathy exhibited visual improvement, reduction in retinovascular permeability to dye, revascularization of ischemic areas with normal vessels and less marked looping (152); the authors state that a remission of this magnitude had been seen previously only in patients after hypophysectomy.

   b. New Haven Study (196): In 10 Type I diabetics near-normalization of glycemia by pump (mean 102 mg/dl) for 3-11 months improved sensory nerve conduction but failed to improve creatinine clearance, reduce proteinuria, or improve retinopathy. In fact, vitreous hemorrhages occurred in 2 patients with proliferative retinopathy after 1 and 3 months on the pump.

   c. Rockefeller University Study (148): Ten patients with Type I diabetes were enrolled in a program of self-adjusted insulin administration based on self-monitored blood glucose determinations plus an exercise program. Six patients showed a decrease in quadriceps MCBM width after 8 months, 3 showed no change, and the only patient in whom hemoglobin A_{1C} remained over 10% throughout the study exhibited an increase in MCBM. However, there was no control group and no attempt was made to determine the effect of exercise on MCBM. Motor nerve conduction velocity (median, ulnar, peroneal and posterior tibial) returned to normal in 3 of the 7 patients who had been abnormal initially and a fourth improved drastically but remained abnormal after 8 months of the regime (149).

   d. Dallas Study (153): Improvement in motor nerve conduction velocity but no change in MCBM, renal function or albuminuria were noted 6 months after near-normalization of glucose and normalization of Hb_{A1C} by insulin pump.

   e. Minnesota Mistake: A diabetic kidney, mistakenly transplanted into a nondiabetic, failed to exhibit any reduction in immunofluorescent staining for albumin after 2 years (Goetz, F.C., unpublished comments).

Conclusion: Functional improvement in nerve function and an isolated report of improvement in retinopathy notwithstanding, near-normalization of glucose with insulin pumps has not yet been shown to cause regression in microangiopathy as of the end of the first year of such studies. While it is far too soon to discount such a possibility, most workers in the field are pessimistic about the possibility of reversing established microangiopathic lesions.

B. Biochemical evidence for a relationship between hyperglycemia and diabetic microangiopathy:

1. Evidence of posttranslational modification of proteins by glucose:

HISTORICAL NOTE: Sixty years ago Bierry and Pathery showed that protein bound carbohydrate was increased in diabetics (14). The demonstration of carbohydrate staining material in the retinal and renal lesions of diabetes caused Friedenwald, McManus, LeCompte and others (58,124,215) to postulate a relationship. In 1968 an Iranian physician, Dr. S. Rahbar, noted a fast-moving hemoglobin on agar gel electrophoresis in two of 1,200 patients studied for hemoglobinopathy (161). Both patients were diabetic. Study of 47 other diabetics revealed this hemoglobin to be present in all. Rahbar then worked with Dr. Helen Ranney (162) and identified the Hb as Hb/A_{1C}, which normally comprises 3-6% of HbA. Tattersall et al. (198) demonstrated that it was not a genetic marker, being present only in hyperglycemic members of monozygotic twins discordant for Type I diabetics, and Koenig and Cerami observed that in db/db mice the increase in Hb A_{1C} appeared about 4 weeks after the appearance of hyperglycemia (102). They also suggested (102) that the increase in HbA_{1C}, known to be a glycohemoglobin (17) might reflect a general glycosylation of protein in hyperglycemic individuals, the thickened capillary basement membranes of renal glomeruli having been shown by Spiro and associates to contain increased amounts of glycoproteins (10,182-186). Since then in a brilliant series of studies the Rockefeller group and others have advanced the concept of diabetic microangiopathy as a disease of posttranslational modification of proteins through hyperglycosylation.

a. The chemistry of glycosylation of proteins: Nonenzymatic addition of glucose to protein is a slow continuous reaction that is a function of the duration of the contact between the reactants and the integrated glucose concentration during the time of contact (Figure 2).

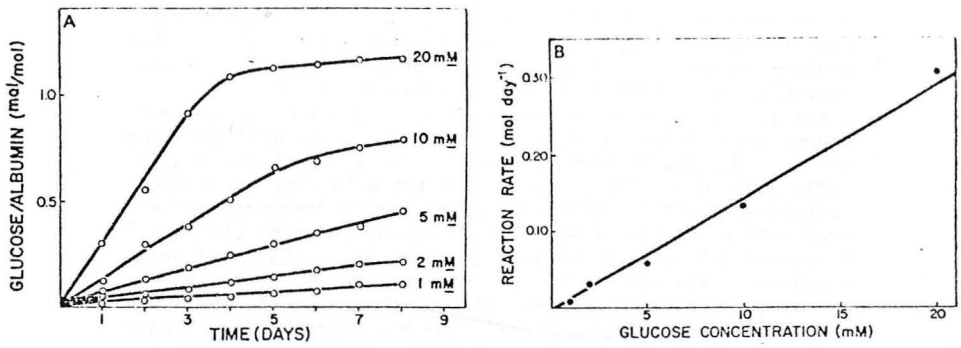

Figure 2: Rate of glycosylation of rat albumin as a function of glucose concentration. Rat albumin, 7 mg/ml of phosphate buffered saline, was incubated with indicated concentrations of glucose made to a final specific activity of 4 x 10^6 cpm/ µmol. A) Acid-precipitable radioactivity in aliquots removed at indicated times was determined. B) Initial reaction rates shown in A) are plotted as a function of glucose concentration (39).

In the case of hemoglobin with N-terminus of the β chain of HbA combines with glucose to form a Schiff base (aldimine) attachment (Figure 3).

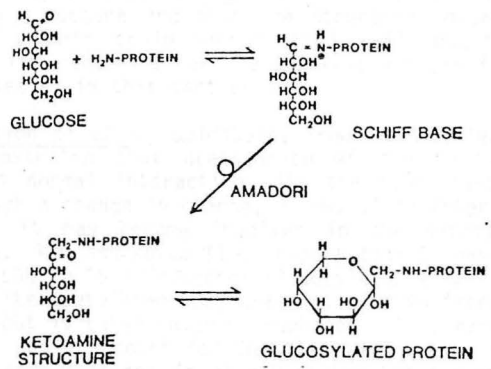

Figure 3: General reaction scheme for the nonenzymatic glycosylation of protein.

Glucose also binds to four lysines of both α and β chains of HbA_0. HbA_{IC} is the most abundant of the glycosylated Hb components and the reaction with the N-terminal valine happens to confer a major decrease in pI which enables its electrophoretic separation. There is nothing specific about this, the formation of glucose adducts with α- and β-amino groups on proteins being a well known phenomenon (1,112,174). The reaction is very slow but the stability of the product after the rearrangement favors the accumulation of those posttranslationally modified proteins that have a relatively slow turnover time (22). The proteins thus far studied for increased glycosylation in diabetes run the gamut from albumin, with a half-time of ~20 days (much shorter than that of Hb), to the very slowly turning over capillary basement membrane proteins and the lens proteins, which don't turn over at all.

NOTE: Sodium cyanate used in sickle cell anemia to modify covalently the HbS, reacts specifically and irreversibly with the N-terminus of certain proteins other than Hb and causes segmental demyelinating polyneuropathy (150) and posterior subcapsular cataract formation (135), both indistinguishable from diabetes!

2. Functional consequences of glycosylation of proteins in the circulation

   a. Glycosylation of albumin: Functional consequences of albumin glycosylation has not been determined. Theoretically, its affinity for fatty acids, bilirubin, clinical dyes, drugs, such as aspirin (which, by acetylating the lysine residue blocks glycosylation)(39) might be altered. Its tertiary or quaternary structure and thus the structural microheterogeneity of albumin could be affected (55) and this could influence its catabolism and perhaps explain the findings of McVerry (see e. in this section).

   b. Glycosylation of LDL: Goldstein, Brown and colleagues (69) have demonstrated that acetylation of the LDL apoprotein blocks its normal interaction with the fibroblast receptor and, through a change in charge, allows it to enter macrophages where it may become involved in the pathogenesis of atheromata. Kim and Kurup (100) report that 14 days of incubation of LDL in 15 mM glucose[1] glycosyl-LDL at a lysyl residue and, like acetylated LDL, does not bind to fibroblast LDL receptors but is taken up by macrophages. This mechanism, if confirmed, could account for the alleged increase in atherosclerotic complications in poorly controlled diabetes. However, the half-time of LDL is too short relative to the glycosylation reaction to permit significant in vivo accumulation of glycosyl-LDL in diabetic plasma.

c. Glycosylation of immunoglobulins: Little is known about the functional consequences, if any, of glycosylation of immunoglobulins.

d. Elevation of acute phase reactants and fibrinogen: However, the glycoproteins α_2-macroglobulin, haptoglobulin, fibrinogen caeruloplasmin and α-1-antitrypsin are high in poorly controlled diabetes and are associated with diabetic complications (87) They are lowered by treatment with regular insulin (18) and normalization of glycemia (87). Kennedy et al. note a relationship between protein-bound hexose levels and Hgb A_{1a+b+c} (95,123).

Fibrinogen levels are increased despite reduced fibrinogen survival during hyperglycemia. There is no evidence that this reflects glycosylation or that the molecule is altered functionally (88) since it is rapidly reversed by normalization of glycemia even though high HgA_{1C} levels are not corrected. Heparin also corrects it, suggesting that thrombin or one of its antagonists is involved. Increased levels of protease inhibitors such as α-2 macroglobulin may contribute to decreased fibrin degradation, together with reduced fibrinolytic activity of diabetic plasma (2,3,20). Fibrin deposits could contribute to small vessel damage (28,204).

Plasma viscosity is increased in diabetes, most strikingly in retinopathy, because of increased acute phase reactant proteins, especially fibrinogen and haptoglobulin. These abnormalities are reduced by good control (18,125,126). This may cause the abnormal red cell aggregation observed in diabetic retinopathy (115,116).

e. Interactions between plasma proteins and glomerular basement membranes: In a remarkable, although preliminary report, McVerry found injection of glycosylated plasma proteins into normal mice weekly for 12 weeks caused glomerular basement membrane thickening, whereas unmodified proteins had no effect (Table III).

TABLE III

PATHOLOGICAL FINDINGS IN RENAL GLOMERULI OF MOUSE KIDNEYS

Group	Type of Injection	No. of Mice	Glomerular Capillary Basement Membrane Thickening	Mesangial Changes
1	Glycosylated proteins	7	5	5
2	Non-glycosylated proteins	4	0	2
3	No injections	10	0	2

f. Glycosylation of red cell components

1) Cell membranes: All major proteins of the red cell membranes are nonenzymatically glycosylated at the lysine residues. Diabetic red cell membranes have twice as many ketoamine linkages as nondiabetics.

2) Hemoglobin: There are several glycosylated hemoglobins: HbA_{1a1}, HbA_{1a2}, HbA_{1b} and HbA_{1c}. HbA_{1c} is the most abundant minor component, about 5% of the total in normals, but >twice this in hyperglycemics. Glucose-6-PO_4 reacts ~20x as fast as glucose to form adducts but its concentration in the red cell is only 1/200 of that of glucose, explaining why G6P-Hb(HbA_{1a2}) is only 1/10 of that of HgA_{1c}. G6P is about 30% higher in diabetes (203).

3) Functional derangements of the hyperglycemic red cell:

a) Oxygen affinity: The N-terminus of the β-chain of Hb is normally involved in binding organic phosphates (6). 2,3-DPG normally forms salt bonds with the positively charged residues on the two β chains, including the N-terminal amine groups. If these sites are blocked by a hexose, acetyl (23) or carbamyl group (98), Hb reactivity with 2,3-DPG is reduced and a slight increase in oxygen affinity occurs (P_{50} of 24-26 mm-Hg vs a P_{50} of 26 in normals) (8). It is doubtful that this small displacement in the O_2 dissociation curve have

functional implications, as had been suggested by Ditzel and Standl (44). [However, 2,3 DPG fluctuates with glycemic change and reduced levels increase O_2 affinity of Hb and decrease its release to tissues (45).]

b) Erythrocyte survival: There is a decrease in red cell survival (27 day half-time in poorly controlled Type 1 diabetics vs 28-36 days in normals). Normalization of glycemia raised survival to 31 days.

c) Rheology: Normally red cells pass through capillaries smaller than their own diameter because of their deformability. An 8 μ red cell can pass through a 3 μ diameter pipette and quickly reassume its original shape. Red cell deformability, which is reduced in thalassemia, sickle cell disease and spherocytosis is also impaired due to increased intra-erythrocyte or membrane viscosity (127). It could be due to increased HbA_{1c}, to reduced elasticity of the glycosylated membrane decreased sialic acid and cholesterol in diabetic red cells (31) or membrane bound Hb (147).

Comment: These combined abnormalities, all of them reversible by correction of hyperglycemia, could lead to stasis in the microcirculation and hypoxia, which appears to be a common factor in development of neovascularization of the retina, nephropathy and neuropathy.

g. Hyperglycemia and the leucocytes: Diabetic abnormalities include decreased chemotaxis, diapedesis, phagocytosis, bactericidal activity and cell-mediated immunity (see 21 for review). The mechanism of these functional derangements and their contribution to the diabetic predisposition to infection is unknown. Cell surface changes secondary to glycosylation or reduced sialic acid in receptors have been suggested (see 21 for review). Such changes could influence the cell surface receptors of hormones, LDL, as well as of mitogens. Indeed, the hepatocyte membranes of diabetic rats exhibit a 25% reduction in conconavalin A binding and a 50% reduction in desialylated thyroglobulin binding suggesting selective loss of sialic acid in specific receptors. Leucocyte adherence to a glass wool column is reduced in poorly controlled diabetics (28% vs. 45-70% in normals). With glucose control it was corrected (to 51%), correlating with normalization of HbA_{1c}. The mechanism is not known. T-cells and B-cells are reduced and show defective response to mitogens - all of which is rapidly restored by normalization of glucose (175).

h. Hyperglycemia and platelets: Increased platelet aggregation platelets has been demonstrated in poorly controlled diabetes (13,46,76,171,172), especially in retinopathy. A concomitant increase in "factor VIII/von Willebrand factor" activity may be one of the causes (4,13,144,172). The carbohydrate portion of this protein determines its interactions with platelets (71) and overglycosylation could be involved here also.

Hyperaggregation of platelets may be caused by altered platelet thromboxane synthetase and blood vessel endothelial prostacyclin synthetase. The former generates thromboxane A_2 from endoperoxides prostaglandins G_2 and H_2, which stimulates platelet clumping and arterial constriction; prostacyclin synthetase converts the same endoperoxides to prostacyclin, which inhibits the thromboxane effects (120). There is recent evidence that an imbalance favoring thromboxane may exist in diabetes, especially when microangiopathy is present (205). Whatever the mechanism of the hyperaggregation of platelets, strict control with normalization of HbA_{1c} restores platelet function to normal (150).

3. Glycosylation of capillary basement membranes (CBM):

a. Normal chemical structure: CBM contains a collagen-like glycoprotein rich in hydroxylysine and two types of carbohydrate units. One is an asparagine-linked heteropolysaccharide containing galactose, mannose, fucose, sialic acid and hexosamine on the more polar regions of the peptide, and the other is a glucose-galactose disaccharide unit attached to hydroxylysine (Figure 4). (Hydroxylysine is formed by enzymatic hydroxylation of the lysine residues of the peptides 80% of which is then linked to galactose and then glucose by transferases to form a disaccharide-hydroxylysine complex.)

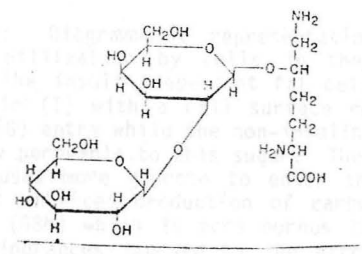

Figure 4: Structure and peptide attachment of the disaccharide unit of the glomerular basement membrane (183).

b. Chemical structure in diabetes: Renal glomeruli isolated from diabetics of long duration exhibit an increase in hydroxylysine and dissacharaide-hydroxylysine complexes compared to nondiabetic glomeruli (9,10,216), whereas glomerular basement membranes from patients with nondiabetic renal disease with proteinuria do not differ from normal (11). If true, this speaks for a distinct chemical change in diabetic nephropathy. An increase in renal lysyl hydroxylase and in glucosyltransferase, the enzyme which completes the dissacharide linkage to hydroxylysine, has been observed in kidneys of alloxan diabetic rats and is reduced towards normal by insulin treatment (96,97,182). Through steric hindrance increased glycosylation could interfere with packing of the polypeptides and increase the pore size of the glomerular CBM (10), explaining the increased excretion of dextran (133) and albumin (146) by the diabetic kidney. Greater availability of glucose substrate would favor enzymatic glycosylation; the kidney does not require insulin for glucose transport or phosphorylation so that glucose concentration may be rate limiting (183) (Figure 5). Indeed, Wahl (212) found that glucose stimulates the incorporation of labeled glucose into glomerular basement membranes in a linear fashion.

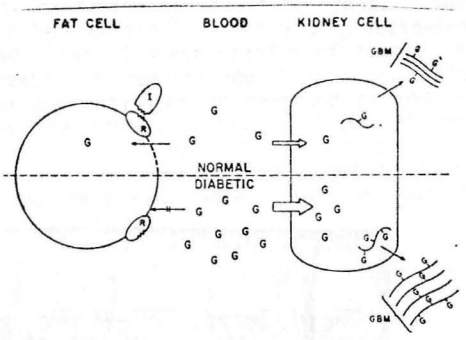

Figure 5: Diagrammatic representation of two patterns of glucose utilization by cells in the normal and diabetic state. The insulin dependent fat cell requires interaction of insulin (I) with a cell surface receptor (R) to permit glucose (G) entry while the non-insulin requiring kidney cell is freely permeable to this sugar. The hyperglycemia of diabetes causes more glucose to enter the kidney cell with a resultant enhanced production of carbohydrate-rich basement membrane (GBM) which is more porous than normal due to the steric hindrances imposed by the extra saccharide units on peptide chain packing (183).

It has also been suggested that increased glycosylation may reduce proteolysis of proteins (15) which could contribute to basement membrane accumulation; glomerular β-glycosidase activity is reduced in diabetic rats (59-61).

3. Glycosylation of the lens: When the crystallin proteins of the lens form aggregates having a mol. wt. >5 x 10^6 daltons, light is scattered and a cataract is formed (181). The aggregation is, in part, the result of sulfhydryl oxidation (43) to form disulfide cross-links (42,74,157,195,206). The disulfide polymers may be early steps in the so-called Maillard reaction or "nonenzymatic browning" of proteins that occurs when proteins are stored with sugars, e.g., foods and in tissues (50,58). Cerami's group at Rockefeller have proposed that in diabetics nonenzymatic glycosylation of the -amino groups of the lysine residues of the lens crystallin proteins results from high tissue levels of glucose and glucose-6-PO_4. (In the lens, as in the kidney, retina, red cells and nerves glucose and glucose-6-PO_4 levels are a function of extracellular concentration of glucose since in these tissues insulin does not influence either transport or phosphorylation of glucose). They suggest that glycosylated crystallins are conformationally altered so as to enhance the susceptibility of the sulfydryl group to oxidation to disulfide bonds. They incubated clear solutions of lens crystallin with 5 mM glucose-6-phophate or 50 mM glucose and observed incorporation of hexose into protein together with formation of opalescence (Figure 6). This was accelerated by oxygen and prevented or reversed by reducing agents, glutathione being the most effective. Brown pigment appeared after 6 months. This nonenzymatic browning of glycosylated crystallins resembles the brown nuclear pigment of the diabetic cataract (29) and may be the ultimate marker for longstanding

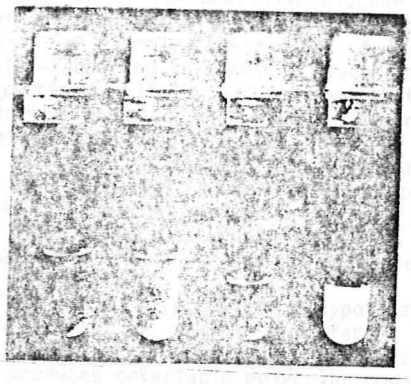

Figure 6: Sterile solutions of bovine crystallin after 28 days of incubation at 37° with 5 mM glucose 6-phosphate or 50 mM glucose. From left: cortical, glucose; cortical, glucose 6-phosphate; nuclear, glucose; and nuclear, glucose 6-phosphate. At this time the control solutions (without hexose) showed no opalescence (29).

glycosylation (183,191). Although unidentified, it could reflect a nondisulfide crosslinking of proteins via a bi-tyrosine moiety.

Cerami suggests that the lack of a clear relationship between cataractogenesis and the duration and magnitude of hyperglycemia may reflect variations in tissue reducing equivalents, such as reduced glutathione, which protect against disulfide crosslinking.

5. Glycosylation in peripheral nerve myelin: In diabetics myelin, constituting 75% of the dry weight of peripheral nerves, is abnormal in virtually all of its measured chemical constituents in association with quantifiable functional impairment. Myelin protein is decreased, amino acid incorporation is reduced (187,188) and its susceptibility to proteolysis increased (189). Although no compositional changes have been clearly established, glucosylysine may be present in basic myelin of nerve protein (54). In any case the functional changes in nerve conduction are readily corrected by careful treatment of diabetes, as first demonstrated in 1971 by Ward et al. (214).

   However, other etiologies may be involved in diabetic neuropathy; sural nerve biopsies in 24 patients with diabetic neuropathy indicate that fibrin plugs within blood vessels is common (204).

6. Glycosylation of other cells: Theoretically, any protein can be modified chemically by condensation with glucose, and this modification may or may not change its structure and function. Those tissues in which insulin does not regulate glucose transport or phosphorylation, the red cells, kidney, nerves, lens and retina are most apt to be victimized by hyperglycemia since intracellular glucose and the more reactive G-6-PO_4 can rise independently of insulin as a function of extracellular glucose concentration and can form condensation products with any protein. The effects may be far-reaching. For example, the B-cells of the islets, are probably not insulin-requiring in terms of glucose transport and glucose phosphorylation. It is well known that their secretory response to glucose is obtunded by chronic hyperglycemia (169) in Type II diabetics and is gradually restored by any maneuver [carbohydrate restriction, insulin rx or tolbutamide rx (107)] that reduces hyperglycemia. The induction of the well-known "honeymoon period" by glucose normalization (132) may also reflect restoration of islet function through reduction of high levels of intracellular glucose. However, this is just speculation.

   Conclusion: The protein glycosylation hypothesis is by far the most attractive concept to emerge thus far. The glycosylation reaction occurs both in vivo and in vitro in relation to glucose concentration, produces detectable molecular modifications both in vivo and in vitro, and is associated with pathologic abnormalities both in vivo and in vitro (lens experiments). It seems improbable

that all of the complexities of clinical microangiopathy can be ascribed to a single chemical reaction when so vast an array of associated abnormalities coexist, but glycosylation could play an essential underlying role by modifying those proteins exposed to high levels of glucose in tissues in which intracellular glucose and glucose-6-PO_4 are a function of extracellular hyperglycemia uninfluenced by insulin.

2. Evidence for a role of the polyol pathway: In the polyol pathway glucose is reduced to sorbitol by aldose reductase, an enzyme located in retina, kidney, papilla, lens, Schwann cells, islets of Langerhans (62-64,209,219) (all tissues in which glucose transport and phosphorylation are independent of insulin and a function of extracellular glucose concentration). Sorbitol is then oxidized to fructose by sorbitol dehydrogenase. Intracellular glucose concentration regulates the rate of both reactions. The osmotic effect of polyol accumulation has been postulated as the cause of diabetic cataracts and perhaps retinopathy, neuropathy, aortic disease (25,63,77,220,221). However, it now seems doubtful that this is the underlying defect, although it may contribute.

   a. Lens: In the lens the polyols probably don't cause cataracts directly but may lower the reducing equivalents and thus increase predisposition for oxidation of sulfhydryl groups and aggregation of crystallin proteins.

   b. Nerves: In peripheral nerves, one of the isomers of sorbitol, myoinositol is decreased, and, as a precursor of membrane phospholipids, this may cause defective nerve impulse transmission. When diabetes is poorly controlled urinary myoinositol is increased and can be corrected by strict insulin control (33,72).

   c. Arterial wall: Morrison et al. find little change in intracellular glucose, or polyols or ultrastructure following exposure of rabbit aortic intima-media to 5 mM and 20 mM glucose (134).

   Conclusion: Evidence for a direct role is not compelling.

C. Evidence of relationship between microangiopathy and abnormalities associated with hyperglycemia: The apparent sine qua non relationship between hyperglycemia and microangiopathy does not exclude the possibility that an abnormality always associated with hyperglycemia, rather than or in addition to hyperglycemia itself, causes or contributes to the pathogenesis of microangiopathy. Identifiable concomitant derangements include both metabolic and hormonal abnormalities.

a. Metabolic abnormalities other than hyperglycemia: There is broad and familiar spectrum of metabolic derangements listed (Table IV) but no clear clinical or chemical relationship with microangiopathy has ever been proposed.

TABLE IV
(from reference 21)

BIOCHEMICAL ABNORMALITIES REPORTED IN DIABETES

I. Lipids and lipoproteins
   A. Serum
      1. Increased cholesterol (blood sugar over 200 mg%)
      2. Increased triglycerides, (?) decreased TG turnover
      3. Decreased lipoprotein lipase activity (postheparin lipolytic activity)
      4. Altered apolipoprotein patterns
      5. Decreased lecithin:cholesterol acyl transferase activity
      6. Increased Type IV hyperlipoproteinemia (VLDL)
      7. Triglyceride-enriched HDL, LDL
   B. Tissue
      1. Decreased retinal arachidonic acid
      2. Decreased cell membrane cholesterol (RBC, hepatocyte)
      3. Increased sat./unsat. ratio in platelet membrane fatty acids
      4. Increased PGE_2-like material in platelets (ADP, epi, coll. arach.)
      5. Decreased myelin cholesterol, (?) decreased acetic thiokinase activity
      6. Increased myelin cholesterol, (?) decreased myelin cholesterol
      7. Decreased myelin cerebrosides with decreased incorporation of saturated fatty acids

II. Carbohydrates
   A. Simple
      1. Increased L-xylulose (reflects glucuronic acid pathway activity)
      2. Increased glucose
         a. Increased sorbitol in lens, retina, peripheral nerve, aortic wall
         b. Decreased free myoinositol in peripheral nerve, increased plasma and urine levels
         c. Increased intracellular glucose in insulin-independent tissues

d. Increased glycogen in WBC, kidney, iris, myocardium, liver
e. Fluctuating levels of 2,3,-diphosphoglycerate

B. Complex
1. Plasma glycoproteins
a. Increased fibrogen, haptoglobin (plus 10 others)
b. Increased vWF activity (other clotting factors ?V)
c. Increased α 2-macroglobulin, α 1-antiprotease
d. Decreased antithrombin III (AOD)
e. Increased CH50, C_1S, C_3, C_4 (complement system)
f. Increased fibrogen. α 2-macroglobulin turnover
2. Tissue glycoproteins
a. Decreased arterial wall mucopolysaccharides
b. Increased GBM with altered composition (increased disacch. units)
c. Increased GBM biosynthetic activity (glycosyltransferase, hydroxylase, peptide synthesis)
d. Decreased urinary GBM fragments
e. Increased glycohemoglobin
f. Decreased sialic acid: RBC, hepatocyte, (?) podocyte cell membrane
g. Increased vitreous humor glycoprotein glucosamine

III. Proteins
A. Decreased mRNA translation + decreased protein synthesis (muscle, heart)
B. Increased mRNA translation + increased protein synthesis (kidney)
C. Altered activities of several hepatic enzymes
D. Decreased glomerular ß-glycosidase activity
E. Increased serum ß-glycosidase activity, urinary glycosidase activity
F. Increased kidney ornithine decarboxylase activity (polyamines, mRNA)
G. Increased actomyosin in glomerular mesangial cells
H. Abnormal peripheral myelin sedimentation and electrophoresis patterns
I. Decreased sciatic nerve total myelin content
J. Decreased AA incorporation into some myelin proteins
K. Decreased susceptibility of peripheral nerve myelin to proteolytic digestion

IV. Nucleotides and nucleic acids
  A. Increased UTP (kidney)
  B. Increased RNA (kidney)
V. Hormones and vitamins
  A. Decreased insulin
  B. Increased GH
  C. Increased glucagon/insulin ratio
  D. Increased PTH (also alk. phos., Ca, P) in rats
  E. Decreased α-OH of 25-OH Vit. D_3 (rat)
  F. Increased serum dehydroascorbic acid

b. Hormonal abnormalities: Secretion of many peptide hormones becomes abnormal during insulin deprivation. Glucagon, pancreatic polypeptide, and somatostatin among pancreatic hormones, and GIP, GLI, somatostatin, secretin and probably others among gut hormones are elevated and do not respond normally to stimuli or suppressants when insulin secretion is impaired. Among the non-GEP peptides growth hormone and PTH are high.

1) Hypersomatotropinemia: Lundbaek has proposed that the elevated hGH levels of Type I diabetics (73) which average three times normal (86,94) may influence basement membrane synthesis (118). hGH levels become normal with near-normalization of glycemia (211). The favorable effects of pituitary ablation on severe microangiopathy (117,167) [60-88% of patients are stabilized or improved (19,67)] and the absence of microangiopathy in hGH-deficient diabetic dwarfs (128,129) has supported the notion. However, microangiopathy also occurs in Type II diabetics in whom hGH is not high and never occurs in acromegaly without hyperglycemia. The Lundbaek hypothesis is schematized in Figure 7.

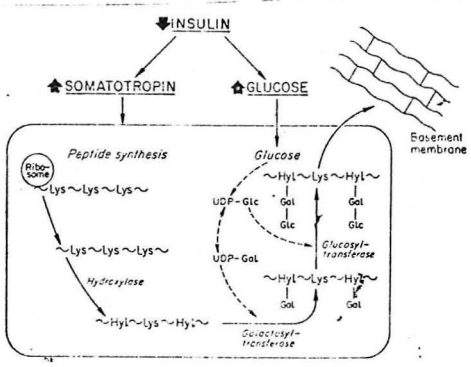

Figure 7: Potential stimulatory influences on glomerular basement membrane synthesis by kidney cells in diabetes (183).

b. Hyperglucagonemia: Spritz and Marinan (190) have reported that altered amino acid incorporation into certain myelin proteins of diabetes is related to the high glucagon, low insulin mixture. The abnormality is simulated in vitro by anti-insulin serum and is inhibited by insulin or antiglucagon serum, suggesting that the Schwann cell is modulated by the relative levels of these two hormones. Glucagon levels can be lowered by normalization of hyperglycemia with insulin (165,166).

Conclusion: Although proof of causal relationships are lacking, the complexity of the microangiopathic lesions, together with large array of metabolic, hormonal, physiologic and molecular derangements that are associated with poor control, make it likely that more than a single abnormality is responsible.

THE NATURAL HISTORY OF CLINICAL MICROANGIOPATHY

A. Development of diabetic retinopathy (Figure 8):

1. The first measurable defect in diabetic retinopathy (143) is breakdown of the blood-retinal barrier in the absence of visible retinopathy. This barrier, formed by retinal capillary endothelial cells connected by tight junctions, prevents organic ions such as fluorescein from moving out of the vascular space. In poorly controlled diabetes fluorescein leaks into the vitreous and this leakage is reduced by normalization of glucose.

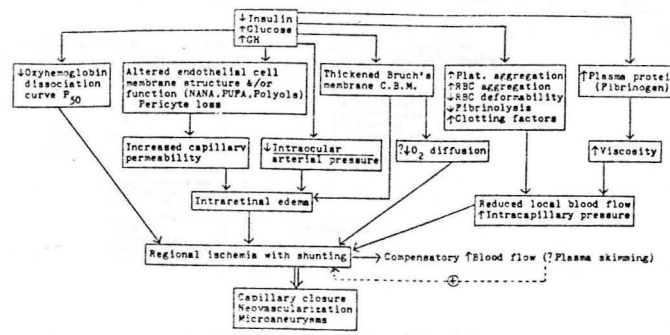

Figure 8: Model of diabetic retinopathy (21).

2. Next, regional ischemia, believed to be the cause of neovascularization, (68,104,105) develops. It may result from the hematologic abnormalities, reduced P_{50}, hyperviscosity and sludging secondary to changes in the red cell and increased fibrinogen and other plasma proteins.

3. This raises intracapillary pressure and reduces local blood flow, causing leakage of serum into tissues, aggregation of platelets and red cells and increased coagulability.

4. This favors capillary closure which causes soft exhudates, a preproliferative sign. Nearly all the hematologic abnormalities are returned to normal by adequate insulinization.

B. Development of Nephropathy (Figure 9):

1. At the onset of Type I diabetes glomerular filtration rate (GFR) and glomerular size increase due to an enlargement of both capillary lumina and glomerular cells (138-140). Roentgenographically kidney size increases (133) and renal plasma flow and albumin excretion are increased at the very onset of Type I diabetes without any increase in basement membrane thickness. Both GFR and kidney enlargement can be reduced towards normal by months of careful insulin treatment.

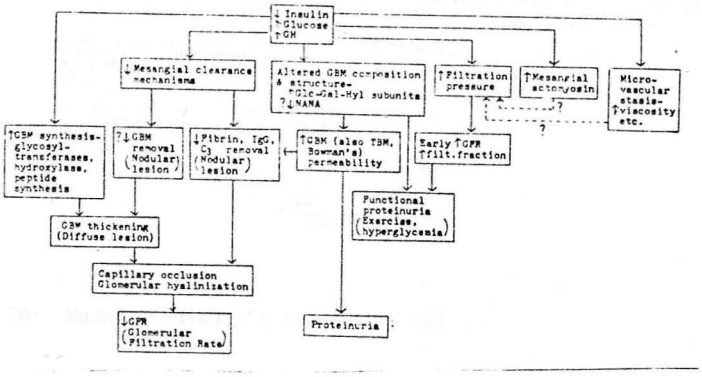

Figure 9: Model of diabetic nephropathy (21).

2. During poor glucoregulation urinary albumin and β_2-microglobulin excretion and whole body transcapillary escape of albumin are increased, due to increased filtration pressure and/or increased capillary permeability (146). Glomerular permeability to anionic proteins may be due to fixed negative charges in the glomerular capillary wall (12). Increase in actomyosin-like material in the mesangium, specific for diabetes (173) may play a role in regulating glomerular blood flow and ultrafiltration (41).

3. Later in the disease, albumin, IgG and other proteins accumulate in the glomerular basement membrane and capillary occlusion and glomerular hyalinization begin. In rats (but not in man), transplantation of an abnormal diabetic kidney into a normal recipient causes disappearance of IgG, IgM and C_3 from the mesangium and albuminuria arrests or reverses mesangial thickening (113), but glomerular basement thickening persists.

C. Development of neuropathy (Figure 10):

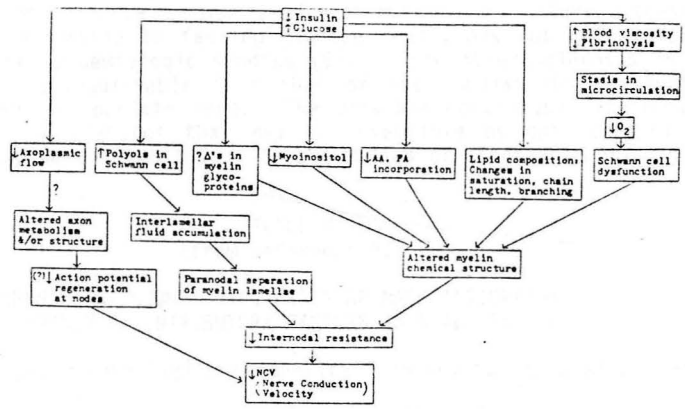

Figure 10: Model of diabetic neuropathy (21).

THE HYPERGLYCEMIA-MACROANGIOPATHY CONNECTION

Atherosclerosis in diabetics is no different than in nondiabetics. Although there is evidence of increased risk of coronary artery disease in diabetes, a relationship to fasting glucose levels has not been substantiated by recent epidemiologic studies (5). Since atherosclerosis in the diabetic is indistinguishable from that of the nondiabetic, no further discussion seems appropriate here. The possible contributory factors to atherosclerosis in diabetes that may be reversible by good control are listed at the bottom of Table V. The prevention of atherosclerosis in the diabetic would include all measures recommended in nondiabetics, plus optimal regulation of hyperglycemia. With good control there is an increase in HDL_2 and a decline in LDL cholesterol.

TABLE VI
(from reference 21)

"RESPONSE-TO-INJURY" HYPOTHESIS OF MACROANGIOPATHY:
POSSIBLE CONTRIBUTORY FACTORS IN DIABETES

I. Increased endothelial permeability ("injury" with focal desquamation
   - A. Altered cell membrane (fluidity, NANA, cholesterol)
   - B. Altered intracellular microskeleton (actin, myosin, tropomyosin
   - C. Hyperlipidemia
   - D. Hyperglycemia (polyols)
   - E. Hypertension
   - F. Vasoactive amines (bradykinin, histamine, serotonin)
   - G. Prostaglandins (?)
   - H. Growth hormone (?)

II. Exposure of subendothelium to platelets and plasma constituents

III. Focal proliferation of smooth muscle cells with connective-tissue formation and lipid deposition.

Possible abnormalities in D.M.:
- platelet membrane
- platelet PG synthetase
- platelet mitogens (SMC, fibroblasts)
- subendothelial MPS
- plasma lipoproteins: lipids, apolipoproteins, LCAT
- hormones (GH, other growth factors)
- number, binding properties of hormone, mitogen, LDL receptors

THE ANSWERS TO THE SIX QUESTIONS

Question 1: Is clinical microangiopathy dependent or independent of hyperglycemia (and/or concomitant abnormalities)?

Answer. Hyperglycemia is essential in the sense that clinical microangiopathy does not occur in its absence. Even if there is a genetic vulnerability for development of microangiopathy, hyperglycemia is a sine qua non; i.e., there is no evidence that even genetically predisposed persons can develop clinically manifest microangiopathy unless hyperglycemia occurs. Conversely, microangiopathy can develop in a presumably normal kidney transplanted into a hyperglycemic person.

Question 2: Are the current methods of management of diabetes satisfactory?

Answer: Obviously not, since clinical microangiopathy is common - even in so-called "well-controlled" patients.

Question 3: If not, why is current therapy not satisfactory?

Answer: It permits undetected chronic hyperglycemia throughout life because:

a) Methods for monitoring glucoregulation are unsatisfactory: Neither of the methods most commonly used to assess degree of control, i.e., occasional fasting glucose levels and home monitoring of urinary glucose, can distinguish between perfect control (round-the-clock normalization of glycemia) and fair or poor control. Fasting glucose levels cannot detect postprandial hyperglycemia, which may be present at least 30% of each day in the life of apparently "well controlled" patients (Figure 10). Qualitative home urines also may not reflect postprandial hyperglycemia and are of value only in signaling very bad control. Both tests are often normal in the face of serious daily hyperglycemia and elevated glycohemoglobins (see Figure 15). The physician who uses only these conventional yardsticks of control has no way of determining if his patient is chronically hyperglycemia (163,176,178). Only 4 of 22 diabetic patients thought to be in good or fair control had glycohemoglobin levels in the normal range (178) (Figure 11).

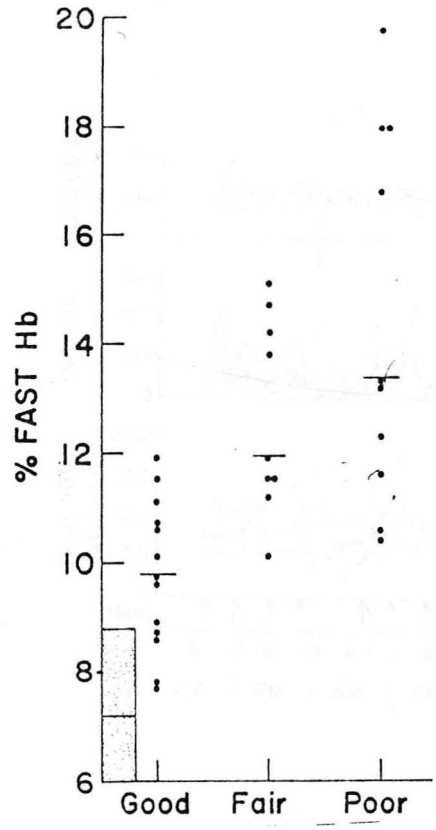

Figure 11: Distribution of glycohemoglobin results from 32 diabetics rated to be in good, fair and poor control compared with the nondiabetic range. Group medians are indicated by the horizontal lines (178).

b. The commonly used insulin regimes encourage both postprandial hyperglycemia and postabsorptive hypoglycemia: The most commonly used insulin regime in the U.S.A. consists of one or two daily injections of an intermediate acting insulin often with supplementary regular insulin. Intermediate acting insulins (NPH, lente) produce an extremely unphysiologic pattern of insulin release.

1) The normal physiologic pattern of insulin secretion: Figure 12 shows the physiologic pattern of endogenous insulin release between, before and after meals in normal subjects. Glucose levels are at all times maintained between 60-170 mg/dl through the appropriate

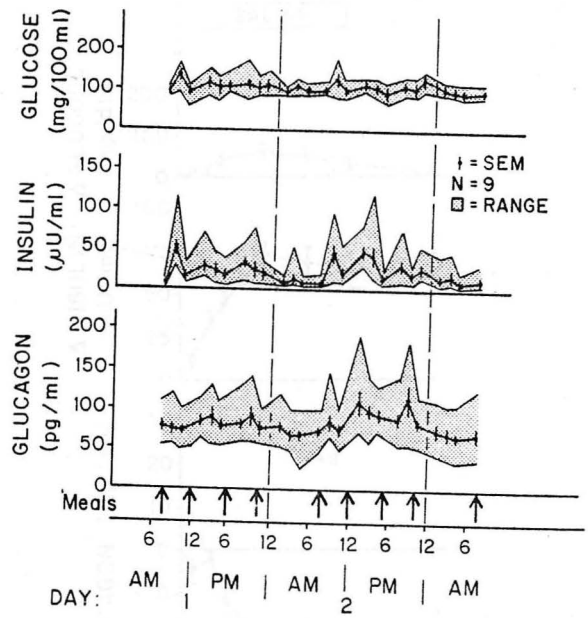

Figure 12: Around-the-clock plasma glucose, insulin and glucagon concentration in normal subjects (207).

interplay of insulin-glucagon secretion. Secretion of insulin consists of two distinct phases: 1) a basal or postabsorptive secretion at a slow constant rate which maintains an insulin level of about 15 μU/ml; its function is to restrain certain effects of glucagon-mediated catabolism, primarily to restrain production of free fatty acids and ketones and amino acids; and 2) a meal-time burst of insulin secretion which begins before the ingested glucose and other nutrients enter the circulation (Figure 13), thereby preventing postprandial hyperglycemia by converting the liver from a glucose-producing to a glucose-storing organ. The meal-induced burst of insulin secretion is a response to signals from the gut since it precedes the postprandial rise in glucose which should be very small if both the release of

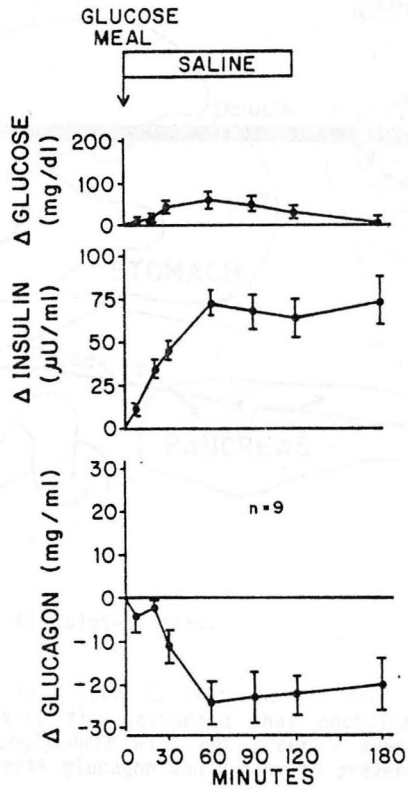

Figure 13: Plasma glucose, insulin and glucagon response to a glucose meal in normal subjects.

gut signals and the insulin response to them are adequate (Figure 14). If this enteroinsular system fails to prevent an undue postprandial rise in glucose levels, then hyperglycemia itself becomes a significant stimulus to insulin secretion, but this is a failsafe. Normally, glucose acts only to set the magnitude of the insulin response to the gut signals.

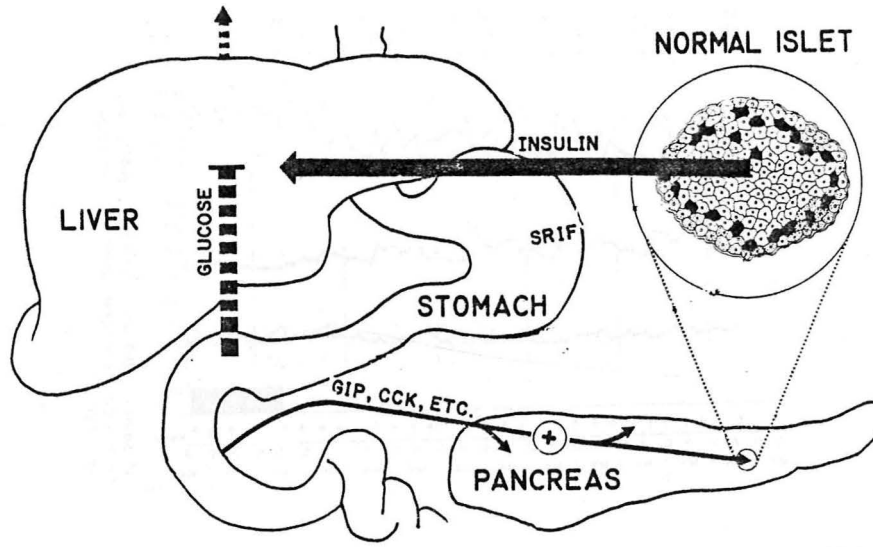

Figure 14: GI-islet-GI axis.

Note, too, in Figure 12 that insulin declines rapidly between meals, thus assuring that postcibal insulin-induced hypoglycemia will not occur - even though in normal subjects glucagon would rise to prevent it.

2) The unphysiologic pattern of insulin delivery in diabetics treated with intermediate acting insulins: The rise in insulin after lente or NPH insulin is too late to prevent postprandial hyperglycemia since only a rapid rise in insulin timed to precede the meal can do this. Unless the insulin level rises at the start of glucose absorption so as to prepare the liver to greet the glucose influx, postprandial hyperglycemia will occur. Figure 15 shows the occurrence of postprandial hyperglycemia despite fasting normoglycemia maintained with 2 and 3 daily injections of NPH supplemented by regular insulin. If higher doses of NPH insulin are used to reduce the hyperglycemia (Figure 16) the persistence of hyperinsulinemia long after meals would cause hypoglycemia if the patients performed muscular work. Nor will

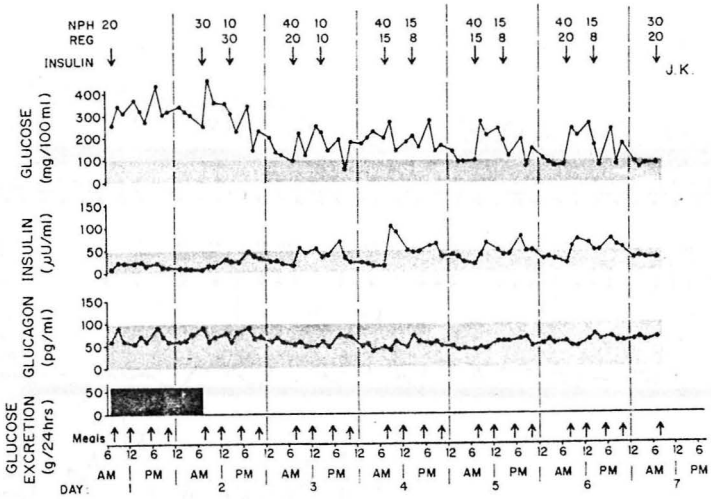

Figure 15: Around-the-clock plasma glucagon, insulin, glucose and glucose excretion in an adult onset diabetic during "optimal" control with insulin.

glucagon come to the rescue and prevent hypoglycemia because the A-cell response to glucose of the Type 1 diabetic is completely inoperative (66) While in some patients two injections of intermediate insulin mixed with regular may achieve a near-normal glucose profile in the confining environment of a General Clinical Research Center (GCRC), outside of the hospital physical activity causes hypoglycemic episodes and prompts a reduction in insulin to a dose which abolishes hypoglycemia but permits postprandial hyperglycemia. In most Type I patients round-the-clock profiles disclose hyperglycemia for >30% of each day despite two or three NPH-containing injections. This usually cannot be normalized by any NPH regime even in the optimal environment of the GCRC. Figure 17 shows the glucose, insulin and glucagon profiles of diabetics during "suboptimal" and "optimal" glucoregulation with NPH insulin.

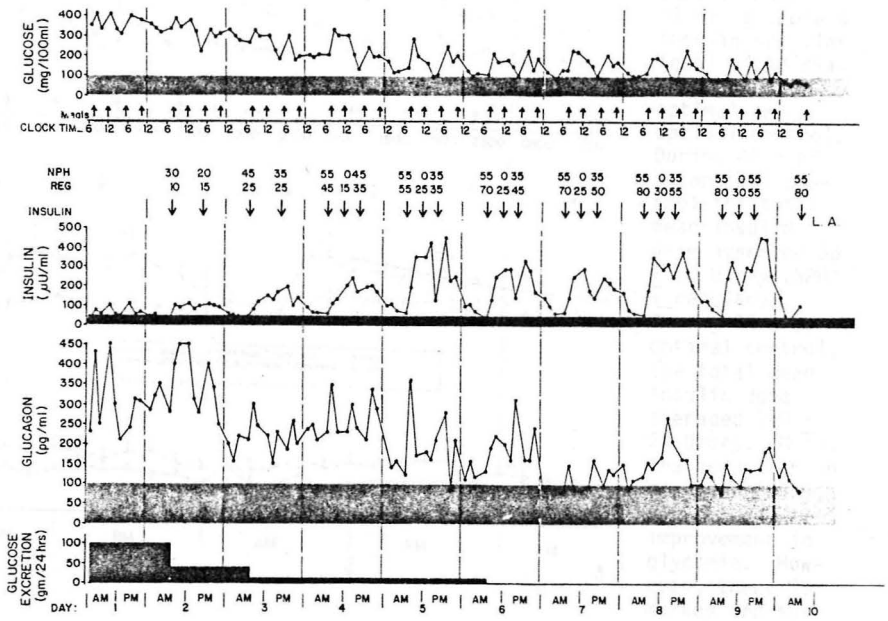

Figure 16:

NOTE: It is ironic and tragic that the very characteristics for which the intermediate insulins were developed in 1935, namely, slower absorption and longer duration intended to obviate the need for multiple injections, may in large part be responsible for the microangiopathy that seems to have been less common before 1935 (87) when multiple injections of regular insulin were the only available therapeutic option available.

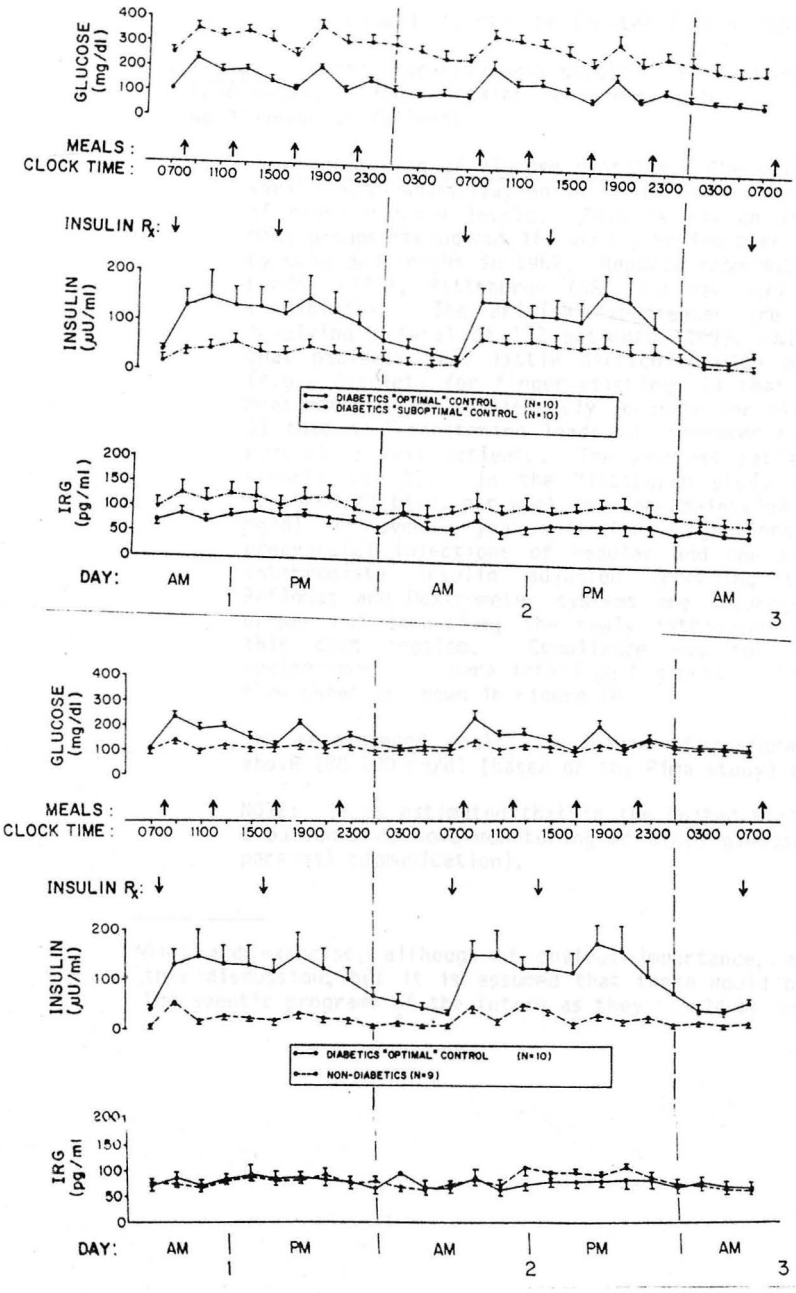

Figure 17, upper panel: Mean plasma glucose & insulin profiles (q2h) of 10 diabetics in suboptimal and optimal control. During 48 h of suboptimal control the total mean insulin dose averaged 56 ± 12 U/day (NPH ± regular). During 48 h of optimal control, the total mean insulin dose averaged 160 ± 24 U/day. Note the reduciton in glucagon (IRG), the marked improvement in glycemia. However, there is marked and sustained hyperinsulinemia between 0700 and 0100; this would almost certainly result in hypoglycemia outside of the physically confining GCRC environment.
Lower panel: The profiles of optimally cor. trolled diabetics are compared with a group of nondiabetics to show that despite marked hyperinsulinemia, significant hyperglycemia was present between 0900 and 2300 h, or over 50% of each day

Question 4: How should diabetes be treated in the 1980s?*

Answer: Chronic hyperglycemia should be eliminated or reduced and protein glycosylation maintained at a normal level. This can, in theory, be achieved as follows:

1. Home monitoring of glucose profiles: The only way to achieve and verify near-normalization of glycemia is through home-monitoring of blood glucose levels. This is now an accepted procedure in many groups throughout the world, having been introduced in London by Keen and Knight in 1962. Reports from Nottingham (213) and in London (180), Pittsburgh (38) and New York (151) indicate its feasibility. The British experiences are the most extensive involving a total of 133 patients (199). All groups agree: 1) that patients have little difficulty with painless new devices (e.g., Autolet) for finger-sticking; 2) that their blood glucose measurements are sufficiently accurate for clinical purposes; and 3) that self-monitoring leads to increased motivation and better control in most patients. The youngest patient in the published reports was 11. In the Pittsburgh study glucose levels were measured 23 times per week and were maintained between 60 and 150 mg/dl for over a year with four injections of insulin (three preprandial injections of regular and one bedtime injection of intermediate insulin adjusted according to glucose levels. Reflomat and Dextrometer systems are accurate if instruction is proper but expensive; the newly introduced Chemstrip may solve this cost problem. Compliance was not confined to higher socioeconomic or more intelligent groups. The Nottingham patient flow sheet is shown in Figure 18.

   The recommended goal: Avoidance of postprandial hyperglycemia above 180-200 mg/dl (based on the Pima study) and hypoglycemia.

   NOTE: It is estimated that in the United States only ~1% of Type I patients do home monitoring of blood glucose (Dr. J. Davidson, personal communication).

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*Diet and exercise, although of obvious importance, are not included in this discussion, but it is assumed that these would be incorporated into therapeutic programs of the future as they should be now.

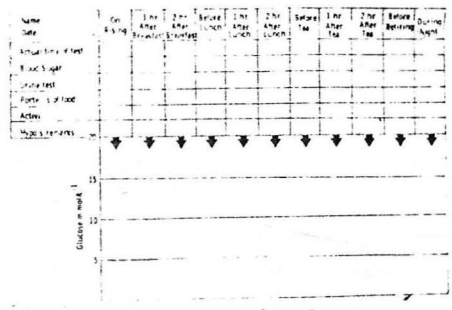

Figure 18: Patient flow sheet used in Nottingham (213).

2. Monitoring of protein glycosylation: The goal of near-normalization of glycemia is to prevent glycosylation of proteins because of the possibility that this is the link between hyperglycemia and clinical microangiopathy. (Someday it may be determined that constant glucose monitoring is unnecessary if glycosyl-protein levels are normal) In addition to glycohemoglobin, glycoalbumin and perhaps measurement of other glycoproteins with a more rapid turnover may be useful. Figure 19 shows the relationship between the "mean blood glucose" (0530 h, 0900 h, 1300 h, 1600 h and 2300 h for the previous 4-5 days) and the glycosyl-albumin, Figure 17 the relationship between glycosyl-albumin and HbA_{Ic}, and Figure 18 the more rapid decline in glycosyl-albumin by virtue of its more rapid turnover. Conceivably by obtaining glycosyl-protein determinations at 1-3 week intervals, glucose profiling can be reduced from a daily requirement to once or twice per week. As soon as a rise in glycosyl-protein occurs, or other evidence of inadequate control appears, full daily profiling would be resumed until near-normalization is reestablished and the glycosylated protein has returned to normal.

NOTE: It is estimated that only ~10% of Type I patients are now followed with glycohemoglobin determinations (Dr. J. Davidson, personal communication).

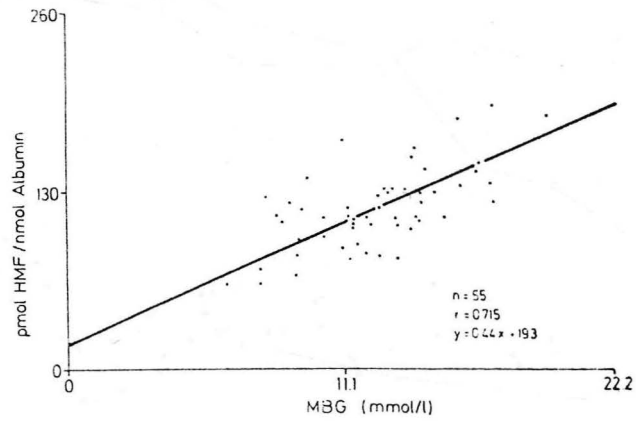

Figure 19: Glycosyl-albumin levels in diabetic subjects. MGB is the mean blood glucose level of the preceding 4 or 5 days (47).

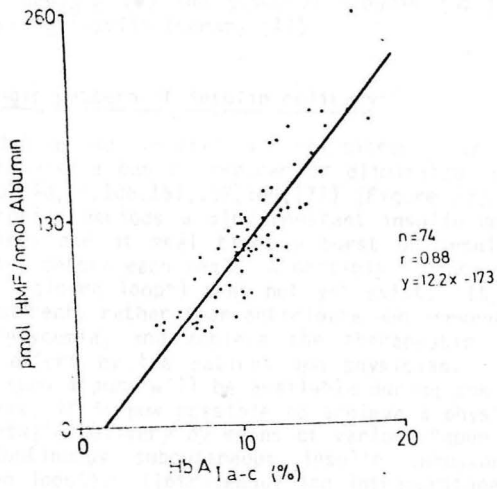

Figure 20: Correlation between the levels of HbA_{1a-c} and glycosyl albumin in 10 normal and 64 diabetic subjects (47).

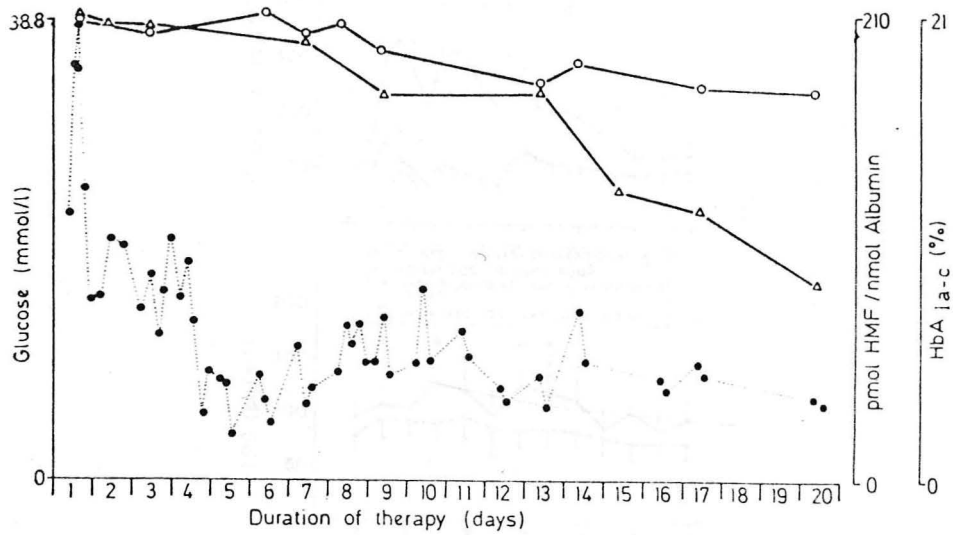

Figure 21: Temporal relationship between the levels of blood glucose (o), HbA_{1a-c} (•) and glycosyl albumin (Δ) in a diabetic patient during insulin therapy (47).

3. A physiologic pattern of insulin delivery:

   a. By delivering insulin in relationship to insulin need, hyperglycemia can be reduced or eliminated in most patients (30,32,40,78,106,151,152,166,177) (Figure 22). During post-absorptive periods a slow constant insulin delivery rate is required and at meal times a burst of insulin is required shortly before each meal. A portable glucose sensing insulin pump ("closed loop") does not yet exist. It would react to and correct, rather than anticipate and prevent, postprandial hyperglycemia, and achieve the therapeutic goals with far less effort by the patient and physician. It is unlikely that such a pump will be available during the 1980s. Nevertheless, it is now possible to achieve a physiologic pattern of insulin delivery by means of various "open loop" systems: 1) Continuous subcutaneous insulin infusion (CSII) pump ("open loop"). (Intravenous and intraperitoneal routes have been used but seem to have increased risks and little advantage.) 2) By multiple injections through the same indwelling catheter or scalp needle used in CSII systems (Paris method). 3) By multiple percutaneous injections - three or four regular insulin injections before meals and a night-time dose of an intermediate insulin. Most patients who have tried CSII prefer it to multiple injections. (See Appendix 1)

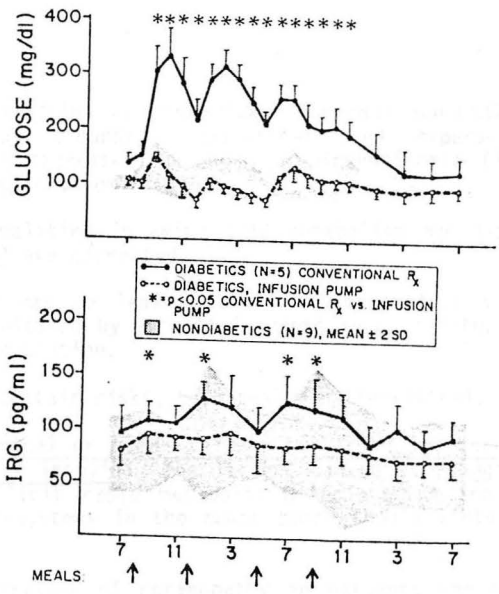

Figure 22: Mean (± SEM) plasma glucose and IRG profiles during 24 h of conventional insulin treatment (o____o) and after 4-5 wk of continuous subcutaneous insulin infusion (o---o) with portable insulin infusion pumps in five juvenile-onset diabetics. Asterisks indicate $p<0.05$ in the diabetics with insulin infusion versus conventional therapy. The shaded area represents the mean ± 2 SD of values from nine nondiabetics on a similar diet included for comparison. Times of meals are indicated by arrows (166).

Question 5: Will elimination of hyperglycemia prevent clinical microangiopathy or provide other benefits; will it impose any risks?

Answer:

a. There is no evidence that elimination of hyperglycemia will prevent clinical microangiopathy, but it is a reasonable expectation.

b. There are other real and expected benefits:

1. Enhanced well-being is striking in poorly controlled patients.

2. Less frequent hypoglycemia is observed because insulin levels decline after meals.

3. A positive psychologic impact (e.g., feeling of mastery over one's glycemia, the feeling of preventing microangiopathy, placebo effect of the pump) is often observed.

4. Normalization of concomitant hormonal abnormalities of poor control occurs: exercise-induced hypersomatotropinemia (196); exercise-induced hyperepinephrinemia (196); relative hyperglucagonemia (166).

5. Abnormalities in amino acid metabolism and lipid metabolism (HDL_2) are corrected.

6. There may be less metabolic vulnerability to ketoacidosis precipitated by stressful events such as infection, but this is speculation.

c. There are certain risks, both real and theoretical, with the pump:

1. Mechanical or human failure resulting in overdose or underdose of insulin: The latter, caused by plugging of inflow, can elicit rapid metabolic deterioration and ketoacidosis. Alarm systems in the newer pumps should virtually eliminate this.

2. Acceleration of retinopathy in patients who have had long-standing underinsulinization: Drash et al. (48) treated four children with long-standing underinsulinization, diabetic dwarfism (Mauriac's Syndrome) and minimal retinopathy on the pump. With good control of glycemia, linear growth resumed, but within 1-9 months there was rapid progression of retinopathy; microaneurysms, hemorrhages, exhudates, macular edema occurred in all four and severe proliferative changes in 3. Tamborlane et al. (197) reported vitreous hemorrhage in two previously poorly controlled patients with proliferative retinopathy 1 and 3 months after starting the pump. Karam and associates (unpublished comment at 1980 ADA Meeting) have had a similar experience. Considering that reversal of clinically severe microangiopathy involving eyes and kidneys is at best unlikely, severe retinopathy may be a relative contraindication for tight control. If undertaken, it should be done as clinical research and only under the most careful opthalmologic scrutiny and should not delay other routine interventions. In treating chronically under-insulinized patients with background retinopathy frequent careful opthalmologic exam should be routine during the first year of near-normalization. At the present time near-normalization of glycemia should be viewed as a preventive, rather than a treatment, of microvascular disease, and it should be employed primarily in otherwise healthy or only minimally microangiopathic diabetics for the purpose of maintaining their health.

b. Precipitation of regional hypoglycemia in patients with cerebrovascular disease: Moderate hyperglycemia may be important to maintain adequate glucose delivery to hypoperfused regions of the brain. Normalization of glycemia in patients with significant

cerebrovascular disease may, therefore, result in regional hypoglycemia to an area with reduced blood flow. Patients with proven or suspected macrovascular disease are not good candidates for this therapy. Moreover, their life expectancy is probably too short to make them candidates.

Note: Patients have any health problem that would limit their life expectancy to <7 years would obviously not be expected to benefit from a program intended to prevent slowly developing microangiopathy which may take one or more decades to develop.

Question 6: If there is no clearcut proof that normalization of glycemia can prevent clinical microangiopathy, is it acceptable to continue current methods of therapy pending scientific proof of superiority of the new therapy?

Answer: No. The clinician is now obligated to make available to candidate patients the extremely demanding and difficult program of near-normalization of glycemia if no absolute or relative contraindication to such management exists. The reasons for this obligation are as follows:

1. The excess morbidity and mortality resulting from diabetic microangiopathy is not known to occur in the absence of hyperglycemia and may be linked clinically, and perhaps chemically, to chronic exposure of proteins to abnormally high levels of glucose.

2. Present methods of managing diabetes are associated with excess morbidity, mortality and health care costs, and they fail to reliably detect or prevent chronic hyperglycemia.

   (Methods for reliable detection of chronic hyperglycemia and excessive levels of glycosylated proteins, though available, are rarely employed; methods for correcting chronic hyperglycemia are currently available in certain centers but are rarely employed.)

3. Lack of scientific data concerning the prophylactic value of near-normalization of glycemia (superior to clinical data now available) cannot be used as a basis for withholding the chance for better control from a patient. Such data will require at least another 10 years, if indeed they are ever obtained. Consider the following points:

   a. Clinical trials for prevention of retinopathy would require a multi-center study of at least 200 diabetics of 0-2 years duration and <22 years of age without retinopathy randomly assigned to a "conventional treatment" group and a "near-normalization" treatment group and followed for 6-9 years (57). The estimated cost is >$60 million. The probability that such a study could be consummated and yield conclusive answers seems far less than the probability that elimination of hyperglycemia is beneficial!

b. Unless the conventionally treated group were maintained in a more hyperglycemic and hyperglycosylated state throughout the decade of study than the "near-normalized" group, failure to find a difference in microangiopathy would be meaningless. But, to maintain the conventionally treated group in poor control for a decade is now considered unethical because it might expose them to the risk of clinical microangiopathy! If it is unethical in research, it must also be unethical in clinical practice.

c. A new round of government-supported clinical trials would tend to perpetuate poor control in clinical practice by implying to clinicians that near-normalization of glycemia is not yet obligatory and that current methods of treatment can still be condoned, thus exposing another generation of diabetics to risk of microangiopathy.

d. There is no burden of proof required to justify near-normalization of glycemia as the standard of management of diabetes in the 1980s for the following reasons:

   1) Insulin is not a new drug but is a naturally secreted substance that has been administered to humans for over half a century.

   2) Programs of near-normalization merely substitute a physiologic pattern of insulin delivery designed to normalize glycemia and glycosylated proteins for an unphysiologic pattern of insulin delivery which permits hyperglycemia and hyperglycosylation of proteins and fails to prevent microangiopathy.

   3) It is probably safe to assume that any environmental change, any deviation from the environment in which evolution of the species transpired, be it cigarette smoke, alcohol, pollutants of atmosphere or water, etc. imposes certain risks to which certain tissues may be vulnerable, and such vulnerability may be expressed as disease. It follows that elimination of the environmental deviation will reduce the risk and prevent the disease. For example, if nonsmokers never get lung cancer there is no burden of proof nor need for clinical trials before recommending elimination of tobacco smoke as a preventative of lung cancer. The chronic hyperglycemia of Type I diabetes constitutes a man-made modification of the internal environment resulting from the discovery of insulin, prior to which such patients did not survive. By this formulation elimination of chronic hyperglycemia would require no further clinical trials. (See Appendix 2 for other examples of man-made pathogenic environmental modifications). Rather, the burden

of proof is to show that it is safe to permit levels of glucose known to alter vital proteins. The evidence strongly suggests that it is not safe.*

It would seem more rational to spend funds, not on clinical trials, but on simplifying methods of near-normalization of glycemia and making them available to the mass of diabetics in the hope that the diabetics of the 1980s will not suffer the same fate as those of the 1935-1970 period.

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*There are only 20 amino acids specified in the genetic code yet some 140 "amino acids", posttranslational derivatives, of course, are found in various proteins. Control of derivitization, at least for some reactions such as glycosylation, may require strict control of the concentration of the modifier (208).

THE CONCLUSIONS

1. There is now an obligation to offer the option of near-normalization of glycemia to all eligible otherwise healthy Type I diabetics without relative or absolute medical contraindication in the hope that it will improve their prognosis - or at least generate interpretable information concerning its efficacy.

2. This obligation cannot be postponed on the grounds that no proof of prophylactic efficacy of this treatment regime in the form of controlled clinical trials is available; for reasons given such trials seem unjustified and would only delay widespread application of improved treatment technics.

THE RECOMMENDATIONS

1. A broad national plan should be instituted to make these new treatment procedures available to all clinically eligible diabetics. This would require public and private support for:

   a. Simplification of monitoring methods and insulin delivery systems required to prevent hyperglycemic overglycosylation of proteins.

   b. Development of specialized private and public centers for hyperglycemic control centers to which such patients could be referred by their physicians for a program combining intensive education and vigorous life-long day-to-day surveillance of glycemic control, protein modification and development of complications. (Most private physicians will be unable to offer such services to their patients because they lack the time, facilities and experience which this demanding program requires; indeed, specialized personnel in designated centers would be necessary for successful implementation and evaluation of such a program.)

   c. Longitudinal collection of clinical data required to prove whether or not a decade of intense effort eliminated or reduced the expected incidence of complications.

If this effort is successful, an important item on the list of irreversible biological catastrophies induced by environmental perturbation (see Appendix) will have been prevented. The poetic portrait by Matthew Arnold of the physician as a helpless namer of incurable illness will no longer be applicable to the diabetologist, who at last will have become a preventor of the "ill he cannot (yet) cure".

"Nor bring to see me cease to live
Some doctor full of phrase and fame
To shake his sapient head and give
The ill he cannot cure, a name."

Matthew Arnold

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Thanks is due to Mrs. Susan Kennedy for the preparation of this protocol and to Drs. Anthony Cerami and Charles Petersen and their colleagues at Rockefeller University for providing much of the reference material, some of it unpublished. Attention is called to the review of this subject by Brownlee and Cahill (21), which is the best of its kind and which served both as a reference source and as a framework for this protocol.

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APPENDIX 1

POLL OF THE 13 DALLAS PATIENTS ABOUT INSULIN PUMP TREATMENT

QUESTIONS:	ANSWERS:
1) Do you prefer the pump to the conventional treatment that you had before?	
Yes	13
No	0
2) If yes, please indicate why (check one or more).	
a) I believe it will prevent diabetic complications.	10
b) I am better controlled now.	13
c) I feel healthier.	12
d) There are fewer injections.	5
e) It makes me feel more confident about the delivery of the needed insulin.	9
f) It's exciting to be part of a new treatment program.	8
3) If you checked a), would you give up the pump if it were clearly established that it does not protect against diabetic complications as compared with the treatment you had before?	
Yes	1
No	12
4) If you could get just as good control with multiple injections of insulin (3-4/day), would you prefer to be on the pump?	
Yes	9
No	4
5) Do you find home monitoring of your blood sugar:	
Painful?	
Yes	5
No	8
Tedious?	
Yes	7
No	4
Helpful?	
Yes	13
No	0

7) Do you think you can continue this regimen of careful monitoring of blood glucose and delivering insulin in the optimal manner (either by pump, multiple injections, or multiple injections via an indwelling catheter) throughout your life?

Yes	13
No	0

8) Do you think that the average juvenile-type diabetic can do so?

Yes	11
No	2

APPENDIX 2

A VIEW OF DISEASE BASED ON CONCEPTS OF DARWIN, WALLACE, AND BERNARD

If the survival and health of a species reflects an evolutionary adaptation to a given external environment, it follows that any environmental change which constitutes a deviation from the evolutionary track of the species may create difficulties in adaptation. Natural changes in environment have been occurring through the ages and often exact a heavy toll, but since ~38,000 B.C. man himself developed the capacity to change both his external environment and his internal microenvironment, thus generating new diseases. It is not unlikely that in those areas of the world in which this capacity is most highly developed, the principal causes of disease and death are man-made and, therefore, preventable. The weight of the evidence presented suggests that diabetic vasculopathy falls into this category.

CHRONOLOGY OF MAN-MADE PATHOGENIC MODIFICATIONS OF ENVIRONMENTS

Date	Event	Change	Disease or Risk
38,000 B.C.	Hunting perfected Meat cooked and preserved	Increased consumption of animal fat	Atherosclerosis
10,000 B.C.	Domestication of goats and later sheep and swine	Consumption of dairy products and further increase in meat consumption	Atherosclerosis
4000 B.C.	Fermentation of grapes	Alcohol ingestion	Cirrhosis Pancreatitis Gastritis Alcoholism CNS disorders etc.
2700 B.C.	Herbal medicine begins; later Nei Ching introduces the first pharmacopoeia (opium, ephedrine, $NaSO_4$)	Drugs are prescribed	Drug-induced diseases of all types

1233 A.D.	Coal mining begins in Newcastle	Use of coal as a fuel leads to the industrial revolution centuries later, then to urbanization and use of other fossil fuels culminating in pollution of the atmosphere, water & soil by industrial by-products	Silicosis and other occupational diseases of miners diseases of general population caused by pollution of air, water and soil
1531 A.D.	Tobacco, discovered earlier by Columbus, is cultivated on a commercial scale by Spanish colonists	Smoking begins	Bronchogenic and other carcinomas; bronchopulmonary disease, higher risk of ASHD
1898 A.D.	Radium is isolated by the Curies	Nuclear age begins	Neoplasia Mutations Nuclear holocausts
1920 A.D.	Increasing inversion of the relationship of come to physical work creates a sedentary lifestyle; cheap easily accessible dispensing of food becomes widely available in U.S.A.	Decline in the caloric cost of food, i.e., the physical work/food ratio become inversely related	Striking change in body composition(obesity) Increased atherosclerotic heart disease diabetes (Type II)
1923 A.D.	Insulin made commercially available	For the first time Type I diabetics now can survive, but in chronically hyperglycemic microenvironment	Glycosylation of proteins exposed to hyperglycemia; diabetic vasculopathy