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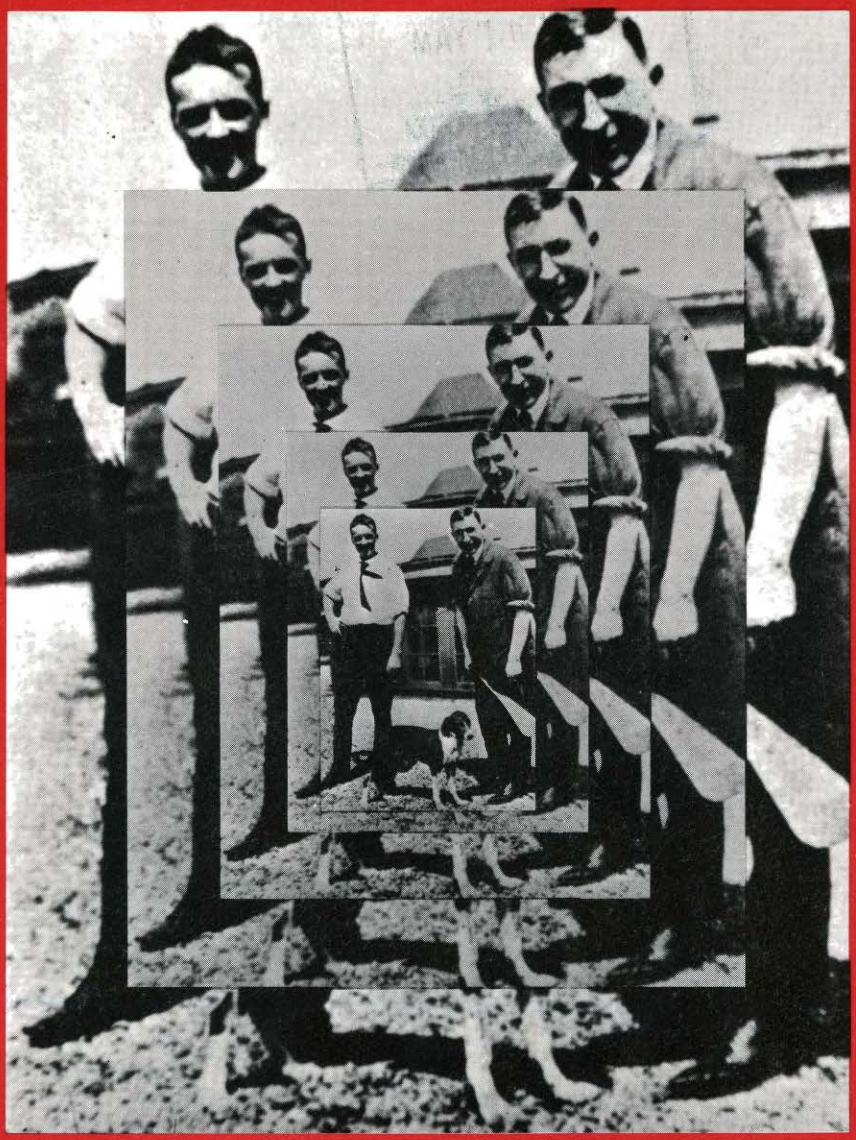

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CONTENTS

Symposium on Diabetes
H. ST. GEORGE TUCKER, M.D., *Guest Editor*

Sponsored by the School of Medicine, Division of Endocrinology and Metabolism and the Department of Continuing Education, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University.

COVER: Photograph of Charles H. Best (left) and Frederick G. Banting on the roof of the Medical Building of the University of Toronto with the first dog which they made diabetic, then cured with insulin. (Photo 1921.)

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INTRODUCTION

A Symposium on Diabetes was presented at the Medical College of Virginia on September 23, 1976, sponsored jointly by the Division of Endocrinology and Metabolism of the Department of Medicine, and by the Department of Continuing Education. The symposium was also sponsored in part by a grant-in-aid for continuing medical education from the Upjohn Company.

The morning portion of the program presented summaries of some of the current important research in the field of diabetes, while the afternoon program was devoted to practical aspects of the treatment of diabetic patients. The emphasis was on the importance of achieving as good chemical control as possible, although some of the practical difficulties in accompanying such control are explained. We are grateful to the members of the MCV faculty and the guest faculty who presented these papers, and to our co-sponsors who made this program possible.

H. ST. GEORGE TUCKER, M.D.

Professor
Department of Endocrinology and Metabolism

Somatostatin: Diverse Physiological Roles and Therapeutic Implications*

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In its brief lifetime as a known peptide, somatostatin has provided a truly remarkable story filled with surprising developments from unexpected quarters. The foundation was set in 1969, when Krulich and McCann[1] reported that fractions of a crude hypothalamic extract inhibited the secretion of growth hormone. In 1973, Guillemin's laboratory reported the sequence and synthesis of a fourteen amino acid peptide with the same inhibitory effect.[2,3] It occurs in both a cyclic and linear form, each displaying equal biological activity. Somatostatin was assigned as its name, but it is also commonly referred to as growth hormone release inhibitory factor (GHRIF) or somatotropin-release inhibiting factor (SRIF).

Characterization of somatostatin's biological activity proceeded rapidly once the synthetic peptide became available. It proved to be a potent inhibitor of thyroid stimulating hormone (TSH).[4] No effect was found upon prolactin or adrenocorticotropic hormone (ACTH) secretion except in the pathological situations of acromegaly[5] and Nelson's syndrome,[6] where inhibition has been described. The secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) is not inhibited by somatostatin.[7] There was great surprise when investigators in Seattle, working with baboons, found that somatostatin inhibited the secretion of two pancreatic hormones, insulin and glucagon,[8] and, as studies expanded to other organs, the inhibition of gastrin,[9] secretin,[10] gastric acid and pepsin secretion,[9] and secretin-stimulated exocrine pancreatic secretion[10] were described. Very little is known about the molecular mechanisms of somatostatin effects, but clues are being provided by the findings that inhibition of insulin secretion can be partially reversed by high calcium concentration[11] and by alpha adrenergic blockade with phentolamine.[12] Furthermore, somatostatin appears to interfere with calcium uptake by islets[13] and may inhibit tissue cyclic adenosine monophosphate (AMP) accumulation.[14] Both events are thought to be important in the secretory process.

Diabetes researchers became interested when it was shown that somatostatin infusions caused a fall of blood glucose in both normal subjects and diabetics.[15] In addition, somatostatin markedly slowed the development of ketoacidosis in insulin-deprived juvenile-type diabetics,[16] even though it did little to reverse established ketoacidosis.[17] Until then there was no convincing evidence that glucagon was anything

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more than a vestigial hormone, only continual debates about the theoretical relative contributions of insulin and glucagon to metabolic processes.

The widespread effects of insulin upon hepatic and peripheral tissues are well known, but glucagon's effects appear to be more limited. Although pharmacological amounts of glucagon can influence many organs, physiological concentrations act primarily on the liver, and probably have minor lipolytic effects on adipose tissue. Hepatic glycogenolysis and gluconeogenesis can both be stimulated by glucagon, thus leading to enhanced hepatic glucose output. It is also now clear that glucagon has a direct stimulatory effect upon hepatic ketogenesis.[18] The issue, however, is whether these effects are trivial and easily nullified by small amounts of insulin or whether they are physiologically important. Somatostatin has become a useful pharmacological tool to help answer these questions, because in addition to being able to block insulin and glucagon secretion, it has no known direct effect upon hepatic glucose output or peripheral glucose utilization. Several laboratories have now shown that the fall of blood glucose during a somatostatin infusion is secondary to a reduction of hepatic glucose output. The interpretation has therefore been made that inhibition of glucagon in these short-term experiments was more important than the inhibition of insulin. A similar fall in glucose was found in a diabetic with hypophysectomy, indicating that growth hormone inhibition did not have much influence.[15]

These unexpected findings made it difficult to understand why patients with pancreatectomies were hyperglycemic. According to the results of the somatostatin experiments, the removal of both insulin and glucagon surgically should have led to hypoglycemia. One finding used to reconcile this puzzle was that insulin-deprived pancreatectomized dogs have abundant amounts of extra-pancreatic glucagon which is known to be primarily of gastric origin.[19] Humans, however, appear to have very little gastric glucagon.[20] The best explanation for the paradox appears to be that glucagon's effects are only transient; thus, a glucagon infusion leads to an increase of hepatic glucose output which lasts for about an hour.[21] Similarly, if glucagon is "removed" with somatostatin, there is a transient reduction in hepatic glucose output.[22] Therefore, during chronic somatostatin infusions, a fall of blood glucose is seen before hyperglycemia, which appears as insulin deficiency dominates.[22] These experiments and others have led some to say that glucagon's influence on blood glucose homeostasis is minor and that the hyperglycemia of diabetes is almost entirely secondary to insulin deficiency. The acceptance of a dominant role for insulin seems inescapable, but glucagon may still have important effects. Daily physiological events bear little relation to infusions of glucagon or somatostatin; glucagon concentrations do not remain constant during the day but fluctuate considerably, particularly with meals. Thus, if each one of these fluctuations led to a transient effect on blood glucose lasting for even 45 mintues, it is clear that glucagon's overall influence throughout the day could be substantial. Furthermore, there is abundant evidence that glucagon secretion is increased in diabetes.[23] To answer some of these critical questions, workers have given glucagon infusions and injections, trying to stay within the concentrations thought to be physiological.[24-26] There is agreement that glucagon has hyperglycemic effects when unopposed by insulin but disagreement that it causes deterioration of control during insulin therapy in diabetics. In addition, there is growing acceptance that endogenous insulin secretion in normal subjects can effectively counter the effects of artificially-raised plasma glucagon concentrations. Therefore, despite large amounts of recent work, there remains much disagreement about glucagon's importance in diabetes.

This rather elaborate background is necessary to understand somatostatin's possible usefulness as a therapeutic agent. Its ability to lower blood glucose is presumed to be mediated primarily via glucagon inhibition. Therefore the question of glucagon's contribution to the hyperglycemia of diabetes is of utmost importance. During debates about somatostatin's potential in diabetes a commonly raised question is, "Why worry about somatostatin? Why not give more insulin?" Somatostatin's advocates argue that following each meal there is a glucagon rise which drives blood glucose to unacceptable postprandial peaks even in so-called well-controlled diabetics. Aborting these glucagon rises with somatostatin would therefore minimize glucose excursions in a way unachievable with conventional insulin therapy. New data adding more confusion to these concepts is the finding that somatostatin can impair carbohydrate absorption by the gastrointestinal tract and may lower postprandial glucose by a mechanism independent of glucagon.[27]

As indicated above, much of the controversy about somatostatin centers on its possible effects

upon glucose control in diabetes. Even though data showing a connection between blood glucose and complications is not conclusive, there is enough circumstantial evidence to warrant the pursuit of optimal control. Another factor linked to complications is growth hormone. Even though large, well-controlled studies are still not available, the weight of evidence suggests that pituitary ablation has a beneficial effect upon diabetic retinopathy. Possibly important as well is the observation that plasma growth hormone levels are increased in diabetics, when control is poor[28] or during exercise.[29,30] Somatostatin therefore may possibly benefit diabetics through two mechanisms—control of blood glucose and suppression of growth hormone secretion.

Obviously much more work is needed before somatostatin's ultimate value as a therapeutic tool can be assessed and there are many hurdles which need to be overcome. Long-acting preparations must be developed, as injections before each meal are obviously impractical. A protamine-zinc somatostatin preparation has been found to increase the duration of action in rats[31] but has been unsuccessful in primates. There were early fears that inhibitory effects upon platelet function would lead to serious bleeding problems, but recent thorough studies have dispelled some of these anxieties.[32] Even though chronic suppression of growth hormone might help some diabetics, growth retardation in children would not be acceptable. Furthermore, it is possible that somatostatin's effects on the gastrointestinal tract will produce discomfort and maldigestion leading to nutritional deficiency. Efforts are underway to develop somatostatin analogues which will selectively suppress glucagon secretion and, although selectivity has not yet been accomplished, a D-tryptophan^8-somatostatin analogue has been synthesized which is eight times more potent than somatostatin in inhibiting growth hormone, insulin, and glucagon secretion.[33] In addition to its potential usefulness in diabetes, somatostatin may be of some benefit in the treatment of acromegaly,[5] peptic ulcer disease, and metastatic insulinomas and glucogonomas.

Another area of research has focused upon the localization of somatostatin. Immunohistochemical techniques, in particular immunofluorescence, have been very useful for accurate localization, and radioimmunoassay has permitted quantitation of somatostatin immunoreactivity. The major obstacle in the development of radioimmunoassays was the problem of labeling somatostatin. Labeling with ^{125}I of the tyrosine of the synthetic analogue l-tyrosinated somatostatin provided the breakthrough and several very specific, highly sensitive assay systems have become available through the research of Arimura et al[34] and by Y.C. Patel, MD and S. Reichlin, MD (unpublished data, 1975).

These techniques led to an unexpected and important discovery in late 1974 when Dubois in France, using immunofluorescence, found somatostatin immunoreactivity in discrete cells of the islets of Langerhans in several species.[35] This finding was rapidly confirmed in isolated islets with a specific radioimmunoassay.[36] Somatostatin was also found scattered throughout the gastrointestinal tract with unusually large amounts in the gastric antrum,[37] and in addition was found in numerous extrahypothalamic areas of the central nervous system,[36,38] with especially large concentrations in the septum and preoptic area, the thalamus, and the cortex. The highest concentration of somatostatin in the hypothalamus has been localized to the median eminence and the arcuate nucleus.[38] Definitive proof that extrahypothalamic material with somatostatin immunoreactivity is identical to hypothalamic somatostatin awaits amino acid sequence determination. The material does appear to have biological activity, however, as extracts of frog pancreas with a high content of somatostatin immunoreactivity have been found to inhibit growth hormone secretion.[39]

Immunofluorescent techniques were also used to show that the cells containing somatostatin immunoreactivity in islets and gut were of the D-type.[40] D cells comprise about 5% to 10% of the mammalian islet cell population and tend to occur in close proximity to glucagon-containing A cells. In the mouse and rat, for instance, A and D cells form a rim of tissue around a central core of B cells.[41] In the horse, however, there is a central mass of A and D cells with peripheral cells being B cells, and in man A and D cells occur together next to capillary walls. The physiological importance of this distribution is as yet unknown, but it seems likely that there is some unique interaction between A and D cells.

An exception to the above pattern is found in birds which have a population of islets, called light islets, with D cells and B cells, but almost no A cells.[42] There are also, however, dark islets containing both A and D cells and few B cells. Birds are also unique because their pancreases contain high concentrations of glucagon and somatostatin compared to mam-

mals,[43,44] but the significance of this can only be speculated upon.

It was assumed by many that normal islet D cells contained gastrin, largely because the cells of gastrin-containing tumors of the Zollinger-Ellison syndrome have D-cell-like morphology. Some workers have found gastrin immunoreactivity in islet tissue,[45] but a recent thorough study has been unable to confirm this.[46] Thus, the origin of the gastrin-containing cells of the Zollinger-Ellison syndrome remains undiscovered.

There has been discussion about whether somatostatin in extrahypothalamic cells is synthesized in situ or taken up following secretion by the hypothalamus. The latter possibility seemed less likely because of somatostatin's instability in blood and the finding that the concentration of somatostatin in islets is comparable to that found in the hypothalamus. Recent studies in the angler fish indicate that somatostatin is synthesized in islets. The angler fish is unique as it has enormous (100 to 200 mg) islets which can easily be dissected free and contain no pancreatic exocrine tissue. Immunohistochemical studies have shown that approximately 30% of the cells contain somatostatin, 20% contain glucagon, and 35% contain insulin. Islets were incubated with the labeled amino acids ^{3}H-tryptophan and ^{35}S-cystine, and islet proteins were separated with column chromatography (P-10) and polyacrylamide gel electrophoresis (pH 9.5). Clear peaks of radioactivity were found in fractions containing the largest quantity of somatostatin immunoreactivity.[47] Synthetic cyclic somatostatin migrated into the same fractions. Thus there is now good evidence that somatostatin is synthesized in situ in islet tissue and there is also preliminary evidence that there is a larger precursor form of somatostatin (prosomatostatin). Precursor forms have been described for a number of other peptides including insulin and glucagon.

There is currently great interest in defining the characteristics of the secretory control of somatostatin as this should help solve some of the puzzles about its physiological role. Data is fragmentary, but studies by G. Patton and co-workers[48] and E. Samols and co-workers[49,50] in the isolated perfused canine and rat pancreas now show that somatostatin secretion can be stimulated by glucagon and arginine. The present interpretation is that glucagon probably has a direct influence upon the D cell, and that the arginine effect may be indirect, acting via local secretion of glucagon by the A cell.

Somatostatin's contribution to the pathophysiology of diabetes has yet to be elucidated, but some provocative information is available. Following the induction of diabetes in rats with the B-cell toxin, streptozotocin, a significant increase of islet somatostatin content was found using radioimmunoassay techniques.[51] This author has observed (unpublished data, 1976) that increased somatostatin in extracts of whole rat pancreas has been found with streptozotocin, as well as with alloxan, a different B-cell toxin. With immunofluorescent techniques it has been possible to show both hypertrophy and hyperplasia of islet D cells not only in streptozotocin diabetic rats but also in tissue from two juvenile-type human diabetics.[52] These findings may be a secondary phenomenon related to the hypersecretion of glucagon, or perhaps the hyposecretion of insulin known to occur in diabetes, or to some other factor. The critical question is whether there is increased islet somatostatin secretion leading to reduced insulin secretion. Also, even though diabetics secrete excessive amounts of glucagon which contribute to hyperglycemia, they might secrete even more glucagon were it not for D-cell secretion of somatostatin. Therefore, the D-cell hypertrophy could have either a beneficial or detrimental influence upon diabetes.

An understanding of somatostatin's physiologic importance may help us make sense of what is occurring in disease states. There is good reason to think that somatostatin's effects are exerted locally. It seems no accident that cells containing somatostatin are located in close proximity to cells capable of responding to somatostatin. Because of this proximity and because of somatostatin's instability in blood, it would seem inefficient for this peptide to be transported through the circulation to distant organs. Unfortunately, it has not yet been possible to measure physiological levels of somatostatin in plasma. Local secretory mechanisms are probably important in many organs, but islets are particularly intriguing. There is now good evidence that local secretion by autonomic nerve terminals of norepinephrine and acetylcholine can influence both glucagon and insulin secretion.[53] Some data suggest that local insulin secretion suppresses glucagon release from A cells[54] and there is now reason to suspect that glucagon may stimulate insulin release,[55] that somatostatin may inhibit the secretion of insulin and glucagon, and that glucagon may stimulate somatostatin secretion. The potential interactions which take place in islet interstitial spaces may be extraordinarily complex and

difficult to clarify. Another possible way in which different islet cells could communicate is through gap junctions. Small molecules have been shown to move from the cytoplasm of one cell into the cytoplasm of another through these structures. Gap junctions have been described as occurring between B cells, and also between A and B cells,[56] but thorough studies of possible D-cell gap junctions are not yet available. Suggestions have also been made that there may be important electrical interactions between islet cells.

Even though one can make many speculations about the possible importance of somatostatin in islets and the gastrointestinal tract, no definitive effects have been elucidated. There is, however, evidence that somatostatin exerts a physiological influence on the pituitary. Injections of somatostatin antiserum into rats led to increases in growth hormone and TSH secretion, presumably by neutralizing somatostatin in the hypothalamic-hypophyseal portal circulation.[57,58] The finding that somatostatin is distributed throughout extrahypothalamic areas of the central nervous system raises important questions about somatostatin's possible role as a neurotransmitter. There is also much curiosity about the evolution and embryology of somatostatin, particularly since its presence is found in such diverse areas as the central nervous system, pancreatic islets, and gut. It is worth noting that another peptide, substance P, has recently been found in various parts of the brain including the hypothalamus, and the gut.[7]

It was predictable that a "somatostatinoma" would eventually be found, and O. P. Ganda and associates[59] indicate that it has been. A 46-year-old woman with an eight-year history of well-documented diabetes mellitus was found to have a pancreatic islet cell tumor during a cholecystectomy. Clinically there was no evidence of its being an insulinoma, glucagonoma, or a tumor of either the Zollinger-Ellison or pancreatic cholera (Verner-Morrison) type. Ultrastructurally the majority of the cells appeared to be of the D-type. The somatostatin content of the tumor was remarkably high (301 ng/mg of tissue), and the content of insulin, glucagon, gastrin, and vasoactive intestinal peptide was negligible.

Pathologically the tumor was judged to be a low-grade malignancy, and metastatic tissue was found in one of the 38 resected lymph nodes. Remarkably, following surgical removal of the entire tumor, glucose values returned to normal and have remained so for 16 months (a recent fasting blood sugar was 73 mg/100ml and two-hour postprandial was 70 mg/100ml). We hypothesized that the patient was diabetic because of the continued secretion of large amounts of somatostatin by her tumor. As mentioned above, experimental infusions of somatostatin in normal subjects produce a fall of blood glucose; this drop, however, is only transitory, lasting for a few hours; with a longer infusion, hyperglycemia develops. Studies of insulin, glucagon, and growth hormone secretion in response to an arginine infusion were done prior to surgery and the response of each hormone appeared to be diminished, as one might expect with chronic somatostatin exposure. Unfortunately, it was not possible to repeat the study following surgery. When a patient is found to have an apparently non-functioning pancreatic tumor of the islet cell type, especially if diabetes is also present, the possibility of a somatostatinoma should be considered.

Somatostatin has proved to be infinitely more interesting than anyone might have imagined at the time of its discovery in 1973. Current interest in this unique peptide is focused upon its potential role in the pathophysiology and therapy of diabetes mellitus. It is probable that somatostatin also will come to occupy an increasingly prominent place in such diverse disciplines as cell biology, the neurosciences, endocrinology, and gastroenterology.

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Insulin Receptors

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Much of the emphasis in the pathogenesis of diabetes mellitus has justifiably been placed on the endocrine gland, the pancreas. Extensive studies on the biosynthesis and release of insulin from the beta cell, bihormonal control of metabolism by insulin and glucagon, and more recently the role of somatostatin have attracted the attention of students of the subject; but considerable evidence exists to suggest at least some role of tissue resistance to insulin in the pathogenesis of this disorder. There have been many advocates for extra-pancreatic factors causing diabetes. One of the first was Mirsky, who proposed that diabetes might be due to excessive amounts of hepatic insulinase, an enzyme which degrades insulin.[1] Vallance-Owen suggested that a circulating insulin antagonist labeled synalbumin might be the cause of insulin resistance in diabetes.[2] This factor was later shown to be an artifact. Others, such as Antoniades, proposed that insulin might circulate predominantly in a bound form in diabetic subjects and thus not exert full biologic activity.[3]

The most articulate spokesman for a role of insulin resistance in diabetes mellitus in recent years has been Gerald Reavan, and his group from Stanford University, who bases his theory on two observations. The first is that a large number, if not the majority, of adult onset diabetics have increased circulating insulin concentrations rather than decreased concentrations as had been expected. This was first observed by Yalow and Berson shortly after the perfection of the radioimmunoassay for insulin.[4] Hyperinsulinism in diabetics has since been confirmed by many investigators. A second observation supporting the role of insulin resistance in diabetes is that of "glucose impedance" in diabetic patients. Glucose impedance was demonstrated by Reaven and colleagues[5] by infusing glucose and insulin at a constant rate in diabetic and non-diabetic subjects whose endogenous insulin release had been shut off by administration of epinephrine and propranolol. New steady states for glucose and insulin were achieved in both groups, with comparable insulin concentrations in diabetics and non-diabetics, whereas the new steady state glucose concentration was considerably higher in diabetic subjects than in non-diabetic subjects. These excellent studies indicated that for a given concentration of insulin, the blood glucose-lowering effect was less in diabetics than in non-diabetic subjects. More recently, Reaven and Olefsky have suggested that insulin resistance in diabetic patients might be due to a decrease in the number of insulin receptors[6] by showing a decrease in the number of insulin receptors on circulating monocytes in diabetic patients compared to those on monocytes of non-diabetic subjects. Additionally, treatment of their diabetic subjects with an oral hypoglycemic agent resulted in a return to normal of the number of insulin receptors on peripheral monocytes.[7] A thorough understanding of these latter observations and their obvious, important implications for the pathogenesis of diabetes requires a certain knowledge of the insulin receptor and of recent advances in the field of receptor technology.

Properties of the insulin receptor are shown in Table 1. As is the case with other polypeptide hormones, the receptor for insulin is located on the cell

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TABLE 1
Properties of Insulin Receptors

A) Located on cell membrane
B) Unevenly distributed and may occur in clumps
C) Protein with mol wt approx. 300,000–Tetramer consisting of monomers of 75,000 mol wt each
D) Continuous synthesis and degradation with relatively slow turnover rate–2%/hr
E) Presence of insulin receptor on cell membrane requires protein synthesis and microfilament integrity

membrane. Evidence for intracellular distribution of insulin receptors is very scant; they are not evenly distributed over the cell surface but rather occur randomly and in clumps at times.[8] Present evidence suggests that the insulin receptor is a protein with molecular weight of approximately 300,000. Studies performed at the National Institutes of Health from Dr. Jesse Roth's laboratory suggest that the insulin receptor is a tetramer consisting of monomers of 75,000 dalton units each. Although the turnover rate is low compared to many biologic processes (2% per hour), continuous synthesis and degradation of the insulin receptor occurs. Studies with inhibitors of protein synthesis and microfilaments such as puromycin and cytochalasin respectively indicate that protein synthesis and microfilament integrity are necessary for the presence of insulin receptors on the cell membrane.[9] Inhibitors of microtubular function surprisingly had no effect on insulin receptors.[9]

Knowledge of three concepts involving the insulin receptor (Table 2) is of critical importance in interpreting studies in which receptor number and affinity have been determined. The number of receptors per cell varies with the particular cell being studied. However, for the peripheral monocyte, which is the most commonly studied cell in man because of its accessibility, the numbers of receptors vary between 15,000 and 30,000 per cell. Clearly, only a fraction of the receptor sites must be occupied for biological activity, and the number of occupied sites required for the different activities of insulin may vary. For example, dose response data suggest that fewer sites must be occupied to inhibit lipolysis than to stimulate glucose oxidation. Thus, many of the insulin receptors on the cell surface will be spare or unused receptors. Recent investigations have even shown that some of these receptors may serve as a peripheral reservoir for insulin, releasing intact insulin under appropriate circumstances.[10]

A second concept which is probably the most important in understanding current receptor studies is that insulin inhibits insulin receptor number. Experiments by Gavin et al[11] demonstrated that preincubation of cultured lymphocytes with physiological concentrations of insulin reduces the number of insulin receptors on these cells. An obvious corollary of this finding would be the presence of decreased insulin receptors in states of hyperinsulinism such as obesity and some forms of diabetes. Although not reported yet, reduced insulin receptors would be anticipated in patients with islet cell tumors. Thus, reduced receptor number might offer some protection to the patient with an islet cell tumor.

The third important concept in understanding insulin receptors is that of negative cooperativity.[12] Simply stated, this concept refers to site interactions on the cell surface by which affinity of the receptor for insulin is decreased as increasing numbers of receptors are occupied. This phenomenon might also be considered a homeostatic mechanism which protects the individual from the effects of hyperinsulinism.

The insulin receptor perceives and either directly or through a transducer substance influences the effector for a specific activity. Insulin binding is the first step in biological activity of the hormone. Although not all receptors are required for biological activity, more receptors increase the likelihood of binding for a given concentration of insulin. Binding of insulin to its receptor is therefore determined by insulin concentration, receptor number, and receptor affinity. Radioimmunoassay techniques for the measurement of insulin have been available for years; now methods are available to measure insulin receptor number and affinity.

As mentioned the most accessible cells for measuring insulin receptors in vivo are peripheral mon-

TABLE 2
Important Concepts Involving Insulin Receptors

A) Spare receptors
  1) Number of receptors per cell varies with cell type Peripheral monocytes have 15,000-30,000 receptors per cell
  2) Only small percentage of receptors must be occupied for biologic activity
  3) Spare receptors may serve as peripheral insulin reservoir
B) Feedback inhibition of insulin receptor number by insulin
C) Negative cooperativity

ocytes. These cells are obtained by Ficoll-Hypaque separation of the buffy coat of centrifuged blood. To determine receptor number and affinity, these cells are incubated with labeled insulin and increasing amounts of cold insulin, resulting in a binding curve (Fig 1). Applying Scatchard analysis to this data results in a curvilinear plot (Fig 2*B*). Similar studies with the growth hormone receptors, or other hormones not showing negative cooperativity, produce a linear Scatchard plot (Fig 2*A*). Although the favored interpretation of the curvilinear Scatchard plot for insulin receptors is negative cooperativity, the possibility that two types of insulin receptor sites (broken lines Fig 2) exist cannot be eliminated from present data. Employing Scatchard analysis, the number of receptors is calculated from the amount of bound insulin where the plot crosses the X axis. The slope of the plot reflects affinity, and new graphic analyses are available to express affinity even from a curvilinear plot.[13]

Employing these techniques, insulin receptor number and affinity can be determined. The factors influencing affinity and receptor number are shown in Table 3. Some of these have already been discussed. One of the most important determinants of affinity is pH; its effect on insulin binding to receptors is shown in Figure 3. For both human monocytes and cultured lymphocytes, reducing pH from 7.4 to 6.8 results in greatly depressed insulin binding and may contribute to the insulin resistance observed in severe diabetic ketoacidosis.

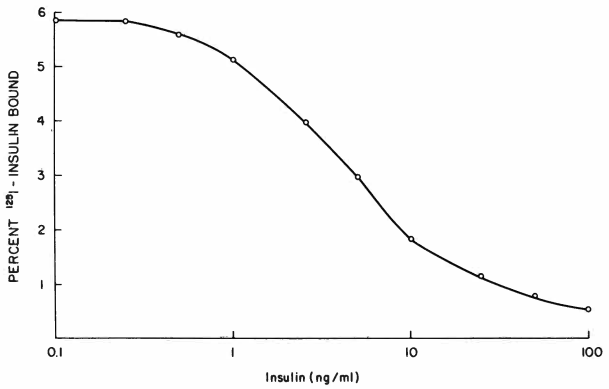

Fig 1—Insulin binding to peripheral monocytes. 20 × 10^6 mononuclear cells (14% monocytes) were incubated in 0.5 ml buffer containing 50–100 pg ^{125}I-insulin and increasing amounts of unlabeled insulin to give the final concentration indicated in the figure. After 3 hours incubation, 200 ul aliquots were centrifuged, aspirated, and the sediment counted.

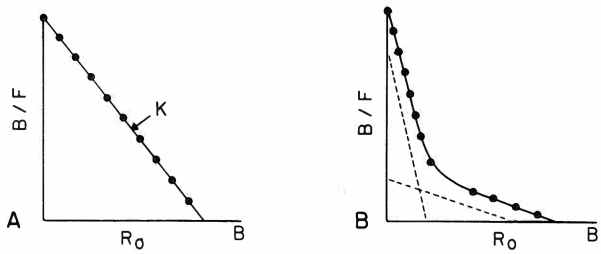

Fig 2—Scatchard analysis of binding data. B/F = bound/free radioactive ligand. Horizontal axis (Ro) is amount of ligand bound to receptor in molar quantities. *A*. Scatchard plot for hormone not exhibiting negative cooperativity; *B*. Plot is for hormone exhibiting negative cooperativity or having two different receptor sites. Insulin receptor studies show curvilinear plot as in *B*.

The major factor so far uncovered altering insulin receptor number is insulin acting in a type of feedback mechanism to inhibit insulin receptor number. Thus, as previously pointed out, reduced insulin receptors are anticipated in obesity where insulin resistance and hyperinsulinism exist. Reduced insulin receptors have indeed been shown to occur in obese humans and animals.[14–16] In addition, dieting and weight reduction result in normalization of the numbers of insulin receptors.[13] It is debatable whether reduced receptor concentration is primary, resulting in insulin resistance and hyperinsulinism, or whether insulin resistance due to some other factor is primary, causing hyperinsulinism and, secondarily, reduced insulin receptors.

Returning to Reavan and Olefsky's observations in non-obese diabetic subjects,[6] it is not clear whether the reduced insulin receptor number is due to the hyperinsulinism exhibited by this group [fasting immunoreactive insulin (IRI) 20 ± 2 versus 10 ± 1 in normals] or whether it might be primary and thus be important pathogenetically. Nevertheless the decreased insulin receptors observed in the diabetic sub-

TABLE 3
Determinants of Insulin Binding to Tissues

I. Receptor affinity
   - PH
   - Temperature
   - Ionic strength
   - Receptor occupancy (Negative cooperativity)

II. Receptor number
   - Insulin decreases receptor number

III. Insulin concentration

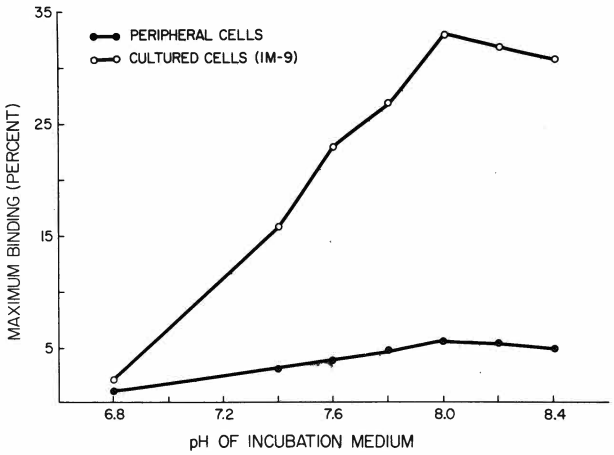

Fig 3—Effect of pH on insulin binding to receptors. Maximum binding refers to percent of ^{125}I-insulin bound to receptors in the absence of unlabeled insulin. For peripheral cells 20×10^6, mononuclear cells (14% monocytes) were used; cultured lymphoblastoid cells (IM-9) were used at 3.0×10^6 cells per ml concentration.

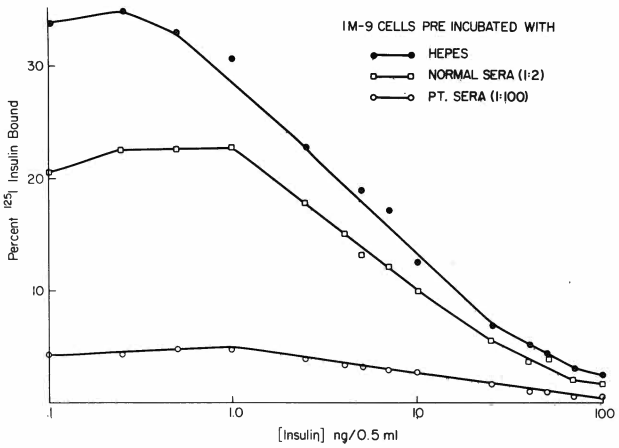

Fig 5—Effect of serum preincubation on insulin binding to cultured lymphoblastoid cells (IM-9). IM-9 cells were preincubated with buffer, normal serum, or serum from insulin-resistant patient for 60 min, washed twice, and resuspended in 0.5 ml buffer at final concentration of 3×10^6 cells per ml. Binding curves were then obtained on these cells.

jects in Reaven and Olefsky's study would certainly contribute to the insulin resistance observed. The return of receptor number to normal with chronic sulfonylurea treatment[7] might in similar fashion be attributed to the reduced insulin concentrations in well-controlled diabetics on chronic sulfonylurea therapy.

Although the role of reduced insulin receptors in the pathogenesis of diabetes mellitus is equivocal, a rare diabetic syndrome recently reported is clearly related to decreased insulin receptors.[17] In several patients with other evidence of immunologic disease associated with severe insulin resistance, an antibody to the insulin receptor has been demonstrated. Since this report we at the Medical College of Virginia have had the opportunity to study two patients with this syndrome. One of the patients requiring over 2,000 units of insulin daily had a strongly positive antinuclear antibody as the only other manifestation of autoimmunity; the second patient had a scleroderma-like illness and required 1200 units of insulin daily. Insulin binding curves by peripheral monocytes from one of these patients is shown in Figure 4. That a serum factor was responsible for the decreased binding was indicated by studies in which cultured lymphocytes (IM-9) were preincubated with the patient's sera (1:100) and then used for binding studies (Fig 5). Scatchard analysis (Fig 6) revealed the decreased binding to be due to a reduction in numbers of insulin receptors. Studies, not shown, in which

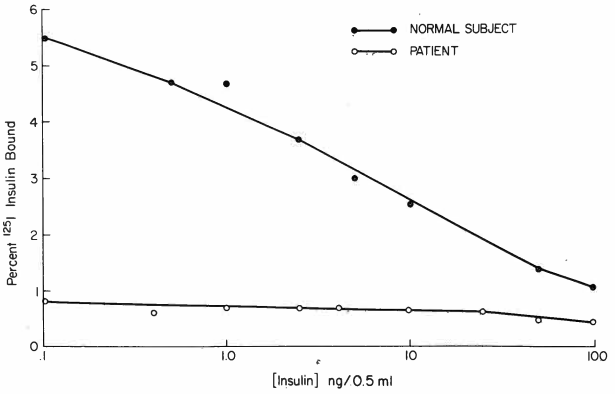

Fig 4—Insulin binding to peripheral monocytes from normal subject and patient with antibodies to the insulin receptors. Details as for Fig 1.

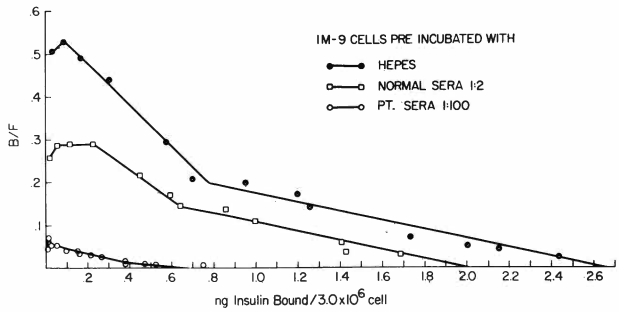

Fig 6—Scatchard analysis from binding data shown in Fig 5.

cultured lymphocytes were preincubated with IgG fraction of the patient's sera exhibited the same phenomenon, suggesting that the serum contained an antibody to the insulin receptor.

In summary, techniques are now available for measuring insulin receptors in vivo. So far, reduced insulin receptors have been observed in obese persons and in a selected group of adult onset diabetic patients. The pathogenetic significance of the latter observation is uncertain and may possibly be a manifestation of the high insulin concentrations in these diabetics. However, a rare diabetic syndrome in which severe insulin resistance due to antibodies to the insulin receptor has been reported and is now corroborated by our findings in two patients.

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Why Control Diabetes?

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The definition of diabetes control varies widely among specialists of the disease. Proponents of "good" control believe that the goals of appropriate therapy for diabetes should include an all-out effort to obtain levels of fasting and postprandial blood glucose as close to those in the non-diabetic as possible. Certainly, good control has been proven to accomplish the following:

1. Prevention of ketoacidosis
2. Prevention of severe hypoglycemia
3. Decrease in perinatal mortality and morbidity
4. Promotion of normal growth and development of the juvenile diabetic
5. Prevention of, or inhibition of, infection

The evidence that hyperglycemia is responsible for vascular complications, particularly microvascular disease, and that good control will prevent or inhibit the rapidity of the development of this pathologic process, or even reverse it, is the basis of this presentation.

At the outset, however, it must be appreciated that retinopathy, nephropathy, neuropathy, and large vessel disease may be the presenting manifestations of diabetes, and that it is possible to have extensive vascular complications in the presence of relatively mild glucose intolerance. Furthermore, 20% of juvenile-onset type, ketosis-prone diabetics of more than 25-years duration with continuous hyperglycemia do not have clinically impressive retinopathy or nephropathy. Also, it has been noted that large vessel disease or neuropathy is absent in 20% to 40% of insulin-dependent diabetics.

The evidence for good control is largely based on the following observations:

1. Clinical studies
2. Animal studies
3. Histologic and electron microscopic data
4. Biochemical studies
5. Altered coagulopathy, viscosity, and hypoxia

Recent studies in well-controlled juvenile diabetics indicate that serial biopsies of quadriceps muscle have demonstrated "thinning" of previously thickened capillary basement membrane, in contrast to persistent and increasing thickening of capillary basement membrane in poorly controlled diabetic patients. In addition, it has recently been demonstrated that diabetic vascular lesions developed in normal kidneys transplanted into patients with diabetes mellitus after a period of two years, while transplanted kidneys in non-diabetic control patients of the same age and type remained unaffected; this also gives great support to the concept of good control.

Animal studies with diabetic Chinese hamsters, mice, rats, dogs, and monkeys have indicated that good control with insulin therapy or islet transplantation prevents or reverses lesions in the eyes, kidneys, or nerves.

Histologic and electron microscopic studies of capillary basement membrane of muscle have given conflicting results. One group of observers indicates that basement thickening is already genetically predetermined, while other investigators relate capillary basement membrane thickening to the duration of diabetes. Methodology of technique, the patient's age, unknown duration of existing diabetes, and cri-

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This is an abstract of the lecture given by Dr. Rifkin at the symposium, Diabetes 1976.

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teria for the diagnosis of diabetes have to be clarified before coming to definitive conclusions.

Biochemical studies indicate that hyperglycemia or insulin deficiency produces alteration in vascular basement membrane composition, as well as accumulation of glucose-derived substances, such as sorbitol in the lens of the eye, Schwann's cell, and aorta. Recently, an elevation of glycolysated hemoglobin (HbAIc) has been noted in the blood of diabetic mice as well as human diabetics. The glycolysation of hemoglobin appears to be a post-synthetic modification of HbA which is dependent on the degree and duration of hyperglycemia.

Recent data reveal that platelet aggregation is more intense in diabetic patients, with increased sensitivity of platelets to aggregation from adenosine diphosphate (ADP) and epinephrine. This sensitivity correlates with elevated levels of Von Willebrand factor, which in turn appears to be influenced by growth hormone. Also, platelets from diabetic subjects are more sensitive to arachidonic acid-induced aggregation, which can be abolished by aspirin, which is a prostoglandin synthetase-inhibitor.

Spontaneous fibrinolytic activity of the blood is abnormally low in persons with diabetes mellitus, and implies a poor defense mechanism against fibrin deposits in the vessel walls, which conceivably may contribute to the development of diabetic microangiopathy. Increased red blood cell aggregation and alterations in plasma protein changes in diabetics, with their effects on blood viscosity and flow, may also play a role in accelerating the rate of progression of diabetic microangiopathy.

In addition, the decreased reactivity of HbAIc with 2,3 diphosphoglycerate might impair oxygen unloading in diabetes, and contribute to tissue hypoxia, which may have pathogenetic implications in the development of diabetic angiopathy.

Whether reversal of hyperglycemia, as well as the use of agents to inhibit some of the above-described adverse effects of the diabetic milieu, may affect the development of diabetic microangiopathy is far from settled. Consideration must be given to more intense regulation of dietary factors; use of the newer oral hypoglycemic agents; administration of insulin three or four times daily for better control; long-term studies on the use of aspirin, and other platelet-aggregating inhibitors, as well as phosphate supplements; availability and effectiveness of growth hormone inhibiting agents; the use of substances which can interfere with the post-ribosomal steps of basement membrane assembly; and finally, improvements in the delivery of insulin, whether it be by better biomechanical engineering or by increased knowledge of immunologic and other biologic defenses in the use of islet or organ transplantation.

Insulin Treatment of Diabetes Mellitus

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Dr. Harold Rifkin*, has previously set forth the reasons why we should try to keep the diabetic patient's blood sugar as near normal as possible. What are the generally accepted standards of good diabetic control?

Marble[1] considered control of the insulin-dependent diabetic good if the whole blood glucose before meals was not over 130 mg% (= serum glucose 150 mg%), the 24-hour urinary glucose did not exceed 5% of the carbohydrate intake, and there was no ketonuria; Oakley et al[2] defined satisfactory control as blood glucose before meals of less than 150 mg% with no hypoglycemia; Lewis et al,[3] in a recent paper dealing with pregnant diabetics, recommended fasting blood glucose below 100 mg% and blood glucose two hours after breakfast below 160 mg%.

In some diabetics we can accomplish this type of control; in others we cannot. The maturity onset diabetic has some endogenous insulin under autoregulatory feedback control and, with a judicious diet or with the addition of a sulfonylurea drug or some exogenous insulin, near normal blood sugars can be achieved. However, in the juvenile or young adult onset diabetic, with little or no endogenous insulin, it is very difficult, even with complicated schedules of administration of insulin, to get blood sugars anywhere near normal without producing hypoglycemia.

A reasonable goal for every diabetic would be to maintain blood sugars as near normal as possible without producing hypoglycemia and without requiring a program so restrictive that it interferes with the quality of life.

Figure 1, taken from a paper by Molnar et al,[4] illustrates the daily variations in plasma insulin and blood glucose throughout a 24-hour period in a normal individual. Meals, snacks, and standard exercise periods are indicated. It is apparent that each feeding is accompanied by a sharp peak in insulin secretion which brings about disposal of ingested glucose and amino acids, and limits the blood sugar rise.

It is obvious that no one injection of intermediate-acting insulin can in any way duplicate the normal insulin pattern. At best an injection of NPH® or Lente® Insulin given before breakfast will be absorbed for the most part over the next 8 to 12 hours when meals will be eaten, with some carry-over effect during the night. As diabeticians we hope to accomplish blood sugar levels that average somewhere near normal, but obviously the blood sugar will rise too high after meals and may fall too low before the next meal. Further leveling out may be accomplished by the addition of between-meal and bedtime snacks. We always insist on midafternoon and bedtime snacks in every patient on NPH® or Lente® Insulin.

Bressler and Galloway[5] some years ago called attention to variations in the timing of effect of various insulins (Fig 2). Diabetics were divided into those showing a normal (B) response, a transient (A) response, or a delayed (C) response to NPH® or Lente® Insulin. Those with a normal response could be moderately well regulated with a single morning injection of NPH® or Lente® Insulin. Those with a transient response might benefit from a second small dose of NPH® or Lente® Insulin before supper, and those with a delayed response might require the addition of regular insulin to the morning dose of NPH® or Lente® Insulin. The reasons for this variation in the timing of effect of the intermediate-acting insulins is not known. One possible factor delaying the action of

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* see preceding abstract.

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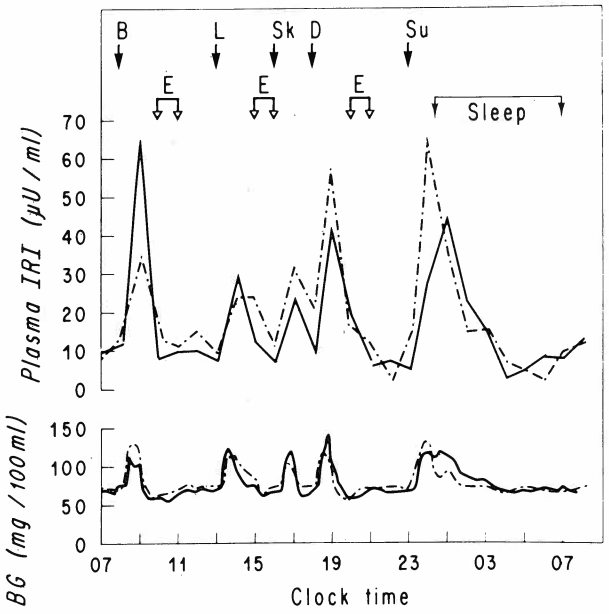

Fig 1—Meal-to-meal and day-to-day variations in blood glucose and immunoreactive insulin in a normal subject on two successive days (solid line represents first day; interrupted line represents second day). B = breakfast, L = lunch, Sk = snack, D = dinner, Su = supper, E = exercise, one hour of walking.

insulin is its binding to antibodies, with subsequent release, which prolongs the insulin effect.

Lukens,[6] Forsham,[7] and others pointed out some time ago that multiple injections of regular insulin before meals, perhaps accompanied by one or more injections of longer-acting insulin to control overnight blood sugar, would more closely resemble normal physiology and would be likely to give better control than a single injection of long- or intermediate-acting insulin. Indeed it is doubtful whether the medical profession did diabetic patients any favor when some years ago it abandoned multiple insulin injections in favor of the once-a-day injection of longer-acting preparations. Once the diabetic public became accustomed to the single morning injection it was difficult to persuade patients to go back to multiple injections and, by and large, doctors have seldom made the effort.

Recently there has been a renewal of interest in the use of multiple injections of regular insulin to accomplish more physiologic control, especially since reports from Paris by Job et al[8] seem to indicate that such regimens in juvenile diabetics result in better control and in less progression of retinopathy. Also, especially good control in pregnant diabetics with multiple injections appears to result in lower infant mortality and morbidity,[3] although better obstetrical techniques for monitoring the pregnancy and timing of delivery are equally important factors in the improved outcome in these patients. Thus a number of schedules of insulin administration have been proposed as illustrated in Table 1. There is good rationale for each of these programs in a given situation, although it is unlikely that any one type of program will be best for all patients.

Attempts have been made to define complicated dosage schedules even more rigidly. Lewis et al[3] recommend the following formula for insulin administration for pregnant diabetics:

$$\frac{\text{Morning NPH®}}{\text{Morning reg. ins.}} = 2:1$$

$$\frac{\text{Evening NPH®}}{\text{Evening reg. ins.}} = 1:1$$

$$\text{Morning total} = 2 \times \text{evening total}$$

I have tried this schedule with several pregnant diabetics with quite varied results. I think the reasoning is right, but it should be remembered that no two diabetic patients will respond in exactly the same

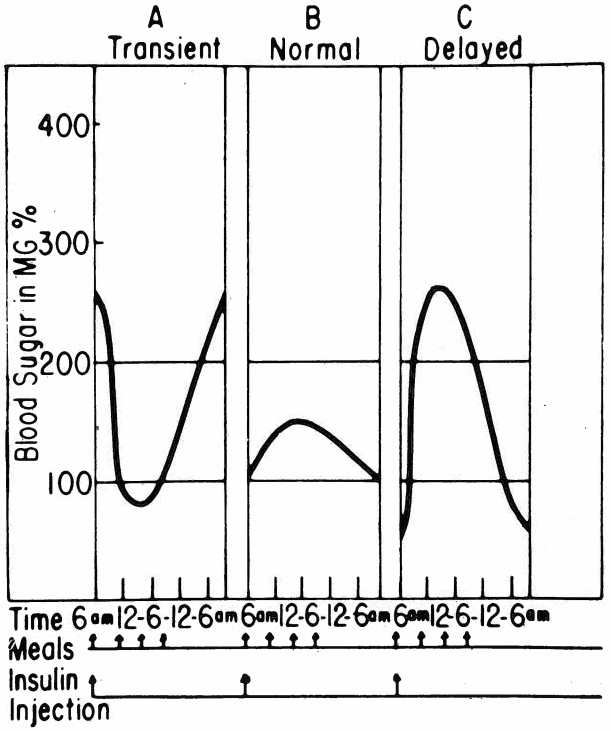

Fig 2—Blood sugar responses to a single daily dose of NPH® Insulin.

TABLE 1
Various Schedules for Insulin Administration

Breakfast	Lunch	Afternoon Snack	Supper	Bedtime Snack
N				
R + N				
N			N	
R	R		N	
R	R		R + N	
R + N			R + N	

(N = NPH® or Lente® Insulin. R = regular or crystalline zinc insulin.)

way—there are just too many variables. Some of these are listed in Table 2.

How then should one approach insulin treatment in any given patient? Two basic principles are helpful: (1) analyze what kind of diabetic the patient is, and (2) keep the variables at a minimum.

The first principle means to try to form some idea of how much endogenous insulin the patient has from evaluation of the age of onset of the diabetes, his or her weight at onset, and the severity of symptoms at onset, especially weight loss. Onset of diabetes early in life, severe polyuria and polydipsia, and especially weight loss from uncontrolled diabetes, usually indicate total or near total insulin deficiency. If the patient has been on treatment, the diabetician should also consider the occurrence of ketoacidosis or frequent hypoglycemic reactions, both of which suggest that the patient has little or no endogenous insulin. If the patient has been controlled in the past on oral agents he or she must have had some endogenous insulin at that time.

TABLE 2
Variable Factors Limiting Good Blood Sugar Control with Exogenous Insulin

1. Variations in food intake
2. Variations in exercise
3. Emotional factors and other stresses
4. Factors affecting the availability and effectiveness of administered insulin:
   (a) variable absorption rates from injection site
   (b) variable antibody titers and uncertain rates of release of insulin from antibodies
   (c) variations in receptor binding sites and in their affinity for insulin
5. Variations in glucagon secretion
6. Hypoglycemia with rebound hyperglycemia caused by counter-regulatory factors
7. Exogenous insulin is delivered into systemic circulation and not primarily into portal circulation and to the liver as is endogenously secreted insulin

The second principle, keeping the variables at a minimum, means trying to get the patient to maintain reasonably constant eating habits and exercise patterns as well as simplifying the insulin schedule until the need for multiple doses becomes apparent. In general, begin with a single dose of NPH® or Lente® Insulin every morning, perhaps 20 units if the patient has never before taken insulin. Instruct the patient in a proper diet, which probably should include both afternoon and evening snacks, for his or her weight and activity. The patient may continue working but should test his or her urine four times daily, before meals and at bedtime. If the patient continues to spill sugar, increase the insulin by 5 units every four or five days until some negative urine tests are obtained. Check the blood sugar at the time of the negative urine test, and if this is still high, continue to increase the insulin until the blood sugar is in the desired range. If any hypoglycemic reactions occur, the insulin dose should be reduced. As the patient approaches control the urine tests should be determined from second voidings whenever the first voiding contains sugar.

Many diabetics will come under satisfactory control with a single morning dose of NPH® or Lente® Insulin. If glycosuria or hyperglycemia persists before breakfast, with negative tests in the afternoon, the patient may be classified as a transient responder, and a second small dose of NPH® or Lente® Insulin should be added before supper. If the morning test is repeatedly negative but glycosuria persists before lunch, the patient may be a delayed responder, and regular insulin can be added to the morning NPH®.

If the desired degree of control is not achieved by these measures, both regular and NPH® Insulin may be given twice daily. In general the indications for administration of such multiple doses would follow a schedule such as in Table 3, as suggested by Oakley et al.[2]

In a highly reliable and highly motivated patient, such as a pregnant diabetic, this type of program may succeed; or a highly obsessive patient may religiously seek perfect control. Unfortunately most patients are not so reliable, and the obsessive ones usually end up making themselves hypoglycemic.

TABLE 3
Management of the Severe Diabetic with Twice Daily Injections of Regular and NPH® Insulin

Insulin	Period of Maximum Action	Timing of urine or blood sugar for dose adjustment
Morning RI	Breakfast to lunch	Pre-lunch
Morning NPH®	Lunch to supper	Pre-supper
Evening RI	Supper to midnight	Bedtime
Evening NPH®	Midnight to breakfast	Pre-breakfast

What do you do with the patient who never shows any consistent pattern? You look for such variables as food intake, exercise, emotional upsets, and other factors. Hypoglycemia, recognized or unrecognized, is the cause of much brittleness. For every recognized hypoglycemic reaction, the brittle juvenile diabetic has ten other occasions when the blood sugar is quite low without his knowledge, and each may be followed by rebound hyperglycemia. You must learn to recognize this pattern and to reduce insulin accordingly. Tables 4 and 5 illustrate two such patients.

Table 4 is a record of the urine sugars on a 23-year-old dental student with diabetes of two years' duration, during which he took 12 units of Semilente® and 20 units of Lente® Insulin daily. He was on a 2500-calorie diet including three main meals and midafternoon and bedtime snacks. He was extremely conscientious and liked to keep all his urine tests negative. To keep himself in physical trim he ran two miles several times a week, and because of his busy schedule chose the late evening as the time for his exercise. He was not conscious of any hypoglycemic reactions, but he invariably showed unexpected sugar in his urine on the mornings after running; when he had not run the night before, his tests were almost all negative. It became apparent that the additional exercise was causing undetected hypoglycemia during the night, followed by rebound hyperglycemia and glycosuria the next day, the so-called Somogyi effect caused by the secretion of epinephrine, glucagon, cortisol, and growth hormone in response to hypoglycemia. The situation was corrected by insisting that he take sizable extra feedings just before and just after his nocturnal exercise periods; this prevented the hypoglycemia and its consequent rebound.

TABLE 4
Insulin Dosage and Urine Tests Showing Glycosuria Following Asymptomatic and Unrecognized Hypoglycemia

J.S. (Male, 23)	Urine sugars			
Insulin	7	11	4	9
12 SL ± 20 L	N	N	N	N
〃 〃	N	N	N	N*
〃 〃	4+	4+	N	N
〃 〃	N	N	N	N*
〃 〃	2+	3+	N	N
〃 〃	N	3+	N	N*
〃 〃	3+	N	N	4+
〃 〃	4+	1+	N	N
〃 〃	N	N	N	N

* Ran approximately 2 miles
(SL = Semilente® Insulin. L = Lente® Insulin.)

The Somogyi rebound hyperglycemia following hypoglycemia is most often seen in the brittle juvenile diabetic, but can be observed in any insulin-dependent diabetic. Table 5 shows the record of urine tests on a 72-year-old diabetic taking 32 units of NPH® Insulin daily. He also was very conscientious and very careful about his diet. After a series of days with all negative urine tests, he showed unexpected 4+ sugar at noon on Aug 29 and Aug 30, with sugar

TABLE 5
Insulin Dosage and Urine Tests in an Older Diabetic Showing Glycosuria, Probably Representing Rebound After Unrecognized Hypoglycemia

J.V.C. (Male, 72)	NPH®	7	12	5	9
8/27	32	N	N	N	4+
8/28	〃	N	N	N	N
8/29	〃	N	4+	1+	3+
8/30	〃	N	4+	4+	4+
8/31	30	N	N	N	N
9/1	〃	N	tr	tr	N
9/2	〃	N	N	3+	3+
9/3	〃	tr	tr	N	4+
9/4	〃	N	N	N	N
9/5	〃	N	N	N	N
9/6	〃	N	N	N	N
9/7	〃	N	N	N	N
9/8	〃	N	tr	tr	4+
9/9	〃	N	2+	N	3+
9/10	〃	3+	3+	N	4+
9/11	〃	N	2+	4+	4+
9/12	〃	3+	tr	N	N
9/13	〃	1+	3+	N	N
9/14	〃	N	N	N	N

persisting during the rest of the day. Questioning revealed no dietary indiscretions, no variation in exercise, and no emotional upsets or other stresses to account for the glycosuria. Although he was unaware of any hypoglycemic symptoms, it seemed inconceivable that the dose of insulin which had kept his tests all negative over the previous days could be so inadequate on these days. I suspected unrecognized hypoglycemia and reduced his NPH® Insulin to 30 units. The urine tests improved and remained all negative until Sep 8 when he again began to show sugar in the evening test. Not shown in Table 5 is the fact that his NPH® Insulin was lowered again to 28 units and later to 26 units with improvement each time and, finally, all negative urine tests. He remained on 26 units of NPH® for several months, then began to spill sugar in all specimens; the NPH® Insulin dose has been gradually raised again to 30 units with good control. The reason for these swings in insulin requirement over periods of several months is unknown. Some patients go through cycles when their insulin requirement goes up or down for no apparent reason; these may be over months or over shorter periods. All we can do is to try to keep pace through adjustments in insulin dose.

When the patient alternates between good days and bad days, with lots of sugar or no sugar, suspect hypoglycemia with rebound. In any event you should adjust the patient's insulin dose to his or her better days and not try to give more for the bad days or you will surely produce hypoglycemia.

The bane of the diabetician's existence is the obese patient on insulin. If these patients could have been made to reduce in the beginning, many of them would not have needed insulin. Once on insulin, lipolysis is inhibited, weight reduction becomes even more difficult, and these patients tend to gain more weight which increases insulin resistance, creating a vicious circle of upward spiraling weight and insulin dosage. In this instance it may be better to cut back the insulin dosage and let the patient spill sugar until somehow he or she can be persuaded to really limit food intake. There is no satisfactory answer to this problem unless the patient loses weight.

In summary, I believe in aiming for the best possible control for each patient through an empirical approach which seeks to arrive at the best schedule for the individual patient. We want especially tight control for the pregnant diabetic, and for this patient it is likely that multiple insulin injections are needed. If further experience confirms the benefits of multiple injections for juvenile patients, this type of treatment may also be indicated for these patients.

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Figure 2 is reproduced with permission from *Medical Clinics of North America* (55:861–868, 1971).

Table 3 is reproduced with permission from *Diabetes* (15:219–222, 1966).

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Oral Hypoglycemic Agents

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The pharmacology of the two general groups of oral anti-diabetic agents, namely the sulfonylureas and biguanides, are briefly discussed here. The sulfonylureas have a common hypoglycemic core. These sulfonylurea compounds can be divided into short, intermediate, and long-acting agents, with varying half-lives, and duration of action. Therapeutic doses of sulfonylureas have produced wide variations in serum levels depending upon the rates of absorption, rates of metabolism, renal clearance, and degree of protein-binding. Tolbutamide is a short-acting agent metabolized by the liver to inactive compounds which are excreted by the kidney; it has a half-life of four to five hours, a duration of action of approximately six to ten hours, and is given in divided daily dosage. Acetohexamide and tolazamide, the intermediate-acting compounds, are metabolized by the liver. Approximately 75% of acetohexamide is metabolized to another hypoglycemic compound, and tolazamide is metabolized to several hypoglycemic products. The duration of the action of these agents is approximately 10 to 16 hours; they are administered either once or twice daily. Chlorpropamide is only slightly altered, less than 1% being metabolized, probably in the liver; it is firmly bound to protein, is dependent on renal excretion for clearance, and, as it has a duration of action varying from 35 to 72 hours, it is given only once daily.

Sulfonylurea compounds appear to exert their primary action through direct stimulation of pancreatic insulin secretion. Peripheral insulin levels correlate with blood sugar levels during acute therapy, but do not appear to correlate with blood sugar-lowering effect of chronic drug therapy. Hence, important extra-pancreatic actions may be responsible for the hypoglycemic effects of these compounds, that is, induced hepatic glycolytic enzymes, impaired insulin sensitivity, increased membrane receptors, lowered insulin resistance, or other as yet undefined mechanisms.

Phenformin, a biguanide, is metabolized to some extent by the liver, but approximately 65% may be excreted unchanged by the kidneys. The hypoglycemic effect of the "short-acting" tablet lasts six to eight hours. In contrast, the timed disintegration capsule has a duration of action from 8 to 14 hours because of prolonged gastrointestinal absorption of the drug. The mode of action of these agents is not completely understood. There is evidence that phenformin lowers blood sugar levels by accelerating anerobic glycolysis and delays the absorption of glucose from the gastrointestinal tract. Phenformin also decreases hepatic gluconeogenesis of diabetic animals, but this has not been confirmed in man. It is also quite possible that the anorexigenic effects of this agent may lower caloric intake, and help exert its hypoglycemic action in this manner.

Side effects and toxic effects are relatively rare with the sulfonylureas. Hematologic, hepatic, and dermatologic reactions have been described. An "antabuse" effect has been noted with chlorpropamide. Rare cases of hypersensitivity angiitis have been described. Dilutional hyponatremia has been noted with chlorpropamide, and very occasionally with tolbutamide. In contradistinction, tolazamide and acetohexamide appear to have a proximal tubular diuretic effect.

Side effects of phenformin consist primarily of

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gastrointestinal reactions. Lactic acidosis, however, is a serious problem, particularly in the phenformin-treated diabetic with liver or renal disease, who is vulnerable to a variety of medical conditions which may lead to tissue hypoxia, that is, myocardial infarction, peripheral vascular disease with gangrene, and gastrointestinal bleeding. In addition, alcohol and phenformin are a particularly dangerous combination.

There are a number of drugs which may interact and potentiate the effects of the sulfonylurea compounds. These include phenylbutazone, probenecid, alcohol, salicylates, and monamine oxidase inhibitors. The list is growing, and the clinician treating the diabetic with sulfonylureas must be constantly on the alert.

The indications for oral agent therapy are relative, depending upon the patient's age, weight, fasting and postprandial blood sugar levels, and intensity of symptoms. Oral agents have a place in the management of the maturity onset diabetic. The aim of therapy of the diabetic patient is to produce normal weight for frame and height, to obtain as close to normal fasting and postprandial blood glucose levels as possible, to maintain normal lipid levels, and to keep the patient free from hypoglycemic episodes. Diet or insulin therapy or both are the bases of treatment for the non-obese patient with fasting blood glucose values above 160 mg/100 ml. A weight reduction regimen, however, is the mainstay of management of the obese maturity onset diabetic. This requires constant dietary counseling by the physician, the dietician, and the nurse-educator. In most cases, if weight reduction is successful, plasma glucose and lipid levels will approach normal values, and the patient will usually become asymptomatic. If the plasma glucose still remains high, and particularly if the patient is still symptomatic, the sulfonylurea agents may be tried. If hyperglycemia is not controlled within a short period of time, by either short- or intermediate-acting agents, as well as long-acting sulfonylurea compounds, then insulin should be administered; the "uncontrolled" patient may also have been taking other drugs during this period which may antagonize the action of the oral agents, for example, corticosteroids in pharmacologic doses, excessive doses of thyroid hormones, diuretics which may produce moderate to severe hypokalemia, oral contraceptives, and nicotinic acid in pharmacologic doses. More significantly, the patient must be warned against increasing weight gain during the administration of these oral agents. All these factors must be borne in mind in the consideration of "secondary failures." Hypoglycemia during oral therapy may result from overdosage (that is, failure to titrate dosage downward), poor choice of the oral therapeutic agent, omission of meals, renal or hepatic insufficiency, and interaction with other drugs, or alcohol.

In conclusion, comment is made on the findings of the University Group Diabetes Program (UGDP). The purpose of the UGDP was to determine whether or not control of blood glucose levels would help to prevent or delay vascular disease in non-insulin-requiring diabetics. After 8½ years of follow-up at 12 university-affiliated treatment centers, "The findings of the study indicate that the combination of diet and tolbutamide therapy is no more effective than diet alone in prolonging life. Moreover, the findings suggest that tolbutamide and diet may be less effective than diet alone or diet and insulin, at least insofar as cardiovascular mortality is concerned." The debate on the validity of this study still rages. A number of the criticisms leveled against the study by diabetologists, other clinicians, epidemiologists, and statisticians are discussed. These include inappropriate patient selection and randomization, higher risk factors at the outset of the study, manipulation of electrocardiographic data, risk factors such as smoking and hypertension not measured or monitored, use of fixed dosage of drug, clinic preponderance regarding mortality (it has been alleged that the bulk of tolbutamide mortality occurred in four clinics, Birmingham, Boston, Cincinnati, and Minneapolis), use of "patient data" against "computerized data," neglect of appropriate "vascular" history, neglect of "co-morbidity," contradictory clinical studies here and abroad, and the danger of extrapolation of the UGDP findings to other sulfonylurea agents.

Diabetes in Children and Adolescents; Observations on Diabetic Microangiopathy

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You may well wonder why I have combined a talk on diabetes in children and adolescents with one on diabetic microangiopathy when as pediatricians we rarely, if ever, see clinical evidence of the sequelae of vascular disease in our patients. If we see proteinuria in a child, we seek another cause. We may see young teen-agers with a microaneurysm or a trace of protein, but we soon refer them to our internist friends who fall heir to the care of problems that doubtless have their beginning in childhood years. Pediatricians are, therefore, concerned about these problems and the adequacy of treatment they are prescribing for their young diabetic patients.

There is a recent movement to place considerable emphasis again on strict control of blood glucose as a means of reducing complications of diabetes. In an effort to achieve better control many physicians are placing their patients on two injections a day.

I believe we can say that there are few, if any, physicians today who could duplicate Dr. Elliott P. Joslin's success in obtaining patient cooperation in his efforts to control their diabetes. Yet data from his clinic show that only a small percentage achieved what he considered excellent control. Nor did we see a striking difference in the incidence of complications in his patients when compared with that in the general diabetic population.

I do not minimize the need for achieving as nearly optimal control as one can with insulin, but I believe a return to the old emphasis on strict control does a disservice to our diabetic children and adolescents. It misleads them into thinking that controlling the blood sugar adequately *is* possible with present day methods of insulin administration; that if this is achieved, it will prevent or slow down complications; and that if this is not achieved, even with the most conscientious efforts, it is the patient's fault. I believe there is evidence that none of the above is true. Also, this re-emphasis on control and what it does or does not do to the basement membrane, diverts our attention from pursuing other productive avenues of research that may uncover clues and therapeutic approaches. It is with some of these avenues that I would like to acquaint you and stir your interest.

I would like first to address myself to important considerations in the care of the child or adolescent who has diabetes.

It is, of course, fundamental that the patient and the parents be properly educated about diabetes and its control at the very beginning if one is to expect acceptance and participation in a program of good management. We feel this should always be initiated on a hospital ward even if the child is not acidotic. Here the patient and parents can work with a teaching team in a relaxed setting where questions can readily be answered before the family is left on its own. The focus of instruction for the very young child and infant is *both* parents; by 7 to 10 years of age the child can help with urine testing; the 11-year-old can learn to give his or her own injections. In the teens the patient becomes the focus of instruction with parents also learning the facts and techniques. Do not be misled on this point: teen-agers very much need and want the support of their parents in these circumstances.

As soon as the patient has intravenous feedings

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discontinued and is ambulatory the routine that he or she will continue at home is initiated. After the acidosis is cleared the patient is not awakened during the night hours for either urine testing or supplemental insulin.

The educational process continues upon discharge from the hospital, first with phone calls to talk over changes in insulin dosage, and then in weekly visits to the clinic. As confidence and understanding increase, visits are less frequent but are continued with regularity.

What do we teach our patients about diabetes and how it should be managed?

Good management of the diabetic begins with the *physician's* smooth management of the initial ketoacidotic episode, for actions speak louder than words. I believe pediatricians have an advantage here because it can be said with certainty, if not with modesty, that pediatricians have been in the forefront in the proper administration of fluids and electrolytes. One need only recall the names of Gamble, Butler, and Darrow to know that we have had good mentors. Fluids play a most important role in recovery from acidosis; no amount of insulin will rid the patient of the accumulation of hydrogen ion and restore his or her base deficit. Briefly, the initial hydrating solution is a mildly hypotonic solution of sodium chloride free of potassium, containing bicarbonate if acidosis warrants its use. This is followed by a more hypotonic solution including potassium and dextrose, usually around the second hour. We discourage the use of isotonic saline as unphysiologic because of its high anion concentration and the absence of free water. Pediatricians also for many years have been administering small doses of insulin at frequent intervals instead of the large doses prescribed in adults. Indeed, the recent introduction of continuous intravenous administration of low levels of insulin has little if any advantage over our long-used methods of administration. We begin with 0.5 to 1.0 units/kg crystalline insulin, giving half intravenously and half deep subcutaneously. Every hour the dose is reduced by 50% unless the interval reveals no progress in reducing the blood glucose. The previous hour's dose is then repeated.

After intravenous therapy is discontinued and oral feedings initiated, regular insulin is given with meals for one or two days. Lente®, a long-acting insulin, comprised of 70% Ultralente® and of 30% Semilente®, is then given once daily prior to breakfast.

Our goal is to allow a trace or 1+ glycosuria in urines tested prior to meals and at bedtime. The Lente® is increased daily by 2 to 4 units until control is achieved.

We teach the patient to monitor his or her diabetic control through daily urine tests: that is, a double-voided specimen before breakfast, before supper, and at bedtime. After discharge from the hospital we allow time for a pattern to develop in response to the child's home and school routine. If the glycosuria falls to negative levels after discharge as so frequently happens, the patient reduces the Lente® Insulin by 2 units after a day of negative tests, or by 4 units if there are symptoms as well. If glycosuria increases uniformly before breakfast and before supper, the patient increases it by 2 units every three to four days until control is again achieved. If a pattern of glycosuria develops in which all the morning urines are 3+ to 4+ and the pre-supper urines are 0 to 1+, the patient increases the Ultralente® or long-acting portion of the Lente® mixture. Conversely, if late afternoon urines are high in sugar, the patient increases the short-acting or Semilente® portion. In this way we achieve as good control over the 24-hour period as with those who use NPH® twice a day and avoid the use of two injections. For this reason we have abandoned the use of NPH® in favor of the Lentes®. As those of you who follow young children know very well, there frequently is no pattern to the glycosuria, and levels will go from negative to 4+. One may be guided in such circumstances by daily urine volumes and adjust the insulin to keep urine volumes in the normal range.

If acetone appears, the patient is to take *regular* insulin at that time. He or she is asked to take 1/5 of his or her daily dose of Lente® for moderate or large and 1/10 for small amounts of acetone. This is repeated at subsequent testings if acetone persists.

If the child becomes ill and is unable to eat or is vomiting, the Lente® is omitted and small amounts of regular insulin are given. Depending on the nature of the problem, the child will be seen in the Emergency Room, admitted, or followed at home by phone instructions.

The diet we propose for the diabetic is no different from one we propose for the non-diabetic. It consists of a good source of protein as the cornerstone of the diet, carbohydrate obtained mainly from starches, a drastic reduction in refined sweets, a mixture of predominantly unsaturated and saturated fats, and, finally, foods containing adequate rough-

age, minerals, and vitamins. We allow the patient to use his appetite as his guide, warning against gorging or excessive snacks, and to use his weight-for-height gains as a monitor of his progress. If overweight is or becomes a problem in the diabetic, he should be approached as a youngster, not a diabetic, with a weight problem. I see no reason to single out the diabetic in his diet therapy when there is no scientific data to suggest this approach is any more harmful to him than to his non-diabetic peers.

We do not attempt to make gross changes in a patient's lifestyle. We recommend regular exercise for all youngsters, but we do not urge all-out vigorous athletics if that is not the child's habit. If exercise can be anticipated, the morning dose of insulin is reduced. If it cannot, increased carbohydrate intake during and after the exercise is required. We explain to teen-agers the serious effects of smoking on the cardiovascular system and urge them not to begin. We explain the hazards of drinking and urge care in this area if they do indulge.

There are a number of special problems we see in children and adolescents.

First of all, the term "brittle" diabetic is frequently used to describe the majority of juvenile diabetics. I believe this is too broad a use of the term and often reflects only a poor understanding of how to manage youngsters whose exercise pattern is unpredictable and whose increased needs with growth are not properly met. I would reserve the term for the juvenile diabetic who is truly difficult to manage; in my experience such patients are few in number and all have been teen-age girls under severe emotional stress.

In the adolescent years there is both an increase in need for insulin and an increase in binding, with insulin resistance of variable degree resulting. Requirements can often rise at an alarming rate to needs of 140 to 160 units per day. The periods of increased need are variable and unpredictable in their length. When the total daily dose reaches 90 to 100 units the dosage is split; 2/3 is given before breakfast and 1/3 before supper. It is frequently advisable to admit the patient to make these adjustments. We admit the teen-ager to the Children's Rehabilitation Center where both physical and school activities can be pursued so that home conditions are more nearly simulated than on a hospital ward. A schedule is set up in which, again, the patient participates. Pre-meal and two-hour post-meal blood glucoses are obtained, urine volumes are measured, and caloric estimates of the food intake that satisfies the patient's appetite in a normal manner are made. Urine volumes are collected in the following manner: patient voids and discards at 7 AM; Urine #1 is voidings up to lunch; urine #2 up to dinner; urine #3 up to 9 PM, and urine #4 overnite to 7 AM. In this manner grams of glucose/hour spilled in respect to a meal can be calculated. Urine volumes collected periodically at home give the diabetic another index of his control.

True insulin resistance is defined rather arbitrarily as a requirement that exceeds 200 units per day, occurring in the absence of ketosis, infections, or other known stresses. The few cases that have occurred in juvenile diabetics have been predominantly among teen-agers 14 to 16 years of age. Most are due to increased antibody formation and respond either to changing to pork insulin, which is less antigenic than beef, or to steroid therapy. We have recently reported the very rare occurrence of severe resistance to insulin in a 17-year-old girl who does not have excessive antibodies either to insulin or to her insulin receptors and showed no improvement on steroids.[1] She was resistant to as much as 5,000 units in pork regular insulin given subcutaneously, but responded normally to a continuous 24-hour infusion of insulin and was well regulated in 60 to 70 units per day. After 5 months of continuous I.V. insulin she remitted spontaneously and is presently regulated on 85 units Lente® in the morning and 35 units Lente® before supper. Since our report, 5 more females of similar age with the same problem have come to our attention.

Over-treatment with insulin can of course result in hypoglycemia, but it can also result in a curious and unexplained phenomenon called the Somogyi reaction, which usually appears after sudden and excessive increases in insulin. The reaction is characterized by polydipsia and polyuria, sometimes to an overwhelming degree. Unlike the patient in acidosis, however, the CO_2 is not elevated though the glucose values may be very high. The explanation given by Somogyi is that the intense hypoglycemia generated by excessive insulin, usually occurring between 2 AM and 4 AM, causes a discharge of adrenalin and a swift rise in blood glucose to excessive levels. The increased glycosuria in the morning and the excessive polyuria suggest the need for more insulin, and a vicious circle ensues. The patient needs to be hospitalized, taken off long-acting insulin, and regulated on regular insulin before returning to Lente®.

One final problem we encounter, especially in

teen-agers, is noncompliance. This must be dealt with in a sympathetic yet firm manner. One can only have deep feelings of empathy for youths who are struggling to establish their own identity in a world generally hostile to teen-agers, and who are aware that in the not-too-distant future they will be on their own.

Our approach,[2] therefore, is to keep the demands on the patient simple but to work closely with him to follow these simple rules:

1) Double void urine twice a day, test for glucose and acetone, and *record* results so they can be reviewed with the patient.
2) Take insulin without fail each morning.
3) Eat a sound diet at regular time intervals and in amounts that satisfy appetite and maintain normal weight.
4) Keep regular medical appointments at 3-month intervals. This provides an opportunity to review the patient's understanding of diabetes and its control.

I would now like to turn to a discussion of abnormalities in serum proteins, platelets, and red blood cells of diabetic subjects discussed at a conference on Diabetic Microangiopathy held by the Kroc Foundation in April, 1976. The conference was arranged by Dr. Donald McMillan of the Sansum Medical Research Foundation and Dr. John Ditzel of Aalborg, Denmark, and the papers have appeared in the November, 1976 supplement of *Diabetes*.[3]

The abnormalities I wish to describe may play a role in the genesis of diabetic angiopathy or are factors that reduce tissue delivery of oxygen by hemoglobin. These mechanisms can lead to tissue hypoxia and subsequent tissue damage.

First, Dr. McMillan,[4] as well as others, describes significant increases in serum proteins in diabetics. No system of proteins is spared. Studies of newly diagnosed diabetics show increases as great as those in established diabetics. Abnormalities are independent of the presence of sequelae of microangiopathy.

The majority of the proteins elevated in diabetics are acute phase reactants: α_1 acid glycoproteins, α_1 antitrypsin, haptoglobin, ceruloplasmin, C-reactive protein, C3C, C4 and C3 activator, and fibrinogen. Albumin levels are lower than normal. By direct measurement Dr. McMillan has shown that diabetic serum has a greatly increased viscosity which is due to the elevation of these proteins. The abnormal viscosity bears no relation to age, sex, body weight or duration or type of treatment of the diabetes.

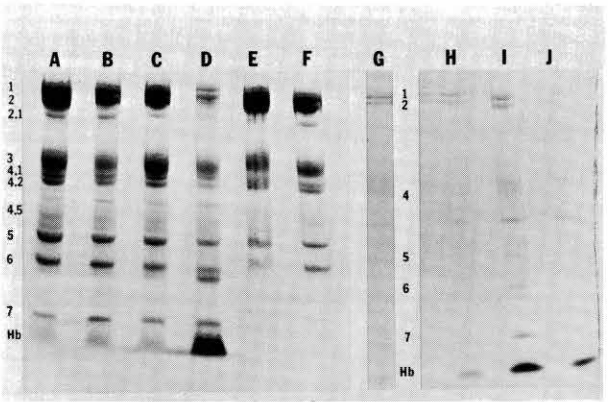
Erythrocyte aggregation is markedly increased due to the increased fibrinogen level as well as to increases in other elongated proteins. Increased erythrocyte aggregation has the added effect of enhancing the coagulation of blood.

Platelet aggregation is also increased in diabetics in the early stages of the disease as well as later. There is a greater sensitivity than normal to aggregating agents such as adenosine diphosphate (ADP), epinephrine, and collagen.

At the same time that intravascular factors are leading to the occlusion of small blood vessels, the fibrinolytic system, the body's defense against vascular occlusion, is decreased. This abnormality is more pronounced in patients with signs of microangiopathy.

We have recently made observations on red cell membranes in diabetics and have noted an abnormal adherence of hemoglobin to the red cell membranes.[5]

Red cell ghosts prepared by the method of Dodge were solubilized in 1% sodium dodecycl sulfate (SDS), separated by gel electrophoresis and stained with Coomassie® Blue (Figure). Increased amounts of hemoglobin were noted in the membranes of diabetics, B, C, and D, compared to those of a non-diabetic child, A. Dislodging the hemoglobin from red cell ghosts with Tris (hydroxymethyl aminomethane), a nonionic buffer, simultaneously



Figure–Membrane proteins of erythrocyte ghosts in 1% SDS separated by polyacrylamide gel electrophoresis and stained with Coomassie® Blue. A to D (untreated ghosts): A, normal 12-year-old; B, 8-year-old on insulin one month; C, top layer of ghosts, pale yellow, D, bottom layer, pink, 15-year-old on insulin five years. E and F (ghosts extracted with 0.01 M Tris, pH 8.2): E ~ A; F ~ B.G to J (Tris eluates): G ~ A, H ~ C, I ~ D, and J ~ B. In D note the doublet of band 6 and the heavy band of hemoglobin, and the appearance of those components in the Tris eluate. The proteins are eluated roughly in proportion to the amount of hemoglobin adhering to the membrane.

removed both peripheral and integral membrane proteins. E and F represent the ghosts after membrane bands 4.1 to 4.5, 5, 6, and 7, as well as the hemoglobin. It is suggested that the tightly adhering and increased amounts of hemoglobin on diabetic membranes interfere with normal erythrocyte deformability because deformability is influenced by the peripheral proteins of the inner aspect of the red cell membrane. Normal deformability is essential for normal blood flow since the diameter of erythrocytes is greater than the diameter of the smallest blood vessels through which they must pass.

Finally there is evidence of disturbance in oxygen transport in diabetics. Rahbar[6] originally described the abnormal increases of the minor hemoglobin components AIa, b, and c in diabetic adults. We have made a similar report in children. In spite of acceptable control of their diabetes, young children, and adults as well, have levels that are twice normal. The important fact about hemoglobin AIc is that it does not react with 2, 3 diphosphoglycerate (2,3 DPG), thus reducing the ability of hemoglobin AIc to yield oxygen to the tissues. Opposing this impairment of oxygen release due to increased levels of AIc, Ditzel has found elevated levels of 2,3 DPG but a lower than normal P_{50}. Phosphate administration to these diabetic children brought their P_{50} to the level of the normal control group. This may be a therapeutic approach toward improving oxygenation of diabetic tissue that warrants further study.[9]

The legitimate question can now be raised as to whether these intravascular abnormalities are not due solely to insulin deficiency and therefore simply reflect inadequate control of the diabetic state with insulin. This may be true. At present we do not know. However, in respect to the increases in acute phase proteins observed in diabetics, studies of protein synthesis of the perfused rat liver by Miller have revealed a very interesting finding.[7] Abnormal alpha cell function is an integral part of the diabetic state.[8] Glucagon secretion continues during periods of glucose plenty and is stimulated by amino acids in the pressure of hyperglycemia. Miller has found that glucagon, either alone or in combination with cortisol, stimulates the acute phase proteins including fibrinogen. The in vitro effects can be abolished with insulin. The possibility thus arises that glucagon excess in a setting of insulin deficiency is the initiating factor of the sequence of events leading to microvascular occlusion. We have begun a prospective study in our young diabetics of the degree of abnormality of glucagon secretion and its progression, and the levels of acute phase proteins, to determine if a correlation exists between them.

I conclude by paraphrasing a well-known slogan, "We want to find a cure for diabetic microangiopathy before our diabetic children leave adolescence," because success of treatment undoubtedly lies in applying it in the trouble-free childhood years.

The figure is reproduced with permission from *Diabetes* (25: 805–930, 1976).

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SCRIPTA MEDICA

Influence of Duration of Homograft on Humoral Responses in Man

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Introduction.

Elevated titers of natural antibodies have been demonstrated in the sera of patients following transplantation.[1-6] These humoral responses were thought to be associated with rejection.[7-10] This concept was challenged by investigators whose studies associated these responses with infection or with injection of heterologous serum.[11-15] Nevertheless, the possible prognostic significance of these relatively simple tests has continued to evoke interest.

This study was undertaken to compare the humoral responses in three different groups of patients with organ transplants in order to evaluate the influence of duration of the homograft and the attendant immunosuppression.

Materials and Methods.

Patient Selection.

Fifty-eight patients who had received homografts were selected for study. The patients were divided into three groups: Group 1, 20 patients tested prior to transplantation and in the immediate post-transplant period with a mean duration of follow-up of 3.3 months; Group 2, 19 patients tested approximately five to seven years following transplantation with a mean follow-up duration of 82.2 months; and Group 3, 19 patients tested approximately ten years following transplantation with a mean follow-up duration of 118.1 months.

Tests for Anti-Rat Erythrocyte Antibodies (Heterophil Antibodies).

Rat erythrocytes were washed three times in saline and reconstituted to a 2% suspension. The test sera were tested at a 1:20 dilution and in serial two-fold dilution for antibody titer. The tubes were allowed to stand at room temperature for 30 mintues, spun for 1 minute at 77 g, and read with the naked eye.

Tests for Rheumatoid Factor (Anti-Fc IgG antiglobulins).

The sensitized human cell test (SHC) was used. A selected DCe/DCe test cell was sensitized with Ripley serum (high-titered antiDC) diluted 1:10. The Rh-positive cells were sensitized for 60 minutes at 37 C and then washed three times in saline. Test sera were titrated in saline in 0.1 ml volumes. Titers of 1:20 or above were considered positive.

Tests for Chymotrypsin Agglutinators (Anti-Fab IgG Antiglobulins) Hydrolysis of IgG globulins.

The IgG globulins of anti-Rh serum (Ripley) were isolated by $(NH_4)_2SO_4$ precipitation followed by chromatography on diethylaminoethyl (DEAE)-cellulose as previously described.[16] Methods of hydrolysis of the IgG globulins with chymotrypsin have been reported.[17]

Sensitizations. One milliliter (containing 5 mg of digested globulin) was added to 0.1 ml of human O, DCe/DCe washed packed cells and sensitized at 37 C for one hour. The sensitized cells were washed three times with saline and reconstituted to a 2% suspension. The cells were tested with goat anti-Fc and anti-Fab (Hyland Laboratories, Los Angeles, California) antisera.

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Methods of testing. Tests for serum agglutinators were performed in tubes by adding 0.1 ml aliquots of the sensitized cells to an equal volume of undiluted and serial twofold dilutions of sera to be tested. The mixture was allowed to stand at room temperature for ten minutes, and then spun for one minute at 77 g and read with the naked eye.

Erythrocyte Sedimentation Rate (ESR).

The Westergren method was used to document the ESR. The fall of the erythrocytes in millimeters in one hour was noted. The normal sedimentation rate with this method is 0 to 15 mm for men with an average of 4 mm, and 0 to 20 mm for women with an average of 5 mm.

Nitroblue-Tetrazolium Test (NBT).

The NBT test was performed according to the modified method of Dorwick[18] on venous blood collected in 4.5 ml vacutainers containing 3.8% sodium citrate. The NBT test was performed as soon as possible after the blood was drawn in order to eliminate morphological changes caused by prolonged exposure to the anticoagulant.

A standard solution of 0.4% NBT was prepared in a 10 ml stoppered volumetric flask by dissolving 20 mg of nitroblue tetrazolium (Grade III reagent, N6876, Sigma Chemical Co., St. Louis, Missouri) in 5 ml of sterile 0.85% isotonic saline. To dissolve the NBT, the stoppered flask was swirled under hot, running tap water until the solution became clear. This solution was then filtered and transferred to a brown bottle and stored at 4°C. The solution was stable for 72 hours.

Citrated blood, 0.2 ml, was placed in a test tube and 0.1 ml of 0.4% NBT was added. The tube was mixed by gently shaking, capped and incubated in a water bath at 37 C for 30 minutes. The tube was removed from the water bath, and one drop of the blood-NBT test mixture was added to two precleaned, labeled glass slides. Special care was taken to avoid damage to the white blood cells. The slides were air-dried and stained with Wright's stain. The stained slides were examined microscopically using an oil immersion lens and 100 neutrophils were counted. Neutrophils were classified as "NBT-positive" if they contained any visible formazan deposits. Cells that were disrupted or clumped together were not counted. The percent of NBT-positive neutrophils was reported as 0% to 15% normal and 16% to 100% normal.

Serum Electrophoresis.

Serum electrophoresis was performed on cellulose acetate strips, using the Microzone cell, Model R-101 (Beckman Instruments, Inc., Fullerton, California).

Results.

Figure 1 shows the humoral responses of the 20 patients in Group 1 during the first five to six months following transplantation. The anti-Fab antiglobulins are designated by dark circles. These antibodies are usually produced as IgG globulins. Only 7 of the 20 patients showed titers at any time in excess of the pretransplant values. Titers below 1:20 are shown as lines without a dot dipping below the 1:20 titer level. Fluctuations in titer are common.

The anti-rat erythrocyte antibodies are designated by open circles. These antibodies are almost always produced as IgM globulins. Fifty percent (10 out of 20) of the patients showed titers in the post-transplant period higher than the pretransplant titer. Once again, fluctuations in titer are common. There

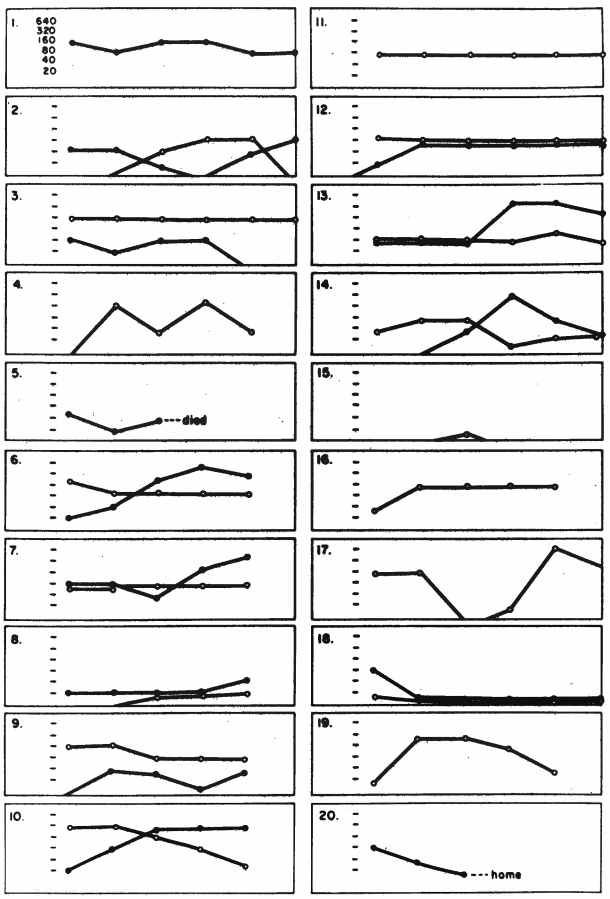

Fig 1—Titers of anti-rat erythrocyte antibodies and chymotrypsin agglutinators in 20 patients in the immediate post-transplant period. *Open circle*: anti-rat erythrocyte; *Dark circle*: chymotrypsin agglutinators.

is a tendency for both antibodies to return toward pretransplant levels 9 to 12 months after transplantation.

Although 4 of these 20 patients gave positive tests for rheumatoid factors, only 3 (patients 2, 7 and 13) showed titers in excess of the pretransplant values. Titers were not constant, as is observed in chronic rheumatoid arthritis, but fluctuated, cresting and then falling, like the other antibodies. The rheumatoid factors were invariably made as IgM globulins.

Modestly elevated titers of anti-sheep erythrocyte antibodies (1:80) were found in only 2 (patients 3 and 11) of the 20 patients.

The blood groups of the 58 patients did not significantly influence the serological responses of the antibodies tested.

Figure 2 shows the titers of the anti-rat erythrocyte antibodies (IgM globulins) and the anti-Fab antiglobulins (IgG globulins) in the three groups of patients in order of increasing duration of the homograft and attendant immunosuppression. The titers of the antibodies in Group 1 were read at three months post-transplant. The titers of Groups 2 and 3 were performed when they appeared for post-transplant check-up. It is apparent from Figure 2 that the number of patients with significant titers of antibodies decreases with duration of the homograft.

Three of the 38 long-term homograft recipients (five to ten years) had positive tests for rheumatoid factors. Neither the presence nor the titers of the rheumatoid factors were related to the duration of the graft. One patient who had shown high titers of rheumatoid factors in the past lost this activity 11 years post-transplant. This patient has developed reticulum cell sarcoma.

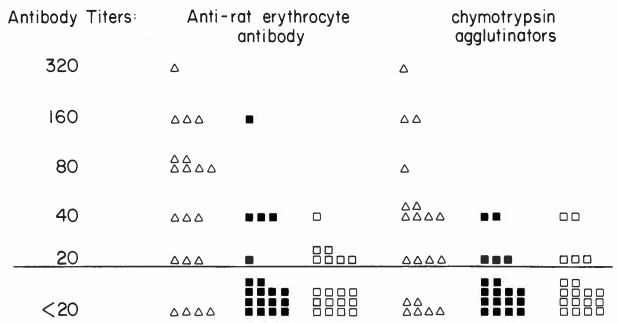

Fig 2—Influence of duration of homograft and attendant immunosuppression on humoral antibodies in 58 patients. *Triangle*: mean 3.3 months; *Dark square*: mean 82.2 months; *Open square*: 118.1 months.

Relationship of level of gamma globulin to the production of humoral antibody.

Electrophoretic patterns of the serum proteins were performed on the three groups of patients. The patients were divided into two groups, those with gamma globulins below 12% and those with gamma globulins above 12% (Table 1). In Group 1, 5 out of the 6 patients with gamma globulin levels below 12% made anti-Fab IgG antibodies as did 9 out of the 14 with gamma globulin levels above 12%. Thus, 70% of Group 1 made these antibodies. However, in Group 2, only 2 out of 11 made these antibodies when the level of gamma globulin was below 12%, while 3 out of 8 made them when the level of gamma globulin was above 12%. In the whole group, 26% made these antibodies. In Group 3, none of the 6 patients with gamma globulin levels below 12% made these IgG antibodies, while 5 of the 13 with gamma globulin levels above 12% did so.

A similar pattern is seen for the IgM anti-rat erythrocyte antibodies. There appears to be no effect of low levels of gamma globulin in the immediate post-transplant group (Group 1), but in the long-term patients, the influence of low levels of gamma globulin on both the IgG and IgM antibodies is demonstrable.

Table 2 shows the relationship between the serum creatinine levels and the failure to show humoral responses in 37 of the long-term transplant recipients. Among the 12 patients with elevated levels of creatinine, the incidence of both IgG and IgM antibodies is decreased. This decrease in antibody production was

TABLE 1

a) Relationship of level of gamma globulins to *IgG antibody production* as chymotrypsin agglutinator

	<12%	>12%	Total
Group 1*	5/6	9/14	14/20 (70%)
Group 2**	2/11	3/8	5/19 (26%)
Group 3***	0/6	5/13	5/19 (26%)

b) Relationship of level of gamma globulins to *IgM antibody production* as anti-rat antibody

	<12%	>12%	Total
Group 1*	6/6	10/14	16/20 (80%)
Group 2**	2/11	3/8	5/19 (26%)
Group 3***	1/6	6/13	7/19 (37%)

* Mean 3.3 months
** Mean 82.2 months
*** Mean 118.1 months

TABLE 2

Thirty-seven patients with long-term homografts showing a relationship between the serum creatinine level and failure to show humoral responses

			IgM Antibody Responses (anti-rat erythrocyte antibody)		
Creatinine levels:	3.1 and above	2.6–3.0	2.1–2.5	1.5–2.0	0.9–1.4
	0/3	0/1	0/2	1/6	11/25
			IgG Antibody Responses (chymotrypsin agglutinators)		
Creatinine levels:	3.1 and above	2.7–3.0	2.1–2.5	1.5–2.0	0.9–1.4
	0/3	0/1	0/2	0/6	10/25

not related to a significantly higher dose of maintenance immunosuppression.

Since infection plays a prominent role in patients maintained for long periods on immunosuppressive agents, the erythrocyte sedimentation rate and the NBT test were performed on 37 of the 38 patients in Group 2 and 3. The erythrocyte sedimentation test was abnormal in 24 of the 37 patients (65%). The numerous factors affecting this test, such as anemia and dysglobulinemia, lessen the value of this test for evaluation of patients with positive tests. The NBT test was only positive in 3 of the 37 long-term patients. Careful review of the discharge summaries corresponding to the period at which the test was done failed to reveal a reason for these abnormal tests. In a further effort to evaluate the significance of these positive tests, C3 and C4 levels were determined on these patients' sera along with 12 other patients who did not show positive NBT tests. The levels of C3 and C4 were not significantly altered in the patients with positive NBT tests.

Discussion.

These studies document humoral antibody activity in the immediate post-transplant period. Rises in titer of both IgG antibodies (anti-Fab IgG antiglobulins) and IgM antibodies (anti-rat erythrocyte antibodies) are demonstrable. The antibody rises are usually inverse to each other, implying that different antigens elicit these responses. The antigens on the rat erythrocyte are also found on dog and rabbit erythrocytes, and related antigens are present on human B erythrocytes. Thus, the antibodies are termed heterophils. McDonald and co-workers[19] recently studied these antibodies and related the absence of the "rat" antigen (HT-A) in the recipients to increased rejection of kidneys from donors with the antigen. Their studies have clinical importance and help to define complex tissue antigens which will receive more attention in the future.

Rises in titer of the anti-Fab IgG antiglobulins could not be closely associated with rejection. These antiglobulins are associated with severe suppurative infection in a hospitalized population.[16] In post-transplant patients, there is a tendency for these antiglobulins to rise when the titers of the anti-rat erythrocyte antibodies are falling,[14] which suggests that these are auxiliary immune responses or responses to antibody-antigen complexes.

The dramatic serological responses in the immediate post-transplant period are not repeated in subsequent years. This is probably due to immunosuppressive therapy. However, we observed no inhibition of polymorphonuclear phagocytosis nor depression of C3 or C4 levels. On the other hand, laboratory tests for infection (sedimentation rate, NBT test) may react nonspecifically, making the diagnosis of occult infection difficult.

As all those interested in transplantation know, the greatest drawback in currently available methods of immunosuppression is their nonspecificity. This fact has stimulated interest in immunological enhancement of the kidney graft. Evidence exists that kidney grafts in rats can survive by virtue of enhancement.[20–25] One approach in humans entailed the production of immune sera to leukocyte antigens, followed by the hydrolysis of the antibody to remove the complement-fixing Fc fragment. The resulting Fab fragments were injected into the recipient to bind to the antigenic sites on the graft.[26] As discussed previously, the serum agglutinators are antiglobulins which have specificity for Fab fragments following hydrolysis. These antiglobulins are naturally occurring IgG globulins commonly found in low titer. However, the serum agglutinators (named homoreactant in rabbits) are not apparently stimulated by the injection of Fab fragments,[27] but once stimulated, their ability to reconstitute the biological potential of the Fab fragment has been established.[28] Therefore, it would be essential to test the recipient for these antiglobulins prior to the injection of the Fab fragments.

In any case, the role of the anti-Fab antiglobulins must be considered when Fab fragments are used to achieve blocking of the antigenic sites of a homograft.

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BOOK REVIEW

Marks, Geoffrey and Beatty, William K. *Epidemics.* New York, Scribner's, xii, 323, illus. 1976. $9.95.
McNeill, William H. *Plagues and Peoples.* Garden City, New York, Doubleday, viii, 369, 1976. $10.00.

Epidemics, like the poor, are always with us; the rapid rise and the equally rapid fall of swine influenza attest this statement. The publication of two books with the common theme of epidemic disease, but with different approaches to it, is therefore welcome and timely, particularly as there are no authoritative books on this subject in print.

Both books rely on established secondary sources and especially on one nineteenth century monument of scholarship: August Hirsch's *Handbook of Geographical and Historical Pathology*[1]; both books describe the chronology of epidemic disease on a worldwide basis; both books are primarily written for the layman; and both books contain extensive reference notes. Where they differ is in their correlation of infectious disease and social history. This, of course, is not surprising as Messrs. Marks and Beatty are a medical journalist and medical bibliographer respectively, while Dr. McNeill is a social historian.

Epidemics presents a broad view of the worldwide pattern of infectious disease, ranging from biblical plagues to recent influenza pandemics. There is no acknowledgement of medical consultation in the preface, and this may account for some of the book's shortcomings, one of which is the absence of comment on the secular changes in the virulence of streptococcal infections. The most glaring omission is rubella, both as an infectious disease and as the cause of birth defects. Rubella would have been an ideal foundation on which to build the last chapter of the book, entitled "The Continuing Scene," which attempts to "provide bridges" between the old and the new epidemiology. One "bridge" used—"Dog Bites: An Unrecognized Epidemic"—is somewhat specious; another, lead poisoning, lacks bibliographical support, ignoring both the Baltimore epidemic of 1932 (probably the best example of a modern epidemic of plumbism[2]) and the possible role of lead poisoning in the fall of Rome.[3] There is also in this chapter a misunderstanding of today's epidemiological approach to the chronic non-infectious diseases, for example, "The term epidemic is currently used loosely in such statements as 'we are faced with an epidemic of heart disease . . .' " As Morris has so frequently pointed out, there is indeed a modern epidemic of heart disease, to which may be added lung cancer and many more syndromes; in fact, modern epidemiological research into chronic disease control is a direct extension of the methods used in epidemic infectious disease investigations.[4]

Two points of historical interest are missed or confused. First, the Black Assizes in England in the sixteenth and seventeenth centuries were six in number, the most dramatic by far being at the old Bailey in London, where the Lord Mayor of London was infected with, and later died from, typhus.[5] Second, the account of John Snow's classic investigation of cholera in Soho is misrepresented, perhaps because it is based on a secondary source paper in *Scientific American* instead of the readily available original work by Snow.[6] The famous Broad Street Pump epidemic occurred in 1854, and not in 1849, the year in which Snow first published his theory of cholera propagation. Although Messrs. Marks's and Beatty's account does not categorically state that the epidemic occurred in 1849, neither does it make it clear that it occurred in 1854; surprisingly, this confusion has appeared several times in epidemiological papers during the past ten years, most notably in the first edition of MacMahon's *Epidemiology* (the date was corrected to 1854 in the second edition).[7] These are, however, minor omissions and the book on the whole is accurate and readable, as one would expect from its authors' credentials. There is a lingering impression that *Epidemics* was written from a previously compiled list of references rather than from primary observations which were verified and added to as the book was written. This must be a fairly common ploy of writing in these "publish or perish" days, the references being used as the point of departure rather than the text and context of the paper.

In summary, *Epidemics* serves its purpose as an interesting, mostly dependable, account of the evolution and ravages of epidemic infectious disease, with

the authors' comments liberally supported by quotations from contemporary documents. Its content is mainly descriptive, although it does not entirely ignore the social consequences of disease outbreaks.

Plagues and Peoples is a more scholarly work, which one would expect from Dr. McNeill, who won the National Book Award for History in 1964 with his book, *The Rise of the West: A History of the Human Community.*[8] Dr. McNeill's book documents and explains the effect of epidemic disease on the structure and evolution of civilization, both through the microcosm of microorganisms and the macrocosm of man's military machinations. The theme of war and disease has been dealt with before by Prinzing[9] and Major,[10] but neither of their works presents the correlation of epidemics and social change which is the basis of *Plagues and Peoples.* Unlike Messrs. Marks and Beatty, Dr. McNeill consulted physicians and medical historians in writing his book, and his accurate medical facts are supported by 55 pages of references and notes. Perhaps a brief description of Pavlovsky's work in landscape epidemiology[11] would have been welcome in the final pages of the book, particularly as it is the antithesis of the Australian experience with myxomatosis, a topic used several times to illustrate man's ecological blunders. As in all works of this type, speculation on early epidemics is replaced by the increasing certainty of documentation and diagnoses after the Black Death; this makes the later epidemics more vivid and interesting to the reader, and of more value to the interpreting social historian.

Neither book contains references to several classical epidemiological texts which should not be ignored in any survey of epidemic disease; the works of Murchison[5] and Drake[12] particularly come to mind. It is surprising, too, to see no reference to America's greatest work on cholera, usually ascribed, wrongly, to Woodworth.[13] The modern works of Ackerknecht[14] and Henschen[15] might have been mentioned, as might the specialized studies of Bell[16] and Ashburn.[17] The social aspects of disease summarized in Zinsser[18] could also have received more recognition, and Hare's *Pomp and Pestilence*[19] might have been noted as an introduction to the theme of *Plagues and Peoples.* Finally, any work describing the world picture of epidemics should contain maps to facilitate the reader's understanding of the geographical and temporal spread of disease; the only map in either volume is one on the Black Death in Dr. McNeill's book. The recent resurgence of interest in medical geography might have been used and some of the many maps in existence copied or modified, if new maps were too costly to prepare.[20] A good example of the use of maps to supplement the text is Siegfried's *Germs and Ideas,*[21] in itself a pioneering precursor of *Plagues and Peoples.*

These two books complement each other; the descriptive content of *Epidemics* is expanded in *Plagues and Peoples* into a fascinating portrait of the interaction of civilization and disease, a subject already dealt with by Sigerist.[22] What McNeill has done for communities needs to be done for individuals, although a book by L'Étang[23] has taken the first step towards relating disease to the decisions affecting history made by a single man or woman. Should this study be made, one could not wish for a more scholarly work than *Plagues and Peoples,* presented perhaps in the familiar style of *Epidemics.*

F.J.S.

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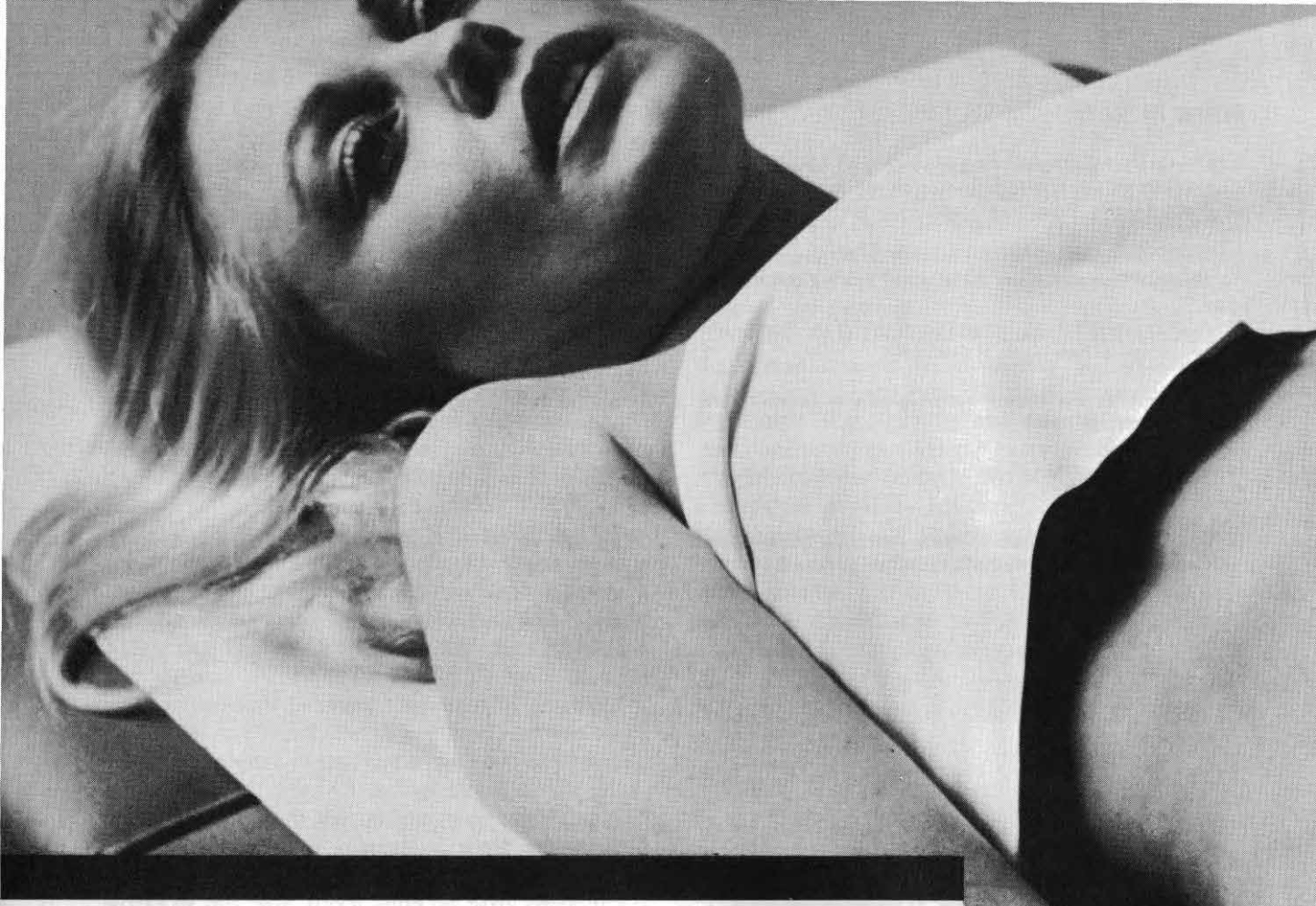

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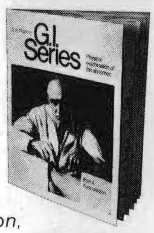

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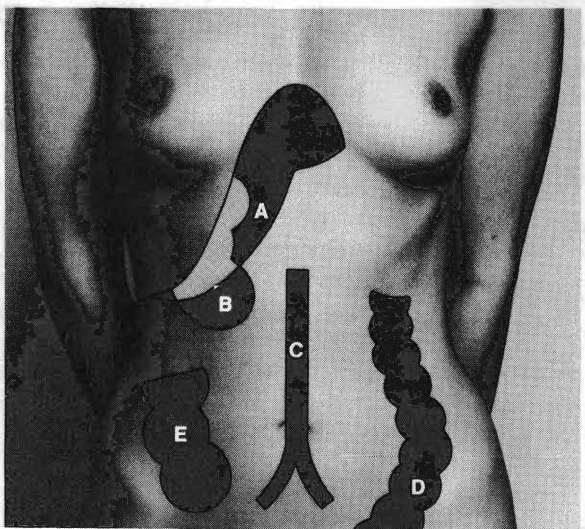
Impossible to outline, unless diseased, distended or enlarged: the gallbladder, pancreas, stomach, small intestine, transverse colon and spleen.



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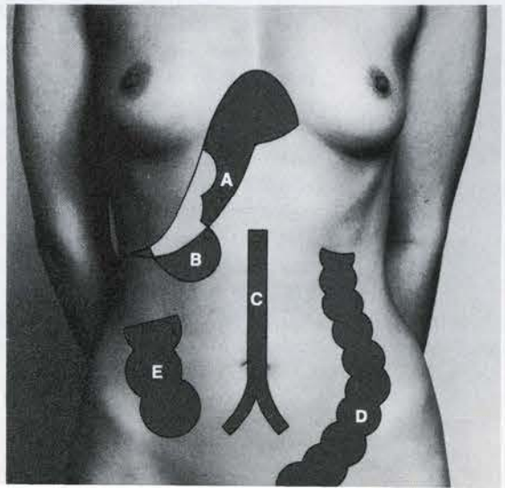

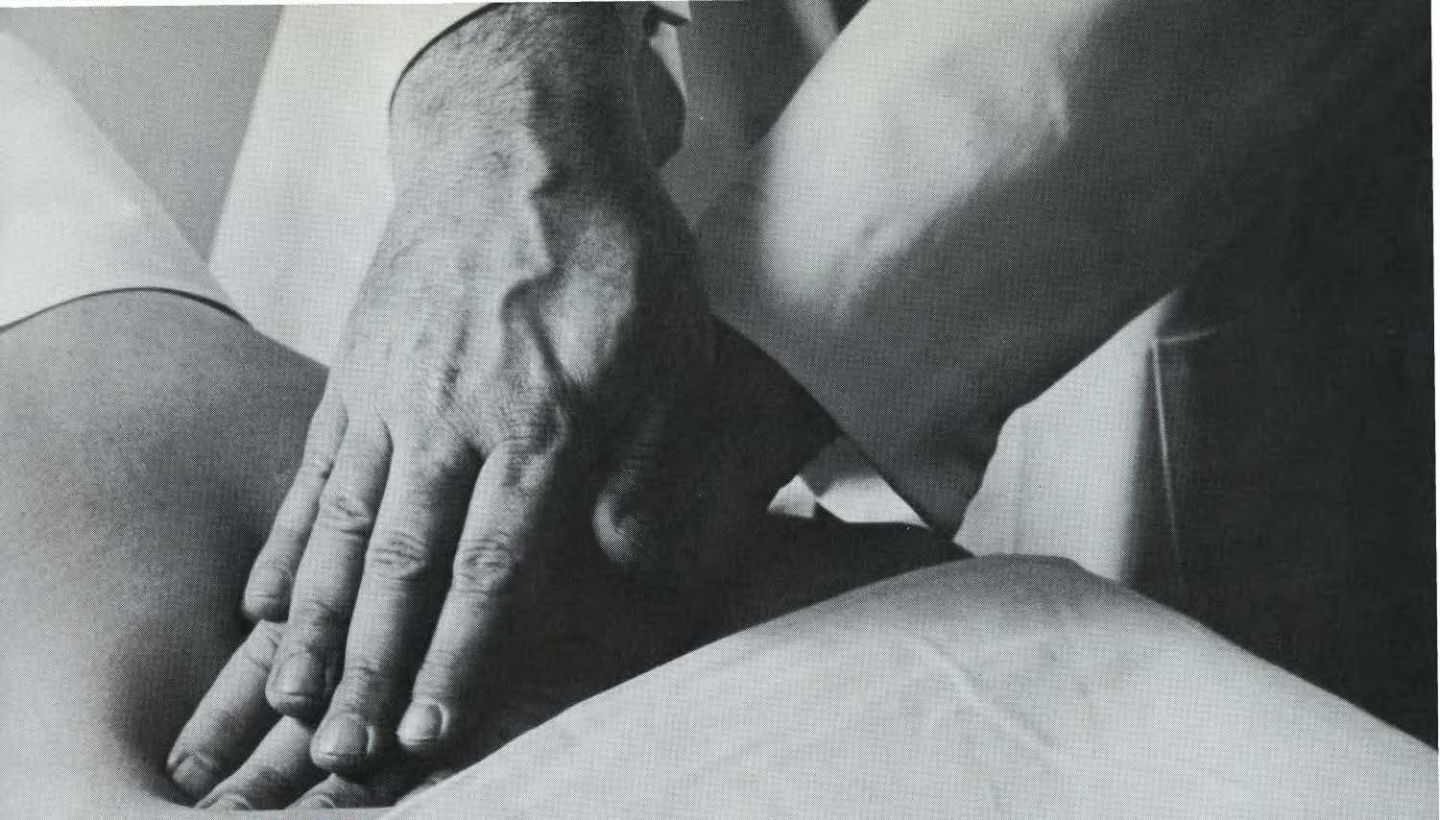

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