THE PATHOGENESIS OF DIABETES MELLITUS*

F. D. W. LUKENS

It was originally maintained by Naunyn that, with very rare exceptions, the underlying cause of diabetes was an inborn biologic inferiority, primarily of the pancreatic reserve. Recently, Joslin[27] has written, "Ever increasing evidence points strongly to the validity of the Naunyn conception of the unity of diabetes with heredity as the common bond." Wilder[41] opens his discussion of the pathogenesis with the heading: "The primary cause of diabetes; inadequate insular reserve." Today most authorities accept this unitary pancreatic hypothesis. It is assumed that upon this inherited tendency there must be superimposed factors which at a given time in the individual's life become prominent enough to precipitate the disease. The development of diabetes in the predisposed is due to a variety of causes. In the first place there are hereditary or acquired conditions which reduce the insular reserve. Secondly, there are hereditary or acquired disorders of metabolism which, by creating a greater demand for insulin, impose an added strain on the island cells. As Long[30] has said, the sum of all this is that insulin deficiency, either relative or absolute, appears to play the central rôle in this disease. In order to define the extent to which the generally accepted theory of diabetes is supported, I shall discuss the inheritance of diabetes and the factors precipitating the disease. It is well to recall that much is still unknown about diabetes, that favorite facts can still be challenged, and that deduction from laboratory experiment has certain limitations. This presentation will, therefore, attempt to draw a picture, which is by no means the final one, of the pathogenesis of this disease.

How is the inheritance of an inadequate insulin reserve manifested? In describing this I shall adopt the view that the knowledge of diabetes must be examined for its relation to the theory of inherited inferiority. In addition to the direct evidence that diabetes is inherited, physiology and pathology will be searched for facts having a possible bearing on the relationship of heredity to pancreatic or pituitary dysfunction.

* Presented before the Yale Medical Society, January 12, 1944.

The inheritance of the disease

Direct evidence for the heredity of diabetes in man

Naunyn based his theory on the finding of diabetes in the relatives of 18 per cent of diabetics. In the pre-insulin days, Joslin observed familial diabetes in 20 per cent of 180 children dying of the disease. The discovery of insulin provided new opportunities which Joslin was quick to grasp. Diabetes usually develops in middle life so that when diabetic children were enabled to live, a far more extensive study of their older relatives became possible. Thus, in 1931, 39 per cent of familial cases were found; and in White's[27] series of 151 children who had lived for 15 years or more the hereditary and familial element reached 52 per cent. After making allowance for errors in diagnosis and for the late age at which diabetes may develop, White was able to show that her data could be explained if diabetes were transmitted as a Mendelian recessive characteristic. Joslin[27] not only observed the number of diabetics whose relatives have the disease, but he has also recorded the *incidence* of diabetes in the relatives of diabetics and non-diabetics. He analyzed a total of 4434 parents and siblings of diabetics and 1290 parents and siblings of non-diabetics. The total incidence of diabetics in the diabetic population was 6.7 per cent, compared with 1.24 per cent in the control non-diabetic population. These findings represent such an advance that a hereditary factor in diabetes may be regarded as established.

In addition to the high incidence of diabetes in the immediate families of diabetics, the recent study of Woodyatt and Spetz[43] is noteworthy. It has long been known that when diabetes occurs in two or more members of the *same generation* of a given family, it may develop in all in the same period of life. There are striking exceptions in a minority group, but in the majority of their cases the ages of onset fell within a span of 15 years. As early as 1865 it was observed by Bence-Jones and others that when diabetes occurred in father and son it might appear at an earlier age in the son than in the father. The same phenomenon may occur in uncle or aunt and nephew or niece and repeat in the following generation. Thus the disease may appear in a first generation in the forties, fifties, or later; in a second generation in the forties, thirties, or twenties; in a third generation in the first or second decade. This phenomenon is known as anticipation. In 100 families that exhib-

ited the disease in two or more generations, Woodyatt and Spetz[43] found evidence of the trend to be definite in 78, probable in 85, possible in 90, and absent or reversed in 10 per cent. The difference between the ages of onset in two succeeding generations varied from 5 to 50 years, but the average in 90 cases was 20 years. When the trend occurs it leads, in the course of two to four generations, to the appearance of the disease in childhood or youth and in such cases the juvenile diabetics are fruits of a *family* diabetes that has already existed in one or more preceding generations. As a continuation of the trend to the progeny of such juvenile diabetics would bring the age of onset into prenatal time (i.e., diabetes in utero or non-conception), the question arises as to whether diabetes is not a self-limited disease which runs its course in a given family and tends to extinction in a limited number of generations.

Further support of these observations and especially the findings in fourth and fifth generations will be awaited with the greatest interest.

The study of diabetes in twins provides further evidence of heredity. White and Pincus[27] review the literature and report their own cases as follows: in 12 of the 19 sets of similar twins both were diabetic (63 per cent) whereas in only 2 (7 per cent) of the 29 pairs of dissimilar twins did each twin have the disease. In Berg's[5] series, after the age of 43 all similar twins were concordant, i.e., all had diabetes.

Turning to the laboratory, the findings of White and Pincus are paralleled by the work of Cammidge and Howard.[7] In 1926 they showed that hyperglycemia in a strain of mice was inherited as a Mendelian recessive characteristic, the results coinciding remarkably with the expected ratios. Hyperglycemia meant blood sugars of 116-120 in contrast to 78-84 in other strains of mice. No other evidence of diabetes was sought for in these small animals. In 1941, Cole, Harned, and Keeler[8] examined the heredity of diabetic characteristics in their rats of the Yale strain. They found that the decreased glucose tolerance was not a simple recessive but was incompletely recessive due to some modifying influence.

Heredity and the pancreas

Having rapidly surveyed the inheritance of *the disease* it is logical to ask what inherited characteristic accounts for or is associated with this familial trend to diabetes. The pancreas first comes to

mind as the organ most likely to be deficient. Pathologists have attempted to relate the size of the pancreas and the number and appearance of the islands to the disease diabetes. Probably the pancreas tends to be small in diabetics but this is not a striking finding. Certainly the variations in pancreatic size have been so great that critical pathologists do not regard them as significant. Pancreatic mass is an inadequate measure of the structure and of the functional capacity of the islands of Langerhans. Efforts to count the islands have not contributed much. The occurrence of lesions in the islands has been a significant item in the development of our understanding and this will be discussed later. At present I should like to note that, to my knowledge, no effort has been made to relate the obvious anatomical defects, such as pancreatic size, to the disease as portrayed by those examining its inheritance. I have, in a preliminary way, attempted to do this by collecting a few cases from the literature (Table 1). The age group of 1 to 30 years corresponds approxi-

TABLE 1

OCCURRENCE OF SMALL PANCREAS IN YOUNG DIABETICS (1-30 YEARS)

	Total cases	*Pancreas 60 gm. or less*	
		Cases	*%*
Diabetic	19	15	79
Normal	13	1	8

mately to the 3rd generation of Woodyatt and Spetz.[43] Only 2 patients, both in the diabetic series, were less than 18 years old. They were included because a small pancreas was estimated from the usual pancreas:body-weight ratio. The pancreatic weight of 60 gm. or less is the lower limit of normal size. From such a small series no conclusions are drawn in spite of the fact that the difference between normal and diabetic is statistically valid. The figures are offered to stimulate this kind of analysis.

At present the number and size of the islands of Langerhans, special stains, etc.[17, 18] seem to add nothing to our understanding of the hereditary defect. Such studies might be revealing if diabetes were found at birth. After birth there is always the likelihood that disease and not heredity has reduced the number of islands. The recent studies in experimental diabetes have emphasized the importance of atrophy of the islands. We have learned again what Allen

saw years ago; namely, that islands may completely disappear, leaving no scar to mark their former presence. The few reports of hypoplasia or atresia of the islands must be accepted with the greatest caution, because the demonstration of a congenital defect is doubtful or impossible in view of what diabetes itself may do to the islands.

Until recently, laboratory evidence that pancreatic deficiency may be inherited amounted to little more than the observation that the amount of pancreatic tissue which had to be removed to cause diabetes varied considerably in different animals. When, as a result of the work of Houssay, of Evans, and especially of Young, the diabetogenic activity of anterior pituitary extracts was elucidated the situation changed. Since it has been possible to inject desired amounts of the same lot of pituitary extract the variable reserve of the intact pancreas has been seen more clearly than before. Following the announcement[44] that permanent diabetes could be produced in dogs by means of anterior pituitary extract, Young[45] reported experiments in other species. He administered the extract to mice, rats, guinea-pigs, rabbits, and cats. Of these animals, the mouse, rat, and guinea-pig were "almost completely insensitive" to the diabetogenic action of the extract. Rabbits and cats showed slight and transitory glycosuria in 50 per cent or less of the animals tested, whereas all but 1 of 25 dogs developed glycosuria. Long, working with rats,[28] and Lukens and Dohan[31] using cats have overcome this species resistance to pituitary extract by partial pancreatectomy. The initial resistance of the normal animal therefore appears to depend on the size of the island reserve. However, the findings in dogs are of greater interest when compared to man. Our results,[12] presented in Table 2, show that in 6 dogs with intact pancreas given increasing doses of extract, there was a large variation in the time required and in the total amount of extract needed to produce permanent diabetes. Two dogs given small constant doses of extract became diabetic. Two other dogs (P 26 and P 29) which failed to develop significant glycosuria from large amounts of extract were easily made diabetic after partial pancreatectomy. The difference in the susceptibility of these animals was not related to body weight or gross pancreatic weight. It appeared to be due to variations in the insular reserve. It seems reasonable to suppose that man may have similar variations in his resistance to the processes which tax his island function.

TABLE 2

ANTERIOR PITUITARY EXTRACT (APE) GIVEN TO PRODUCE PERMANENT DIABETES IN DOGS

Dog No.	*Initial body weight*	*Period* of injection*	*APE†*	
			Total	*Average daily*
	kg.	*days*	*gm./kg./period*	*gm./kg./day*
Dogs given increasingly larger doses				
P16	8.5	43	103.2	2.4
P19	5.7	33	70.4	2.1
P21	9.7	30	81.2	2.7
P22	8.7	24	35.6	1.5
P23	10.4	24	42.0	1.8
P27	4.9	14	26.6	1.9
Dogs given small doses				
P30	19.4	62	16.8	0.3
P36	9.2	98	48.0	0.5
Partially depancreatized dogs				
P26	7.0	24	14.6	0.6
P29	12.9	27	15.5	0.6

* Injections were omitted for as many as 8 consecutive days within these periods.

† Expressed as grams of anterior pituitary gland from which the extract used was made.

(Reproduced from Dohan, Fish, and Lukens. Endocrinology, 1941, *28*, 341-57)

The inheritance of pituitary dysfunction

Having considered the heredity of the disease and the possible inheritance of pancreatic deficiency, it is well to inquire what other abnormal characteristics might be transmitted in the families of diabetics. For simplicity, the inheritance of pituitary hyperfunction, for which there is some evidence, will be cited. Here, also, one can begin with the clinical observations of White. She found[40] that 86 per cent of 227 children measured at the *onset* of the disease were taller than the standards in common use. This excess in stature amounted, on the average, to 2.2 inches. Other clinicians have noted this tendency. Although cases measured at the onset of the disease were over-height, children who had diabetes of several years' duration were below the expected height. The cause of this initial

overgrowth is not clear, as overfeeding and the possible trend of American children to be taller in recent years cannot be fully evaluated. The facts remain that increased growth in diabetics is probable, that it coincides with the onset of the disease, and that the pituitary is the organ which dominates the regulation of growth.

Certain experimental findings pertain to this subject. One of the most striking is the work of Harned and Cole.[20] In their rats with hereditary low glucose tolerance, they observed an increased rate of growth (Fig. 1). This was of such magnitude that the average adult weight of the diabetic Yale strain was 50 per cent

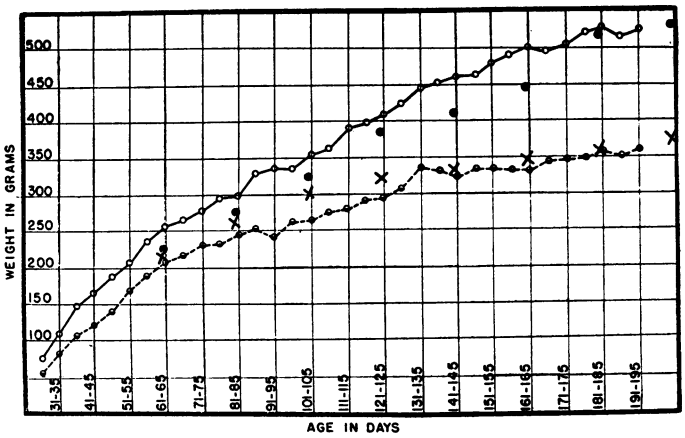


FIG. 1. GROWTH OF MALES, 1938. O—O Yale strain; ⊙----⊙ Wistar strain; x Long-Evans strain; ● Long-Evans strain administered anterior pituitary (Evans and Simpson '31). (From Harned and Cole.[20])

heavier than that of the normal Wistar strain on the same diet. The normal growth curve of the Long-Evans strain paralleled that of the Wistar strain, but when treated with pituitary extract the curve followed that of the Yale strain. This suggested that growth hormones were operating at a higher level in the Yale strain. Furthermore, Harned and Cole described[20] in the Yale strain a greater hyperglycemic response to epinephrine, a slightly higher fasting blood sugar, possibly some delay in the response to insulin, a larger volume of urine per unit body weight, a greater incidence of sterility, and a higher percentage of body fat than in the Wistar rats. Their suggestion that hyperfunction of the anterior pituitary in the Yale strain is the common denominator for these differences seems quite acceptable.

Another indication that the degree of pituitary function may be an inheritable characteristic is found in the species differences seen after pancreatectomy. An excellent tabulation of these variations has been compiled by Long.[30] The wide range of response to the removal of the pancreas in different species is well recognized. Last year Nelson, Elgart, and Mirsky described[34] pancreatectomy in the owl. They found that this carnivorous bird developed a severe diabetes like that of the cat and quite unlike the mild diabetes seen in the duck. They call attention to the rôle played by food habit as well as species in the differences which have been observed in various animals. In agreement with them, I imagine that the carnivora which must hunt their prey, which commonly bolt their food and gorge themselves when opportunity permits, are very dependent on insulin, which facilitates the storage of this food for possible lean days ahead. They are equally dependent on the anterior pituitary, which mobilizes protein and fat from the tissues during starvation. The characteristics thus described are not acquired but are inherited in the several kinds of animals. Because insulin was absent in all cases the results suggest that the level of pituitary function is the feature which is inherited in the several species. For this reason, these oft-quoted findings are cited here as further evidence that the inheritance of an abnormal pituitary function may play a rôle in human diabetes. An increased pituitary activity in man might be especially significant, because Houssay et al.[23] have found that extracts of human pituitaries were the most diabetogenic of any species studied.

In concluding these remarks about the pituitary I must emphasize the tentative nature of conclusions derived from such general observations. There is good evidence that the disease diabetes is inherited in man. There is some evidence that a deficient pancreatic reserve, an overactive pituitary function, or both, may be the particular factors involved. Further analysis of these mechanisms is not warranted at this time.

Factors taxing the functional capacity of the islands and damaging pancreatic tissue

The anatomical causes of insulin deficiency as outlined by Warren[39] are shown in Table 3. Gross destruction of pancreatic tissue (section I) accounts for so few cases of diabetes and its mechanism is so obvious that it needs no comment. The third item

(III), congenital deficiency of the islands, is, as I have said, quite probable on the indirect evidence of heredity. However, its direct

TABLE 3

ANATOMICAL CAUSES OF INSULIN DEFICIENCY

I. *Destruction of pancreatic tissue (both insular and acinar)*
   1. Pancreatitis (a) Acute or (b) Chronic
   2. Malignant disease (a) Primary or (b) Metastatic
   3. Hemochromatosis
   4. Toxic injury

II. *Selective destruction of insular tissue*
   1. Hyaline infiltration
   2. Fibrosis
   3. Toxic injury and lymphocytic infiltration
   4. Hydropic degeneration

III. *Inadequate insular tissue (congenital deficiency)*

IV. *Inadequate blood supply*
   1. Arteriosclerosis

demonstration is impossible at present because agenesis cannot be adequately distinguished from the atrophy which may result from the disease. Item IV, arteriosclerosis, as a cause of diabetes has a few advocates, but most people think this is seldom important because of the frequency of advanced arteriosclerosis in the pancreas of non-diabetics. This leaves the second heading (II), the selective destruction of insular tissue, as the major problem. The subheadings, hydropic degeneration, fibrosis, and hyaline degeneration, are probably stages of the response of the island cells to certain types of injury. Whatever type of lesion is found, several questions arise:—

1. Are these island lesions consistently related to diabetes?
2. When the insular reserve is reduced by any means, what factors impose a strain on the islands?
3. Does such functional stress lead to injury of the islands?
4. Are there noxious agents, other than functional strain, which injure the islands?

The answer to all these questions is yes, and the evidence supporting this affirmative will be reviewed.

The question of the relation of island lesions to diabetes is an old one and is answered by a glance at Table 4, abbreviated from Warren's monograph.[39] Island lesions were observed in 80 per cent of his collected series of diabetic autopsies; they were found in only 16 per cent of the non-diabetic group. There is a fair degree of

correlation between injury of the islands and diabetes. However, there remain the "irritating and puzzling" 20 per cent of diabetics

TABLE 4

LESIONS OF THE ISLANDS OF LANGERHANS (WARREN)

	Total cases	*Lesions*	*"Normal"*
		%	%
Diabetic	842	80	20
Normal	200	16	84

in which the islands are described as normal. For the moment let us say that there is visible evidence for the pancreatic hypothesis in the majority of diabetics and omit comment about the cases with so-called normal islands at this time.

The second and third questions may be answered together. Functional stress of the islands by means of hyperglycemia appears to lead to the development of most of the lesions which have been observed in diabetes. The means by which hyperglycemia may be induced will be discussed by reviewing certain studies in experimental diabetes.

Hyperglycemia and the development of injury of the islands

Experimental diabetes in which some pancreatic tissue is present for study may be produced in 3 ways: (1) by the removal of a sufficiently large portion of the pancreas; (2) by the injection of crude saline anterior pituitary extract into suitable animals; and (3) within the past year alloxan has been found to damage the islands specifically enough so that animals may survive the acute poisoning and be permanently diabetic with no other obvious sequelae. From the standpoint of pathogenesis the first two of these have hyperglycemia as a probable common denominator. Alloxan diabetes will be regarded as a direct toxic agent, i.e., a new pathogenic mechanism.

The use of partial pancreatectomy, first studied by Minkowski, was greatly advanced by F. M. Allen.[1] Using partially depancreatized dogs he showed that the islands developed hydropic degeneration within a week after sugar was found in the urine. This change progressed until the 4th or 6th week of glycosuria. Thereafter the islands of Langerhans underwent atrophy, becoming few and small.

This change was not accompanied by any inflammatory reaction or fibrosis. In summarizing so briefly Allen's description of the islands it must be remembered that the time required for atrophy to develop varied somewhat, but its occurrence was inevitable. In these experiments Allen also demonstrated the protective influence of dietary reduction.

At the same time Copp and Barclay[9] and Bowie[6] demonstrated morphological recovery of hydropic islands in partially depancreatized dogs during treatment with insulin. Although the hydropic islands were restored, recovery of the animals was not possible because the pancreatic remnants had been originally too small to

TABLE 5

INSULIN CONTENT OF THE PANCREAS
(Results of Haist, Campbell, and Best[19])

Normal insulin content	*Hyperglycemia: Diabetes produced*	*Decreased insulin content*
Balanced diet	No	1. Fasting* 2. Low carbohydrate: high fat diet* 3. Insulin*
Not increased after partial pancreatectomy without diabetes	No	
	Yes	4. Partial pancreatectomy and diabetes 5. Anterior pituitary extract

* 1, 2 exert this effect after hypophysectomy; insulin content returns to normal after carbohydrate.

* 2, 3 prevent diabetes when used with anterior pituitary treatment.

support them. In their one dog with atrophy of the islands, no anatomical recovery followed insulin treatment.

Recently Haist, Campbell, and Best[19] determined the *insulin content* of the pancreas after partial resection. They found that the insulin content per gram of pancreas did not rise, but remained normal if the animals were not diabetic. However, if diabetes followed the operation, the insulin content of the pancreas was greatly

reduced. Table 5 outlines the results of Haist et al.[19] concerning the relationship of insulin content of the pancreas and of hyperglycemia to the development of diabetes. They show that the presence of hyperglycemia and not the insulin content of the pancreas is the factor that has been associated with the development of island lesions. Houssay et al.[22] studied the rate of insulin secretion by grafting pancreatic remnants into the necks of totally depancreatized dogs and by recording the fall in blood sugar. They found a diminished secretion in grafts from diabetic animals. In agreement with Allen's histological data these studies by Haist et al. and by Houssay et al. show that partial pancreatectomy is not followed by damage of the remaining islands unless hyperglycemia has intervened.

In the case of partial pancreatectomy, then, it has been established that island lesions do not occur unless hyperglycemia has developed. Although there must be a primary dysfunction to permit the hyperglycemia, the severe structural injury seems to be a consequence of the high blood sugar. Further information about this vicious cycle has been obtained from the study of pituitary-diabetes.

Since Young,[44] in 1937, produced permanent diabetes in dogs by a short course of pituitary extract much work has been done with this method. Here it is only necessary to recall those results especially relevant to the pathogenesis of diabetes.

I have mentioned that certain species do not respond to pituitary extract with hyperglycemia and glycosuria. In these animals there is no injury to the islands. Furthermore, among susceptible animals there are many which become refractory to pituitary extract. The refractory state induced by the protein anterior pituitary hormones has been reviewed by Thompson.[38] After their hyperglycemia has subsided, refractory animals show no island lesions in spite of the continued administration of extract.[31] Finally, pituitary extract fails to produce diabetes in fasted, or fat-fed, or insulin-treated animals.[19, 31] In all of these conditions in which there was no hyperglycemia, there was no island damage. It seems to be generally agreed that pituitary extract, of itself, is not toxic to the islands and that the damage which follows pituitary treatment is associated with the physiological action of its blood-sugar-raising principle.

Knowing that you are acquainted with the work of Long[29] and of Russell[35] it is necessary only to mention that the adrenal cortex is one of the most important organs through which the pituitary exercises its diabetogenic effect. Studies on the diabetogenic activity

have not been extended to the production of all stages of island damage so that I have limited this outline to the results with crude extract. It is probable that in the near future results with adrenal cortical hormones and purified pituitary hormones will be substituted for the results with crude extract which have been described here.

The rôle of the blood sugar level in the reversal of hydropic degeneration

Having considered the factors involved in the production of experimental island damage the companion studies on *the recovery of island injury* may be outlined. I have referred to the early observations of Copp and Barclay[9] and of Bowie[6] which showed that insulin repaired the hydropic degeneration of the islands in partially depancreatized dogs. Dohan and I[31] have extended, with minor modifications, the study of pituitary-diabetes to the cat. The course of pituitary-diabetes in the cat has been studied in more than 30 animals during the past 4 years and the recovery of animals given early treatment has been described. Such recovery has occurred when the islands of Langerhans were in the stage of hydropic degeneration. When treatment was delayed for 3 months the island lesions had progressed to atrophy and fibrosis and no recovery occurred. In connection with these results it must be emphasized that after permanent diabetes was established, it has persisted for an observed duration of 9 months. However, during the first 3 months of glycosuria, the cat with pituitary-diabetes has proved to be a useful preparation in which to study the response to therapeutic measures. Four measures; namely, insulin, low diet, adrenalectomy, and phlorhizin have been tried and found beneficial to the diabetes. Of these, the findings with insulin and phlorhizin will be illustrated by single experiments.

A control experiment showing the induction of diabetes and its persistence after extract is stopped is shown in Fig. 2. In the cat, hydropic degeneration is the conspicuous lesion of the islands for the first three months. After this, atrophy and fibrosis of the islands develop. In the permanent phase, there is a period from the end of the extract treatment until about the 90th day, when the cat has a stable diabetes unless treatment is instituted. This is the period which has been selected for these studies. After atrophy and fibrosis have developed, treatment with insulin produced only temporary control of the metabolism (Fig. 2).

Nine recoveries under insulin treatment have been observed when the treatment was begun in the first three months of the diabetes. In the example shown in Fig. 3, insulin controlled the

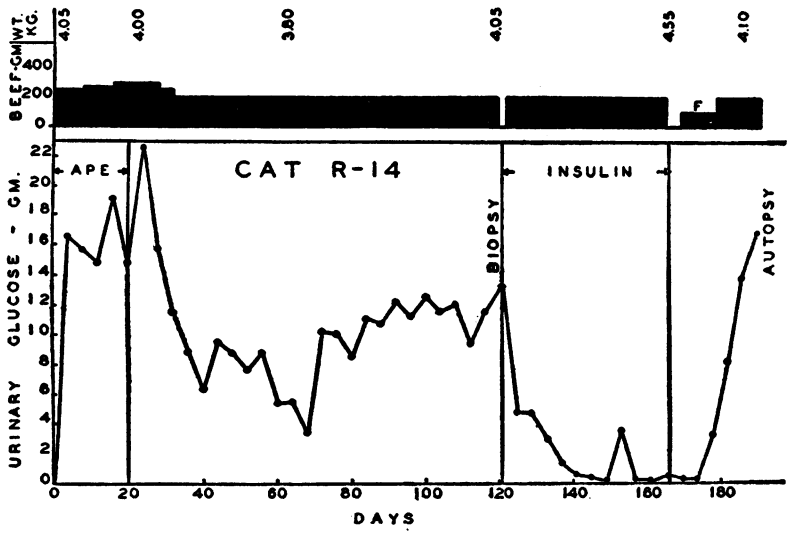


FIG. 2. INDUCTION AND COURSE OF DIABETES IN Cat R-14. Partial pancreatectomy was done 6 days before the first point on the chart. The curve shows the average daily glycosuria of consecutive 4-day periods, plotted on the last day of each period. F over the black area indicates a period on 100 gm. of beef and 10 gm. of lard. APE represents the total period of anterior pituitary treatment, including trial omissions of extract. Biopsy showed island atrophy. (From Lukens and Dohan.[31])

glycosuria and the blood sugar soon became normal. After 23 days of insulin, it was discontinued and the animal remained sugar-free, with a normal blood sugar for the rest of the experiment.

At the time insulin was begun, this animal had severe diabetes, excreting 90 per cent of the available glucose of its diet. In other animals which have recovered, the severity of the diabetes, measured in this manner, varied from 43 to 94 per cent. With regard to the high figures 90 and 94 per cent, it is notable that such severe diabetes has been capable of recovery. It seems that diabetes of any degree of *severity* may be reversed by insulin treatment, and that the *duration* of the diabetes is the conspicuous factor limiting the restoration of the islands and the recovery of the animal.

The hydropic islands of another animal before treatment are shown in Fig. 4, and Fig. 5 from a biopsy taken 65 days after stopping insulin shows the restoration of normal island structure.

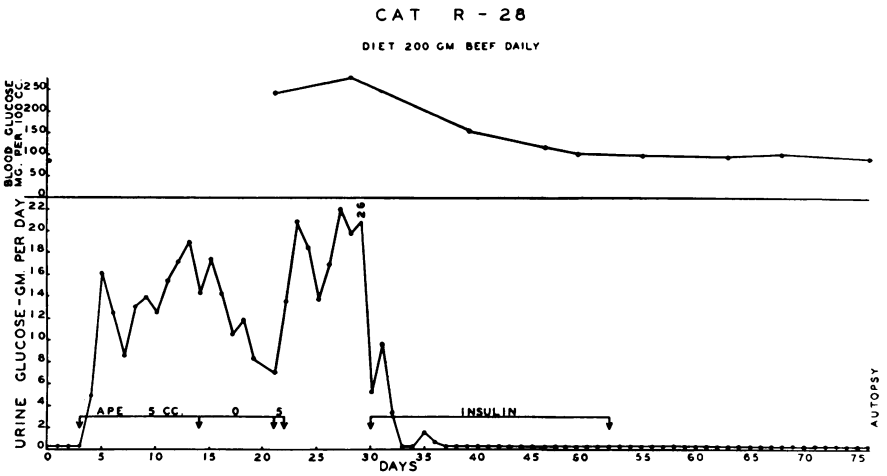


FIG. 3. RECOVERY FOLLOWING INSULIN TREATMENT. Cat R-28. Partial pancreatectomy performed 23 days before the first injection of APE. Extract was given daily for the periods designated by the arrows. The dose of insulin was adjusted to control the diabetes and was gradually decreased in the days before its withdrawal. (From Lukens and Dohan.[41])

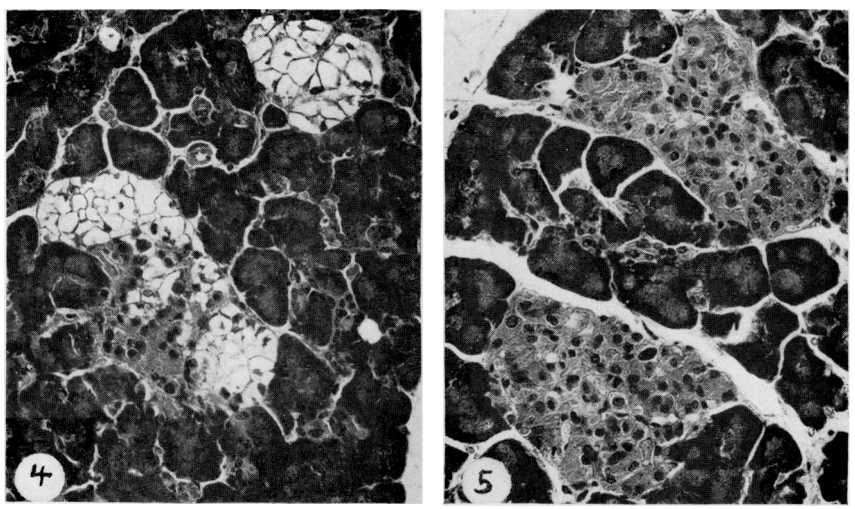


FIG. 4. Cat R-3. BIOPSY OF PANCREAS AFTER GLYCOSURIA FOR 72 DAYS and before insulin treatment. × 240.

FIG. 5. Cat R-3. PANCREAS, 66 days after the end of insulin treatment. × 240. From Lukens and Dohan.

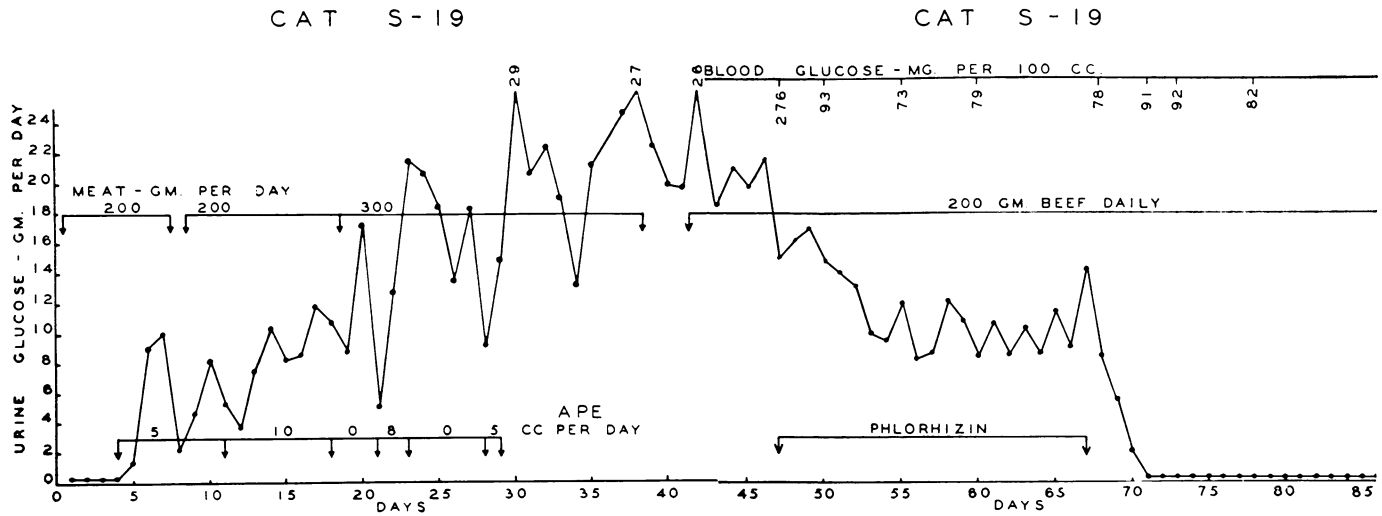


FIG. 6. Cat S-19. Partial pancreatectomy was performed 3 weeks before the first day of the chart. Diabetes was produced by anterior pituitary extract (APE) as shown by the first group of arrows. The first charted blood sugar of 276 was determined just before the first dose of phlorhizin, which was given in dosage of 0.2 gm. daily for the period indicated. The recovery was observed for 19 days beyond the last point on the chart at which time the experiment was terminated.

In connection with these photographs I should repeat that atrophy of the islands which ensues after 3 months of uncontrolled diabetes[31] is an irreversible lesion.

In addition to insulin, we have studied the influence of phlorhizin in pituitary-diabetes. Phlorhizin was employed by Allen[2] in his studies on the pathology of the islands. He showed clearly that the administration of phlorhizin for from 3 to 4 months produced no abnormality in the islands of normal dogs. The glycosuria disappeared in the usual manner after the drug was withdrawn. Allen also studied the action of phlorhizin on the development of hydropic islands in partially depancreatized dogs. These experiments were not conclusive, but their importance to us was the fact that phlorhizin kept the blood sugar low in his diabetic animals. With this knowledge we[32] employed it in cats early in the permanent phase of pituitary-diabetes.

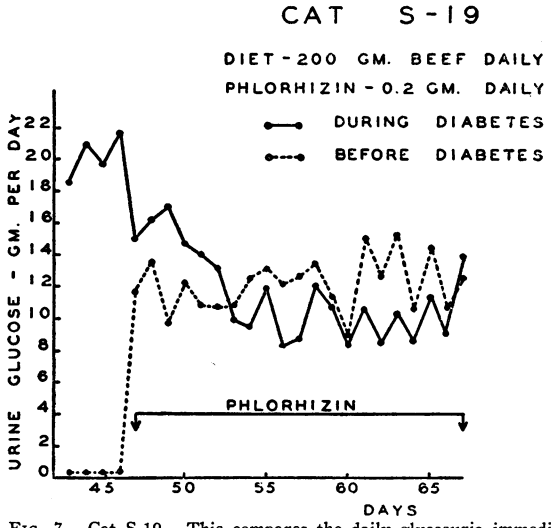


FIG. 7. Cat S-19. This compares the daily glycosuria immediately before and during treatment of the diabetes by phlorhizin and the response of the same animal on the same diet and dose of phlorhizin before it had been made diabetic. The time scale of Fig. 6 has been used.

The induction of pituitary-diabetes is shown in Fig. 6. After the injection of extract was terminated the glycosuria remained at a very high level and, after the standard diet of 200 gm. of beef had been resumed, represented 94 per cent of the available glucose of the diet, calculated in the usual way. The animal was losing weight so rapidly that treatment was begun early The blood sugar, which was 276 before treatment, promptly fell to normal levels. In this, and in another severely diabetic cat, phlorhizin resulted in a reduction of the glucose excretion. The glycosuria diminished until it reached the amount caused by that diet and dose of phlorhizin in the normal cat. When the drug was stopped, the glycosuria disappeared and the cat remained without glycosuria or

hyperglycemia for the remaining 35 days of the experiment. The curves of phlorhizin glycosuria before and after diabetes on the same diet were studied in this animal (Fig. 7). They demonstrate the *initial* effect of the drug on the islands as well as the final recovery which has been seen in all animals treated.

The morphological restoration of the islands observed after phlorhizin treatment[32] is similar to the results obtained with insulin.

As phlorhizin and insulin may each bring about recovery from pituitary-diabetes in the cat, a comparison of the actions of these drugs is in order. For this purpose Table 6 is presented. Such a summary is not designed to portray an exact picture of two substances

TABLE 6

COMPARISON OF THE ACTION OF PHLORHIZIN AND INSULIN

PHLORHIZIN	INSULIN
A. Similar Effects	
1. Reduction of Hyperglycemia	
2. Restoration of Hydropic Islands	
B. Different or Opposite Effects	
1. Impairs reabsorption of glucose by kidney-primary action	1. No such action on kidney is known
2. Increases protein catabolism (N excretion)	2. Decreases protein catabolism (N excretion)
3. Increases ketogenesis	3. Decreases ketogenesis
4. Amount of food assimilated by diabetic cats may be increased, *decreased* or unchanged	4. Food assimilated by diabetics is increased
5. Slight gain or loss of weight occurs	5. Marked gain in weight is usual
C. Uncertain Effects, Common Assumptions Listed	
The following effects are probably secondary to loss of glucose:	The following may include the primary action of insulin:
1. Decreases COH oxidation (secondarily)	1. Increases COH oxidation
2. Liver and muscle glycogen is usually decreased but may be increased by added COH or insulin	2. Increases liver and muscle glycogen in the diabetic

(Reproduced from Lukens, Dohan, and Wolcott: Endocrinology, 1943, *32*, 475-87)

whose actions are far from fully understood, for the comparison in this table refers chiefly to their effects in pituitary-diabetes. There

have been two obvious similarities between phlorhizin and insulin in our diabetic animals. Each lowered the level of blood sugar and each one led to recovery from hydropic degeneration of the islands of Langerhans. Except for these features, the tables present only contrasts between insulin and phlorhizin. Some items have been well established, others are admittedly uncertain. They are presented with the following comments: (1) The reduction of hyperglycemia. It is agreed that this is the result of the effect of phlorhizin on the kidney. That it is not due to the stimulation of insulin secretion is indicated by the work of Houssay and Foglia,[21] who have reported a slightly diminished secretion of insulin during phlorhizin. This may be compared with the finding of Cori[10] that the insulin content of the pancreas is normal in phlorhizinized cats. In contrast, a distinct decrease in insulin content occurs when insulin is administered.[19] (2) The action of phlorhizin is on the kidney. This represents the generally accepted conclusion. The possible influence of phlorhizin on other organs is beyond the scope of this discussion (Nash,[33] Beck,[4] Soskin, Levine, and Lehmann[36]). (3) "Increases protein catabolism." Our experiments[32] have confirmed the increased nitrogen excretion of normal cats and we have found that the increased urinary nitrogen of depancreatized cats is maintained under treatment with phlorhizin. In contrast to this is placed the sparing action of insulin on protein metabolism as measured by nitrogen excretion. (4) The antiketogenic action of insulin has been contrasted with the frequent appearance of ketonuria in the phlorhizin experiments. Most writers[11, 33, 36] regard this ketogenic effect of phlorhizin as secondary to its action on the kidney; i.e., secondary to the loss of glucose or to excessive gluconeogenesis. The table is not meant to imply that phlorhizin is primarily ketogenic, merely that it is far from being as antiketogenic as is insulin. (5) Fat formation, measured as gain in weight, occurred to a limited degree in a few cats during phlorhizin treatment. Almost all animals except those severely diabetic kept a fairly constant weight during treatment. Most of the insulin-treated diabetic animals gained weight rapidly. (6) Common postulates about the oxidation of glucose are given.[11, 33, 36] As in the case of phlorhizin ketonuria this is regarded as being secondary to the deficiency in the supply of glucose because the R.Q. of the phlorhizinized dog can be raised by glucose administration.[11] (7) It is agreed that muscle glycogen is increased by insulin and is usually lowered by phlorhizin,[33] although it may be

maintained during phlorhizin if the animal is well fed. The effect on liver glycogen is less clear. Phlorhizin lowers liver glycogen in normal animals[33] and insulin increases it in diabetic animals. The difference in circumstances makes the comparison highly approximate and the effects of phlorhizin on glycogen are generally regarded as secondary manifestations.

With due allowance for the controversial elements in such a table, the apparent contrast between insulin and phlorhizin, except in their effect on the diabetic blood-sugar level, affords a new type of evidence that the normal level of blood glucose is the factor promoting the recovery of the islands of Langerhans.

At this point the facts to be considered in estimating the rôle of hyperglycemia in the pathogenesis of diabetes may be briefly summarized. Neither partial pancreatectomy nor anterior pituitary extract cause island lesions in the absence of hyperglycemia. Partial pancreatectomy and anterior pituitary extract each lead to hydropic degeneration after hyperglycemia has been present for a week or two. While the lesions are still reversible (cat), the island damage, in the presence of hyperglycemia, *progresses to irreversibility, but may not advance in severity*. The elevation of the blood sugar by perfusion of glucose has resulted in early lesions of the islands.[42] If treated early, the pancreatic islands are restored by various procedures; viz., insulin, low diet, phlorhizin, and adrenalectomy (one experiment), which return the blood sugar to normal levels. In particular, the contrast between phlorhizin and insulin, save for their effect on the blood sugar, supports the hypothesis that hyperglycemia plays a part in the pathogenesis of diabetes. This hypothesis must be regarded as tentative until such facts as the following are more fully understood. Hydropic degeneration has not been seen in the islands of the diabetic rat. Other species have not been studied and ought to be better understood before the results in the cat and dog are accepted as final. Mild diabetes in animals (and man) may persist for long periods without rapid progression in the severity of the disease. This is in contrast to the severe glycosuria produced during the period of pituitary treatment. The minor changes after glucose perfusion are not comparable to the frank lesions of experimental diabetes. In a study of insulin secretion Houssay et al.[24] state "the intensity of the lesions was almost parallel to the level and duration of hyperglycemia." Yet in their conclusions they say, "A high blood sugar lasting 4 days does not alter the

islets. The hypophyseal extract acts, therefore, by some other mechansims." It seems to me that they are showing how long it takes hyperglycemia to act, not demonstrating another mechanism. However, I agree with them that it is well to consider factors other than hyperglycemia. Within the last year this caution has been emphasized by two case reports. One was by Sprague, Priestley, and Dockerty,[37] the other by Howard.[25] In both cases the diabetes was of three years' duration and hyperglycemia had persisted despite insulin therapy. Following the removal of adrenal tumors, these patients recovered completely. By comparison with animal experiments, three years of hyperglycemia should have produced irreversible damage.

In spite of these difficulties, however, the weight of evidence appears to support the concept that the level of blood glucose is a factor of importance in the pathogenesis of diabetes.

The story of alloxan diabetes can be briefly stated. In April, 1943, Dunn and his co-workers[13, 14] of Glasgow reported the production of experimental necrosis of the islands of Langerhans in rabbits by the intravenous injection of alloxan. Although Jacobs[26] in 1937 had found that alloxan produced fatal hypoglycemia, he did not examine the islands. The necrosis of the islands was first produced by lethal doses of alloxan. In August, 1943, Bailey and Bailey[3] in Joslin's Clinic found that by giving glucose along with alloxan the rabbits survived and were diabetic. In September, 1943, Dunn and McLetchie[15] described permanent diabetes in the rat following the subcutaneous injection of alloxan, and in November Goldner and Gomori[16] reported the production of diabetes in the dog. Dr. Kennedy and I have confirmed the results in preliminary studies in the rabbit. Lesions of the islands similar to those described by others[14, 16] were readily obtained.

The significance of these results in the pathogenesis is simple and striking. The long-postulated toxic injury of the islands has become a reality. The time required to produce the damage is a matter of hours, the course of the lesions is one of necrosis progressing to atrophy in a few days. One would assume that diabetes with this type of lesion is irreversible. As far as the islands are concerned there may be little to add to these initial results. On the other hand a verbal report that many diabetic rabbits have cataract and the finding of fatty livers in dogs which did not have ketonuria

suggest that this new method of producing diabetes may have a great future.

Having noted the means by which island stress and damage may be produced experimentally I should return to the clinical causes. Obesity is present in 85 per cent of adult diabetes at or before the diagnosis of the disease. Obesity is the result of overeating; i.e., of overeating relative to the energy requirements. The extent to which obesity may be linked to heredity and to environmental, racial, and family habits is not easy to define. Much more puzzling, however, is the physiological significance of obesity. The failure of pituitary extract to cause hyperglycemia in fasting, and the influence of fat and carbohydrate diets on the glucose tolerance test are not yet part of an explanation of the well-recognized danger of obesity. The more fundamental concept that fat and carbohydrate may be substrates competing for oxidation is still too obscure to explain how any clinical condition,—obesity, infection, endocrinopathy—strains or injures the islands. Before the metabolism is seriously disturbed obesity may commonly have been present for from 5 to 20 years, and such long periods are not reproducible in the laboratory.*

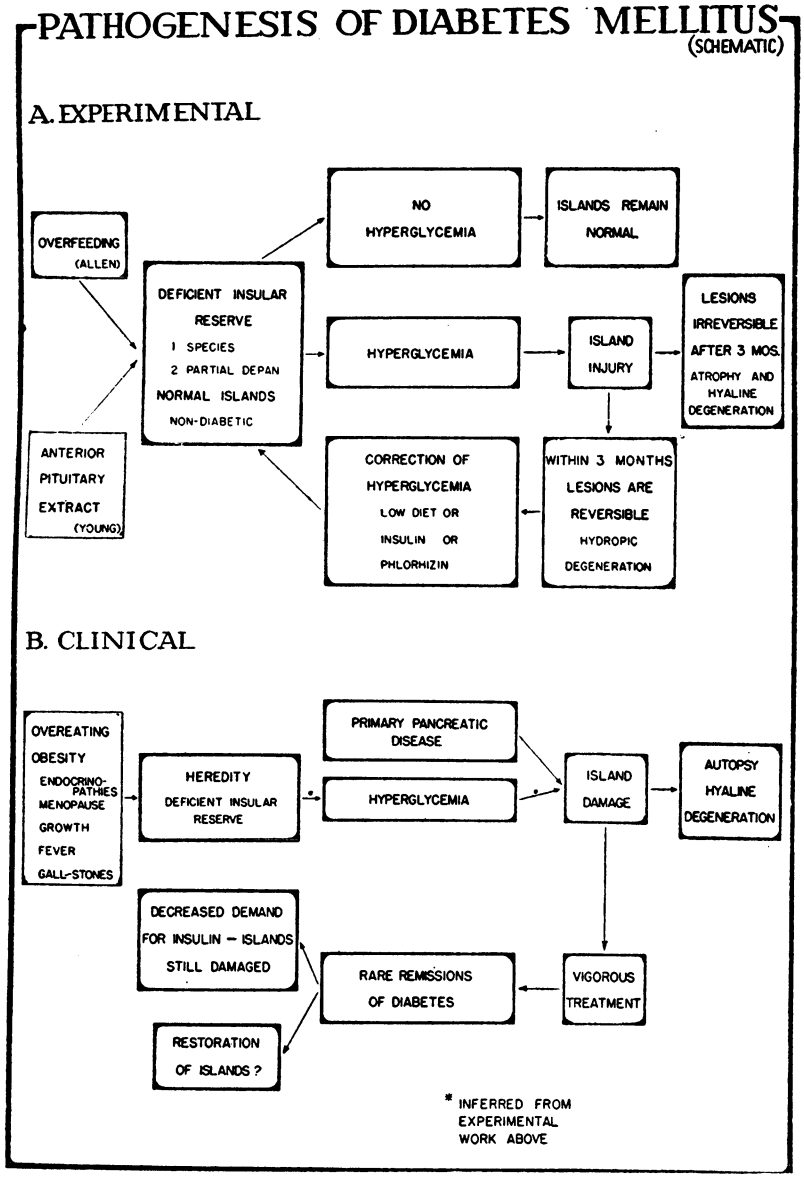
In an effort to condense what has been said, a schematic diagram (Fig. 8) has been prepared. In Section A, the information derived by experimental methods is outlined. The present conception of alloxan diabetes could be included by the addition of a separate line: alloxan → toxic necrosis of islands → atrophy. In Section B, the attempt to apply these experimental conclusions to clinical diabetes offers at best a concept emphasizing the lack of direct information in this field. Should any agent like alloxan be concerned with diabetes mellitus in man it would add fact to the heading "primary injury of pancreas."

In conclusion, the development of diabetes mellitus in man is related to some hereditary element which behaves as a Mendelian recessive characteristic and manifests the phenomenon of anticipation. Clinical and laboratory evidence has been cited to show that the anatomical and functional deficiency so inherited may involve the pancreas, the pituitary, or both. Other types of dysfunction may well share in the background of diabetes.

In *man,* disease of the islands may arise from a variety of

* The experimental hypothalamic obesity described by Brobeck, Tepperman, and Long (Yale J. Biol. & Med., 1943, *15,* 893-904) provides a method of attacking this problem.

unknown causes. In *experimental* diabetes there have appeared two distinct mechanisms by which the islands of Langerhans may be damaged. The first of these is hyperglycemia; the second, alloxan.



The former is thought to cause functional exhaustion or strain of the islands. It is recognized that hyperglycemia may have to be accompanied by other factors as yet unknown in order to produce island damage. The second mechanism of island damage from alloxan is an immediate primary chemical injury. It appears to be quite distinct from hyperglycemia in the speed with which it acts and in the course of the lesions. In the cases associated with hyperglycemia there is hydropic degeneration which progresses to atrophy in weeks or months; in the case of alloxan, primary necrosis is followed by atrophy in a few hours or days. Clinical observation tells us most about heredity; the laboratory indicates possible mechanisms of island injury. The experimental work provides useful analogies for a few dabetics such as the acromegalic, but in the majority of diabetics the *direct* demonstration of cause remains a challenge.

References

1 Allen, F. M.: J. Metab. Res., 1922, *1*, 5-41.
2 Allen, F. M.: J. Metab. Res., 1923, *3*, 623-39.
3 Bailey, C. C., and Bailey, O. T.: J. Am. Med. Asso., 1943, *122*, 1165-66.
4 Beck, L. V.: Proc. Soc. Exper. Biol. & Med., 1942, *49*, 435-39.
5 Berg, H. T.: J. Am. Med. Asso., 1939, *112*, 1091.
6 Bowie, D. J.: Unpublished data.
7 Cammidge, P. J., and Howard, H. A. H.: J. Genetics, 1926, *16*, 387-92.
8 Cole, V. V., Harned, B. K., and Keeler, C. E.: Endocrinology, 1941, *28*, 25-32.
9 Copp, E. F. F., and Barclay, A. J.: J. Metabol. Res., 1923, *4*, 445-51.
10 Cori, G. T.: Am. J. Physiol., 1925, *71*, 708-13.
11 Deuel, H. J., Wilson, H. E. C., and Milhorat, A. T.: J. Biol. Chem., 1927, *74*, 265-97.
12 Dohan, F. D., Fish, C. A., and Lukens, F. D. W.: Endocrinology, 1941, *28*, 341-57.
13 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B.: Lancet, 1943, *i*, 484-87.
14 Dunn, J. S., Kirkpatrick, J., McLetchie, N. G. B., and Telfer, S. V.: J. Path. & Bact., 1943, *55*, 245-57.
15 Dunn, J. S., and McLetchie, N. G. B.: Lancet, 1943, *ii*, 384-87.
16 Goldner, M. G., and Gomori, G.: Endocrinology, 1943, *33*, 297-309.
17 Gomori, G.: Am. J. Path., 1941, *17*, 395-406.
18 Gomori, G.: Arch. Path., 1943, *36*, 217-32.
19 Haist, R. E., Campbell, J., and Best, C. H.: New England J. Med., 1940, *223*, 607-15.
20 Harned, B. K., and Cole, V. V.: Endocrinology, 1939, *25*, 689-97.

21 Houssay, B. A., and Foglia, V. G.: Rev. Soc. argent. de biol., 1937, *13*, 370-84.
22 Houssay, B. A., Foglia, V. G., and Smyth, F. S.: J. Exper. Med., 1941, *74*, 283-95.
23 Houssay, B. A., Smyth, F. S., Foglia, V. G., and Houssay, A. B.: J. Exper. Med., 1942, *75*, 93-106.
24 Houssay, B. A., Foglia, V. G., Smyth, F. S., Rietti, C. T., and Houssay, A. B.: J. Exper. Med., 1942, *75*, 547-66.
25 Howard, J. E.: Personal communication.
26 Jacobs, H. R.: Proc. Soc. Exper. Biol. & Med., 1937, *37*, 407-09.
27 Joslin, E. P., Root, H. F., White, P., and Marble, A.: *The Treatment of Diabetes Mellitus*, ed. 7, Philadelphia, Lea & Febiger, 1940.
28 Long, C. N. H.: Harvey Lect., 1937, *32*, 194-228.
29 Long, C. N. H., Katzin, B., and Fry, E. G.: Endocrinology, 1940, *26*, 309-44.
30 Long, C. N. H.: The Etiology of Diabetes Mellitus. In: Duncan, G. G., *Diseases of Metabolism*, W. B. Saunders Co., Philadelphia, 1942, Chap. 16, 711-21.
31 Lukens, F. D. W., and Dohan, F. C.: Endocrinology, 1942, *30*, 175-202.
32 Lukens, F. D. W., Dohan, F. C., and Wolcott, M. W.: Endocrinology, 1943, *32*, 475-87.
33 Nash, T. P.: Physiol. Rev., 1927, 7, 385-430.
34 Nelson, N., Elgart, S., and Mirsky, I. A.: Endocrinology, 1942, *31*, 119-23.
35 Russell, J. A.: Physiol. Rev., 1938, *18*, 1-28.
36 Soskin, S., Levine, R., and Lehmann, W.: Proc. Soc. Exper. Biol. & Med., 1938, *39*, 442-47.
37 Sprague, R. G., Priestley, J. T., and Dockerty, M. B.: J. Clin. Endocrinology, 1943, *3*, 28-33.
38 Thompson, K. W.: Physiol. Rev., 1941, *21*, 588-631.
39 Warren, S.: *The Pathology of Diabetes Mellitus*, Philadelphia, Lea & Febiger, 1938.
40 White, P.: *Diabetes in Childhood and Adolescence*, Philadelphia, Lea & Febiger, 1935, p. 46.
41 Wilder, R. M.: *Clinical Diabetes Mellitus and Hyperinsulinism*, W. B. Saunders Co., Philadelphia, 1940.
42 Woerner, C. A.: Anat. Rec., 1938, *71*, 33-57.
43 Woodyatt, R. T., and Spetz, M.: J. Am. Med. Asso., 1942, *120*, 602-05.
44 Young, F. G.: Lancet, 1937, *ii*, 372-74.
45 Young, F. G.: Biochem. J., 1938, *32*, 513-23.