Ph.D. Pathology.

GLYCOGEN INFILTRATION (SO-CALLED "HYDROPIC DEGENERATION") IN THE PANCREAS IN EXPERIMENTAL AND HUMAN DIABETES MELLITUS

(The effects of treatment with insulin and with crude anterior pituitary extract on the course and on the pancreatic lesions in alloxan diabetes in rabbits).

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PREFACE

These studies were made during the four years beginning in September 1946 while the author was a graduate student at McGill University under the direction of Professor G. Lyman Duff and a junior number of the staff at the Pathological Institute. To Dr. Duff I am indebted for his original conception of the experiments in which restoration of the affected pancreatic cells by insulin therapy was studied. Prevention of development of these lesions was a naturally concomitant experiment. For his guidance in the accomplishment of these aspects of the project and for his keen interest in the other two projects I am indeed very grateful. The demonstration of glycogen in each of several varieties of "hydropic" pancreatic cells in several forms of experimental diabetes was the original and independent effort of the author. Experimental administration of crude anterior pituitary ("pancreatropic") extract to alloxan-diabetic rabbits was a project conceived by the director and the author together; it was lent impetus by the publication during these studies of titles for papers presented in England by R. F. Ogilvie suggesting similar studies.

Dr. John R. Mote, Armour & Company, Chicago, courteously provided the fresh frozen ox pituitary glands from which "A.P.E." was prepared.

Dr. E. H. Venning, the University Clinic, kindly accepted pieces of pancreas for biochemical analysis of glycogen content in the presence of "hydropic degeneration" and Dr. Revis Lewis of the

Montreal Neurological Institute made several attempts to provide the author with frozen-dehydrated pancreas embedded directly in paraffin. Dr. David R. Murphy generously contributed skilled surgical assistance in the difficult task of resecting most of the pancreas from several dogs. Through the courtesy of Doctors S. D. Kobernick and R. H. More pancreatic sections from a cortisone-treated rabbit were obtained, and from Dr. S. Bencosme a few sections in series from a rabbit given massive infusions of glucose.

Various associates at the Institute helped the author perfect a technic for surgical resection of small pieces of pancreas from severely diabetic and insulin-treated rabbits. Dr. R. H. More was especially helpful. Amongst the technical staff of the Institute Phyllis Valére consistently accomplished the vast numbers of chemical determinations of blood and urine glucose and assisted with the mathematical calculations of data presented in the protocols. Miss Lois Dickie and Miss Sheila Jaques made many of the chemical determinations during the earlier years of the study. Mrs. Betty Gallagher, Miss Evette Latondresse and Miss Vera Kostelowa made most of the histological and many of the histochemical microscopic preparations. Mr. Harold Coletta made all of the photomicrographic illustrations presented in the appendix and many kodachrome transparences of similar subjects.

These studies were assisted by grants-in-aid from the National Research Council, Canada.

PART I

Glycogen Infiltration (so-called "Hydropic Degeneration") in the Pancreas in Experimental and Human Diabetes Mellitus

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Review of Literature

In 1901 Weichselbaum and Stangl described unique changes in the islets of Langerhans of 18 pancreases from comatose diabetic human subjects. Having found the affected islet cells uncolored by methods designed to demonstrate hyaline and mucoid forms of degeneration, they (1902) later interpreted the lesion as a manifestation of cytoplasmic vacuolation and liquefaction, and applied the term hydropic degeneration to indicate its presumably aqueous character. Allen (1913) accomplished the first experimental production of a similar lesion in the islets and ductules of the pancreatic remnants of dogs made diabetic by resection of nine-tenths of the pancreas. At about the same time Homans (1914, 1915) demonstrated that in the islets of such experimental material degranulation and vacuolation developed only in the beta cells. Both Allen and Homans failed to find fat, mucin or glycogen in the affected cells; both concluded, as Weichselbaum did, that the vacuoles represented aqueous fluid. Richardson (1939-40), Ham and Haist (1941), Lukens and Dohan (1940, 1942) and Lukens, Dohan, and Wolcott (1943) described the pancreases of dogs and cats treated with anterior pituitary extract; these authors apparently accepted the idea of aqueous vacuolation as the basis of "hydropic degeneration" in their material. Kennedy and Lukens (1944), Duff, McMillan and Wilson (1947) and Ogilvie (1949) reported "hydropic degeneration" in the pancreas in alloxan diabetes of rabbits, and Goldner and Gomori (1943) observed it in alloxan diabetes of dogs. Duff, McMillan and Wilson did not find fat, mucin or glycogen in the vacuolated islet and ductule cells, while Ogilvie

attributed the lesion to artefactual loss of excessive intracytoplasmic accumulations of serous fluid. Dohan and Lukens (1948) produced permanent diabetes and pancreatic islet "hydropic degeneration" in cats by repeated intraperitoneal injections of glucose. Kadota (1950) recently reported diabetes of permanent duration, precisely simulating alloxan diabetes, produced by single intravenous injections of oxine or of dithizone. After many weeks "hydropic degeneration" was apparent in the islets but not in the ductules of one oxine-diabetic rabbit. Kobernick and More (1950) recently observed a severe diabetic state in rabbits given repeated injections of cortisone; in the pancreases of some of these animals extreme "hydropic" appearances were evident in the beta islet cells, ductular epithelium and centro-acinar cells. Pathology textbooks generally attribute the cytoplasmic vacuolation of "hydropic degeneration" to an exaggerated process of aqueous imbibition qualitatively similar to the supposed mechanism of cloudy swelling.

Homans (1914, 1915) noted the presence of mitochondria in "hydropic" islet cells and Bencosme (1950) has confirmed this observation. Bencosme has also demonstrated the presence of the Golgi apparatus and the macular area in such cells. The structural features of various cells known to contain large quantities of cytoplasmic glycogen (e.g., vaginal epithelium, parathyroid "clear" and Wasserhelle" cells, heart muscle fibers adjacent to areas of infarction, renal tubular epithelium in Armanni's lesion, and liver cells in von Gierke's

disease; see Figures 1-6, incl.) are similar to those of pancreatic islet, ductular epithelial and centro-acinar cells affected by so-called "hydropic degeneration". Except for the presence of glycogen in fetal pancreatic duct epithelium, as reported by Ohohashi (1924), the normal human pancreas has not been reported to contain histologically demonstrable glycogen. Warren (1938) has observed glycogen deposits in the cuboidal and columnar epithelium of pancreatic ducts in human diabetes mellitus; he has never found it in any other type of pancreatic cell nor did he associate the deposits observed with "hydropic degeneration" in the pancreas.

The vacuolated islet cells of partially depancreatized dogs, of APE-treated diabetic dogs and cats and, according to Weichselbaum (1910), of diabetic humans gradually disintegrate and disappear. However, the affected pancreatic ductular epithelial cells of diabetic dogs and both the islet and ductular cells of diabetic rabbits persist in the vacuolated state for many weeks or months. Such cells may be restored to normal appearance by appropriate manipulation of the carbohydrate content of the diet (Allen, 1922[a]; Lukens and Dohan, 1942), by poisoning with phloridzin (Lukens, Dohan and Wolcott, 1943) or by the provision of exogenous insulin (Copp and Barclay, 1923; Duff, McMillan and Wilson, 1947; Lukens and Dohan, 1940; Lukens and Dohan,1942; Part II, this thesis).

Summary

All authors have agreed that the cytoplasmic vacuoles of pancreatic islet and ductular epithelial cells affected by so-called "hydropic degeneration" are devoid of any histologically demonstrable content. Hence, it has been presumed hitherto, such vacuoles contain only excessive amounts of water or serous fluid.

Although the affected beta islet cells have been considered functionally and structurally degenerate, their cytoplasm has been shown to contain several organelles. Restoration of "hydropic" pancreatic cells to normal morphological appearance has been accomplished repeatedly in various kinds of experimental diabetes.

Glycogen has been observed in the pancreatic duct epithelium of the normal human fetus and of the diabetic human. No one has related such deposits to the lesion known as "hydropic degeneration".

Demonstration of Glycogen in Pancreatic Cells Affected by so-called "Hydropic Degeneration".

1. Materials and Methods.

Large numbers of blocks of pancreas were obtained from thirty domestic albino rabbits made permanently diabetic by single intravenous injections of alloxan. (The details of the metabolic changes consequent to various experimental manipulations are reviewed in Parts II and III of the thesis; detailed protocols with histological studies are included in the appendix.) The pancreases of two dogs

which had received repeated injections of crude alkaline APE*, prepared and used according to the method of Young (1938) were studied. Pancreatic remnants were also available from two dogs subjected to extensive resection of the pancreas** in the manner of Allen's (1913) and Homans' (1915) experiments. (Protocols for the two more successful experiments (K 14 and J 56) are presented in the appendix.) Drs. S. D. Kobernick and R. H. More, Pathological Institute, McGill University, kindly allowed me to study sections of pancreas from a cortisone-treated diabetic rabbit. From Dr. S. Bencosme, Faculty of Graduate Studies, McGill University (Pathological Institute) a few sections in series from a rabbit given massive injections of glucose were secured.

Most of the animal tissues were secured at autopsy performed immediately after killing each animal but a few were biopsy specimens. Thin, small slices of pancreas were immersed in freshly prepared Helly's fluid (5% formalin in Zenker base), allowed to fix for 8 to 24 hours, washed in tap water for 8 to 24 hours, dehydrated in alcohol, cleared in toluol and embedded in paraffin. Multiple sections were cut at thicknesses of from 3 to 5 microns and mounted on glass slides with gelatin. The pancreas of the cortisone-diabetic rabbit was fixed in 20% formalin in Zenker base and that of the glucose-treated rabbit in Bouin-Hollande. Helly-fixed, paraffin-embedded rabbit kidney blocks from alloxan-diabetic animals provided "known positive" control

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*The pituitary glands were courteously provided by Dr. John R. Mote, Armour and Company, Chicago, Illinois.

**I am indebted to David R. Murphy, M.D.,F.R.C.S., Diploma in Surgery (McGill 1950) for the successful surgical outcome of these efforts.

material for glycogen staining reactions and for enzymatic digestion tests. Digestion was carried out for half to one hour at 37°C. in buffered 1% malt diastase U.S.P. prepared according to the method of Lillie (1948). All other technical manipulations of control and test sections were made identically and simultaneously.

All of the animal tissues studied herein were stained by the following methods:

1. Hematin, phloxin and saffron (HPS); this was a "routine" stain for general histological assessment of all tissues.

2. Gomori's chromic alum hematoxylin. This was modified from time to time during the years these studies were in progress. Generally, the ponceau mixture of Masson's trichrome was substituted for phloxin. The stain gave clear differentiation of alpha and beta types of islet cells on the Helly-fixed pancreas; it did not differentiate D-cells.

3. Mayer's mucicarmine; use of this stain was discontinued when it was noted to give no more information about the mucin content of pancreatic duct epithelium and duodenal glands than could be equally readily and certainly secured from the Gomori preparations. The latter colored mucin an intense shade of light, bright blue.

4. Best's carmine stain for glycogen. This stain usually provided brilliantly colored photogenic preparations. However, on several occasions (e.g., D 75, see protocol), the stain failed completely with some sections although it subsequently gave positive results with the same blocks. When one of the other glycogen stains

used in these studies stained positively, Best's carmine could always be made to give an identical picture if sufficient repetitions were attempted.

5. Bauer's chromic acid-Schiff's reagent technic for glycogen (Lillie, 1948). Although used frequently, this method was abandoned in the latter course of these studies because of the longer time required and the necessity to maintain a special water-bath at 60°C. It is open to theoretical objection that glycogen might be rendered undemonstrable if hydrolysis were prolonged.

6. The periodic acid-Schiff's reagent reaction as described by McManus (1948) and the Hotchkiss (1948) version of the same test. Except for the use of alcoholic solutions in the latter instead of the aqueous ones recommended in the former and slight differences of timing in the various stages, these methods are basically similar. In these studies the McManus technic came to be preferred for quickness, for economy and stability of reagents and because it appeared to give equally good results in identical tissues. Although neither method colors glycogen selectively, these methods were found less capricious than the traditional carmine method of Best. When used in conjunction with metanil yellow as a background stain, or light green for the same purpose, and omitting the hematoxylin nuclear stain recommended by McManus, the preparations were unusually brilliant.

7. The iodine-reactions of Mancini (1946). The author's claim for facility, clarity, and precision could not be confirmed for any of the three versions he described. The glycogen was stained

but numerous artefacts marred the preparations so that reproducibility and microphotography were unsatisfactory. Since these methods give temporary preparations, their use was abandoned. This is not meant to imply that the method could not be perfected nor that its originator's special use of it with frozen-dehydrated tissues should be questioned.

Human material was obtained from twenty-six autopsies performed between 1926 and 1950 on diabetic patients who died in coma. Most of these pancreases had been fixed in 10% formalin and embedded in paraffin. Acetic-Bouin's fluid, Helly's fluid and absolute alcohol had been used for one case each. After the original micro-sections had been reviewed, new sections were cut from the old paraffin blocks, at a thickness of 5 microns; after deparaffinizing the mounted slides a thin film of celloidin was applied according to the suggestion of Lillie (1948). The stains utilized regularly comprised a less pretentious battery than for the animal tissues, although several cases were studied by each method. Efforts to stain islet cell cytoplasmic granules were usually not made; Mancini's method gave a positive reaction in the one instance when it was employed. Masson's trichrome was used in one case.

Of the twenty-six human pancreases studied, "hydropic degeneration" could be recognized in eleven. (None had been diagnosed at the time of original study). Relevant data are appended in Tables I and II:

Table I

Clinical Data for Eleven Cases of Diabetic Coma

Autopsy Number	Age (yrs.)	Sex	Duration of Diabetes (years)	Terminal Blood Sugar (Mg.%)	Other Diseases or Complications
12333	63	Mc	1 week	1008	None
6194	49	F	1	400	Poor Nutrition
5284	33	M	2	?*	None
12708	65	F	?	?	Infarct Heart, recent
5793	57	F	11	462	Fatty Myocardium
10776	59	F	5	?**	Ascariasis
6088	3	F	1½	450;70	Acute Mastoiditis
11124	72	F	2	?*	Gangrene of Foot
11799	57	F	6	302	Rheumatoid Arthritis
8562	36	Mc	?	516;336	Acute Pneumonia
5901	65	F	2	498	Tbc. Spine and Kidneys

C = colored race

* = no insulin for coma

** = insulin reaction

Table II

Autopsy Data for Eleven Cases of Diabetic Coma

Autopsy Number	Weight of Pancreas (Gms.)	Other Islet Lesions	P.M.hours after death	Hydropic Degeneration	Glycogen Infiltration
12333	80	Hemorrhage Hyperplasia E.I. Mitoses	3	+++	+++
6194	80	None	?	+++	+
5284	50	E.I.	12	++	++
12708	110(Fatty)	None	9	++	++
5793	120(Fatty)	Sclerosis	2	++	+
10776	45	Sclerosis Hyalinization	7	++	+
6088	16	E.I.	22	++	0
11124	"Normal"	E.I.	10	+	+
11799	65	E.I.	$3\frac{1}{2}$	+	+
8562	80	E.I.	15	+	+
5901	90	Sclerosis	11	+	0

E.I. = Elektive Inselleiden of Weichselbaum (1910).

The glycogen content of the pancreas of an alloxan-diabetic rabbit in which severe "hydropic change" was present was determined chemically by the Good, Kramer and Somogyi method as utilized by Venning*, Kazmin and Bell (1946). An attempt was made to employ the method of frozen-dehydration upon other portions of the pancreas of this same rabbit (number B 98) as had been previously attempted with another (number D 75). The author is most grateful to Dr. Revis Lewis, the Montreal Neurological Institute, for accepting the tissues. However, due to the limited facilities available to him, it has not been possible for him to pursue this technical method to the point where the pancreatic blocks are sufficiently perfect for close study. The two attempts secured pancreatic blocks which were neither histologically nor histochemically acceptable.

Observations:

In sections stained by the HPS, Gomori and Masson methods (see Figures 5, 17, 21 and 24) pancreatic cells affected by so-called "hydropic degeneration" appeared slightly to extremely swollen, being apparently distended by accumulation in their cytoplasm of a substance not visible in such preparations. The cytoplasmic membranes were sharply defined and the nuclei usually had well preserved, apparently normal structural patterns. In some of the human material these features were more or less obscured by post mortem autolytic

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* Dr. E. H. Venning of the University Clinic, Royal Victoria Hospital, kindly made this analysis.

changes. In the APE-treated dog occasional hydropic cells appeared to be disintegrating, having pyknotic nuclei (see Figure 20). In most instances the nuclei were situated at the centers of the cells or were only slightly displaced peripherally; they never appeared compressed in the semilunar fashion of cells containing fatty cytoplasmic masses. Often the demonstrable cytoplasm was represented by occasional delicate wisps of lacy, cobweb-like material extending in radial fashion from the nucleus to the cytoplasmic membrane. However, there was present sometimes a more or less vesicular cytoplasm devoid of demonstrable specific beta granules but containing mitochondria, Golgi apparatus and macular area. In some cells one or several small, round or irregular, homogeneous bodies, the Körner of Weichselbaum (1910), were evident.

Mucin was frequently encountered in epithelial cells lining the larger pancreatic ducts. Gomori's method demonstrated this material in the same situations and quantities as did Mayer's mucicarmine technic but depicted it as fine, closely packed, blue granules. Both the McManus and the Hotchkiss versions of the periodic acid-Schiff's reagent technic colored mucin less brilliantly red than they did glycogen. Mucin-filled columnar cells of small ducts were often found together with cells having vacuolated cytoplasm. In no instance was mucin detected in cells of the islets (see Figure 15). Exposure to enzymatic digestion did not remove mucin.

When the pancreases of the alloxan-treated rabbits were stained by the several technics capable of demonstrating glycogen, this substance was always found in the cells affected by so-called "hydropic degeneration" (see Figures 11, 12 and 13). It was present not in traces but rather in abundance and its location coincided accurately with the cytoplasmic vacuolar change in beta cells of the islets, ductular epithelium and centro-acinar cells. Ordinarily there was an obvious ipsilateral shift of the material referable to the artefact generally attributed to the diffusion current of the fixative. Although the material was often represented as granular deposits of variable dimensions, in many cells the whole cytoplasmic area was occupied by a single, homogeneous mass which partially obscured the nucleus. In some instances there appeared to be partial loss of glycogen so that the remnant occupied only a fraction of the cytoplasmic area of the affected cells. This appearance was not more frequent than the similar and familiar aspect of glycogen infiltration of vaginal epithelium, myocardial fibers, renal tubular epithelium and parathyroid cells (see Figures 7, 8, 9 and 10).

The pancreases of the APE-treated and the partially pancreatectomized dogs showed glycogen infiltration of beta cells of the islets, epithelial cells of the ductules and centro-acinar cells. The lesion was similar in every qualitative respect to that of the alloxan-diabetic rabbit pancreas (see Figure 18). However, the lesions observed in the dogs' pancreases were of much milder degree and co-existed with pyknotic and cytolytic changes (see Figure 20).

The presence of glycogen in the vacuolated pancreatic cells was displayed in flamboyant manner in the preparations from the cortisone-diabetic rabbit (see Figure 22). The affect was comparatively exaggerated by the presence of more numerous beta cells than appeared in alloxan-diabetic rabbits, so that the islet lesion as well as the ductular one was obvious at a glance.

If allowance be made for the poor technical quality of the available human material, the appearance of apparent vacuolation of islet cells in it closely resembled the experimental lesions (see Fig. 24). In this material, however, the glycogen appeared much more granular and was less strictly confined to cytoplasmic vacuoles (see Figure 25). Some of it appeared to have been deposited upon the islet rather than within swollen beta cells. Ductular epithelium was relatively uninvolved, even in the presence of marked infiltration of islets. Among the 26 human pancreases studied, "hydropic degeneration" was apparent in eleven (42.3%). Glycogen was demonstrable in abundance in one case, in moderate amounts in two and in small amounts in six. No positive staining reaction could be elicited in the remaining two instances. In no case was glycogen found in the absence of definite cytoplasmic vacuolation (see Tables I and II).

Exposure of control sections to diastase always removed the demonstrable substance from the various cells (see Figures 14, 19, 23 and 26) in which it could regularly be demonstrated if the tissues

were not exposed to diastase (see Figures 11, 18, 22 and 25). Mucin in columnar duct epithelium remained demonstrable despite treatment.

The pancreas of the alloxan-diabetic rabbit contained 0.0025 gram of glycogen per gram of tissue (= 0.25%).

Although some of the beta cells of the islets of the rabbit given massive infusion of glucose appeared to have lost their cytoplasmic granules and a few appeared to have large vacuoles in their cytoplasm, attempts to stain glycogen in the limited number of available serial sections failed. None of the stains for glycogen colored the vacuolar cytoplasmic defects, nor did mucin (Gomori's and Mayer's) stains show differential staining of these cells. Tissue suitable for fat stains was not available (see Figure 16).

Discussion

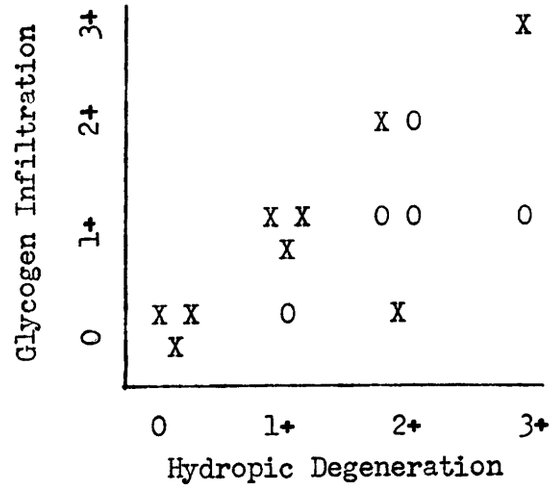
The appearance of the lesion as described is qualitatively similar in every morphological respect in all the diabetic pancreases studied. This is true without regard to species or to mode of production of diabetes.

The apparent absence of glycogen from the cytoplasmic vacuoles of the beta cells of a single rabbit treated with massive infusions of glucose is anomalous; before this result can be accepted as factual, much more thorough studies on additional material should be made.

Woerner (1938,1939) reported similar vacuoles produced by massive continuous infusions of glucose in the guinea pig. In the same species Menten and King (1935) found "hydropic" islet lesions associated with vitamin C deficiency and injections of diphtheria toxin. Critical analysis of Woerner's studies casts grave doubt upon the reported "hydropic" character of the vacuoles observed: in the presence of marked agranularity of the cells only a single animal in the two studies was found to show a few small discrete vacuoles in the cytoplasm. The lesions described by Menten and King seem to be, like those observed earlier by Thomas (1924), unmistakably similar to the classic lesion of diabetic animals. Thomas found the islets affected in guinea pigs dead of an epizootic infection with enteritidis--paratyphoid B organisms. No histochemical studies were made by Thomas or by Menten and King.

Modifications of the lesion called "hydropic degeneration" are known to occur in the islets in diabetic cats, dogs and probably in diabetic humans; these are referable to gradual pyknotic and lytic changes in the affected cells, terminating in numerical atrophy of beta cells. Changes of this type were observed in the APE-diabetic dog (see Figure 20) at a time when the islets showed degranulation of beta cells, vacuolation of their cytoplasm, mitoses in both beta and alpha cells, and apparent increases in number of centro-acinar cells. Weichselbaum (1910) described as <u>elektive Inselleiden</u> a peculiar state of atrophy of islets of Langerhans in human diabetic

pancreases. He was convinced that this appearance developed only in the wake of previous "hydropic change". It seems very likely that this special atrophic lesion could be accounted for by numerical atrophy of beta cells of the islets. However, it would be an unjustifiable presumption to attribute the atrophy in every instance to previous "hydropic degeneration", as he did (Weichselbaum, 1910). The association of "hydropic degeneration", glycogen infiltration and "elektive Inselleiden" in the human diabetic pancreases studied herein have been tabulated (see Tables I and II). There were found in addition to these instances three others in which the atrophic appearance was present but there was neither cytoplasmic vacuolation nor glycogen infiltration. Rearranged in checkerboard histiogram, these data appear to lend support to the idea that so-called "hydropic degeneration" may terminate in numerical atrophy of beta cells.



X = Elektive Inselleiden present

0 = " " absent

Of course, this apparent consequential relationship does not prove that all such atrophic lesions result from "hydropic degeneration".

The atrophic appearance of the islets of Langerhans in permanent alloxan diabetes during the interval before "hydropic change" becomes apparent closely resembles the condition Weichselbaum called elektive Inselleiden when seen in routine preparations not designed to differentiate islet cells by types. In the later stages, when "hydropic change" has developed, the absolute decrease of beta type cells is responsible for the relatively inconspicuous numbers of affected islets as compared to the widespread ductular affect. Of course, in this type of material, the search for islets containing many glycogen-filled cells (such as those selected for microphotographic illustration) is prolonged. The cortisone-diabetic rabbit pancreas, not having received the necrotizing damage of alloxan, provides the most abundant and flagrant lesions; in this instance, however, the details of the development, progress and termination of the lesion are not yet available.

The amount of glycogen determined by chemical analysis of the alloxan-diabetic rabbit pancreas is small but it is similar to the amounts found in human kidneys in glycogen nephrosis (Warren, 1938). This single observation, unsupported by analyses of the normal rabbit pancreas, confirms the existence of some glycogen in the abnormal tissue, but is not otherwise meaningful.

Artefactual removal of the abnormal content of glycogen from the cytoplasm of swollen pancreatic islet, ductular epithelial and centro-acinar cells showing "hydropic degeneration" is probably more responsible for the characteristic vacuolation apparent in ordinary histological preparations than is the concomitant presence of excessive intracytoplasmic water. The granular appearance of glycogen in chemically fixed preparations and the ipsilateral disposition of more homogeneous crescentic masses of intracytoplasmic glycogen represent fairly constant artefacts (Maximow and Bloom, 1948). Fixation by freezing, dehydration in vacuo and subsequent direct embedding in paraffin permit demonstration of glycogen evenly distributed throughout the cytoplasm of various types of cells which normally contain or store it (Mancini, 1946). Microscopic demonstrations of glycogen prepared by other technics inevitably misrepresent the amounts and locations of the material to some extent. Because in the normal process of glycogen storage in the liver, each gram of glycogen stored is accompanied by the simultaneous entrance of 1.16 to 2.33 mls. of water into the liver cells (Fenn and Haege, 1940), it is reasonable to suppose that a similar imbibition may occur when abnormal deposits of glycogen accumulate in the pancreatic islet, ductular epithelial and centro-acinar cells. Nevertheless, this lesion of the pancreas would be more distinctively designated "glycogen infiltration", in keeping with customary usage for pathological accumulations of glycogen in other tissues.

Although so-called "hydropic degeneration" in the pancreas has been accorded unique status as the histopathological common denominator of human and experimental diabetes ever since Allen (1913) first produced the experimental lesion, certain recognition of its presence in diabetic human material has always posed a difficult diagnostic problem. Post mortem autolytic phenomena may produce simulated vacuolar appearances in islet cytoplasm; on the other hand, shrinkage referable to fixation artefact may so distort cytological detail that actual vacuolar affects may become obscured. Warren (1938) found the incidence of the lesion to be no greater in material from the pre-insulin era than in that obtained subsequently. However, in experimental alloxan diabetes of rabbits provision of exogenous insulin, even in amounts insufficient to affect appreciably the level of hyperglycemia and to cause only moderate diminution of glycosuria, may prevent development of the lesion or restore affected cells to normal structural appearance (see Part II). Thus, apparently anomalous observations on routinely prepared human material are to be expected. Weichselbaum (1910) reported vacuolated islet cells in 67 of 183 pancreases of diabetic patients (36.6%), whereas Warren (1938) found only 22 instances amongst 484 autopsies (4.5%). As reported by various authors cited by Kraus (1929) the incidence has varied from 48% to zero in small series. The true incidence of the lesion in human material is probably insusceptible to exact analysis. However, utilization of histochemical technics for demonstrating glycogen in suspected lesions should permit an increased accuracy of diagnosis. The presence of histologically demonstrable glycogen in

human islets of Langerhans affected by characteristic cytoplasmic vacuolation apparently constitutes positive morphological evidence of diabetes mellitus. Failure to find glycogen present in characteristically vacuolar islet cytoplasm need not necessarily mean the absence of the lesion; such failure might also result from either post mortem glycolysis or from imperfections of fixation and staining technics.

The significance of accumulations of glycogen in various pancreatic cells in diabetes remains obscure. It may possibly represent merely one component of the more widespread pathological deposits found in subjects whose diabetes has been poorly controlled. One alternate explanation which may be suggested tentatively is that in the diabetic pancreas glycogen infiltration may be an indication of deranged regeneration of islets. This idea derives from the occurrence of the material in the islet cells, centro-acinar cells and ductule epithelium. These cells are the principal histogenetic sources of differentiated islet cells in fetal and probably also in extrauterine life. Furthermore, the pancreatic duct epithelium of the normal human fetus has been reported to contain glycogen (Ohohashi, 1924) and it is known that embryonic tissues in general contain more chemically determinable glycogen than their mature derivatives. On the other hand, mitotic activity as an indicator of proliferative regeneration of islets is not a common feature of the histopathology of diabetic pancreases showing glycogen infiltration except in APE-

treated animals. A second alternative is suggested by the renal lesion known as glycogen nephrosis. The renal tubular epithelial cells normally resorb and transmit glucose from the glomerular filtrate but they do not normally contain histologically demonstrable glycogen. When their functional capacity to handle glucose is exceeded, as in severe spontaneous or experimental diabetes mellitus and in phloridzin diabetes, these renal cells do accumulate glycogen in their cytoplasm. Allen (1913; 1922) considered the pancreatic islet lesion a regressive morphological response to prolonged excessive functional stimulation; because of its presumed aqueous character and for want of analogous phenomena in other organs or tissues he thought so-called "hydropic degeneration" unique. The demonstration of glycogen in pancreatic cells in diabetes establishes a degree of parallelism between the morphological responses of the over-strained pancreatic islets and the renal tubular cells. However, a normal physiological process provides a basis for the development of the renal lesion whereas no analogous pancreatic function has been determined.

Within the endocrine system there exists another pathological lesion which develops apparently in relation to prolonged excessive functional stimulation and results in hyperplasia of cells which accumulate intracytoplasmic masses of glycogen. Reference is being made here to the increased numbers of clear cells in hyperplastic parathyroid glands associated with chronic renal failure (Brines and Fritz, 1950). It would be interesting to attempt experimental

reproduction of this observation (along lines parallel with those to be reported in Parts II and III); i.e., to produce functional overstrain of the parathyroid glands by inducing chronic renal failure and observe the development of glycogen accumulation in the hyperplastic parathyroid cells. Provision of exogenous parathormone might prevent the anticipated lesion from developing.

Handbooks are replete with cautions regarding the histological demonstration of glycogen; one of the outstanding amongst these is that alcoholic fixation is essential and another, that water must be avoided. Lillie (1947) has studied the advantages and disadvantages of various fixatives and stains in great detail. It would appear from the above demonstrations that fixation in Helly's fluid offers several advantages for the histological demonstration of glycogen infiltration in the pancreas: (1) As with other chrome-containing solutions, comparable amounts of glycogen are found in the central and in the peripheral regions of blocks fixed in Helly's fluid. (2) The use of celloidin for embedding or application of a celloidin film to paraffin-embedded sections mounted on slides is unnecessary. (3) Furthermore, pancreatic tissue so fixed permits utilization not only of methods recommended for histological demonstration of glycogen and enzymatic digestion of control preparations but also provides excellent material for concurrent assessment of islet cytology with respect to cytoplasmic granulation and relative numbers of cell types. Although the periodic acid-Schiff's reagent

reaction colors many substances other than glycogen, its reproducibility, simplicity and brilliant differentiation make it especially valuable for screening old material of doubtful technical quality. Confusion of glycogen with mucin, fibrin, hyaline, basement membranes and other substances and structures colored by the periodic acid-Schiff's reagent reaction can be circumvented at least partially by enzymatic digestion of control sections. Even so, anomalous results occur with all technics employed in this study; therefore, careful utilization of sections known to contain glycogen as controls for both the staining and digesting processes is essential to accurate interpretation.

Summary and Conclusions

1. In so-called "hydropic degeneration" of the pancreatic islets and ductular epithelium of experimental and human diabetes glycogen is demonstrable in the vacuolated cells by common histological technics.

2. The characteristic vacuolar appearance of swollen pancreatic cells affected by "hydropic degeneration" is referable to artefactual removal of intracytoplasmic accumulations of glycogen rather than of excessive quantities of water or serous fluid.

3. This pancreatic lesion would be more precisely and distinctively designated by the term glycogen infiltration.

4. Failure to demonstrate glycogen in suggestively vacuolated islet cells does not necessarily preclude the possibility of its having been present at the time of death.

5. Glycogen infiltration of human islets of Langerhans constitutes positive morphological evidence of diabetes mellitus.

6. The pathogenetic significance of glycogen infiltration in the pancreas in diabetes is obscure.

PART II

The Effects of Treatment with Insulin on the Course and on the Pancreatic Lesions in Alloxan Diabetes in Rabbits.

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Introduction

The occurrence of "hydropic degeneration" in the islets of Langerhans in the pancreases of human diabetic subjects was first reported by Weichselbaum and Stangl in 1901. Homans and Allen independently produced similar lesions in experimental animals made diabetic by partial pancreatectomy in 1913. Warren's comprehensive studies indicated the rarity of the lesion in diabetic human pancreases. Allen suggested that "hydropic degeneration" is a unique lesion representing physical deterioration of the cell consequent to prolonged stimulus to excessive function and Homans showed that in the islets only the beta cells are affected. Thus a firm basis was established for the hypothesis that insulin is secreted by the beta cells. Later investigators have confirmed these findings in animals made diabetic with anterior pituitary extracts, alloxan, glucose and oxine. Allen's concept of "overstrain" enabled him to predict reversal of the lesion by hormone substitution therapy, a feat first accomplished by Copp and Barclay in 1923. Until recently Lukens has doubted the validity of the overstrain theory; he formerly attributed the lesion to a deleterious effect of simple hyperglycemia. Whereas Allen imagined a decreased level of insulin in the blood of the diabetic might be the stimulus to excessive function, Lukens long asserted that hyperglycemia per se (without hyperfunctional stimulation) might damage the islet cells. Recently Lukens has agreed that hyperglycemia may not be the sole pathogenetic factor of importance.

The studies reported in Part II of this thesis were undertaken with the principal intent of attempting an evaluation of the effects of insulin treatment on the course and pancreatic lesions of alloxan diabetes in rabbits.

Survey of the Literature

Weichselbaum and Stangl (1901) gave the first description of so-called "hydropic degeneration" of the islets of Langerhans in their study of 18 pancreases from persons between the ages of 14 and 75 years who died in diabetic coma after known durations of diabetes varying from 3 weeks to 19 years. In some islets they saw epithelial cells characterized by delicate, thin cytoplasm or by a few delicate, single filaments and small granules. The appearance was sketched in an accompanying figure but it was not designated by the term hydropic degeneration. These authors presented another report (Weichselbaum and Stangl, 1902) in which they re-described the lesion in greater detail and proposed the name hydropic degeneration to indicate a process of liquefaction and vacuolation. Weichselbaum's comprehensive analysis of 183 cases of diabetes, which is frequently but incorrectly cited as the first description of the lesion, was not published until 1910. Weichselbaum (1910) also observed a peculiar atrophy of islets, which he termed elektive Inselleiden; he considered this lesion to be a consequence of "hydropic degeneration" only. In the light of present knowledge concerning the

different cell types in the islets, one may reasonably assume that Weichselbaum had observed islets from which most of the beta cells had disappeared. However, his assumption that only "hydropic degeneration" could lead to such a condition is unjustifiable. Therefore, his reported incidence of "hydropic degeneration" may be revised downward from 98/183 to 67/183. Even so, the resultant frequency (36%) is amongst the highest on record (Kraus, 1929). Warren (1938) could find only 22 examples in 484 diabetic pancreases, an incidence of only 4.5%. Because he found the lesion to be nearly as rare amongst cases from the pre-insulin era as amongst those obtained thereafter, Warren thought the great disparity could not be attributed to insulin therapy. Warren also found that he could attain a deceptively similar appearance by allowing the gland to remain at room temperature for eight to ten hours before fixation. In Part I of this thesis I have indicated the probability that the actual incidence of so-called "hydropic degeneration" (i.e., glycogen infiltration) in the human pancreas is insusceptible to exact analysis.

In 1913 Homans reported the failure of Bensley's granule-staining technic to demonstrate beta granules in the islet cells of partially depancreatized dogs. He considered this agranular state a manifestation of exhaustion from functional overactivity. In the same year Allen (1913) noted a similar phenomenon under precisely the same circumstances. However, having approached the problem from a metabolic viewpoint, he showed that removal of 9/10 of the

pancreas produced a diabetic state in dogs. This was associated with a sequence of changes in the pancreatic remnant terminating in "hydropic degeneration" similar to that described by Weichselbaum in human diabetes. Allen found that not only the islets but also the intralobular ductules were affected by the degenerative phenomenom. In later reports Homans (1914, 1915) described production of the same appearance in the pancreatic remnants of cats made diabetic by removal of about three-fourths of the organ. These studies confirmed the work of Allen and also asserted that in the islets only the beta cells were affected. Neither Allen nor Homans recorded the effect of the time factor in cats, but Allen (1922[a]) later showed that at least four days of severe diabetic symptoms were essential to the first unmistakably hydropic appearance in dogs. In this species the lesion attained maximum severity in about three weeks; thereafter, the affected cells of the islets gradually disintegrated until, after five to six weeks, only a few islets composed entirely of alpha cells could be found.

Mice, rats, guinea pigs and rabbits have not been extensively studied, from the histopathological point of view, by the method of partial pancreatectomy because of the unusual difficulty of the surgical procedure in these species. Greeley (1937-38) apparently found no "hydropic" cells in the pancreatic remnants of extensively depancreatized diabetic rabbits. Friedman and Marble (1941) noted marked hyperplasia and proliferation in partially depancreatized diabetic rats; in a single one of their 23 animals they found a few "hydropic" cells.

Richardson and Young (1938) published the first observation of "hydropic degeneration" in the islets of dogs rendered permanently diabetic by treatment with crude anterior pituitary extract. Richardson (1939-40) made the first thorough study of the pancreatic histopathology in such animals. In the early stages he noted islets with agranular beta cells; later, "hydropic changes" were apparent in ductules and beta cells, while terminally the islets consisted of small groups of alpha cells with a few agranular or hydropic beta cells. Ham and Haist (1941) made a similar study. These observers found moderate "hydropic degeneration" in some islets and ductular epithelial cells during the stage of temporary APE-diabetes. In permanent diabetes no normal islets were found; one pancreas contained only scattered alpha cells, another showed islets composed almost exclusively of alpha cells and a third had some "hydropic" beta cells. In each instance the ductular epithelium showed extensive "hydropic degeneration". The reports of Richardson (1939-40) and of Ham and Haist (1941) established the fact that in the dog made diabetic by APE injections the development and progress of the lesions were very similar to those following resection of 9/10 of the pancreas of the dog or 3/4 of the pancreas of the cat. Although the most terminal lesions in the islets were usually devoid of "hydropic" beta cells, these did sometimes persist. Lukens and Dohan (1946) have observed "hydropic change" in beta cells of the APE-diabetic dog after five years of permanent symptoms.

In 1940 Lukens and Dohan found that cats subjected to operative excision of one-half of the pancreas (an amount insufficient to render them diabetic) demonstrated marked susceptibility to the diabetogenic action of APE. In subsequent papers Lukens and Dohan (1941, 1942) showed that "hydropic degeneration" in these experiments developed more slowly than in pancreatectomy-diabetes of dogs or cats or pituitary-diabetes of dogs. The change first appeared in about two weeks, remained florid for three months and then gradually diminished (through disappearance of affected cells). The final picture was one of atrophy of beta cells; fibrosis, hyalinization and lymphocytic infiltration of islets were sometimes found.

In the normal rabbit Ogilvie (1944) was unable to produce permanent pituitary diabetes, but one rabbit, after temporary diabetic symptoms consequent to 30 daily injections of APE, showed "hydropic vacuolation" of ductular epithelial cells but not of islets.

Since Dunn, Sheehan and McLetchie (1943) discovered the necrotizing action of alloxan on the islets of Langerhans, almost every species of animal ever used for laboratory experimentation has been subjected to injection of alloxan. Many of these studies have only indirect relevance, or none, with respect to the question of "hydropic degeneration"; many others have dealt only with the morphology of the early necrotic lesions in the islets. Occasional reports included mention of the histopathology visualized in one or

a few animals after intervals of diabetes of several months. Review of those studies concerned only with the earliest phases of pancreatic histopathology will not be attempted here. However, it should be noted that there exists no consensus agreeing upon the persistence of some beta cells after a single large dose of alloxan.

Goldner and Gomori (1943) found marked "hydropic degeneration" of intralobular ductules on the 16th day following administration of alloxan to dogs but in none of their animals were "hydropic changes" evident in islet cells. Beta cells, if such they were, occurred in very small numbers and were characterized by completely agranular cytoplasm. Bailey, Bailey and Leech (1944) studied the effects of repeated small doses of alloxan in rabbits. By this device they produced unique lesions of the islets: normal, pyknotic, mitotic and "hydropic" cells were evident together after only 7 and 13 injections in two animals. Although Bailey, Bailey and Hagan (1944) examined the pancreases of alloxan-diabetic rabbits after periods of as long as three months, they found no "hydropic change". Duffy (1945) found the decreased islet tissue to contain very few beta cells ten days after injection of alloxan in rabbits; some of the cells were normal and some were agranular. Between the 20th and 270th days islets were decreased in both size and number and contained beta cells in the proportion of 1:1 as compared with alpha cells. Some beta cells were normal, others enlarged and others

showed pyknotic nuclei. Seven animals showed agranularity of beta cell cytoplasm. Of these, three showed coarsely reticulated, vacuolated cytoplasm. Two animals showed hydropic change in beta cells. One of them was examined after 97 days of diabetes; the other had persistent normoglycemia following 120 days of moderate hyperglycemia and had been aglycosuric for 70 days before autopsy. Duffy did not observe "hydropic" ductular epithelium; he did see one mitotic figure in a beta cell. Kennedy and Lukens (1944) saw "hydropic" beta cells in the islets of two alloxan-diabetic rabbits which had been diabetic 48 and 58 days.

Hard and Carr (1944) studied the islets of Langerhans in nine rabbits made diabetic with alloxan, 7, 11 and 25 days before autopsy. In the earlier studies, they found the number of islets to be normal but their size to be reduced. Although most of the islet cells were of alpha type, some "undifferentiated cells" practically devoid of cytoplasm but having normal nuclei were also seen. Later the islets seemed normal in number and size; they were composed of a majority of alpha cells, numerous agranular cells and some normally granular beta cells. In many islets normal alpha:beta ratio prevailed. Hence, Hard and Carr concluded, complete necrosis of beta cells did not occur and regeneration of a functional beta cell with amelioration of diabetes was possible.

Several authors have reported "cytoplasmic vacuoles", "hydrops" and "hydropic change" affecting the beta cells of rats

(Lackey, Bunde, Gill and Harris, 1944), pigeons, a barn owl and ducks (Scott, Harris and Chen, 1945) and monkeys (Bannerjee,1944) after very brief intervals following injection of alloxan. Sometimes there was no associated disturbance of carbohydrate metabolism. Usually the descriptive material was so meager that one may reasonably doubt the accuracy of the interpretations as reported.

Duff, McMillan and Wilson (1947) first reported that "hydropic degeneration" was the characteristic lesion in the islets of Langerhans and intralobular ductules of the pancreas in permanent alloxan-diabetes in rabbits. After 45 to 90 days of diabetes the lesion was always present if the average fasting blood sugar level remained above 303 mg.%. Furthermore, the lesion persisted without histological evidence of further change for periods up to one year. The authors acknowledged the uncertain origin of the "hydropic" islet cells and could not demonstrate mucin, fat or glycogen in the vacuoles. They also found that adequate insulin therapy was associated with restoration of all the affected cells to normal appearance. In the "restored" islets indifferent, agranular cells and typical beta cells were present with alpha cells.

In a recent publication Kadota (1950) has compared the diabetic response of rabbits to several organic reagents. In a very striking manner evidence is presented to indicate that many of the phenomena of alloxan diabetes can be reproduced precisely by both oxine

(8-hydroxyquinoline) and by dithizone (diphenylthiocarbazone). Not only were all of the typically triphasic changes of the blood sugar level observed at comparable intervals following single intravenous injections, but the early necrotic and subsequent atrophic histological phenomena were reproduced exactly. Furthermore, in one rabbit autopsied after 120 days of oxine-induced permanent diabetes, typical "hydropic degeneration" was observed in the islets of Langerhans. The affinity of alloxan, oxine and dithizone for heavy metals, including zinc was emphasized by Kadota as a significant factor in the theoretical explanation of the mechanism of action of these reagents.

Dohan and Lukens (1947, 1948) have presented a study of the production of unquestionable "hydropic degeneration" in the beta cells of the islets of Langerhans associated with permanent diabetes in response to prolonged, repeated, intraperitoneal injections of glucose in cats. In one instance the blood sugar level returned to normal and glucosuria disappeared three months after glucose injections were discontinued; at autopsy two weeks thereafter the pancreatic islets still retained many "hydropic" cells. The authors considered the relation of development of "hydropic degeneration" to duration and degree of hyperglycemia most important; caloric intake, gain in weight and local action of the intraperitoneally-injected glucose had no important role.

Warren (1938) recorded 22 instances of "hydropic degeneration" in the islets of 484 human diabetic subjects and 2 instances in 200 non-diabetic "controls"; he stated that in most instances "apparent hydropic degeneration" in human islets was simply post mortem change. Apparently Sneeden(1946) fell into the error of misinterpretation of autolytic phenomena as "hydropic change": he found spaces without stainable material between the islet cells and small, homogeneous, edematous islet cell cytoplasm in 25 of 40 men who had been subjected to prolonged malnutrition often complicated by pulmonary tuberculosis. Glucosuria was not found in these men; blood sugar levels and glucose tolerance tests were not done. Gomori (1941) reported vacuolation of the cytoplasm of beta cells in five pancreases from non-diabetic patients and marked "hydropic degeneration" of the beta cells in a pancreas resected for benign islet cell adenoma. He thought the lesion might be attributable to massive infusions of glucose required to control hypoglycemic seizures.

Allen (1913, 1922[b]) was unable to show any alteration in the islet cells of normal dogs given large amounts of glucose intravenously and orally; most investigators have reported cytological changes of significance in various species. Gomori, Friedman and Caldwell (1939) noted increasing degrees of degranulation of beta cells as the blood sugar rose and reappearance of granulation during the ensuing fall. The authors inferred a direct relationship between the presence of granules and the insulin in the beta cells. Barron

(1948) concluded from studies on white rats that beta granules actually represent a precursor of insulin. Richardson (1939-40) found that partial loss of beta granules might be produced by injecting non-diabetogenic fractions of APE in dogs. The same fractions, however, always induced some degree of vacuolation in ductular epithelium. Woerner (1938), 1939) claimed to have produced (and is frequently cited as actually having done so) "hydropic degeneration" of beta cells in the islets of guinea pigs by continuous intravenous administration of dextrose. However, the lesion he described and portrayed is scarcely comparable, at least in degree, with that found in various forms of experimental diabetes. In fact, from his report it appears that only one animal was found to show a few small vacuoles in the cytoplasm of an agranular cell. Also, Woerner stated that alpha cells were difficult to distinguish from beta cells in these studies, for alpha cells were also vacuolar. In his first report, hyperglycemia rarely occurred and glucosuria was absent. In the second study blood sugar levels often exceeded 200 mg.% (even attaining values of 1300 and 1670 mg.%) and glucosuria was constantly present. Nevertheless, even after 11 days no beta cell appeared "hydropic": agranularity of some cells and swollen globular mitochondria were the sole manifestations of degenerative reaction to glucose treatment.

To date the experiments of Dohan and Lukens (1947, 1948) are the only ones describing actual "hydropic change" in the islets in

response to glucose infusions; the amounts of glucose given over periods of several months by thrice-daily intraperitoneal injections were heroic.

Thomas (1924) observed "hydropic change" in the islets of guinea pigs which had died of an epizootic. He recovered Enteritidis-Paratyphoid B organisms from these animals and, by experimental infection of other guinea pigs, successfully reproduced the lesion. He noted apparent correlation of increased blood sugar levels in the infected animals with the presence of the lesion and, therefore, suggested a similarity to the diabetic dogs of Allen and Homans. Menten and King (1935) produced "hydropic" beta cells in the islets of vitamin C-deficient guinea pigs by injecting sublethal doses of diphtheria toxin; their animals exhibited hyperglycemia and impaired glucose tolerance. It is difficult to appraise the reports of Thomas and of Menten and King. The photographic reproductions of the lesions produced by Thomas are convincing. However, these are the only reports of "hydropic degeneration" in the islets of Langerhans in guinea pigs. Neither pancreatectomy nor APE injections nor alloxan have been reported to produce such lesions in this species.

By restricting the dietary carbohydrate content Allen (1913, 1922 a,b) prevented the development of "hydropic degeneration" in partially pancreatectomized dogs. In this way dogs which showed glucosuria and hyperglycemia on full feedings could be brought to

a state in which they showed continuous hyperglycemia without glucosuria for as long as one month. Such dogs did not have "hydropic change" on pancreatic biopsy. If they were then given a full diet, the lesion developed in degree corresponding to the duration of aggravated symptoms. Allen (1922a) demonstrated that dietary relief allowed cells in earlier stages of vacuolation to recover form and cytoplasmic granulation; to a considerable degree carbohydrate tolerance was also recovered. Haist, Campbell and Best (1940) and Campbell, Haist, Ham and Best (1940) reported sudden, marked decrease of insulin content in the pancreas in dogs subjected to partial pancreatectomy or injected repeatedly with anterior pituitary extract. In both instances the dogs later manifested permanent diabetes with typical "hydropic degeneration" in the pancreas. Fasting the pituitary-diabetic dogs and feeding them with excess fat tended to prevent both the fall in insulin content and the development of "hydropic degeneration" in the pancreas. Lukens and Dohan (1941b, 1942) found that dietary restriction for 24 days was sufficient to cure the mild diabetic state in partially pancreatectomized, anterior pituitary extract injected cats. For six months thereafter the animals had normal a:c. and p:c blood sugar levels, remained free from glycosuria and gained weight while on standard diets. However, such recovered cats were more susceptible to a second course of APE injections than they had been to the first course. Treatment, if it was to be effective, had to be instituted within the first three months after the injections;

otherwise, atrophy of the degenerate islets brought about an irreversible state. In these studies Lukens and Dohan did not establish the existence of "hydropic degeneration" by biopsy before dietary treatment.

In other forms of experimental diabetes comparable dietary restrictions designed for the purpose of amelioration of symptoms or beneficial effect upon the islets of Langerhans have not been attempted.

Allen (1922b) studied the possibility that hyperglycemia stimulated the functional hyperactivity preceding "hydropic degeneration" by poisoning partially pancreatectomized dogs with phlorhizin. His studies have been cited by Lukens and Dohan (1941a) and by Lukens, Dohan and Wolcott (1943), who have stated that Allen's phlorhizinized dogs did not show hyperglycemia or develop "hydropic degeneration". On the other hand, Ham and Haist (1941) cite Allen's general conclusion, based in part on his studies in the phlorhizinized dog, that hyperglycemia played no role in the development of the lesion. Review of the recorded protocols of the four dogs which Allen studied shows that one dog developed "incipient vacuolation", a second developed "advanced vacuolation of islets and ducts"; a third dog showed no vacuolation of islets but did show "widespread vacuolation of small ducts and cell cords which seemed to be ramifying in unusual numbers throughout the tissue". The fourth dog apparently did not become diabetic. It should also be noted that continuous normoglycemia

was assumed in these studies from relatively few, widely spaced blood sugar analyses.

Lukens and Dohan (1941a, b) and Lukens, Dohan and Wolcott (1943) made extensive studies of the effects of phlorhizin and of insulin therapy upon pituitary-diabetic cats. They found that the renal poison had two effects upon such animals similar to the effects of insulin: reduction of hyperglycemia and restoration of "hydropic" islets to normal appearance. Diabetic cats treated with phlorhizin regained functional and morphological normality if treatment was begun during the first three months of diabetes. Concurrent administration of phlorhizin and anterior pituitary extract to partially depancreatized, non-diabetic cats prevented injury to the islets. Because these phlorhizin-treated cats did not show hyperglycemia, the authors attributed a definite deleterious quality to elevated blood sugar per se with respect to "hydropic changes" in the pancreatic islets. Goldner and Gomori (1944) treated two dogs with phlorhizin and injected them with alloxan in studies concerning the mechanism of the early phases of alloxan-diabetes; they did not attempt to assess the possibility of prevention of "hydropic degeneration" of ductules previously observed by them in the alloxan-diabetic dog (Goldner and Gomori, 1943). Houssay (1947) found that repetition of daily injections of phlorhizin for several weeks was associated with "cure" of the diabetic state in a few dogs. Histological studies and detailed analyses were not presented.

Other forms of experimental diabetes have not been studied by means of phlorhizin injection.

Allen's extensive studies on the pathogenesis of "hydropic degeneration" in partially pancreatectomized diabetic dogs enabled him to suggest that when the hormone produced by the islets was discovered a means would be at hand for the experimental restoration of "hydropic" islets to normal appearance. This prediction, based upon Allen's concept of a distinctive, unique, temporarily reversible lesion resulting from functional overstrain, was realized by Copp and Barclay in 1923. Theirs was the first demonstration of morphological recovery of "hydropic islets" in partially depancreatized dogs by treatment with insulin. These investigators found that 25 to 74 units of insulin per day for dogs weighing between 21 and 36 pounds was sufficient to produce recovery in about two weeks. With time the dogs showed fatal hypoglycemia on dosages formerly barely adequate to maintain normal blood sugar levels. Bowie (cited by Lukens, 1946) accomplished similar restoration in the same animal by the same method. Functional recovery was not permanent, for cessation of insulin therapy was accompanied by relapse into the diabetic state. The relative severity of diabetes before and after therapy was not studied.

Lukens and Dohan (1941a, b) and Lukens, Dohan and Wolcott (1943) secured complete morphological and functional recovery from both severe and mild diabetes in APE-diabetic (partially depancreatized)

cats. As seemed usual with this type of diabetic animal, recovery was contingent upon beginning therapy during the first three months of diabetes, before the hydropic islet cells had disappeared. Neither Copp and Barclay (1923) nor Lukens and associates observed evidence of regeneration of islets. Insulin treatment of permanently APE-diabetic dogs for periods of 58 to 198 days did not restore the characteristically low insulin content of the pancreas (Best, Campbell and Haist, 1939-40) although concurrent treatment with insulin and APE hindered the fall of insulin content and the development of hydropic degeneration which occurred when APE was given alone (Campbell, Haist, Ham and Best, 1940).

The administration of insulin to alloxan-diabetic animals has been designed, hitherto, to test its effect upon the initial hyperglycemia, to reduce mortality in the early days of secondary hyperglycemia or to determine its effect upon the early course of alloxan diabetes. Houssay (1947) reported "curing" three alloxan-diabetic dogs of a group of 23 treated with insulin but he did not present ancillary data with respect to metabolic changes or histopathology. Most investigators have found slight increase of dosage necessary to control the secondary hyperglycemia in alloxan diabetic animals (Bailey and Bailey, 1943; Bailey, Bailey and Leech, 1944; Kennedy and Lukens, 1944; Duffy, 1945). The last author found that glucose tolerance was improved following insulin therapy in alloxan-diabetic rabbits; he did not assess any possible relationship between

treatment and the "hydropic" lesions he observed late in the course of diabetes. Herbut, Watson and Perkins (1946) found that insulin sensitivity increased continuously in the alloxan diabetic rabbit; even on the very small dosage of one unit per day (of protamine zinc insulin) they found that most of their animals dying between two weeks and thirty days had succumbed to insulin overdosage. This result is directly opposite to the observations of Bailey and Bailey (1943) and appears inexplicable on the basis of information at hand.

Duff, McMillan and Wilson (1947) are the only authors who have observed the effect of adequate insulin therapy on "hydropic degeneration" of the islets of Langerhans and intralobular ductules of the alloxan-diabetic rabbit. They described a sequence of changes in the "hydropic" islet cells characterized by loss of the swollen, "hydropic" state, assumption of clear cytoplasm, appearance of varying amounts of beta granularity and finally, in some cells, the resumption of granularity quite indistinguishable from that in the islets of the normal rabbit.

No descriptions have been published describing the effects upon "hydropic degeneration" in the pancreas of hypophysectomy or adrenalectomy in partially pancreatectomized or alloxan injected diabetic animals. Lukens, Dohan and Wolcott (1943) have reported restoration of islets to normal following adrenalectomy in one pituitary-diabetic, partially depancreatized cat.

Summary:

Although "hydropic degeneration" of the islets of Langerhans rarely occurs in humans, it appears more consistently than any other lesion in all forms of permanent experimental diabetes. In dogs and cats made diabetic by partial pancreatectomy or by treatment with crude anterior pituitary extract "hydropic degeneration" of the islets appears to occur as a prolonged but transitory phase of a process which terminates in disappearance of almost all beta cells; "hydropic degeneration" of small ductules persists indefinitely in diabetic dogs. Before the advanced (atrophic) islet lesion has developed, recovery from the metabolic disturbance and restoration of the affected cells to normal structural appearance may be attained by restricting carbohydrate consumption, fasting, feeding excess fat, administering insulin and adrenalectomy. Similar measures may prevent development of the lesion and secure recovery from metabolic abnormalities if timed appropriately. Phlorhizin intoxication may achieve both prevention and "cure" of the islet lesion. The "hydropic" lesions in the islets and ductules of alloxan diabetic rabbits are also amenable to restoration to normal structural appearance by adequate insulin therapy. The "hydropic" cells of the islets come to resemble beta cells upon restoration. The hypothesis that insulin is elaborated and secreted by the beta cells of the islets of Langerhans is supported by the fact that alloxan and oxine produce necrosis of these cells accompanied by rapid onset of an insulin-deficiency type of diabetes.

There is no direct evidence to prove that hyperglycemia *per se* acts in a deleterious manner upon the islet cells, nor is there direct evidence that diminished concentrations of insulin in the blood stimulate the beta cells to elaborate and/or secrete further quantities of hormone. The possibility that hyperglycemia might not act directly upon the beta cells but by reducing the level of insulin the blood must be considered. A definite answer to this problem awaits a reliable and simple method for estimating the blood insulin level.

If "hydropic degeneration" of the pancreatic islets is a consequence of prolonged excessive functional strain, provision of exogenous insulin in amounts inadequate to lower blood sugar levels appreciably might prevent development of the lesion or allow restoration of the affected cells to normal appearance.

Experimental Study

Materials and Methods:

Adult, white, domestic rabbits were purchased locally and housed separately in individual cages. During initial periods of one to two weeks observations of general health and two determinations of fasting blood sugar level were made on each animal. During most of the study blood sugar was estimated by a modified Folin micromethod described in "Notes on the Operation of the Evelyn Photo-electric Colorimeter", Rubicon Company, Philadelphia. However, for a diabetic group given many small doses of protamine zinc insulin in an attempt to prevent the development of glycogen infiltration, Nelson's (1944) photometric adaptation of the Somogyi method for determination of glucose was employed; this method was also used for studying the untreated alloxan-diabetic animals B 98 and D 75. Appreciable differences in blood sugar values determined by the two methods on identical samples were not found when the blood sugars were markedly elevated. All blood samples were collected from the central artery of the pinna. In the earlier phases of this study, blood samples were taken following fasting intervals of 16 hours. During insulin treatment values for such estimations were found to vary so markedly (see Graphs C49, C89 and D48, and protocols) that fasting analyses were abandoned; it was considered more valid to gauge hyperglycemia by random (i.e., non-fasting)

blood sugar levels in association with daily estimations of glucosuria determined by Benedict's quantitiative titration method. During the earlier studies metabolism cages were available in very limited numbers, so that only occasional quantitative determinations of urine glucose excretion could be attempted. Later it became possible to house each animal in a separate metabolism cage; then daily urine glucose analyses were made. For most of the animals body weights were measured only at infrequent intervals (one week to one month) but in several instances daily records were made. There was no attempt to measure food and water consumption during these experiments except for numbers B98 and D75. All the animals had constant access to Purina Rabbit Chow and fresh water. Ketone bodies in the urine were tested for by using "Acetonetest", a product of Denver Chemical Manufacturing Company, Montreal. Although obvious variations of intensity of purple color were readily recognizable, facilities were not available to justify the arbitrary grading of ketonuria from "zero" to "4 plus". A similar test powder, "Galatest", was utilized for rough estimation of glucosuria as a guide to insulin dosage. With this powder my experience has shown that levels of one gram per 100 mls. or more are readily distinguishable by the intense black color; however, lesser values, including the absence of glucose, could not be consistently estimated, even after months of trials, despite the manufacturer's claim. No records were kept for degree of gross lipemia or its persistence

and only scattered estimations of blood lipids were attempted. The development of grossly apparent cataracts was noted from time to time but was not consistency recorded. Nitrogen excretion could not be studied for want of facilities.

After preliminary observations had established the apparent normality of the health of the rabbits, and when their body weights were between 1.80 and 3.50 kilograms, they were given intravenous injections of alloxan (Alloxan monohydrate, Eastman Kodak Co.). A freshly prepared, non-sterile solution of 5 grams of alloxan per 100 mls. of water was used. Each rabbit received the full dose via the marginal vein of the pinna in 30 seconds to 2 minutes. The usual dose was 200 mg. of alloxan per kilogram of body weight, but a few animals (including T53 and T55) received 175 mg. and several others were given 150 mg. (including T64, T67, C89 and C91). In the beginning an empirical treatment (Duff, McMillan and Wilson, 1947) was employed in an attempt to reduce the mortality incidence of the first two weeks following alloxan injection. This consisted of the administration of 20 mls. of 20% glucose (4 grams) intravenously and of 4 units of protamine zinc insulin subcutaneously once each day for about 10 days to 2 weeks after injection of alloxan. In my experience this device had no very certain effects upon the high mortality of the corresponding period; occasionally it seemed to precipitate hypoglycemic convulsions. Later in the course of these

studies, the plan was abandoned. Instead, 25 grams of glucose in 50 mls. of water were given by stomach tube about six to eight hours following injection of alloxan. This seemed to avoid all but occasional deaths from hypoglycemia during the first 24 hours. No effort was made thereafter to treat the diabetic and azotemic consequences of alloxan action.

The plan for the first group of experiments was to establish by biopsy that "hydropic degeneration" existed in the pancreas after several months of severe diabetic symptoms, then to give varying amounts of insulin over varying periods of time and re-assess the pancreatic histopathology. In the second group of experiments, very small doses of insulin were to be given for three or more months before examining the pancreatic changes; re-examination was contemplated following readjustment of the insulin dosage. Accordingly, the significant experiments could be arranged in small groups as follows:

A. 1. The restorative effects of a few units of insulin given in a brief period of postoperative survival.

2. The restorative effects of heavy insulin dosage with good "therapeutic control" maintained for several weeks.

3. The restorative effects of very small doses of insulin over several weeks during which hyperglycemia and glycosuria were constantly present.

B. 1. The preventitive effects of very small doses of insulin begun immediately after alloxan injection and maintained over periods of weeks to months with constant severe hyperglycemia and glucosuria.

Although these diabetic animals were "poor operative risks," when intravenous nembutal and local procaine were substituted for atropine and general ether anesthesia, operative mortality was not a problem. Several subcutaneous infections occurred despite aseptic technique, and two rabbits had localized peritonitis. Sections of pancreas were fixed in Helly's fluid and stained by the hematin-phloxin-saffron method, by Gomori's chromic alum hematoxylin method and by several methods for demonstrating glycogen. At autopsy, all of the organs were examined grossly and histological preparations of most organs were studied microscopically.

In an earlier account of part of these studies (M.Sc. thesis, McGill University, 1948), an attempt was made to grade separately the severity of glycogen infiltration in the pancreatic islets and ductules (then thought to be "hydropic degeneration") both at biopsy and at autopsy. In the light of continued efforts to accomplish relative ranking of severity, I have come to believe that the arbitrariness of the procedure renders it invalid for small biopsy fragments. Study of the larger blocks from autopsies probably enhances the validity of the assumptions. The principal

difficulty and source of error is the inability to determine accurately whether a cluster of affected cells represents a ductule in tangential section, or an islet, without the use of serial sections. The known occurrence of alpha cells in and about ductules (as well as in proper islets) renders utilization of their presence to recognize islets impossible. Although the effort was always made to distinguish beta cytoplasmic granules, the material was not satisfactory for generalization from such observations. Sections were usually cut at 6 microns and repetitive attempts to stain granules in cells not obviously containing them were not made.

It should be noted that these studies were made upon a selected group of alloxan-injected, severely diabetic rabbits since most of the injected animals did not survive long enough to be included in this study. A vast literature dealing with the manifold expressions of physiopathological response to alloxan has been accumulating for several years. It is not pertinent to review all of this information here. Instead, a few factors partially responsible for post-alloxan deaths in my studies will be briefly excerpted from the M.Sc. thesis (see Tables III, IV and V) and from incidental observations made since then (Table VI).

TABLE III

Summary of Mortality Experience

	No. of Rabbits	% of Group	Cumulative Mortality, %
Injected with Alloxan	63	100	---
Died, 1 -14 days	25	39.7	39.7
Died, 15-29 days	11	17.5	57.2
Died, 30-59 days	4	6.4	63.6
Died, 60-89 days	1	1.6	65.2
Died, 90- days	6	9.5	74.6
Alloxan - recovered	7	11.1	---
Survived, diabetic	9	14.3	---

TABLE IV

Causes of Death

Accident (fractured spine)	4
Infectious disease (diarrhoea, respiratory)	10
Gangrene of bowel	1
Anesthesia	1
Postoperative hemorrhage	1
Hypoglycemia, coma and loss of weight	30

TABLE V

Loss of Weight Manifested by Eight Diabetic Rabbits Dying During Third Week Following Alloxan Injection

Day of Alloxan Diabetes	Initial Weight (Kgm.)	Final Weight (Kgm.)	Loss (Kgm.)	$\frac{\text{Loss}}{\text{Initial Weight}} \times 100$
12	3.35	3.225	0.125	3.7
12	3.05	2.860	0.190	6.2
14	3.14	2.485	0.655	20.8
15	2.56	1.720	0.840	32.8
15	3.42	3.020	0.400	11.7
16	3.26	2.400	0.860	27.6
16	3.47	2.210	1.260	36.3
17	2.33	1.670	0.660	28.3
Average	3.07	2.450	0.620	20.9

TABLE VI

Effect of Alloxan on Blood Urea Level

	Days after Alloxan (150 mg./Kg.)				
Rabbit No.	0	1	2	3	4
J 93	10.2	15.1	19.0	21.2	25.6
J 94	10.1	Died	-	-	-
J 95	11.3	15.0	Died	-	-
J 96	14.2	23.5	54.0	68.7	Died
J 97	8.3	11.0	20.6	26.3	30.9
J 98	9.2	12.2	18.0	26.2	31.3
J 99	13.0	Died	-	-	-
J 100	9.8	10.9	14.8	25.6	31.4
K 1	16.5	27.9	Died	-	-
K 2	13.0	17.8	26.9	33.8	35.3
E	115.6	113.4	153.3	201.8	154.5
n	10	8	6	6	5
E/n	11.6	16.7	25.6	33.6	30.9
Minimum	8.3	10.9	14.8	21.2	25.6
Maximum	16.5	27.9	54.0	68.7	35.3

Seventeen alloxan-injected diabetic rabbits observed for periods of 8 to 48 weeks showed obvious glycogen infiltration in the pancreas at biopsy or autopsy. Rabbit number T 4 was also observed for 16 weeks in "relapse" following a 5-week course of insulin treatment. The metabolic data gathered during these intervals preliminary to insulin treatment have been arranged in tabular form (see Table VII). It is apparent that the diabetic state provoked by alloxan was accompanied by no consistent trend in change of body weight: half the animals gained and half lost; the range of gain or loss was about the same and the average in either case was almost identical. The duration of diabetes appeared to be unrelated to the degree of loss or gain in body weight, to the average fasting or random blood sugar level, average urine volume or average glucose excretion. The absolute values for gain or loss of body weight, urine volume and glucose excretion presented in the table provide sufficient corroboration of the apparently severe diabetic state which existed in each rabbit. Data for random and fasting blood sugar levels conceal to some extent the sometimes marked fluctuations observed in each single animal but allow an approximate, though crude comparison of severity amongst the different animals of the group. The figures for average urine volume and glucose excretion also conceal wide variations noted from day to day; since they have been expressed in absolute values, comparisons of severity amongst the group are at best very crude in contrast to what might have been achieved by expressing glucosuria in terms of grams of glucose per kilogram of body weight per day. This aspect of the data could not be pursued further because values for food consumption, basic to estimation of "available carbohydrate", were not recorded.

TABLE VII

Diabetic Characteristics of Rabbits Surviving

Injection of Alloxan from 8 to 48 weeks

Rabbit Number	Duration of Diabetes Weeks	Change in Body Weight (Kgm.) Gain	Loss	Average Blood Sugar Level (mg.%) Fasting	Random	Average Urine Excretion ccs./day Volume	Gms./day Glucose
T53-A	8		0.92	365	557		
T4-B1	12	0.35		414			
T6-B1	12		1.22	442			
T37-A	13	1.00		463	549		
T64-B1	13	0.46		440	547	700	31.1
T67-B1	13	0.40		354	458	528	28.4
T15-A	15		0.31	376		875	24.7
T55-A	15		0.47	458	533	616	25.6
T4-B3	16*		0.39	555	469	490	15.2
T16-B1	17	0.67		470		950	25.7
T17-B1	17		0.50	463	645	1000	15.0
T40-A	17		0.15	495	576	458	21.1
T25-B1	20	0.31		518		460	16.6
T20-B1	21	0.61		546		800	25.6
T21-B1	22	0.73		420		850	
T23-B1	26	0.39		485		525	15.0
D75-A	28	0.03		246	390	601	21.5
B98-A	48		0.42	595	579	878	37.6
E		4.95	4.38	8105	5303	9731	305.1
n		10	8	18	10	14	13
E/n		0.50	0.55	450	530	695	23.4
Minimum	8	0.03	0.15	246	390	458	15.0
Maximum	48	1.00	1.22	595	645	1000	37.6

* Values represent 18th to 34th weeks, inclusive, i.e., following discontinuation of insulin treatment given from 13th to 17th weeks inclusive.

It is interesting to note that rabbit D75 showed an average fasting blood sugar level of only 246 mg.% but had severe glycogen infiltration in the pancreas after 28 weeks (see Figures 11 and 12). Duff, McMillan and Wilson (1947) did not find the lesion if the average blood sugar (fasting) level was less than 303 mg.%. After one year during which the blood sugar level averaged 256 mg.% one of their rabbits did show the pancreatic lesion.

In most of these studies daily tests for ketonuria were not attempted. However this phenomenon was rarely encountered by occasional testing after the first week or two of diabetes. Rabbit B98 was exceptional in that ketonuria was never absent throughout the entire 48 weeks of observation.

Biopsies (11 instances) and autopsies (7 instances) disclosed glycogen infiltration in obvious degrees in each of these experiments. Islets and ductules were always affected, although sometimes with apparently different degrees and extents; cells occupying centroacinar positions were seen in nine pancreases, including six biopsies. Mitotic figures in ductular epithelial cells were seen in three biopsies; in one of these pancreases mitoses were also present in centroacinar cells, acinar exocrine cells and agranular islet cells (apparently beta cells). Lobules of pancreatic tissue having a hyperplastic, embryonic resemblance (i.e., supposedly "proliferating") were encountered in each of the three samples which had apparent mitotic activity and

in one other biopsy. Glycogen could be demonstrated in the renal tubular epithelium of each of three autopsy samples; in four other instances kidney blocks were not studied microscopically at autopsy. Microphotographic illustrations of these features will be cited in context with observations on the effects of insulin treatment.

Observations

A. Restoration of glycogen-infiltrated pancreatic cells to normal structural appearance by treatment with insulin.

1. Three rabbits received a few units of protamine zinc insulin during one or two days following pancreatic biopsy. Metabolic observations were not made during the brief postoperative survival periods. Sections of pancreas studied at autopsy were not significantly different in any way from those studied at biopsy. All of these rabbits showed ketonuria at death and all had Armanni renal lesions. Infarcts of the spleen apparently contributed to the causes of death in two instances (T16 and T6); one rabbit had lost more than a kilo of body weight before operation, and one (T21) had an incisional herniation of the small bowel. The scant pertinent data appear in Table VIII. A microphotograph of the pancreas of rabbit T 16 is presented in the Appendix (see Figure 27).

TABLE VIII

Rabbit No.	T21	T6	T16
Duration of diabetes (weeks)	22	12	17
Average Glycemia before Rx (fasting, mg.%)	420	442	470
Total Units of Prot. Zinc Insulin	4	10	32
Duration of treatment (days)	1	1	2
Glycogen infiltration before Rx			
islets	some	some	extreme
ductules	some	some	extreme
centro-acinar cells	some	none	some
Glycogen infiltration after Rx	(no appreciable differences)		

2. Three experiments in which insulin therapy secured good control of hyperglycemia and glucosuria and marked increase of body weight are considered here together with the available data of similar nature regarding an experiment previously performed by Drs. Duff, McMillan and Wilson (1947), to whom I am indebted for permission to recount their observations (rabbit number 74W). Rabbit number T4 received two courses of insulin treatment separated by an interval of 17 weeks, thus accounting for two attempts to produce experimental restoration of "hydropic" pancreatic cells to normal. Data summarizing these studies appear in Table IX.

TABLE IX

Rabbit Number	74W	T17	T4-B_1B_2	T4-B3A
Duration of diabetes (weeks)	27	17	12	17
Total Insulin given (units)				
protamine zinc	440	420	1684	2072
crystalline zinc	843	-	776	-
Duration of Treatment (days)	30	14	27	59
Average Glycemia (mg./100 mls.)				
before Rx fasting	416	463	414	555
random*	-	645	-	469
during Rx random*	320**	133	164	217
Average Glucosuria (gms./day)				
before treatment	-	15.0	-	15.2
during treatment	-	-	-	-
Change in body weight				
during treatment (Kgm.)	+0.95	+0.34	+0.43	+0.63
Glycogen infiltration (biopsy)				
before treatment islets	some	extreme	some	extreme
ductules	some	extreme	some	extreme
centro-acinar	some	some	none	some
after treatment islets	none	none	none	none
ductules	none	some	none	none
centro-acinar	none	none	none	none

* random = sample taken before morning dose of insulin.
**average daily level during first twenty days of treatment.

Rabbit number 74W was the first alloxan-diabetic animal to be treated with insulin for the purpose of attempting adequate symptomatic control and restoration of "hydropic" lesions in the pancreas. During the first 20 days of insulin therapy daily determinations of blood sugar level showed that hyperglycemia was almost constantly present although larger and larger doses of both crystalline and protamine insulin were given. During the last 10 days of treatment the blood sugar level was not estimated; instead urine glucose was tested semiquantitatively by "Galatest". The use of

larger quantities of protamine insulin supplemented by crystalline insulin three times daily achieved all but complete absence of glucosuria during this period of ten days. For the entire therapeutic period of observation the average daily dose of insulin was 15 units of protamine supplemented by 28 units of crystalline preparation; the greater portion was administered during the last 15 days of therapy. Although islets, ductules and centro-acinar cells contained obvious accumulations of glycogen in their cytoplasm before therapy, no trace of glycogen was identifiable afterward. Many of the islets in autopsy sections contained numerous agranular cells and other cells with some beta granules. Some islets contained cells indistinguishable from normal beta cells.

Rabbit number T 17 received 420 units of protamine zinc insulin during a period of 14 days. The blood sugar fell to normal and subnormal levels; three times, on different days, hypoglycemic convulsions occurred, necessitating administration of glucose by vein. Only the least appreciable discoloration of "Galatest" powder suggested some persistence of glucosuria. When pancreatic biopsy sections were studied <u>after</u> insulin therapy, no glycogen could be found in the islets or centro-acinar cells; however, a few definite deposits were seen in scattered ductular epithelial cells. These appearances were in sharp contrast to the extreme lesions noted in sections prepared <u>before</u> treatment.

Rabbit number T 4 responded to treatment with protamine zinc insulin supplemented by the crystalline preparation by attaining blood sugar levels seldom exceeding the upper limits of "normal" range. However, preposterous dosage was necessary to secure this effect. Subsequent experience suggests that the utilization of soft tissues on the dorsum of the pinna as the sites for twice-daily injections of both types of insulin in large volumes constituted a basic error; probably these tissues permitted increasingly poor absorption of the hormone. Over a period of 27 days 1684 units of protamine and 776 units of crystalline insulin were given. The average daily dose was 62 units of protamine and 30 units of crystalline insulin divided in equal morning and evening injections. Animals subsequently injected in the soft tissues of the back or abdomen have not required such huge doses to achieve control of hyperglycemia and glucosuria. Pre-treatment pancreatic biopsy had shown glycogen infiltration both in islets and in ductules; post-treatment biopsy did not reveal any trace of glycogen (see Figures 28, 29, 30).

After the second pancreatic biopsy rabbit T 4 was largely neglected for several weeks, only widely spaced random blood sugar estimations were made. These, however, were found interesting in that they only gradually rose to more than 500 mg.%, whereafter fasting blood sugar levels were higher (see Table IX, column T4-B3,A). In the 17th week after insulin therapy had been discontinued, the

average daily urine glucose excretion was found to be 15.2 grams. At this point a third biopsy was successfully accomplished, a large piece of pancreas being secured together with the entire spleen. The pancreatic islets, ductules, centro-acinar cells and the large ducts (those having obvious cuffs of stromal support and columnar epithelial membranes) showed extreme glycogen infiltration. Many areas of apparent active proliferation and differentiation of pancreatic exocrine and endocrine tissue were noted, and mitoses were numerous in islet cells, ductular epithelial cells, centro-acinar cells and acinar cells (See Figures 31, 32 and 33). A second course of protamine zinc insulin was given, again utilizing the pinnas as injection sites. Over a treatment period of 59 days, 2,072 units of insulin were administered; this was given in a single morning injection averaging 35 units. Daily estimations of the random blood sugar level (i.e., the level before each morning injection) showed poor control of hyperglycemia during the first 10 days of treatment. Thereafter, more widely spaced determinations usually gave values below the upper limit of "normal" range. However, the average value for the entire period was 217 mg.%. Occasional urine samples contained more than 1 gram of glucose per 100 mls. Plans for a fourth biopsy on the 60th day of therapy study were thwarted by the unexpected death of the rabbit after 59 days of treatment (296 days after injection of alloxan). Death was attributed to intercurrent respiratory infection. Autopsy sections of pancreas revealed no trace of glycogen and no vacuoles could be found in the

cytoplasm of any pancreatic cells. There were no apparent areas of proliferating pancreatic tissue and there were no mitoses. The kidneys contained no demonstrable glycogen or cytoplasmic vacuoles but revealed extensive calcification of proximal convoluted tubules and loops of Henle (see Figure 34).

3. In two experiments (see Table X, columns T23 and T20) treatment with insulin for 10 days and 36 days failed to achieve control of hyperglycemia and glucosuria despite average daily doses of 18 units and 48 units of protamine zinc insulin given each morning in the soft tissues of the pinna. Although rabbit T23 showed no significant change of body weight, T20 gained 590 grams. Infection of the operative wound with localized extension to the peritoneum led to death of the former animal, and terminal ketonuria was detected at autopsy. A similar wound infection in the other rabbit was controlled by penicillin therapy; after a second pancreatic biopsy treatment was continued for eight more days but wound infection and peritonitis did not respond to penicillin therapy. This animal was killed for autopsy. Despite average blood sugar levels, during treatment, of 367 and 415 mg.%, very little glycogen could be demonstrated in the pancreases of these two rabbits after 10 and 36 days of insulin therapy. Before insulin was given, the biopsy of one of them (T23) had shown obvious accumulations of glycogen in islets and ductules and the other (T20, see Figure 35) had shown extreme lesions in islets, ductules and centro-acinar cells.

TABLE X

Rabbit No.	T23	T20	T64	T67-B_1B_2	T67-B_1A*
Dur. of Diabetes (weeks)	26	21	13	13	13
Total insulin (units)	180	1722	140	114	210 (96)
Dur. of Treat. (days)	10	36	20	29	49 (20)
Av. Glycemia (mg.%)					
before Rx, fasting	485	546	440	354	354
before Rx, random	-	-	547	458	458
during Rx, random	367	415	489	374	355 (321)
Av. Glucosuria (Gms./day)					
before Rx	15.0	25.6	31.1	28.4	-
during Rx	6.8	7.4	13.2	7.1	-
Change in Weight during Rx (Gms.)	-0.08	+0.59	+0.29	-0.03	-0.01 (+0.02)
Glycogen Infiltration					
before Rx, islets	some	extreme	some	some	--
ductules	some	extreme	some	some	--
centro-acinar	none	some	none	none	--
after Rx, islets	some	none	some	some	none
ductules	none	none	some	some	none
centro-acinar	none	none	none	none	none

* Parenthetic values are averages for the 20 days of treatment additional to that previously given (column T67-B_1B_2). Quantititive urinalyses were seldom made during the additional period of insulin therapy.

Afterwards, T23 showed no affected cells in the ductules but the islets still contained a few (see Figure 36). The sections from both the second pancreatic biopsy of number T20 and from the autopsy were devoid of glycogen or suggestive cytoplasmic vacuoles in any cells (see Figure 37). Although both rabbits had marked hyperglycemia and glucosuria until death, glycogen nephrosis was found only in number T23. In neither instance were mitoses or lobules of proliferating pancreatic tissue apparent.

Having observed partial and complete restoration of "hydropic degeneration" in the pancreas in response to insulin injections which did not overcome hyperglycemia and glucosuria, two attempts were made to corroborate this result. Data pertinent to these experiments are presented in Table X, columns T64, T67-B_1B_2 and T67-B_1A.

Rabbit number T64 had shown severe hyperglycemia and glucosuria for 13 weeks. Pancreatic biopsy established the presence of obvious glycogen infiltration. For 20 days a single daily injection of protamine zinc insulin (average 7 units per day) was given. During treatment daily determinations of glycemia and of glucosuria were made. The average values of these observations were 489 mg.% and 13.2 grams per day as compared to 547 mg.% and 31.1 grams per day during the preceding untreated interval. Post-treatment biopsy sections of the pancreas showed the merest traces of glycogen in the cytoplasm of islet cells but a few larger deposits were noticeable in the ductules. Autopsy sections, prepared the day after post-treatment biopsy, confirmed the biopsy observations. Glycogen nephrosis was apparent; some glycogen was demonstrable in the cytoplasm of liver cells (see Figure 38).

Rabbit number T67 responded to treatment with about 4 units of insulin per day over a period of 29 days (see Table X, column T67-B_1B_2) by showing diminution of average random blood sugar level from 458 mg.% to 374 mg.% and of average glucosuria from 28.4 to

7.1 grams per day. The pre-treatment pancreatic biopsy sections had shown many glycogen-filled islet and ductule cells (see Figure 39) but after treatment a second pancreatic biopsy showed only traces of glycogen in scattered islet cells with a few more conspicuous deposits in ductule epithelium. Even more prominent in these preparations than the trifling amounts of demonstrable glycogen was the presence of apparently proliferating lobules of pancreatic tissue in which some islets seemed to be differentiating (see Figure 40). Insulin therapy was continued at about the same dosage for 20 additional days. In Table X, column T67-B_1A, data for the entire treatment period (49 days) have been summarized and values for the last 20 days of treatment have been inserted parenthetically. For the whole period of therapy the average blood sugar level was 355 mg.%; for the last 20 days it was 321 mg.%. Daily quantitative analyses of glucosuria were not made, but "Galatest" reaction consistently indicated values greater than one gram per 100 ml. The body weight of this rabbit was not appreciably modified by the initial or the continued administration of therapy. When the animal was killed and autopsied no trace of glycogen could be found in the pancreas. There were numerous areas of active proliferation containing well differentiated islets with many beta cells; mitoses were apparent in beta cells, ductule epithelial cells, centro-acinar cells and acinar cells. These appearances are shown in Figures 41, 42 and 43. Some glycogen was demonstrable in the renal tubular epithelium but some was also present in the cytoplasm of the liver cells; in neither instance were the amounts seen present either extensively or in abundance.

B. Prevention of development of glycogen infiltration in the pancreas by prolonged treatment with small doses of insulin.

Five rabbits made diabetic with single intravenous doses of alloxan were given small doses of insulin each morning over periods of several weeks to several months. Daily observations of body weight, glucosuria and glycemia were made for several weeks in each instance (see protocols C49, C50, C89, C91 and D48). Later, blood sugar levels were determined at weekly intervals. The relevant data are summarized in Table XI.

Rabbit number C 91 received about 5 units of protamine zinc insulin daily for 21 days. Terminally a large fluctuant mass appeared in the right upper leg; penicillin did not prevent the progress of this mass which was incised and drained on the 35th day. Next morning the rabbit was found dead. Ketonuria did not occur. Very little insulin was given during the last two weeks of study. The average blood sugar level for the entire period was 354 mg.% and the average glucosuria 14.6 grams per day. Body weight decreased 0.53 kilogram. Autopsy disclosed no evidence of glycogen infiltration in the pancreas although small deposits could be demonstrated readily in the renal tubular epithelium.

Rabbit number C 50 developed obvious widespread glycogen infiltrations in pancreatic islets, ductules and centro-acinar cells despite daily administration of 5 units of protamine zinc insulin.

The average daily blood sugar level and glucose excretion during 39 days of observation were 413 mg.% and 23.4 grams. Although this animal was in "good health" and gained 0.49 kilogram of body weight in 35 days, a 24-hour fast precipitated marked ketonuria, loss of weight and a moribund state which necessitated premature termination of the experiment. At autopsy on the 39th day glycogen infiltration of islets, ductules and centro-acinar cells was obvious in the pancreas and glycogen nephrosis was apparent in the kidney sections.

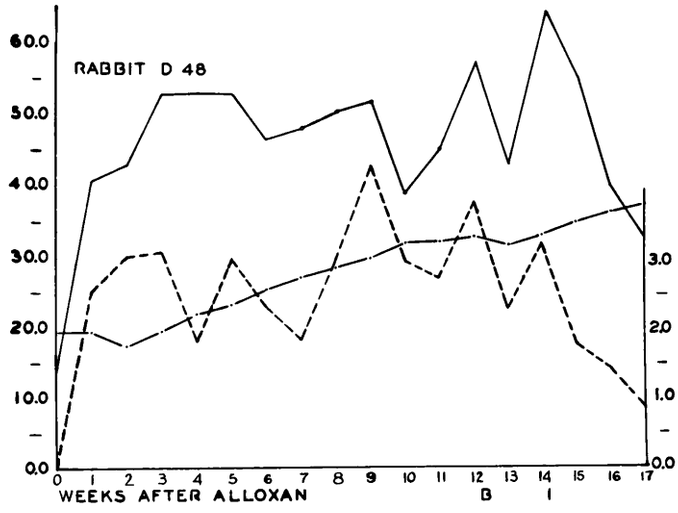
TABLE XI

Rabbit Number	C91	C50	D48	C49	C89
Duration of Treatment (weeks)	5	6	12	16	15
Average daily Insulin (units)	3	5	5	4	5
Average Blood Sugar (mg.%)	354	413	496	488	457
Average Glucosuria (Gms./day)	14.6	23.4	29.4	16.6	21.3
Change in Body Weight (Kgm.)	-0.53	+0.49	+1.46	+0.74	+2.16
Glycogen Infiltration					
islets	none	some	extreme	none	none
ductules	none	some	extreme	none	none
centro-acinar cells	none	some	some	none	none
Changed Insulin dose (units)			9	0	0.8
Duration of Changed Rx (weeks)			3	3	3
Average Blood Sugar (mg.%)			433	477	606
Average Glucosuria (Gms./day)			10.1	18.3	37.1
Change in Body Weight (Kgm.)			+0.39	-0.15	-0.77
Glycogen Infiltration					
islets			none	some	some
ductules			some	some	some
centro-acinar cells			none	some	some

Rabbit number D 48 gained 1.46 kilograms of body weight during 12 weeks of treatment with 5 units of insulin daily. The average blood sugar level was 496 mg.% and the average glucose excretion 29.4 grams per day. In addition to the summary of the data presented in Table XI, a graph of the weekly observations (see graph D48) has been prepared. Pancreatic biopsy demonstrated the existence of extreme glycogen infiltration in the islets, ductules and centro-acinar cells. For two weeks following the biopsy the administration of insulin was continued at the usual dosage. After the biopsy sections had been studied the dosage was revised so that the average daily dose during the 15th, 16th and 17th weeks was 9 units. During these weeks glucose excretion averaged 10.1 grams per day. Blood sugar levels tended to decrease (see graph D48 and protocol) although only two of eight were less than 340 mg.% and the average was 433 mg.%. Body weight increased by 0.39 kilogram. Autopsy showed persistence of the lesion in obvious degree in the ductules but no evidence of glycogen could be found in the islets or centro-acinar cells. The kidneys showed glycogen nephrosis and the cytoplasm of the liver cells contained abundant quantities.

Rabbits number C49 and C89 received 4 and 5 units of insulin daily, respectively, throughout 4 months of observation. Their average blood sugar levels varied somewhat from week to week (see graphs C49 and C89). (Number C49 stopped eating temporarily, for completely obscure reasons, in the 14th week, when a fasting level was 84 mg.% and the scant urine excretion was free of sugar.)

(continued - Page 74)

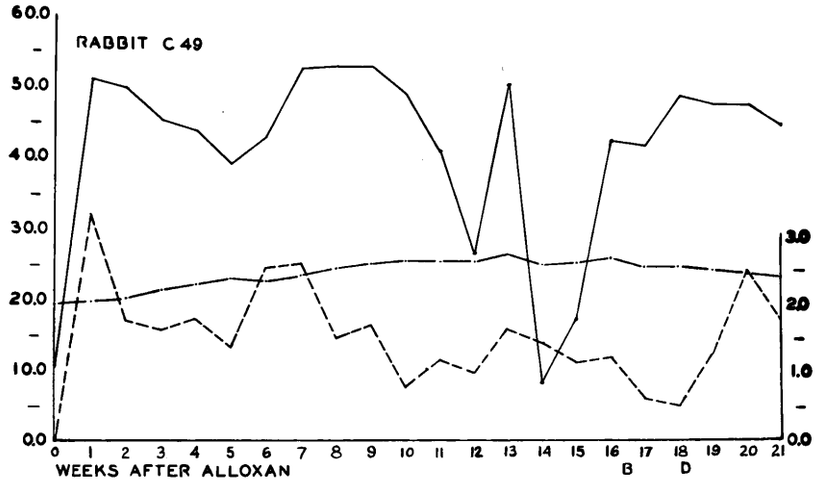


Graph D48. The effects of treatment with small doses of insulin on the course of alloxan diabetes in the rabbit. Five units of insulin were given daily until the 99th day, when the dose was increased to nine units daily.

Legend:

Left vertical scale: Blood sugar level in centigrams per 100 mls. (———) and urine glucose in grams per day (-----).
Right vertical scale: Body weight in kilograms (-·-·-).
B: pancreatic biopsy on 84th day.
I: increased daily dose of insulin on and after 99th day.

Note: In each instance the point plotted represents the average for the week of observations made daily, except that blood sugar determinations were not made daily after the fourth week. Blood sugar values of the seventh to eleventh weeks, inclusive, represent single determinations on fasting samples; all others were random samples.



Graph C49. The effects of treatment with small doses of insulin on alloxan diabetes in the rabbit. Five units of insulin were given daily until the 128th day, when insulin treatment was discontinued completely.

Legend:

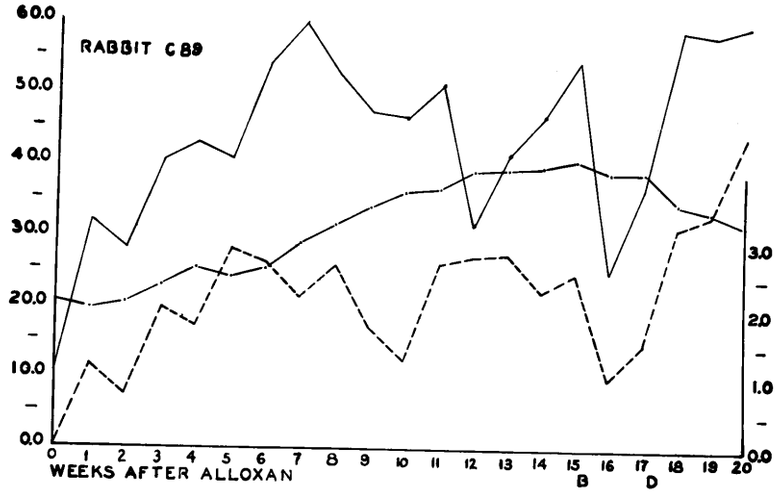
Left vertical scale: blood sugar in centigrams per 100 mls. (———) and urine glucose in grams per day (– – – –).

Right vertical scale: Body weight in kilograms (–·–·–).

B: pancreatic biopsy on 113th day.

D: insulin therapy discontinued on and after 128th day.

Note: In each instance the point plotted represents the average for the week of observations made daily except that blood sugar determinations were not made daily after the ninth week. Blood sugar values of the eleventh to sixteenth weeks, inclusive, represent single determinations on fasting samples; all others were random samples.



Graph C89. The effects of treatment with small doses of insulin on alloxan diabetes in the rabbit. Five units of insulin were given daily until the 118th day, when the dose was decreased to 0.8 unit daily.

Legend:

Left vertical scale: Blood sugar level in centigrams per 100 mls. (———) and urine glucose in grams per day (----).

Right vertical scale: Body weight in kilograms (-·-·-).

B: pancreatic biopsy on 103rd day.

D: decreased daily dose of insulin on and after 118th day.

Note: In each instance the point plotted represents the average for the week of observations made daily except that blood sugar determinations were not made daily after the seventh week. Blood sugar values of the tenth to fourteenth weeks, inclusive, represent single determinations on fasting samples; all others were random samples.

For the entire periods their average blood sugar levels were 488 and 457 mg.% and they gained 0.74 and 2.16 kilograms of body weight. In both instances the pancreatic biopsy sections were completely devoid of evidences of glycogen infiltration (see Figure 44). During the two weeks following biopsy the usual daily dose of insulin was continued. After the sections had been studied insulin treatment was discontinued. Number C89 thereupon manifested ketonuria and severe loss of weight of such magnitude that very small doses of insulin were given as a precaution against loss of the animal. Similar changes of much less severe degree were noted in number C49. Hyperglycemia and glucosuria were markedly increased during the three-week period of reduced insulin dosage in the case of number C89 but only slight changes were manifested by C49. Autopsy of these rabbits revealed readily apparent and widespread glycogen infiltration in the pancreatic islets, ductules and centro-acinar cells (see Figures 45 and 46). Glycogen was demonstrable in the kidneys of both and in a few liver cell nuclei in one (see Figure 47).

In none of the above experiments were lobules of proliferating pancreatic tissue found nor were mitotic figures in evidence except for a single metaphase figure in an alpha cell in one instance (D48, autopsy section).

C. The effect of alloxan on glycogen-infiltrated and "restored" pancreatic cells.

(All but one of the rabbits used in this study were made available to me through the courtesy of Dr. G. C. McMillan.)

Four "normal" cholesterol-fed rabbits; one alloxan-diabetic rabbit treated with small doses of insulin for 20 days to secure "partial restoration" of the pancreatic "hydropic" lesions; one "alloxan-recovered", cholesterol-fed rabbit; and two alloxan-diabetic, cholesterol-fed rabbits comprised the experimental group. The previously insulin-treated rabbit was given 200 mg. of alloxan per kilo of body weight four hours after a second operation for pancreatic biopsy (see protocol number T64). Each of the others was given 150 mg. of alloxan per kilo of body weight.

Three and one-half hours after injection of alloxan the blood sugar levels of the previously normal rabbits were found to be 320, 385, 109 and 91 mg.%. They were given 20 ccs. of 20% glucose intravenously and 4 units of protamine zinc insulin subcutaneously. Pancreatic sections taken at autopsy ten hours after injection of alloxan showed typical necrotic changes in the beta cells of the islets.

The previously insulin-treated rabbit had had a blood sugar level of 260 mg.% immediately before the second dose of alloxan was given. Ten hours later, just before autopsy, the blood sugar

level was found to be 100 mg.%; the general filthiness of the rabbit's fur suggested the probability of unobserved hypoglycemic convulsions. The pancreatic sections were indistinguishable from the biopsy preparations; no evidence of necrotic changes was apparent.

The post-alloxan blood sugar level of the alloxan-recovered rabbit was lost through technical error. Pancreatic sections prepared at autopsy ten hours after injection of alloxan showed many islets in which profound necrotic changes were apparent in almost all the beta cells. Some islets contained numerous undamaged, apparently normal beta cells.

During two months of cholesterol feeding the alloxan-diabetic rabbits had average fasting blood sugar levels of 373 and 309 mg.%. Three and one-half hours after the second injection of alloxan the values were 496 and 380 mg.%. Autopsy sections of pancreas, prepared ten hours after re-administration of alloxan, disclosed "hydropic degeneration" of moderate severity in the islets and ductules. There were no changes which could be interpreted as necrosis in either instance (see Figure 48).

Discussion

The experimental observations recounted above provide new evidence bearing on several aspects of the histopathology of experimental diabetes.

In general, the findings of Duff, McMillan and Wilson (1947) are corroborated by these studies. "Hydropic degeneration", i.e., glycogen infiltration, in the pancreatic islets, ductules and centro-acinar cells was found to be the typical lesion of permanent, severe alloxan diabetes in the rabbit. The lower limit of severity of diabetic state as judged by hyperglycemia and the lower limit of duration of diabetes required for the development of the pancreatic lesion in the alloxan-diabetic rabbit were slightly less than the former authors' report indicated: 246 instead of 303 mg.% and 39 days instead of 45, respectively. Once fully developed, the lesion did not appear to progress to atrophic dissolution of the affected islet cells in periods as long as eleven months.

Control of hyperglycemia and glucosuria with large doses of protamine zinc insulin over periods of about 30 days was associated with restoration to normal appearance of the previously affected pancreatic cells. Exact bracketing of dosages and treatment intervals necessary to achievement of this histological result has not been accomplished. It is apparent that thirty days of therapy may suffice.

In the islets, cells resembling agranular and normal beta cells became apparent. While it was not proven that these cells were the identical ones previously found filled with glycogen, it is probable that they were. There was no evidence that the affected cells disappeared by dissolution under insulin therapy or that new cells arising in islets or elsewhere proliferated to replace them. Furthermore, it would be reasonable to anticipate the disappearance of glycogen from the cytoplasm of these pancreatic cells under the impetus provided by insulin treatment. Such behavior would be in keeping with known effects of insulin on other abnormal glycogen deposits, e.g. in the liver cell nuclei, in leucocytes, and in renal epithelium. If these cells are actually beta cells, then the histopathological similarities between the pancreatic islet lesions of alloxan diabetes, other forms of experimental diabetes and some cases of the human disease are precise.

A feature of these experimental observations was the striking restorative and preventitive effects of prolonged treatment with very small doses of insulin. These results show that both restoration and prevention can be achieved despite continuance of severe hyperglycemia and glucosuria. It is the more remarkable that restoration occurred with small doses of insulin in about the same time as it did when normal blood sugar levels were maintained by large amounts of insulin. The latter observation may have been a chance result; for, insufficient studies have been accomplished

to permit generalized evaluation of the time factor. However, it is noteworthy that withdrawal of insulin was attended by development of obvious lesions in the several types of pancreatic cells in only 3 weeks. The minimum time observed when insulin therapy failed to prevent the development of the lesion was 6 weeks (rabbit C50) and when no preceding therapy was given it was 8 weeks (rabbit T53).

Assessment of the possibility that hyperglycemia played a significant role in the development of the lesions observed in these studies must be accomplished cautiously. The average random blood sugar levels of ten untreated alloxan diabetic rabbits which showed glycogen infiltrations in the pancreas were presented in Table VII (see Page 56). The highest average was 645 mg.%, the lowest was 390 mg.% and the mean for the group was 530 mg.%. Of four rabbits showing some degree of restoration subsequent to administration of small (or "inadequate") doses of insulin, all had average random blood sugar levels lower than the mean of the untreated group and two had average levels lower than the lowest of the untreated group. On the other hand, in both of the studies in which development of glycogen infiltrations in the pancreas was prevented by administering small doses of insulin over several months, the average random blood sugar levels were higher than the lowest observed in the untreated rabbits although they were lower than the mean. Of two studies in which rapid development of typical

pancreatic lesions upon cessation of insulin therapy was observed, one was attended by no significant increases of hyperglycemia or glucosuria and the other by marked elevations of both symptoms. Thus it would appear debatable whether any valid conclusion can be drawn from these studies denying any role to the factor of hyperglycemia in the pathogenesis of the pancreatic lesions. Nevertheless, it is apparent that severe, continuous hyperglycemia is not incompatible with complete restoration of the glycogen-infiltrated cells to normal appearance or with complete prevention of the development of the lesion. The observation of these phenomena under the influence of small doses of insulin tends to support the original suggestion of Allen (1913) that a deficiency or depression of blood insulin level may be the stimulus to beta cell hyperfunction. If such were the case, it would be reasonable to expect that a dose of insulin which proved sufficient to relieve the excessive strain in one diabetic animal might be quite ineffective in another. Likewise, very different periods of treatment with a uniform dose of insulin might be found necessary to achieve restoration of the fully developed pancreatic lesion. The observations made in these studies do not support the long-asserted view of Lukens and associates that hyperglycemia per se may directly influence the development of the typical pancreatic lesion; instead, they favor the more recent modifications of that group's opinion, that hyperglycemia has to be associated with some other factor. It could be that hyperglycemia leads to diminished blood insulin levels and the latter to islet stimulation.

The observation of a gradual ascent of the blood sugar level of one rabbit (number T4) during several months following discontinuation of a course of insulin treatment which had attained morphological restoration of the previously affected pancreatic cells suggests that a slight degree of amelioration of the diabetic state may have been achieved. The fact that lesser quantities of protamine zinc insulin without supplementary crystalline insulin were required to attain adequate control of hyperglycemia during a second course of treatment than had been found necessary during the first course lends support to this idea of possible functional amelioration. From the histopathological studies on this animal it might be inferred that proliferation of pancreatic tissue to form new islets had occurred during the first course of insulin treatment and that during the subsequent relapse these structures temporarily retained some degree of endocrine function. However, it has already been indicated that the injections of insulin were probably so poorly absorbed from the sites into which they were injected as to render erroneous the sizes of the doses apparently required to secure control of hyperglycemia. The occurrence of "apparent proliferation" and mitotic activity in the various biopsies and autopsies was so irregular that these appearances noted in the case of number T4 cannot justifiably be attributed to the insulin given. Furthermore, in the absence of comparative data regarding food consumption and glucosuria, one cannot eliminate the possibility that

the gradual ascent of the blood sugar level noted in this study may have been attributable to these factors. On the other hand, if glycogen infiltration in the epithelial cells of the pancreatic ductules represented an hypothetical strain exerted by some unrecognized stimulus to proliferation (Allen, 1913) and if new-formed islet cells of such origin had functional capacity, it might be possible to accept the observed peculiarity of the blood sugar level during relapse as evidence of temporary amelioration of the diabetic state. According to the view of Lukens that hyperglycemia is one factor in a "vicious cycle", the increasing level of the blood sugar could be attributed simply to progressive severity of the recurrent pancreatic lesion.

The ineffectiveness of re-administration of a diabetogenic dose of alloxan upon glycogen-infiltrated pancreatic islet cells and upon similar cells after restoration by insulin treatment is not at present explicable. This finding is the more puzzling in view of indirect indications of hypoglycemic convulsions manifested by the previously insulin-treated rabbit. It is possible that necrotic changes might have appeared after ten hours had the diabetic animals been permitted to live longer. If this were the case, the glycogen-filled and restored cells could be said to be only relatively less susceptible to alloxan necrosis than the normal beta cells, which did show necrosis within ten hours. In view of preceding argument concerning the nature of the hydropic islet cells,

it seems only a remote possibility that the affected and restored islet cells of the permanently diabetic rabbit might be other than beta cells. It has been observed in several species and in several types of diabetes that glycogen-infiltrated pancreatic ductular epithelium persists indefinitely, whereas the affected beta cells disappear in weeks or months in pancreatectomy-diabetes and pituitary diabetes of dogs and cats. If such ductule cells were the origin of the affected islet cells of the alloxan-diabetic rabbit, the persistence of these islet cells in the alloxan-diabetic rabbit would appear more explicable. Furthermore, necrosis of ductule epithelium in response to alloxan has not been reported to occur. Thus, if glycogen-filled and restored islet cells of the alloxan-diabetic rabbit are so derived, their apparent insensitivity to alloxan damage might be reasonably anticipated. Many observers have noted "indifferent" or "agranular" cells in the islets at various intervals after alloxan injection. Some of these cells probably represent D type islet cells but it is possible that many of them are agranular forms of beta cells. If some beta cells are insusceptible to alloxan necrosis on first exposure, it is possible that they or their progeny might be insusceptible when alloxan is re-administered. Phenomena of this order are known in the responses of liver cells to a first and repeated doses of carbon tetrachloride and of renal tubular epithelium to various organic reagents. If the implication of the studies of Kadota (1950) are acceptable evidence that the necrotizing actions of alloxan as well as of oxine and dithizone are functions of the affinity of these reagents for

zinc, the decreased amounts of this substance in the islets of the diabetic animals might offer a possible explanation for the apparent lack of alloxan-sensitivity observed in the studies reported above.

Summary and Conclusions

1. Treatment of alloxan diabetic rabbits with large doses of insulin for periods of about 30 days may achieve restoration to normal structural appearance of pancreatic islet, ductule and centro-acinar cells which have previously become infiltrated with glycogen. The restored islet cells have the cytological characteristics of beta type cells. Upon cessation of insulin treatment, symptoms of diabetes rapidly reappear and glycogen infiltration again develops in the pancreas.

2. Treatment with small doses of insulin for very brief periods does not modify appreciably the appearance of glycogen infiltration in the pancreas.

3. Treatment of alloxan diabetic rabbits with small doses of insulin for periods of several weeks may achieve restoration to normal structural appearance of pancreatic islet, ductule and centro-acinar cells which have previously become infiltrated with glycogen. This result may be attained despite persistence of continuous hyperglycemia and glucosuria during treatment.

4. Treatment of alloxan-diabetic rabbits with small doses of insulin for periods of several months may prevent the development of glycogen infiltration in the pancreas. When insulin therapy is discontinued the typical lesions develop more rapidly than is the case when no insulin is given. The former result may be attained despite the persistence of continuous hyperglycemia and glucosuria during treatment. The latter result may occur without concomitant further elevations of the blood sugar level and the amount of glucose excreted in the urine.

5. Severe, continuous hyperglycemia is not incompatible with either restoration or prevention of the typical pancreatic lesion by experimental administration of small doses of insulin to alloxan diabetic rabbits.

6. Hyperglycemia per se is not apparently the sole factor responsible for the development of glycogen infiltration in the pancreas in experimental diabetes. It is possible that diminished blood insulin level is a more important factor than hyperglycemia.

7. The insusceptibility of glycogen-infiltrated or restored islet cells of alloxan-diabetic rabbits to readministration of alloxan may be related tentatively to the possible origin of such cells from alloxan-insensitive beta islet or duct epithelial cells.

PART III

The Effects of Treatment with Crude APE on the Course and on the Pancreatic Lesions in Alloxan Diabetes in Rabbits

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Introduction

Houssay and associates established the existence of a hormonal relationship between the anterior lobe of the pituitary gland and the pancreatic islets of Langerhans in 1930. Seven years later Young (1937) reported the first production of permanent diabetes by injecting crude anterior pituitary extracts. Soon afterward Richardson (1939-40) demonstrated the morphologic identity of the pancreatic lesions of permanent diabetes produced by crude APE and by partial pancreatectomy.

Anselmino and Hoffman (1933) were the first to use the term pancreatropic with reference to a special substance in their anterior pituitary extract. Young (1937), Richardson and Young (1938-39), Richardson (1939-40) and Ham and Haist (1941) subscribed to the belief that a pancreatropic factor acting directly upon the pancreas existed in APE as an entity separate from the indirect-acting diabetogenic factor(s). Ogilvie (1944) likewise concluded that such a pancreatropic substance existed. In a recently published study Ogilvie (1949) attributed to this factor the evidences of amelioration of symptoms and of proliferative regeneration of islets which he observed in alloxan-diabetic rabbits treated with crude APE.

That alloxan-diabetic rabbits show an absolute reduction of insulin-producing islet cells need not be re-documented. The response

of Ogilvie's rabbits to APE injections is not an expected one in view of previous studies of the effects of APE on animals with decreased "islet reserves". Long (1939-40) observed that 70% of rats with surgically decreased "islet reserve" could be made diabetic by treatment with APE whereas normal rats are insusceptible to its diabetogenic action. Lukens and Dohan (1940) found that surgical resection of about half of the pancreas of the cat rendered this normally APE-resistant species highly susceptible to APE-diabetes. In the experiments of Long and of Lukens the animals did not have diabetes before APE was given; thus they must have had an adequate, although diminished, islet reserve. Even the refractoriness to APE occasionally encountered in the dog was overcome readily by partial pancreatic resection (Lukens, Fish and Dohan, 1941). Rats treated with subdiabetogenic doses of alloxan show increased diabetogenic effectiveness of APE (Shipley and Rannefeld, 1945).

The recent reviews of Lukens (1946) and of Anderson (1948) emphasize that the mechanism of the diabetogenic effects of APE are obscure. These authors concluded that pure growth and adrenocorticotropic hormones are the only pure pituitary substances thus far isolated which have been shown to possess diabetogenic properties. The presence of these two hormones may account for the pathogenic character of crude APE preparations. Recent studies (e.g. Bennet et al, 1947 and 1948) in which pure growth and adrenocorticotropic hormones were given to alloxan-diabetic rats showed that glucose

excretion and ketonuria were increased or unaffected; although nitrogen storage was increased when growth hormone was injected, no other manifestation of amelioration of symptoms was noted.

Hypophysectomy has no effect upon the islets of Langerhans in normal animals; if they are fed amounts of food comparable to those eaten by normal animals, hypophysectomized animals do not show a reduction of pancreatic insulin content (Haist et al, 1949). A specific pancreatropic factor has not been proved to exist amongst the galaxy of pituitary hormones (Selye, 1947).

It is apparent that researchers to date have failed to secure convincing evidence to support the expectation of beneficially influencing alloxan-diabetes by treating rabbits with APE.

Survey of Literature

Although Johns, O'Mulvenny, Potts and Laughton (1927) reported their observation of polyuria, glucosuria and hyperglycemia in dogs treated with injections of crude extract of anterior pituitary glands, Houssay and Biasotti (1930a) emphasized the interrelationship between the pituitary gland and the pancreatic islets by demonstrating that hypophysectomy prevents the development of pancreatectomy-diabetes in toads. Subcutaneous implantation of anterior pituitary lobes reestablishes diabetes in such experimental animals. Houssay and Biasotti (1930b) also observed that hypophysectomized-pancreatectomized diabetic dogs exhibited milder symptoms and lived longer than did dogs subjected to pancreatectomy only. Soon afterward, Evans, Meyer, Simpson and Reichert (1932), using a partially purified solution of their "growth hormone" prepared from an extract of acetone-precipitated anterior pituitary lobes, produced hyperglycemia and polyuria in normal dogs. At about the same time Baumann and Marine (1932) observed severe but transient polyuria and glucosuria in rabbits given repeated injections of crude saline extract of anterior pituitary lobes. The following year Anselmino and Hoffmann (1933) prepared such an extract by acetone-desiccation, adjustment of pH to a weakly acid value, ultracentrifugation and aqueous solution. Their extract had some physical and chemical properties which differed from those of other early preparations of

pituitary hormones; it produced insulin-like physiological effects during several hours immediately following a single dose given to various species of experimental animals; and, when repeated injections were given to rats, it seemed to produce a "pancreatropic effect" upon the histological picture in the islets. This effect was not studied quantitatively but, in their report, Anselmino, Herold and Hoffman (1933) described and attempted to photograph enlargement of the islets of Langerhans, fusion of individual islet complexes and the appearance of young, newly formed islets. In their biochemical studies of metabolic responses to single injections of their extract in various species, they noted the following effects:

1. Transitory depression of blood sugar level (in dogs).
2. Lesser elevation of post-cibal blood sugar level (in dogs); lesser elevation and shorter duration of post-cibal blood sugar levels (in rabbits).
3. Lesser elevation of blood sugar level in response to injection of adrenaline (in rabbits).
4. No appreciable influence on insulin-induced hypoglycemia (in rabbits).
5. Decreased liver glycogen content without hyperglycemia (in rats).
6. No change in the hyperglycemic level of pancreatectomized dogs given APE on the third postoperative day; no change in the post-cibal blood sugar level of pancreatectomized dogs given APE on the sixth postoperative day.

These histological and physiological data together with their few physicochemical data constituted the basis of the author's conclusion that their APE contained a special pancreatropic substance.

The first reported permanent diabetes produced by prolonged injection of APE in dogs was published by Young in 1937. Only one of twenty-five treated dogs (a pregnant bitch) failed to manifest permanent symptoms. However, Young (1938) found that only 25% to 50% of his normal rabbits and cats developed diabetes (which was transitory) when treated similarly, while mice, rats and guinea pigs were apparently insensitive to the diabetogenic activity of various crude preparations. According to Young's studies, acetone desiccation of fresh glands diminished but did not completely destroy diabetogenic activity. Having observed an unusual number of mitoses in the islets of the resistant (pregnant) dog, Young (1938) imagined that the extract contained a pancreatropic factor causing islet hypertrophy and increased secretion of insulin; if a time differential were assumed, he considered, the presence of two different factors was possibly responsible for the predominance of resistance (pancreatropic effect) in some animals of a species (i.e., the resistant dog) and for the varying degrees of resistance and susceptibility (diabetogenic effect) evident amongst the different species studied.

Richardson and Young (1937-38) employed Ogilvie's (1937) quantitative planimetric and statistical device for assessing islet/acinar ratios in rats treated with APE. In the Wistar strain they

observed a statistically significant doubling of the mean value of this ratio when the acinar as well as the islet tissue was found increased. However, they could not confirm histologically the pancreatropic activity of the acetone-desiccated preparation described by Anselmino, Herold and Hoffmann (1933) nor were "giant islets" and "proliferative activity" frequently associated with increased islet/acinar ratios. Richardson (1939-40) thoroughly described the histological effects of APE on the pancreas of the diabetic dog. He found that "hydropic degeneration" of the islet cells appeared after most of the beta cells had become agranular but during a phase when mitoses were evident in some beta cells. Accordingly, he conceived the sequence: degranulation and mitotic proliferation of beta cells (= stimulation) followed "hydropic degeneration" (= exhaustion) followed by atrophic dissolution of exhausted cells and collagenous hyalinization of the remnant of the islets. "Hydropic degeneration" was found in the ductule epithelium of the pancreas more frequently and consistently than in the islet cells; it did not disappear after prolonged periods of diabetes. Curiously, both the degranulation of beta cells and the vacuolation of ductule cells occurred in the pancreases of dogs treated with fractions of APE which did not possess diabetogenic activity. This study by Richardson (1939-40) was especially significant because it established that both partial pancreatectomy-diabetes and APE-diabetes were associated with a single morphological alteration in the same types of pancreatic cells, viz. the beta islet cells and the ductular epithelium.

Ham and Haist (1941) studied all of the organs of dogs made temporarily and/or permanently diabetic with APE by Best, Campbell and Haist (1939). They confirmed Richardson's findings with respect to pancreatic histopathology and added pertinent observations on the thyroid, adrenals, kidneys and liver as well as other organs. In a neatly-reasoned argument they presented an interpretation of the mechanisms whereby APE treatments bring about progression from temporary to permanent diabetes. Accepting the overstrain hypothesis of Allen (1913, et seq.), they noted that the pancreatic remnants of partially depancreatized diabetic dogs were called upon to produce as much as 10X the amount of insulin as that portion of the islet mass had produced before operation. Since the APE treated dog possessed the full complement of islet tissue, 10X the normal production of insulin would be required before sufficient overwork excessively strained the pancreatic islets. During the early phase of APE treatment such a strain must have been attained before hyperglycemia was manifest. Why such a marked overproduction of insulin did not lead to hypoglycemia during this early phase was explained by the authors in accordance with a relatively greater influence by the numerator than by the denominator of the ratio:

$$\frac{\text{diabetogenic activities of (or controlled by) anterior pituitary}}{\text{antidiabetogenic activity of beta cells (insulin secretion)}}$$

Ham and Haist (1941) assumed the occurrence of overproduction of insulin in this argument on obviously logical grounds; however, on the actual dogs studied, Best, Campbell and Haist (1939) reported no

such increase. On the contrary, they observed a prompt fall in the insulin content of the pancreas of treated dogs. There was a recovery to normal values on cessation of treatments after a few days but, if injections were continued until permanent diabetes existed, no subsequent increase in pancreatic insulin content occurred. In rats, however, Marks and Young (1940) did find that APE treatment was associated with double normal values for both the total amount of islet tissue and the insulin content of the pancreas. Recent studies of Haist, Evans, Kinash, Bryans And Ashworth (1949) re-emphasized the lack of changes in islets and the insulin content of the pancreas following hypophysectomy.

Ham and Haist (1941) pursued the mechanisms whereby APE produces permanent diabetes in their discussion of the cause of excessive stimulation of beta cells by APE. Four indirect influences, they thought, operated through increasing the metabolic need for insulin. These were: (1) Thyroid hyperfunction, (2) adrenal cortical hyperfunction (gluconeogenesis), (3) a von Gierke-like "hydropic" storage of glycogen in the liver, and (4) reduced hypoglycemic response to insulin. The last of these influences of APE was observed by Cope and Marks (1934), Young (1936) and Marks (1936). It is an important observation; for, if secretion of insulin were controlled by the blood insulin level, increased resistance to insulin would place an additional strain upon the islets. Ham and Haist (1941) assumed, as others have done, that degranulation of

beta islet cells indicated a functional state of stimulated secretion. Such degranulation was observed in their dogs and in those of Richardson (1939-40) at a time when the blood sugar was not yet elevated to hyperglycemic levels. Furthermore, Allen (1922b) observed the development of "hydropic change" in phlorizinized partially depancreatized dogs. Barron (1949) observed beta-degranulation in response to both glucose-infusion and repeated injection of insulin; he concluded that the beta granules are actually precursors of insulin. If this be true, degranulation of beta cells might represent either stimulation of these cells with loss of granules by secretion or, alternatively, it might represent functional inactivity.

Milman and Russel (1949) recently observed apparent profound and prolonged secretory stimulation of the beta islet cells in response to intraperitoneal injections of pure growth hormone in the fasted normal rats. Fatal hypoglycemia occurred without there having been any hyperglycemic phase. In France, Loubatières (1944) has observed fatal hypoglycemia in rabbits and dogs in response to single doses of "2254 R.P." (p-aminobenzenesulfamidothiodiazol). In North America a similar direct response was noted by Chen, Anderson and Maze (1946) when sulfanilamido-cyclopropylthiazole was given to rabbits. Both compounds were shown to stimulate the islets directly to secrete insulin. These several observations

indicate that hyperglycemia is not the sole stimulus to insulin secretion. They are in apparent disagreement with the bulk of reported evidence. The intricate perfusion experiments of Anderson and Long (1947) and experiments with pancreatofemoral anastomoses such as those performed by Foa, Smith and Weinstein (1948) seem to prove that hyperglycemia, rather than blood insulin concentration, controls the secretion of insulin by the islets. Nevertheless, it would seem impossible to dissociate hyperglycemia and hypo-insulinemia in the experiments cited, so that the evidence is not completely decisive. It still remains true that development of an accurate method for the estimation of insulin in the blood is a critically important problem (Waters and Best, 1947).

Although Ham and Haist (1941) considered the indirect influences acting upon the islets as of great importance, they could not rule out the possibility that an actual pancreatropic factor existed in APE. Especially noteworthy in their histopathological descriptions was the presence of mitotic activity in acinar tissue as well as in islet cells. They remarked upon the difficulty of defining the term trophic with precision because most hormones having trophic activities exert both a growth-effect and a secretion-stimulating effect upon their target tissues. They accepted the possibility of a pancreatropic (direct-acting) principle in their material.

Long (1939-40) resected most of the pancreatic tissue from immature rats. When these animals had attained maturity, they did not show glucosuria. Upon administration of APE about 70% of the rats promptly developed diabetes. Normal rats injected with APE remained symptom-free. Shipley and Rannefeld (1945) have noted increased diabetogenic effectiveness of APE in rats given repeated subdiabetogenic doses of alloxan. Lukens and Dohan (1940) found that surgical resection of half of the pancreas of cats did not produce diabetes. But, when these cats received APE injections, diabetes appeared and it persisted if sufficient doses were given over prolonged periods. Similar procedures readily evoked a diabetic state in dogs found previously to have been refractory to APE (Lukens, Fish and Dohan, 1941). These experiments were interpreted as indicating that if sufficient islet tissue is removed the diabetogenic effects of APE predominate over the insulin-secreting effects of the islet reserve. Hyperglycemia, Lukens concluded, was per se the damaging agent responsible for "hydropic degeneration" thus produced; for, various procedures which lowered the blood sugar level such as reduced caloric intake, low carbohydrate diet, insulin treatment, and phlorizin treatment prevented the development of the pancreatic lesion in response to crude APE injections.

Cavallero (1947-48) studied mitotic activity in the islets of Langerhans, adrenal medulla and anterior pituitary lobe of alloxan-diabetic rats from the first to twelfth post-alloxan days.

He gave colchicine to the animals nine hours before killing them. A "burst" of mitotic divisions was observed in the islet cells from the third to fifth days following the alloxan injections. This was wholly suppressed by controlling hyperglycemia with insulin. Anterior pituitary extracts, whether "whole" or "pancreatropic" (i.e. prepared according to Anselmino and Hoffman, 1933) increased the number of mitotic divisions observed in the islets to values twice as great as those enumerated in animals given alloxan only:

TABLE XII

Number of mitoses observed in 100 cross-sections of Islets of Langerhans (adapted from Cavallero, 1947-48).

Days after Alloxan	Alloxan only	Alloxan plus Insulin	Alloxan plus APE
1	8	0	3
2	7	1	24
3	44	2	132
4	81	0	185
5	31	1	86
9	2	0	8
12	7	1	7

Cavallero found a parallel increase and decline in the incidence of mitoses encountered in the anterior lobe of the pituitary glands of these animals. This increase was also suppressed by insulin treatment and augmented by APE injections. One might suggest that a pancreatropic agent of endogenous origin was made evident by destruction of the insulin-secreting beta cells with alloxan. It would,

however, be curious if exogenous pancreatropic substance not only exaggerated the islet-cell proliferative response but also evoked exaggerated proliferation (instead of suppression) of the pituitary cells responsible for secretion of the endogenous principle. The numerical parallelism indicates caution in attributing the results of Cavallero's experiments to a special pancreatropic factor. Cavallero attempted no concurrent assessment of metabolic changes.

Ogilvie (1944) observed the effects of crude saline APE on normal rabbits. By quantitative estimations (Ogilvie 1937) he found twice as much islet tissue in rabbits given APE for 30 days as in a control group. Almost all of the treated animals experienced temporary phases of diabetes with decreased sugar tolerance and decreased insulin sensitivity. The islets of the treated group were found to measure about twice the size of the control group, but their number remained the same. One rabbit (of 28) also showed proliferation of small ducts and growth of new islets. No mitoses were seen. Ogilvie attributed these effects to a pancreatropic factor.

More recently Ogilvie (1949) treated five of six severely alloxan-diabetic rabbits with crude APE. He attempted to portray metabolic changes in these rabbits during very long studies. The APE was given in repeated courses of a few days to a few weeks, separated by intervals of a few days to more than a month. Unfortunately (apparently for lack of adequate space) the published reports

are not susceptible to close scrutiny, periods of about a year being compressed into very small graphs. However, if one accepts the author's descriptions, only one of the treated rabbits showed no metabolic changes and no histological effects in the pancreas. Four rabbits responded by temporary periods of diminished severity. Later, three of these became completely unresponsive to APE and were as severely diabetic at the termination of the experiment as they had been before APE was administered. The fourth, however, made a dramatic response to each of eleven courses of APE; at one time a period of 22 days elapsed without any manifestation of diabetes. Nevertheless, this rabbit also became unresponsive eventually (about 16 months after alloxan had been injected) and it was likewise as severely diabetic finally as it had been originally. Ogilvie found that those animals which had responded by temporary improvement following APE showed regeneration of islet tissue. The evidence for this consisted of enlargement of islets, "budding" of islets and a suggestive new growth of islets from the ducts. All of these criteria are open to grave doubt. Nevertheless, Ogilvie concluded that the temporary improvement exhibited by four of his APE-treated rabbits could be attributed to the pancreatropic action of pituitary extract.

It is noteworthy that "hydropic degeneration" of islets and ductules was present in all of Ogilvie's alloxan-diabetic rabbits; because they were killed after several weeks of severe diabetic symptoms following discontinuation of APE treatment and many months after alloxan injection, this finding was to be expected (Duff, McMillan and Wilson, 1947).

By means of the ultracentrifuge, electrophoresis and other relatively modern methods for protein chemistry six major hormones have been obtained in pure form from the pituitary gland. Thyrotropic, lactogenic and the two gonadotrophic hormones have been obtained as single proteins, in which state they manifest their respective activities with little or no overlapping of other pituitary functions. As a result, it is possible to exclude them from any major part in the diabetogenic activity of the pituitary (Lukens, 1946). Thus, by elimination, the growth hormone and adrenocorticotropic hormone must be largely responsible for the metabolic effects characteristic of diabetes of pituitary type. Although the separate metabolic effects of these two hormones have not been studied completely, to date increasing evidence has become available to indicate that their combined actions may account for all the diabetic effects observed in animals treated with crude extracts. It is not an obligatory task to review all of the studies supporting this conclusion here; it must suffice to cite several pertinent observations bearing more directly upon the problem of the effects of APE upon established diabetes. In the studies of Bennet and associates (1947, 1948) ACTH always increased the severity of diabetes in alloxan-diabetic rats regardless of their maintenance on either stock diet or carbohydrate-free diet and regardless of the amelioration induced by hypophysectomy. Growth hormone given to similarly prepared animals occasionally increased the amount of glucose excreted while animals were on stock

diet, but did not significantly alter glucose excretion of animals maintained on a carbohydrate-free diet or subjected to hypophysectomy. The nitrogen storage values for such rats were increased by growth hormone injections when rats were maintained on either stock or carbohydrate-free diets; nitrogen loss was found decreased when hypophysectomized diabetic rats were treated with growth hormone. On the carbohydrate-free diet both growth hormone and ACTH increased the severity of ketonuria if diabetes was severe. Gaarenstroom, Hublé and de Jongh (1949) found that growth hormone increased the glucose excretion of mildly alloxan-diabetic rats but not of "alloxan-resistant" or of severely diabetic animals. Growth hormone did not arrest decreased sugar excretion when hypophysectomized, alloxan-diabetic rats were treated with it by the same investigators. Cotes, Reid and Young (1949) have produced diabetes by administering pure growth hormone to adult cats. Working with the perfused rat pancreas (maintained in the special apparatus of Long, 1938), Anderson and Long (1947b) studied the effects of various pure hormones on the secretory activity of the pancreatic islets in the perfused rat pancreas. Their finding was not consonant with the above-mentioned observation of fatal hypoglycemia in response to growth hormone in intact rats (Milman and Russel, 1949). They found that growth hormone did not directly stimulate the secretion of insulin and that both growth and adrenocorticotropic hormones inhibited insulin secretion induced by raising blood glucose levels. Neither thyroxin nor adrenal cortical extract inhibited insulin secretion. Conn, Louis and Wheeler

(1948) and Conn, Lewis and Johnston (1949) observed temporary diabetes in men experimentally injected with ACTH. Both Lukens (1946) and Anderson (1948) in reviewing the pertinent data have concluded that growth hormone is ketogenic and that it inhibits utilization of carbohydrate while ACTH mobilizes both protein and carbohydrate. Despite the newer knowledge of the effects of pure pituitary hormones, both reviewers agreed, the mechanism of action of crude APE remains poorly understood. Colowick, Cori and Slein (1947) published studies indicating that anterior pituitary and adrenal cortical extracts inhibited the enzyme hexokinase as tested in the reaction glucose + adenosine triphosphate = glucose 6 phosphate + adenosine diphosphate. The inhibitions exerted by these extracts could be overcome by addition of insulin. Lactogenic, thyrotropic and adrenotropic hormones did not inhibit hexokinase reactivity. However, the demonstrations reported are intricate and are susceptible to vagaries of instability of hexokinase and of APE. Furthermore, they utilize a highly artificial control to represent "normals." In any event, these results have not been substantiated.

Summary:

There is no doubt that crude extracts of anterior pituitary lobes have a diabetogenic effect upon some species of normal animals. The mechanism of this action is not clear despite accumulative evidences that it may be attributed to either growth hormone or to adrenocorticotrophic hormone or to both of these factors. Although

many investigators have attributed some of the metabolic changes observed in animals treated with APE to a special pancreatropic factor, all of their evidence is indirect; much of it consists of possibly equivocal interpretations of histological changes in the pancreas. When permanent diabetes results in APE-treated animals, the pancreatic islets and ductules show so-called "hydropic degeneration", a lesion common also to partial pancreatectomy-diabetes, alloxan-diabetes, oxine diabetes and glucose infusion-diabetes. In each of these types except the last two and in one cortisone-diabetic rabbit as well, the author has demonstrated (Part I) that the vacuolation commonly attributed to aqueous or plasmatic dissolution of the cytoplasm is actually referable to artefactual removal of intracytoplasmic accumulations of glycogen.

Only one author has observed temporary amelioration of the symptoms of diabetes upon treatment of alloxan-diabetic rabbits with crude APE. All other studies employing pure growth hormone and adrenocorticotropic hormone have either failed to modify the diabetic state significantly or have evoked increases in the severity of glucosuria and ketonuria. Growth hormone apparently alters nitrogen balance favorably, but it may inhibit the secretion of insulin, increase glucosuria and increase ketonuria. Despite the beneficial effects of APE on the course and lesions of alloxan-diabetes in rabbits as reported by Ogilvie (1949) no firm rationale exists upon which to predicate beneficial effects resulting from an hypothetical

pancreatropic factor in crude APE. On the contrary, it would be more appropriate to anticipate a worsened metabolic state or a lack of any effect.

The experiments which are reported herewith were undertaken for the purpose of attempting to evaluate some of the effects of APE treatment on the pancreatic lesion and on the course of alloxan diabetes in rabbits.

Experimental Study

Preparation of APE

Fresh frozen ox pituitary glands were obtained from Armour and Company, Chicago, Illinois, through the courtesy of Dr. John R. Mote. Preparation of crude alkaline extract of the anterior lobes was carried out in the manner of Young (1938). Upon thawing to a semi-frozen state, the glands were maintained at a temperature near zero degrees centrigrade by working in a cold room with all vessels immersed in ice water. The capsules and posterior lobes were dissected away by scissors and forceps to provide well-cleaned anterior lobes only. These anterior lobes were then weighed. They were minced with a knife and ground with clean sterile sand in a large mortar immersed in ice water. During the grinding process cold

physiological saline solution was added in the proportion of 3 ml. of saline per gram of anterior lobes. At frequent intervals small quantities of N/5-NaOH were added to maintain a pH of about 8.5 according to pH test paper. Extraction was allowed to proceed overnight in the cold. The extract was then centrifuged in chilled 250 ml. bottles at 1500 r.p.m. for 30 minutes. The supernatant (extract) was transferred to a large Erlenmeyer flask by suction and the solid residue discarded.

Up to this stage in the preparation every effort was made to preserve cleanliness and avoid further contamination of the extract. The fluid was subsequently passed through a Seitz bacteriological filter (which was set up in a cold room), collected in sterile 50 ml. bottles sealed with perforated screw-caps utilizing rubber interliners. Bacteriological cultures of the filtered extract yielded neither aerobic nor anaerobic growths. The bottles were stored at -22°C. until required for injection.

For use, a sufficient number of bottles for the day's treatments was removed from storage; the extract was thawed rapidly at about 37°C in a pan of water. With sterile precautions the extract was transferred to 20 ml. hypodermic syringes, one for each animal to be treated. The backs of the rabbits were shaved smoothly. The skin was sterilized with iodine followed by 70% alcohol. The total dose was injected subcutaneously at a different site each day.

Since it was desired to minimize the diabetogenic effect in hope of favoring the pancreatropic effect, a rather small constant dosage was used. This was approximately "one gram-equivalent" per kilogram of body weight per day. This volume of crude extract represents one gram of fresh anterior pituitary lobes. For the preparation made and used in this study, 5.5 mls. of crude alkaline APE constituted one "gram-equivalent."

Preliminary determination of diabetogenic activity of APE.

The diabetogenic effect of APE on a normal dog: A mongrel adult male dog was given six subcutaneous injections of APE during a period of seven days, using approximately one gram-equivalent per kilogram of body weight. Because of the large volume required for this dosage and the difficulty in handling this ill-tempered animal, sterile technique could not be maintained; a formidable subcutaneous hemolytic streptococcal infection supervened and necessitated premature sacrifice of the dog. Nevertheless, glucosuria was apparent on the second to seventh days; on the second, fourth and sixth days the fasting blood sugar levels were 190, 152 and 240 mg.%, respectively; ketonuria was present on the seventh day. This dog refused to eat any kind of food, vomited frequently and lost more than a kilo of body weight.

It was realized that this test did not constitute a suitably exact assessment of the diabetogenic capacities of the extract to be

used. However, methods currently available for testing the diabetogenic activity of pituitary preparations (Reid, 1949) entail procedures of greater complexity and require greater amounts of extract than were available. Accordingly, it was necessary to forego a more precise demonstration of the qualities of the APE preparation.

To a degree the use of two normal rabbits in conjunction with the alloxan diabetic experimental group was considered sufficient demonstration of the diabetogenic activity of the extracts. Two normal adult domestic albino rabbits, one male and one female, were treated simultaneously with APE in daily doses of about one gram-equivalent per kilogram of body weight over an interval of twenty-two days. One of them, number J26, was killed within twenty-four hours of the last dose; the other was kept for a post-treatment interval of twelve days before autopsy was performed. During treatment both animals were allowed free access to food. The amount eaten each day was measured by weighing the remnant, after twenty-four hours, from a fixed excess allowance. The only food permitted was "Miracle Baby Rabbit Pellets", manufactured by Ogilvie Flour Mills Co., Limited, Montreal. According to the manufacturer's analysis, kindly provided by Mr. D. R. Kennedy of that firm, this food has the following composition:

Protein	19.3 - 21.3%
Fat	4.5 - 5.2%
Carbohydrate*	40.0 - 50.0%
Fiber	- 18.0%

* Assuming ash residue = 6% and moisture = 11.5%.

The approximate amount of "available glucose" was calculated from these figures in the manner of Kennedy and Lukens (1944). This calculation assumes that all of the carbohydrate and half of the protein could be metabolically transformed to glucose. The rabbit may digest 22% of cellulose, according to Lusk (1932). This proportion was neglected in the calculation because of the inexact knowledge of the cellulose content of the food (the manufacturer's claim of a very low fiber content not being guaranteed beyond the statement that there would not be more than 18% of fiber). The maximal error resulting from neglecting this fraction of "available glucose" may be calculated as approximately 7%; e.g.:

Food consumed -	100 grams
Protein	20
Carbohydrate	45
Cellulose	less than 18

Available glucose: $(20 \div 2) + 45 = 55$ grams
" glucose from cellulose: $0.22 \times 18 = 4$ grams
Error: $4 \div (4+55) \times 100 = 7\%$

It was found useful to express the daily "available glucose" and the urine glucose excretion in terms of kilograms of body weight (i.e. grams/kilo/24 hours). By this maneuver, one may calculate the proportion of available glucose excreted in the urine, thus attaining a kind of measure of the severity of the diabetic state of the fed animal. One may also compare severity for the same animal at different times, different body weights and different diets, or one may roughly assess the difference in severity of diabetes in different animals. The device eliminates some of the

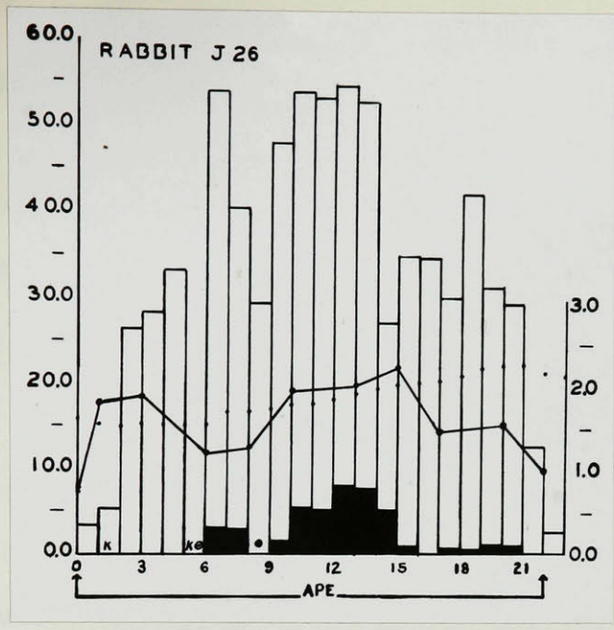
fallacies inherent in comparisons of severity of diabetes based only on quantitative analyses of glucosuria.

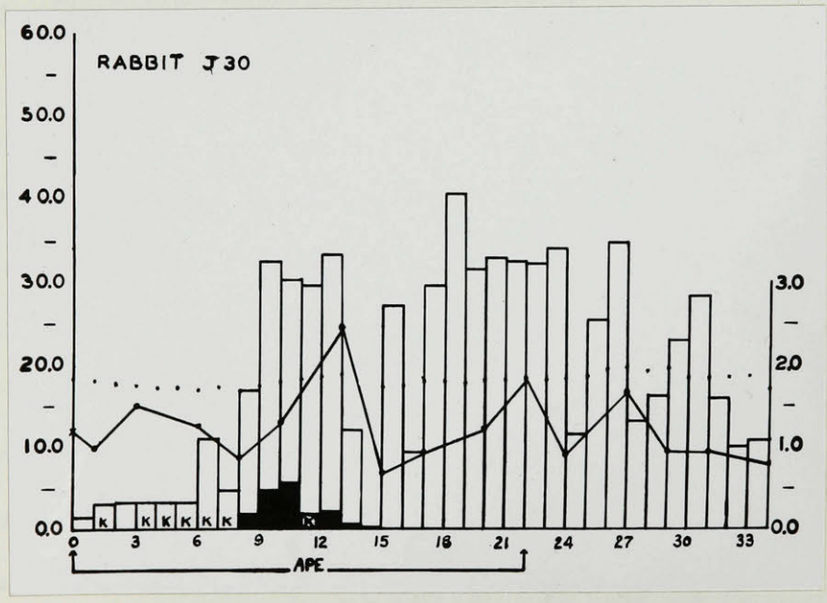
Daily urine glucose excretion was determined by Benedict's method of titration on samples of collected 24-hour specimens of urine.

Blood glucose levels were determined by the Nelson (1944) photometric adaptation of Somogyi's method. These were made three times per week throughout the course of the experiment. After APE treatment was discontinued, the determinations of blood sugar levels of number J30 were made after fasting periods of about 16 hours, the animal having food removed from the cage overnight on each of the twelve additional days. Blood samples were taken from the central artery of the ear.

The effects of APE treatment on these two normal rabbits, numbers J26 and J30, are depicted graphically.

Comparison of these graphs reveals that both J26 and J30 responded similarly to APE treatments. Initially, food consumption fell off sharply, body weight decreased slightly and ketonuria appeared early. The blood sugar level, having shown an early slight elevation was found to be less than 150 mg. per ml. when glucosuria first appeared. There was a second rise of glycemia during the second week of treatment coincident with marked polyphagia, glucosuria, slightly increased body weight and disappearance of ketonuria.





Graphs J 26 and J 30. The diabetogenic effects of crude APE on the normal rabbit. Dose: 1 gram-equivalent/kilo body weight/day.

Legend

Left vertical scale: Blood sugar level in centigrams/100 mls. (solid line); available glucose (open bars) and urine glucose (solid bars) in grams/kilo of body weight/day.

Right vertical scale: Body weight in kilograms (. . . . .).

K: ketonuria; ⊙ food not measured; • urine glucose not determined.

During the third week, although body weight continued to increase, blood glucose levels diminished, glucosuria became much reduced (J26) and disappeared completely (J30). During this third week J26 was eating less than during the second week but J30 showed no significant change in food consumed.

The metabolic changes portrayed provide strong evidence of the metabolic effectiveness of the APE preparation. The temporary diabetes thus produced in these rabbits together with its gradual diminution and disappearance are in agreement with the reported experiences of Young (1938), and of Ogilvie (1944). The differences which did appear were minor: (1) both J26 and J30 showed polyuria during the phase of treatment when glucosuria was most pronounced, and (2) urine glucose excretion by J26 reached a peak of 15.8 grams per 24 hours which is slightly higher than the highest value observed by Young (1938). However, comparisons of severity of glucosuria would be more meaningful if expressed in accordance with the device of percentage of available glucose excreted. It is not possible to make such a computation from the reports published by Young. The graphs presented show that J26 made a more exaggerated response to APE as judged by severity and duration of glucosuria, peak level of hyperglycemia, and polyphagia. However, ketonuria was more severe and prolonged in J30, and this animal also gained less body weight than did the other. When the calculated percentages of available glucose excreted were compared for the two animals (see Table XIX) it was found that the severity of their diabetic states was much the

same: each attained an average value of about 9% over one six-day interval.

To assess the cytologic changes in the pancreatic islets in a satisfactory quantitative way was beyond the scope of the present efforts. No attempt was made to confirm by quantitative methods the occurrence of hypertrophy and hyperplasia of the islets of Langerhans as described by Richardson and Young (1937-38) for the rat and by Ogilvie (1944, 1944-46) for the normal rabbit. The experiences of Jaffe (1949), who undertook quantitative investigations of the islets of the normal and of the alloxan-diabetic rabbit, amply warn the researcher away from facile techniques and random impressions, indicating the necessity for scrupulous and exhaustive preparation and analysis of histological material. While Jaffe's studies provide data for such quantitative efforts, it is apparent that many months of experience would be necessary for a proper background upon which to attempt a similar essay. Tejning (1947) has found for the rat that the presence of large numbers of unusually large islets is positively correlated with an absolute increase in total volume of islets, while a large number of unusually small islets is positively correlated with an absolute decrease in total volume of islets. If true, this observation would sometimes obviate the necessity of complicated and exhaustive quantitative technics such as Ogilvie (1944) and Jaffe (1949) have utilized. Nevertheless, Tejning's method would not disclose the relative numbers

of special types of islet cells. Hence, it would not be applicable to rabbits previously treated with alloxan, in which few islets are found to contain beta cells, the total number of islets is reduced, and most of them are of smaller size than normal. Since such rabbits constituted the main subjects of these experiments, Tejning's method was invalid.

Therefore, only a simple histopathological examination of the islets was undertaken. For numbers J26 and J30, no islet abnormalities could be appreciated. Mitoses were seen but were not more abundant than usual. The tissue after a twelve-day period without APE (J30) was indistinguishable from that prepared within 24 hours after the last dose of APE (J26). In every morphological respect, these pancreases appeared quite similar to those of normal rabbits examined by the same histopathological routine methods.

Thus there was no obvious morphological confirmation of the suggested existence in crude APE preparations of a special pancreatropic factor. This interpretation does not suffice to exclude the possible reality of a pancreatropic effect having been present.

Preparation of the alloxan-diabetic rabbits.

Preliminary observations: Fifteen adult domestic albino rabbits were housed individually in metabolism cages. After a few days of preliminary observation, each of these normal rabbits plus one mature alloxan-recovered female were submitted to simplified

glucose tolerance tests. The test consisted of determinations of blood sugar levels in the fasting state and at one and two hour intervals after intravenous administration of 20% dextrose solution in the amount of one gram of dextrose per kilogram of body weight. The results of these tests are tabulated in Table XIII.

TABLE XIII

Glucose Tolerance* of Rabbits

Rabbit Number	Fasting Blood Sugar Level#	1 hour Blood Sugar Level	2 hours Blood Sugar Level
I 88	100	134	148
I 89	60	148	128
I 91	86	196	144
I 92	100	242	156
I 93	104	120	148
I 94	100	134	114
I 95	108	246	120
I 96	114	214	172
I 97	124	134	180
I 98	98	242	128
I 99	104	194	-
I 100	96	84	106
J 1	66	144	86
J 2	76	304	106
J 3	86	114	120
E	1422	2650	1856
N	15	15	14
E/N	95	177	133
D 73##	80	146	92

* 1 gram dextrose per kilo of body weight, 20% aqueous solution intravenous.

\# Arterial blood samples estimated by Nelson's photometric adaptation of Somogyi's method for true glucose.

Alloxan recovered adult doe.

It is apparent from the table that the arterial blood sugar level of the normal rabbit two hours following intravenous administration of one gram of dextrose per kilo of body weight was usually less than 150 mg./100 ml., exceeding this level in only three of the fifteen tests. The test levels of the alloxan-recovered rabbit lie within the average values for the fifteen normals. It may be presumed that more elaborate techniques of testing glucose tolerance, such as that recently proposed by Zucker (1949) might have disclosed an altered tolerance in this animal.

Response to injection of alloxan: The fifteen normal rabbits were injected intravenously with 5% aqueous alloxan after a fasting period of 16 hours. The dose was 150 mg. of alloxan monohydrate (Eastman) per kilogram of body weight. One rabbit was found dead one and a half hours after the injection. The other fourteen were given 50% dextrose solution by stomach tube about 6 hours after alloxan administration; each animal received 25 grams of dextrose. On the following day one rabbit was found dead. The urine samples of the remaining animals gave positive reactions with "Galatest", indicating severe glucosuria. During the ensuing eleven days body weight, urine volume and glucose content, ketonuria and food consumption were measured each day. Food was made available during eight hours of each day, being removed each evening. Thus fasting blood sugar levels were attainable. These were made three times per week on each animal. Utilizing the conventional calculation for "available glucose", this figure was computed from the

daily records for each animal; the percent of available glucose excreted was also calculated. By the twelfth day after the alloxan injections only seven of the fifteen injected rabbits remained alive. Metabolic data for each of these have been plotted in graphic form together with subsequent data for changes attending APE treatment. Average values for intervals of six days have also been tabulated.

From the graphic and tabulated data it can be readily determined that a severe state of diabetes resulted from alloxan treatment of each surviving rabbit. It is also apparent that no single criterion may be used to assess the relative severity of diabetes in this group (see Table XX). For instance, if one uses the height of the blood sugar level to judge the severity of the metabolic disturbance, during the second six-day interval, the order of decreasing severity is I 97, I 100, I 89, I 92, I 93, J 2 and I 99. However, if the percentage of available glucose excreted is utilized in like manner for the same six-day interval, then the order becomes I 97, I 89, I 99, I 93, I 100, J 2 and I 92. Similarly, utilizing loss of body weight, ketonuria, polyphagia and polyuria no consistent order of severity of diabetes can be discovered (see Table XX). It becomes apparent, however, that numbers I 97 and I 100 were probably the most severely affected, for they showed the greatest weight loss, most persistent ketonuria and highest fasting blood sugar elevation. These two rabbits ate less than the others and excreted less urine. Number I 97 excreted a higher percentage of the available glucose than any other of the group, but three rabbits in addition to I 97 excreted higher percentages of available glucose than did number I 100.

(continued - page 124)

TABLE XIV

Average Daily Body Weight (Kilograms)

by 6-day intervals

	Alloxan			Anterior Pituitary Extract					
	Before	After		During				After	
	0	1	2	1	2	3	4	1	2
J 26	-	-	-	1.55	1.74	2.03	2.19	-	-
J 30	-	-	-	1.75	1.77	1.79	1.83	1.91	1.81
I 100	2.55	2.50	2.30	2.14	-	-	-	-	-
I 89	2.29	2.50	2.16	2.15	-	-	-	-	-
J 2	2.42	2.42	2.22	2.01	-	-	-	-	-
I 92	1.89	1.94	1.98	2.05	2.24	2.46	2.78	-	-
I 93	2.19	2.23	2.30	2.17	2.26	2.45	2.66	2.58	2.37
I 97	1.91	1.93	1.64	1.35	1.47	1.50	-	1.36	-
I 99	2.51	2.55	2.62	2.55	2.71	2.86	2.78	-	-

TABLE XV

Average Blood Sugar Level

by 6-day intervals

	Post. Alloxan*		During APE Rx**				Post. Rx.*	
	1	2	1	2	3	4	1	2
J 26	-	-	163	158	189	129	-	-
J 30	-	-	126	110	133	132	127	88
I 100	458	447	-	-	-	-	-	-
I 89	449	431	390	-	-	-	-	-
J 2	308	293	318	-	-	-	-	-
I 92	416	356	406	445	504	585	-	-
I 93	334	343	474	342	389	464	220	70
I 97	545	493	362	450	401	-	342	-
I 99	348	246	384	398	212	106	-	-

* Fasting

**Random

TABLE XVI

Average Daily Available Glucose

by 6-day intervals

	Post-Alloxan		APE Rx.				Post-Rx.	
	1	2	1	2	3	4	1	2
J 26	-	-	19.4	46.8	39.4	29.1	-	-
J 30	-	-	2.9	20.8	18.5	34.0	20.7	16.5
I 100	16.5	11.2	(2.7)	-	-	-	-	-
I 89	20.8	22.5	0.5	-	-	-	-	-
J 2	21.9	24.2	0.6	-	-	-	-	-
I 92	32.5	36.3	29.9	54.3	51.9	44.6	-	-
I 93	22.5	29.6	10.2	55.1	58.3	56.2	22.1	25.0
I 97	17.1	19.0	23.6	47.5	31.2	-	20.0	-
I 99	22.9	25.2	18.1	43.6	24.8	13.4	-	-

TABLE XVII

Average Daily Urine Volume

by 6-day intervals

	After Alloxan*		During APE Treatment#				After APE#	
	1	2	1	2	3	4	1	2
J 26	-	-	162	432	347	188	-	-
J 30	-	-	125	323	137	125	220	190
I 100	190	71	160					
I 89	159	195	125					
J ?	135	125	90					
I 92	158	145	240	612	670	698		
I 93	168	172	133	398	543	703	297	152
I 97	100	115	173	230	137		143	
I 99	162	160	227	707	292	248		

* ccs./kilo/day

ccs./day

TABLE XVIII

Average Daily Excretion of Glucose(1)

by 6-day intervals

	Post-Alloxan		During APE Rx.				Post-Rx	
	1	2	1	2	3	4	1	2
J 26	-	-	0.0	3.8	3.9	0.6	-	-
J 30	-	-	0.0	2.3	0.5	0.0	0.0	0.0
I 100	7.1	2.9	(2.7)*	-	-	-	-	-
I 89	7.3	8.8	2.3	-	-	-	-	-
J 2	6.6	5.5	1.8	-	-	-	-	-
I 92	8.5	8.5	8.1	17.3	18.1	16.6	-	-
I 93	6.9	7.8	4.2	11.7	14.9	15.4	5.1	0.8
I 97	5.7	8.1	7.3	12.5	5.5	-	1.5	-
I 99	6.9	6.7	5.5	13.8	2.3	0.4	-	-

(1) Grams per kilo per day

* Two observations

TABLE XIX

Average Daily Excretion of Glucose*

by 6-day intervals

	Post-Alloxan		During APE Treatment				Post-Treatment	
	1	2	1	2	3	4	1	2
J 26	-	-	0.0	7.4	9.1	1.8	-	-
J 30	-	-	0.0	9.0	2.3	0.0	0.0	0.0
I 100	72.5	26.4	-	-	-	-	-	-
I 89	43.0	42.2	127.0	-	-	-	-	-
J 2	39.9	24.4	110.3	-	-	-	-	-
I 92	34.5	14.6	33.7	31.9	35.4	41.5	-	-
I 93	33.0	26.6	97.1	21.3	25.3	28.5	27.3	3.9
I 97	123.3	47.5	19.5	24.9	17.2	-	0.3	-
I 99	40.9	29.5	42.7	32.6	9.4	1.7	-	-

* % Available Glucose Excreted:

(Excreted ÷ Available) X 100

TABLE XX

Attempted Comparison of Severity of Diabetes

during 7th to 12th days following Alloxan

*Weight Change	Ketonuria**	Fasting Bl.Sugar	Food Consumption	Urine Excretion ccs./day	Urine Excretion ccs./kilo/day	Glucose Excretion gms./kilo/day	Glucose Excretion % AGE
I 97	I 100	I 97	I 92	I 99	I 89	I 89	I 97
I 100	I 97	I 100	I 93	I 89	I 93	I 92	I 89
J 2	I 89	I 89	I 99	I 93	I 99	I 97	I 99
I 92	I 99	I 92	J 2	I 92	I 92	I 92	I 93
I 93	I 93	I 93	I 89	J 2	J 2	I 99	I 100
I 99	I 92	J 2	I 97	I 97	I 97	J 2	J 2
I 89	J 2	I 99	I 100	I 100	I 100	I 100	I 92

* Second post-alloxan interval - pre-alloxan interval

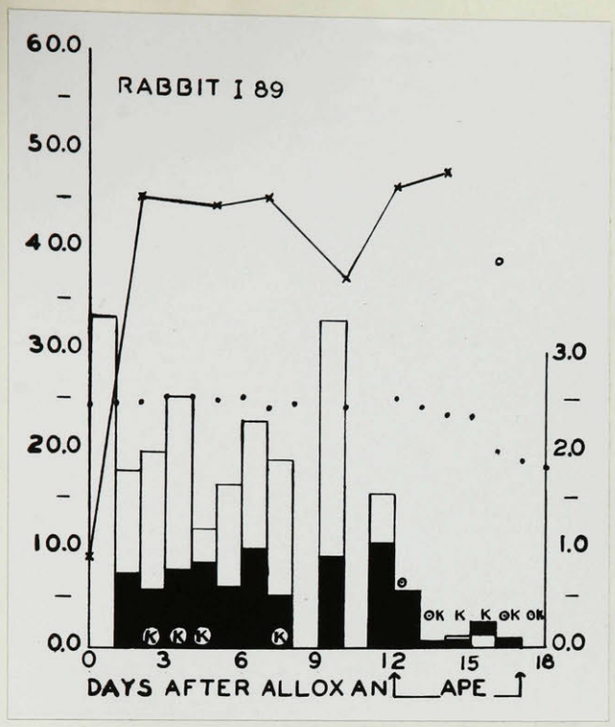
**Persistence during 1st and 2nd post-alloxan intervals.

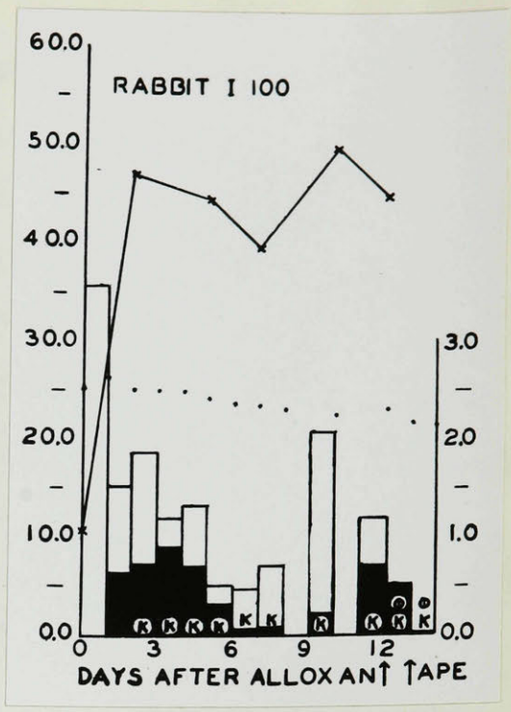
Other effects of alloxan than those dependent upon necrosis of beta cells complicate the metabolic alterations attributable to diabetes. Consequently, simple indirect estimations such as have been attempted herein are not wholly reliable measures of severity of diabetes. A precise method for estimating blood insulin levels might provide a sound basis for gauging severity of diabetes, but reliable methods currently available, such as that utilized by Anderson et al (1947) are too complicated for such a large scale experiment as has been attempted here.

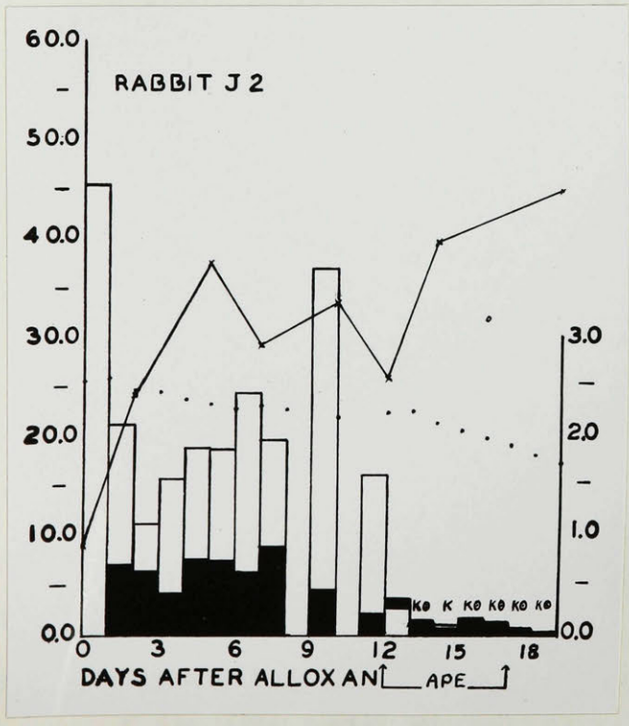
Effects of APE-treatment on Alloxan-diabetic Rabbits

Data pertinent to metabolic changes attending injections of APE in alloxan-diabetic rabbits have been portrayed graphically for each of the animals except number D 73, the alloxan-recovered one. In addition, the data have been tabulated as average values for successive six-day intervals, again excluding number D 73.

Comparative reference to graphs for numbers I 89, I 100 and J 2 shows that each of these diabetic rabbits responded similarly to APE treatment. Food consumption was suddenly reduced to almost nothing, with the result that, in the first twenty-four hours following the first dose of APE, glucose excretion exceeded the amount of "available glucose." Ketonuria appeared following the second injection. The body weight declined rapidly. Blood sugar levels of numbers I 89 and J 2 remained elevated; the blood serum was visibly lipemic.







Legend:

Left vertical scale: Blood sugar level in centigrams/100 mls. (x, fasting; o random); available glucose (open bars) and urine glucose (solid bars) in grams per kilo of body weight per day.

Right vertical scale: Body weight in kilograms (. . . . .).

K: ketonuria; ⊙ no food eaten.

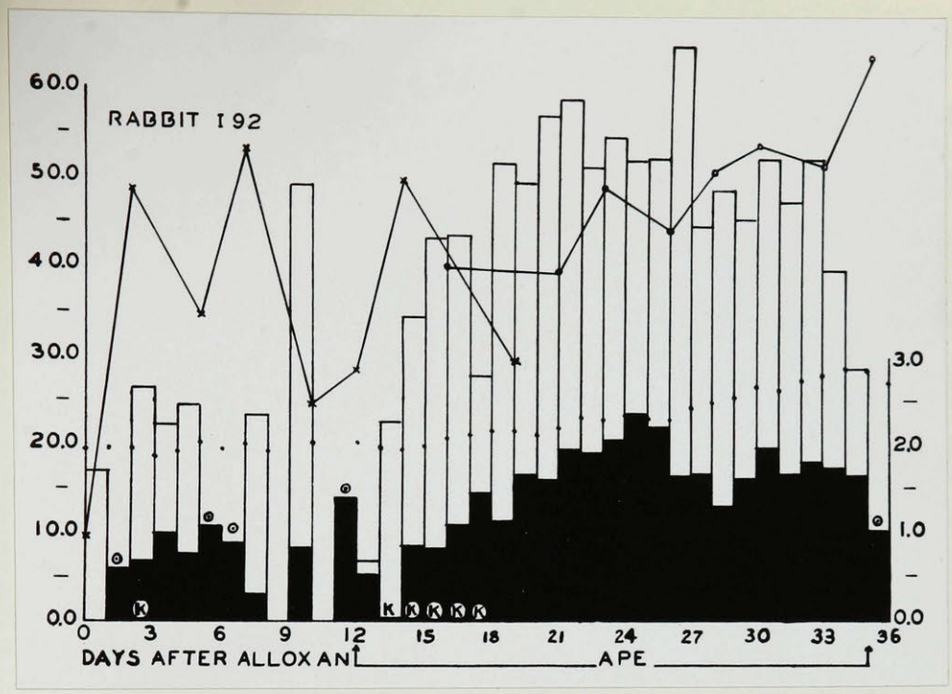
Graphs I 89, I 100 and J 2. The effects of crude APE treatment on the course of alloxan diabetes in the rabbit. Dose: 1 gram-equivalent/kilo body weight/day.

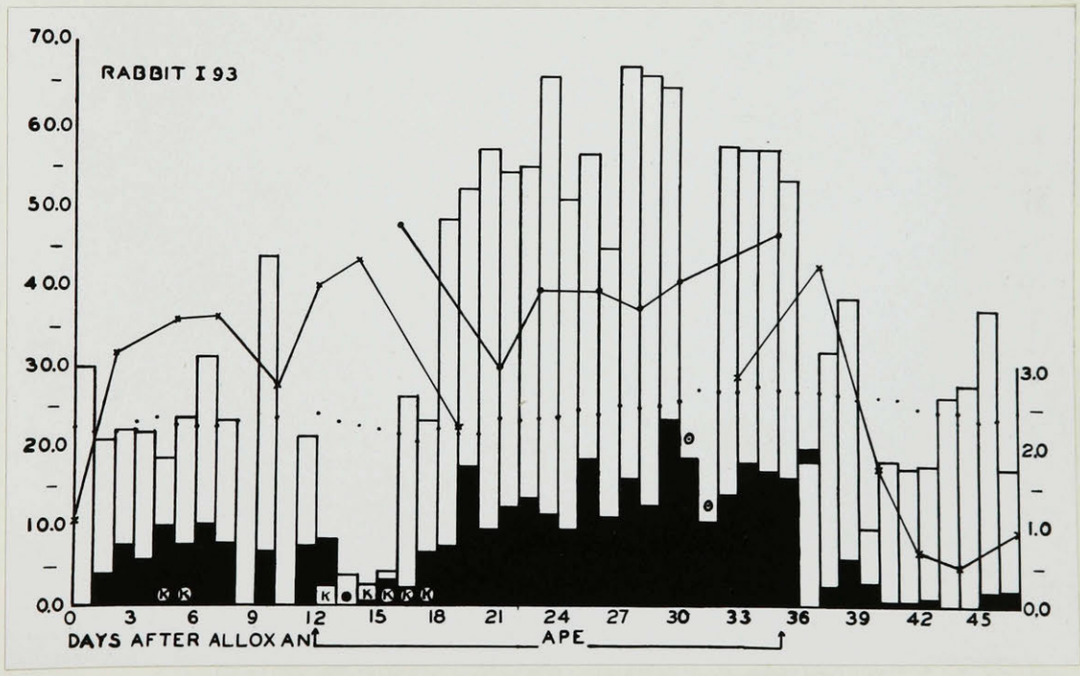
Number I 100 died after the second injection and number I 89 after the sixth. Number J 2, whose course was practically identical with that of I 89, was killed after the sixth injection in order to obtain histological studies unobscured by post mortem changes. Such changes were so advanced in the body of number I 100 that autopsy examination was omitted. The pancreases of both I 89 and J 2 (see Figures 49 and 50) showed obvious accumulations of glycogen in the islets, ductular epithelium and centro-acinar cells. Mitoses were present in agranular (beta) islet cells of number J 2 but could not be found in number I 89. A slight degree of glycogen infiltration of renal tubular epithelium was present in number J 2 but not in number I 89.

Reference to graph number I 92 shows that this rabbit responded initially but transiently to the first few APE treatments as did numbers I 100, I 89 and J 2. Ketonuria appeared after the second injection and was evident for the next four days. Although food consumption was much less during the first twenty-four hours after the first APE injection, it rose abruptly immediately afterwards and eventually, during the second and third six-day APE treatment intervals (see Table XVI) attained an average value almost twice as great as the averages of the two six-day post-alloxan intervals. While glucose excretion was much depressed on the first and second days of treatment, the percent of available glucose excreted on the first day was markedly elevated. During the balance of the APE treatment

period, the absolute amount of glucose excreted, in terms of grams per kilo per day was about double what it had been following alloxan; however, in terms of percent of available glucose excreted, there was only a slightly increased severity of the diabetic process. Body weight rose by small irregular increments during APE treatment. There was a striking continuous elevation of the random blood sugar levels. This rabbit was killed on the twenty-fourth day of APE treatment. Its pancreas showed widespread glycogen infiltration affecting agranular (beta) islet cells, ductular epithelium and centro-acinar cells (see Figure 51). No mitoses were discovered in the pancreas. The kidneys were the sites of severe Armanni lesions.

Rabbit number I 93 (see Graph I 93 and Tables XIV to XIX) reacted initially to the first few APE injections as did all the others, with respect to food consumption, ketonuria and glucosuria. In the first twenty-four hours following the first injection of APE the percent of available glucose excreted spiked dramatically to more than 300%; during the second day no urine was excreted. After five injections the food consumption rose rapidly so as to attain, during the second, third and fourth six-day intervals of APE therapy, average daily values nearly double those of the two post-alloxan six-day intervals. During APE treatments the random blood sugar levels were somewhat higher than the fasting levels of the preceding period, but they did not show any distinct rising trend of appreciable degree or continuity. Although there was an absolute increase of severity of diabetes as gauged by glucosuria in terms of grams per kilogram of body weight per day (being nearly double what it had





Graphs I 92 and I 93. The effects of crude APE treatment on the course of alloxan diabetes in the rabbit. Dose: 1 gram-equivalent per kilo body weight per day.

Legend. Left vertical scale: Fasting (x——x) and random (o——o) blood sugar levels in centigrams/100 mls.; available glucose (open bars) and urine glucose (solid bars) in grams/kilo body weight/day.

Right vertical scale: body weight in kilograms (. . . . .)

K: ketonuria; ⊙ Food not measured; • no urine excreted.

formerly been, see Table XVIII), in terms of percent of available glucose excreted the severity of the metabolic abnormality was not very different. There was a slow, irregular gain of body weight during the treatment period, amounting to about 500 grams.

On the 36th day after the alloxan had been injected, which was the twenty-fourth day of APE treatment, injections of APE were discontinued. Within the first twenty-four hours thereafter, food consumption decreased abruptly, so that again the glucose excreted exceeded the calculated available glucose. Although food consumption was irregular during the succeeding eleven days, the average values by six-day intervals were almost identical with those of the two pre-APE treatment (post-alloxan) intervals (see Table XVI). Nevertheless, glucosuria decreased abruptly (see Graph). During the first six-day interval following discontinuation of APE treatment the glucose excreted (in grams per kilo per day) fell to less than it had been before treatment. In the second six-day interval the average daily excretion of glucose was only 0.8 gram per kilo; on two of these days no glucose was excreted. In terms of percent of available glucose excreted, diabetes during the first post-treatment interval was approximately as severe as it had been throughout but during the second interval it was about one-seventh as severe (see Table XIX). When APE injections were discontinued, the fasting blood sugar levels plunged to sub-normal values which were maintained despite slight persistent glucosuria. On the forty-seventh day after

alloxan injection a glucose tolerance test was made. The blood sugar values for this test are presented together with those obtained by the identical method before alloxan was given.

TABLE XXI

Blood Sugar Values for Glucose Tolerance Tests (mg./100 ml.) Rabbit I 93

	Fasting	1 hour	2 hours
Before Alloxan	104	120	148
After APE	90	215	140

After several days of irregular fluctuation, the body weight fell sharply during the last four days. This rabbit was killed on the forty-seventh day after the alloxan injection (which was the twelfth day after APE treatment was discontinued). The pancreas contained no trace of glycogen in any type of cell. The islets were not obviously different from those of any other alloxan-treated animal in regard to apparent numbers and sizes of islets. Well-granulated beta islet cells were readily demonstrable in a small proportion of islets which otherwise were composed principally of alpha cells (see Figure 52). Mitoses were not evident in any type of cell.

The metabolic data determined during the course of the experiment for rabbit I 97 are depicted graphically by days and are

tabulated by average values for six-day intervals (see graph I 97 and Tables XIV - XIX). It is apparent from these data that the early changes attending the first few doses of APE resemble those previously described for the other treated rabbits. As the others had done, this rabbit, too, stopped eating at first and later ate more than it had eaten before APE treatment. During the first 6-day interval of injections glucosuria in average daily grams per kilogram of body weight remained about the same as it had been before treatment with APE, but calculation of the percent of available glucose excreted gave only about half the value of the figure for the 6-day interval preceding treatment. During the second six-day interval of therapy with APE the average daily food consumption increased twofold, as did the figure for glucosuria in grams per kilo of body weight per day. In this same period, however, there was only a slight increase in the calculated value for percent of available glucose excreted. During the third treatment interval all of these measurements again decreased disproportionately. Following the seventeenth and eighteenth injections the urine contained only 0.9 gram and and 1.8 gram of glucose per 24 hours, respectively, despite approximately 30 gms. of available glucose per kilo on both of those days. Ketonuria, which had been present almost constantly throughout the twelve days following alloxan, disappeared following the fourth APE injection. This rabbit had regained a few of the several hundred grams of body weight lost following the injection of alloxan, but when

APE injections were begun there occurred a further precipitous loss of body weight amounting to about 500 grams. Nevertheless, after four injections of APE the weight curve began to show a slight but steady rise which attained a maximum after twelve injections. The blood sugar levels during the course of APE treatments were not as high as those following alloxan.

The curves and tabulated values for the latter portion of APE treatment period are untrustworthy indices of the severity of the diabetic state during this time because the rabbit contracted a severe subcutaneous infection which could not be controlled by surgical drainage and large doses of penicillin. Despite this complication, both glucosuria and blood sugar levels declined and ketonuria did not appear.

The APE injections were discontinued after the eighteenth day of treatment. Despite a continued high food intake during the first and second days thereafter, the urine became free of glucose on the second day. On the last two days of life the rabbit ate nothing. Glucosuria reappeared and terminal ketonuria was noted when the animal was found dead. A single (fasting) terminal blood sugar level was 346 mg.per 100 mls.

Histological examination of the pancreas of rabbit I 97 showed glycogen present in islet cells but no evidence of its presence in ductule epithelium or in centro-acinar cells. No mitotic figures were noted in any type of cell. The kidneys did

not contain demonstrable glycogen, nor did vacuoles attributable to its former presence appear in routine preparations.

Within the first 24 hours following the administration of the first dose of APE to rabbit I 99 more glucose was excreted than was available (by calculation) in the markedly decreased amount of food consumed (see graph I 99). Ketonuria appeared promptly and persisted for nine days. During this interval body weight showed a slight, transitory depression. However, the food consumption rose steeply, being almost double during the second six-day therapy interval what it had been during the pre-treatment intervals (see Table XVI). During the same APE-therapy interval almost double the previous amount of glucose appeared in the daily urine output, but this increase did not represent a substantial difference in severity as judged by the percent of available glucose excreted. During the first twelve days of APE treatment blood sugar values were much higher than they had been during the six days just prior to treatment.

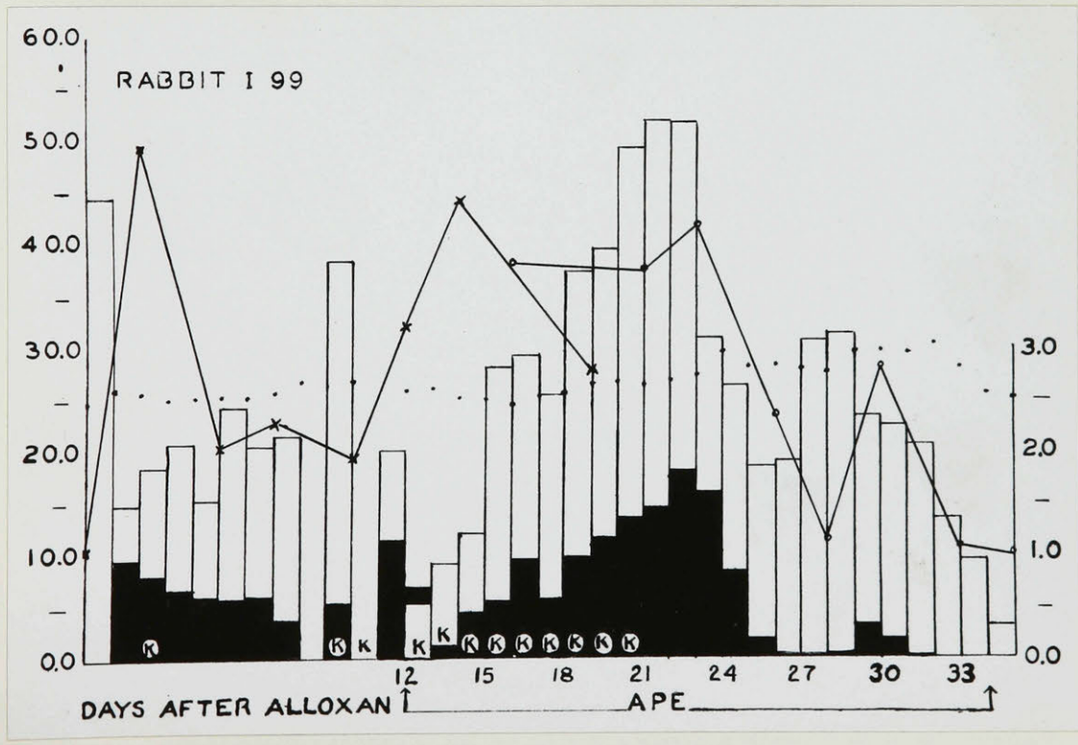
When this rabbit developed a severe subcutaneous infection there occurred a sudden precipitous fall of food consumption attended by decreased glucosuria, lowered blood sugar level and slight loss of body weight (see graph I 99, days 25, 26 and 27). Although surgical drainage and penicillin therapy were attended by increased appetite, and ascent of the blood sugar level, glucose excretion remained depressed. Terminally, the rabbit developed pneumonia.

again the food consumption decreased, falling steadily to less than five grams per kilogram of body weight on the last day of life. This fatal infection was attended by marked loss of body weight, descent of blood sugar levels to normal values and complete absence of glucose from the urine. Ketonuria did not occur.

Autopsy examination confirmed the existence of a severe lobar pneumonia and disclosed a widespread bilateral chronic pyelonephritic process. The pancreas contained no traces of glycogen nor were vacuolated cells apparent in routine preparations. Many islets consisting of beta cells with well-granulated cytoplasm were present (see Figure 53). There were no mitotic figures in the pancreatic cells. Glycogen was not demonstrable in the renal tubular epithelium.

The alloxan-recovered rabbit, number D 73, had been injected with the diabetogenic dose thirty-two weeks before APE treatment was started (see Protocol D 73). This animal had born two litters, in the eighth and seventeenth weeks after receiving alloxan. As part of a separate project not relevant here, it had also received 0.125 gram of "Antabus" (tetraethyl thiuram disulfide) by mouth daily for four weeks. Although a mild diabetic state persisted, as evidenced by slight glucosuria, the random blood sugar levels were frequently within normal limits. A glucose tolerance test was done immediately before starting APE therapy; it resembled the tests of normal rabbits performed at the same time in the same way

(see Table XIII). Immediately upon receiving APE, this rabbit ceased eating; the blood sugar level rose, ketonuria, lipemia and loss of body weight were severe. Death occurred after the sixth injection. At autopsy there were large numbers of hemorrhagic corpora in the ovaries. Microscopic study of the pancreas showed large, bizarre islets containing numerous well-granulated beta cells in addition to the presence of numerous islets comprised of alpha cells only or predominantly. Glycogen accumulations in islet cells, ductule epithelium and centro-acinar cells could not be demonstrated by special histochemical stains or by inference from routine histological preparations. Mitoses were not evident in the pancreas. In the kidney tubules vacuoles referable to fatty change were found in the proximal convolutions but neither glycogen nor fat was demonstrable elsewhere in the nephron.



Graphs I 97 and I 99. The effects of crude APE treatment on the course of alloxan diabetes in the rabbit. Dose: 1 gm.- eq./kilo body weight/day.

Legend. Left vertical scale: Fasting (x——x) and random (o——o) blood sugar levels in centigrams/100 mls.; available glucose (open bars) and urine glucose (solid bars) in grams/kilo body weight/day.

Right vertical scale: Body weight in kilograms (. . . .).

K: ketonuria; ⊙ no food eaten.

Discussion

When APE treatment was commenced all of the experimental group showed an immediate reduction of food intake, loss of body weight and ketonuria; similar changes were observed in two normal rabbits given the same amounts of APE. Three of the severely diabetic rabbits and the alloxan-recovered one failed to resume eating; their urine volumes and glucose excretions diminished rapidly, gross lipemia appeared and a moribund state developed. Hyperglycemia remained present in the three severely diabetic rabbits and showed an abrupt reappearance in the alloxan-recovered one. One rabbit died after only two injections; two died after the sixth injection and the remaining one was killed at this time in order to secure technically excellent tissues for microscopic examination. Glycogen infiltration was apparent throughout the pancreatic islets, ductules and centro-acinar cells of the two severely diabetic rabbits examined; it could not be demonstrated in the pancreas of the alloxan-recovered rabbit. A few mitoses were found in the beta islet cells of one rabbit, but no signs of proliferation of pancreatic cells attributable to APE injections were seen. The glycogen infiltrations in the pancreases of these rabbits are the earliest appearances of this lesion observed in rabbits; the lesion appeared only 18 days after the alloxan injections were made. Hitherto, the earliest appearance of glycogen infiltration noted in

an alloxan-diabetic rabbit was 36 days (see Part II, rabbit number C 50). Previous investigators (Duff, McMillan and Wilson, 1947) never found this morphological change in alloxan-diabetic rabbits until intervals of at least forty-five days had elapsed. It seems highly probable that development of glycogen infiltration in these two rabbits was accelerated in response to APE treatment. Since there was no exaggeration of hyperglycemia during APE administration, one may not attribute the accelerated development of glycogen infiltration to any change in the blood sugar level. The insulin contents of the blood and of the pancreas of the alloxan-diabetic rabbit have not been determined, but these values are undoubtedly very low. In other species, the pancreas has been found to contain some insulin. Therefore, one may tentatively accept the possibility that APE depressed the pancreatic insulin content and the blood insulin level even further than alloxan had done, consequently exerting a greater strain upon the few existing beta islet cells and accelerating the development of glycogen infiltration.

Four alloxan-diabetic rabbits resumed eating, began to gain weight, excreted larger volumes of urine and showed complete disappearance of ketonuria within a week to ten days after APE treatment was begun. One of them, number I 92, showed continuously increasing food consumption, body weight, urine volume, glucosuria and hyperglycemia. However, there was no appreciable change in the calculated proportion of available glucose lost in the urine from day to day

as shown by average values for intervals of six days each. On examination of the pancreas after twenty-four days of APE therapy, a very extensive and severe degree of glycogen infiltration was observed; this had developed during the period of 36 days subsequent to injection of alloxan. If one judges the severity of diabetes by the blood sugar level, this animal became more severely diabetic while receiving APE injections than it had been before; however, according to the calculated values for percentage of available glucose excreted in the urine there was no change in severity of diabetes. This experiment seems to support, in the usual indirect manner, the idea that hyperglycemia per se produces the morphological alterations in the pancreatic islet and ductule cells. However, it should be noted that the same appearance has been observed before in a rabbit (Part II, No. C 50) 36 days after alloxan administration. The increasing hyperglycemia of rabbit number I 92 during the course of the experiment need not necessarily have been responsible for any degree of accelerated development of the pathological process in the pancreas: it is not certain that acceleration occurred.

Another rabbit, number I 93, had shown diabetic symptoms of approximately equal severity to those of number I 92. After the initial period of reduced food intake, loss of weight and ketonuria, attendant upon commencement of APE injections, this rabbit also ate ravenously, gained weight, and excreted a larger volume of urine containing a greater amount of glucose than it had formerly done.

Nevertheless, the percent of available glucose excreted in the urine was not dissimilar to the former values. The blood sugar levels (considering their random nature) were not appreciably altered. When APE treatment was discontinued after 24 days, this rabbit showed a prompt fall in blood sugar levels (fasting values), urine volume and urine glucose content. The values for food consumed during the twelve days following cessation of APE injections were, on the average, almost identical to those for the twelve days preceding treatment. Therefore, the striking decrease in glucosuria, both in absolute value and in percentage of available glucose excreted, associated with return of fasting blood sugar levels to normal and lower values provide clear evidence that the severity of diabetes was much less after APE therapy, both with respect to the pre-treatment period and to the period of treatment. Terminally, even the glucose tolerance test gave values quite similar to those of the same animal before it had received alloxan. Microscopic examination of the pancreas of this rabbit not only failed to disclose any trace of glycogen, but revealed excellent granulation of most of the few beta cells present. There were, however, no mitotic figures to be seen in the pancreas nor were any proliferative phenomena resembling "budding" of islets or "new formation" of islets from ductules found. It is difficult to avoid attributing the amelioration of diabetic symptoms encountered in this experiment to some influence of APE treatment. Although no biopsy of the pancreas was taken to establish

the existence of glycogen infiltration in the pancreas at the time when APE treatment was discontinued, the lesion may have existed then, as would be expected from the experiments recounted above. However, it would appear unlikely that resolution of such a lesion could occur in only twelve days, since much longer periods of partial or complete control of diabetes by insulin therapy have been found necessary to achieve restitution of cytoplasmic structure and reappearance of beta granules (Part II). If a pancreatropic factor in APE was associated with the observed decrease in severity of diabetes and the apparent restitution or preservation of the cytoplasm of the pancreatic cells, evidences of its growth-promoting effects were not found. An alternate explanation of the changes seen in this experiment may be suggested. It is that the rabbit might have recovered spontaneously from alloxan injury in a shorter time if the APE injections had not exerted a deleterious effect. Although delayed recovery from alloxan injections is encountered very rarely, such a phenomenon has been observed in these studies once before. The animal concerned, number D 73, promptly exhibited severe diabetic symptoms and died when six APE injections were given following a prolonged,gradual, apparently spontaneous amelioration of diabetes observed for 30 preceding weeks.

The metabolic changes which accompanied administration of APE to rabbits number I 97 and I 99 were qualitatively similar to those previously observed in the other experimental animals immediately after treatment was begun and during the ensuing ten days.

at that time subcutaneous infections developed in the injection sites of both animals; when these did not respond favorably to penicillin therapy, surgical drainage was attempted. Curiously, although these rabbits regained their appetites, glucosuria decreased almost continuously and their blood sugar levels fell. Ketonuria was not apparent in these animals during this time. Injections of APE were given to number I 99 continuously despite the infectious process. During the last six days this animal developed pneumonia, later confirmed at autopsy; it practically stopped eating, random blood sugar levels remained very low and glucosuria disappeared completely. Although body weight fell precipitously, ketonuria and lipemia were not apparent. On the other hand, when APE injections were discontinued on the 30th post-alloxan day, number I 97 showed a further increase of food consumption and continuing hyperglycemia but glucosuria fell to zero on the 32nd day. This rabbit ate no food on the last two days of its life during which the body weight fell even lower. There was loss of six and one-half grams of glucose (4.75 grams per kilo of body weight) on the 33rd day and ketone bodies were present in the urine excreted before death on the 34th day. Histological examination of the pancreases of these two rabbits showed no glycogen in the islets, ductules or centro-acinar cells of number I 99 but some was evident in the islets of number I 97. Pancreatic islets containing beta cells with full complements of cytoplasmic granules were numerous in number I 99.

Interpretations of the results of these two experiments are even more intricate than those previously attempted. The presence of disastrous infections appear to have modified the experimental observations in such manner as to preclude any precise evaluations. The disastrous effects of infectious processes upon diabetic subjects are commonly associated with obvious metabolic changes indicative of increased severity, including exaggerated levels of blood sugar, glucosuria and ketonuria as well as dehydration, acidosis, coma, and decreased effectiveness of insulin therapy. Nevertheless, these animals showed progressive diminution of glucosuria and lowered blood sugar values which were maintained briefly despite later increases of food consumption. Only terminally did they appear moribund and even then only one of them showed ketonuria. The normal blood sugar levels of rabbit I 99 are in contrast to the diminished but continuing elevations of I 97, which remained high even after APE was discontinued. It might be suggested that this difference in the two experiments provided the basis for the occurrence of glycogen infiltration in the islets of the rabbit with hyperglycemia but not in the other. It should be noted, however, that the fasting blood sugar levels, the percentages of available glucose excreted, the changes of body weight and the durations of ketonuria during the pre-treatment (post-alloxan) period indicated a much severer diabetic state in number I 97 than in I 99. Furthermore, after the initial metabolic disturbances consequent to administration of APE but before the development of the severe

infections (i.e. about the 6th to 12th days of APE treatment) the metabolic data of the two animals were practically equivalent. These comparisons, if valid, might indicate that the severity of diabetes of number I 99 was temporarily increased by APE treatment, but that number I 97 was more severely diabetic over a longer interval after alloxan injection than was I 99. In this rather tenuous way, it would appear possible to correlate the development of glycogen infiltration in the pancreas of number I 97 with severe diabetes of 34 days duration and to associate the absence of the lesion from the pancreas of number I 99 with the lesser severity of diabetes during most of its 34 days duration. Accordingly, if this presumptive reasoning were valid, it would not be necessary to accept hyperglycemia as the factor responsible for glycogen infiltration in the pancreas of rabbit number I 97.

Summary

1. Crude alkaline extract of fresh frozen ox anterior pituitary glands was prepared according to the method of Young. Repeated injections of approximately one gram equivalent per kilo of body weight per day evoked typical diabetic responses in two normal rabbits and a normal dog.

2. A group of alloxan-diabetic rabbits was observed by daily estimations of various metabolic data for an interval of twelve days following injection of alloxan. An attempt was made to grade the relative severity of diabetes within the group of survivors.

3. Seven severely diabetic rabbits and one alloxan-recovered one were treated daily with constant doses of APE. Three animals, including the alloxan-recovered one, died after two or six injections; one was killed on the sixth day of treatment. Four rabbits received from 18 to 24 injections; two died with severe complicating infections. Of the two remaining, one was killed after 24 injections; the other was kept for twelve days of observation after treatment was discontinued.

4. Despite the absence of appreciable exaggerations of hyperglycemia during APE treatments, glycogen infiltration in the pancreas was found in two of the animals (I 89, J 2) which were

examined after six injections of APE (18 days after alloxan). The accelerated development of this lesion was considered to be attributable to probable further decrease of blood insulin level.

5. One rabbit (I 92) exhibited severe and extensive glycogen infiltration following 24 injections of APE which were associated with increasing hyperglycemia although no change was noted in the proportion of available glucose excreted in the urine. Another very severely diabetic animal (I 97) showed only mild fluctuation of blood sugar level but marked reduction of glucosuria when a severe subcutaneous infection developed during the course of treatment. Glycogen infiltration of the islets was found when the pancreas was examined four days after APE was discontinued following 18 injections. The role of hyperglycemia in the production of the pancreatic lesion in these two experiments was not clear; increased severity of the diabetic state was not obvious. The development of glycogen infiltration in the pancreas was probably not accelerated.

6. One rabbit (I 99) exhibited no glycogen in the pancreas when autopsied after twenty-three injections of APE. Although this animal showed a temporary increase of severity of diabetes as judged from further elevation of blood sugar level and increased proportion of available glucose excreted in the urine, these manifestations disappeared completely when a severe subcutaneous infection and pneumonia complicated the experiment. The absence of

glycogen from the pancreatic cells was attributed to a relatively lesser severity of diabetes during most of the course of the experiment rather than to the normoglycemia of the terminal interval.

7. The alloxan-recovered rabbit (D 73) showed no evidence of pancreatic glycogen infiltration. Its absence was attributed to the brief duration of severe diabetic symptoms.

8. One rabbit (I 93) showed no appreciable change of severity of diabetes during 24 days of APE therapy but closely simulated an alloxan-recovered animal when APE was discontinued. The apparent beneficial effects of APE treatment might have been referable to an hypothetical pancreatropic factor in the crude extract. However, it was thought to be more likely that APE treatment had merely delayed spontaneous recovery from the alloxan effects.

9. Histological analyses of quantitative nature were not attempted. Efforts to discover morphological evidences of islet regeneration in the routine preparations did not disclose any of the commonly-reported pictures.

Conclusions

1. Treatment of alloxan-diabetic rabbits with crude alkaline anterior pituitary extract accelerated the development of glycogen infiltration in the pancreas without conclusively altering the severity of the metabolic aberrations estimable by simple methods.

2. Treatment of an alloxan-recovered rabbit with crude alkaline anterior pituitary extract evoked prompt manifestation of severe diabetes terminating in death.

3. Amelioration of diabetic symptoms following a course of treatment with crude alkaline anterior pituitary extract might have represented a delayed spontaneous recovery from the effects of alloxan rather than a beneficial therapeutic effect.

4. The existence of a special pancreatropic factor in crude anterior pituitary extract was not confirmed by metabolic data or histological study of the pancreas.

5. Hyperglycemia *per se* did not appear to be the only factor of importance in the pathogenesis of glycogen infiltration in the pancreas.

Claims for Originality

1. The author first noticed glycogen infiltration in the pancreas of the alloxan-diabetic rabbit in the Fall of 1948. This observation was corroborated from materials then at hand and since derived from subsequent experiments. In the Winter of 1948-49 the author encountered an excellent example of "hydropic degeneration" of the islets of Langerhans in the slide file of McGill University Pathological Institute (A 12333). After demonstrating glycogen in these vacuolated islet cells, a search was made for similar lesions in all of the material on file from cases of diabetic coma. The courteous provision of ox pituitary glands by Dr. John R. Mote of Armour and Company enabled the author to extend this observation to the APE-diabetic dog and Dr. David R. Murphy's surgical skill assisted in furthering the demonstration to include the partially-depancreatized diabetic dog. Both of these experiments were accomplished during the Winter of 1950. At about this same time Drs. Kobernick and More found "hydropic degeneration" in the pancreas of a rabbit made diabetic with cortisone; with their kind consent the author reported the presence of glycogen in the affected cells in this instance also. Dr. Bencosme kindly diverted some of his serial sections of pancreatic islets from a rabbit given massive infusions of glucose to my benefit, thus permitting a few unsuccessful attempts to find glycogen in the cytoplasmic vacuoles of this material.

Denying all previous published reports, the author claims complete originality for each of the observations cited.

2. During the academic year 1947-48 the author first observed complete restoration to normal structural appearance of pancreatic cells previously affected by "hydropic degeneration" in alloxan-diabetic rabbits treated with such "inadequate" doses of insulin that hyperglycemia remained present continuously. This observation was the direct result of the author's failure to achieve full control of the symptoms of diabetes in an experimental project conceived and directed by Dr. G. Lyman Duff. In cooperation with Dr. Duff the author then intentionally administered small doses of insulin to alloxan-treated rabbits to test both restorative and preventitive effects of such therapy during continuous hyperglycemia. The resultant prevention and restoration despite continuous hyperglycemia, as described in Part II, are original observations new to the literature at large. However, the author can fairly claim only to share in their originality together with the director of the project, Dr. Duff.

The author's observation of the ineffectiveness of a second injection of alloxan upon glycogen-infiltrated islet cells and restored islet cells in alloxan-diabetic rabbits is an original experiment accomplished in the Spring of 1948.

3. The demonstration of accelerated development of glycogen infiltration in the pancreas of the alloxan diabetic rabbit in response to treatment with crude APE is an observation for which the author claims originality. The finding that APE treatment probably increased the severity of the diabetic state in the alloxan-diabetic rabbit is a new observation of a result previously described in other

species and in other kinds of experimental diabetes., Failure of these experiments to confirm the possible existence of a special pancreatropic factor is a new observation at variance with the interpretations of similar experiments reported by Ogilvie. His experiments and mine were accomplished completely independently, his report having reached me only after my experiments had ended. This portion of the project, Part III, was designed in cooperation with the Director, Dr. G. Lyman Duff, and was carried out during the early months of 1950.

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APPENDIX

Photomicrographic Illustrations, Plates I to XIV.

Protocols of Experimental Animals.

The swollen, vacuolar appearance of various types of cells after artefactual removal of glycogen from their cytoplasm by routine histological methods.

Figure 1. Human vaginal epithelium. Formalin; H.and E.; x530.

Figure 2. Human parathyroid adenoma. Formalin; H.and E.; x 530.

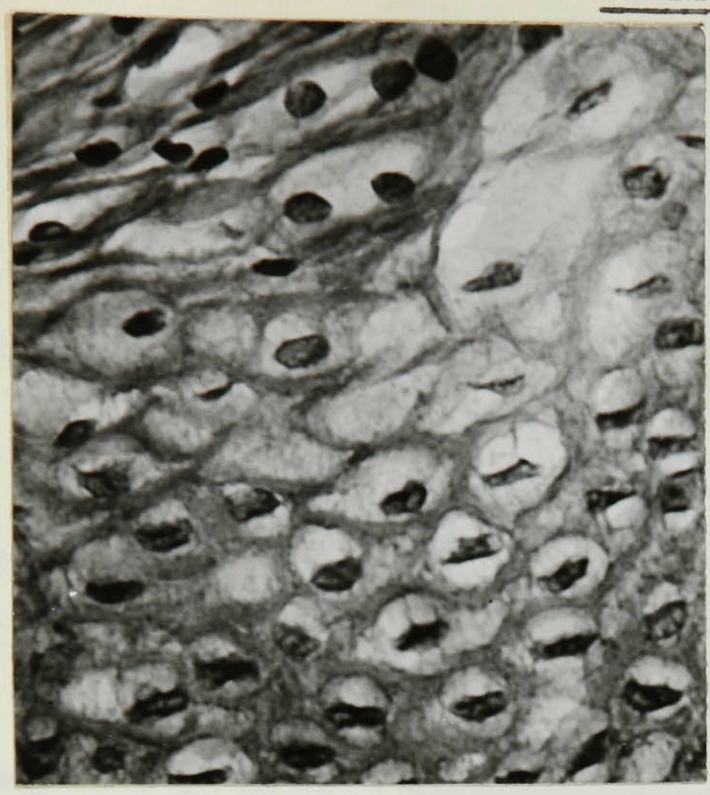
Figure 3. Human heart muscle fibers adjacent to an area of infarction. Formalin; H.and E.; x530.

Figure 4. Armanni lesion in rabbit renal tubular epithelium (number D 75) in permanent severe alloxan diabetes. Helly's: HPS; x530.

Figure 5. So-called "hydropic degeneration" in rabbit pancreatic islet and in a centro-acinar cell in permanent, severe alloxan diabetes. Helly's; Gomori's chromic alum hematoxylin; x530.

Figure 6. Human liver in von Gierke's disease. Formalin; H.and E.; x530.

PLATE I



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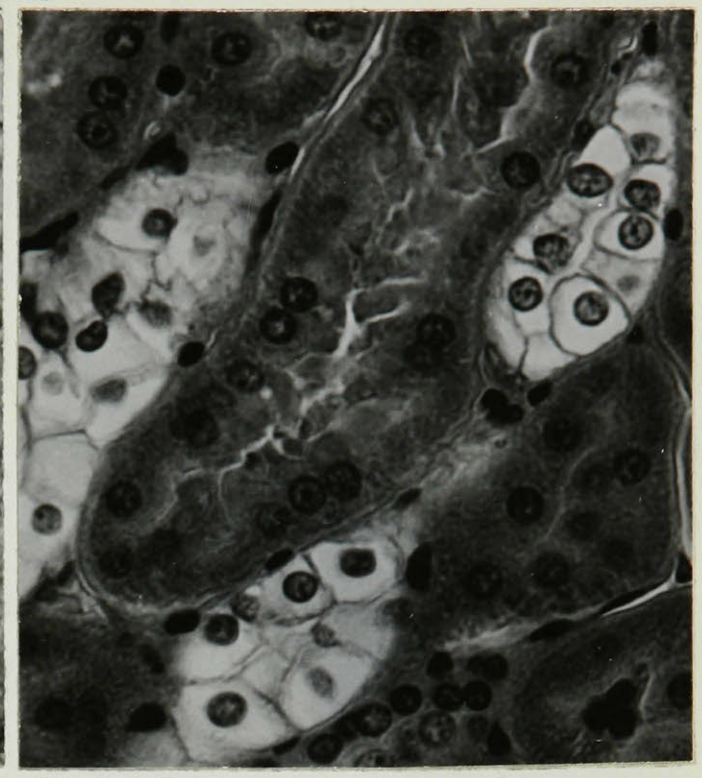

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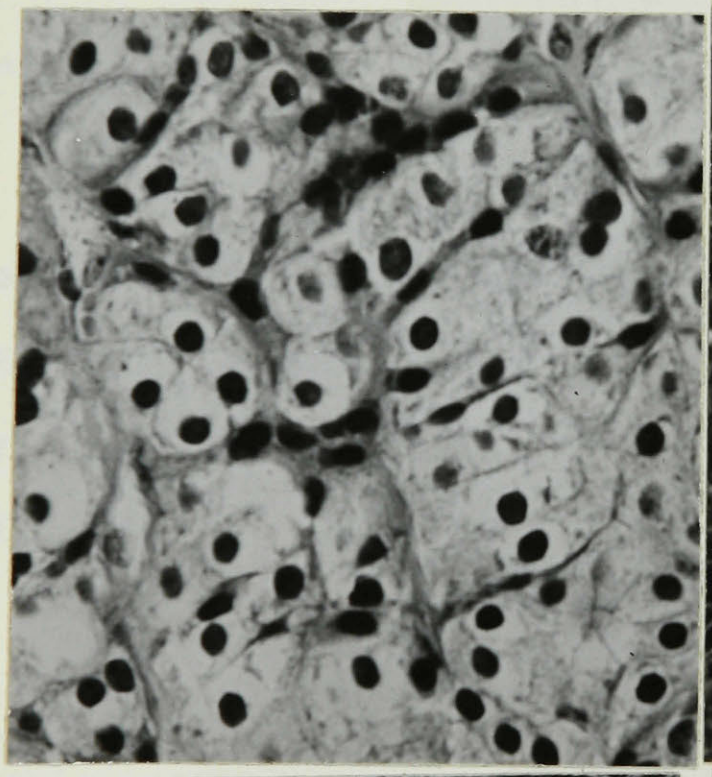

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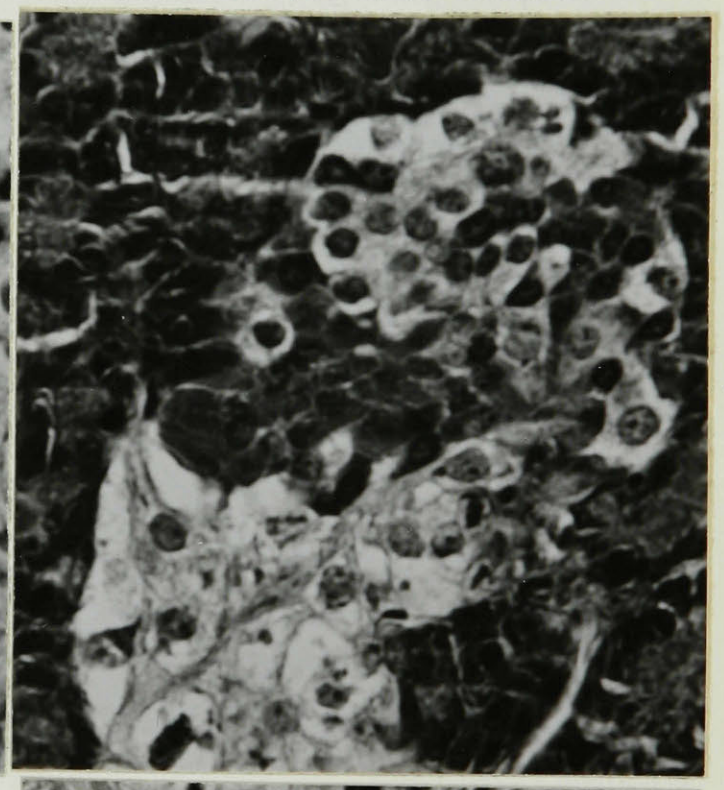

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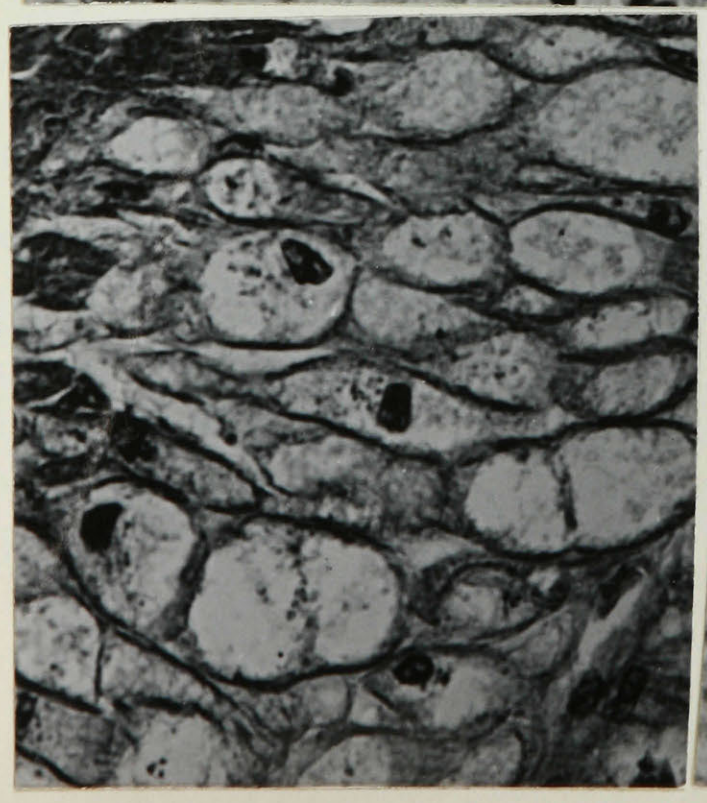

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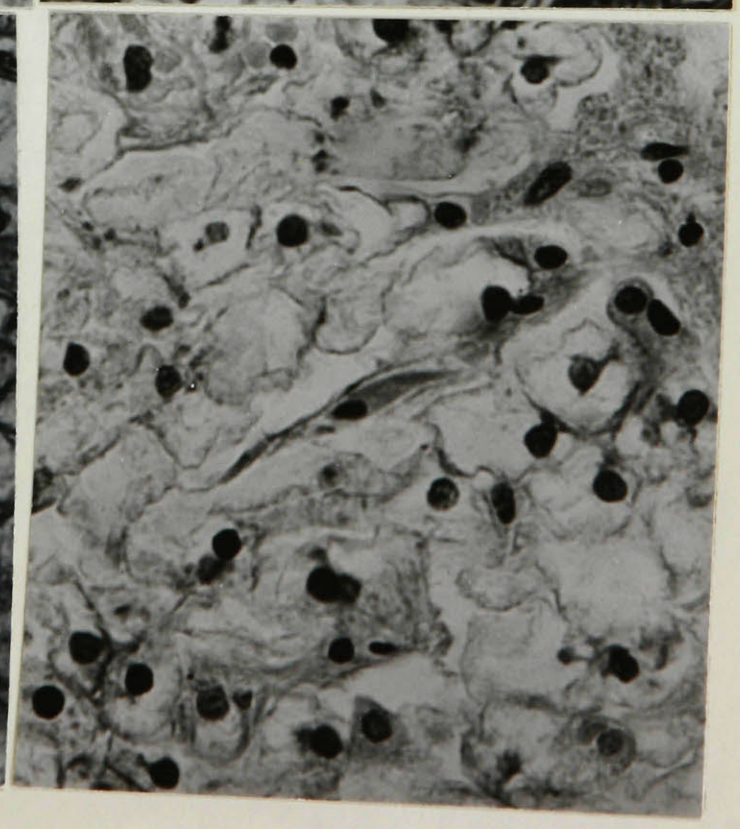

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Artefactual ipsilateral displacement of histologically demonstrated glycogen.

Figure 7. Human vaginal epithelium. Formalin; Best's carmine; x530.

Figure 8. Human "hydropic" heart muscle fibers adjacent to an area of infarction. Formalin; Bauer's chromic acid-Schiff's reagent; x530.

Figure 9. Armanni lesion in rabbit renal tubular epithelium in alloxan diabetes(number D 75). Helly's; Hotchkiss' alcoholic periodic acid-Schiff's reagent; x530. (Note the peculiar sparing of the macula densa, first called to my attention by Dr. G. C. McMillan.)

Figure 10. Human parathyroid adenoma. Formalin; celloidin-embedded; Best's carmine; x530. (Note that the thickness of the section obscures the definition of glycogen, which appears here as accentuations of the cell walls.)

PLATE II

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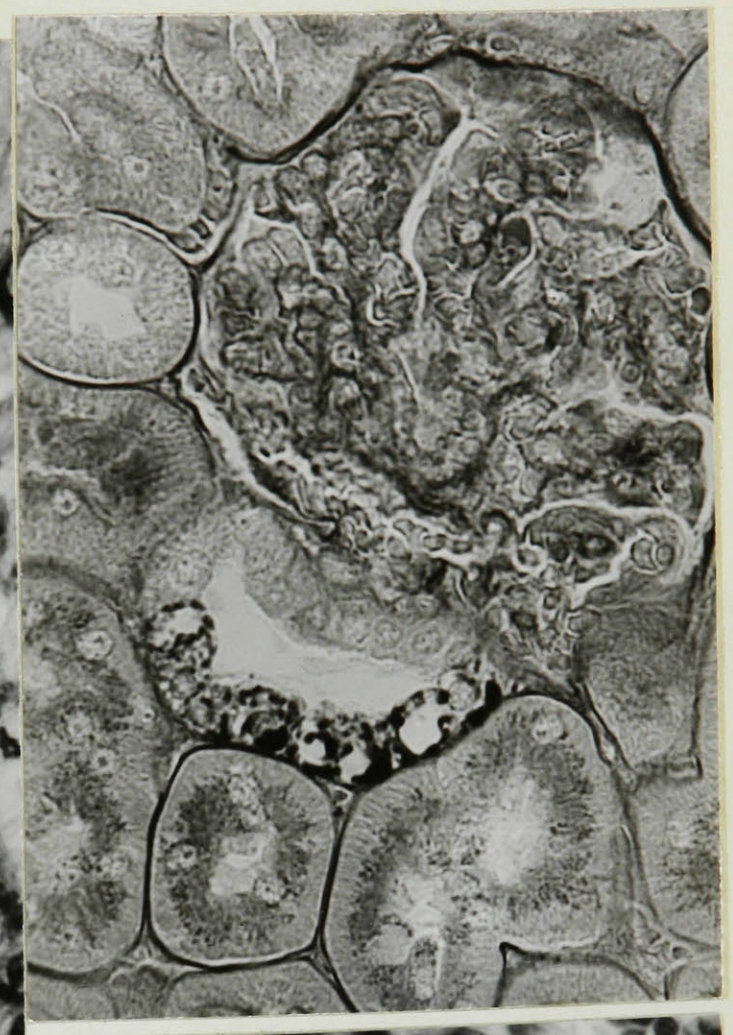

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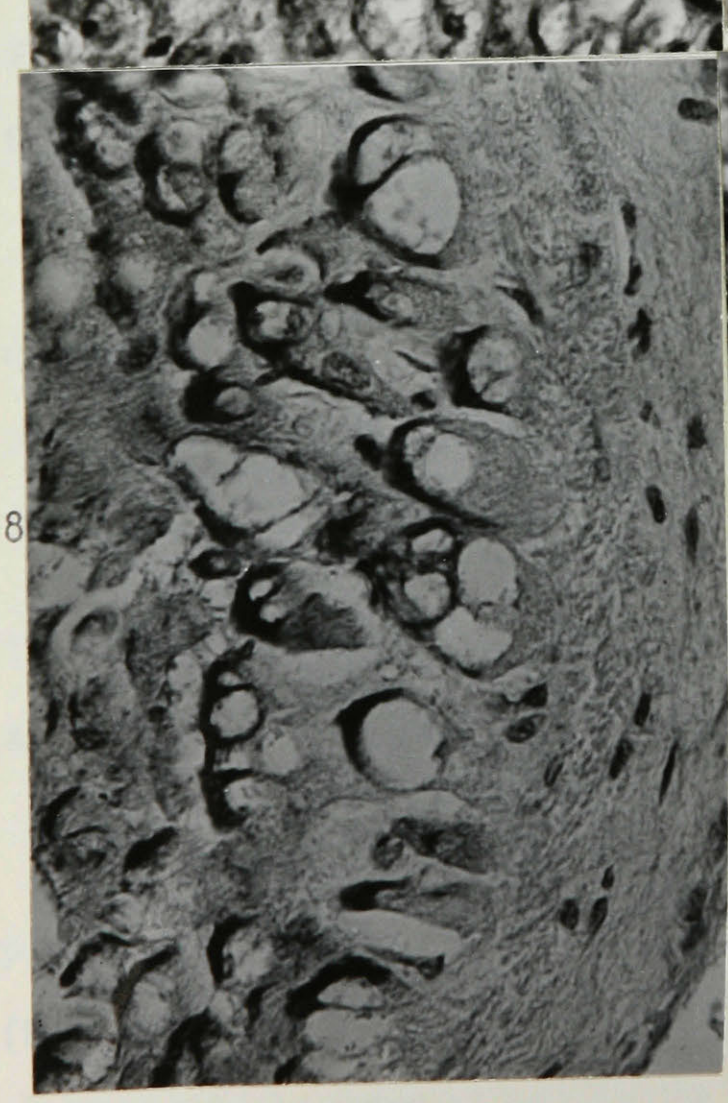

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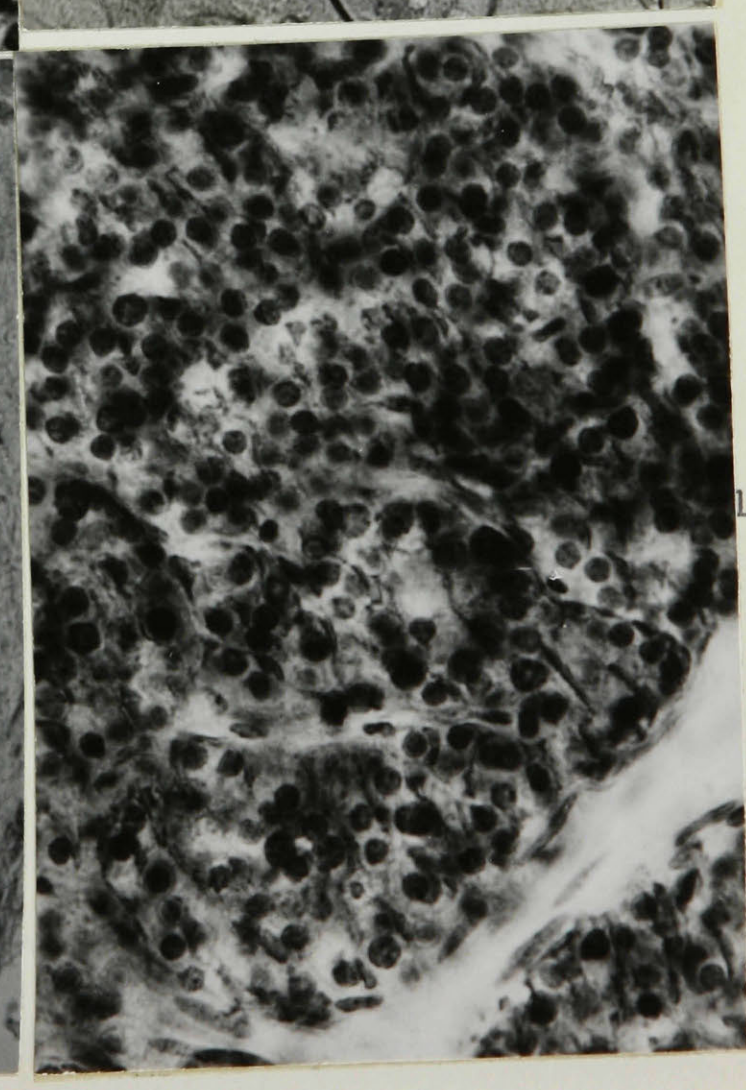

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Glycogen infiltration in pancreas of alloxan diabetic rabbit.

Figure 11. Glycogen in pancreatic islet cells and in centro-acinar cell (upper right) of number D 75. Helly's; Best's carmine; x530.

Figure 12. Another islet and ductule (lower right) of number D 75. Helly's; Best's carmine; x530.

Figure 13. Extreme glycogen infiltration in pancreatic islet of number 72W. Helly's; Best's carmine; x530. (Note: This rabbit was one of group previously studied by Duff, McMillan and Wilson.)

Figure 14. Removal of glycogen from islet and ductule cells by diastase. Helly's; diastase; Hotchkiss' alcoholic periodic acid-Schiff's reagent; x530. (Rabbit number D 75).

Figure 15. Absence of demonstrable mucin from pancreatic islet and centro-acinar cells. Helly's; Mayer's mucicarmine; x530. (Rabbit number D 75).

Figure 16. Absence of glycogen from vacuolar cytoplasm of rabbit treated with massive infusions of glucose. (Dr. Bencosme's experiment, number A-54-A). Bouin-Hollande; Best's carmine; x530.

PLATE III

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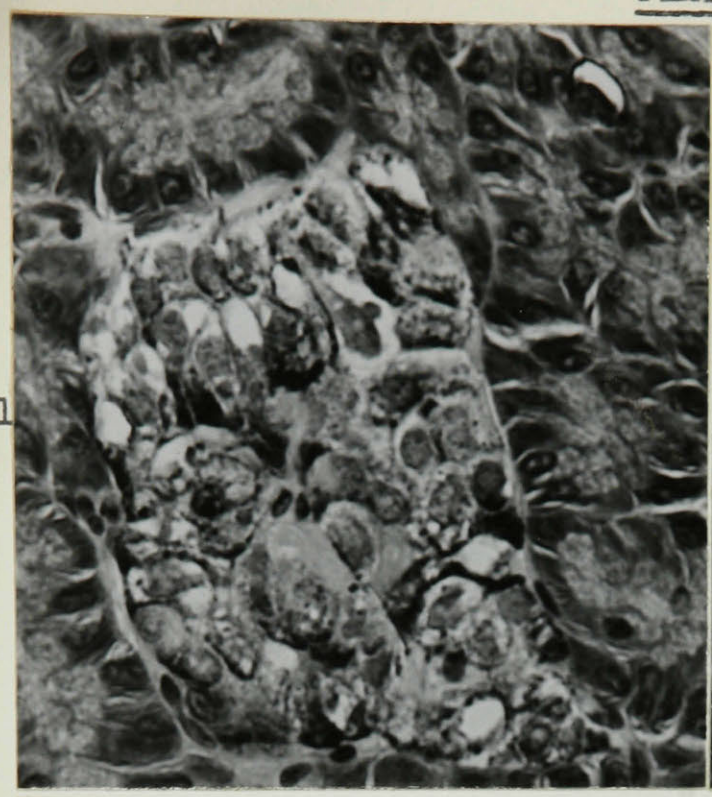

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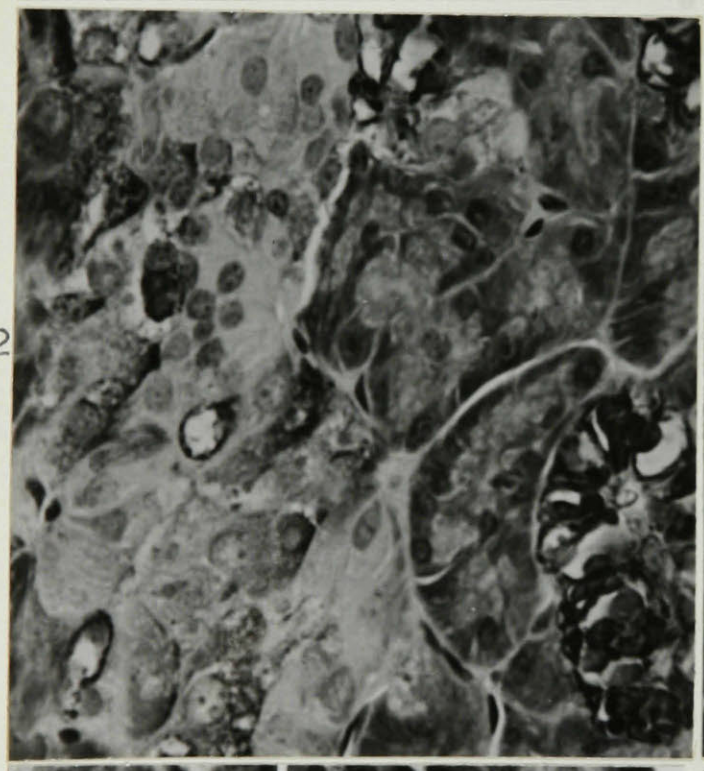

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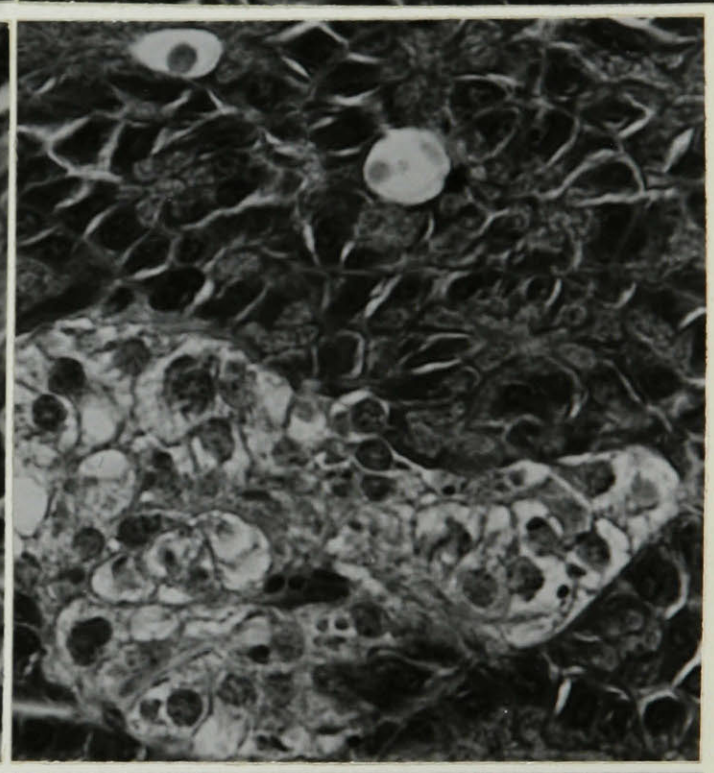

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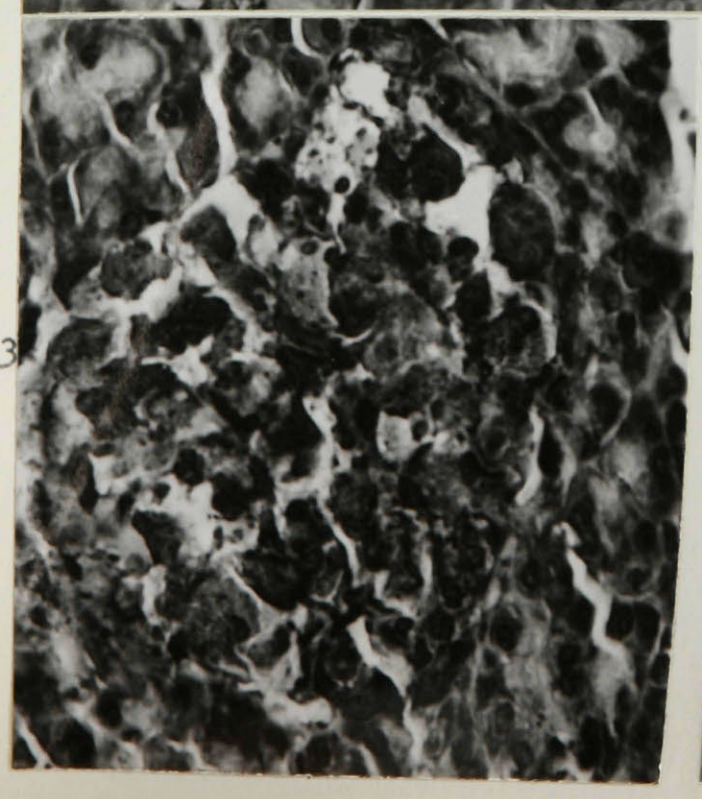

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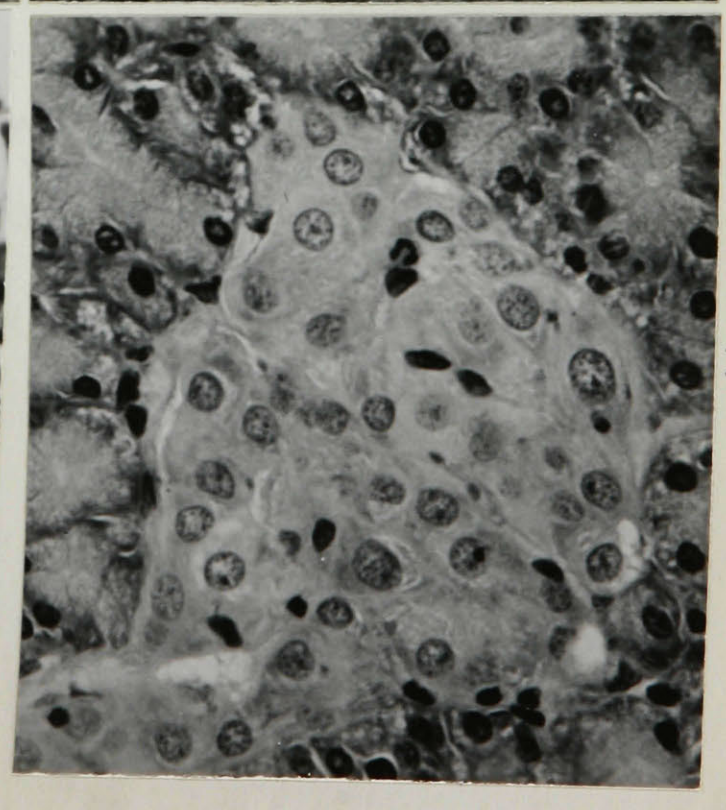


Glycogen infiltration in the pancreas of the APE-treated dog.

Figure 17. Extensive degranulation and slight vacuolation of the cytoplasm of the beta cells of the APE-treated dog (number K 14). Helly's; modified Gomori's chromic alum hematoxylin; x530.

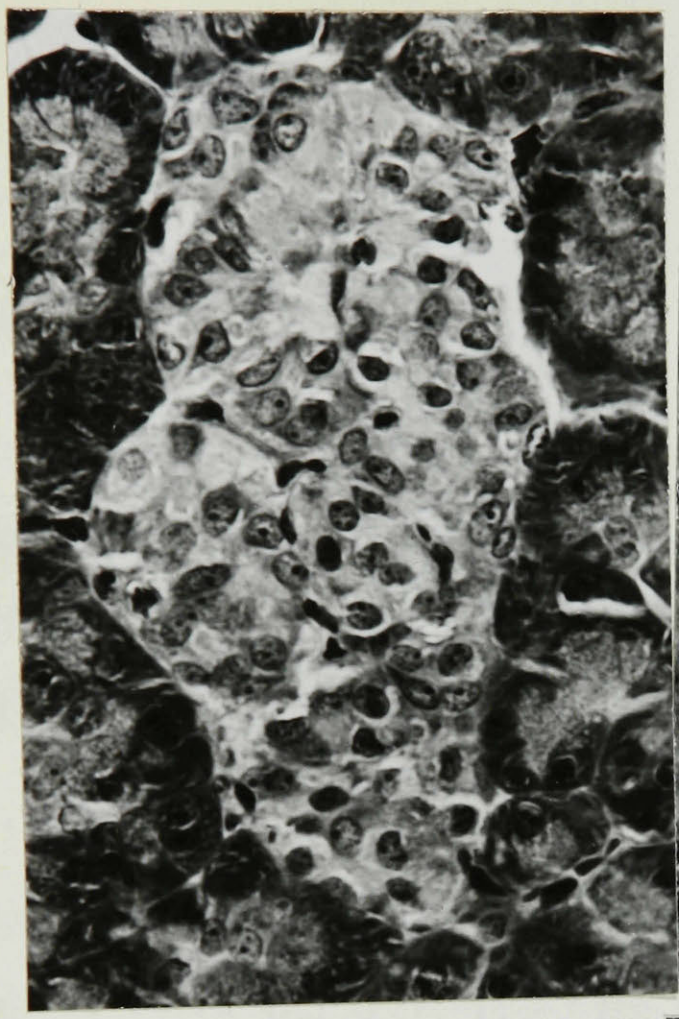
Figure 18. Small crescentic bands of glycogen in a few islet cells in the APE-treated dog (number K 14). Helly's; Best's carmine; x530.

Figure 19. Removal of glycogen from an islet and ductule by diastase. APE-treated dog (number K 14). Helly's; diastase; Best's carmine; x530.

Figure 20. Pyknosis and cytolysis affecting two glycogen-infiltrated islet cells in the APE-treated dog (number K 14). Helly's; Best's carmine; x530. Note: photographed from same slide as Figure 18; glycogen faintly visible microscopically in both disintegrating cells does not appear in photomicrograph.

PLATE IV

17



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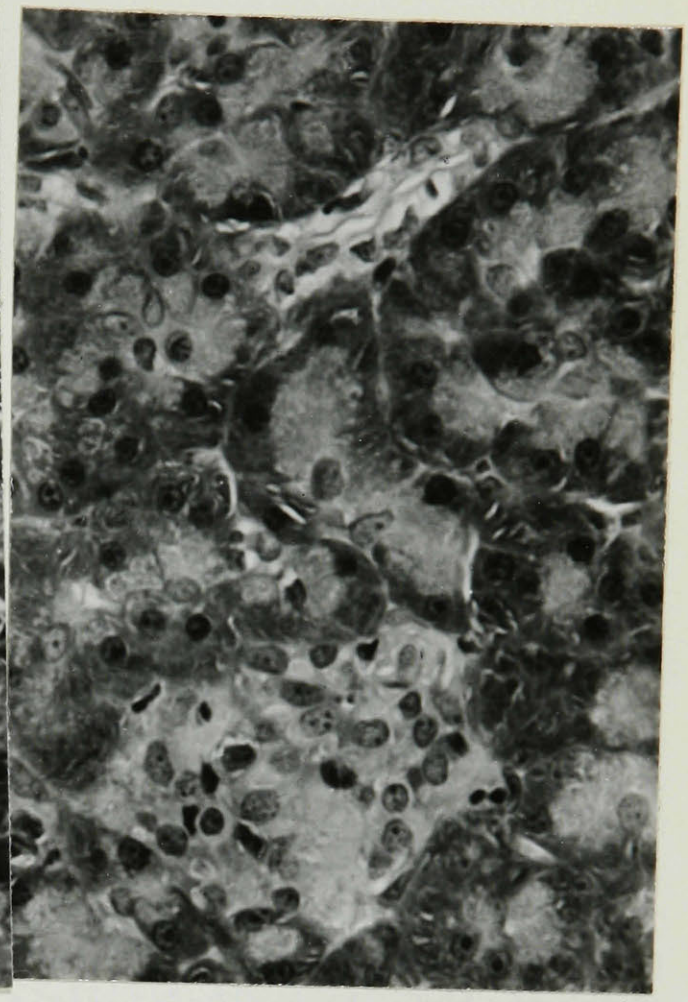

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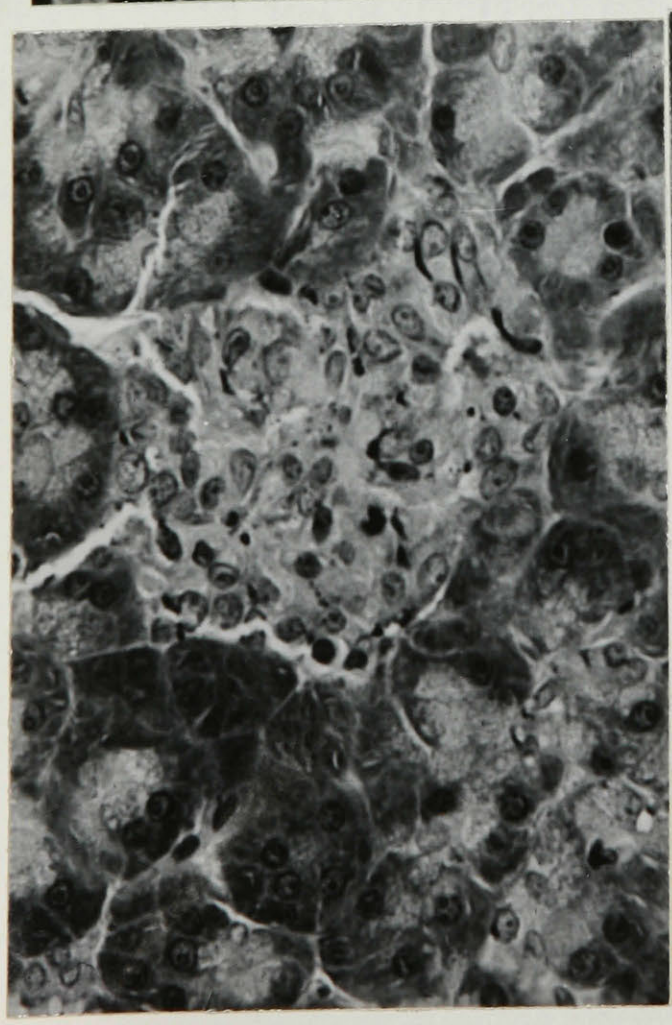

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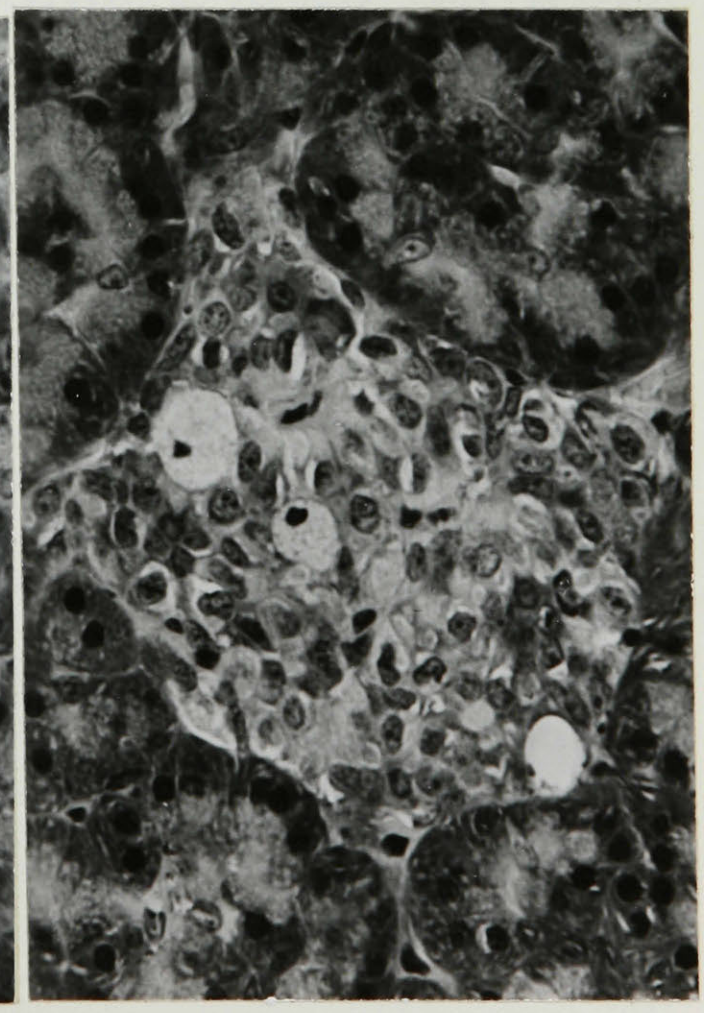


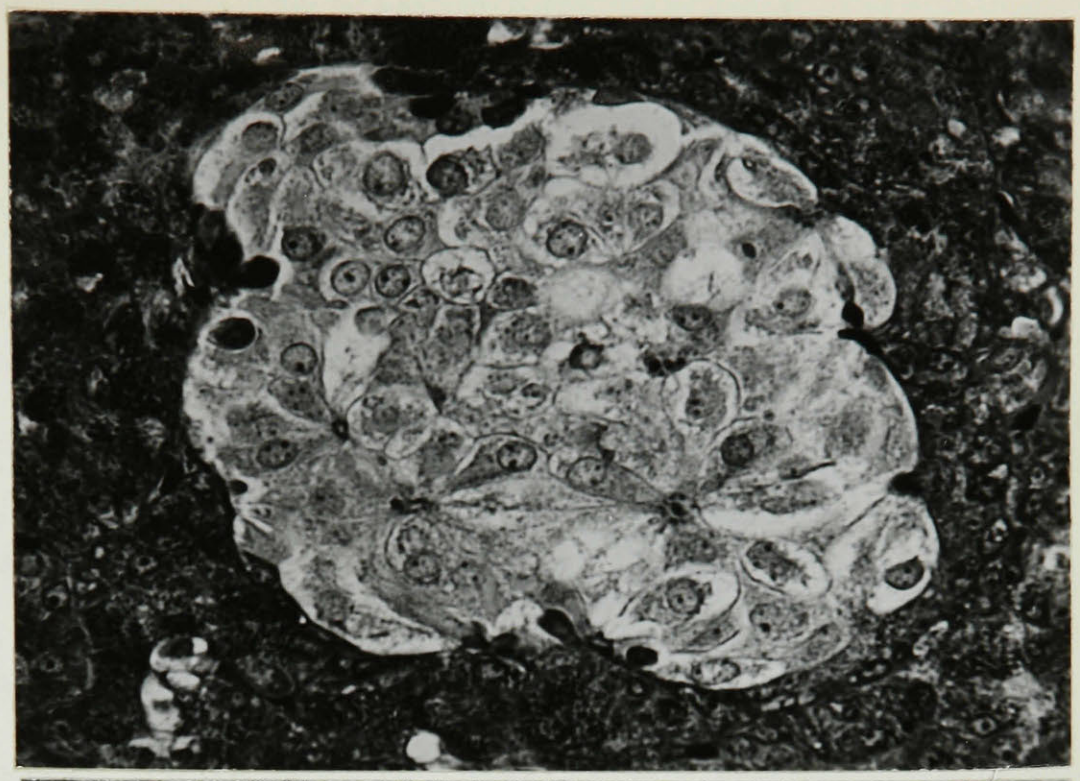
Glycogen infiltration in the pancreas of the cortisone-treated rabbit (number K 37, Drs. Kobernick and More).

Figure 21. Marked swelling and vacuolation of beta islet cells without regressive nuclear changes. Vacuolation of centro-acinar cells. Zenker-formol; Masson's trichrome; x530.

Figure 22. Abundant quantities of glycogen in the cytoplasm of beta islet cells. Several islet and centro-acinar cells appear incompletely filled by peripherally displaced glycogen. Zenker-formol; Best's carmine; x530.

Figure 23. Removal of glycogen from beta islet cells by diastase. Zenker-formol; diastase; Best's carmine; x530.

PLATE V



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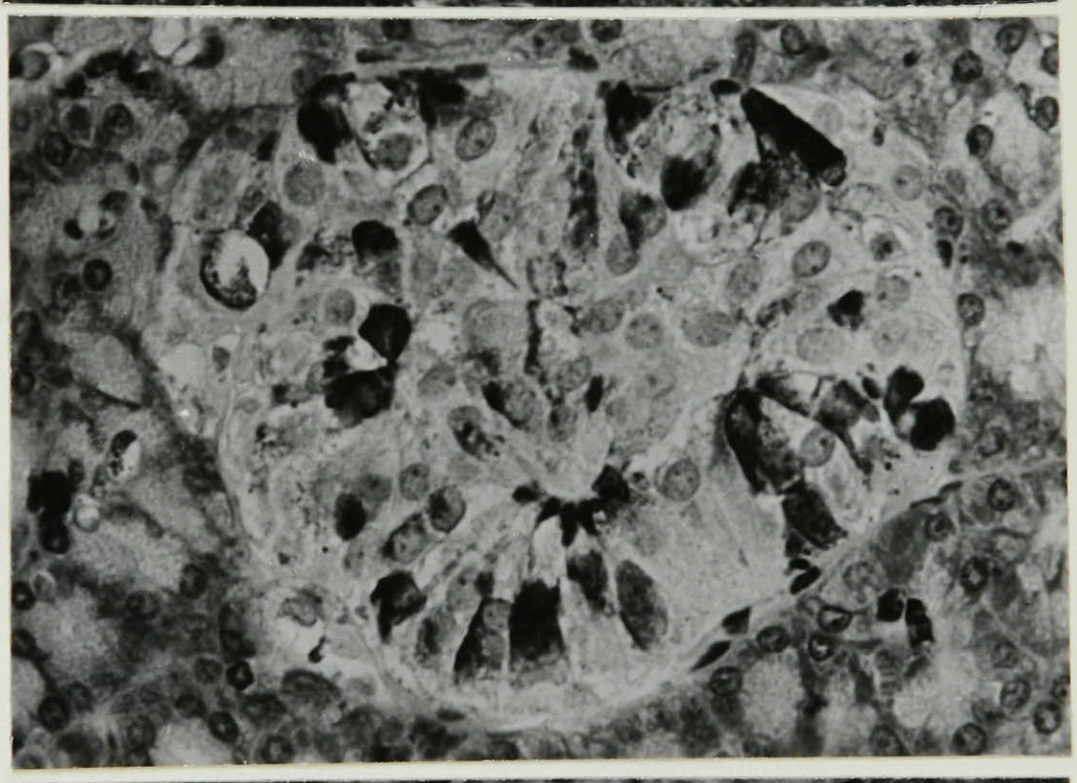

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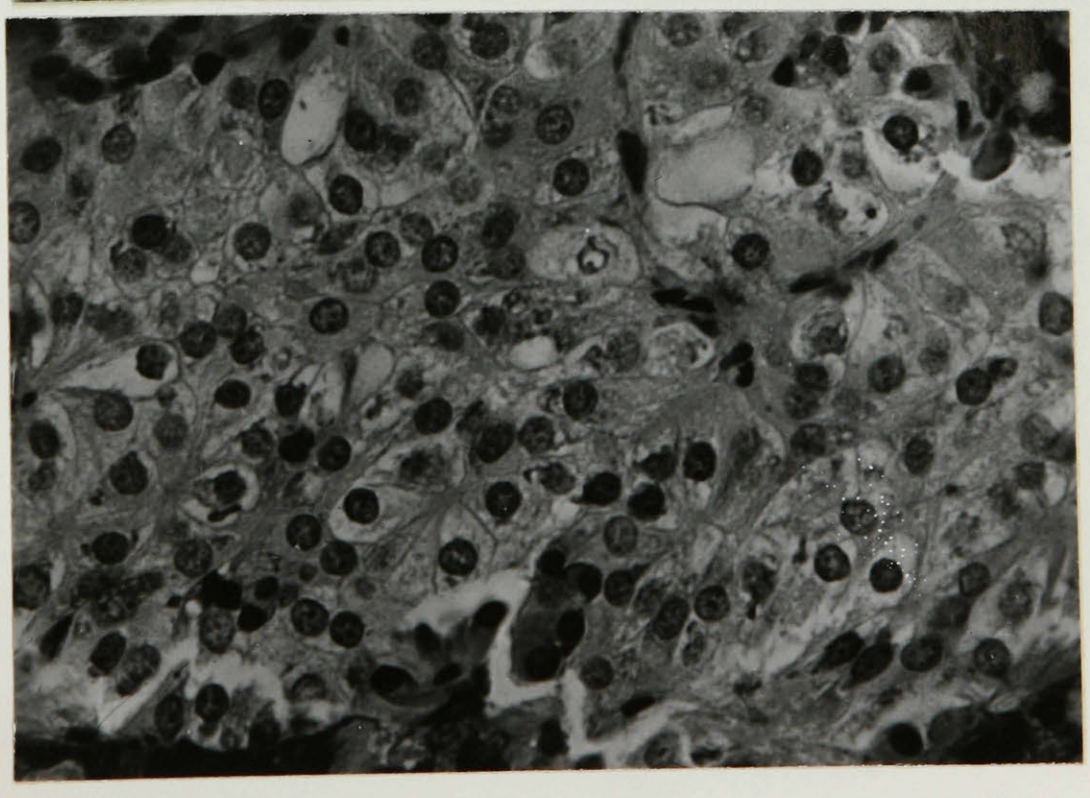

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Glycogen infiltration in the pancreas of a comatose diabetic human. (A 12333, Pathol. Inst., McGill Univ.)

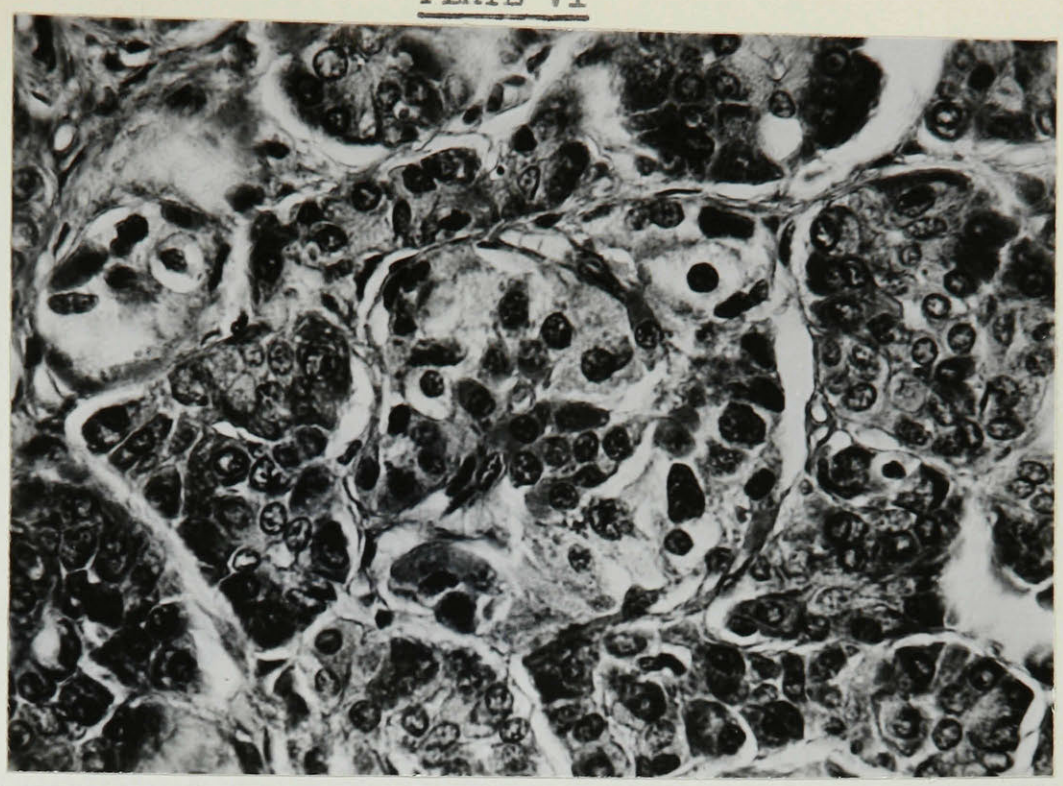
Figure 24. Swollen, vacuolated beta islet cells in two small islets. Acetic acid-Bouin's; Masson's trichrome; x530.

Figure 25. Widespread deposition of granules of glycogen in or upon many cells of a large islet. Acetic acid-Bouin's; Best's carmine; x530.

Figure 26. Removal of glycogen from the islet previously shown in Figure 25 by diastase. Acetic-Bouin's; diastase; Best's carmine; x530. Note: This section is many microns removed from previous section.

PLATE VI

24



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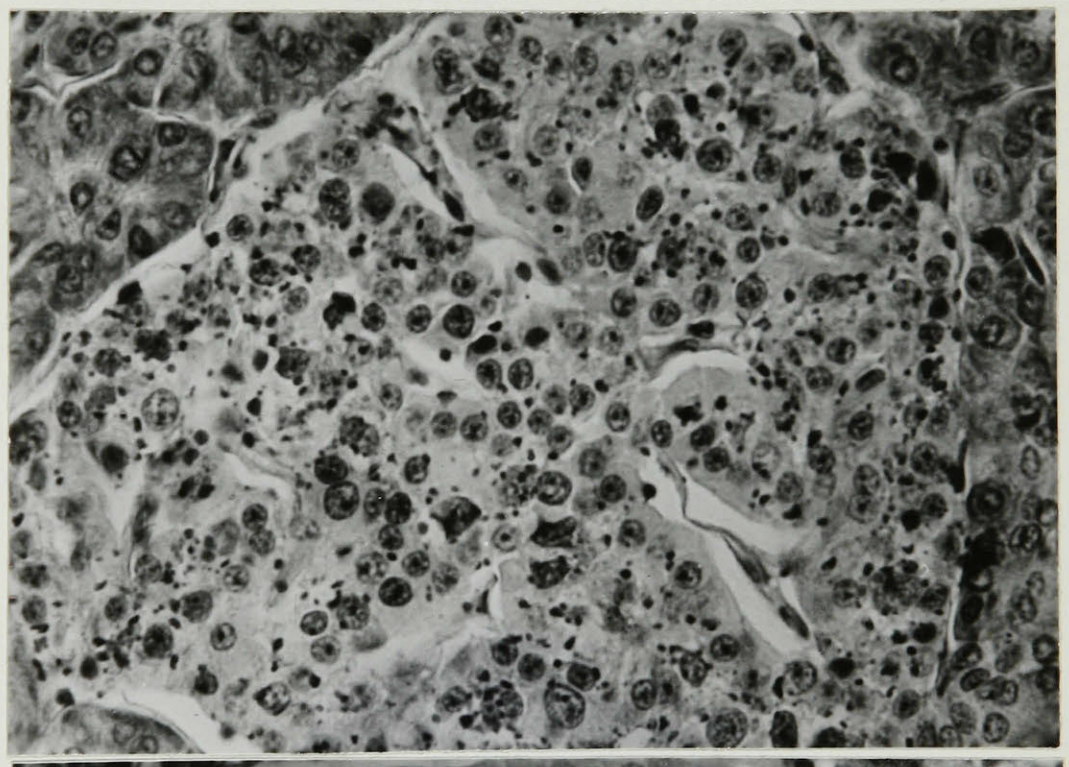

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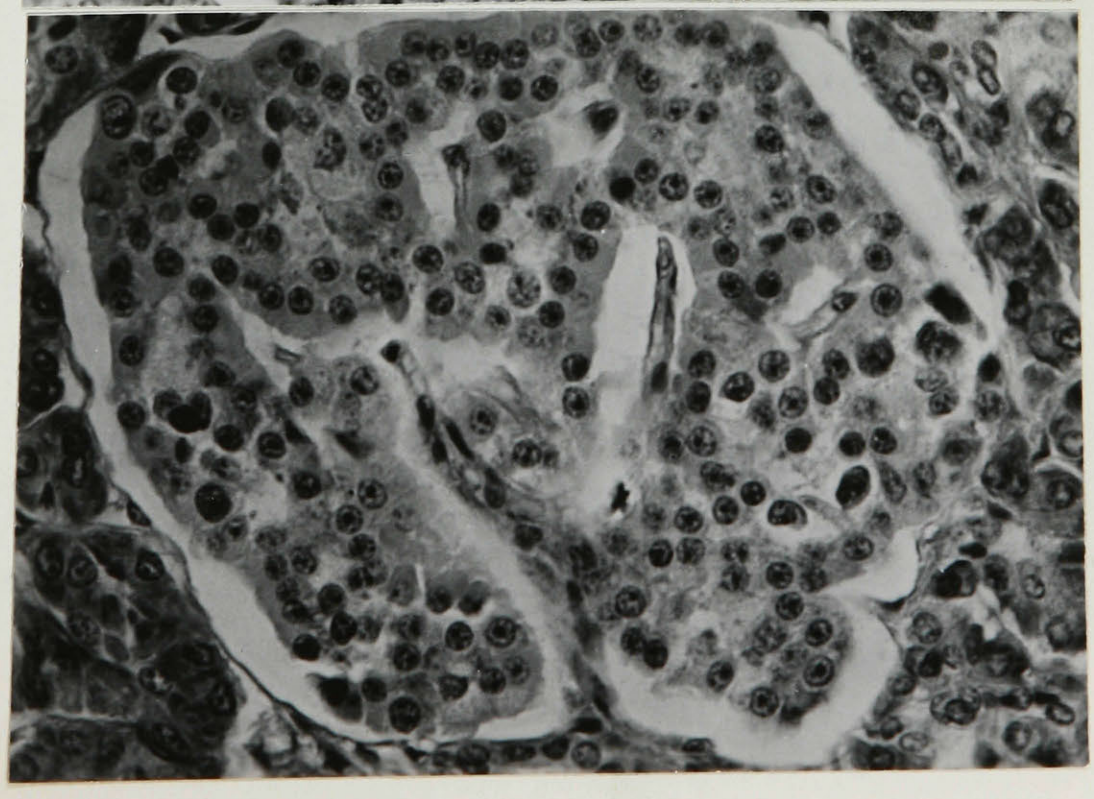


The development of glycogen infiltration in the pancreas of the alloxan-diabetic rabbit and restoration of the affected cells to normal appearance by treatment with insulin.

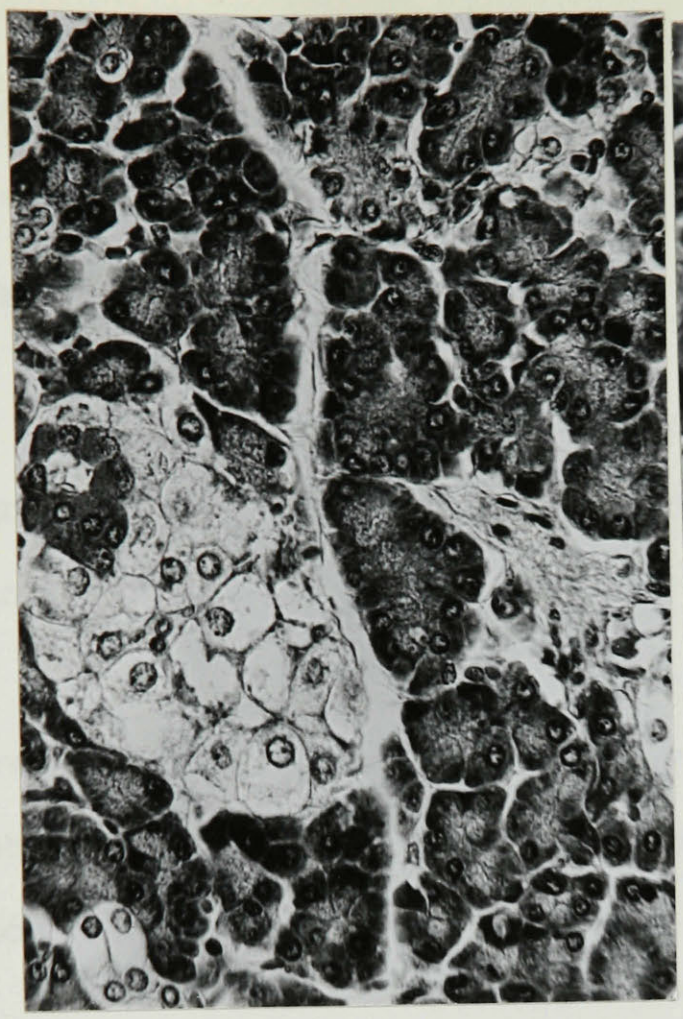
Figure 27. Persistence of glycogen infiltration in islet, ductule and centro-acinar cells after administration of 32 units of insulin during 2 days following pancreatic biopsy. Rabbit number T16, autopsy section; Helly's; Gomori; x340.

Figure 28. Apparently slight degree of glycogen infiltration in an islet and ductule found at biopsy after 86 days of severe diabetes. Rabbit number T4, first biopsy section; Helly's; Gomori; x660.

Figure 29. Restoration of affected cells of an islet and ductule to normal appearance by administration of massive doses of protamine and crystalline insulin for 27 days. Note a few beta granules in one islet cell and the apparent contiguity with the ductule. Rabbit number T4, second biopsy section; Helly's; Gomori; x710.

Figure 30. A larger islet from the same slide as Figure 29. Note "agranular beta" cells and many alpha cells. x590.

PLATE VII



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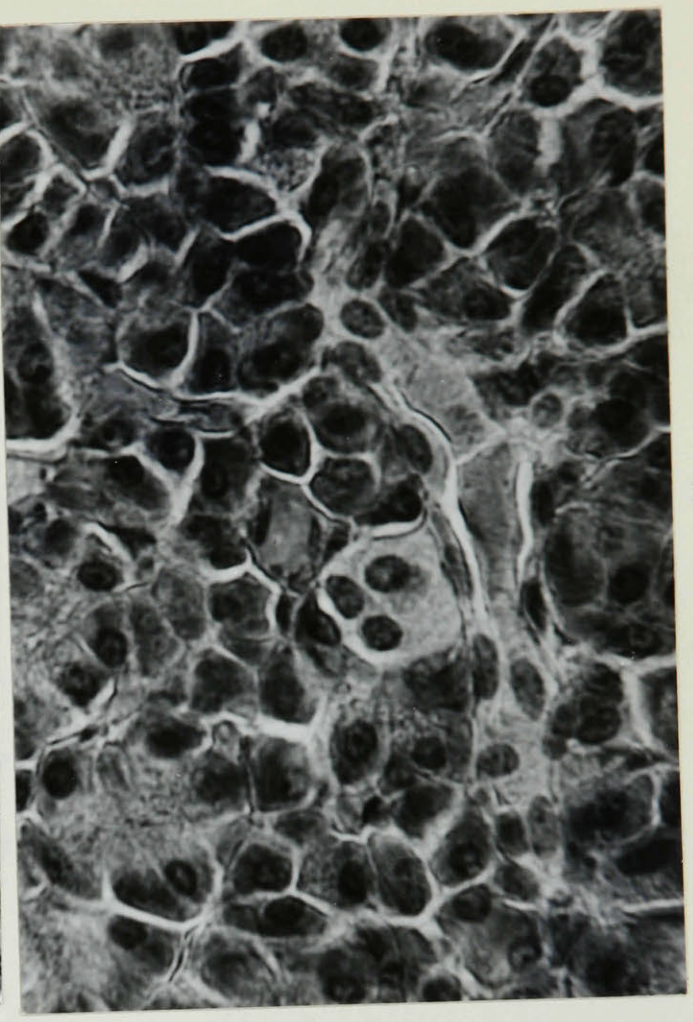

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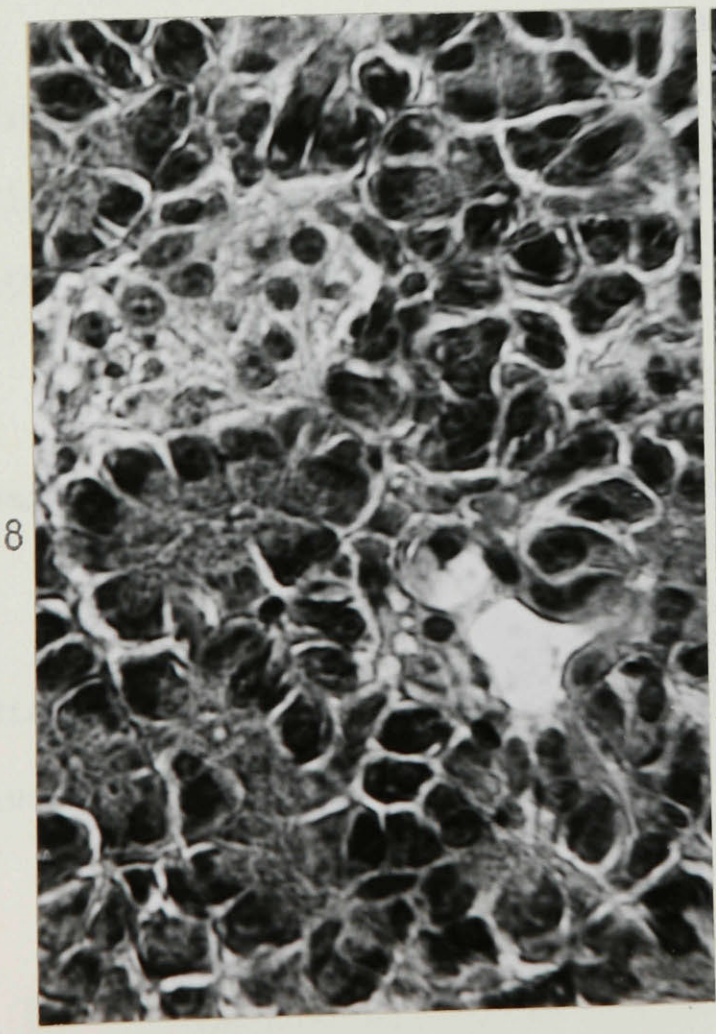

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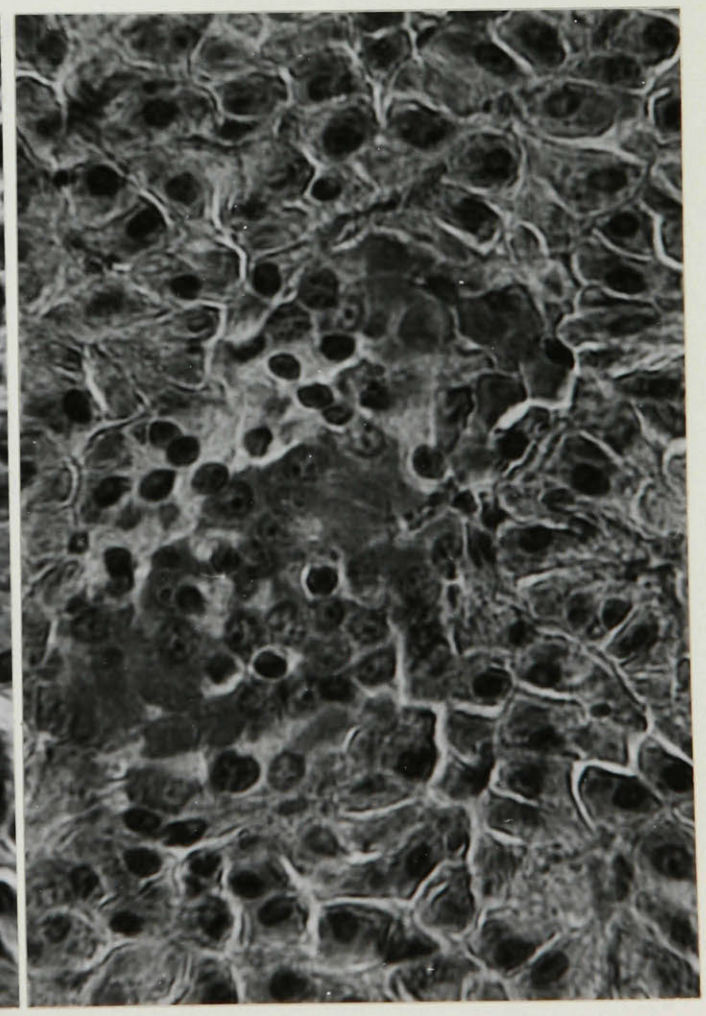

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Recurrence of glycogen infiltration in the pancreas of an alloxan-diabetic rabbit following discontinuation of insulin treatment.

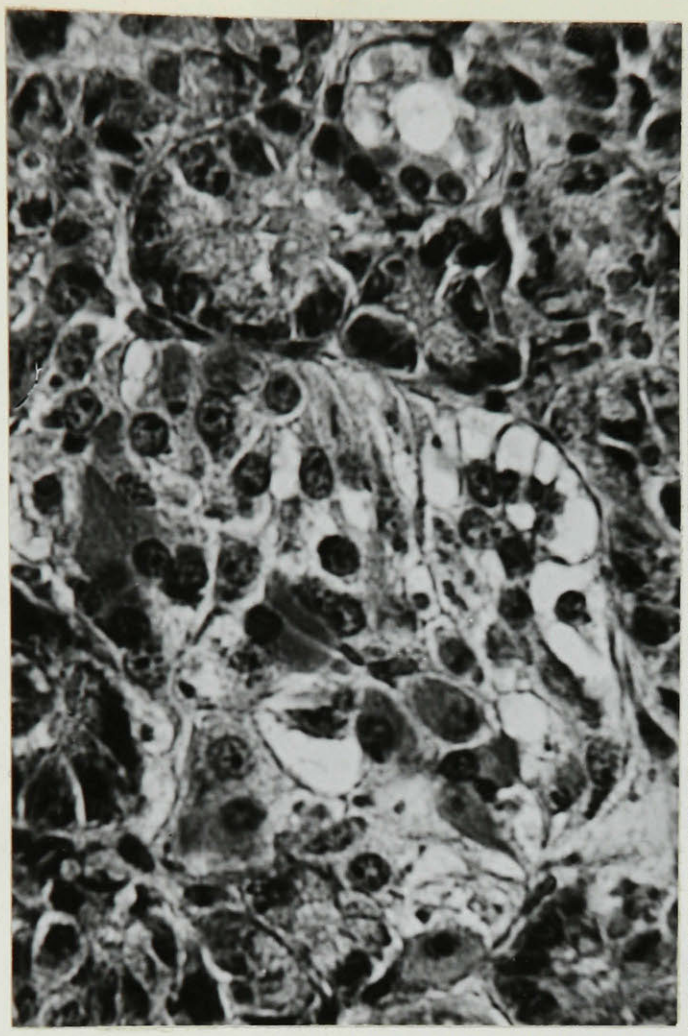
Figure 31. A large islet composed of alpha cells, agranular cells and glycogen-filled cells situated near a ductule which also shows glycogen infiltration in a lobule of proliferating pancreatic tissue. This appearance has developed during 120 days of exacerbated diabetic symptoms. Rabbit number T 4, third biopsy section; Helly's; Gomori; x560.

Figure 32. A ductule and sympathetic ganglion from a field adjacent to the islet shown in figure 31. Note mitotic figure to the left and slightly below the larger ductule. x590.

Figure 33. Glycogen infiltration in a large duct in the same slide as figures 31 and 32. x190.

Figure 34. Calcification in the kidney of alloxan diabetic rabbit (number T 4) after a second course of insulin treatments. Autopsy section; Helly's; H.and E.; x 130.

PLATE VIII



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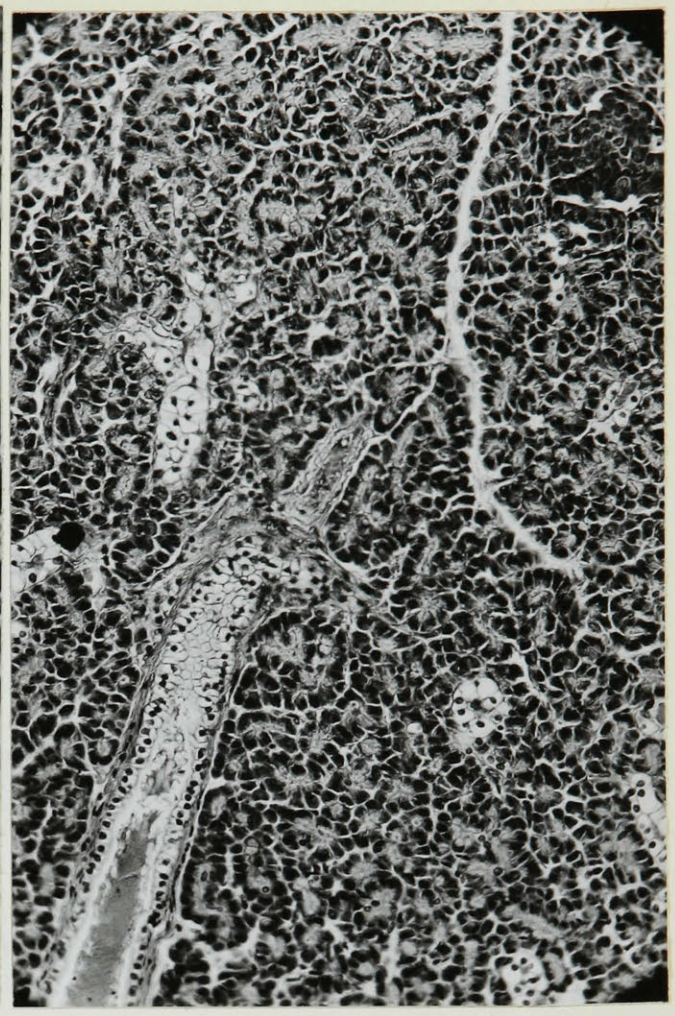

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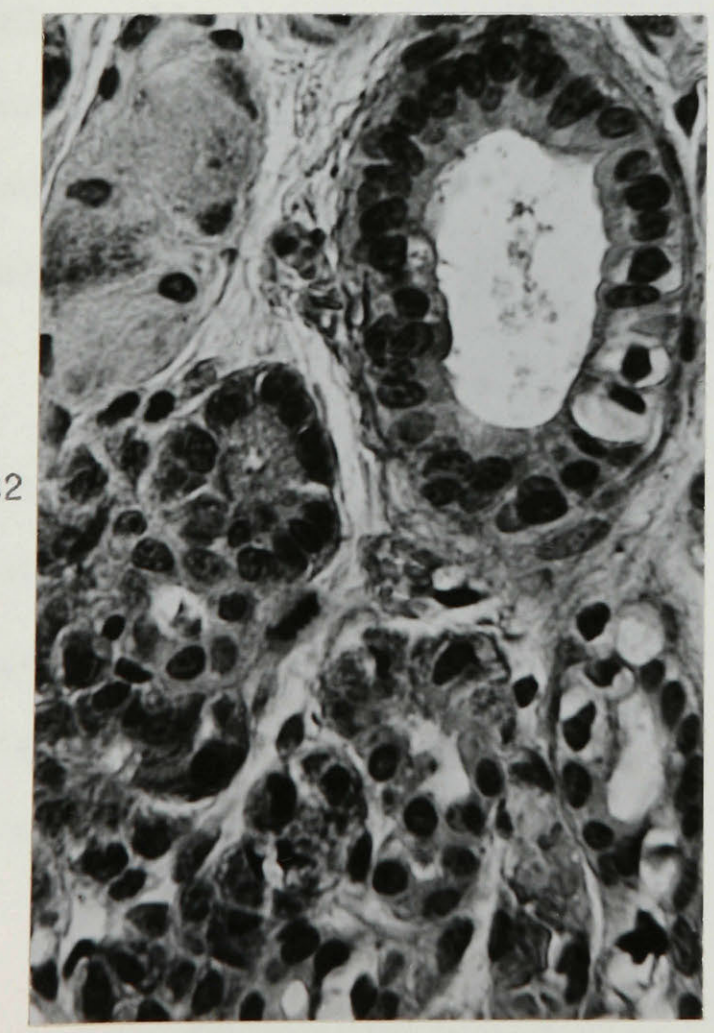

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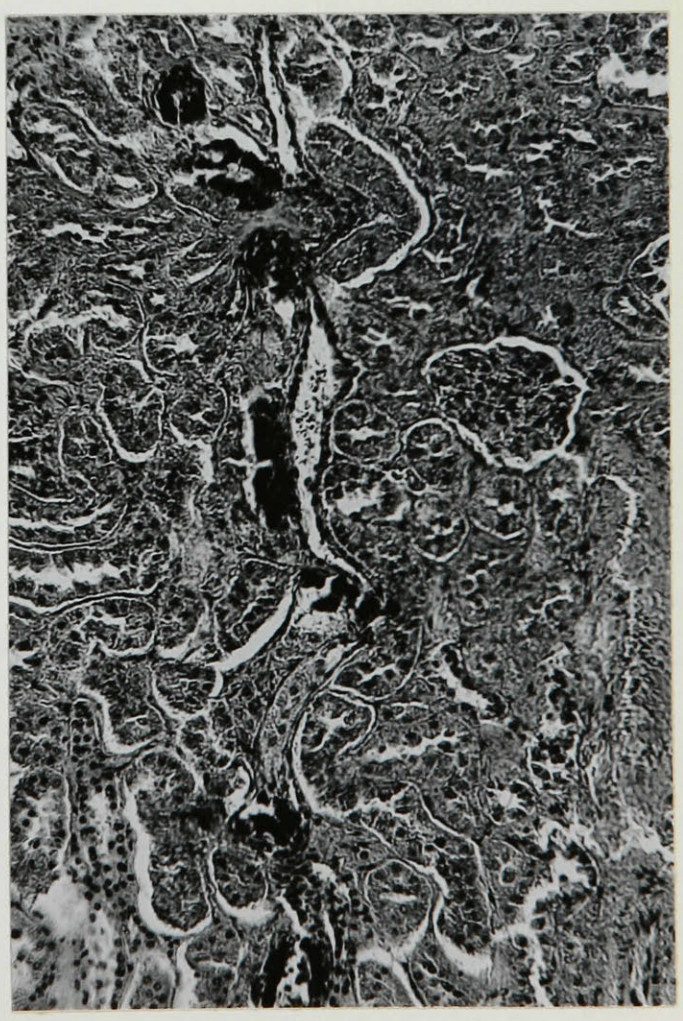

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Development of glycogen infiltration in the pancreas of the alloxan-diabetic rabbit and restoration of affected cells to normal appearance by treatment with insulin.

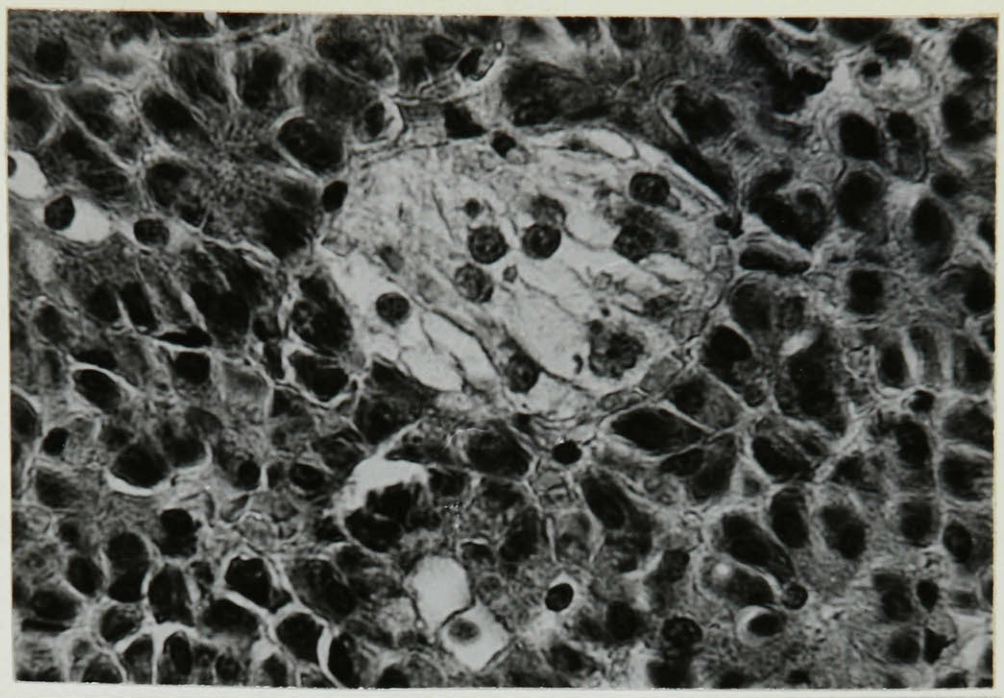
Figure 35. Glycogen-filled islet and ? centro-acinar cells as seen at biopsy after five months of severe diabetes. Note the "Körner" of Weichselbaum in three affected islet cells. Rabbit number T 20, first biopsy section; Helly's; Gomori: x570.

Figure 36. Persistence of glycogen infiltration in a few islet cells despite administration of 180 units of insulin during 10 days following pancreatic biopsy. No glycogen could be found in ductule cells after this treatment. Rabbit number T 23, autopsy section; Helly's; Gomori; x550.

Figure 37. Restoration of islet cells to normal appearance by treatment with 1,722 units of insulin during 36 days following pancreatic biopsy. This islet contained only agranular cells. (Previous appearance is demonstrated in figure 35.) Rabbit number T 20, autopsy section; Helly's; Gomori; x870.

PLATE IX

35



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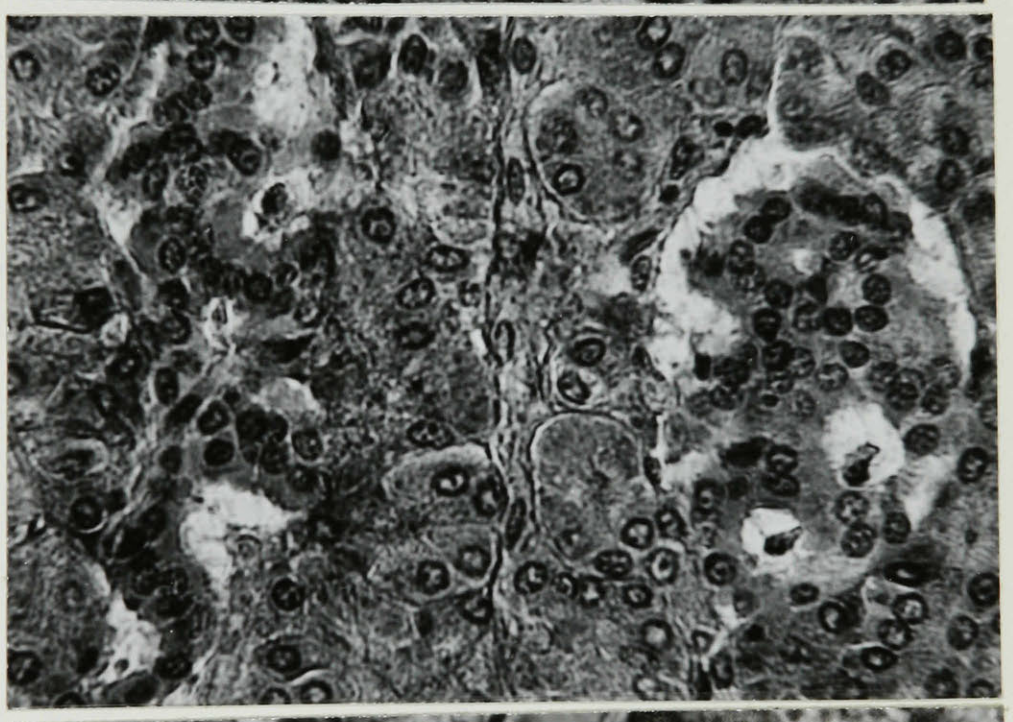

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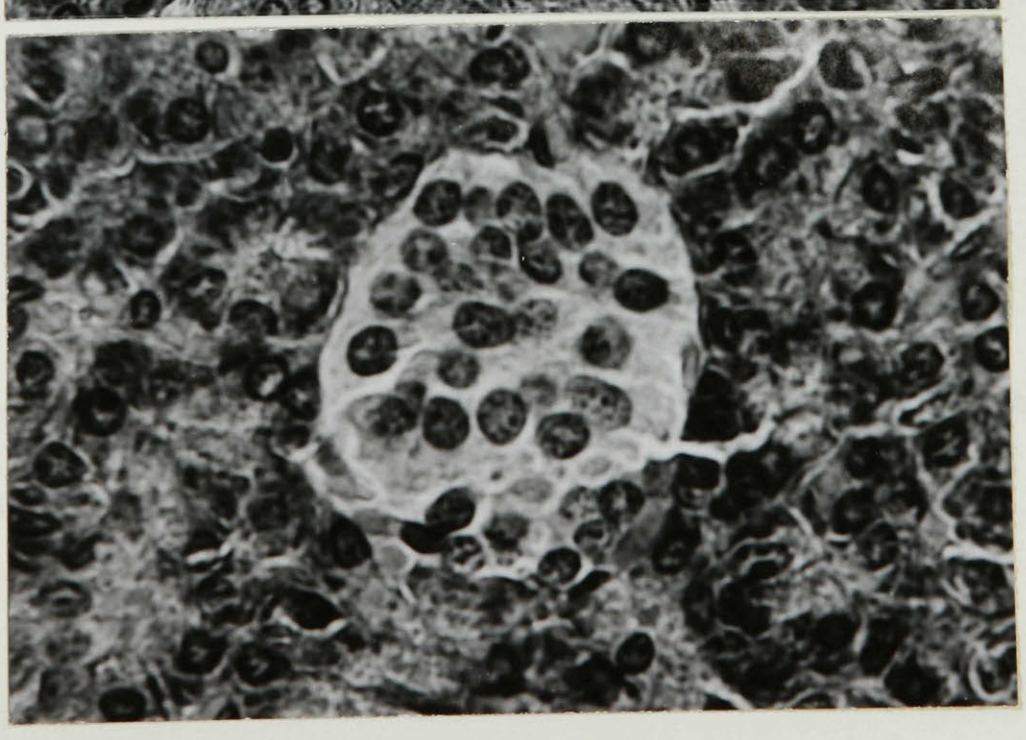


Development of glycogen infiltration in the pancreas of the alloxan-diabetic rabbit and restoration of glycogen-filled pancreatic cells to normal appearance despite hyperglycemia by treatment with small doses of insulin.

Figure 38. Abundant glycogen in the cytoplasm of liver cells in the middle and central zones of a hepatic lobule in an alloxan-diabetic rabbit treated with 140 units of insulin during 20 days following pancreatic biopsy. (Some glycogen remained demonstrable in the pancreatic islets and ductules after therapy.) Rabbit number T 64, autopsy section; absolute alcohol; Best's carmine; x325.

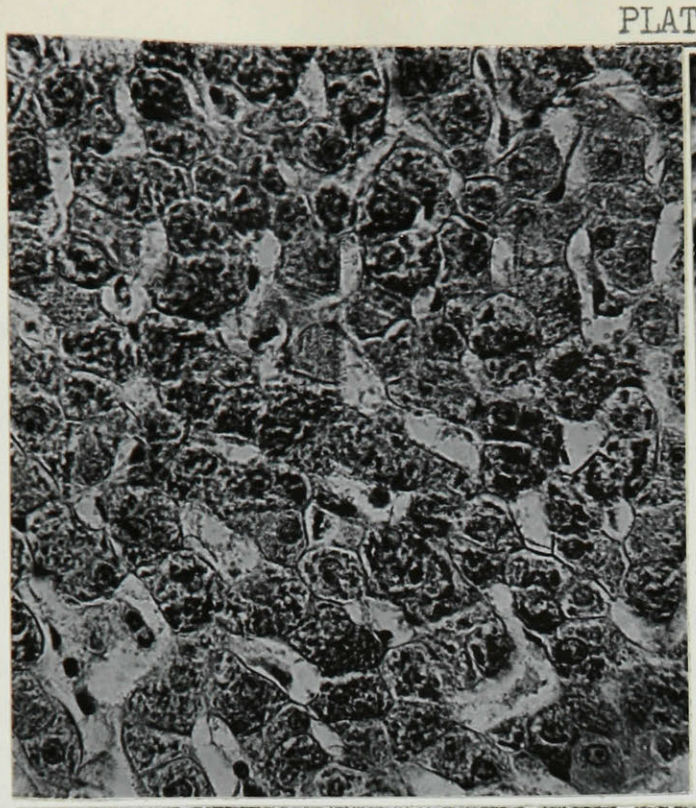
Figure 39. Development of glycogen infiltration in the pancreas of an alloxan diabetic rabbit during 96 days of severe diabetes. Rabbit number T 67, first biopsy section; Helly's; Gomori; x 300.

Figure 40. Absence of glycogen from an islet (just above center of field) and ductules in a lobule of proliferating pancreas following injection of 114 units of insulin during 29 days after pancreatic biopsy. Traces of glycogen remained demonstrable in other areas of this slide. Rabbit number T 67, second biopsy section; Helly's; Gomori; x305.

Figure 41. Restoration of islet cells to normal appearance by continued treatment with insulin; 96 units were given during 20 days. (Following previous administration of 114 units during 29 days some islet cells still contained glycogen.) Most of the cells portrayed are well-granulated beta cells. Rabbit number T 67, autopsy section; Helly's; Gomori; x500.

Figures 42 and 43. The appearance of active proliferation in the pancreas of an insulin-treated alloxan-diabetic rabbit. Number T 67, autopsy section; Helly's; Gomori; x500 and x690, respectively.

PLATE X



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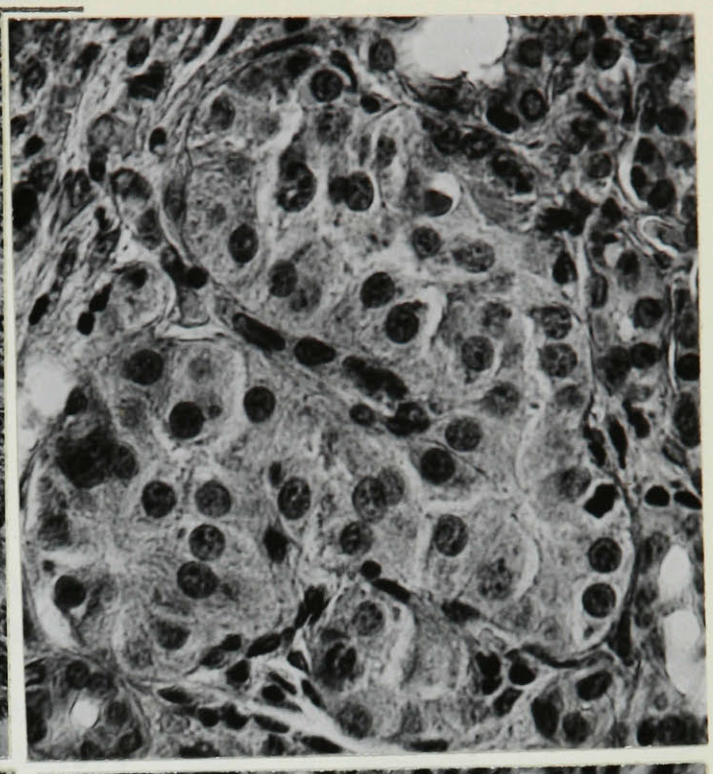

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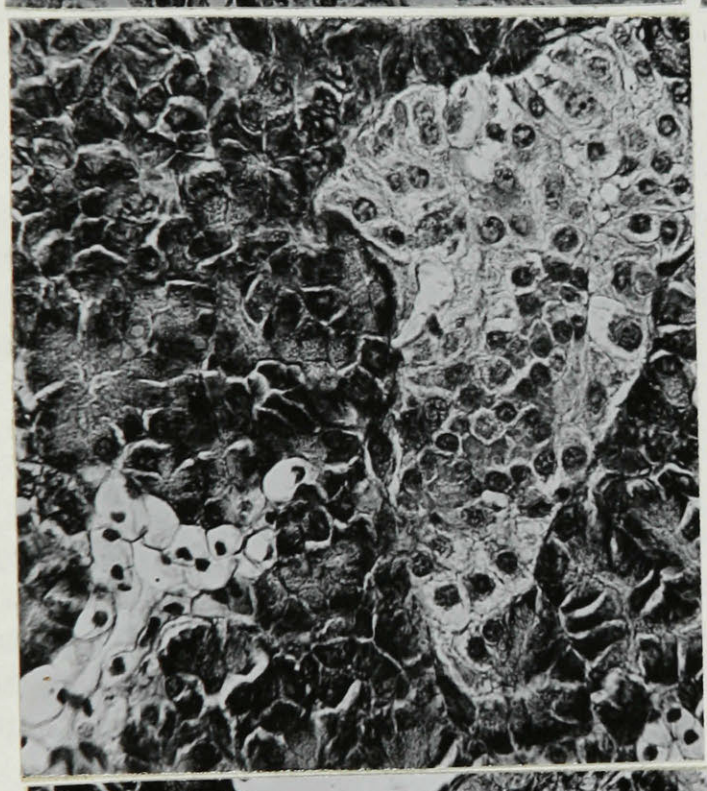

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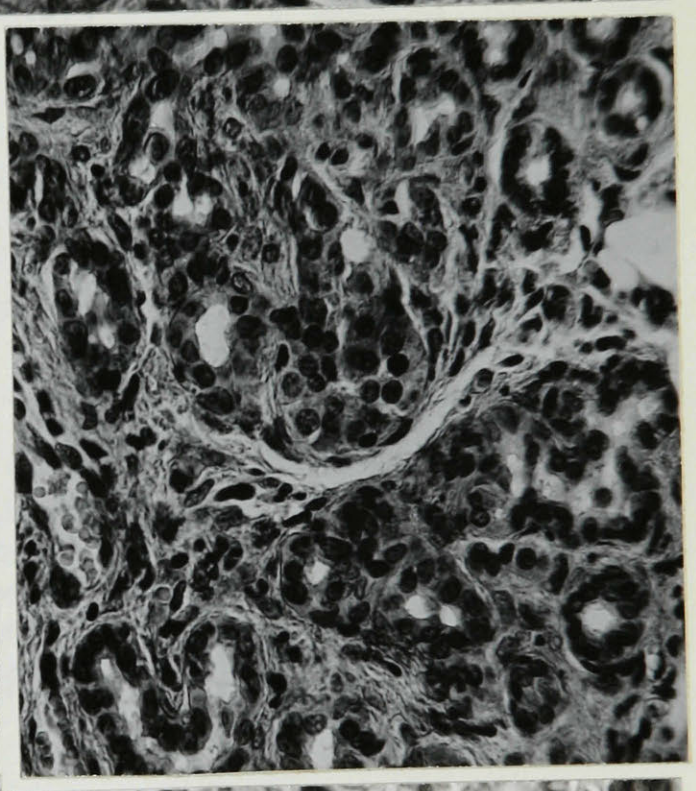

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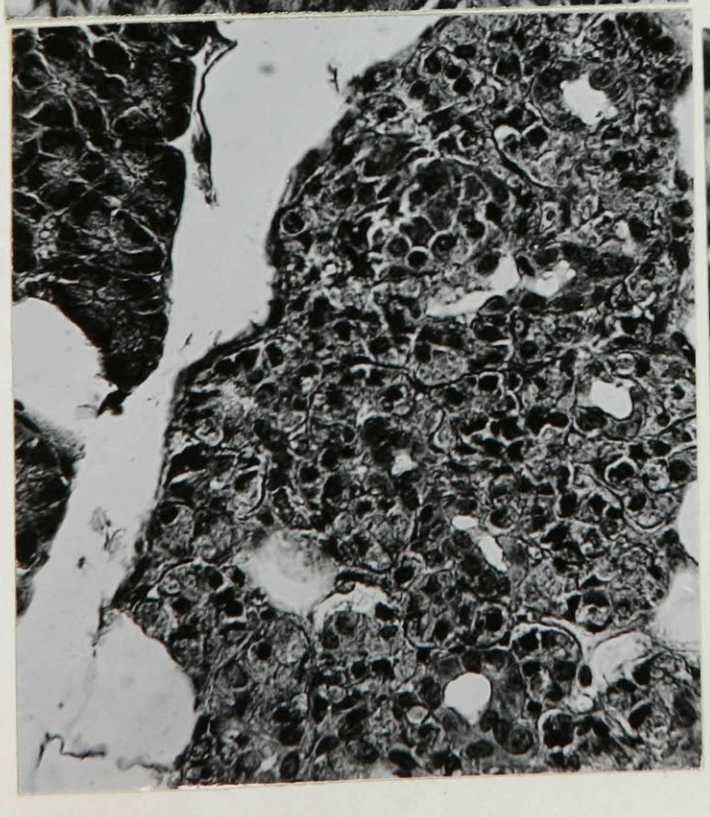

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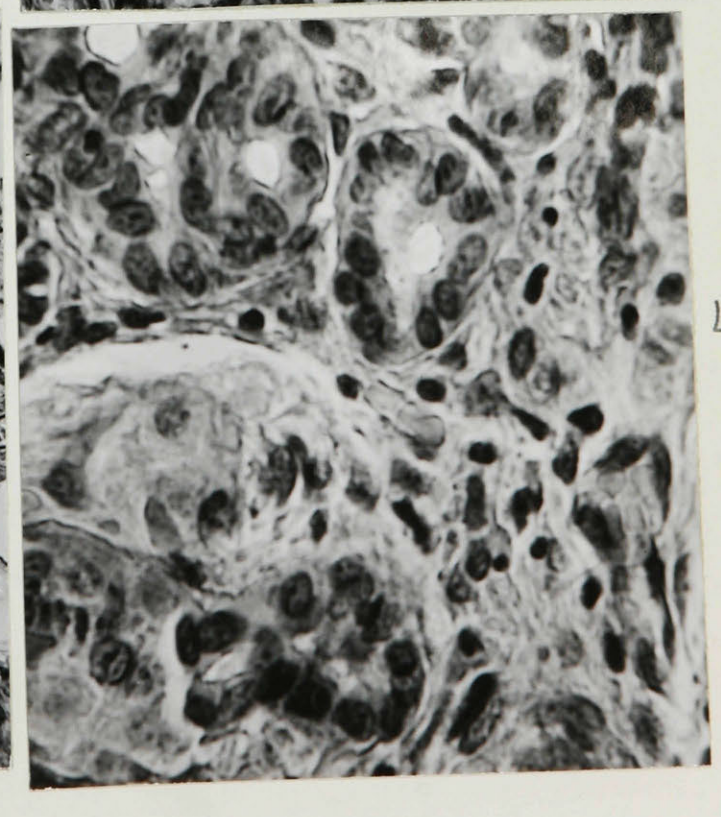

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Prevention of the development of glycogen infiltration in the pancreas of the alloxan-diabetic rabbit, despite continuous hyperglycemia, by prolonged treatment with small doses of insulin, and rapid development of the lesion after discontinuation of insulin treatment.

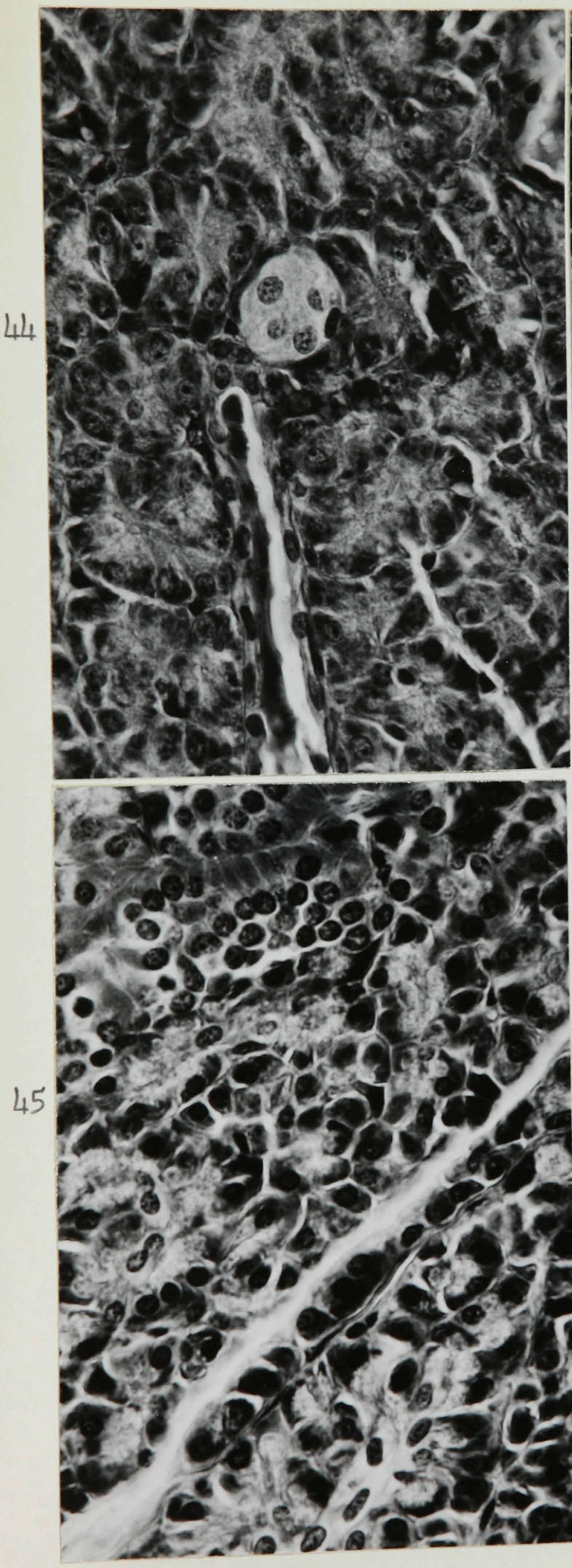
Figure 44. Absence of glycogen from a small islet composed of agranular cells and from a ductule and a few centro-acinar cells as seen at biopsy after 103 days of severe diabetic symptoms; five units of insulin were given daily. Rabbit number C 89, biopsy section; Helly's; Gomori; x530.

Figure 45. Rapid development of glycogen infiltration in an islet (upper left) and a ductule (lower center) and in scattered centro-acinar cells upon discontinuation of insulin treatment. Rabbit number C 89, autopsy section; Helly's; Gomori; x530.

Figure 46. Rapid development (3 weeks) of glycogen infiltration in an islet upon discontinuation of insulin treatment; after 113 days of severe symptoms, despite 5 units of insulin daily, no glycogen-filled cells were demonstrable by pancreatic biopsy. Note the peculiar central position of alpha cells. Rabbit number C 49, autopsy section; Helly's; Gomori; x530.

Figure 47. Glycogen infiltration of a liver cell nucleus in rabbit number C 49. This lesion was seldom found in any of the alloxan-diabetic rabbits irrespective of insulin treatment or severity and duration of diabetes. Rabbit number C 49, autopsy section; Helly's; H.and E.; x530.

PLATE XI



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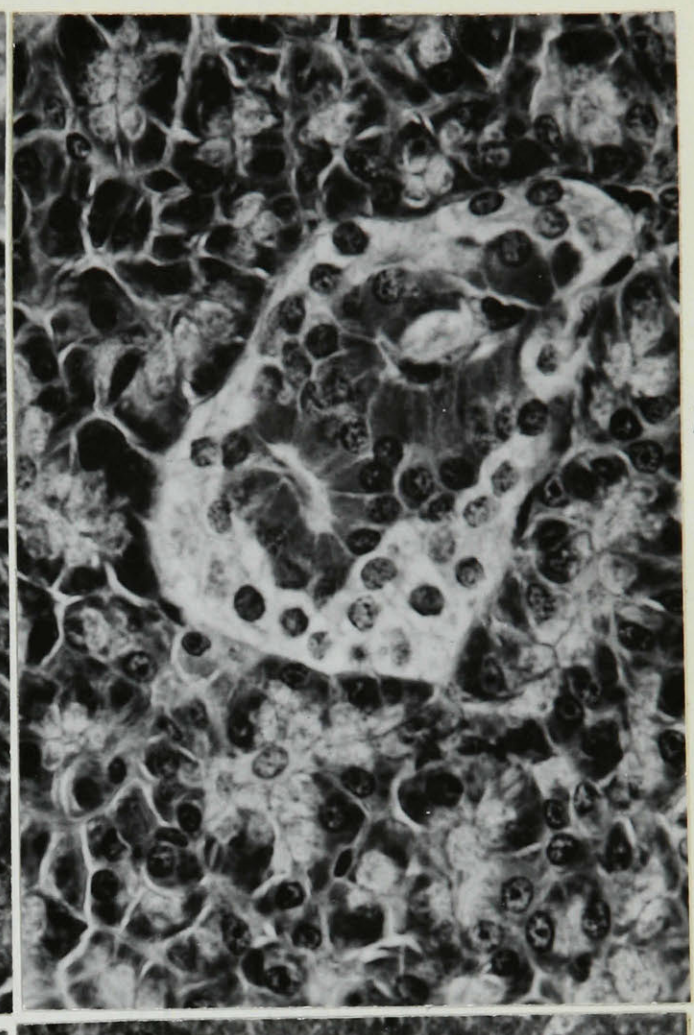

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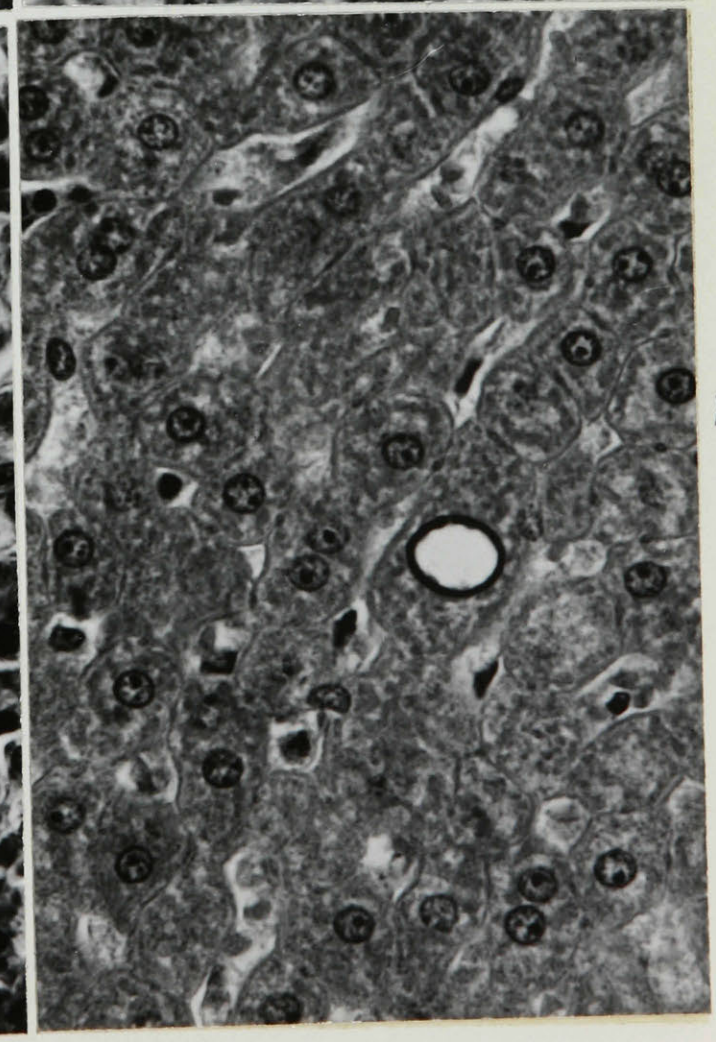

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PLATE XII

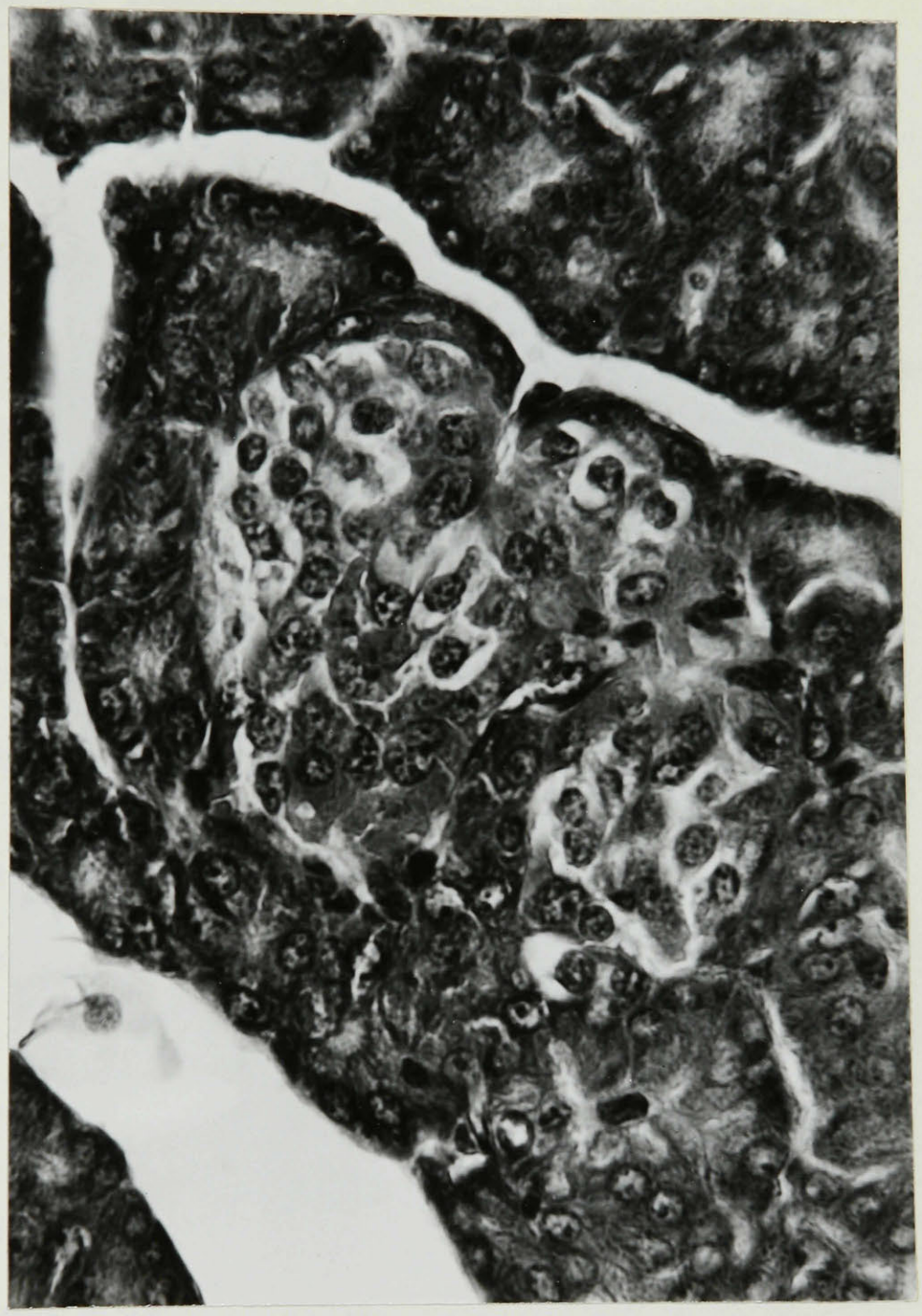


Figure 48. Ineffectiveness of a second injection of alloxan upon the glycogen-filled islet cells of the diabetic rabbit 59 days after a first diabetogenic dose was given. Rabbit number McM 172, autopsy section; Helly's; H.and E.; x630.

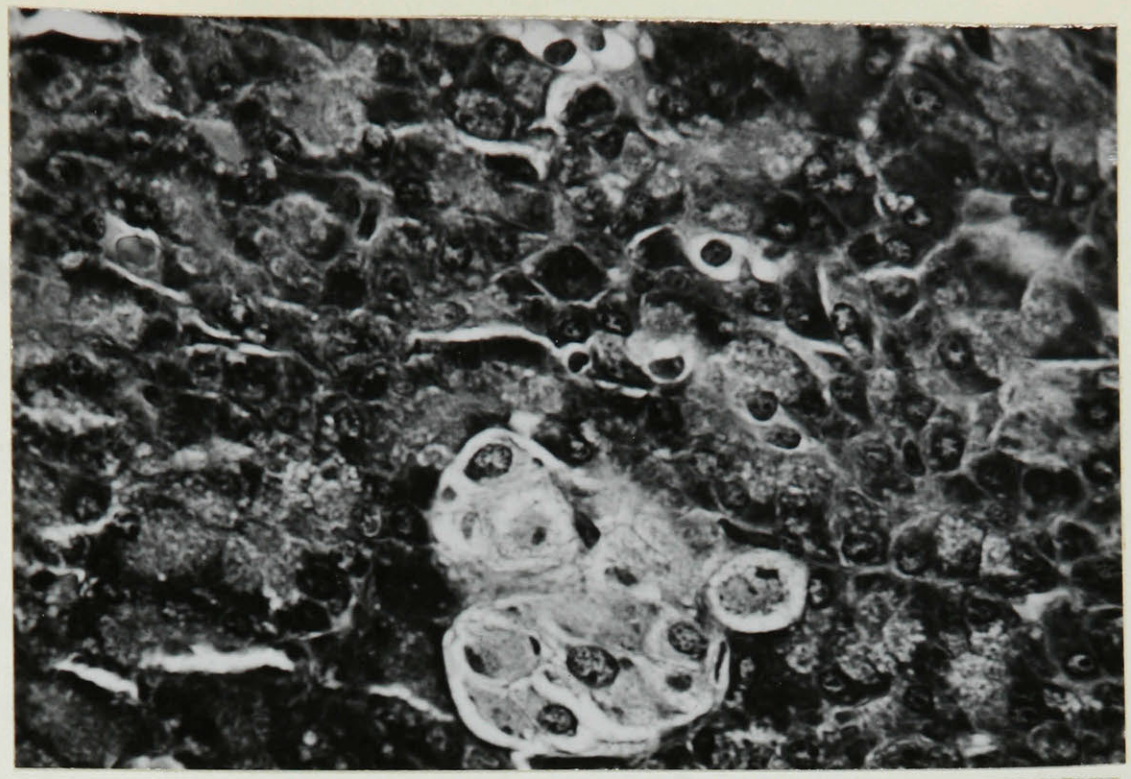
Accelerated development of glycogen infiltration in the pancreas of the alloxan-diabetic rabbit treated with crude APE.

Figure 49. Apparently mild but definite glycogen infiltration in the islet cells and ductules of an alloxan-diabetic rabbit given six daily injections of crude APE as found at autopsy on the nineteenth day after injection of alloxan. Note mitotic figure at the upper right in an agranular islet cell. Rabbit number J 2, autopsy section; Helly's; Gomori; x530.

Figure 50. Photomicrograph of a Best's carmine preparation showing very faintly small granules of glycogen in several islet cells. Rabbit number J 2, autopsy section; Helly's; Best's carmine; x530.

Figure 51. Apparently severe glycogen infiltration in a small islet, a ductule (lower edge, center) and in several centro-acinar cells of a rabbit treated with crude APE for 24 days, beginning 12 days after injection of alloxan. Rabbit number I 92, autopsy section; Helly's; Gomori: x530.

PLATE XIII



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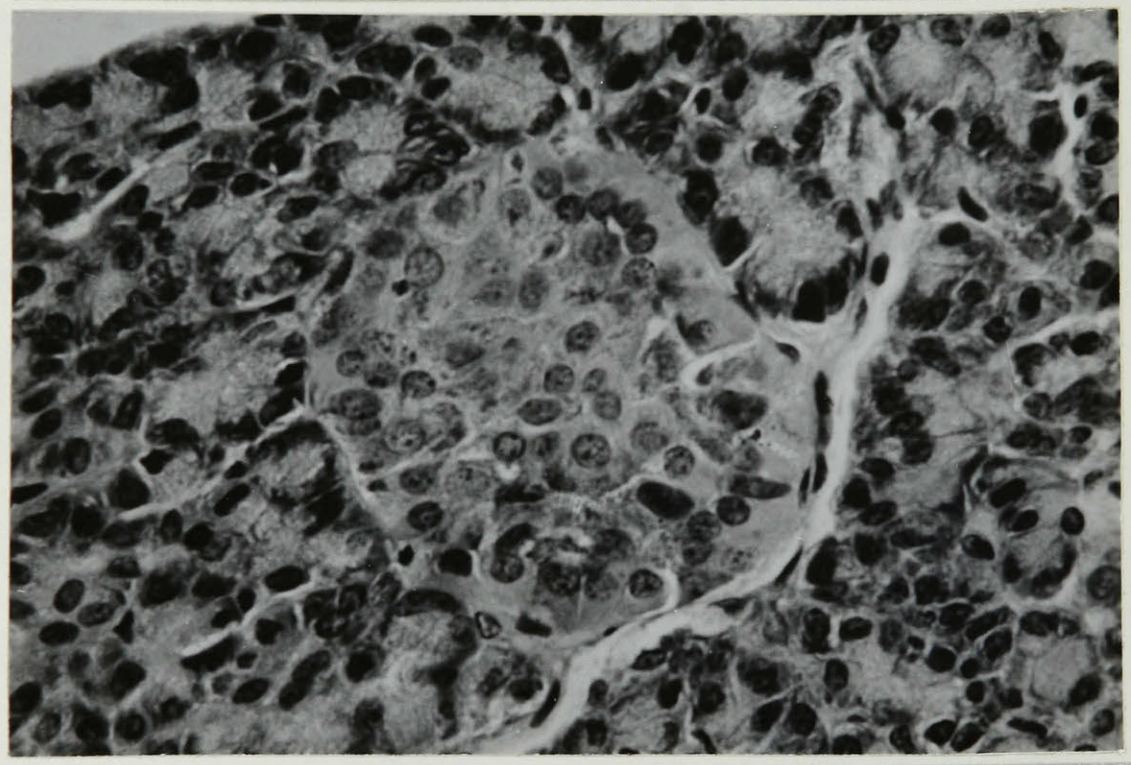

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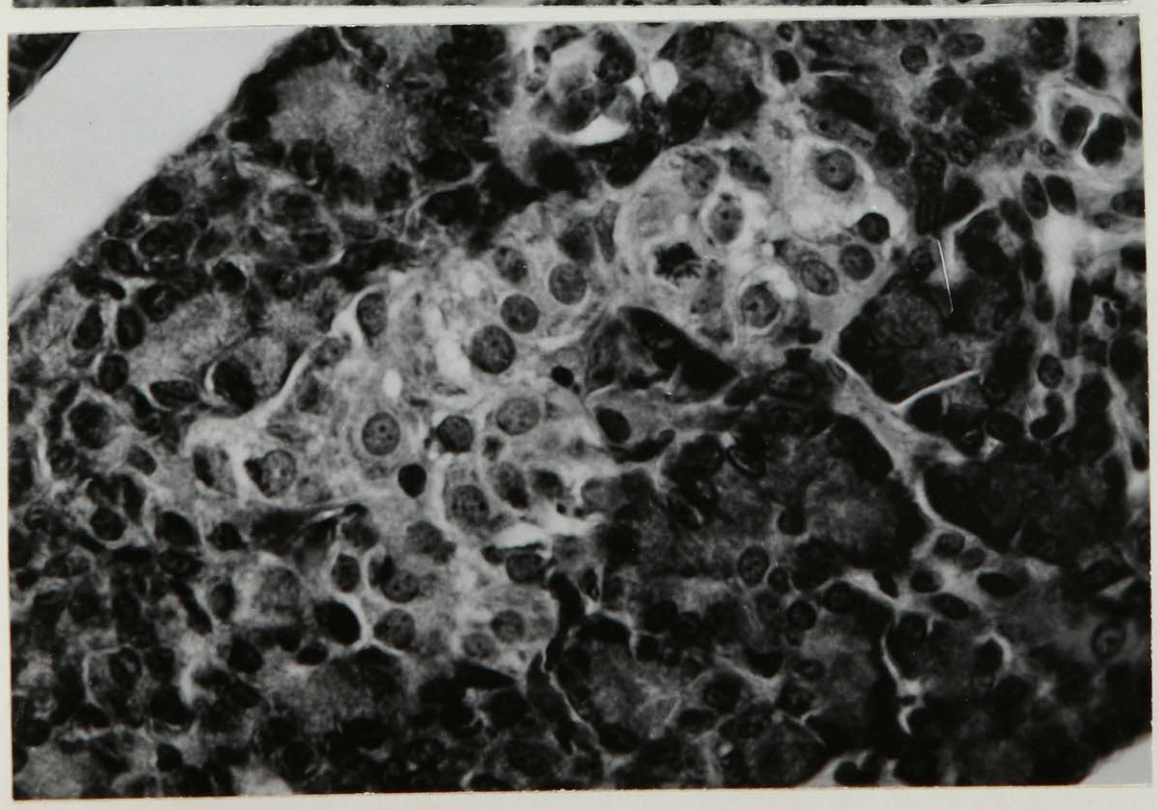


51

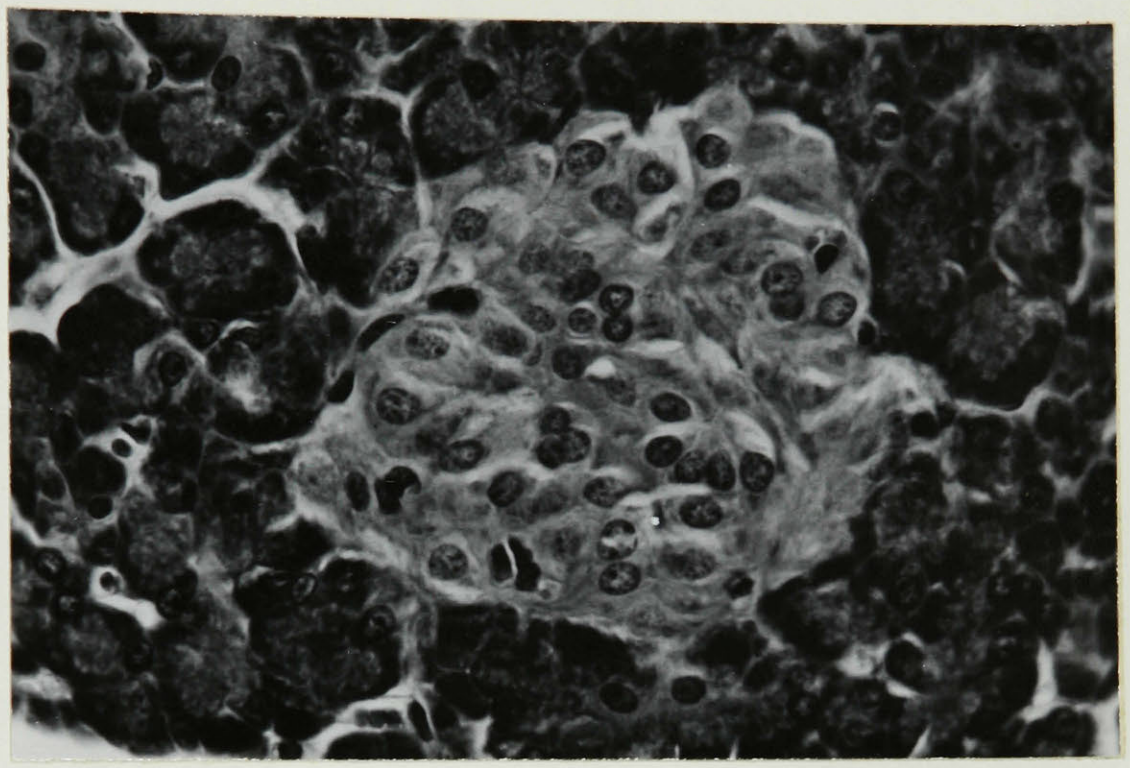
Absence of glycogen infiltration in the pancreas of the alloxan diabetic rabbit following and during treatment with crude APE.

Figure 52. Small islet composed of numerous beta cells having varying degrees of cytoplasmic granularity; autopsy appearance twelve days after discontinuation of APE treatment which was given from the 12th to 36th days after alloxan was injected. (Suggestions of cytoplasmic vacuolation are apparently referable to fixation artefact; no glycogen could be demonstrated in other sections of this islet.) Rabbit number I 93, autopsy section; Helly's; Gomori; x530.

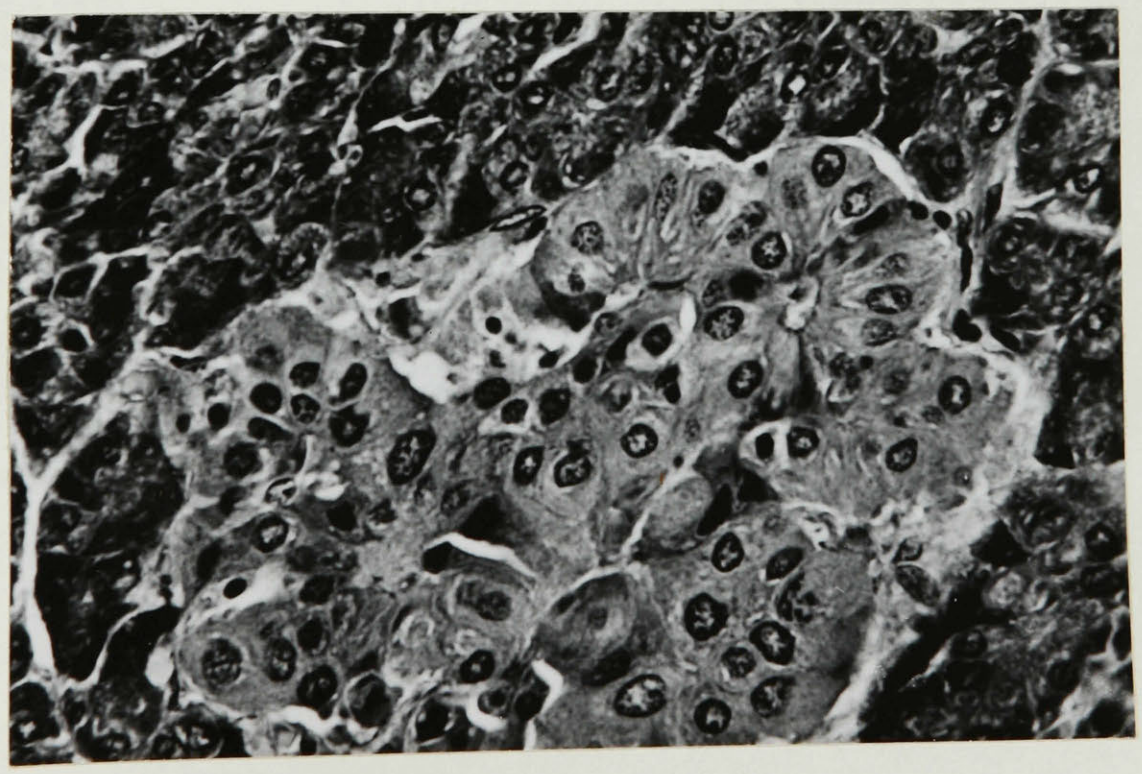
Figure 53. Large islet composed of alpha cells, agranular cells and apparently normal beta cells; (several pseudo-necrotic cells show post mortem autolysis). Rabbit died of pneumonia and subcutaneous infection after 23 daily injections of APE given from the 12th to 34th days after injection of alloxan.

PLATE XIV

52



53



Protocols:-

1. A protocol is presented for each animal described in the text. These records are arranged in alphabetical sequence and subdivided by number, e.g., B 98, C 49, C 50 etc.

2. Average values of the daily observations, summarizing each week of study, are indicated by the inclusive days in spaced lines.

3. The notation "Alloxan 200", for example, indicates the dose 200 mg. of alloxan per kilogram of body weight.

4. The letter 'f' immediately after any value recorded signifies that the observation refers to the fasting state.

Number :— B 98 Male

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
0	2.25	96						0	Alloxan 200 Non-fasting 6:00 P.M.
1		92f						0	9:00 A.M.
2	2.17	390	640			4	0	0	
3	2.24	434	570	4.3	24.5	4	2	0	
4	2.18	420	660	3.2	21.1	4	4	0	
5	1.95	348	970	3.5	34.0	4	4	10	
6	2.10	448	400	2.7	10.8	4	4	0	
7	1.92	396	940	4.2	39.6	4	4	0	
1-7	2.09	406	693	3.6	26.0			1.4	
8	2.09	460	710	3.4	24.1	4	4	0	
9	2.10	466	870	4.5	39.2	4	4	0	
10	2.10	756	840	3.5	29.4	4	4	10	
11	2.18	280	470	3.0	14.1	4	4	0	
12	2.03	600	750	2.3	17.3	4	4	0	
13	1.98f	534f	190f	3.6f	6.8f	4	1	0	Fasting past 24 hours
14	1.93f	430f	150f	2.3f	3.5f	4	0	0	Fasting past 48 hours
8-14	2.10	512	728	3.3	14.8			1.4	
15	1.97	340	820	3.3	27.1	4	1	0	
16	2.07		610	3.7	22.6	4	4	0	
17	2.16		980	3.3	31.4	4	4	0	
18	2.05		840	3.8	31.9	4	4	0	
19	1.95	396	740	3.5	25.9	4	4	0	
20	1.93f	348f	200f	3.0f	6.0	4	2	0	Fasting past 24 hours.
21	1.97f	324f	0					0	Fasting past 48 hours
15-21	2.04	368	798	3.5	33.2			0	
22	1.98	370	650	3.6	23.4	4	0	0	
23	2.05	442	1000	3.8	38.0	4	2	0	
24	1.95	360	1090	3.6	39.2	4	4	0	
25	2.09	400	970	2.9	28.1	4	4	0	
26	2.00	406	1100	3.5	38.5	4	4	0	
27	1.99	384	700	3.7	25.9	4	4	0	
28	2.07	440	1040	3.6	37.4	4	4	0	
22-28	2.02	420	937	3.5	32.9			0	

Number :— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
29	2.13	412	1040	3.6	37.4	4	4	0	
30	2.13	394	1080	4.2	45.4	4	4	0	
31	2.19	412	940			4	4	0	
32	2.10	374	1110	4.0	44.4	4	4	0	
33	2.00		760	4.1	31.2	4	4	0	
34	2.10		740	4.2	31.1	4	4	0	
35	2.03		740	3.8	28.1	4	4	0	
29-35	2.10	398	916	4.0	36.3			0	
36	2.20		1090	3.5	38.2	4	4	0	
37	2.23	576	960	3.7	35.5	4	4	0	
38	2.26		1170	3.2	37.1	4	4	0	
39	2.25		1050	2.9	30.5	4	4	0	
40	2.07		760	2.9	22.0	4	4	0	
41	2.21		730	3.9	28.5	4	4	0	
42	2.30		1220	3.2	39.0	4	4	0	
36-42	2.22	576	997	3.3	33.0			0	
43	2.20		1190	4.4	52.4	4	4	0	
44	2.32	407	1280	4.8	61.4	4	4	0	
45	2.14		1230	4.5	55.4	4	4	0	
46	2.27		1140	5.4	61.6	4	4	0	
47	2.11		1090	4.0	43.6	4	4	0	
48	2.19	483	810	3.7	30.0	4	4	0	
49	2.12	558f	280f	4.5f	12.6f	4	4	0	Fasting past 24 hours
43-49	2.20	445	1123	4.5	60.7			0	
50	1.83f	435f	170f	1.4f	2.4f	4	0	0	Fasting past 48 hours
51	1.78f	350f	110f	0.5f	0.6f	3	0	0	Fasting past 72 hours
52	2.00	467	650	4.4	28.6	4	0	0	
53	1.98		790	4.5	35.6	4	3	0	
54	1.96		780	4.3	33.5	4	4	0	
55	2.08		800	2.8	22.4	4	4	0	
56	2.13		790	3.8	30.0	4	4	0	
50-56	2.03	467	762	3.9	25.0			0	

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
57	2.17	500	910	3.7	33.7	4	4	0	
58	2.00f	480f	270f	3.0f	8.1f	4	1	0	Fasting past 24 hours
59	1.91f	295f	280f	0.4f	1.2f	2	0	0	Fasting past 48 hours
60			820	4.3	35.3	4	2	0	
61	1.73		660	5.0	33.0	4	3	0	
62	1.78	467	770	4.5	34.7	4	3	0	
63	1.92	560	1100	4.1	45.1	4	4	0	
57-63	1.90	509	852	4.3	36.4			0	
64	1.92f	450f	690f	2.8f	19.1f	4	3	0	Fasting past 16 hours
65	1.99		1200	3.3	39.6	4	4	0	
66	2.05		1150	3.5	40.3	4	4	0	
67	2.01	580	1050	5.1	53.6	4	4	0	
68			910	3.5	31.9	4	4	0	
69	1.97		740	3.6	26.6	4	4	0	
70	1.97		1010	3.0	30.3	4	4	0	
64-70	2.00	580	1010	3.7	37.1			0	
71	2.10	610	1200	3.8	45.6	4	4	0	
72	2.12		1200	3.5	42.0	4	4	0	
73	2.16					4	4	0	
74	2.13		1090	3.3	36.0	4	4	0	
75	2.05		790	2.9	22.9	4	4	0	
76	2.11		780	4.1	32.0	4	4	0	
77	2.15		1260	3.4	42.8	4	4	0	
71-77	2.12	610	1063	3.5	36.8			0	
78	2.14		1150	2.4	27.6	4	4	0	
79	2.13		1120	3.3	37.0	4	4	0	
80	2.20		1120	4.4	49.3	4	4	0	
81	2.16		1140	4.6	52.4	4	4	0	
82	2.16		980	5.0	49.0	4	4	0	
83	2.12	648	940	4.1	38.5	4	4	0	
84	2.10		930	4.1	38.1	4	4	0	
78-84	2.14	648	1054	4.0	41.5			0	

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
85	2.08		970	4.6	44.6	4	4	0	
86	2.10		820	4.1	33.6	4	4	0	
87			870	4.0	34.8	4	4	0	
88	2.11	670	1020	4.1	41.8	4	4	0	
89	1.92f	593f	960f	2.4f	23.0f	4	4	0	Fasting past 16 hours
90	2.13		800	3.9	31.2	4	4	0	
91	2.18		1090	4.4	48.0	4	4	0	
85-91	2.12	670	1054	4.0	41.7			0	
92	2.15		1130	4.5	50.9	4	4	0	
93	1.97f	660f	510f	2.6f	13.3f	4	4	0	Fasting past 16 hours
94			820	3.8	31.2	4	4	0	
95			640			4	4	0	
96	2.04		960	4.0	38.4	4	4	0	
97	2.04		830	3.0	24.9	4	4	0	
98	1.99		950	2.8	26.6	4	4	0	
92-98	2.06	660f	888	3.6	34.4			0	
99	2.09		1000	3.7	37.0	4	4	0	Urine choline 19.8 per min.
100	2.01f	660f	490f	3.3f	16.2f	4	4	0	Fasting past 16 hours
101	2.00		1070	2.6	27.8	4	4	0	
102	2.00		990	3.6	35.6	4	4	0	
103	1.90		910	2.8	25.5	4	4	0	
104	1.98		790	3.7	29.2	4	4	0	
105	2.02		1060	4.5	47.7	4	4	0	
99-105	2.00	660f	970	3.5	33.8			0	
106	2.05		980	5.2	51.0	4	4	0	
107	1.93f	660f	660f			4	4	0	Fasting past 16 hours
108	2.13		970	4.2	40.7	4	3	0	
109	2.05		950	4.1	39.0	4	4	0	
110	2.09		930	3.7	34.4	4	4	0	
111	2.02		990	4.6	45.5	4	4	0	
112	1.92		690	4.4	30.4	4	4	0	
106-112	2.04	660f	918	4.4	40.2			0	

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
113	1.97		970	5.2	50.4	4	4	0	
114	1.82f	630f	500f	4.3f	21.5f	4	1	0	Fasting past 16 hours
115	1.85		680	4.6	31.3	4	4	0	
116	1.91		910	5.1	46.4	4	4	0	
117	1.91		870	4.6	40.0	4	3	0	
118	1.99		930	5.3	49.3	4	4	0	
119	1.94		870	4.3	37.4	4	4	0	
113-119	1.93	630f	872	4.9	42.5			0	
120	1.96		770	5.0	38.5	4	2	0	
121	1.90	608f	600	4.1	24.6	4	1	0	Fasting past 16 hours
122	1.97		920	4.6	42.3	4	4	0	
123	2.00		1000	5.2	52.0	4	4	0	
124	1.94		900	5.2	46.8	4	4	0	
125			920	4.6	42.3	4	4	0	
126			960	4.5	43.2	4	4	0	
120-126	1.96	608f	867	4.7	41.4			0	
127	1.85f	558f	540f	4.3f	23.2f	4	4	0	Fasting past 16 hours
128	1.91		1030	4.2	43.3	4	4	0	
129	1.99		900	4.8	43.2	4	4	0	
130	2.00		1030	6.9	71.1	4	4	0	
131	1.97		1010	4.6	46.5	4	4	0	
132	2.09		1020	4.8	49.0	4	4	0	
133	2.06		1050	4.7	49.4	4	4	0	
127-133	2.00	558f	1070	5.0	50.4			0	
134	2.01		960	4.5	43.2	4	4	0	
135	1.80f	776f	470f	3.6f	16.9f	4	4	0	Fasting past 16 hours
136	1.97		830	4.4	36.5	4	4	0	
137	1.98		810	4.7	38.1	4	4	0	
138	1.83		790	5.0	39.5	4	4	0	
139	2.02		810	4.5	36.5	4	4	0	
140	2.02		890	4.0	35.6	4	4	0	
134-140	1.97	776f	848	4.5	38.2			0	

Number :— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
141			960	3.9	37.4	4	4	0	
142	1.93		890	4.8	42.7	4	4	0	
143	1.95		870	4.8	41.8	4	4	0	
144	2.00		940	4.3	40.4	4	4	0	
145	1.84		830	4.0	33.2	4	4	0	
146	1.80f	728f	570f			4	2	0	Fasting past 16 hours
147	1.94		950	3.9	37.1	4	4	0	
141-147	1.93	728f	907	4.3	38.8			0	
148	2.00		950	4.6	43.7	4	4	0	
149	1.89		780	4.3	33.5	4	4	0	
150	2.02		460	4.3	19.8	4	4	0	
151	2.06		520	4.2	21.8	4	2	0	
152	1.99		520	5.2	27.0	4	3	0	
153	2.02		610	4.0	24.4	4	1	0	
154	2.02	628	920	4.3	39.6	4	1	0	
148-154	2.00	628	680	4.4	30.0			0	
155	2.02		930	4.1	38.1	4	4	0	
156	2.01		1020	3.9	39.8	4	4	0	
157	1.99		850	4.6	39.1	4	4	0	
158	1.93		850	4.3	36.6	4	4	0	
159	1.92		970	2.9	28.1	4	4	0	
160	1.97	660	820	4.1	33.6	4	4	0	
161	1.98		650	6.5	42.3	4	4	0	
155-161	1.99	660	899	4.3	36.8			0	
162	1.99		820	3.8	31.2	4	4	0	
163	2.05		830	3.9	32.4	4	3	0	
164	2.03		750	3.5	26.3	4	3	0	
165	2.00		870	4.5	39.2	4	2	0	
166	2.02		920	4.3	39.6	4	2	0	
167	1.93	668	780	3.8	29.6	4	4	0	
162-168	2.00	668	833	3.9	32.6			0	

Number :— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)
169	1.96		800	3.9	31.2	4	2	0	
170	1.98		770	4.8	37.0	4	2	0	
171	1.97		820	4.6	37.7	4	2	0	140
172	1.97		560	3.7	20.7	4	1	0	
173	2.00		790	4.3	34.0	4	3	0	
174	1.94		720	3.4	24.5	4	3	0	140
175	1.96	676	770	4.1	31.6	4	3	0	165
169-175	1.97	676	747	4.1	31.0			0	
176	1.93		890	3.9	34.7	4	3	0	120
177	1.96		910	3.5	31.9	4	3	0	170
178	1.82		780	3.4	26.5	4	3	0	145
179	1.91		960	3.9	37.4	4	3	0	185
180	2.00		870	3.7	32.2	4	3	0	180
181	1.90		860	4.3	37.0	4	3	0	155
182	1.93	660	990	3.7	36.6	4	3	0	175
176-182	1.92	660	894	3.8	33.8			0	167
183	1.98		880	3.8	33.4	4	3	0	165
184	2.00		990	4.0	39.6	4	3	0	180
185	1.95		1020	4.0	40.8	4	4	0	180
186	1.99		1010	2.1	21.2	4	4	0	175
187	1.95		920	4.3	39.6	4	4	0	180
188	1.87		820	4.2	34.4	4	4	0	150
189	1.88	640	980	3.4	33.3	4	4	0	185
183-189	1.95	640	946	3.7	34.6				174
190	1.93		840	3.4	28.6	4	3	0	180
191	1.94		840	3.9	32.8	4	3	0	165
192	1.95		1090	4.0	43.6	4	4	0	195
193	1.94		950	3.8	36.1	4	4	0	180
194	2.10		830	3.6	29.9	4	0	0	185
195	1.93		740	4.1	30.3	4	1	0	130
196	1.89	728	880	4.9	43.1	4	4	0	180
190-196	1.95	728	881	4.0	34.9				174

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)
197	1.92		890	4.3	38.3	4	4	0	180
198	1.92		950	4.1	39.0	4	3	0	190
199	1.95		1010	4.5	45.5	4	3	0	190
200	1.96		970	4.0	38.8	4	4	0	170
201	1.98		1100	3.5	38.5	4	3	0	180
202	2.20		650	4.1	26.7	4	2	0	180
203	1.99	688	960	3.7	35.5	4	2	0	185
197-203	1.98	688	933	4.0	37.5				182
204	1.80		830	4.5	37.4	4	4	0	130
205	1.98		830	4.1	34.0	4	4	0	170
206	1.86		810	4.8	38.9	4	4	0	155
207	1.84		920	4.1	37.7	4	4	0	190
208	1.99		970	6.1	59.2	4	4	0	185
209	1.88		690	5.1	35.2	4	4	0	190
210	1.93	660	950	4.7	44.7	4	4	0	190
204-210	1.90	660	857	4.8	41.0				173
211	1.89		960	4.7	45.1	4	3	0	180
212	1.87		970	4.2	40.7	4	4	0	190
213	1.91		910	4.2	38.2	4	4	0	180
214	2.00		940	4.2	39.5	4	4	0	170
215	1.96		1050	3.9	41.0	4	4	0	190
216	1.98		860	4.2	36.1	4	4	0	185
217	1.94	510	930	4.6	42.8	4	4	0	180
211-217	1.94	510	946	4.3	40.5			0	182
218	2.04		870	4.5	39.2	4	4	0	190
219	2.10		1030	4.7	48.4	4	4	0	205
220	2.20		940	5.0	47.0	4	4	0	190
221	1.96		860	5.1	43.9	4	4	0	180
222	1.91		1000	5.7	57.0	4	4	0	200
223	1.99		870	5.0	43.5	4	4	0	185
224	1.91	435	970	5.2	50.4	4	4	0	165
218-224	2.02	435	934	5.0	47.1			0	188

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)
225	1.92		940	5.0	47.0	4	4	0	190
226	1.97		880	4.6	40.5	4	4	0	190
227	1.96		920	4.0	36.8	4	4	0	180
228	1.91		860	5.0	43.0	4	4	0	175
229	1.99		900	3.5	31.5	4	4	0	185
230	1.94		960	4.5	43.2	4	4	0	185
231	1.97	590	810	5.0	40.5	4	4	0	170
225-231	1.95	590	896	4.5	40.4			0	182
232	1.94		1010	4.4	44.4	4	4	0	190
233	1.99		790	4.7	37.1	4	4	0	180
234	1.91		860	4.9	42.1	4	4	0	175
235	1.93		980	4.6	45.1	4	4	0	180
236	1.84		830	2.9	24.1	4	4	0	170
237	1.98		830	4.2	34.9	4	4	0	180
238	1.99	576	950	4.6	43.7	4	4	0	190
232-238	1.94	576	893	4.3	38.8			0	181
239	2.01		830	4.3	35.7	4	4	0	175
240	2.03		860	3.9	33.5	4	4	0	180
241	2.01		930	4.4	40.9	4	4	0	190
242	1.97		960	4.5	43.2	4	4	0	170
243	2.01		540	5.4	29.2	4	4	0	180
244	1.95		920	4.2	38.6	4	4	0	180
245	2.02	652	930	4.4	40.9	4	4	0	190
239-245	2.00	652	853	4.4	37.4			0	181
246	2.03		850	4.9	41.7	4	4	0	175
247	2.01		880	5.5	48.4	4	4	0	180
248	2.02		920	5.2	47.8	4	4	0	180
249	2.00		820	4.9	40.2	4	4	0	190
250	1.92		850	5.0	42.5	4	4	0	185
251	1.92		870	4.7	40.9	4	4	0	185
252	1.94	740	860	4.4	37.8	4	4	0	185
246-252	1.98	740	864	4.9	42.8			0	173

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	
253	1.89		840	4.9	41.2	4	4	0	175	
254	1.89		900	3.2	28.8	4	4	0	190	
255	1.99		750	4.8	36.0	4	4	0	180	
256	1.91		960	4.3	41.3	4	4	0	180	
257	2.00		980	4.8	47.0	4	4	0	190	
258	1.96		860	4.7	40.4	4	4	0	190	Given new food type
259	1.93	482	570	4.9	27.9	4	4	0	125	
253-259	1.94	482	837	4.5	37.5			0	176	
260	1.96		580	4.4	25.5	4	4	0	130	
261	1.99		500	4.9	24.5	4	4	0	160	Returned to former
262	2.00		820	5.5	45.1	4	4	0	180	food
263	2.04		760	4.7	35.7	4	4	0	175	
264	1.95		920	5.0	46.0	4	4	0	185	
265	1.92		1000	4.9	49.0	4	4	0	185	
266	2.02	530	790	5.1	40.3	4	4	0	160	
260-266	1.98	530	767	4.9	38.0			0	168	
267	1.97		860	5.4	46.4	4	4	0	190	
268	1.91		880	5.1	44.9	4	4	0	180	
269	1.98		830	4.8	39.8	4	4	0	170	
270	1.96		1000	4.5	45.0	4	4	0	200	
271	1.93		830	4.1	34.0	4	4	0	160	
272	1.97	760	830	5.0	41.5	4	4	0	160	
273	1.97		850	5.2	44.2	4	4	0	200	
267-273	1.96	760	869	4.9	42.3			0	180	
274	1.89		900	4.6	41.4	4	4	0	190	
275			990	4.4	43.6	4	4	0	180	
276	1.90		730	5.5	40.2	4	4	0	185	
277			890	4.8	42.7	4	4	0	190	
278	1.84		890	2.6	23.1	4	4	0	150	
279		440	690	5.0	34.5	4	2	0	150	
280	1.87		790	4.9	38.7	4	4	0	180	
274-280	1.88	440	840	4.5	32.0			0	175	

Number :— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)
281	1.80		820	5.7	46.7	4	4	0	185
282	1.75		740	5.6	41.4	4	3	0	170
283	1.81		700	5.4	37.8	4	3	0	170
284	1.77		900	5.5	49.5	4	4	0	160
285	1.80		800	4.2	33.6	4	2	0	225
286	1.81		850	5.0	42.5	4	4	0	185
287	1.83	462	690	5.5	38.0	4	4	0	160
281-287	1.80	462	776	5.3	41.4			0	179
288	1.83		830	5.7	47.3	4	4	0	190
289	1.80		850	4.3	36.6	4	4	0	--
290	1.87		800	4.4	35.2	4	4	0	185
291	1.79		810	5.3	42.9	4	4	0	160
292	1.80	508	810	5.2	42.1	4	4	0	180
293	1.78		820	5.1	41.8	4	4	0	180
294	1.82		780	6.7	52.3	4	4	0	185
288-294	1.81	508	814	5.2	42.6			0	180
295	1.82		800	3.3	26.4	4	4	0	200
296	1.87		900	4.7	42.3	4	4	0	160
297	1.93		790	6.0	47.4	4	4	0	185
298	1.91		840	5.3	44.5	4	4	0	190
299	1.66		760	5.5	41.8	4	4	0	135
300	1.80	602	820	4.3	35.2	4	4	0	170
301	1.84		840	5.1	42.8	4	4	0	190
295-301	1.83	602	821	4.9	40.1			0	176
302	1.81		850	4.9	41.7	4	4	0	185
303	1.89		800	5.4	43.2	4	4	0	180
304			850	4.6	39.1	4	4	0	185
305	1.85		900	5.4	48.6	4	4	0	140
306			570	5.3	30.2	4	4	0	150
307	1.81	686	950	5.1	48.5	4	4	0	195
308	1.90		790	5.3	41.9	4	4	0	185
302-308	1.85	686	816	5.1	41.9			0	174

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	
309	1.83		920	4.5	41.5	4	4	0	185	
310	1.94		700	4.5	31.5	4	4	0	175	
311	1.90		920	4.5	41.5	4	4	0	---	
312	1.73		770	5.8	44.7	4	4	0	150	
313	1.81		730	5.2	38.0	4	4	0	155	
314	1.90		800	4.4	35.2	4	4	0	180	
315	1.82	574	950	4.0	38.0	4	4	0	185	
309-315	1.85	574	827	4.7	38.6			0	172	
316	1.85		760	5.1	42.5	4	4	0	165	
317	1.85		820	5.8	47.5	4	4	0	205	
318	1.82		840	4.8	40.4	4	4	0	195	
319	1.84		950	5.0	47.5	4	4	0	190	
320	1.84		880	4.1	36.1	4	4	0	180	
321	1.94	680	670	5.5	36.9	4	4	0	160	
322	1.89		790	4.3	34.0	4	4	0	170	
316-322	1.86	680	816	4.9	40.7			0	181	
323	1.95		720	5.0	36.0	4	4	0	165	
324	1.83		960	5.1	49.0	4	4	0	190	
325	1.78		740	5.8	43.0	4	4	0	165	
326	1.86		700	4.8	33.6	4	4	0	165	
327	1.81		870	5.0	43.5	4	4	0	195	
328	1.82	654	890	5.6	49.8	4	4	0	170	
329	1.84		800	4.6	36.8	4	4	0	200	
323-329	1.84	654	811	5.1	41.7			0	179	
330	1.82		880	4.8	42.2	4	4	0	140	
331	1.86		760	5.0	38.0	4	4	0	185	
332	1.80		810	5.1	41.4	4	4	0	185	
333	1.76		770	4.5	34.7	4	4	0	160	
334	1.90		710	4.4	31.2	4	4	0	170	
335	1.79	654	770	4.4	33.9	4	4	0	155	
336	1.86		700	4.8	33.6	4	4	0	165	
330-336	1.83	654	771	4.7	36.4			0	166	
337	1.86		800	4.8	38.4	4	4	0	165	Killed & autopsied.
1-337		579	878		37.6				177	See Protocol attached

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

(1) Pancreas to M.N.I. for Frozen-Dehydration by Dr. Lewis.

(2) Test Tube A + cork + 50 ccs. 40% KOH weighs 19.436 Grams
" " " " + Pancreas 19.661 "

0.225 Gram A

Test Tube B + cork + 50 ccs. 40% KOH weighs 19.028 Grams
" " " " + Pancreas 19.418

0.390 Gram B

Autopsy

(Killed by exsanguination from carotid and jugular vessels after taking samples of pancreas for frozen-dehydration and for chemical analysis of glycogen content under intravenous nembutal anesthesia.)

Gross Examination:

The right testis cannot be found; the left is small, grey and firm. The lungs appear stippled with grey on external and cut surfaces. The liver shows an exaggerated lobular pattern referable to yellow linear midzonal markings.

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: Glycogen infiltration of pancreatic centro-acinar cells, ductular epithelium and beta islet cells is apparent in extreme degree and widespread distribution. There are no evidences of cellular proliferation by mitosis.

Kidneys: Slight calcification is notable in the cortical convoluted tubules. Glycogen nephrosis is extreme.

Liver: Hepatic cells in the mid-zones show moderately severe fatty change. Scattered hepatic cells are enlarged, pale and show peripheral displacement of cytoplasm leaving a paranuclear clear area which contains a round or irregular, brightly eosinophilic, homogeneous mass.

Adrenals: Not remarkable.

Pituitary: Not remarkable.

Testis: Cellular division is noted in two or three layers of cells in the seminiferous tubules, but mature spermatocytes are not present. The interstices are exaggerated and contain loose fibrous tissue in excessive amounts.

Spleen: There are very large amounts of granular yellow pigment in the reticulo-endothelial cells.

Number:— B 98 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Lungs: Peribronchial and perivascular nodules of lymphocytes are large and numerous. Bronchial epithelial cells and other cells lying free in alveolar spaces or in contact with their septa show large numbers of acidophilic cytoplasmic inclusions. The larger bodies appear as single masses of spherical contour with a clear peripheral halo. Some of these appear vesicular rather than homogeneous.

ROYAL VICTORIA HOSPITAL

Montreal 2, Canada

UNIVERSITY CLINIC

January 21st, 1950.

Dr. Toreson,
Department of Pathology.

Dear Dr. Toreson,

Following are the results on the specimens sent to us:

(1) 1 gm. tissue contains 0.00266 gm. glycogen = 0.266%

(2) 1 gm. tissue contains 0.0025 gm. glycogen = 0.250%.

Yours sincerely,

(signed) E. H. Venning

Eleanor H. Venning, Ph.D.

EHV/KB.
Copy/GS

Number:— C 49 Male

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
0	1.96	111f							Alloxan 200. Fasting past 24 hours
1	1.99	195f	220f			2	0	0	
2	2.00	550	710	4.2	29.8	4	0	0	
3	2.05	442	760	4.8	36.5	4	0	0	
4	1.95	452f	130f			4	tr	0	Fasting past 24 hours
5	1.85f		140f			4	0	0	
6	1.93	556	600			4	0	0	
7	1.97	500	780	3.9	30.4	4	0	0	
1 - 7	1.98	512	713	4.3	32.2			0	
8	2.04	472	380	4.5	17.1	4	0	0	
9	2.00	500	330	4.5	14.9	4	0	5	
10	2.02	492	360	3.4	12.2	4	0	5	
11	2.04	526	420			4	0	5	
12	2.05	528	490	3.8	18.6	4	0	5	
13	2.00		530	4.2	22.1	4	0	5	
14	2.07	475	540	3.5	18.9	4	0	5	
8 - 14	2.03	499	436	3.6	17.3			4.3	
15	2.04		540	3.4	18.4	4	tr	5	
16	2.15	467	530	2.9	15.4	4	1	5	
17	2.14	500	490	3.2	15.7	4	1	5	
18	2.15	400	450	3.7	16.7	4	tr	5	
19	2.19	454	410	2.7	11.1	4	0	5	
20	2.13	443	670	2.8	18.8	4	0	5	
21	2.18	500				4	0	5	
15 - 21	2.14	456	498	3.1	16.0			5	
22	2.20	388	550	3.3	18.2	4	0	5	
23	2.19	388	370	3.6	13.3	4	0	5	
24	2.24	395	360	3.4	12.2	4	0	5	
25	2.27	555	380	3.4	12.9	4	0	5	
26	2.25	523	530	4.2	22.3	4	0	5	
27	2.21	421	460	4.8	22.1	4	0	5	
28	2.31	420	450	4.7	21.2	4	0	5	
22 - 28	2.24	441	443	3.9	17.5			5	

Number:— C 49 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
29	2.30	282	580	5.1	29.6	4	0	5	
30	2.30	485	390	4.4	17.2	4	0	5	
31	2.37	450	390	3.9	15.4	4	0	5	
32	2.40	435	400	3.5	14.0	4	0	5	
33	2.36		340			4	0	5	
34	2.30	370	120	1.6	1.9	4	0	5	
35	2.33	435	220	2.0	4.4	4	0	5	
29 - 35	2.34	393	349	3.4	13.8			5	
36				3.4		4	0	0	
37	2.40	450	430	3.5	15.1	4	0	0	
38	2.20f	435f	130f			4	0	0	Fasting past 24 hours
39	2.14f		60f			3	0	0	Fasting past 48 hours
40			390	6.4	25.0	4	0	0	
41	2.30		580	4.2	24.4	4	0	0	
42	2.20	420	610	5.5	33.6	4	0	0	
36 - 42	2.30	435	503	4.6	24.5			0	
43	2.35	483	600	4.0	24.0	4	0	0	
44	2.23f	405f	410f			4	0	0	Fasting past 16 hours
45	2.37		690	3.4	23.5	4	tr	5	
46	2.41	614	720	3.3	23.8	4	tr	5	
47	2.40	555	700	4.3	30.1	4	1	5	
48	2.27	467	750	4.2	31.5	4	tr	5	
49	2.43	533	480	4.1	19.7	4	tr	5	
43 - 49	2.37	530	657	3.9	25.4			3.6	
50	2.40	520	440	2.4	10.6	4	1	5	
51	2.44	560	580	3.7	21.5	4	1	5	
52	2.47	510	550	3.9	21.5	4	0	5	
53	2.44	518	430	2.8	12.0	4	0	5	
54	2.48	510	500	3.2	16.0	4	0	5	
55	2.49	570	360	2.9	10.4	4	0	5	
56	2.48	556	480	2.6	12.5	4	0	5	Cellulitis right
50 - 56	2.46	535	477	3.1	14.9			5	

Number:— C 49 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
57	2.51	494	340	3.1	10.5	4	0	5	Penicillin 60,000 U.
58	2.51	540	370	3.8	14.1	4	0	5	Penicillin 30,000 U.
59	2.53	570	450	5.0	22.5	4	0	5	
60	2.52		340	4.4	15.0	4	0	5	
61	2.57	528	480	4.0	19.2	4	0	5	
62	2.57		400	4.6	18.4	4	0	5	
63	2.59	540	390	3.8	14.8	4	0	5	
57 - 63	2.54	534	396	4.1	16.4			5	
64	2.55		160	2.5	4.0	4	0	5	Severe diarrhoea
65	2.62		160	2.2	3.5	4	0	5	Slight diarrhoea
66	2.59		250	3.1	7.8	4	0	5	No diarrhoea
67	2.60		130	3.1	4.0	4	0	5	
68	2.61	496	270	2.8	7.6	4	0	5	
69	2.60		340	3.2	10.9	4	0	5	
70	2.63		430	4.1	17.6	4	0	5	
64 - 70	2.60	496	249	3.0	7.9			5	
71	2.67		360	3.8	13.7	4	0	5	
72	2.62		540	4.0	21.6	4	0	5	
73	2.53f	420f	140f			4	0	5	
74			300	2.3	6.9	4	0	5	
75			510			4	0	5	
76	2.54		500	1.5	7.5	4	0	5	
77	2.60		440	2.4	10.6	4	0	5	
71 - 77	2.61	420f	442	2.8	11.7			5	
78	2.60		280			4	0	5	
79	2.56		400	3.1	12.4	4	0	5	
80	2.51f	266f	140f			4	0	5	Fasting past 16 hours
81	2.59		360	2.3	8.3	4	0	5	
82	2.62		290	3.0	8.7	4	0	5	
83	2.63		390	2.5	9.8	4	0	5	
84	2.64		350	3.0	10.5	4	0	5	
78 - 84	2.61	266f	345	2.8	9.9			5	

Number:— C 49 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
85	2.60		520	4.6	23.9	4	0	5	
86	2.69		300	3.6	10.8	4	0	5	
87	2.58f	513f	160f			4	0	5	Fasting past 16 hours
88	2.67		300	4.6	13.8	4	0	5	
89	2.68		390	4.4	17.2	4	0	5	
90	2.66		460	4.0	18.4	4	0	5	
91	2.69		330	3.9	12.9	4	0	5	
85 - 91	2.67	513f	383	4.2	16.2			5	
92	2.51f		60f	3.2f	1.9f	4	0	5	Not eating
93	2.45f		60f	0	0	0	0	0	Not eating
94	2.42f	84f	35f	0	0	0	0	0	Not eating
95	2.48		120	5.6	6.7	4	0	2.5	
96	2.51		290	5.9	17.1	4	0	2.5	
97	2.54		330	5.2	17.2	4	0	5	
98	2.58		310	4.7	14.6	4	0	5	
92 - 98	2.53	84f	263	5.3	13.9			3	
99	2.60		390	4.4	17.2	4	0	5	
100	2.57		310	3.8	11.8	4	0	5	
101	2.51f	176f	140f			4	0	5	Fasting past 16 hours
102	2.63		260	2.3	6.0	4	0	5	
103	2.63		380	3.9	14.8	4	0	5	
104	2.64		350	2.9	10.2	4	1	5	
105			290	3.5	10.2	4	1	5	
99 -105	2.61	176f	330	3.5	11.6			5	
106			500	4.7	23.5	4	1	5	
107	2.54f	433f	150f			4	0	5	Fasting past 16 hours
108	2.63		360	4.0	14.4	4	0	5	
109	2.58		410	4.4	18.0	4	0	5	
110	2.67		370	4.1	15.2	4	0	5	
111	2.68		310	3.7	11.5	4	0	5	
112	2.67		480	4.1	19.7	4	0	5	
106-112	2.65	433f	405	4.2	17.1			5	

Number:— C 49 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
113	2.70	534	310	4.6	14.3	4	0	2.5	2 units crystalline insulin i-v. Biopsy, local procaine & nembutal
114	2.57	316	70	7.1	5.0	4	0	5	
115	2.46		60	1.5	0.9	3	0	5	
116	2.44		20			2	0	0	Incision infected
117	2.45		30	6.4	1.9	4	0	0	Penicillin 50,000 U. i.-m.
118	2.46		110	8.3	9.1	4	0	5	Penicillin 50,000 U. i.-m.
119	2.48		130	4.8	6.2	4	0	5	Penicillin 25,000 U. i.-m.
113-119	2.51	425	104	5.5	6.2			3.5	
120	2.54	454	120	3.1	3.7	4	0	5	Sutures out. Penicillin 20,000 i.-m.
121			290	3.9	10.3	4	0	5	Incision draining.
122	2.51		140	3.1	4.3	4	0	5	Penicillin 35,000 U. i.-m.
123	2.52		120	0.9	1.1	3	0	5	
124	2.49		190	2.7	5.1	4	0	5	
125	2.52		200	3.0	6.0	3	0	5	
126	2.52	540	80			4	0	5	
120-126	2.52	497	163	2.8	5.1			5	
127	2.55		180	3.2	5.8	4	0	5	
128	2.51		190	2.5	4.8	4	0	0	
129	2.45		450	4.4	19.8	4	1	0	
130	2.45	454	410	3.9	16.0	4	1	0	
131	2.43		380	4.5	17.1	4	1	0	Subcut. abscess incised
132	2.41		150	5.5	8.3	4	0	0	Penicillin 30,000 local area
133	2.40	520	350	4.7	16.5	4	1	0	
127-133	2.46	487	301	4.1	12.6			0.7	

Number:— C 49 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
134	2.42		400	4.3	20.4	4	0	0	
135	2.42	482	430	5.4	23.2	4	1	0	
136	2.41		420	6.2	26.0	4	1	0	
137	2.40	474	470	5.8	27.3	4	1	0	
138	2.43		410	6.2	25.4	4	1	0	
139	2.40		440	6.3	27.7	4	1	0	
140	2.42	506	460	5.0	23.0	4	1	0	
134-140	241	487	433	5.6	24.7			0	
141	2.34		340	6.1	20.7	4	2	0	
142	2.37	448	120	5.0	6.0	4	0	0	
143	2.45		240	6.6	15.8	4	0	0	
144	2.40	460	280	4.8	13.4	4	1	0	Penicillin G.(i-m)20,000 U.
145	2.37		350	6.1	21.4	4	1	0	Penicillin G.(i-m)20,000 U.
146	2.39		350	6.2	21.7	4	1	0	Penicillin G.(i-m)20,000 U.
147	2.38	466	440	5.5	24.2	4	0	0	Penicillin G.(i-m)20,000 U.
141-147	2.38	458	303	5.8	17.6			0	
148	2.40		500	5.1	25.5	4	1	0	

Biopsy of Pancreas

There is no evidence of glycogen infiltration of islet, ductule or centro-acinar cells. The islets are of irregular shapes and sizes, comprised mostly of alpha cells, but often contain some agranular cells. No mitoses are seen.

Number:—C 49 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

(Killed by exsanguination under intravenous nembutal anesthesia.)

Gross Examination:

The root of the aorta shows slight medial calcification. Both kidneys are superficially marked by numerous small scars. The intermediate lobe of the pituitary contains a small cyst (2 mms.). There are small locules of purulent exudate beneath the surgical incision in the subcutaneous layer.

Microscopic Examination:

Pancreas: "Hydropic" vacuolar change is readily apparent in the ductular and centro-acinar cells. In the islets there is a lacy, cobweb-like appearance of the cytoplasm of the beta cells together with numerous examples of moderately vacuolated cells. All of these types contain demonstrable glycogen. There is no evidence of mitotic cellular activity.

Kidneys: There is a moderate deposition of calcium in the cortical convoluted tubules, associated with patchy areas of collagenous fibrosis. Glycogen infiltration is moderately severe.

Number:— C 49 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (cont'd.)

Liver: The striking feature never seen in any other animal of this entire work is the presence in a few widely scattered liver cell nuclei of characteristic glycogen infiltrations. Such cells are located in the midzone of the lobules.

Adrenals: The fasciculate zone is very wide and is rich in lipid.

Pituitary: There is a small "colloid" cyst in the intermediate lobe; it is lined by cuboidal and flattened epithelium. The basophiles and eosinophiles are well differentiated.

Testis: Not remarkable.

Lungs: Not remarkable.

Number :— C 50 Male

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
0	3.05f	113f						0	Fasting past 24 hours. Alloxan 200
1	3.04	133	150			2	0	0	
2	3.04	344	300	4.2	12.6	4	0	0	
3	2.80	492	300	3.0	9.0	4	4	5	+ Crystalline Z. Insulin 5 units
4	2.70	424	130	0.8	1.0	3	4	5	
5	2.62	494	60			3	0	5	
6	2.58	360	100			4	0	5	
7	2.73	330	320	6.6	21.1	4	0	5	
1 - 7	2.79	368	194	3.7	10.9			3.6	
8	2.85	446	340	3.8	12.9	4	0	5	
9	2.87	354	510	4.5	23.0	4	0	5	
10	2.99	370	460	3.3	15.2	4	0	5	
11	3.00	402	430			4	0	5	
12	3.02	374	560	3.9	21.8	4	0	5	
13	3.03	446	700	2.7	18.9	4	0	5	
14	3.04	342	620	3.9	24.2	4	0	5	
8 - 14	2.97	391	517	3.7	19.3			5	
15	3.01		520	3.6	18.7	4	tr	12.5f	
16	3.20	274	200	1.2	2.4	3	0	5	
17	3.13	470	480	3.6	17.3	4	0	5	
18	3.14	395	490	3.2	15.7	4	0	5	
19	3.15	400	640	3.6	23.0	4	0	5	
20	3.25	416	500	4.2	21.0	4	0	5	
21	3.27	417	590	5.2	30.7	4	0	5	
15 - 21	3.16	395	489	3.5	18.4			6.1	
22	3.30	408	310	2.6	8.1	4	0	5	
23	3.31	394	850	5.4	45.9	4	0	5	
24	3.32	465	770	5.3	40.8	4	0	5	
25	3.05	420	730	3.8	27.7	4	0	5	
26	3.40	434	830	6.1	50.6	4	0	5	
27	3.28	408	710	3.9	27.7	4	0	5	
28	3.42	435	510	5.6	28.6	4	0	5	
22 - 28	3.30	423	673	4.9	32.8			5	

Number:— C 50 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
29	3.44	590	970	5.7	55.3	4	0	5	
30	3.47	493	530	3.5	18.6	4	0	5	
31	3.48	468	850	5.2	44.2	4	0	5	
32	3.50	533	660	3.7	24.4	4	0	5	
33	3.50		790			4	0	5	
34	3.26	427	700	4.6	32.2	4	0	5	
35	3.54	610	780	5.2	40.6	4	0	5	
29 - 35	3.46	520	754	4.7	35.9			5	
36						4	0	5	
37	3.43	440	1050	3.9	41.0	4	2	0	
38	2.98f	430f	410f	3.2f	13.1f	4	4	10	Fasting past 24 hours.Weak
39	2.72	320	150	3.5	5.3	4	4	5	Moribund*
36 - 39	3.08	380	600	3.7	23.2			5	
1 - 39		413	538	4.0	23.4			5	

* Moribund all day; has neither eaten nor drunk. Given 10 gms. Glucose by stomach tube at 1:00 P.M.: without benefit.

Autopsy

(Killed by severing carotid and jugular vessels after a stunning blow on the neck.)

Gross Examination:

No lesions are evident in any of the viscera. The blood is extremely lipemic.

Number:— C 50 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (Cont'd.)

Microscopic Examination:

Pancreas: There is obvious glycogen infiltration of moderate degree in the ductular epithelium and the centro-acinar cells. Numerous islets composed exclusively of alpha type cells are noted. A few contain vacuolated cells in very small numbers. No mitotic figures are encountered in any type of cell.

Kidneys: Glycogen infiltration of the distal convoluted tubules is evident in moderate degree. A few calcific deposits are noted in the proximal convoluted tubules.

Liver: The central three-fourths of each hepatic lobule shows obvious accumulations of cytoplasmic glycogen. There is not any evidence of fatty change.

Number:— C 89 Male

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
	2.20								
	2.04								
	2.03								
	2.10								
	2.07								
	2.00f	129f	600f						
0	2.04f	92f	230f						Fasting past 24 hours
	2.09	111f							
1		129	410			4	0	0	
2	1.90	448	560	2.7	15.1	4	0	5	
3	1.87	416	290			4	0	5	
4	1.88	92	310	0.3	0.9	4	0	5	
5	1.87		380	0.4	1.5	4	0	0	
6	1.91	387	610	2.8	17.1	4	0	0	
7	1.75	440	840	2.7	22.7	4	1	0	
1 - 7	1.96	320	486	1.8	11.5			2.1	
8	1.91	375	900	2.6	23.4	4	3	5	
9	2.00	215	460	2.8	12.9	4	1	5	
10	2.08	295	260	1.1	2.9	4	0	5	
11	2.09	336	210	1.4	2.9	4	0	5	
12	2.17	175	190	0.7	1.3	3	0	5	Convulsions 12 noon.
13	2.10	316	200			1	0	5	8 Gms. Glucose (o)
14	2.16	256	160	0.6	1.0	3	0	5	
8 - 14	2.09	281	340	1.5	7.4			5	
15	2.22	400	280	1.9	5.3	4	0	5	
16	2.22	413	600	4.2	25.2	4	0	5	
17	2.18	382	570	3.3	18.8	4	0	5	
18	2.32	395	540	3.7	20.0	4	0	5	
19	2.37	500	680	4.1	27.9	4	0	5	
20	2.47	370	460	3.8	17.5	4	0	5	
21	2.45	393	580	4.2	24.4	4	0	5	
15 - 21	2.32	408	530	3.6	19.9			5	

Number:— C 89 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
22	2.53	408	530	3.4	18.0	4	0	5	
23	2.52		630	2.1	13.2	4	0	5	
24	2.54	420	580	4.4	25.5	4	0	5	
25	2.71	450	420	1.8	7.6	4	0	5	
26				3.2		4	0	0	
27	2.60	448	740	3.1	22.9	4	0	0	
28	2.46f	420f	430f			4	0	0	Fasting past 24 hours
22 - 28	2.60	432	580	3.0	17.4			2.9	
29	2.38f	465f	320f			4	2	5	Fasting past 48 hours
30			370	5.8	21.5	4	1	0	
31	2.45		660	5.3	35.0	4	0	0	
32	2.45	420	660	4.7	31.0	4	4	0	
33	2.46	405	770	3.9	30.0	4	4	0	
34	2.31f	420f	380f			4	4	0	Fasting past 16 hours
35	2.30		800	3.2	25.6	4	4	5	
29 - 35	2.42	412	652	4.6	28.6			1.4	
36	2.58	550	670	4.6	31.0	4	3	5	
37	2.67	502	550	3.9	21.5	4	1	5	
38	2.50	544	830	2.7	22.4	4	0	5	
39	2.62	544	730	2.6	19.0	4	0	5	
40	2.40	494	1280	2.4	30.7	4	tr	5	
41	2.50	596	1250	3.1	38.8	4	4	5	
42	2.78	576	690	3.5	24.2	4	4	5	
36 - 42	2.58	544	857	3.2	26.8			5	
43	2.79	616	630	2.6	16.4	4	0	5	
44	2.80	596	850	2.8	23.8	4	0	5	
45	2.91	740	630	2.3	14.5	4	0	5	
46	2.98	500	400	2.6	10.4	4	0	5	
47	2.99	474	520	2.5	13.0	4	0	0	
48	2.79	700	1350	3.3	44.6	4	0	5	
49	3.05		760	3.7	28.1	4	0	5	
43 - 49	2.91	604	734	2.8	21.5			5	

Number :— C 89 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
50	3.01		1000	3.5	35.0	4	0	5	
51	3.11	574	1050	3.1	32.6	4	0	5	
52	3.14		840	3.7	31.1	4	0	5	
53	3.31	492	610	3.3	20.1	4	0	5	
54	3.26		890	2.8	24.9	4	0	5	
55	3.28		630	2.9	18.3	4	0	5	
56	3.33		780	2.8	21.8	4	0	5	
50 - 56	3.21	533	829	3.2	26.3			5	
57	3.39		610	2.5	15.3	4	0	5	
58	3.42	480	680	2.7	18.4	4	0	5	
59	3.34		500	2.3	11.5	4	0	5	
60	3.50		640	3.3	21.1	4	0	5	
61	3.50		570	2.5	14.3	4	0	5	
62	3.57		850	2.8	23.8	4	0	5	
63	3.42f	400f	280f			4	0	5	Fasting past 16 hours
57 - 63	3.45	480	642	2.7	17.4			5	
64			490	1.4	6.9	4	0	5	
65			580			4	0	5	
66	3.53		900	1.6	14.4	4	0	5	
67	3.72		560	1.9	10.6	4	0	5	
68	3.62		750	1.2	9.0	4	0	5	
69	3.70		920	2.5	23.0	4	0	5	
70	3.67f	474f	460f			4	0	5	Fasting past 16 hours
64 - 70	3.64	474f	700	1.7	12.8			5	
71	3.70		980	2.3	22.5	4	0	5	
72	3.64		1030	2.3	23.7	4	0	5	
73	3.66		700	2.3	16.1	4	0	5	
74	3.87		510	2.0	10.2	4	0	5	
75	3.90		1320	3.5	46.2	4	0	5	
76	3.66		1120	3.6	40.3	4	0	5	
77	3.92f	523f	360f			4	0	5	Fasting past 16 hours
71 - 77	3.74	523f	943	2.7	26.5			5	

Number:— C 89 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
78	4.05		1200	3.2	38.4	4	0	5	
79	4.03		1120	3.4	38.1	4	0	5	
80	4.03		640	2.4	15.4	4	0	5	
81	4.00		700	3.1	21.7	4	0	5	
82	3.89		940	3.1	29.1	4	0	5	
83	4.02		770	3.0	23.1	4	0	5	
84	4.06f	320f	130f			4	0	5	Fasting past 16 hours
78 - 84	4.00	320f	895	3.0	27.6			5	
85	3.93		930	2.9	27.0	4	0	5	
86	4.00		990	3.5	34.7	4	0	5	
87	4.00		730	3.6	26.3	4	0	5	
88	4.08		970	3.8	36.9	4	0	5	
89	4.12		620	2.7	16.7	4	0	5	
90	4.07		650	4.0	26.0	4	0	5	
91	4.19f	425f	30f			4	0	5	Fasting past 16 hours
85 - 91	4.03	425f	815	3.4	27.9			5	
92	4.13		810	2.5	20.3	4	0	5	
93	4.04		810	2.7	21.9	4	0	5	
94	4.13		600	2.7	16.2	4	0	5	
95			920	3.4	31.3	4	1	5	
96			600	3.4	20.4	4	1	5	
97	4.25f	483f	160f			4	1	5	Fasting past 16 hours
98	4.07		1120	2.3	25.8	4	0	5	
92 - 98	4.09	483f	810	2.8	22.7			5	
99	4.16		850	3.6	30.6	4	0	5	
100	4.11		970	3.0	29.1	4	0	5	
101	4.10		730	2.0	14.6	4	0	5	
102	4.17		870	2.9	25.2	4	0	5	
103	4.20	560	870	3.3	28.7	4	0	2.5	2 U. crystalline insulin i.v. Biopsy local procaine & nembutal
99 - 103	4.15	560	858	3.0	25.6			5	

Number:— C 89 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
104	4.03	194	80	3.3	2.6	4	0	5	
105	3.96		260	1.1	2.9	4	0	5	
106	4.02		400	2.4	9.6	4	0	5	
107	3.94		770	4.1	31.6	4	1	5	
108	4.02		300	2.7	8.1	4	tr	5	
109	4.02		400	2.8	11.2	4	1	5	Incision infected
110	3.90	320	100	0.4	0.4	2	0	5	Sutures out. Penicillin
111			280			4	0	5	50,000 U.
112	3.99		450	3.6	16.2	4	0	5	Penicillin 35,000 U. i-m.
104-112	3.99	257	487	2.6	10.3			5	
113	4.02		400	3.5	14.0	4	0	5	
114	3.94		600	3.5	21.0	4	0	5	
115	3.99		390	3.5	20.7	4	1	5	
116	4.07	380	150			4	0	5	
117	4.06		560	2.3	12.9	4	0	5	
118	4.04		640	2.9	18.6	4	0	0	
119	3.93f		1490f	4.2f	62.6f	4	0	0	
113-119	4.02	380	490	3.1	15.4			3.6	
120	3.91	598	1280	3.3	42.1	4	1	0	
121	3.77		1170	3.8	44.5	4	1	0	
122	3.33		550	3.0	16.5	4	4	5	
123	3.34	620	350	2.1	7.4	4	4	2.5	Penicillin 25,000 U. i-m.
124	3.69		400	2.9	11.6	4	0	0	
125	3.67	602	1000	4.9	49.0	4	0	0	Penicillin 25,000 U. i-m.
126	3.42		1350	3.8	51.3	4	4	1.25	
120-126	3.58	607	763	3.4	31.8			1.25	
127	3.53	588	1130	4.0	45.2	4	4	1.25	
128	3.59		1190	3.8	45.2	4	1	0	
129	3.55		1080	4.5	48.6	4	4	0	
130	3.29	604	950	2.9	27.6	4	4	1.25	
131	3.28		450	4.6	20.7	4	4	1.25	
132	3.41	606	850	3.3	28.1	4	0	1.25	
133	3.45		540	3.9	22.1	4	3	1.25	
127-133	3.44	599	884	3.9	33.9			0.9	

Number:— C 89 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
134	3.54	620	490	3.9	19.1	4	2	1.25	Penicillin G (i-m)20,000 U.
135	3.58		720	4.0	28.8	4	1	0.0	Penicillin G (i-m)20,000 U.
136	3.24		1270	4.7	59.7	4	1	0.0	Penicillin G (i-m)20,000 U.
137	3.27	606	1000	5.4	54.0	4	3	0.0	Penicillin G (i-m)20,000 U.
138	3.25		1470	4.5	66.2	4	2	0.0	
134-138	3.27	613	990	4.5	45.6			0.3	

Biopsy of Pancreas

No vacuolar change is recognizable in any type of cell. Glycogen is not demonstrable. The beta cells of the islet are devoid of demonstrable granules; these cells are present in very scant numbers. Most islets are made up exclusively of alpha cells. No mitotic activity is evident.

Autopsy

(Killed by exsanguination under intravenous nembutal anesthesia.)

Gross Examination:

Two small subcutaneous abscesses are present in the line of surgical incision. Both kidneys are large and pale; they are uniformly streaked with linear pale yellow lines in their cortices as seen on section.

Number:— C 89 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (Cont'd.)

Microscopic Examination:

Pancreas: There is widespread glycogen infiltration of ductular epithelium and centro-acinar cells. The majority of islets have weird, sinuous forms; alpha types comprise all but a few of the islet cells. Agranular islet cells are numerous. On searching, numerous moderately swollen islet cells with vacuolar cytoplasm are readily apparent, but only one or two can be found in most islets. No mitoses are evident in any cell type.

Kidneys: There is extreme calcification of renal proximal convoluted tubules and of Henle's loop. Glycogen is present in the proximal tubules in abundance.

Liver: Neither fat nor glycogen are evident in the liver cells.

Adrenals: The widened cortex is rich in lipid. A single mitotic figure is found in a medullary cell.

Pituitary: Both basophiles and acidophiles are well differentiated by Mann's stain.

Testis: Not remarkable.

Spleen: Not remarkable.

Lungs: Not remarkable.

Number:— C 91 Male

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
	2.65								Received
	2.62		380						
	2.63		400						
	2.64		300						
	2.69		170						
	2.45f	107f	640f						Fasting past 24 hours
0	2.45f	102f	520f						Fasting past 48 hours. Alloxan 150
	2.65	105f	313						
1		154f	220f			4	0	0	
2	2.47	506	740	3.0	22.2	4	0	5	
3	2.46	290	630	2.2	13.9	4	0	5	
4	2.45	220	460	2.1	9.7	4	0	5	
5	2.47		380	1.9	7.2	4	0	5	
6	2.50	382	680	1.9	12.9	4	tr	0	
7	2.46	422	770	3.2	24.6	4	tr	0	
1 - 7	2.47	364	610	2.4	15.1			2.9	
8	2.44	360	610	3.6	22.0	4	4	5	
9		325	420	3.3	13.9	4	4	5	
10	2.52	300	420	3.8	16.0	4	0	5	
11	2.57	435	500	4.6	23.0	4	0	5	
12	2.65	153				4	0	5	
13	2.63	348	400	3.9	15.6	4	0	5	
14	2.62	383	430	3.6	15.5	4	0	5	
8 - 14	2.57	329	463	3.8	17.7			5	
15	2.66	475	350	3.8	13.3	4	0	5	
16	2.70	365	600	5.4	32.4	4	0	5	
17	2.65	435	430	6.2	26.7	4	0	5	
18	2.60	360	190	4.2	8.0	4	0	5	
19	2.70	408	170	6.5	11.1	4	0	5	
20	2.58	380	380	1.9	7.2	4	0	5	
21	2.57	88	130	5.9	7.7	4	0	5	
15 - 21	2.64	359	321	4.8	15.2			5	

Number:— C 91 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
22	2.58	68	190	3.5	6.7	4	0	5	
23	2.68		60			2	0	2	
24	2.50	450	330	3.9	12.9	4	0	0	
25	2.43	533	350	4.6	16.1	4	0	0	
26				4.1		4	0	0	
27	2.42	328	330	3.9	12.9	4	0	0	
28	2.34f	430f	90f			4	0	0	Fasting past 24 hours
22 - 28	2.52	345	252	4.0	12.3			1	
29	2.25f	265f	160f			3	0	5	Fasting past 48 hours
30			240			2	0	0	
31	2.23		120			1	0	0	
32	2.23	360	280	4.0	11.2	4	0	0	
33	2.20	386	380	3.5	13.3	4	0	0	
34	1.94f	190f	220f			4	0	0	Fasting past 16 hours
35	2.02		390	3.6	14.0	4	0	5	(1)
29 - 35	2.17	373	282	3.7	12.8			1.4	
36	1.92					4	0		(2)

(1) 4:30 P.M.:- Incised large abscess medial aspect right foreleg: 100 ccs. thick, odorless pus.

(2) 8:00 A.M.:- Found dead. Refrigerated until 11:00 A.M.

Autopsy

Complete autopsy disclosed only the "abscess" which was really the scapulo-humeral joint.

No fatty tissue was seen in any depot.

Pancreas and spleen appeared partially autolysed.

Number:— C 91 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: Despite early autolytic changes apparent in the acinar cells and in the alpha type islet cells, it can be readily ascertained that no vacuolar cytoplasmic abnormality is present in the beta islet cells or in the ductular epithelium. Glycogen stains of this tissue are negative. No evidence of proliferation of ductular cells to form new islet and acinar elements is apparent.

Kidneys: There are small amounts of demonstrable glycogen in the cytoplasm of cells in Henle's loops. The convoluted tubules show hyaline droplets in their cytoplasm.

Liver: No glycogen is demonstrable in the hepatic cell cytoplasm or nuclei. Fatty change is not apparent.

Adrenals: Not remarkable.

Testes: Not remarkable.

Number:—D 48 Female

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
	1.84f								Fasting past 24 hours.
0	1.90f	137f	220f						Fasting past 48 hours. Alloxan 200
1	1.90	205	190			4	0	0	
2	1.85	434	360			4	tr	0	
3	1.73f	395f	510f			4	4	0	Fasting past 16 hours
4	2.00		530			4	4	5	
5	1.82	557	520	5.8	30.2	4	0	5	
6	1.87	435	530	3.6	19.1	4	0	0	
7				4.1		4	0	0	
1 - 7	1.89	408	426	4.5	24.7			1.43	
8	1.87	486	770	4.3	33.1	4	1	0	
9	1.77f	380f	560f			3	1	0	Fasting past 24 hours
10	1.64f	118f	340f			1	1	5	
11			130	3.4	4.4	1	1	0	
12	1.74		480	6.8	32.6	4	0	0	
13	1.66	420	700	7.3	51.1	4	4	0	
14	1.78	384	780	3.6	28.1	4	4	0	
8 - 14	1.73	430	572	5.1	29.9			0.7	
15	1.73f	382f	350f			4	4	0	Fasting past 16 hours
16	1.82		820	4.7	38.5	4	4	5	
17	1.87	630	740	5.6	41.4	4	2	5	
18	1.86	512	820	6.0	49.2	4	3	5	
19	1.90	533	510	417	24.0	4	0	5	
20	2.03	544	390	3.3	12.9	4	0	5	
21	2.02	420	540	3.4	18.4	4	tr	5	
15- 21	1.92	528	637	4.6	30.7			4.27	
22	2.00	630	1000	4.4	44.0	4	tr	5	
23	2.04	786	480	3.8	18.2	4	0	5	
24	2.18	576	450	4.4	19.8	4	0	5	
25	2.14	465	490	3.5	17.2	4	0	5	
26	2.18	320	240	3.4	8.2	4	0	5	
27	2.30	450	400	3.3	13.2	4	0	5	
28	2.32	486	260	2.0	5.2	4	0	0	
22- 28	2.17	530	474	3.5	18.0			4.27	

Number:— D 48 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
29	2.22	560	650	6.2	40.3	4	0	5	
30	2.31		420	2.2	9.2	4	0	5	
31	2.28		550	4.6	25.3	4	0	5	
32	2.34	540	680	5.7	38.8	4	1	5	
33	2.42		590	5.3	31.3	4	0	5	
34	2.45	484	540	4.8	25.9	4	0	5	
35	2.43		730	4.7	34.3	4	0	5	
29- 35	235	528	597	4.8	29.6			5	
36	2.47		610	4.4	26.8	4	0	5	
37	2.60		400	3.6	14.4	4	0	5	
38	2.59		480	4.6	22.1	4	0	5	
39	2.58	466	550	4.5	24.8	4	1	5	
40	2.49		600	3.6	21.6	4	1	5	
41	2.63		570	4.6	26.2	4	1	5	
42	2.66		570	4.5	25.7	4	2	5	
36- 42	2.57	466	540	4.2	23.1			5	
43	2.68		700	3.2	22.4	4	1	5	
44	2.56f	484f	410f			4	0	5	Fasting past 16 hours
45			630	2.8	17.6	4	0	5	
46			640			4	0	5	
47	2.67		800	2.0	16.0	4	2	5	
48	2.83		580	3.0	17.4	4	2	5	
49	2.60		890	2.2	19.6	4	1	5	
43- 49	2.70	484f	707	2.6	18.6			5	
50	2.81		720	3.2	23.0	4	1	5	
51	2.74f	510f	450f			4	0	5	Fasting past 16 hours
52	2.84		1090	2.8	30.5	4	0	5	
53	2.91		820	3.6	29.5	4	0	5	
54	2.85		790	2.4	19.0	4	0	5	
55	2.91		580	3.8	22.0	4	1	5	
56	2.97		1170	4.6	53.8	4	0	5	
50- 56	2.88	510f	862	3.4	29.6			5	

Number:— D 48 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
57	2.94		1070	5.4	57.8	4	0	5	
58	2.83f	523f	610f			4	0	5	Fasting past 16 hours
59	3.11		840	4.5	37.8	4	0	5	
60	3.00		780	4.1	32.0	4	0	5	
61	3.05		810	4.7	38.1	4	0	5	
62	2.94		920	4.6	42.3	4	0	5	
63	3.03		1050	4.6	48.3	4	0	5	
57 - 63	3.00	523f	911	4.7	42.7			5	
64	3.21		1170	4.7	55.0	4	0	5	
65	3.04f	390f	560f			4	0	5	Fasting past 16 hours
66	3.23		600	3.2	19.2	4	0	5	
67	3.18		1130	3.8	42.9	4	0	5	
68	3.21		680	3.6	24.5	4	1	5	
69	3.28		600	3.2	19.2	4	1	5	
70	3.18		500	3.1	15.5	4	1	5	
64 - 70	3.22	390f	780	3.6	29.4			5	
71	3.19		830	4.3	35.7	4	0	5	
72	3.13f	460f	530f			4	1	5	Fasting past 16 hours
73	3.22		660	2.9	19.1	4	1	5	
74	3.19		810	3.3	26.7	4	1	5	
75	3.34		700	3.0	21.0	4	1	5	
76			890	3.7	32.9	4	0	5	
77			770	3.6	27.7	4	0	5	
71 - 77	3.24	460f	777	3.5	27.2			5	
78	3.25f	574f	600f			4	0	5	Fasting past 16 hours
79	3.38		1100	4.3	47.3	4	0	5	
80	3.40		690	3.6	24.8	4	1	5	
81	3.40		910	4.7	42.8	4	1	5	
82	3.25		760			4	1	5	
83	3.28		870	3.9	33.9	4	1	5	
84	3.35	580	950	4.4	41.8	4	1	2.5	2 u. Crystalline insulin i.v. Biopsy local procaine & nembutal.
78 - 84	3.34	580	880	4.2	38.1			5	

Number:— D 48 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
85	3.10	348	190	4.1	7.8	4	0	5	
86	3.10		450	4.3	19.4	4	0	5	
87	3.15		550	3.8	20.9	4	0	5	
88	3.22		690	4.4	30.4	4	1	5	
89	3.18		480	3.6	17.3	4	1	5	Incision infected
90	3.26		650	4.3	28.0	4	1	5	Incision drained.
91	3.26	524	850	4.1	34.9	4	1	5	Sutures out. Penicillin 60,000 U. i-m.
85 - 91	3.18	436	551	4.1	22.7			5	
92			560	4.3	24.1	4	0	5	Penicillin 50,000 U.i-m.
93	3.28		880	4.6	40.5	4	0	5	
94	3.32		940	4.5	42.3	4	0	5	Penicillin 50,000 U.i-m.
95	3.37		750	4.2	31.5	4	0	5	
96	3.32		570	3.9	22.2	4	1	5	
97	3.38	650	500			4	0	5	
98	3.44		730	4.1	29.9	4	0	5	
92 - 98	3.35	650	704	4.3	31.8			5	
99	3.40		540	3.5	18.9	4	0	10	
100	3.46		540	3.0	16.2	4	1	10	
101	3.50	580	570	2.6	14.8	4	tr	10	
102	3.53		700	5.1	35.7	4	1	10	
103	3.63		380	1.3	4.9	4	0	7.5	
104	3.56	540	390	3.0	11.7	4	0	10	
105	3.57		540	4.2	22.7	4	0	10	
99 -105	3.52	560	523	3.2	17.8			10	
106	3.59	510	350	2.2	7.7	4	0	10	
107	3.65		460	3.1	14.3	4	0	10	
108	3.67	484	560	3.3	18.5	4	tr	10	
109	3.76		350	2.9	10.2	4	tr	10	
110	3.61		540	3.8	20.5	4	0	10	
111	3.70	224	330	1.2	4.0	4	0	10	
112	3.68		560	3.9	21.8	4	0	10	
106-112	3.67	406	450	2.9	13.9			10	

Number:— D 48 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)
113	3.72	340	250	0.9	2.3	4	0	10.0
114	3.74		320	2.6	8.3	4	0	10.0
115	3.75	236	230	0.5	1.2	3	0	10.0
116	3.79		190	0.0	0.0	1	0	7.5
117	3.78		450	1.9	8.6	4	0	5.0
118	3.77	424	580	3.8	22.0	4	0	7.5
119	3.77		610	2.9	17.7	4	0	
113-119	3.79	333	379	1.8	8.6			8.3

Biopsy of Pancreas

There is diffuse severe glycogen infiltration of the ductular epithelium, centro-acinar cells and islets. Islets are numerous and composed of abundant alpha cells and small numbers of glycogen-filled cells. No mitotic activity is apparent.

Autopsy

(Killed by exsanguination under intravenous nembutal anesthesia.)

Gross Examination:

The operative field is clean and well healed. No gross lesions are encountered except a mild degree of medial calcification in the root of the ascending aorta.

Number:— D 48 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (Cont'd.)

Microscopic Examination:

Pancreas: Although moderately severe glycogen infiltration is obvious in the ductular epithelium, the islets are notable for a large component of agranular, pallid, non-vacuolated cells; none of these contains demonstrable beta granules. There is no evidence of mitotic activity.

Kidneys: Calcification is apparent in mild degree in the convoluted tubules. Glycogen is readily demonstrable in moderate amounts in the distal convoluted tubules.

Liver: Cytoplasmic glycogen is abundant in the liver cells of the central two-thirds to three-fourths of the lobules.

Adrenals: These glands are characterized by very wide cortices rich in lipid.

Ovary: Not remarkable.

Pituitary: There are many well differentiated granules in acidophile and basophile cells. The overall dimensions of the anterior lobe are large.

Lungs: Not remarkable.

Spleen: Not remarkable.

Number:— D 73 Female -

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
	2.53	138	100			0	0		
	2.53	110	90			0	0		
	2.38f	118f	80f			0	0		Glucose tolerance
	2.50		120			tr	0		
	2.52		130			0	0		
	2.54		100			0	0		
			90			0	0		
	2.52	124	105						
	2.63		100			0	0		
	2.50		60			0	0		
	2.74		200			0	0		
	2.75	147	140			0	0		
	2.60f	89f	210f			0	0		Fasting past 16 hrs.
	2.79		90			1	0		
	2.80		170			1	0		
	2.70	147	127						
	2.72		150			0	0		
	2.76		170			0	0		
	2.73	98	140			1	0		
	2.73		180			0	0		
	2.74	98	160						
1	2.70		90			3	0	0	
2	2.64	340	200	2.3	4.6	4	0	0	
3	2.64	460	410	4.1	16.8	4	0	0	
4	2.61	420	650	3.8	24.7	4	1	0	
5	2.65	394	580	3.9	22.6	4	2	0	
6	2.55f	356f	380f			4	0	0	Fasting past 16 hrs.
7	2.60		700	3.2	22.4	4	0	0	
1 - 7	2.64	404	438	3.5	18.2				

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed/24 hours Grams	
8			600	3.3	19.8	4	0	0		
9	2.61	396	870	3.0	26.1	4	1	0		
10	2.46		710	2.2	15.6	4	1	0		
11	2.65		710	3.0	21.3	4	0	0		
12	2.61		640	3.2	20.5	4	1	0		
13	2.62		1150	2.9	33.4	4	1	0		
14	2.49f	264f	530f			4	0	0		Fasting past 16 hrs.
8 - 14	2.59	396	780	2.9	22.8					
15			690	2.0	13.8	4	0	0		
16			550			4	0	0		
17			980	1.5	14.7	4	0	0		
18	2.63		660	2.4	15.8	4	1	0		
19	2.58		870	1.6	13.9	4	1	0		
20	2.64		740	2.6	19.2	4	0	0		(Urine choline 2.0 g. per min.)
21	2.54f	236f	320f			4	0	0		Fasting past 16 hrs.
15 - 21	2.62	236f	748	2.0	17.5					
22	2.65		550	2.8	15.4	4	1	0		
23	2.64		860	2.9	24.9	4	1	0		
24	2.60		680	2.0	13.6	4	1	0		(Mated with #C49)
25	2.64		640	3.0	19.2	4	1	0		
26	2.66		890	3.2	28.5	4	1	0		
27	2.69		890	4.1	36.5	4	0	0		
28	2.62f	227f	430f			4	0	0		Glucose Tolerance
22 - 28	2.65	227f	752	3.0	23.0					
29	2.70		810	3.3	26.7	4	0	0		
30	2.72		830	3.1	25.7	4	1	0	215	
31	2.75		980	3.9	38.2	4	1	0	235	
32	2.72		910	4.1	37.3	4	0	0	235	
33	2.74		580	3.5	20.3	4	0	0	180	
34	2.79		320	3.2	10.2	4	0	0	145	
35	2.71f	180f	140f			4	0	0	40f	Fasting past 16 hrs.
29 - 35	2.74	180f	738	3.5	26.4				205	

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed/24 hours Grams	Antabus Grams (0)	
36	2.71		430	4.1	17.6	4	0	0	165		
37	2.84		630	3.8	23.9	4	0	0	200		
38	2.82		660	4.3	28.4	4	0	0	195		
39	2.82		560	3.9	21.8	4	0	0	170	0.125	
40	2.86		750	3.6	27.0	4	0	0	215	0.125	
41	2.88		690	4.3	29.7	4	0	0	165	0.125	
42	2.85	380	450	4.5	20.3	4	0	0	160	0.125	Purulent nasal discharge
36 -42	2.83	380	596	4.1	24.1				181	0.07	
43	2.83		510	4.3	21.9	4	0	0	125	0.125	
44	2.81		220	2.4	5.3	4	0	0	75	0.125	
45	2.80		170	0.0	0.0	tr	0	0	35	0.125	
46			80	0.2	0.2	1	0	0	15	0.125	
47			220	0.0	0.0	0	0	0	35	0.125	
48	2.92	294	250	1.0	2.5	3	0	0	195	0.125	
49	2.84		460	3.9	17.9	4	0	0	300	0.125	
43 -49	2.85	294	316	2.4	9.6				111	0.125	
50	2.90		360	3.2	11.5	4	0	0	185	0.125	
51	2.93		500	3.9	19.5	4	0	0	200	0.125	
52	2.96		540	3.2	17.3	4	0	0	175	0.125	
53	2.92		330	3.0	9.9	4	0	0	130	0.125	
54	2.90		70f	2.7f	1.9f	4	0	0	65	0.125	Bore 6 living young.
55	2.71		470	2.4	11.3	4	0	0	145	0.125	
56	2.74	436	730	2.9	21.2	4	0	0	205	0.125	
50 -56	2.87	436	476	3.1	13.4				158	0.125	
57	2.80		500			4	0	0	170	0.125	
58	2.81		560	3.4	19.0	4	0	0	225	0.125	1 of young dead
59	2.73		520	3.5	18.2	4	0	0	205	0.125	
60	2.85		550	2.4	13.2	4	0	0	240	0.125	
61	2.88	660	360	2.8	10.1	4	0	0	220	0.125	
62			430	3.5	15.1	4	0	0	270	0.125	
63	2.96		810	3.4	27.5	4	0	0	300	0.125	
57 -63	2.84	660	533	3.2	17.2				233	0.125	

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"		Protamine Zinc Insulin (units, s.c.)	Food consumed/24 hours Grams	Antabus Grams (0)	
64	2.98		770	3.4	26.2	4	0	0		310	0.125	
65	2.95		730	3.1	22.6	4	0	0		400	0.125	(Eyes open)
66	2.90		490	3.0	14.7	4	0	0		270	0.125	
67	2.86f	130f	550f			4	0	0		65f	Disc'd. Glucose	
68	3.04		650	3.0	19.5	4	0	0		330	Tolerance	
69	2.99		300	2.6	7.8	4	0	0		315	(New large cage)	
70	2.97	292	210	2.9	6.1	4	1	0		220		
64 -70	2.97	292	525	3.0	17.8					308	0.054	
71	3.02		90	2.7	2.4	4	0	0		300		
72	3.02		200	2.3	4.6	4	tr	0		240		
73	3.02		170	0.5	0.9	3	0	0		290		
74	3.02		130	0.6	0.8	3	0	0		190		
75	3.03	157	150	0.7	1.1	2	0	0		305		
76	2.97		70	0.0	0.0	0	0	0		225		
77	2.90		140	0.0	0.0	0	0	0		140		
71 -77	3.00	157	136	0.1	1.4					241		
78	2.88		90	0.0	0.0	0	0	0		150		
79	2.90		100	0.0	0.0	0	0	0		240		
80			140	0.0	0.0	2	0	0		270	Returned to own cage.	
81		130f	130f			1	0	0		( )	Fasting p.16 hrs.	
82	2.91		280	0.2	0.6	3	0	0		270		
83	2.83f	95f	430	0.4	1.7	3	0	0		( )	Fasting p.16 hrs.	
84	2.89		420	0.7	2.9	3	0	0		270		
78 -84	2.90	113f	243	0.2	0.9			0		240		
85	2.81		340	0.7	2.4	4	0	0		140		
86	2.80		430	2.3	9.9	4	0	0		150		
87	2.76		340	3.3	11.2	4	0	0				
88	2.69	304	130	1.7	2.2	4	0	0				
89	2.70		240	0.2	0.5	3	0	0		85		
90	2.64		90	0.0	0.0	0	0	0		20	Mated ♂ D75	
91	2.62		120	0.0	0.0	0	0	0		20		
85 -91	2.72	304	241	1.2	3.7					83		

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	
92	2.58		95	0.0	0.0	0	0	0	10	
93	2.56		160	0.0	0.0	0	0	0	20	
94	2.55		140	0.0	0.0	0	0	0	25	
95	2.53		240	0.0	0.0	0	0	0	80	
96	2.58	242	250	0.7	1.8	3	0	0	140	
97	2.59		250	1.2	3.0	4	0	0	170	
98	2.67		280	1.5	4.2	4	0	0	115	
92 -98	2.58	242	202	0.5	1.3				80	
99	2.71		330	2.3	7.6	4	0	0	170	
100	2.68		380	2.3	8.7	4	0	0	180	
101	2.73		420	2.7	11.3	4	0	0	195	
102	2.77		220	2.4	5.3	4	0	0	185	
103	2.83	248	380	2.0	7.6	4	0	0	185	
104	2.84		330	2.3	7.6	4	0	0	185	
105	2.83		520	2.1	10.9	4	0	0	195	
99-105	2.77	248	369	2.3	8.4				185	
106	2.87		360	1.3	4.7	4	0	0	140	
107	2.87		200	2.2	4.4	4	0	0	200	
108	2.86		330	1.2	4.0	4	0	0	180	
109	2.97		220	1.7	3.7	4	0	0	165	
110	2.97	152	210	1.7	3.6	4	0	0	135	
111	3.10		250	0.7	1.8	4	0	0	145	
112	3.30		230	1.4	3.2	4	0	0	130	
106-112	2.99	152	257	1.5	3.6				157	
113	3.06		220	0.6	1.3	4	0	0	165	
114	3.08		270	0.2	0.5	3	0	0	130	
115	3.08		230	0.0	0.0	0	0	0	135	
116	3.13		220	0.0	0.0	0	0	0	130	
117	3.11	113	240	0.0	0.0	0	0	0	115	
118	2.69		20	0.0	0.0	0	0	0	40	7 Rabbits born;2 dead
119	2.63		60	0.0	0.0	0	0	0	300	2 young surviving
113-119	2.97	113	180	0.1	0.3				145	

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	
120	2.75		140	0.0	0.0	0	0	0	125	
121	2.73		230	1.1	2.5	3	0	0	160	
122	2.75		210	1.6	3.4	3	0	0	170	
123	2.78		240	1.9	4.6	3	0	0	170	
124	2.73	179	320	2.2	7.0	4	0	0	190	
125	2.77		330	3.0	9.9	4	0	0	210	
126	2.79		270	2.5	6.8	4	0	0	210	
120-126	2.76	179	249	1.8	4.9				176	
127	2.82		280	2.0	5.6	4	0	0	210	
128	2.83		390	2.5	9.8	4	0	0	245	
129	2.84		360	2.0	7.2	3	0	0	220	
130	2.84		390	2.4	9.4	4	0	0	225	
131	2.87	138	360	2.5	9.0	4	0	0	260	
132	2.89		310	1.5	4.7	4	0	0	250	
133	2.87		390	2.5	9.8	4	0	0	270	
127-133	2.85	138	354	2.2	7.9				240	
134	2.94		320	1.6	5.1	4	0	0	260	
135	2.93		390	2.0	7.8	4	0	0	260	
136	2.92		230	1.8	4.1	4	0	0	190	
137	2.94		270	1.9	5.1	4	0	0	230	
138	2.92	181	460	2.6	12.0	4	0	0	290	
139	2.97		400	2.4	9.6	4	0	0	315	
140	2.99		330	2.7	8.9	4	0	0	305	
134-140	2.94	181	343	2.1	7.5			0	264	
141	3.10		320	2.2	7.1	4	0	0	420	
142	3.04		380	2.2	8.4	4	0	0	340	
143	3.15		290	1.6	4.6	4	0	0	340	
144	3.03		260	1.6	4.2	4	0	0	335	Young removed from cage
145	3.05	145	100	1.6	1.6	3	0	0	220	
146	3.07		280	3.3	9.2	4	0	0	235	
147	3.07		420	2.2	9.2	4	0	0	205	
141-147	3.07	145	293	2.1	6.7			0	299	

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed Grams/24 hours
148	3.05		340	2.3	7.8	4	0	0	150
149	3.02		160	0.0	0.0	0	0	0	90
150	2.89		140	0.0	0.0	0	0	0	35
151	2.83		80	0.0	0.0	0	0	0	35
152	2.81	92	140	0.0	0.0	0	0	0	30
153	2.80		180	0.0	0.0	0	0	0	100
154	2.75		110	0.0	0.0	0	0	0	90
148-154	2.88	92	164	0.3	1.1			0	76
155	2.80		130	0.0	0.0	0	0	0	100
156	2.83		140	0.2	0.3	2	0	0	120
157	2.84		160	0.7	1.1	4	0	0	135
158	2.88		170	0.7	1.2	4	0	0	150
159	2.90	140	190	0.1	0.2	4	0	0	150
160	2.91		210	0.3	0.6	4	0	0	155
161	2.92		210	0.4	0.8	4	0	0	155
155-161	2.87	140	173	0.3	0.6			0	138
162	2.96		200	1.6	3.2	4	0	0	155
163	2.98		230	0.9	2.1	4	0	0	160
164	2.95		180	0.5	0.9	2	0	0	130
165	3.05		190	0.0	0.0	2	0	0	170
166	3.00	98	120	0.6	0.7	2	0	0	130
167	3.02		230	0.9	2.1	3	0	0	155
168	3.03		240	0.9	2.2	4	0	0	160
162-168	3.00	98	199	0.8	1.6			0	151
169	2.99		160	0.0	0.0	0	0	0	90
170	2.99		160	0.0	0.0	0	0	0	120
171	2.93		140	0.0	0.0	0	0	0	120
172	2.99		110	0.0	0.0	2	0	0	120
173	2.99	220	130	0.0	0.0	2	0	0	140
174	3.00		130	0.0	0.0	1	0	0	120
175	3.05		160	0.3	0.5	2	0	0	145
169-175	2.99	220	141	0.0	0.1			0	122

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed Grams/24 hours	
176	3.00		140	0.0	0.0	2	0	0	120	
177	3.04		150	0.0	0.0	2	0	0	110	
178	3.05		140	0.0	0.0	1	0	0	110	New type of food
179	3.05		150	0.0	0.0	0	0	0	110	
180	3.04	67	140	0.0	0.0	0	0	0	100	
181	2.96		140	0.0	0.0	0	0	0	10	Returned to former
182	2.91		80	0.0	0.0	0	0	0	50	food
176-182	3.01	67	134	0.0	0.0			0	87	
183	3.00		180	1.1	2.0	3	0	0	100	
184	2.88		180	4.3	7.7	4	0	0	180	
185	2.95		300	1.6	4.8	4	0	0	135	
186	2.97		340	3.1	10.5	4	0	0	140	
187	3.01		380	4.6	17.5	4	0	0	180	
188	3.01		330	2.4	7.9	4	0	0	160	
189	3.02	305	320	2.0	6.4	4	0	0	135	
183-189	2.98	305	290	2.7	8.1			0	147	
190	3.02		220	1.7	3.7	3	0	0	125	
191	3.03		230	0.8	1.8	3	0	0	130	
192	3.06		240	1.7	4.1	4	1	0	160	
193	3.05	236	400	1.5	6.0	4	1	0	150	
194	3.06		120	0.0	0.0	2	0	0	80	
195	3.04		270	0.0	0.0	2	0	0	135	
196	3.05		230	1.3	3.0	4	0	0	100	
190-196	3.04	236	244	1.0	2.7			0	126	
197	3.07		210	0.0	0.0	2	0	0	130	
198			150	0.0	0.0	2	0	0	135	
199	3.04		190	0.0	0.0	1	0	0	125	
200		155	200	0.3	0.6	2	0	0	125	
201	3.09		240	2.4	5.8	4	0	0	180	
202			190	0.2	0.4	2	0	0	130	
203	3.00		150	0.3	0.5	3	0	0	100	
197-203	3.05	155	190	0.5	1.0			0	132	

Number:— **D 73 Female - continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	Crude Alkaline APE, ccs. (5.5 ccs.= 1 Gm. Equiv.)	
204	3.07		160	0.6	1.0	3	0	0	125		
205	3.05		170	1.2	2.0	4	0	0	120		
206	3.09		150	0.0	0.0	2	0	0	120		
207	3.10		210	1.4	2.9	3	0	0	140		
208	3.13	155	190	1.7	3.2	4	0	0	155		
209	3.13		180	1.3	2.3	4	0	0	135		
210	3.14		170	0.0	0.0	0	0	0	105		
204-210	3.10	155	176	0.9	1.6			0	129		
211	3.16		180	0.0	0.0	0	0	0	135		
212	3.15	1	170	0.0	0.0	4	0	0	120		
213	3.15		170	0.0	0.0	2	0	0	115		
214	-		-	-	-	-	-	-	---		
215	3.14	140	180	0.0	0.0	2	0	0	120		
216	3.15		200	0.8	1.6	3	0	0	135		
217	3.14		200	0.0	0.0	2	0	0	115		
211-217	3.15	140	183	0.3	0.5			0	123		
218	3.13f		200f	0.0	0.0	0	0	0	80f		
219	3.13f		160f	0.0	0.0	1	0	0	135f		
220	3.19f		180f	0.0	0.0	3	0	0	165f		
221	3.07f	162f	200f	4.2	8.4	2	0	0	140f		
222	3.15f		200f	0.0	0.0	0	0	0	80f		
223	3.10f	106f	200f	0.0	0.0	0	0	0	110f		
224	3.09f		160f	0.0	0.0	0	0	0	75		
218-224	3.12	134f	186	0.6	1.2			0	112		
225			140	0.0	0.0	0	0	0	100f		
226	3.02f	104f	30	0.0	0.0	1	0	0	85f		
227			100f	0.0	0.0	3	0	0	110f		
228	3.08f	180f	110f	0.0	0.0	2	0	0	70f	17.0	
229	3.06f		70f	0.3	0.2	3	0	0	0f	16.8	
230	3.00f	354f	30f	1.4	0.4	4	3	0	0f	16.5	
231	2.96f		15	6.1	0.9	4	3	0	5	15.0	
225-231	3.02	213	71	1.1	0.2			0	53	16.3	
232	2.87	376	80	2.7	2.2	4	4	0	5	14.0	Purulent nasal
233	2.68		110	2.7	2.9	4	4	0	0	13.0	discharge
234	2.66		0	-	-	-	-	-	0	-	

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

(Hours after death unknown. Post mortem decomposition slight to moderate.)

Gross Examination:

The blood is extremely lipemic. The adrenals appear large and pale, weighing 0.6 and 0.7 grams. The kidneys are soft and very pale. The liver is large, soft, friable and light tan.

Microscopic Examination:

Pancreas: There are many very large and oddly shaped islets. Rarely do alpha types comprise the majority of cells in any islet. The beta cells appear pale and do not contain demonstrable granules; none is vacuolated. Glycogen is not demonstrable in any cell and mitoses are not evident.

Kidneys: There is an extreme degree of calcification of proximal convoluted tubules associated with a very abundant collagenous fibrous replacement of atrophied cortical tubules. Autolytic changes are advanced, but fatty change is widespread and severe.

Number:— D 73 Female - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (Cont'd.)

Liver: The lobular architecture is very obscure due to severe fatty change affecting all hepatic cells in all zones of the lobules.

Adrenals: Aside from unusually wide, lipid-rich cortical layers these glands are not unusual.

Number:— D 75 Male

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
	2.17	103f	720f			0	0		Fasting past 16 hrs.
	2.28	140	520			0	0		
	2.34		460			1	0		
	2.35		210			2	0		
	2.40		450			1	0		
	2.29f	73f	630f			1	0		Glucose Tolerance
0	2.34		490			2	0	0	Alloxan 200
	2.34	140	426						
1	2.39		620	1.7	10.5	4	0	0	
2	2.31	540	960	3.0	28.8	4	1	0	
3	2.27	400	980	3.0	29.4	4	0	0	
4	2.33	510	960	2.5	24.0	4	0	0	
5	2.32	450	1040	2.5	26.0	4	0	0	
6	2.29f	400f	600f			4	0	0	Fasting past 16 hrs.
7	2.30		670			4	2	0	
1 - 7	2.32	475	872	2.5	23.7				
8			760	2.4	18.2	4	1	0	
9	2.35f	196f	490f			4	2	0	Fasting past 16 hrs.
10	2.34		320			4	1	0	
11	2.32		520	1.3	6.8	4	1	0	
12	2.31	450	2.0	9.0	4	0	0		
13	2.33		520	1.5	7.8	4	0	0	
14	2.27f	150f	430f			4	0	0	Glucose Tolerance
8 - 14	2.33	173f	514	1.8	10.5				
15			420	0.9	3.8	4	0	0	
16			690			4	0	0	
17			650	1.5	9.8	4	0	0	
18	2.39		610	1.7	10.4	4	1	0	
19	2.25		680			4	1	0	
20	2.40		680	1.8	12.2	4	0	0	(Urine choline 1.0 g. per min.)
21	2.24f	186f	370f			4	0	0	Fasting past 16 hrs.
15- 21	2.35	186f	622	1.5	9.1				

Number:— D 75 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"		Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	Antabus Grams (O)	
22	2.33		500	1.6	8.0	4	0	0				
23	2.35		740	2.3	17.0	4	0	0				
24	2.38		650	1.9	12.4	4	1	0				
25	2.36		610	2.1	12.8	4	1	0				
26	2.34		690	3.0	20.0	4	0	0				
27	2.35		660	3.5	23.1	4	0	0				
28	2.30f	247f	310f			4	0	0				Glucose Tolerance
22- 28	2.35	247f	642	2.4	15.6							
29	2.36		630	3.0	18.9	4	0	0				
30	2.48		430	3.3	14.2	4	0	0		145		
31	2.37		660	2.7	17.8	4	0	0		130		
32	2.40		350	3.0	10.5	4	0	0		135		
33	2.37		460	2.9	13.3	4	0	0		145		
34	2.36		460	3.3	15.2	4	0	0		165		
35	2.27f	146f	310f			4	0	0		30f		
29- 35	2.39	146f	498	3.0	15.0					144		
36	2.36		290	2.8	8.1	4	0	0		145		
37	2.36		610	3.1	18.9	4	0	0		170		
38	2.38		520	4.0	20.8	4	0	0		145		
39	2.44		440	2.8	12.3	4	0	0		125	0.125	
40	2.45		380	3.2	12.2	4	0	0		110	0.125	
41	2.30		500	3.1	15.5	4	0	0		100	0.125	
42	2.32	278	390	2.5	9.8	4	0	0		100	0.125	
36- 42	2.37	278	447	3.1	13.9					114	0.071	
43	2.33		250	1.9	4.8	4	0	0		85	0.125	
44	2.27		200	0.8	1.6	3	0	0		45	0.125	
45	2.22		70	0.0	0.0	tr	0	0		15	0.125	
46	2.23		240	0.0	0.0	tr	0	0		55	0.125	
47			370	1.2	4.4	3	0	0		90	0.125	
48	2.31	420	500	3.1	15.5	4	0	0		155	0.125	
49	2.31		720	3.7	26.6	4	0	0		165	0.125	
43- 49	2.28	420	336	1.5	7.6					87	0.125	

Number:— D 75 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"		"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	Antabus Grams (0)	
50	2.37		670	3.0	20.1	4	0	0		160	0.125	
51	2.37		700	3.7	25.9	4	0	0		160	0.125	
52	2.31		620	3.7	22.9	4	0	0		140	0.125	
53	2.35		480	3.5	16.8	4	0	0		145	0.125	
54	2.36		670	3.7	24.8	4	0	0		150	0.125	
55	2.38		710	3.1	22.0	4	0	0		150	0.125	
56	2.27f	226f	280f			4	0	0		25f	0.125	Fasting past 16 hours
50- 56	2.36	226f	642	3.5	22.1					151	0.125	
57	2.35		680	2.3	15.6	4	0	0		175	0.125	
58	2.38		600	3.2	19.2	4	0	0		130	0.125	
59	2.31		610	3.3	20.1	4	0	0		200	0.125	
60	2.31		530	3.4	18.0	4	0	0		110	0.125	
61	2.34	340	500	3.0	15.0	4	0	0		125	0.125	
62	2.37		590	2.9	17.1	4	0	0		150	0.125	
63	2.42		620	3.3	20.5	4	0	0		150	0.125	
57 -63	2.35	340	590	3.1	17.9					149	0.125	
64	2.38		740	3.7	27.4	4	0	0		150	0.125	
65	2.37		710	3.9	27.7	4	0	0		155	0.125	
66	2.32		720	4.2	30.2	4	0	0		140	0.125	
67	2.28f	295f	280f			4	0	0		(40)	Disc.	Glucose Tolerance
68	2.36		600	4.4	26.4	4	0	0		140		
69	2.49		610	3.7	22.6	4	0	0		165		
70	2.40	454	750	3.8	28.5	4	0	0		150		
64- 70	2.39	454	688	4.0	27.1					150		
71	2.40		710	3.1	22.0	4	0	0		155		
72	2.46		620	3.2	19.8	4	0	0		140		
73	2.33		760	3.7	28.1	4	0	0		135		
74	2.41		550	3.7	20.4	4	0	0		120		
75	2.41	410	630	3.9	24.6	4	0	0		145		
76	2.43		550	3.1	17.1	4	0	0		110		
77	2.42		550	3.3	18.2	4	0	0		125		
71- 77	2.41	410	624	3.4	21.5					133		

Number:— D 75 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	
78	2.43		520	3.9	20.3	4	tr	0	130	
79	2.43		570	3.8	21.7	4	0	0	160	
80	2.41		660	3.9	25.7	4	0	0	155	
81		315f	340f			4	0	0	( )	Fasting past 16 hrs.
82	2.46		560	3.2	17.9	4	0	0	145	
83	2.37		560	3.7	20.7	4	1	0	125	
84	2.41		520	3.5	18.2	4	0	0	110	
78 - 84	2.42	315f	565	3.7	20.8			0	138	
85	2.40		330	3.2	10.6	4	1	0	100	
86	2.33		400	3.5	14.0	4	0	0	90	
87	2.35		400	4.0	16.0	4	0	0		
88	2.37	348	510	4.8	24.5	4	0	0		
89	2.45		530	4.0	21.2	4	0	0	145	
90	2.45		510	3.6	18.4	4	0	0	120	
91	2.36		620	2.8	17.4	4	0	0	105	
85 - 91	2.39	348	471	3.7	17.4			0	112	
92	2.43		420	3.6	15.1	4	0	0	120	
93	2.40		560	3.7	20.7	4	1	0	140	
94	2.41		450	2.9	13.1	4	0	0	230	
95	2.39		350	3.8	13.3	4	0	0	100	
96	2.31	358	450	3.6	16.2	4	0	0	100	
97	2.41		420	3.5	14.7	4	0	0	125	
98	2.39		520	4.0	20.8	4	0	0	120	
92 - 98	2.39	358	453	3.6	16.3			0	134	
99	2.41		510	3.3	16.8	4	0	0	140	
100	2.41		610	3.9	23.8	4	0	0	150	
101	2.44		590	4.2	24.8	4	0	0	150	
102	2.38		640	4.3	27.5	4	0	0	140	
103	2.43	406	650	2.7	17.6	4	0	0	160	
104	2.43		730	3.2	23.4	4	0	0	160	
105	2.41		610	3.5	21.4	4	0	0	145	
99 -105	2.42	406	620	3.6	22.2				149	

Number:— D 75 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)
134	2.47		530	3.8	20.1	4	0	0	150
135	2.55		650	2.7	17.4	4	1	0	150
136	2.55		720	3.6	25.9	4	1	0	135
137	2.50		660	3.2	21.1	4	0	0	170
138	2.54	390	600	4.4	26.4	4	1	0	170
139	2.48		650	4.9	31.9	4	0	0	160
140	2.53		620	4.5	27.9	4	0	0	180
134-140	2.52	390	633	3.9	23.4			0	159
141	2.58		670	3.8	25.5	4	0	0	180
142	2.53		730	4.9	35.8	4	0	0	190
143	2.47		700	4.6	32.2	4	0	0	170
144	2.46		730	4.7	34.3	4	0	0	175
145	2.44	335	650	4.8	31.2	4	0	0	155
146	2.58		700	4.4	30.8	4	1	0	250
147	2.51		790	4.3	34.0	4	1	0	170
141-147	2.51	335	710	4.5	32.0			0	184
148	2.56		640	4.1	26.2	4	tr	0	155
149	2.55		800	3.9	31.2	4	tr	0	180
150	2.60		740	2.9	21.5	4	1	0	155
151	2.43		710	3.8	27.0	4	1	0	155
152	2.47	390	590	4.3	25.4	4	3	0	140
153	2.51		610	3.6	22.0	4	2	0	160
154	2.48		680	4.6	31.3	4	1	0	170
148-154	2.51	390	681	3.9	26.4			0	159
155	2.48		630	4.6	29.0	4	tr	0	160
156	2.48		730	4.6	33.6	4	1	0	160
157	2.50		730	3.8	27.7	4	1	0	170
158	2.49		560	3.4	19.0	4	1	0	130
159	2.49	386	640	3.6	23.0	4	1	0	155
160	2.53		520	2.8	14.6	4	0	0	130
161	2.53		670	4.0	26.8	4	0	0	150
155-161	2.50	386	640	3.8	24.8			0	151

Number:—D 75 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	
162	2.55		780	3.6	28.1	4	1	0	180	
163	2.40		830	4.1	34.0	4	0	0	160	
164	2.50		840	4.0	33.6	4	1	0	175	
165	2.53		830	4.2	34.9	4	1	0	175	
166	2.45	498	900	3.6	32.4	4	1	0	170	
167	2.50		770	4.3	33.1	4	1	0	165	
168	2.47		830	4.4	36.5	4	0	0	180	
162-168	2.49	498	826	4.0	33.2			0	172	
169	2.50		820	4.0	32.8	4	1	0	180	
170	2.43		830	4.7	39.0	4	2	0	165	
171	2.43		680	4.9	33.3	4	1	0	160	
172	2.46		640	4.8	30.7	4	0	0	160	
173	2.42	388	440	4.9	21.6	4	0	0	110	
174	2.39		280	4.8	13.4	4	0	0	75	
175	2.34		300	5.7	17.1	4	0	0	80	
169-175	2.42	388	570	4.8	26.7			0	133	
176	2.36		300	5.8	17.4	4	0	0	100	
177	2.36		430	5.3	22.8	4	0	0	125	
178	2.33		540	5.2	28.1	4	1	0	180	
179	2.32		520	4.8	25.0	4	1	0	180	New type food
180	2.40	370	490	4.7	23.0	4	0	0	140	
181	2.39		510	4.8	24.5	4	0	0	130	
182	2.45		440	5.6	24.2	4	0	0	150	Returned to old type food
176-182	2.37	370	461	5.2	23.6			0	144	
183	2.23		500	4.7	23.5	4	0	0	120	
184	2.46		620	5.4	33.5	4	0	0	100	
185	2.44		480	4.9	23.5	4	0	0	135	
186	2.36		570	5.3	30.2	4	0	0	125	
187	2.44		640	5.4	34.6	4	0	0	160	
188	2.40		700	5.3	37.1	4	0	0	180	
189	2.42	406	770	4.3	33.1	4	0	0	170	
183-189	2.39	406	611	5.0	30.8			0	141	

Number:— D 75 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Food consumed (Grams/24 hours)	
190	2.36		710	4.2	29.8	4	0	0	155	
191	2.39		670	5.3	35.3	4	0	0	175	
192	2.33		600	4.8	28.8	4	tr	0	130	
193	2.36	450	620	4.7	29.1	4	1	0	155	
194	2.37		537	5.0	26.5	4	1	0	145	Killed 3:00 P.M.
190-194	2.36	450	626	4.8	29.9			0	152	

Autopsy

(Killed by stunning blow and severing carotid vessels.)

Sections taken from splenic portion of pancreas stat:

(A) piece immersed instantly into liquid air for frozen-dehydration; (B) piece immersed instantly into very hot 40% KOH for tissue glycogen analysis; (C) (D) retroperitoneal sections immersed in Helly's fluid and in 10% alcoholic formalin for special stains.

Balance of autopsy resumed at 4 P.M.: No gross lesions were to be seen in thoracic or abdominal viscera. Aorta and heart (no Ascl seen) fixed in 10% neutral formalin and given to Dr. Payne for lipid analysis. Routine sections of lung, esophagus, both adrenals and both kidneys, both testes, liver fixed in Helly's fluid. Cranial contents not examined.

Number:— D 75 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (cont'd.)

Microscopic Examination:

Pancreas: Glycogen infiltration is present in extreme severity throughout the ductular epithelium, centro-acinar cells and islets; columnar cells of major ducts are also prominently infiltrated. In one group of Best's carmine preparations the stain fails to color the glycogen; this is also true of a group of PAS preparations. The alcoholic formalin-fixed block yields very mediocre results as compared to the Helly-fixed block. There are several large lobules of atrophic pancreas.

Kidneys: Glycogen nephrosis is very marked. A few calcified proximal tubules are noted.

Liver: No glycogen is demonstrable in cytoplasm or nuclei.

Testis: Spermatogenesis is very active.

Lungs: Not remarkable.

Number:— I 89, Male New Zealand white; age 2 months

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
-5	2.30											
-4	2.14								180	46.3		
-3	2.13	120f							135	34.9		
-2	2.36								125	29.1		
-1	2.36	60f										
0	2.44											
-5 to 0	2.29	90f							147	36.8		
1	2.47					4	0		150	33.4	?	?
2	2.48	454f	420	4.4	18.5	4	0		80	17.8	7.5	42.0
3	2.51		250	5.9	14.8	4	1		90	19.8	5.9	29.8
4	2.51		410	4.8	19.6	4	2		115	25.2	7.8	31.0
5	2.50	444f	440	5.0	22.0	4	1		55	12.1	8.8	72.7
6	2.52		470	3.6	16.9	4	0		75	16.4	6.5	39.6
1 - 6	2.50	449f	398	4.7	18.4				94	20.8	7.3	43.0
7	2.42	454f	550	4.4	24.2	4	0		100	22.7	10.0	44.0
8	2.48		280	4.8	13.4	4	1		85	18.9	5.3	28.1
9			460	4.6	21.2	4	0		95	?	?	?
10	2.42	374f	450	4.8	21.6	4	0		145	33.0	9.0	27.2
11			620	5.2	32.2	4	0		150	?	?	?
12	2.51	466f	640	4.2	26.9	4	0	13.8	70	15.4	10.7	69.5
7 - 12	2.46	431f	500	4.7	23.3				108	22.5	8.8	42.2
13	2.47		310	5.0	15.5	4	0	13.6	0	0.0	6.3	
14	2.36	480f	50	3.2	1.6	4	4	13.2	0	0.0	0.7	
15	2.34		100	2.0	2.0	4	4	12.0	5	1.2	0.9	75.0
16	2.00	390	200	2.5	5.0	4	4	10.0	5	1.4	2.5	179.0
17	1.88		90	2.3	2.1	4	4	9.0	0	0.0	1.1	
18	1.83		0									
13- 18	2.15	390	125	3.0	5.2				2	0.5	2.3	127

Autopsy

Gross Examination:

(Hours after death unknown.) Reaction of bladder mucosa to "Galatest" 4 plus and to "Acetonetest" 4 plus. Viscera are congested.

Post mortem decomposition is incipient.

Number:— I 89 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (Cont'd.)

Microscopic Examination:

Pancreas: Islets are scarce and small but contain numerous agranular cells as well as alpha cells. Most islets are round in outline. Mitoses are not evident. Rare cytoplasmic vacuoles are found in agranular islet cells. Ductular epithelium shows obvious cytoplasmic vacuolation, and occasional cells in centro-acinar position also appear to have large cytoplasmic vacuoles. There are no manifestations of cellular proliferation of ductules or centro-acinar cells.

Kidneys: Convoluted tubules are lined by epithelium which shows cytoplasmic vacuolation referable to fatty change. Calcification of tubules apparently restricted to the convoluted segments is abundant. Glomeruli, larger vessels, collecting tubules and calyces appear intact. No inflammatory lesions are evident.

Liver: There is extreme hyperemia of the central lobular venules and sinusoids. The liver cells of the central three-fourths of the lobules contain numerous large fat vacuoles. Glycogen is not apparent in cytoplasm or nuclei. Necrosis of liver cells is not evident.

Adrenals: Fasciculate and reticular zones appear widened and rich in cytoplasmic lipid.

Number:— I 92, Male New Zealand White; age 2 months

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
-5	1.91											
-4	1.88	94f							135	39.5		
-3	1.82											
-2	1.87	100							80	23.6		
-1	1.93											
0	1.93											
-5 to 0	1.89	97f							108	31.6		
1	1.95					4	0		60	16.9	?	?
2	1.97	486f	200	6.5	13.0	4	0		?	?	6.6	?
3	1.86		230	5.0	11.5	4	1		90	26.6	7.0	26.2
4	1.90		370	5.2	19.2	4	0		75	21.7	10.1	46.4
5	2.02	346f	280	5.5	15.4	4	0		90	24.6	7.6	31.0
6	1.94		450	4.8	21.6	4	0		?	?	11.1	?
1 - 6	1.94	416f	306	5.4	16.1				79	22.5	8.5	34.5
7	2.00	533f	370	4.9	18.1	4	0		?	?	9.1	?
8	1.89		100	5.5	5.5	4	0		80	23.3	2.9	12.5
9			180	4.6	8.3	4	0		85	?	?	?
10	2.01	248f	300	5.3	15.9	4	0		180	49.3	8.2	16.7
11			640	5.5	35.2	4	0		150	?	?	?
12	2.02	286f	490	5.5	27.0	4	0	11.1	?	?	13.9	?
7 - 12	1.98	356f	347	5.2	18.3				124	36.3	8.5	14.6
13	1.98		200	5.3	10.6	4	0	10.9	25	6.9	5.4	78.3
14	1.94	500f	40	2.1	0.8	4	4	10.4	80	22.6	0.4	1.8
15	1.99		200	8.5	17.0	4	4	10.0	125	34.6	8.6	24.9
16	2.09	406	210	8.3	17.4	4	4	10.0	165	43.5	8.3	19.1
17	2.14		380	6.2	23.6	4	3	11.1	170	43.7	11.0	25.2
18	2.17		410	7.8	32.0	4	1	0.0	110	27.9	14.7	52.7
13- 18	2.05	406	240	6.4	16.9				113	29.9	8.1	33.7
19	2.16	296f	300	8.3	24.9	4	0	11.0	205	52.2	11.5	22.1
20	2.15		690	5.2	35.9	4	0	11.0	195	49.8	16.7	33.6
21	2.20	400	540	6.5	35.1	4	0	11.0	230	57.5	16.0	27.8
22	2.32		690	6.6	45.5	4	0	12.0	250	59.3	19.6	33.1
23	2.28	490	860	5.1	43.9	4	0	11.0	215	51.8	19.3	37.4
24	2.34		590	8.2	48.4	4	0	12.0	235	55.3	20.7	37.4
19- 24	2.24	445	612	6.7	39.0				222	54.3	17.3	31.9

Number:— I 92 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
25	2.30		870	6.3	54.8	4	0	12.0	220	52.5	23.8	45.4
26	2.29	448	760	6.6	50.2	4	0	11.0	220	52.8	22.0	41.8
27	2.42		630	6.4	40.3	4	0	12.0	290	66.0	16.6	25.2
28	2.50	518	710	5.9	41.9	4	0	13.0	205	45.1	16.8	37.2
29	2.58		500	6.9	34.5	4	0	13.0	230	49.1	13.4	27.3
30	2.69	546	550	7.9	43.5	4	0	13.0	225	46.0	16.2	35.2
25-30	2.46	504	670	6.7	44.2				232	51.9	18.1	35.4
31	2.65		680	7.7	52.4	4	0	13.0	255	52.9	19.8	37.4
32	2.75		720	6.4	46.1	4	0	14.0	240	48.0	16.8	35.0
33	2.80	524	870	5.8	50.5	4	0	14.0	270	53.0	18.0	33.9
34	2.88		800	6.3	50.4	4	0	14.0	210	40.1	17.5	43.7
35	2.86	646	670	7.1	47.5	4	0	14.0	150	28.8	16.6	57.7
36	2.72		450	6.4	28.8	4.	0	0.0	?	?	10.6	?
31-36	2.78	585	698	6.6	46.0				225	44.6	16.6	41.5

Autopsy

(Killed by severing carotid and jugular vessels under intravenous nembutal anesthesia.)

Gross Examination:

Both eyes show severe cataracts. The subcutaneous injection sight appears moderately inflamed. Both lungs contain poorly demarcated small consolidations of dark red color. The liver is large and shows diffusely distributed, irregular, patchy areas of pallor. Both kidneys are large, pale and swollen.

Number:— I 92 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: There is severe glycogen infiltration of islet, ductular and centro-acinar cells. Islets are scarce, small and irregular; most contain only a few glycogen-filled cells together with many alpha cells. Mitoses are not seen in any type of cell. No typically granular beta cells are evident but a few agranular cells without glycogen accumulations are encountered. Proliferating lobules are not seen.

Kidneys: The distal convoluted tubules are the site of severe Armanni lesions. Fatty change is extensive and severe in the proximal tubules. These tubules and the loops of Henle are also the sites of marked calcification. Renal glomeruli, large vessels and collecting tubules are intact.

Liver: There are abundant quantities of glycogen in the liver cells about the central lobular venules, involving about the central half of each lobule. In addition there is a moderate degree of cytoplasmic fatty change in the middle third of these lobules. Widespread patchy areas of focal necrosis are seen, irregularly distributed with respect to lobular pattern.

Adrenals: The fasciculate zone is very broad and contains abundant quantities of lipid.

Thyroid: Not remarkable. Parathyroid likewise not unusual.

Number:— I 92 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (cont'd.)

Pituitary: There is excellent demonstration of both acidophile and basophile granules. No lesion is obvious.

Spleen: The pulp is congested and heavily infiltrated by polymorphonuclear leukocytes.

Number:— I 93, Male. New Zealand White, age 2 months.

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
-5	2.10								-			
-4	2.15								-			
-3	2.17	114f							300			
-2	2.27								155			
-1	2.24	104f							125			
0	2.22								-			
-5 to 0	2.19	109							193			
1	2.15		-	-	-	4	0		115	29.4	-	-
2	2.15	313f	240	3.4	8.2	4	0		80	20.5	3.8	18.5
3	2.17		320	5.3	17.0	4	0		90	21.8	7.5	34.4
4	2.32		380	3.5	13.3	4	0		90	21.3	5.7	26.8
5	2.24	354f	510	4.3	21.9	4	1		75	18.4	9.8	53.3
6	2.22		440	3.8	16.7	4	3		95	23.5	7.5	32.1
1 - 6	2.23	334f	378	4.1	15.4				91	22.5	6.9	33.0
7	2.22	360f	520	3.9	20.3	4	0		125	31.0	10.0	32.3
8	2.27		410	4.2	17.2	4	0		95	23.0	7.6	33.0
9	-		370	4.9	18.1	4	0		100	?	?	?
10	2.34	272f	320	4.7	15.0	4	0		188	43.5	6.4	14.7
11	-		610	4.5	27.5	4	0		140	?	?	?
12	2.37	396f	320	5.4	17.3	4	0	13.0	90	20.9	7.3	26.3
7 -12	2.30	343f	425	4.6	19.2				123	29.6	7.8	26.6
13	2.27		350	5.3	18.6	4	3	12.5	10	2.4	8.2	341.9
14	2.22	430f	0	-	-	-	-	12.1	15	3.7	-	
15	2.18		60	1.9	1.1	4	4	12.0	10	2.5	0.6	24.1
16	2.13	474	100	6.7	6.7	4	4	11.0	15	3.9	3.2	82.1
17	2.02		60	7.4	4.4	4	4	10.0	95	25.9	2.2	8.4
18	2.18		230	6.2	14.3	4	4	0.0	90	22.8	6.6	28.9
13-18	2.17	474	133	5.5	9.0				39	10.2	4.2	97.1
19	2.12	220f	250	6.2	15.5	4	0	11.0	185	48.0	7.3	15.2
20	2.13		630	5.8	36.5	4	0	11.0	200	51.7	17.2	33.1
21	2.33	294	350	6.2	21.7	4	0	12.0	240	56.7	9.3	16.4
22	2.30		440	6.3	27.7	4	0	12.0	225	53.8	12.1	22.5
23	2.32	390	400	7.5	30.0	4	0	12.0	230	54.5	13.0	23.8
24	2.34		320	8.2	26.2	4	0	12.0	280	65.9	11.2	17.0
19-24	2.26	342	398	6.7	26.3				227	55.1	11.7	21.3

Number:— I 93 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms/kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
25	2.40		430	5.0	21.5	4	0	12.0	220	50.5	9.0	17.7
26	2.35	390	400	10.6	42.4	4	0	12.0	240	56.2	18.0	32.0
27	2.48		520	5.2	27.0	4	0	12.0	200	44.4	10.9	24.5
28	2.45	372	670	5.8	38.8	4	0	12.0	300	67.4	15.8	23.4
29	2.49		510	6.0	30.6	4	0	12.0	300	66.3	12.3	18.5
30	2.55	406	730	8.1	59.1	4	0	13.0	300	64.8	23.2	35.8
25-30	2.45	389	543	6.8	36.6				260	58.3	14.9	25.3
31	2.65		530	9.1	48.2	4	0	13.0	?	?	18.2	?
32	2.63		670	4.0	26.8	4	0	13.0	?	?	10.2	?
33	2.64	284f	760	4.8	36.5	4	0	13.0	275	57.4	13.8	24.0
34	2.72		770	6.2	47.7	4	0	14.0	275	57.0	17.6	30.9
35	2.66	464	730	6.0	43.8	4	0	14.0	275	57.0	16.5	28.9
36	2.64		760	5.5	41.8	4	0	0.0	255	53.2	15.9	29.8
31-36	2.66	464	703	5.9	40.8				270	56.2	15.4	28.5
37	2.62	424f	680	7.5	51.0	4	0		85	17.9	19.5	108.9
38	2.61		110	5.3	5.8	4	0		150	31.6	2.2	7.0
39	2.57		490	3.0	14.7	4	0		180	38.6	5.7	14.8
40	2.62	172f	240	2.9	7.0	4	0		45	9.4	2.7	28.4
41	2.61		130	0.5	0.7	2	0		85	17.9	0.3	1.7
42	2.44	65f	130	0.6	0.8	3	0		75	16.9	0.3	1.9
37-48	2.58	220f	297	3.3	13.3				103	22.1	5.1	27.3
43	2.41		50	0.4	0.2	3	3		75	17.2	0.9	5.2
44	2.41	49f	120	0.0	0.0	1	0		115	26.2	0.0	0.0
45	2.29		240	0.0	0.0	2	0		115	27.6	0.0	0.0
46	2.32		190	1.9	3.6	4	0		155	36.8	1.6	4.3
47	2.41	90f	160	2.6	4.2	4	0		75	17.1	1.7	9.9
48												
43-48	2.37	70f	152	1.0	1.6				107	25.0	0.8	3.9

Number:— I 93 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

(Killed by severing carotid and jugular vessels under intravenous nembutal anesthesia.)

Gross Examination:

The subcutaneous injection sites appear moderately inflamed. No other noteworthy lesions are recognizable. The kidneys are large, pale and swollen, and the liver is soft.

Microscopic Examination:

Pancreas: Islets are rather widely separated and most are very small. Single alpha cells are numerous, as are single beta cells or clusters of four to six. In several sections unusually irregular islet patterns are noted in association with ductules. Mitoses are not found in any type of cell. Well granulated cytoplasm characterizes the majority of beta cells, but some appear pale and are apparently devoid of granules. The Golgi apparatus is large. No glycogen is demonstrable in the islets or ductules or centro-acinar cells even though the latter two varieties appear swollen and pale (but not vacuolated).

Number:— I 93 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"	
				Grams/100 ccs.	Grams/24 hours			

Autopsy (cont'd.)

Kidneys: No glycogen is demonstrable in the kidneys. There is obvious but mild fatty change in the proximal convoluted tubular epithelium. This portion of the nephrons is also the site of moderate calcification. Glomeruli and larger vessels are not remarkable. The macula densa is a prominent feature throughout the sections.

Liver: There is abundant glycogen in the hepatic cells throughout the lobular zones. A slight fatty change is apparent in midzonal cells.

Adrenals: The cortex appears very wide and rich in lipid.

Pituitary: There is excellent differentiation of the acidophile and basophile cells. The latter infiltrate the posterior lobe in moderate numbers. A single mitotic figure is noted in an agranular anterior lobe cell.

Spleen: The red pulp and sinusoids are heavily infiltrated with polymorphonuclear leukocytes.

Testis: Not remarkable.

Number:— I 97, Female New Zealand White; age 2 months

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
-5	1.95											
-4	1.85								100	29.8		
-3	1.80	133f							130	39.7		
-2	1.95											
-1	1.98	124f							160	44.5		
0	1.91											
-5 to 0	1.91	129f							130	38		
1	2.03					4	0		180	48.8	?	?
2	2.03	498f	120	5.3	6.4	4	0		60	16.3	3.2	18.5
3	1.94		270	5.9	15.9	4	1		50	14.2	8.2	56.5
4	2.01		160	5.7	9.1	4	4		0	?	4.5	?
5	1.82	592f	240	5.9	14.2	4	4		10	3.0	7.8	260.0
6	1.75		160	5.3	8.5	4	0		10	3.1	4.9	158.0
1 - 6	1.93	545f	190	5.6	10.8				52	17.1	5.7	123.3
7	1.73	560f	130	4.2	5.5	4	4		20	6.4	3.2	50.0
8	1.53		150	5.9	8.8	4	4		40	14.4	5.8	40.2
9			100	9.0	9.0	4	4		55	?	?	?
10	1.63	440f	230	7.4	17.0	4	4		110	37.1	10.4	28.0
11			350	8.0	28.0	4	4		85	?	?	?
12	1.68	478f	310	7.1	22.0	4	4	9.2	55	18.2	13.1	71.9
7 -12	1.64	493f	212	6.9	15.1				61	19.0	8.1	47.5
13	1.60		240	7.5	18.0	4	4	8.8	0	0.0	11.3	
14	1.37	456f	190	2.4	4.6	4	4	7.7	10	4.0	3.3	8.3
15	1.22		180	2.4	4.4	4	4	6.0	40	18.1	3.6	19.9
16	1.26	362	60	6.4	3.8	4	0	6.0	105	45.9	3.0	6.5
17	1.30		170	9.6	16.3	4	0	7.0	105	44.5	12.6	28.4
18	1.33		200	6.6	13.2	4	0	0.0	70	29.0	9.9	34.2
13-18	1.35	362	173	5.8	10.1				55	23.6	7.3	19.5
19	1.38	210f	170	6.2	10.5	4	0	7.0	155	62.0	7.6	12.2
20	1.44		330	7.9	26.1	4	0	7.0	?	?	18.1	?
21	1.44	460	260	9.8	25.4	4	0	7.0	145	55.5	17.6	31.8
22	1.48		270	9.6	26.0	4	0	7.0	105	39.1	17.6	45.1
23	1.52	440	120	10.0	12.0	4	0	8.0	125	45.0	7.9	17.6
24	1.53		230	4.2	9.7	4	0	8.0	100	36.0	6.4	17.8
19-24	1.47	450	230	8.0	18.3				126	47.5	12.5	24.9

Number:— I 97 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
25	1.56		260	4.8	12.5	4	0	8.0	115	40.5	8.0	19.8
26	1.51	498	220	7.6	16.7	4	0	8.0	110	40.1	11.1	27.6
27	1.53		150	8.5	12.7	4	0	8.0	75	27.0	8.3	30.8
28	1.48	414	100	6.6	6.6	4	0	7.0	55	20.5	4.5	21.9
29	1.45		50	0.9	0.5	3	0	8.0	75	28.6	0.4	1.4
30	1.46	290	40	1.8	0.7	3	0	0.0	80	30.2	0.5	1.6
25-30	1.50	401	137	5.0	8.3				85	31.2	5.5	17.7
31	1.41		70	0.4	0.3	3	0		115	44.9	0.2	0.5
32	1.41		330	0.0	0.0	3	0		90	35.1	0.0	0.0
33	1.37	342f	130	5.0	6.5	4	0		0	0.0	4.8	
34	1.25		40	2.5	1.0	4	2		0	0.0	0.8	
35												
36												
31-36	1.36	342f	143	2.0	1.6				51	20.0	1.5	0.3

Autopsy

(Hours after death unknown. Post mortem decomposition slight to moderate.)

Gross Examination:

Reaction of bladder mucosa to "Galatest" 2 plus and to "Acetonetest" 3 plus. Subcutaneous injection site appears extremely inflamed, containing loculated purulent exudate. The lungs appear hyperemic and edematous.

Microscopic Examination:

Pancreas: Mild post mortem autolysis invalidates precise evaluation of the severity of changes noted. However, it is obvious that the

Number:— I 97 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

PANCREAS (Cont'd)

number of islets is much less than normal and that the majority are very small. Almost all the cells of each islet are alpha type, but every islet contains several agranular cells which show traces of glycogen in their pallid cytoplasm. The ductular epithelium is prominent due to pallor and swelling, but no glycogen is demonstrable in it. Mitoses are not in evidence in any type of cell. No proliferative formation of new islets from ductules is seen.

KIDNEYS: Glycogen and fat vacuoles are not apparent. Neither substance can be demonstrated by special techniques. There are moderate calcific deposits in the proximal convoluted tubules. Glomeruli and larger vessels are intact.

LIVER: Neither glycogen nor fat is demonstrable in the cytoplasm of hepatic cells. The central lobular venules and the sinusoids are distended and congested.

ADRENALS: The cortex is narrow; it contains lipid in droplets and in long narrow crystals.

THYROID: Not remarkable.

PITUITARY: Basophile and eosinophile cell types are well demonstrated.

OVARY: Not remarkable.

LUNGS: There are patchy areas of edema and hyperemia.

Number:— I 99, Female New Zealand White; age 2 months.

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
-5	2.33											
-4	2.41								115	26.3		
-3	2.39	118f							100	23.0		
-2	2.55								155	33.5		
-1	2.52	104f										
0	2.49											
-5 to 0	2.45	111f							123	27.6		
1	2.62					4	0		210	44.1	?	?
2	2.57	492f	450	5.4	24.3	4	0		70	14.9	9.5	63.8
3	2.51		470	4.3	20.2	4	1		85	18.6	8.1	43.6
4	2.52		400	4.2	16.8	4	0		95	20.8	6.7	32.1
5	2.53	204f	320	4.8	15.4	4	0		70	15.2	6.1	40.1
6	2.53		410	3.7	15.2	4	0		110	24.0	6.0	25.0
1 - 6	2.55	348f	410	4.5	18.4				107	22.9	6.9	40.8
7	2.57	227f	360	4.5	16.2	4	0		95	20.4	6.1	29.8
8	2.67		230	4.1	9.5	4	0		105	21.6	3.6	16.7
9			640	4.9	31.4	4	0		100	?	?	?
10	2.64	190f	420	3.5	14.7	4	1		185	38.6	5.6	14.5
11			830	3.9	32.4	4	1		180	?	?	?
12	2.61	322f	660	4.5	29.7	4	0	14.4	100	20.0	11.4	57.0
7 - 12	2.62	246f	523	4.2	22.3				128	25.2	6.7	29.5
13	2.63		360	5.2	18.2	4	1	14.5	25	5.2	6.9	133.0
14	2.53	442f	70	5.7	4.0	4	4	13.7	40	8.7	1.4	16.1
15	2.51		180	5.6	10.1	4	4	13.0	55	12.1	4.0	33.0
16	2.44	384	170	7.8	13.2	4	4	12.0	125	28.2	5.4	19.2
17	2.56		270	9.0	24.3	4	4	13.0	135	29.0	9.5	32.8
18	2.60		310	4.8	14.9	4	4	0.0	120	25.4	5.7	22.4
13- 18	2.55	384	227	6.4	14.1				83	18.1	5.5	42.7
19	2.64	278f	490	5.2	25.5	4	1	13.0	180	37.5	9.7	25.9
20	2.65		540	5.6	30.2	4	4	13.0	190	39.4	11.4	29.0
21	2.62	376	600	5.5	33.0	4	3	13.0	235	49.3	13.4	27.2
22	2.68		790	4.8	38.0	4	0	13.0	255	52.3	14.2	27.1
23	2.74	420	880	5.6	49.3	4	0	14.0	260	52.2	18.0	34.5
24	2.94		940	5.0	47.0	4	0	15.0	165	30.9	16.0	51.8
19- 24	2.71	398	707	5.3	37.2				214	43.6	13.8	32.6

Number:— I 99 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
25	2.83		750	3.1	23.2	4	0	14.0	135	26.2	8.2	31.3
26	2.85	234	170	3.0	5.1	4	0	14.0	95	18.4	1.8	9.7
27	2.81		160	0.3	0.5	4	0	14.0	95	18.7	0.2	1.1
28	2.78	116	200	0.2	0.4	2	0	14.0	155	30.8	0.1	0.3
29	2.92		220	0.4	0.9	3	0	14.0	165	31.2	0.4	0.1
30	2.96	286	250	3.8	9.5	4	0	15.0	125	23.2	3.2	13.8
25-30	2.86	212	292	1.8	6.6				128	24.8	2.3	9.4
31	2.94		280	1.9	5.3	4	0	15.0	120	22.4	1.8	8.0
32	3.04		70	0.4	0.3	3	0	15.0	100	18.1	0.1	0.6
33	2.82	110	340	0.0	0.0	2	0	14.0	70	13.6	0.0	0.0
34	2.57		350	0.0	0.0	2	0	13.0	45	9.6	0.0	0.0
35	2.53	102	200	0.0	0.0	1	0	0.0	15	3.3	0.0	0.0
36												
31-36	2.78	106	248	0.5	1.1				70	13.4	0.4	1.7

Autopsy

(Hours after death unknown. Post mortem decomposition very slightly appreciable.)

Gross Examination:

The subcutaneous injection sites contain small amounts of purulent exudate. The lungs are extensively consolidated by pneumonia. The liver is large, soft and dark red. The kidneys appear large, swollen, pale and show diffusely pitted surfaces. The adrenals are very large and bright yellow. Internal lymph nodes and spleen appear large and soft.

Number:— I 99 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

MICROSCOPIC EXAMINATION:

PANCREAS: No vacuolar affect can be appreciated in any type of cell. Islets are variable in size and number from section to section. Beta cells are abundant and all of them appear well granulated. Mitoses are not evident in any type of cell. The ductular epithelium is very slightly swollen but neither it nor centro-acinar cells nor islets contain any demonstrable glycogen.

KIDNEYS: Both show extensive moderately severe chronic pyelonephritis. Tubular casts, hyaline droplets and fatty changes are obvious in all sections. No evidence of glycogen infiltration can be demonstrated.

LIVER: Neither fat nor glycogen is demonstrable. The central lobular venules and the sinusoids appear congested.

ADRENALS: The cortex is very wide and very rich in lipid.

THYROID: Not remarkable. PARATHYROID: Not remarkable.

PITUITARY: Both eosinophile and basophile cell types are readily demonstrable by Mann's stain.

OVARY: The bulk of the non-follicular tissue appears comprised of lipid-rich lutein-like cells.

Number:— I 99 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy (Cont'd.)

SPLEEN AND LYMPH NODES: Lymphocytes are scarce; sinusoids are wide and heavily infiltrated by polymorphonuclear leucocytes.

LUNGS: The sections are almost completely and uniformly consolidated by a fibrinopurulent exudate centered upon bronchi and bronchioles.

Number:— I 100, Female New Zealand White; age 5 months.

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food Consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Glucose Excreted (Gms./kilo/24 hours)	% Available Glucose Excreted
-5	2.56											
-4	2.42								145	32.9		
-3	2.58	126f							155	33.0		
-2	2.64								160	33.4		
-1	2.58	96f										
0	2.54											
-5 to 0	2.55	111f							153	33.1		
1	2.67					3	0		175	36.0	?	?
2	2.52	472f	460	3.6	16.6	4	0		70	15.3	6.6	43.1
3	2.51		430	4.4	18.9	4	1		85	18.7	7.5	40.2
4	2.50		560	4.0	22.4	4	3		55	12.1	9.0	74.4
5	2.42	444f	500	3.5	17.5	4	4		60	13.6	7.3	53.7
6	2.38		390	3.2	12.5	4	4		15	3.5	5.3	151.0
1 - 6	2.50	458f	468	3.7	17.6				77	16.5	7.1	72.5
7	2.37	396f	90	2.5	2.3	4	4		20	4.7	0.9	19.2
8	2.29		130	1.9	2.5	4	4		30	7.2	1.1	15.3
9			80	3.1	2.5	4	4		35	?	?	?
10	2.24	496f	150	5.3	7.9	4	3		85	20.9	2.4	11.5
11			360	5.1	18.3	4	3		85	?	?	?
12	2.28	448f	280	5.9	16.5	4	4	12.5	50	12.1	7.2	59.5
7 - 12	2.30	447f	182	4.0	8.3				51	11.2	2.9	26.4
13	2.15		280	4.0	11.2	4	4	11.8	0	0.0	5.2	
14	2.12		40	0.8	0.3	4	4	0.0	0	0.0	0.1	
15												
16												
17												
18												
13 - 18	2.14		160	2.4	5.8				0		2.7	

Autopsy

Discarded without post mortem examination because of advanced decomposition.

Number:— J 2, Female. New Zealand White; age 4 months

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Grams/24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
-5	2.30											
-4	2.35								190	44.4		
-3	2.40	98f							155	35.6		
-2	2.51								185	40.5		
-1	2.43	76f										
0	2.56											
-5 to 0	2.43	87f							177	40.2		
1	2.60					4	0		215	45.5	?	?
2	2.47	240f	330	5.5	18.2	4	0		95	21.2	7.4	34.8
3	2.44		360	4.4	15.8	4	0		50	11.3	6.5	57.4
4	2.41		220	4.2	9.3	4	0		70	16.0	3.9	24.4
5	2.35	376f	350	5.0	17.5	4	0		80	18.7	7.8	41.6
6	2.25		350	4.9	17.1	4	3		75	18.4	7.6	41.3
1 - 6	2.42	308f	322	4.8	15.6				98	21.9	6.6	39.9
7	2.28	290f	330	4.5	14.8	4	0		100	24.2	6.3	26.1
8	2.25		360	5.5	19.8	4	0		80	19.6	8.8	45.0
9			330	4.8	15.8	4	0		80	?	?	?
10	2.16	334f	150	6.4	9.6	4	0		145	36.9	4.5	12.2
11			410	4.3	17.6	4	0		105	?	?	?
12	2.20	254f	280	5.0	14.0	4	0	12.1	65	16.2	2.3	14.2
7 - 12	2.22	293f	310	5.1	15.3				96	24.2	5.5	24.4
13	2.23		170	4.5	7.6	4	0	12.3	10	2.5	3.4	136.0
14	2.13	396f	80	4.7	3.8	4	4	11.5	0	0.0	1.8	
15	2.07		30	7.6	2.3	4	4	10.0	5	1.3	1.1	84.6
16	1.98	318	100	3.8	3.8	4	4	10.0	0	0.0	1.9	
17	1.88		80	3.5	2.8	4	4	9.0	0	0.0	1.5	
18	1.79		80	2.0	1.6	4	4	0.0	0	0.0	0.9	
13- 18	2.01	318	90	4.4	3.7				2.5	0.6	1.8	110.3
19	1.70	474f	10	2.7	0.3	4	4	0.0	0	0.0	0.2	

Number:— J 2 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

(Killed by severing carotid and jugular vessels.)

Gross Examination:

The blood shows extreme lipemia. Urinary bladder mucosa gives strongly positive "Galatest" and "Acetonetest?" reactions. Liver is soft and friable and gall-bladder distended and attached by fibrous adhesions to adjacent viscera. Kidneys are soft, pale and swollen. Ascending aorta and arch show severe spontaneous arteriosclerosis (medial calcification). Ovaries are enlarged, being occupied by many corpora hemorrhagica.

Microscopic Examination:

Pancreas: Islets are numerous and have many irregular sinuous forms as well as many large ones. In the small islets alpha cells are the preponderant type but in the large ones agranular cells frequently comprise almost the entire islet. Moderate to severe cytoplasmic vacuolation characterizes the agranular cells and in several instances mid-phase and later stages of mitotic figures are encountered. The ductular epithelium and centro-acinar cells are much less affected by the vacuolar appearance than are the agranular islet cells. Mitoses are not evident in these "exocrine" cell types.

Number:— J 2 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination - (Cont'd.)

KIDNEYS: Convoluted tubules show both fatty change and moderate glycogen infiltration. The proximal convoluted tubules and the loop of Henle show severe calcification. Glomeruli appear intact. Many protein casts are apparent in tubular lumens at all levels.

LIVER: The central lobular venules and sinusoids are distended. Moderate to severe fatty change is apparent in the hepatic cells of the central four-fifths of the liver lobules. The gall-bladder mucosa is deficient at two points where necrotic debris of inflammatory nature fills and covers the muscularis and serosa. Adjacent liver cells are necrotic and a small artery is completely occluded by unorganized thrombus.

ADRENALS: Fasciculate and reticular zones appear widened and rich in cytoplasmic lipid.

THYROID: Not remarkable.

PITUITARY: The basophile granules are very poorly demonstrated or are possibly, in the vast majority of cells, absent. Mitoses are not seen. There are no obvious degenerative lesions.

SPLEEN: The Malpighian corpuscles are not remarkable. The red pulp and sinusoids are heavily infiltrated by polymorphonuclear leukocytes.

Number:— J 26, Female; New Zealand White, age 2 months.

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Gms./24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
0	1.61	76f	280			0	0	8.9	15	8.3	0.0	0.0
1	1.56	180	70			0	0	8.2	10	3.5	0.0	0.0
2	1.51		100			0	1	8.0	15	5.5	0.0	0.0
3	1.56	188	170			0	0	8.0	75	26.5	0.0	0.0
4	1.56		200			0	0	8.0	80	28.2	0.0	0.0
5	1.56		?	0.0		1	0	0.0	95	33.5	0.0	0.0
6	1.56	120	270	0.0		1	1	8.0	?	?	0.0	0.0
1 - 6	1.55	163	162						55	19.4	0.0	0.0
7	1.68		460	1.2	5.5	4	0	8.0	165	54.0	3.3	6.1
8	1.68	126	440	1.2	5.3	4	0	8.0	125	40.9	3.2	7.8
9	1.71		?	0.5	?	3	0	9.0	90	29.5	?	?
10	1.76	190	200	1.3	2.6	4	0	10.0	155	48.5	1.5	3.1
11	1.78		520	1.9	9.9	4	0	9.0	175	54.1	5.6	10.4
12	1.85		540	1.8	9.7	4	0	9.0	180	53.5	5.2	9.7
7 -12	1.74	158	432	1.3	6.6				148	46.8	3.8	7.4
13	1.90	200	510	3.1	15.8	4	0	10.0	190	55.0	8.3	15.1
14	1.96		490	3.1	15.2	4	0	10.0	190	53.3	7.8	14.6
15	2.01	220	330	3.4	11.2	4	0	10.0	100	27.4	5.6	20.4
16	2.03		240	0.7	1.7	3	0	10.0	130	35.2	0.8	2.2
17	2.04	148	260	0.0	0.0	2	0	10.0	130	35.0	0.0	0.0
18	2.12		250	0.5	1.3	2	0	11.0	135	30.3	0.6	2.0
13-18	2.03	189	347	1.8	7.5				146	39.4	3.9	9.1
19	2.20		260	0.3	0.8	3	0	11.0	170	42.5	0.4	0.9
20	2.24	158	230	1.1	2.5	4	0	11.0	130	31.5	1.1	3.5
21	2.24		170	1.1	1.9	4	0	11.0	120	29.5	0.8	2.7
22	2.14	100	90	0.0	0.0	2	0	11.0	50	12.9	0.0	0.0
23	2.12		(0)					0.0	(10)	(2.6)		
24												
19-24	2.19	129	188	0.6	1.3				118	29.1	0.6	1.8

Number:— J 26 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

(Killed by severing carotid and jugular vessels under intravenous nembutal anesthesia.)

Gross Examination:

The subcutaneous injection sites appear slightly inflamed. No other gross abnormalities are appreciable.

Microscopic Examination:

PANCREAS: In every respect the pancreatic exocrine and endocrine tissues appear normal.

KIDNEYS: There is calcification of the proximal convoluted tubules in mild degree. The tubular epithelium shows prominent bulging pale cytoplasmic masses projecting into the lumens. A few pale casts are noted.

LIVER: Several large focal areas of necrosis are evident at variable sites with respect to zonal distribution. The cytoplasm of hepatic cells elsewhere contains abundant glycogen and moderate numbers of small fat vacuoles.

ADRENALS: The adrenal cortex is very rich in lipid.

THYROID: Not remarkable.

PITUITARY: There is beautiful differentiation of acidophile and basophile cells. A single mitosis is noted in a chromophobe cell.

OVARY AND UTERUS: Not remarkable.

SPLEEN: The red pulp is congested and contains numerous polymorphonuclear leukocytes.

LUNGS: No abnormalities are evident.

Number:— J.30, Male. New Zealand White; age 2 months.

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Gms./24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
0	1.86	120f	160			0	0	10.2	10	3.0	0.0	0.0
1	1.83	100	30	0.0	0.0	0	0	9.9	5	1.3	0.0	0.0
2	1.77		130	0.0	0.0	0	1	9.0	10	3.1	0.0	0.0
3	1.74	152	40	0.0	0.0	0	0	9.0	10	3.2	0.0	0.0
4	1.73		150	0.0	0.0	0	3	9.0	10.	3.2	0.0	0.0
5	1.73		60	0.0	0.0	0	1	0.0	10	3.2	0.0	0.0
6	1.70	126	340	0.0	0.0	1	1	9.0	10	3.2	0.0	0.0
1 - 6	1.75	126	125		0.0				9	2.9	0.0	0.0
7	1.73		160	0.0	0.0	1	2	9.0	35	11.2	0.0	0.0
8	1.65	88	130	0.0	0.0	0	2	9.0	15	4.7	0.0	0.0
9	1.74		400	0.8	3.2	3	0	9.0	65	16.8	1.8	10.8
10	1.78	132	610	1.4	8.5	4	0	10.0	105	32.5	4.8	14.4
11	1.83		430	2.4	10.3	4	0	9.0	100	30.1	5.6	19.3
12	1.87		210	1.5	3.2	4	1	9.0	100	29.4	1.7	5.8
7 -12	1.77	110	323	1.0	4.2				70	20.8	2.3	9.0
13	1.88	240	210	1.8	3.8	4	0	10.0	115	33.6	2.0	6.0
14	1.80		100	1.3	1.3	4	0	9.0	40	12.2	0.7	5.7
15	1.72	70	50	0.2	0.1	2	0	9.0	0	0.0	0.1	-
16	1.74		190	0.0	0.0	0	0	9.0	85	26.9	0.0	0.0
17	1.80	90	120	0.0	0.0	0	0	9.0	30	9.2	0.0	0.0
18	1.79		150	0.0	0.0	0	0	12.0	95	29.2	0.0	0.0
13-18	1.79	133	137	0.6	0.9				61	18.5	0.5	2.3
19	1.76		110	0.0	0.0	2	0	9.0	130	40.6	0.0	0.0
20	1.82	124	100	0.0	0.0	2	0	9.0	105	31.5	0.0	0.0
21	1.83		130	0.0	0.0	2	0	9.0	110	33.1	0.0	0.0
22	1.86	184	130	0.0	0.0	2	0	9.0	110	32.5	0.0	0.0
23	1.84		130	0.0	0.0	1	0	0.0	110	32.3	0.0	0.0
24	1.86	88	150	0.0	0.0	2	0	0.0	115	33.8	0.0	0.0
19-24	1.83	132	125	0.0	0.0				113	34.0	0.0	0.0
25	1.87		250	0.0	0.0	1	0	0.0	40	11.8	0.0	0.0
26	1.94		170	0.0	0.0	1	0	0.0	90	25.5	0.0	0.0
27	1.98	158	200	0.0	0.0	2	0	0.0	125	34.7	0.0	0.0
28	1.90		260	0.0	0.0	0	0	0.0	45	13.1	0.0	0.0
29	1.94	95	140	0.0	0.0	0	0	0.0	60	16.2	0.0	0.0
30	1.81		300	0.0	0.0	2	0	0.0	75	22.8	0.0	0.0
25-30	1.91	127	220	0.0	0.0				73	20.7	0.0	0.0

Number:— J. 30 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)	Food consumed (Gms./24 hours)	Available Glucose (Gms./kilo/24 hours)	Excreted Glucose (Gms./kilo/24 hours)	% Available Glucose Excreted
31	1.83	93	180	0.0	0.0	1	0	0.0	95	28.6	0.0	0.0
32	1.84		230	0.0	0.0	0	0	0.0	55	16.2	0.0	0.0
33	1.85		110	0.0	0.0	0	0	0.0	35	10.2	0.0	0.0
34	1.71	82	240	0.0	0.0	0	0	0.0	35	11.0	0.0	0.0
35												
36												
31-36	1.81	88	190	0.0	0.0				55	16.5	0.0	0.0

Autopsy

(Killed by severing carotid and jugular vessels under intravenous nembutal anesthesia.)

Gross Examination:

PANCREAS: Both exocrine and endocrine tissue are normal in every respect.

KIDNEYS: Rare calcific deposits are noted in proximal convoluted tubules. There are many hyaline droplets in the tubular epithelium and pale protein casts are moderately numerous. There is no evidence of glycogen infiltration or of fatty change.

LIVER: The hepatic cells are rich in glycogen.

ADRENALS: The cortical cells are rich in lipids.

PITUITARY: Acidophile and basophile cells are well differentiated.

TESTIS: The seminiferous tubules are lined by Sertoli cells and spermatogonia. Rarely meiosis is noted; maturation of these cells is extremely depressed. No mature spermia are evident.

SPLEEN: The sinusoids are wide and full of red cells. The pulp is intact.

Number:— K 13 Dog. Male Adult Mongrel.

Days	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Crude Alkaline APE (ccs.)
0	11.22	136	200	0	0	0	0	5.0
1	11.41		300			1	0	5.0
2	11.53		180			1	0	7.5
3	11.32	156	900			1	0	7.5
4	11.82		1600	tr		1	0	10.0
5	11.40		1950	0		1	0	10.0
6	11.60		1600	0.2	3.2	3	0	15.0
7	11.76	200	1500	0.2	3.0	2	0	15.0
8	10.93		1750	0.1	1.8	2	0	15.0
9	12.54		1250	0.1	1.3	2	0	10.0
10	12.38	240	1560	0.2	3.1	3	0	----

Autopsy

(Killed by intravenous nembutal anesthesia and exsanguination.)

Gross Examination:

The only grossly visible abnormality is a deep ulcer with rolled elevated edges on the lesser gastric curvature near the pylorus. The liver does not appear fatty. There is no suggestion of peritonitis.

Number:— K 13 Dog - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: Vacuolation of ductular epithelial cells is obvious in routine preparations. Centro-acinar cells are very numerous, appearing as masses of pale agranular or vacuolar cells surrounded by exocrine acinar cells and communicating with vacuolar duct epithelium. Infrequent mitoses are present in such areas in all types of cells. The islets are numerous. In granule stains (Gomori) alpha cells are rather pale; beta cells are agranular and frequently mildly vacuolar. In glycogen preparations all the vacuoles appear to contain definite deposits of glycogen displaced peripherally in ipsilateral manner. Mitoses are readily observed in beta cells and rarely in alpha cells.

Kidneys: There is obvious cloudy swelling with a few pale casts in convoluted tubules. No glycogen is demonstrable. Fatty change is not present.

Liver: There is a marked degree of cytoplasmic vacuolation referable to both fatty change and glycogen accumulation. Occasional mitoses are evident.

Adrenals: Not remarkable.

Thyroid: Not remarkable.

Pituitary: Both eosinophile and basophile types of cells are well differentiated in the anterior lobe.

Testes: Not remarkable.

Stomach: The ulcer is a flask-shaped lesion of subacute inflammatory character.

Number:— K 14 Dog. Adult Male Mongrel.

Days after partial resection of pancreas	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
0	12.94	74						6	23.0 Gms. of pancreas resected; estimated weight of remnant, less than 1.5 Grams.
1						2	0	6	
2	12.97		400	0.15	0.60	3	0	6	
3	12.94					4	0	4	
4	13.10		420	0.43	1.81	3	0	0	
5	12.68					4	0	0	
6	13.41		250	0.54	1.35	3	0	0	
7		232				3	0	0	
8	12.19		200	0.20	0.40	3	1	0	
9	13.24		250	0.41	1.03	3	0	0	
10	13.06	112	550	0.19	1.05	3	0	0	
11	13.07		450	0.23	1.04	2	1	0	
12	13.84					3	1	0	
13	13.74		450	0.50	2.25	4	2	0	
14	13.66	94	350	0.70	2.45	4	2	0	
15	14.24		85	2.03	1.72	4	2	0	
16		80	200	0.55	1.10	3	2	0	9:30 A.M.
16		120	65	0.36	0.23	2	0	0	12:30 P.M.
17			160	0.12	0.19	2	0	0	8:30 A.M.
17			100	2.38	2.38	4	2	0	2:30 P.M.
17			80	0.22	0.17	2	0	0	6:00 P.M.

Number:— K 14 Dog - continued

Days after partial resection of pancreas	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
18	12.61		270	0.23	0.62	2	1	0	10:00 A.M.
18			190	0.63	1.20	3	1	0	4:30 P.M.
19	14.51		270	2.72	7.34	4	2	0	9:30 A.M.
19			130	0.21	0.27	2	1	0	10:30 A.M.
19			180	0.56	1.01	3	1	0	2:30 P.M.
20			1030	0.69	7.10	3	1	0	11:00 A.M.
21	14.17	130	690	1.63	11.24	4	0	0	10:30 A.M.

Autopsy

(Killed by exsanguination under intravenous nembutal anesthesia.)

Gross Examination:

A few peritoneal fibrous adhesions are present in the operative area. When dissected relatively free from fibrous tissue the pancreatic remnant weighs 4.0 grams. There are no grossly visible abnormalities in any of the abdominal or thoracic viscera.

Microscopic Examination:

Pancreas: (Multiple blocks of remnant were separately embedded and subsequently cut and stained.) In all sections fibrous septa separate lobules of pancreas. A few small round cells and hemosiderin-filled macrophages are found along such bands of tissue. These septa do not subdivide lobules. Vacuolation of ductular epithelium, centro-acinar cells and islet cells is present but very inconspicuous. No

Number:— K 14 Dog - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Pancreas -(cont'd.)

mitotic activity is detected. Granule stains differentiate alpha cells readily but fail to show beta granules. In the glycogen preparations, traces of glycogen are found displaced toward the cell membrane of about three to six cells in almost every islet.

Kidneys: Not remarkable. No glycogen is demonstrable.

Liver: Not remarkable except that glycogen is demonstrable in the cytoplasm of liver cells in small quantities only.

Adrenals: Not remarkable.

Thyroid: Not remarkable.

Pituitary: Basophiles and eosinophiles are readily differentiated by Mann's stain.

Testes: Not remarkable.

Number:— **T 4 Male**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Crystalline Zinc Insulin (units, s.c.)	
-7 to 0	3.15	113f								Alloxan 200 5/12/46
1-7	3.15	418f				4	2	4		
8-14		445f				4	2			
15-21						4	0			
22-28						4	0			
29-35						4	0			
36-42	3.25	265f				4	0			
43-49	3.35	465f				4	0			
50-56						4	0			
57-63		445f				4	0			
64-70						4	0			
71-77						4	0			
78-84		445f*				4	0			
		414f				4				
85	3.50	445f				4	0	0		Biopsy #1
86	3.50							10	20	
87		98	0					10	20	
88						3		10	12	
89		368	140			3		40	28	
90		187	80			4		50	32	
91		127	180			3		60	32	
85-91	3.50	195	133			3		26	21	
92	3.60	125	100			1		60	32	
93		110	90			1		60	32	
94			160			0		60	32	
95			210			0		60	24	
96		171	160			3		30	16	
97			120			4		60	32	
98		300	140			4		60	32	
92-98	3.60	185	140			2		56	29	

*Value taken from 85th day and <u>not</u> included in week #13

Number:— **T 4 Male - Continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	Crystalline Zinc Insulin (units, s.c.)	
99		165	60			1		60	32	
100		122	50			0		70	32	
101		223	60			2		80	32	
102	3.86		120			0		80	32	
103		123	50			0		80	32	
104		138	50			0		80	32	
105			60			1		80	32	
99-105	3.86	154	64			1		76	32	
106			60			0		80	32	
107			160			2		80	32	
108			50			1		88	32	
109			80			1		96	32	
110		119	70			0		96	32	
111			80			2		96	32	
112	4.06		160			1		48	16	Biopsy #2
106-112	4.06	119	94			1		83	30	
113			120			2		20	0	
114			130			2		80	32	
115			110			2		96	32	
116			60			0		96	32	
117	3.93					2		96	32	
118						1		0	0	Insulin Rx
119						4		0	0	Discontinued
113-119	3.93		105			2		55	18	
120-126	3.97	378								
127-133	4.01									
134-140	3.90	486								
141-147	4.03	462								

Number :— **T 4 Male - Continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				
148-154	3.95	550							
155-161									
162-168	4.01	484f							
169-175	3.80	542f							
176-182									
183-189		572f							
190-196	3.80	572f							
197-203									
204-210	3.81	576f							
211-217									
218-224									
225-231	3.72	584f							
232-238	3.54		490	3.1	15.2				
120-238		469 555f							
239	3.54		400			4	0		Biopsy #3
240	3.23	340	130			4	4		
241	3.41	380	90			2	8		
242	3.49	424	190			4	12		
243	3.47	460	390			4	16		
244	3.65	520	290			4	20		
245	3.59		370			4	24		
239-245	3.48	425	266			4	12		

Number:— **T 4 Male - continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
246	3.63	300	140			4		28	
247	3.74	200	200			2		32	
248	3.73	228	200			1		32	
249	3.71		60			2		36	
250	3.72		180			2		36	
251	3.73		290			3		36	
252	3.80	115	250			3		36	
246-252	3.72	211	189			2		34	
253	3.80		400			1		36	
254	3.78	300	360			3		36	
255	3.72		400			4		40	
256	3.80		240			1		40	
257			400			3		40	
258		206	420			4		40	
259			420			4		40	Abscess drained. Rx. penicillin
253-259	3.78	253	377			3		39	
260			300			2		40	
261	3.80		300			2		40	
262	3.91		190			2		40	
263		152	300			1		40	
264	3.89		320			1		40	
265	3.91		200			2		40	
266	3.98		240			1		40	
260-266	3.90	152	264			2		40	
267	4.02		150			2		40	
268	3.93		200			2		40	
269	3.93		150			1		40	Penicillin discontinued
270	3.91		180			1		40	
271			170			1		40	
272	3.93		180			2		40	
273	3.90		140			1		40	Suture removed; wound draining
267-273	3.94		167			1		40	

Number :— **T 4 Male - continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
274			160			4		40	
275			200			3		40	
276			180			2		40	
277			160			2		40	
278			110			1		40	
279			150			2		40	
280			130			1		40	
274-280			156			2		40	
281			160			1		40	
282			120			3		40	
283						1		40	
284		111				1		40	
285						1		40	
286	4.01		230			1		40	
287						1		40	
281-287	4.01	111	170			1		40	
288	4.03		150			1		40	
289	4.03	147	80			1		40	
290	4.06		110			3		40	
291	4.03		120			3		40	
292	4.03		100			4		40	
293	4.05		130			3		40	
294	4.04		160			2		40	
288-294	4.04	147	121			2		40	
295	4.00		130			1		40	
296	4.17		150			1		40	Found dead 8:30 A.M. Autopsy
297									
298									
299									
300									
301									
295-301	(4.09)	-	(140)			(1)		(40)	

Number:— T 4 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

First Biopsy of Pancreas

Glycogen infiltration of ductules is readily apparent. In the few islets present, cells filled with glycogen are rare while agranular types are common. There are no mitoses.

Second Biopsy of Pancreas

No trace of glycogen is demonstrable. There are few well granulated beta cells, most being agranular. There are no evidences of mitotic activity.

Third Biopsy of Pancreas

There is extensive glycogen infiltration of marked degree affecting islets, ductules, centro-acinar cells and even columnar cells of large ducts. No granular beta cells are evident. Numerous areas of mitotic proliferation are evident. These show small ductules leading to acinar and islet structures, all containing glycogen-filled cells.

Number:— T 4 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

Gross Examination:

The trachea and bronchi appear markedly inflamed and the lungs contain scattered foci of consolidation. Urinary bladder contains 30 ccs. of yellow urine giving 4 plus "Galatest" and zero "Acetonetest" reactions.

Microscopic Examination:

Pancreas: No trace of glycogen is demonstrable; no cells with vacuolar cytoplasmic change are found. Numerous islets are present; often they contain only beta cells. Rarely do these cells contain readily demonstrable beta granules. Mitoses are rare but are found in agranular islet cells and in ductules.

Kidneys: There is marked calcification of proximal convoluted epithelium.

Liver: There are large quantities of cytoplasmic glycogen without nuclear infiltration or fatty change.

Adrenals: The cortical cells contain small amounts of lipid.

Thyroid, Parathyroid: Not remarkable.

Pituitary: Not remarkable.

Number:— **T 6 Male**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				
-6 to 0	3.00	112f							Alloxan 200 5/12/46.
1-7	2.70	490f				3	4		
8-14	2.45	415f				4	2		
15-21	2.18	428f				4	0		
22-28	2.04	460f				4	0		
29-35						4	0		
36-42	1.90	335f				4	0	4	Daily insulin started
43-49	2.06	540f				4	0	4	" " cont'd.
50-56	2.02	475f				4	0	4	" " discont'd.
57-63						4	0		
64-70	1.82	365f				4	0		
71-77	1.80					4	0		
78-84	1.78	470f							82nd day: Biopsy
1-84		442f							83rd day: Died

Biopsy of Pancreas

Glycogen infiltration is present in moderate degree in the ductules, while the islets rarely contain affected cells. Numerous islets are seen to be composed almost entirely of alpha cells with a few agranular cells. Several lobules of proliferating ductules and newly-formed acini and islets are encountered. Mitotic figures are numerous in the ductular epithelium. In such lobules there is no suggestion of glycogen vacuolation of ductular epithelium or of islet cells.

Number:— T 6 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

Gross Examination:

Hemoperitoneum of 50 ccs. is localized to the area of the spleen which appears infarcted. Omentum is caught in the peritoneal suture. Urinary bladder is empty; its mucosa gives a 4 plus "Galatest" and a zero "Acetonetest" reaction.

Microscopic Examination:

Pancreas: Not substantially different from the biopsy result except that glycogen infiltration of the ductular epithelium is more widespread than heretofore appreciated.

Kidneys: Calcification and glycogen infiltration are noted in moderate degrees.

Liver: The liver cells near the central lobular venules contain small amounts of cytoplasmic glycogen while there are a few glycogen-infiltrated liver cell nuclei toward the peripheries of the lobules.

Adrenals: The widened fasciculate zone contains moderately rich deposits of lipids.

Number:— T 15

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	3.11	123f							Alloxan 200
1-7	3.06	430f				4	0		
8-14						4	0		
15-21						4	0		
22-28	2.93	435f				4	0		
29-35						4	0		
36-42						4	0		
43-49						4	0		
50-56	2.75					4	0		
57-63		510f				4	0		
64-70	2.91	596				4	0		
71-77	2.80	552				4	0		
78-84	2.73	665				4	0		
85-91	2.54	492				4	0		
92-98	2.67	494f				4	0		
99-105	2.80	376f	875	2.79	24.7	4	0		Anesthetic death at biopsy.
106-112									

Autopsy

Gross Examination:

Except for extreme emaciation and visceral hyperemia, no abnormalities are noted.

Microscopic Examination:

Pancreas: Severe glycogen infiltration is apparent in the ductular epithelium, centro-acinar cells and islets. No beta granules are demonstrable in islet cells but some pallid agranular forms are present in addition to those showing extreme vacuolation. No mitoses are evident in any type of cell.

Number :— T 16

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	2.05	124f							Alloxan
1-7						4	4		
8-14	2.30	440f				4	4		
15-21						4	0		
22-28	2.49	490f				4	0		
29-35						4	0		
36-42						4	0		
43-49						4	0		
50-56	2.92	480f				4	0		
57-63						4	0		
64-70		668f				4	0		
71-77	3.00	630f				4	0		
78-84	2.45	556f				4	0		
85-91						4	0		
92-98	2.56	440f				4	0		
99-105						4	0		
106-112	2.40	422f				4	0		
113-119	2.67	470f	950	2.7	25.7	4	0		
120						4	0	8	Biopsy
121						4	0	20	
122									Died

Biopsy of Pancreas

Appearance is identical with later autopsy sections (v.i.).

Autopsy

Gross Examination:

The spleen appears infarcted and there is a slight localized hemoperitoneum. Viscera appear hyperemic. Urinary bladder contains 50 ccs. of clear urine which gives markedly positive reactions with both "Galatest" and "Acetonetest".

Number:— T 16 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: Extremely severe degrees of glycogen infiltration are manifested in the islets, ductules and centro-acinar cells. Many small islets are composed entirely of such affected cells, but the larger ones are composed almost entirely of alpha cells. There is no evidence of proliferative activity.

Kidneys: A severe degree of "glycogen nephrosis" is apparent.

Liver: Central lobular zones are well filled with cytoplasmic glycogen while in the peripheral zones the hepatic cell nuclei are often infiltrated by glycogen.

Adrenals: In the outer portion of the fasciculate zone the cortical cells are very rich in lipid.

Testes: There is a single layer of inactive epithelium lining the seminiferous tubules.

Number :— T 17

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	3.17	114f							Alloxan 200
1-7						4	1	4	
8-14	3.05	418f				4	4	0	
15-21						4	0	0	
22-28	2.95	480f				4	tr	0	
29-35						4	0	0	
36-42						4	0	0	
43-49						4	0	0	
50-56	2.93	464f				4	0	0	
57-63	2.26					4	0	0	
64-70		654				4	0	0	
71-77	2.70	688				4	0	0	
78-84	2.70	596				4	0	0	
85-91	2.50	640				4	0	0	
92-98	2.44	370f				4	0	0	
99-105	2.50	480f				4	0	0	
106-112	2.67	566f				4	0	0	
113-119			1000	1.5	15.0	4	0	0	
1-119		463f 645							
120	2.67							8	Biopsy
121	2.65							20	
122								40	
123								48	
124	2.85		35			2		48	
125	2.99		50			2		44	
126			65			2		48	
120-126	2.79		50			2		37	
127	2.98	90				2		48	
128		107				2		48	
129						2		48	
130								0	Convulsions.Rx Glucose
131		200				4		0	(4 Gms.I-V
132	3.01	100				4		20	" (10 Gms.O
133	2.98	160						0	Rx.Glucose as above. Biopsy.
127-133	2.99	133						23	

Number:— T 17 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
134	2.82	572	10			2		0	
135	2.82	708	20			3		0	
136			100						Found dead 9:00 A.M. Autopsy.

First Biopsy of Pancreas

Glycogen infiltration of the pancreatic ductular epithelial cells is widespread and severe; centro-acinar cells showing this change are numerous. In the islets there are many cells containing glycogen, a few agranular cells and no cells with demonstrable beta granules.

Second Biopsy of Pancreas

There is no trace of demonstrable glycogen in the islets but a few small accumulations are encountered in ductular epithelial cells. None of the islets contains beta cells having well differentiated beta granules.

Autopsy

Gross Examination:

Peritoneal cavity at operative site contains a few ccs. of blood-tinged fluid and a small granular deposit of fibrin. Urinary bladder contains 20 ccs. of clear urine which gives 4 plus reactions to both "Galatest" and Acetonetest".

Number:— **T 17 - continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: Appearance confirms that seen in second biopsy. No proliferating lobules of pancreatic tissue are seen.

Kidneys: No glycogen can be demonstrated.

Liver: Liver cells appear to be devoid of glycogen.

Adrenals: Not remarkable.

Testes: Not remarkable.

Number:— T 20

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-9 to 0	2.25	99f							Alloxan 200
1-7	2.47	350				4	0	4	
8-14	2.60	496				4	0	0	
15-21	2.71	494f				4	0		
22-28	2.74	640f				4	0		
29-35						4	0		
36-42	2.60	460f				4	0		
43-49	2.69	482f				4	0		
50-56						4	0		
57-63		538f				4	0		
64-70	2.93	594f				4	0		Bilateral cataracts.
71-77						4	0		
78-84	2.88	548f				4	0		
85-91						4	0		
92-98	2.75					4	0		
99-105		568f				4	0		
106-112	2.68					4	0		
113-119	2.70	584f				4	0		
120-126						4	0		
127-133	2.65	548f				4	0		
134-140						4	0		
141-147	2.86	602	800	3.2	25.6	4	0		
1-151		546f							
148									
149									
150									
151	2.96								
152	2.87							4	Biopsy
153						4	3	6	
154	2.95	782				4	2	12	
148-154	2.93	782						7	

Number:— T 20 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				
155	2.96	810	180			4	2	32	
156	3.05		130			4	0	32	
157	3.03		50			4	0	28	
158	3.18		90			3	0	28	
159	3.19	284	80			3	0	32	Sutures removed
160	3.20		80			4	0	32	
161	3.12		280			4		36	
155-161	3.10	547	127					31	
162	3.27		100			4		40	Infected skin. Rx.
163	3.17	375	340			4		40	penicillin
164	3.23		210			4	2	40	
165	3.23		340			4		44	
166	3.33		90			4		44	
167		259	60			4		40	
168			80			4		40	
162-168	3.25	317	174					42	
169			200			4		44	
170			150			4		44	
171		340	120			4		44	
172	3.39		240			4		44	
173	3.39		160			4		48	
174	3.44	294	160			4		48	
175	3.48		260			4		52	
169-175	3.43	317	184					46	
176	3.49		200	4.0	8.0			52	
177	3.46		260					52	
178	3.54	368	170	3.3	5.6			44	
179	3.54	362	150	1.1	1.7			44	
180	3.58	214	90	0.3	0.3			44	
181	3.56	310	90			4		44	
182	3.56	203	50			4		44	
176-182	3.53	291	144	2.2	3.9			46	

Number :— **T 20 - continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
183	3.61		80			4		40	
184	3.56		100			4		40	
185	3.58	420	70			4		40	
186	3.56	331	80			4		40	
187	3.46	362	350	3.8	13.3			40	Biopsy
188	3.32		30			4		40	
189	3.35	414	250	3.4	8.5			40	
183-189	3.49	382	137	3.6	10.9			40	
190	3.40		380			4		40	
191	3.46		80			4		40	Infected Wound Drained.
192	3.40	294				3		40	
193	3.24		80			3		40	
194	3.41		20			2		40	
195	3.11	226	50			3		40	Moribund. Killed.
190-195	3.34	260	122					40	

First Biopsy of Pancreas

The islets, ductules and centro-acinar cells show extreme glycogen infiltration in widespread distribution. No non-vacuolated cells other than alpha cells are to be found in the islets. Mitoses are not evident.

Second Biopsy of Pancreas

No trace of glycogen is demonstrable in any type of cell. The islets appear to be made up of large numbers of alpha cells with a lesser proportion of agranular types which rarely contain well differentiated beta granules. No evidence of mitotic proliferation is found.

Number:— T 20 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

Gross Examination:

A large subcutaneous incisional abscess can be traced into the left upper portion of the peritoneal cavity where fibrino-purulent exudate coats the liver, spleen and stomach. The urinary bladder contains 70 ccs. of urine which gives a 2 plus "Galatest" reaction and a zero "Acetonetest" reaction.

Microscopic Examination:

Pancreas: Essentially the same appearance is noted as was found in the second biopsy, but there is the complication of sero-fibrinous inflammation.

Kidneys: No glycogen can be demonstrated. The cytoplasm of the convoluted tubules shows hyaline droplets and fatty vacuoles are evident in Henle's loop.

Liver: The subcapsular zones are distorted by inflammatory edema related to the adjacent peritonitis. Elsewhere the lobules are intact. Scant cytoplasmic glycogen is demonstrable.

Adrenals: Not remarkable.

Testes: Not remarkable.

Number:— T 21 Male

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-8 to 0	2.65	108f							
1-7	2.73	349f				4	3		
8-14	2.89	395f				4	0		
15-21	3.00	338f				4	0		
22-28	3.30	518				4	0		
29-35						4	0		
36-42	3.24	422f				4	0		
43-49	3.26	392f				4	0		
50-56						4	0		
57-63		422f				4	0		
64-71	3.32	416f				4	0		
72-77						4	0		
78-84	3.54	464f				4	0		
85-91						4	0		
92-98						4	0		
99-105	3.50	466f				4	0		
106-112	3.75					4	0		
113-119	3.78	512				4	0		
120-126						4	0		
127-133	3.30	460f				4	0		
134-140						4	0		
141-147			850			4	0		
148-154	3.46	496f				4	0	4	Biopsy 153rd day Died 154th day.

Biopsy of Pancreas

Glycogen infiltration is readily apparent in islets, ductules and centro-acinar cells. Some islets are comprised entirely of alpha cells while others contain a few vacuolated cells or agranular types. Two small lobules show apparent proliferation by mitotic division of ductular epithelium to form new acinar and islet elements.

Number:— T 21 Male - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

Gross Examination:

An incisional hematoma is noted in association with herniation through the peritoneal layer of a 6-inch length of hyperemic small bowel. The bladder is distended by over 200 ccs. of urine. This reacts 2 plus with "Acetonetest" and 4 plus with "Galatest."

Microscopic Examination:

Pancreas: Identical with biopsy (v.s.)

Kidneys: There is marked "glycogen nephrosis" and a very mild degree of calcification in the cortex.

Liver: Liver cells in the central lobular zones contain cytoplasmic glycogen: in the mid-zones there is conspicuous but mild fatty change. Peripherally there are a few scattered glycogen-infiltrated nuclei.

Adrenals: The cortical cells contain but little lipid.

Pituitary: Not remarkable.

Testes: Not remarkable.

Number :— T 23

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-7 to 0	1.97	124f							Alloxan 200
1-7	2.36					4		4	
8-14	2.50	448f				4		4	
15-21	2.63	402f						0	
22-28	2.77	480f						0	
29-35								0	
36-42	2.72	436f						0	
43-49	2.77	442f						0	
50-56								0	
57-63		480f						0	
64-70	2.81	562f						0	
71-77								0	
78-84	2.80	484f						0	
85-91								0	
92-98	2.84	464f						0	Bilateral cataracts
99-105								0	
106-112	2.68							0	
113-119	3.13	572f						0	
120-126								0	
127-133	2.44	484f						0	
134-140								0	
141-147	2.69		910					0	
148-154	2.56	568f						0	Marked diarrhoea.
155-161	2.70		450					0	
162-168	2.43		390					0	
169-175	2.36		350					0	
176-182						15.0		0	
1-185		485f	525						
183								0	
184								0	
185								0	
186	2.43	485						8	
187	2.61	414	270	3.8	10.3			12	
188	2.97	400	330	3.4	11.2			16	Biopsy
189	2.76		120					20	Ascites. Splenomegaly
183-189	2.69	425	240	3.6	10.8			8	

Number:— T 23 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
190	2.71	176	240	0.6	1.4			24	
191	2.83		220	0.2	0.4			24	
192	2.77		330					24	
193	2.53	470	220	2.8	6.2			28	
194	2.45	280						24	
195	2.35		200			4			Found dead 9:00 A.M.
190-196	2.61	309	240	(1.2)	2.7			25	

Biopsy of Pancreas

(There was marked ascites and a large spleen.)

There is glycogen infiltration of the ductular epithelial cells of moderate degree. The islets are composed of about equal numbers of alpha cells and others. These are characteristically devoid of beta granules for the most part, while some of them contain obvious small deposits of glycogen. There is no mitotic proliferation.

Autopsy

Gross Examination:

A subcutaneous inflammatory reaction beneath the surgical excision leads to the left upper portion of the peritoneal cavity where fibrinopurulent exudate coats the viscera. A hemorrhagic extravasation extends in the tissues along the spleen toward the pancreas. The urinary bladder is empty; its mucosa reacts strongly to both "Galatest" and "Acetonetest".

Number:— T 23 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: No glycogen can b e demonstrated in the ductular epithelium while traces of it are present in islet cells which appear slightly vacuolar in the Gomori and H.P.S. stained sections. Some well differentiated beta cells with typical granules are found while numerous agranular forms are present. There is no suggestion of mitotic proliferation.

Kidneys: Widespread, minimal accumulations of glycogen are apparent in the loops of Henle. Hyaline droplets are seen in many cells of the convoluted tubules.

Liver: Glycogen is not apparent in the cytoplasm or nuclei of the hepatic patenchymal cells. The peritoneal exudate is beginning to become organized by granulation tissue arising in the subcapsular portions of the liver.

Adrenals: Not remarkable.

Pituitary: Not remarkable.

Testes: Spermatogenesis is active.

Number :— T 25

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	2.03	132f							Alloxan 200
1-7	2.66	130				4	0		
8-14	2.77	497				4	0		
15-21	2.72	460f				4	0		
22-28	2.80	610				4	0		
29-35						4	0		
36-42	2.51	392f				4	0		
43-49	2.32	368f				4	0		
50-56						4	0		
57-63		604f				4	0		
64-70	2.49	586f				4	0		
71-77						4	0		
78-84	2.50	580f				4	0		
85-91						4	0		
92-98						4	0		
99-105	2.25	564f				4	0		
106-112	2.24					4	0		
113-119						4	0		
120-126	2.25	592f				4	0		
127-133	2.16		460	3.6	16.6	4	0		
134-140	2.34	624						0	Biopsy. Post-op. death
1-140		518f							

Autopsy

Gross Examination:

Peritoneal cavity contains in excess of 60 ccs. of fluid and clotted blood. Pancreatic stump appears hemorrhagic. The lungs are edematous and hyperemic, with small patches of atelectasis. There is slight hydrothorax, bilateral. The urinary bladder contains 150 ccs. of urine; this reacts strongly positive for glucose by "Galatest".

Number:— T 25 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: (Biopsy and Autopsy appearances are identical and are described together.) The islets contain many glycogen-filled cells, numerous agranular cells and many (a majority of) alpha cells. The ductules are massively infiltrated by glycogen and many centro-acinar cells are similarly affected. Several lobules of proliferating pancreatic tissue are found. These are characterized by apparent new formation of acinar and islet cells from ductules which show numerous mitoses. In the newly formed islets about half the cells are of alpha type while the remainder appear agranular but not vacuolated.

Number:— T 37

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-0	1.70	108f							200 Alloxan
1-7	2.00	452				4	4		
8-14	2.09	472f				4	4		
15-21						4	0		
22-28	2.32	444f				4	0		
29-35	2.18					4	0		
36-42	2.56	664				4	0		
43-49						4	0		
50-56	2.60	532				4	0		
57-63						4	0		
64-70						4	0		
71-77	2.70	472f				4	0		
78-84						4	0		
85-91						4	0		Died
1-91		463f 549							Autopsy

Autopsy

Gross Examination:

No structural abnormalities are evident.

Microscopic Examination:

Pancreas: The islets are moderately severely infiltrated by glycogen while the ductular epithelium is everywhere severely affected. No evidence of mitotic activity is demonstrated.

Number:— T 53

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	3.28	109f							Alloxan 175
1-7		665				4	4		
8-14	3.24	464f 648				4	4		
15-21	2.67	608				4	0		
22-28	2.53	454f 428				4	0		
29-35	2.26	210f 370				4	0		
36-42	2.13	375f				4	0		
43-49	2.23	320f 626				4	0		
50-56	2.36					4	4		Died
1-56		365f 557							

Autopsy

Gross Examination:

Both kidneys and all the abdominal, thoracic and subcutaneous lymph nodes are enlarged and extensively replaced by a caseous, necrotic reaction. The urinary bladder contains 50 ccs. of urine which gives 4 plus reactions with both "Galatest" and "Acetonetest".

Number:— T 53 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Microscopic Examination:

Pancreas: Islets are very rare; each is composed almost entirely of alpha cells, but rare vacuolated cells (glycogen infiltrated) are encountered. The ductules show moderately severe glycogen infiltration. There are no mitoses.

Number:— T 55

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	3.18	114f							Alloxan 175
1-7		(476)				4	4		
8-14	3.37	336f 540				4	4		
15-21	3.04	(456)				4	1		
22-28	3.18	496f 552				4	0		
29-35	2.82	376f 454				4	0		
36-42	2.89	457f				4	1		
43-49	3.12	474f 562				4	1		
50-56	2.90	461f 509	370	4.4	16.3	4	0		
57-63			600	3.2	19.2	4	0		
64-70	3.00	570f							
71-77									Cataracts, bilateral
78-84									
85-91	2.63	491f	670				2		
92-98	2.66		825	5.0	41.3	4	1		
99-105	2.71	(712)							Died
1-105		458f 533	616		25.6				

Number:— T 55 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

Gross Examination:

Urinary bladder mucosa reacts 4 plus to "Galatest" and 2 plus to "Acetonetest". Peritoneal cavity contains 75 ccs. of clear fluid. Eyes show obvious cataracts. There is slight visceral post mortem decomposition.

Microscopic Examination:

Pancreas: Glycogen infiltration of the pancreatic islets, ductules and centro-acinar cells is obvious in moderate to severe extent. Numerous agranular islet cells are present. There are no evident mitotic figures.

Kidney: "Glycogen nephrosis" is present in severe degree.

Number:— T 64

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	2.39	101f							Alloxan 150
1-7	2.27	571							
8-14	2.40	526f							
15-21	2.54	387f 454							
22-28	2.90	(660)							
29-35	2.76	479f 532							
43-49									
50-56	2.83	441f							
57-63	2.80	425f							
64-70	2.74	335f							
71-77									
78-84	2.78	(518)	620	4.4	27.0				Cataract, left
85-91	2.85	466f	770	4.6	35.1				
1-91		440f 547	700	4.5	31.1				
92		772	950	4.5	42.8			8	Biopsy (+4 CZ1)
93	3.06	148	510			4		4	
94		244	90	2.2	2.0			4	
95	2.87	256	50			4		4	
96	2.92	524	120	1.9	2.3			4	
97	3.11	503	130	1.2	1.6			4	
98		476	570	2.3	13.1			4	
92-98	2.99	418	346	2.4	12.4			5	

Number:— T 64 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
99	2.92	608	240	4.3	10.3			4	
100	2.70	642	710	3.6	25.6			8	
101	3.10	508	410	5.2	21.3			12	
102	3.18	360	140	3.6	4.0			12	
103	3.24	464	360	2.3	8.7			12	
104	3.14	630	470			4		12	
105	3.17	292	290	4.5	13.1			12	
99-105	3.06	501	374	3.9	13.8			10	
106	3.25	396	30			4		4	
107	3.36	474	410	3.3	13.5			4	
108	3.08	629	290	4.0	11.6			4	
109	3.10	638	420	3.9	16.4			6	
110	3.15	570	320	4.4	14.1			6	
111	3.21	575	290	3.8	11.0			8	
112	3.35	260f	140			4	4		(Biopsy 2:30 P.M.
106-112	3.21	547	271	3.9	13.3			5	(Alloxan 200 9:30 P.M.
113	2.94	100	170			4			Convulsions. Killed.

First Biopsy of Pancreas

Glycogen infiltration of pancreatic ductular epithelial cells and of islets is apparent in moderate severity. There is no evidence of mitotic proliferation.

Second Biopsy of Pancreas

The ductules contain conspicuous but small deposits of cytoplasmic glycogen. Rare islet cells contain traces of glycogen while the majority of beta cells are agranular.

Number:— T 64 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

Autopsy

Gross Examination:

The visceral tissues show no lesions. In the urinary bladder a putty-like mass weighing 85 grams is found; there is no urine. The bladder mucosa gives a 4 plus "Galatest" reaction.

Microscopic Examination:

Pancreas: The appearances of all sections are identical with those of the second biopsy. No effects of the second dose of alloxan are apparent.

Kidneys: Small traces of glycogen are seen in the cells of Henle's loop and the distal convolution.

Liver: A "normal" distribution of small amounts of glycogen is noted.

Adrenals: Not remarkable.

Pituitary: Not remarkable.

Number :—T 67

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
-6 to 0	2.45	127f							Alloxan 150
1-7	2.50	471							
8-14									
15-21	2.57	305f							
22-28	2.70	460							
29-35	2.72	344f							
36-42	2.79	359f 458							
43-49	2.75	394f							
50-56	2.75		490	4.7	23.0				
57-63	2.78	352f							
64-70			550	6.6	36.3				
71-77	2.83	364f							
78-84	2.82	438	540	4.2	22.7				
85-91	2.85	282f	420	5.5	23.1				
1-95		354f 458	528	5.4	28.4				N.B. These values incl. 1-95!
92		520	640	5.8	37.1			0	
93	2.77f	424f						4	
94		403						0	
95	2.83f	362f					4	2 (+4 CZI)	Biopsy 2:00 P.M.

Number :— **T 67 - continued**

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
96	2.77	300	120					2	
97	2.69	327	110	2.0	2.2	4		4	
98	2.65	591	160	2.0	3.2	4		2	
92-98	2.70	428	130*	2.0*	2.7*			3	*Excluding 92nd day
99	2.62	510	400	5.2	20.8			2	
100	2.56	508	440	6.3	27.7			4	Sutures removed.
101	2.71	484	220	6.2	13.6			12	
102	2.77	242	230	5.0	11.5			12	
103	2.74	344	140	5.5	7.7			10	
104	2.71	418	160			4		10	
105	2.71	418	120	5.1	6.1			10	
99-105	2.69	418	244	5.6	14.6			9	
106	2.75	236	60			4		4	
107	2.77	312	100	3.7	3.7			4	
108	2.78	442	90	3.2	2.9			4	
109	2.79	423	100	2.6	2.6			4	
110	2.78	362	130	5.4	7.0			4	
111	2.81	380	30			4		4	
112	2.86	205	60			4		4	
106-112	2.79	323	81	3.7	4.1			4	
113	2.78	226	50			4		0	
114	2.71	330	170	4.3	7.3			2	
115	2.75	368	90	4.9	4.4			2	
116	2.74	401	150	3.3	5.0			2	
117	2.76	390	140	5.4	7.6			2	
118		421	150	6.3	9.5			0	
119	2.74	460				4		2	
113-119	2.75	371	125	4.8	6.8			1	

Number:— T 67 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours) Volume (ccs.)	Glucose Grams/100 ccs.	Glucose Grams/24 hours	"Galatest"	"Acetonetest"	Protamine Zinc Insulin (units, s.c.)	
120	2.73	376	200			4		2	
121	2.73	414	30			4		2	
122	2.74	360	170			4		6	
123	2.80	212	70			4		10	Biopsy
124	2.71		100			4		4	
125								0	
126	2.68	368	150			4		4	
120-126	2.73	346	120			4		4	
127	2.72	406	70			4		4	
128								0	
129								0	
130	2.69	454	40			4		12	
131	2.78	406	10			4		10	
132	2.81	174						10	
133	2.81	113	60					10	
127-133	2.75	311	45					7	
134	2.89	168	40			4		6	
135	2.84	127	50			4		6	
136			60			4		0	
137	2.79	290	110			4		4	
138	2.80	346	80			4		4	
139	2.80	374	250			4		4	
140	2.81	384	150			4		4	
134-140	2.82	282	106			4		4	
141	2.82	352	120			4		6	
142	2.82	414	160			4		4	
143									Killed
(141-142)	(2.82)	(383)	(140)			(4)		(5)	

Number:— T 67 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

First Biopsy of Pancreas

Moderate to marked glycogen infiltration of islets and ductules is obvious. No mitoses are seen.

Second Biopsy of Pancreas

Definite persistence of glycogen deposits is noted in all of the previously affected types of cells but in very much smaller quantities. In addition, numerous lobules of proliferating pancreatic tissue show newly formed islets and acini arising from ductules in which mitotic figures are numerous. Both alpha cells and beta cells are apparently differentiating; the latter do not contain cytoplasmic glycogen. Rare mitoses in islet cells are seen.

Autopsy

Gross Examination:

No lesions are found in any organ or system with the exception of delicate fibrous adhesions in the operative field.

Microscopic Examination:

Pancreas: No traces of glycogen are demonstrable in any type

Number:— T 67 - continued

Days after alloxan	Body Weight (kilograms)	Blood Sugar (mg./100 cc.)	URINALYSIS (preceding 24 hours)					Protamine Zinc Insulin (units, s.c.)	
			Volume (ccs.)	Glucose		"Galatest"	"Acetonetest"		
				Grams/100 ccs.	Grams/24 hours				

(Pancreas - cont'd.)

of cell. The beta cells do not contain demonstrable granules. Marked proliferation of ductules to form acini and islets, as previously described in the second biopsy, is apparent.

Kidneys: Traces of glycogen are demonstrable in the distal convoluted tubules.

Liver: Some glycogen is present in the cells of the central zones of the liver lobules.

Adrenals: Not remarkable.

Pituitary: Not remarkable.