INFORMATION TO USERS

This dissertation was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again – beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

A Xerox Education Company

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

EXPERIMENTAL DIABETES MELLITUS IN BABOONS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

BY

CHIU HSING TSENG, M.D.

Oklahoma City, Oklahoma

1972

EXPERIMENTAL DIABETES MELLITUS IN BABOONS

APPROVED BY

Clarke Kent

Jacqueline Condon

Robert Coulson

M Jack Kyle

Jo R. Ryan

DISSERTATION COMMITTEE

ACKNOWLEDGMENTS

It is with sincerest appreciation that I acknowlege the assistance rendered to me by all those with whom I have been associated during the period of this project at the Renal Pathology Laboratory of the University of Oklahoma.

I am deeply grateful to Dr. L. Clarke Stout, Interim Chairman of the Department of Pathology, for his kindness in serving as major advisor and chairman of the reading committee. Appreciation is also expressed to the members of my examination and dissertation committee, Dr. Jacqueline J. Coalson, Dr. Robert E. Coalson, Dr. A. L. Dee, Dr. M. Jack Keyl, Dr. Fay K. Myers, for their assistance.

Special thanks is due my former advisor, the late Dr. Paul Kimmelstiel, for giving me the ability to understand the concepts of Renal Pathology. Also, special thanks is due Dr. Benjamin Spargo, visiting Professor of Renal Pathology from the University of Chicago, for his valuable time and guidance.

Appreciation is extended to Dr. D. E. Parker, statistician from the Department of Biostatistics, and also to the Nephrologists, Drs. Robert D. Lindeman, James Wenzl, and T. Morita, for their interest and help.

This investigation was supported by the John A. Hartford Foundation, Inc.

Finally, my deep appreciation goes to my parents, brother, sisters and daughter for their patience and encouragement, and to my wife for her support and helpfulness in the preparation and typing of the dissertation.

TABLE OF CONTENTS

LIST OF TABLES

EXPERIMENTAL DIABETES MELLITUS IN BABOONS

CHAPTER I

INTRODUCTION

Significance of Proposed Research

The microangiopathic changes which have been described in capillaries of muscle and skin (1, 3, 26, 58) in diabetics are morphologically similar to the changes found in diffuse, and possibly also nodular glomerulosclerosis in diabetics. Although proof is lacking, the concept that the microangiopathic changes in muscle capillaries and renal glomeruli in diabetics have a similar pathogenesis is an appealing one. Recent studies (52, 53) in humans have suggested that the microangiopathic changes in muscle capillaries in diabetes may be due to a separate hereditary trait rather than to poor therapeutic control of the diabetic process. This hypothesis has not as yet been universally accepted. If it is proven to be true, then the rigid control of the blood sugar level in human diabetic patients might be unnecessary. On the other hand, if the microangiopathic changes are due to the chronic elevation of blood sugar level or other factors present in uncontrolled diabetes mellitus, then the careful regulation of the diabetic process would be justified. It is difficult to approach this problem directly in humans, since the necessary renal biopsy procedures are too risky to be done repeatedly,

and it is almost impossible to satisfactorily identify individuals who will develop diabetes in the future, and to obtain "before and after" observations. The above problem has been approached through the study of animals with artifically induced diabetes mellitus. Observation of the glomeruli of the kidney are made before and after the creation of diabetes by pancreatectomy. Only one such study (24) has previously been reported in non-human primates, and the results suggested a relationship between both poor diabetic control and a high carbohydrate diet and microangiopathy.

Although the microangiopathic changes which occur in human diabetic patients have been clearly documented (1, 3, 8, 10, 11, 15, 19, 26, 35, 36 37, 48, 51, 53, 58), the way in which these lesions develop is not well understood. The problem of "before and after" sampling is again a serious impediment to the study of this process in humans. Means of circumventing this problem are limited to the study of human tissues shortly after the onset of diabetes. It seems unlikely that diabetes has a sudden onset, however, even though the first overt manifestation of the disease may appear during a short period of time. It is now clear that microangiopathic changes do occur in dogs (12) and monkeys (24) with experimental diabetes, even though some disagreement exists as to whether or not the lesions are exactly similar to those in human diabetics. The sequential examination of renal biopsies in diabetic baboons should allow us to observe the microangiopathic changes in all stages of their development.

Review of History

Glomerular Structure in Human Diabetes

The first description of a glomerular lesion characteristic of diabetes was made by Kimmelstiel and Wilson (36), who observed what they called "intercapillary glomerulosclerosis". Since this important publication, a multitude of papers concerning the pathology of the kidney in diabetes has appeared.

Diabetic glomerulosclerosis comprises three groups of lesions: the diffuse, the nodular, and the exudative.

The term exudative lesion has been used for the fibrinoid cap alone, and for the fibrinoid cap as well as the capsular drop and arteriolar hyaline change. Salinas-Madrigal (50) suggested "insudative" lesion instead of "exudative", because he thought there was no true exudation of material; rather, the process represented insudation into the capillary or arteriolar walls.

The ultrastructure of human diabetic glomerulosclerosis has been elucidated by a great number of investigations reported during the late 1950's and 1960's, the work of Irvine et al. (31), and of Bergstrand and Bucht (9, 10) being among the first, followed by Farquhar et al. (19), Kimmelstiel et al. (34, 35), Bloodworth (11), and Dachs et al. (15).

It was early ascertained that a thickening of the peripheral basement membrane was characteristic of diabetes. This thickening can be uniform or irregular. A thickening of up to ten times greater than normal has been reported. The amount of mesangial matrix is also increased and this is most often described as a deposition of a basement membrane-like material in the mesangial region. When these changes are

of moderate degree, they seem to correlate with the light microscopic picture of diffuse glomerulosclerosis. The mesangial lesion, however, can progress, and when the deposition of basement membrane-like material in this region has reached a high degree, a nodular lesion is formed. The general point of view is that these processes, the peripheral and the mesangial, progress side by side. Kimmelstiel et al. (35), however, think that they develop independently, and state that nodular glomerulosclerosis may occur without thickening of the peripheral basement membrane. In the most severe lesions, collagenous fibrils can be identified in the condensed mesangial regions.

It is generally accepted that it takes several years for nodular glomerulosclerosis to develop in patients with diabetes. Opinions are divided however, as to the time of appearance of the diffuse glomerular lesion. During the last few years some investigators have claimed that a thickening of the basement membrane could be observed by electron microscopy very early in the disease, and in some cases at the time of initial diagnosis (11). A few reports on basement membrane thickening in so-called prediabetes have also appeared (16).

Since the duration of newly discovered diabetes in old patients is very uncertain, investigations of the basement membrane have been conducted in patients with recent onset juvenile diabetes. Investigations by Kimmelstiel et al. (35), on the contrary, found the peripheral basement membrane of normal thickness in cases of recent diabetes in young patients.

There are also some investigations of glomerular structure in pancreatic diabetes. Lonergan and Robbins (39) studied postmortem renal

sections from sixty two patients with hemochromatosis and diabetes, the duration of diabetes being five to nineteen years in twenty one of the patients. They did not observe intercapillary glomerulosclerosis in any of these cases. Nevertheless, Becker and Miller (6), in twenty two patients with hemochromatosis and associated diabetes, found four with diffuse and nodular glomerular lesions and three with only diffuse changes. There were no cases of glomerulosclerosis in thirty cases of hemochromatosis without diabetes.

Electron microscopic studies of glomerular capillaries were made by MacDonald and Ireland (43) on five cases of pancreatic disease with accompanying diabetes. The glomeruli were normal by light microscopy in all cases except one, where there was an early diffuse lesion. A recent study by Ireland et al. (30), showed a definite increase in thickness of the basement membrane in seven of the secondary diabetics.

Glomerular Lesions in Experimental Diabetes

Permanent diabetes can be produced in animals in several ways (21, 55). A few of these methods have been used in investigations of renal pathology in experimental diabetes and only those will be mentioned in the following.

Alloxan was introduced as a substance producing permanent hyperglycemia by Bruschwig et al. (13), based on the discovery by Dunn et al. (18), that alloxan was a selective beta cell toxin. In a number of species, alloxan produces a permanent diabetes. The fact that alloxan is definitely toxic to renal tissue, especially the tubular epithelium, is of importance in evaluating the effect of alloxan diabetes

on the kidney. Alloxan is rapidly eliminated from the blood (38) and this fact can be used in a procedure aimed at protection of the kidney during the short phase of beta cell destruction (45). Alloxan has been the most extensively used substance in producing diabetes in studies of glomerulosclerosis. In a single study, Foglia et al. (21), used pancreatectomy. The main reason for its limited use in experimental diabetes seems to be the technical difficulties associated with removal of the pancreas in many species.

Many hormones cause temporary alterations in carbohydrate metabolism. In 1937, Young (57) reported the production of a permanent diabetic state in dogs following a limited number of injections with anterior pituitary extract. Later investigations showed that the active agent was growth hormone. The action of this hormone is thought to be the provocation of a temporary hyperfunction of the beta cells in the pancreatic islets, which is followed by degeneration and, eventually, necrosis. When this phase has been reached, only alpha cells can be identified in the islets and a permanent diabetic stage has been produced.

Most studies of experimental diabetic nephropathy have been performed in rats.

In the study of Foglia et al. (21), diabetes was induced in rats by pancreatectomy. After two to twelve months of hyperglycemia, 88% of the rats had glomerular lesions. The mesangial areas were increased and the walls of the glomerular capillaries were thickened.

Gerritzen et al. (23), made five rats diabetic with alloxan for ten months. They were not able to observe any changes in the glomeruli

(or other organs) comparable to those observed in human diabetes. Nevertheless, their report describes and illustrates hyaline swelling of the capillary walls in the glomeruli.

Alloxan diabetes in the golden hamster was studied by Beaser et al. (4, 5). The animals were treated with protamine zinc insulin. Different degrees of glomerulosclerosis were observed, the most severe of which were PAS-positive (Periodic acid-Schiff) irregular thickenings of the peripheral capillary walls. Nodular lesions of the Kimmelstiel-Wilson type were not seen.

Glomerulosclerosis in the diabetic dog was first described by Lukens and Dohan (41). One dog, made diabetic by injections of pituitary extract, survived for five years. The dog received several short courses of insulin therapy which consisted of thirty to thirty six units of an unspecified type of insulin. The glomerular lesions resembled the early changes of human intercapillary glomerulosclerosis.

Bloodworth (12) made dogs diabetic with growth hormone (five animals) and with alloxan (five animals). The duration of the diabetes was from about one to six years. The diabetic dogs received from ten to twenty five units of neutral protein Hagedorn (NPH) insulin (Lilly) daily. All of the diabetic dogs developed diffuse glomerulosclerosis, that is, PAS-positive thickening of the mesangial region or, in the most severe cases, almost complete obliteration of the glomerular area. By electron microscopy, the capillary basement membrane was estimated as being from two to four times thicker than in the controls. This change was seen in all diabetic animals and in almost every capillary loop studied, but the thickening was not uniform. The next phase in the

development of the glomerular lesion was the formation or proliferation of irregular interdigitating branches of basement membrane-like material in the mesangial areas. Bloodworth considered the process to advance as follows. There was a gradual formation of irregular projections of basement membrane-like material or "basement membrane branches" inward from the basement membrane into the cytoplasm of the underlying cell. The process occurred in the peripheral as well as the mesangial parts of the loops. As the process continued, the "mesangial matrix" was gradually incorporated into the basement membrane-like material in this area. The last step was the formation of the typical nodule, which was described as a meshwork of interdigitating basement membrane-like branches in which nuclei and small islands of cytoplasm were trapped. Endothelial cells and mesangial cells showed swelling and proliferation, and this change was correlated directly with the amount of basement membrane thickening and proliferation of basement membrane-like branches.

The glomerular ultrastructure in alloxan diabetic monkeys was studied by Gibbs et al. (24). Lesions of the glomeruli consisted of accumulations of basement membrane-like material in the glomerular tufts. There was thickening of the capillary basement membrane and some swelling of endothelial cytoplasm.

The ultrastructure of the alloxan diabetic rat was studied by Osterby et al. (46). An increase in the mesangial area was seen by electron microscopy. A diffuse thickening of the peripheral basement membrane was noted in the capillaries of several glomeruli, but the thickening varied. None of basement membrane changes were present in control animals. The epithelial cells showed some cytoplasmic changes,

that is, the presence of large dense bodies and giant bodies with myelin figures. A pronounced fusion of foot processes was only occasionally observed.

General Objective and Specific Aims

In reviewing the history, it is apparent that the published ultrastructual observations of the glomeruli in experimental diabetes are incomplete, especially in the primate. The aim of this study is to sequentially document the early changes which occur in the renal glomeruli in depancreatized baboons.

The previous studies of glomerular changes in experimental diabetes have been concerned with determining whether or not changes develop, and with a comparison of carefully versus poorly managed (diabetic) animals. In the latter study, the inclusion of too many variables precluded a satisfactory pathogenetic interpretation of the structual changes.

Present knowledge suggests that diabetic glomerulosclerosis is produced by a diffuse thickening of the glomerular capillary basement membrane, or by proliferation of basement membrane-like material in the mesangium (or both). In the present study, six animals who have been depancreatized for twenty two to twenty five months are examined. These animals have had renal biopsies at intervals of six to twelve months, so that control and sequential experimental materials are available. The sequential study of renal glomeruli in an adequate sample of similarly managed animals should allow us to observe the quantitative and qualitative changes in the depancreatized primate model.

CHAPTER II

MATERIALS AND METHODS

The colony has nineteen pancreatectomized baboons whose ages vary from four to more than ten years, and who have been diabetic for one to four years. Six of these animals with similar duration of diabetes, varying from twenty two months to twenty five months, were selected for study in order to concentrate on early changes. The age of these six animals varied from four and one half years to old age.

These animals were maintained on similar diets of 200 grams Purina chow twice daily, plus apples, carrots and oranges with water ad lib, and insulin. The insulin dosage was adjusted as necessary to maintain three + to four + glycosuria at 8:00 A.M. and one + to two + glycosuria at 4:00 P.M. The urine sugars were checked daily by using dip reagent strips (14). The measurement of blood glucose (49) was obtained from each animal at approximately three to four month intervals.

The average blood glucose varied from 277 mgs% to 494 mgs% with an average of 395 mgs% for the colony, and 360 mgs% to 494 mgs% with an average of 418 mgs% for the six animals to be studied. The urine sugar varied from three + to four + at 8:00 A.M. and one + to two + at 4:00 P.M. for the colony, and varied from three + to four + at 8:00 A.M. and one + to two + at 4:00 P.M. for the six animals. Each baboon received

daily Insulin (NPH) varying from six to twenty two units and two units of regular insulin to maintain the urine glucose of three + to four + at 8:00 A.M. and one + to two + at 4:00 P.M. The various examinations including serum urea nitrogen (28), serum creatinine (22), urine protein (44) and blood pressure were performed at appropriate time period. The blood pressure was measured in the right arm using a pediatric sphygmomanometer cuff and stethoscope, six minutes after injection of Sernylan (phencyclidine HCl), ten to sixteen mgs intramuscularly.

Renal biopsies were obtained from each animal at the time of pancreatectomy or during the time when one kidney of each animal was translocated underneath the skin. The serial needle biopsies were obtained from the translocated kidney at nine to twelve month intervals for a duration of two years. The pancreatectomy was performed three weeks after the translocation of the kidney. To control for the possible effect of translocation on renal morphology, two animals received bilateral renal biopsies after approximately two years of diabetes. One baboon whose kidney was translocated without pancreatectomy also served for control purposes. In this animal the kidney was biopsied one and two years after translocation.

Tissues for light microscopy were fixed in Carnoy II solution, embedded in paraffin, sectioned at two micra and stained with Hematoxylin-eosin (H & E) and Periodic acid-Schiff (PAS) stain.

Tissues for electron microscopy were fixed in osmium tetroxide (42), embedded in Epon (20, 25, 40), sectioned at 0.1 micra and stained with uranyl acetate and Reynold's lead stain (47). The details of technique of preparation for electron microscopy were as follows:

Preparation of Stock Buffer Solutions

The s-collidine buffer (0.2M) was prepared by putting s-collidine (2, 4, 6-trimethyl pyridine) (especially purified for EM) 2.67 cc and double distilled water 60-70 cc into 100 cc volumetric flask. It was adjusted to pH 7.42-7.43 by addition of 1 N HCl.

Preparation of Fixing Solution

Equal parts of the buffer solution and the 4% osmium tetroxide solution were mixed. For one biopsy specimen 1 cc of each was sufficient. The solution was kept on ice from the time of preparation to the end of the fixing period.

Embedding in Epon 812

1. The tissue was fixed for two hours.
2. Rinsed two to three times in s-collidine buffer.
3. Placed directly into dehydrating solutions of increasing percentages of alcohol, beginning with 50% if embedding procedure is followed directly.
   a) 50% ethyl alcohol ..... 10 minutes
   b) 70% ethyl alcohol ..... 10 minutes
   c) 95% ethyl alcohol ..... 10 minutes
   d) 100% ethyl alcohol ..... 10 minutes
4. Infiltration:
   a) Propylene oxide ..... 15 minutes
   b) Propylene oxide ..... 15 minutes
   c) Propylene oxide-epon Epoxy mixture 1 : 1 for one hour.
   d) Propylene oxide-epon Epoxy mixture 1 : 2 for six hours. If

necessary the tissue can be left overnight.

e) Epon epoxy mixture for one hour.

f) Embed in Epon epoxy mixture in capsule.

Epon epoxy resin mixture:

Mixture A	5.0cc
Mixture B	5.0cc
2,4,6-tri (dimethylaminomethyl)-phenol..... (DMP-30)	0.2cc

A and B mixtures, propylene oxide and each resin were kept in the refrigerator in air and moisture tight containers. They were removed and left at room temperature for at least three hours before use. Any solution that appeared cloudy or showed any moldy growth was not used.

The embedding mixture of A & B was stirred for five minutes, then added to the DMP and stirred for five more minutes. The capsules were filled three fourths full and the tissue embedded. The specimens were allowed settle to the bottom of each capsule and then centered. The specimens were then placed in the oven for polymerization.

5. Polymerization:

a) 35 degrees C. ..... 12 - 24 hours

b) 40 degrees C. ..... 12 - 24 hours

c) 60 degrees C. ..... 12 - 24 hours

The specimens were removed from oven after complete polymerization and put into containers for storage. Moisture exposure was avoided.

Staining, Examination and Photography

The specimens for electron microscopic observation were sectioned with the Porter-Blum ultramicrotome fitted with glass knives.

Ultra-thin sections were mounted on uncoated copper 300 mesh specimen screens for electron microscopic study.

Ultra-thin sections were double stained with uranyl acetate and Reynold's lead citrate stain.

The stained, ultra-thin sections were examined with an RCA EMU-3F electron microscope.

When desired fields were viewed in the electron microscope, photographic plates were exposed to the electron beam to secure appropriate electron micrographic data.

Procedure for Micrograph Interpretation

The following procedure was designed to reduce the number of subjective factors involved in the evaluation of the electronmicrographs.

As previously described, the tissue from each renal biopsy was embedded individually in Epon. A technician labeled each block with a numerical tag. The tag number and respective date of biopsy were recorded for later reference. The blocks were then mixed in a container and randomly selected for sectioning by the investigator, who was unfamiliar with the correlation between tag number, identity of animal and duration of diabetes. This step was initiated to ensure a typical representation of each case and to reduce the possibility of any selective sectioning.

One glomerulus from each serial biopsy was subsequently selected

for sectioning according to size, with the largest receiving priority. This criterion was based on the observation that the equator of a glomerulus presents the optimal level for evaluation. Once selected, the glomeruli were randomly sectioned and all of the mesangial areas and capillary loops were photographed.

The newly labeled micrographs were then presented for evaluation to a panel of pathology reviewers (Dr. Stout, Dr. Spargo and the investigator). Each pathologist made his individual interpretations independently. Remarks or significant findings were recorded according to the labels on the photographs. The evaluations of each investigator were then compared and a consensus was reached as to the interpretation of each case, through deliberation and reexamination of the photographs. The final step involved breaking the code and correlating the interpretations with the duration of diabetes.

Methodology of Quantitative Study of Glomeruli

In addition to the above studies, which were conducted primarily at the ultrastructural level, light microscopic evaluation of the glomeruli was done using the quantitative methods recently developed by Iidaka *et al.* (29). These methods allowed the objective measurements of the area occupied by the various structural components of the glomerulus, and consequently, the identification of abnormalities in these components.

Thin paraffin sections of kidney tissue, fixed in Carnoy II solution and stained with PAS, were observed under oil immersion (X1250), Bowman's capsule, the capillary basement membrane, the mesangium, and all nuclei were identified accurately, and a camera lucida drawing was

made. The nuclei of mesangial cells were identified as those cells lying within the network of the PAS-positive fibrils of the matrix; the endothelial cells were situated within the capillary lumen; and the epithelial cells were next to the basement membrane in the urinary space. Polymorphonuclear leukocytes, lymphocytes, and monocytes were omitted. Free cells in capillary blood were also not included in the count. The total glomerular area was determined by guiding the planimeter along the inner surface of Bowman's capsule. Five to ten of the largest glomeruli per case were selected for quantitative analysis.

CHAPTER III

OBSERVATIONS

Quantitative Analysis

The mean total area, mesangial area and nuclei per glomerulus for the six baboons before and approximately one and two years after pancreatectomy are shown in Tables 1, 2 and 3. This research project involves three sets of data. Therefore the T test, which is often used for a two data system, was not applicable in this situation. Instead all of the measurements were statistically tested and analyzed as a repeated measure design with three measurement periods (before, and approximately one and two years after pancreatectomy) (56). This method allows the best interpretation of the data.

The mean differences among the total area per glomerulus for the six baboons are as follows:

Time 1[a]	-	Time 2[b]	1764.3 sq. micron
Time 3[c]	-	Time 2	8172.2 sq. micron
Time 3	-	Time 1	6407.8 sq. micron

The differences between time 3 and time 2, and between time 3 and time 1 are statistically significant ($P < 0.05$). The difference between time 1 and time 2 is not.

a - Time 1: Control observations prior to pancreatectomy
b - Time 2: Observations six to nine months after pancreatectomy
c - Time 3: Observations twenty two to twenty five months after pancreatectomy

TABLE 1

NORMAL CONTROL OBSERVATIONS: TOTAL AREA, MESANGIAL AREA AND NUCLEI PER GLOMERULUS

Baboon	Total area (sq.micron)	Mesangial area (sq.micron)	Mesangial area (% of Total area)	Mesangial Nuclei per 1000sq.micron of Mesangial area	Number of Total Nuclei
#1	14558	2087.5	14.3	13.1	86.4
#2	14427	1565.7	10.9	14.9	68.9
#3	10510	1935.7	16.8	11.6	66.1
#4	12078	1982.0	15.8	14.5	78.9
#5	12556	1794.1	14.4	11.5	74.1
#6	11538	1772.9	15.8	24.1	107.0
Mean	12611.2	1856.3	14.7	14.95	80.21
Standard Deviation	1608.8	184.8	2.1	4.7	15.0

TABLE 2

DIABETIC BABOONS AT SIX TO NINE MONTHS: TOTAL AREA, MESANGIAL AREA AND NUCLEI PER GLOMERULUS

Baboon	Total area (sq.micron)	Mesangial area (sq.micron)	Mesangial area (% of Total area)	Mesangial Nuclei per 1000sq.micron of Mesangial area	Number of Total Nuclei
#1	13345	1858.4	13.5	10.8	62.6
#2	11380	1278.5	11.3	15.8	57.7
#3	11145	1448.0	13.6	10.6	52.9
#4	9423	1535.7	16.0	12.3	61.3
#5	8945	1114.2	12.3	10.2	46.9
#6	10843	1338.1	12.2	16.7	79.6
Mean	10846.8	1428.8	13.1	12.73	60.08
Standard Deviation	1565.8	744.2	1.6	2.8	11.1

TABLE 1

NORMAL CONTROL OBSERVATIONS: TOTAL AREA, MESANGIAL AREA AND NUCLEI PER GLOMERULUS

Baboon	Total area (sq.micron)	Mesangial area (sq.micron)	Mesangial area (% of Total area)	Mesangial Nuclei per 1000sq.micron of Mesangial area	Number of Total Nuclei
#1	14558	2087.5	14.3	13.1	86.4
#2	14427	1565.7	10.9	14.9	68.9
#3	10510	1935.7	16.8	11.6	66.1
#4	12078	1982.0	15.8	14.5	78.9
#5	12556	1794.1	14.4	11.5	74.1
#6	11538	1772.9	15.8	24.1	107.0
Mean	12611.2	1856.3	14.7	14.95	80.21
Standard Deviation	1608.8	184.8	2.1	4.7	15.0

TABLE 2

DIABETIC BABOONS AT SIX TO NINE MONTHS: TOTAL AREA, MESANGIAL AREA AND NUCLEI PER GLOMERULUS

Baboon	Total area (sq.micron)	Mesa… (sq…		…gial per micron …ngial	Number of Total Nuclei
#1	13345				62.6
#2	11380	12…		…8	57.7
#3	11145	1448.…		10.6	52.9
#4	9423	1535.7	16.0	12.3	61.3
#5	8945	1114.2	12.3	10.2	46.9
#6	10843	1338.1	12.2	16.7	79.6
Mean	10846.8	1428.8	13.1	12.73	60.08
Standard Deviation	1565.8	744.2	1.6	2.8	11.1

TABLE 3

DIABETIC BABOONS AT TWENTY TWO TO TWENTY FIVE MONTHS:
TOTAL AREA, MESANGIAL AREA AND
NUCLEI PER GLOMERULUS

Baboon	Total area (sq.micron)	Mesangial area (sq.micron)	Mesangial area (% of Total area)	Mesangial Nuclei per 1000sq.micron of Mesangial area	Number of Total Nuclei
#1	22768	3792.6	16.5	11.9	120.8
#2	20241	3261.0	16.6	13.2	123.0
#3	22608	2730.6	14.3	10.8	94.2
#4	16041	3053.7	19.3	12.8	110.6
#5	18630	2820.0	12.2	12.2	102.9
#6	13826	2519.7	18.2	13.9	92.4
Mean	19019	3029.6	16.1	12.47	107.28
Standard Deviation	3589.3	453.8	2.6	1.1	13.0

The mean differences among the mesangial area per glomerulus for the six baboons are as follows:

Time 1	-	Time 2	427.5 sq. micron
Time 3	-	Time 2	1600.8 sq. micron
Time 3	-	Time 1	1173.3 sq. micron

Each of these differences are significantly different ($P < 0.05$).

The mean differences among the mesangial nuclei per 1000 square micron of mesangial area for the six baboons are as follows:

Time 1	-	Time 2	2.21
Time 2	-	Time 3	0.26
Time 3	-	Time 1	2.48

None of these differences are statistically significant.

The mean differences among the total nuclei (disregarding cell types) per glomerulus for the six baboons are as follows:

Time 1	-	Time 2	20.13
Time 3	-	Time 2	47.20
Time 3	-	Time 1	27.07

Each of these differences are significantly different ($P < 0.05$).

The differential count of nuclei per glomerulus for the six baboons before and approximately one and two years after pancreatectomy are shown in Tables 4, 5 and 6. For statistical purposes the data of Tables 4, 5 and 6 are analyzed as a 3 X 3 factorial arrangement of treatments with repeated measures (56). The three types of cells act as the three levels of one factor and the three measurement periods act as the three levels of the other factor. The primary interest is upon the simple effects of time periods within each cell type.

The means for the mesangial cells are significantly different ($P < 0.05$) at the three time periods. This difference is primarily due

TABLE 4

NORMAL CONTROL OBSERVATIONS: DIFFERENTIAL COUNT OF NUCLEI PER GLOMERULUS

Baboon	Mesangial Cells		Epithelial Cells		Endothelial Cells	
	Number	% of Total	Number	% of Total	Number	% of Total
#1	26.4	30.5	19.6	22.7	40.4	46.8
#2	21.2	30.8	19.1	27.7	28.6	41.5
#3	20.7	31.3	17.6	26.7	27.8	42.0
#4	28.6	36.3	21.9	27.7	28.4	36.0
#5	20.6	27.8	24.7	33.4	28.8	38.8
#6	42.5	39.7	32.6	30.5	31.9	29.8
Mean	26.7	32.7	22.6	28.1	31.0	39.2
Standard Deviation	8.4		5.5		4.8	

TABLE 5

DIABETIC BABOONS AT SIX TO NINE MONTHS: DIFFERENTIAL COUNT OF NUCLEI PER GLOMERULUS

Baboon	Mesangial Cells		Epithelial Cells		Endothelial Cells	
	Number	% of Total	Number	% of Total	Number	% of Total
#1	19.1	30.5	14.4	23.0	29.1	46.5
#2	19.7	34.2	17.4	30.2	20.6	35.6
#3	15.2	28.7	22.2	41.9	15.5	29.2
#4	18.4	30.0	22.8	37.2	20.1	32.8
#5	11.4	24.4	18.3	39.0	17.2	36.6
#6	21.9	27.5	32.1	40.3	25.6	32.2
Mean	17.6	29.2	21.2	35.3	21.4	35.5
Standard Deviation	3.7		6.1		5.1	

TABLE 6

DIABETIC BABOONS AT TWENTY TWO TO TWENTY FIVE MONTHS:
DIFFERENTIAL COUNT OF NUCLEI PER GLOMERULUS

Baboon	Mesangial Cells		Epithelial Cells		Endothelial Cells	
	Number	% of Total	Number	% of Total	Number	% of Total
#1	44.8	37.1	16.1	13.3	59.9	49.6
#2	44.3	36.0	20.9	17.0	57.8	47.0
#3	31.3	33.7	18.4	19.5	44.1	46.8
#4	39.6	35.8	22.0	19.9	49.0	44.3
#5	33.1	32.2	25.0	24.3	44.8	43.5
#6	34.1	36.9	25.9	28.0	32.4	35.1
Mean	37.9	35.3	21.4	20.3	48.0	44.4
Standard Deviation	5.8		3.8		10.1	

to the large number of mesangial cells present at time 3 (twenty two to twenty five months diabetic).

The means for the epithelial cells are not significantly different at the three time periods.

The means for the endothelial cells are significantly different ($P < 0.05$). Again, the primary reason for the difference is due to the large number of endothelial cells present at time 3 (twenty two to twenty five months diabetic).

To test for the possible effect of translocation of the kidney, a quantitative analysis of the glomeruli was done on each kidney in two animals which had been diabetic for approximately two years. The results are shown in Table 7. There was no significant difference between the specimens obtained from the translocated kidney and the non-translocated kidney in the two animals.

To test for the possible long term effects of translocation of the kidney, a quantitative analysis of the glomeruli was done in a non-pancreatectomized baboon. Biopsies were done at one year and two years after translocation. Unfortunately, the biopsy taken at the time of translocation was lost. The results are shown in Table 8. There was no increase in total glomerular size, mesangial area or mesangial cells, and no decrease in epithelial cells, such as were seen in all of the diabetic animals. The changes which were apparent in the quantitative analysis between one and two years in the control animal are difficult to evaluate at the present time. Another analysis at three years after translocation should allow the determination as to whether translocation produces any changes in the glomeruli.

TABLE 7

EFFECT OF TRANSLOCATION OF KIDNEY ON QUANTITATIVE ANALYSIS OF GLOMERULI IN DIABETIC (TWO YEARS) BABOONS

Baboon	Total Area (square micron)	Mes. Area (square micron)	Mes. Area (% of Total)	Mes. N. Density	Mes. Cells Number	Mes. Cells % of Total	Epi. Cells Number	Epi. Cells % of Total	End. Cells Number	End. Cells % of Total	Total Nuclei
Baboon #1, translocated kidney	22768	3792.5	16.5	11.9	44.8	37.1	16.1	13.3	59.9	49.6	120.8
Baboon #1, non-translocated kidney	20509	3582.1	17.2	10.9	37.4	37.6	15.2	15.8	47.0	46.6	98.4
Baboon #4 translocated kidney	16041	3053.7	19.3	12.8	39.6	35.8	22.0	19.9	49.0	44.3	110.6
Baboon #4 non-translocated kidney	17179	2905.2	16.2	13.0	36.6	33.8	25.6	23.0	48.0	43.2	110.4

Mes. - Mesangial; Mes. N. Density - Mesangial Nuclei per 1000 sq.micron of Mesangial Area; Epi. Cells - Epithelial Cells; End. Cells - Endothelial Cells; Mes. Cells - Mesangial Cells.

TABLE 8

EFFECT OF TIME ON QUANTITATIVE ANALYSIS OF GLOMERULI OF TRANSLOCATED KIDNEY IN A NONDIABETIC BABOON

Duration of Renal Translocation	Total Area (square micron)	Mes. Area (square micron)	Mes. Area (% of Total)	Mes. N. Density	Mes. Cells Number	Mes. Cells % of Total	Epi. Cells Number	Epi. Cells % of Total	End. Cells Number	End. Cells % of Total	Total Nuclei
One Year	15288	2088	13.4	13.2	27.6	33.8	14.8	20.9	35.0	45.3	77.4
Two Year	11040	1137	10.3	14.3	24.7	26.1	22.8	26.6	41.5	46.3	89.0

Mes. - Mesangial; Mes. N. Density - Mesangial Nuclei per 1000 sq.micron of Mesangial Area; Mes. Cells - Mesangial Cells; Epi. Cells - Epithelial Cells; End. Cells - Endothelial Cells.

Micellaneous Findings

Various miscellaneous examinations including the fasting blood sugar, serum urea nitrogen, serum creatinine, urine protein and blood pressure are listed in Table 9. Baboon #9, Linus, is a control animal whose pancreas was not removed and whose kidney was not translocated. Baboon #7, Alvin, had the highest serum urea nitrogen (twenty one mg%) with a serum creatinine of 2.4 mg% and a moderate amount of urine protein. He was proven to have pyelonephritis in repeated renal biopsies. Baboon #15, Ora, had a serum creatinine of 1.4 mg%. Her urine cultures showed a significant growth of E. coli. She was treated with antibiotics.

Crystalline structures containing virus-like particles were seen in the cytoplasm of the endothelial cells in both control and diabetic baboons (Figure 22 - Appendix). The presence of these crystalline structures could not be correlated with clinical events.

Fine Structure of the Normal Baboon Glomerulus

Epithelium of the Glomerulus

The visceral epithelial cell of Bowman's capsule is continuous with the parietal epithelial cell of Bowman's capsule at the vascular pole of the renal corpuscle. Ultrastructurally, the epithelial cell cytoplasm has broad extensions of trabeculi with foot processes producing occasional arcades as they cover the external portion of the lamina densa. The cytoplasm of the nuclear region of the epithelial cell contains mitochondria, Golgi apparatus and endoplasmic reticulum. The cytoplasm is occasionally vacuolated and has a spongy

TABLE 9

MISCELLANEOUS EXAMINATIONS

Baboon		Sex	Fasting Blood Sugar(mg%) Before Pancreatectomy	Fasting Blood Sugar(mg%) After Pancreatectomy	Serum Urea Nitrogen (mg%)	Serum Creatinine (mg%)	Urine Protein[b]	Blood Pressure (mm Hg)
# 1	Dona	F	53	402.6	10	0.9	± to +	124/90
# 2	Janice	F	24	392.3	10	1.0	− to ±	116/80
# 3	Clark	M	26	435.0	7	1.1	−	158/100
# 4	Marina	F	46	424.0	11	1.0	± to +	100/82
# 5	Vivian	F	75	359.8	12	1.0	−	140/104
# 6	Hailey	M	79	493.5	9	1.0	−	148/106
# 7	Alvin	M	118	445.6	21	2.4	± to 3+	140/108
# 8	Jerry	M	66	428.4	12	1.1	−	132/104
# 9	Linus	F	85	a	16	0.9	−	106/88
#10	Susan	F	57	393.6	7	1.0	− to +	118/90
#11	Aretha	F	32	339.0	5	1.1	− to ±	120/74
#12	Patty	F	56	367.7	13	1.2	−	152/100
#13	Peddy	M	52	489.3	8	1.3	−	94/64
#14	Gayle	F	22	464.5	9	0.8	± to 2+	100/72
#15	Ora	F	47	444.5	15	1.4	± to +	118/92
#16	Nita	F	73	422.5	12	0.9	± to +	114/78
#17	Pauline	F	42	336.3	13	1.1	− to ±	108/80
#18	Vera	F	43	309.8	9	0.9	− to ±	100/60
#19	Ruby	F	20	277.0	7	1.0	− to ±	122/98
#20	Caledonia	F	43	285.0	10	1.2	−	118/74

a - No pancreatectomy

b - ± : 30 mg/dl; + : 30 mg/dl; 2+ : 100 mg/dl; 3+ : 300 mg/dl; 4+ : 1000 mg/dl

appearance with a fibrillar network. Sometimes the cytoplasm has a beaded appearance.

Capillary Basement Membrane

The basement membrane forms the principal framework of the glomerulus (Figure 9 - Appendix). It completely encloses the glomerular capillaries except at the inner side of the capillary loop where the nucleus of the endothelial cell is situated (Figures 10 and 11-Appendix).

The glomerular capillary basement membrane in the normal baboon is a structure with a central dense layer having a layer of lesser density on either side. The basement membrane has a fine reticular structure. The thickness of the basement membrane varies slightly between different capillary loops of the same glomerulus. On the epithelial side there are occasional focal thickenings which have a hemispherical shape and are composed of material which is indistinguishable from the basement membrane.

Endothelium of the Glomerular Capillary

The inner surface of the glomerular capillary is covered with endothelium. Except for the portion near the nuclear region, the cytoplasm of the endothelial cell appears as a sheet-like extension applied directly to the basement membrane. Occasional discontinuities are noted. In the perinuclear region there are cytoplasmic organelles such as mitochondria, Golgi apparatus and endoplasmic reticulum. The endothelial cells present a free surface at the capillary lumen. They are not separated from each other by bundles of basement membrane-like bars. The cytoplasmic matrix of the endothelial cell is clear in

appearance (Figure 11 - Appendix).

The mesangial cells occupy the center of the axial zones and are surrounded by loose textured basement membrane-like material (Figure 12 - Appendix). The mesangium is composed of mesangial cells embedded in ground substance containing parallel fine filaments. Occasionally the fine filaments are in continuity with fibrils of equal density and thickness within the peripheral portion of the cytoplasm of the mesangial cells.

Fine Structure of the Diabetic Baboon Glomerulus

The ultrastructural changes in the glomeruli after six to nine months of diabetes consist of a variable degree of definite accumulations of basement membrane-like material in the glomerular tufts. At fifteen months of diabetes there is moderate glomerulosclerosis with electron dense deposits in the mesangial area (Figures 13, 14, 15 - Appendix). Increased mesangial matrix with collagenous fibrils is also evident at this stage (Figure 16 - Appendix). After twenty one months of diabetes, the glomerular lesion resembles a lobular sclerosis (Figure 17 - Appendix). There is alteration of the peripheral capillary basement membrane consisting primarily of widening of the lamina rara interna (Figure 18 - Appendix). The peripheral capillary basement membrane also contains irregular densities and has a granular appearance, but is without actual holes. It appears as if the normal basement membrane is "stretching and popping out". The texture is smooth on both sides of the capillary basement membrane (Figure 19 - Appendix). Occasionally there are irregular identations of the capillary basement membrane at the endothelial edge. These irregular identations are excep-

tional rather than usual. Focal collections of electron dense, granular material were seen in the subendothelial area in one of the animals. These lesions resembled trapped complexes (Figure 24-Appendix).

After twenty four months of diabetes, there is a prominent central lobular glomerulosclerosis with lipid and electron dense deposits in the mesangial area (Figures 20, 21 - Appendix). There is a decreased amount of normal mesangial cytoplasmic material and an increased amount of mesangial matrix (Figure 23 - Appendix).

CHAPTER IV

DISCUSSION

The results of the quantitative analyses show a significant increase in the number of glomerular mesangial and endothelial cells, and a significant increase in total glomerular and mesangial area after approximately two years of diabetes. These findings are consistant with the results of a quantitative study of human diabetic glomerulosclerosis (29, 33), and with the observation of Dachs et al. (15), showing an increase of mesangial cells in human diabetic nephropathy.

Although there is a consistant relative increase of epithelial cells (from 28.1% to 35.3% of total nuclei) in the six to nine month diabetic animals (Tables 4, 5), and a consistant relative decrease of epithelial cells (from 35.3% to 20.3% of total nuclei) in the twenty two to twenty five month diabetic animals (Tables 5, 6), the absolute number of epithelial cells in both control and experimental diabetic baboons remains approximately the same. The relative changes in the percentage of epithelial cell are due to the changes in absolute numbers of endothelial and mesangial cells.

The absolute decrease in the total glomerular and mesangial area in association with translocation of the kidney in the control animal is conspicuous. It is difficult to assess this finding in the absence

of any significant light or electronmicroscopic changes in the glomeruli. Moreover, the fact that only two quantitative analyses (at one and two years after translocation) were available makes the evaluation of these absolute figures unfeasable at this time. A repeat quantitative analysis at three years after translocation will hopefully resolve this question.

In view of the historic debate (2, 7, 27, 32) concerning the existence of mesangial cells in the human glomerulus, it was important to examine the normal baboon glomerulus in this regard. This study confirms the existence of distinct mesangial cells within the glomeruli of normal baboons.

A correlation between the intensity of renal damage, as observed in morphological studies, with impairment of renal function can not be made in this study because most of the available renal functional data are within the normal limits (17). We have hesitated to carry out more sophisticated measurements of renal function in the diabetic baboons for fear of inducing pyelonephritis by instrumentation, etc. Other investigators (19) have shown that alterations in glomerular structure in humans are not necessarily correlated with the severity of diabetes as judged by insulin requirement, frequency of diabetic coma, altered renal function or degree of proteinuria.

The ultrastructual changes in the glomeruli of diabetic baboons are progressive. At first there is a hyaline thickening of the mesangial area with a conspicuous increase of basement membrane-like material. This material is arranged in an irregular anastomosing network, and is in continuity with the basement membrane proper. The homogeneous

basement membrane becomes focally thickened. The epithelial cells in some areas show distortion of their foot processes. Gradually, the mesangial matrix is focally replaced by collagen fibrils. Collagen is observed only in the mesangium. The experimental lesion increases in severity with increasing duration of diabetes. A definite glomerulosclerosis, extending from the central lobular area toward the periphery is seen in all of the animals after two years of diabetes. The full blown Kimmelstiel-Wilson nodule was not observed.

The basic lesion in diabetic glomerulosclerosis appears to be a progressive increase in substances which morphologically resemble the normal constituents of the mesangial matrix and the capillary basement membrane. However, it is conceivable that the basement membrane-like material deposited in the mesangium may have a different chemical composition than that which is deposited in the basement membrane proper. From embryologic observations, Suzuki (54) has suggested that mesangial matrix is produced by the mesangial cells. Since the matrix does contain carbohydrate, Dachs et al. (15), have suggested that the overproduction of mesangial matrix in diabetic glomerulosclerosis is related to the disturbances in carbohydrate metabolism. Others have felt that the deposition of abnormal blood constituents in the mesangium may be important. The exact mechanism by which the mesangial matrix becomes increased in diabetic glomerulosclerosis remains unknown. There is a correlation between the number of glomerular mesangial cells and the amount of mesangial area after two years of diabetes in baboons. This correlation is not apparent in the animals which have been diabetic for six to nine months. It is possible that the increase in mesangial

matrix is related to the increased number of mesangial cells, with the latter change being somehow influenced by the disturbance in carbohydrate metabolism.

This study suggests that the development of glomerulosclerosis in baboons with total pancreatectomy is directly related to the chronic hyperglycemia and glycosuria which is produced. The glomerulosclerosis which accompanies experimental diabetes in the baboon closely resembles, both qualitatively and quantitatively, the diffuse glomerulosclerosis which accompanies diabetes mellitus in man. These results in baboons may imply that diabetic glomerulosclerosis in man is due to the diabetic state rather than a separate hereditary defect of the capillaries.

CHAPTER V

SUMMARY

A progressive glomerulosclerosis was demonstrated in baboons made diabetic by total pancreatectomy. The glomerular changes were present in all six animals after twenty two to twenty five months of diabetes. In addition, quantitative analyses of glomeruli showed statistically significant increases in mesangial nuclei, endothelial nuclei, mesangial area, and glomerular area after two years of diabetes in all baboons.

Ultrastructural changes were substantiated by the light microscopic findings. The glomerulosclerosis extended from the central lobular area and involved primarily the mesangium. Some peripheral capillary basement membrane alterations were observed. These consisted of widening with irregular density and a granular appearance of the basement membrane after approximately two years of diabetes. The control baboons (prior to pancreatectomy) showed uniformity of peripheral capillary basement membranes and no evidence of glomerulosclerosis.

One kidney was translocated to the subcutaneous tissue of the flank in each animal to facilitate the acquisition of serial needle biopsy specimens. To test for the possible effects of translocation on the kidney, two baboons had bilateral biopsies after two years of

diabetes. There was no significant quantitative or qualitative difference between the specimens obtained from the translocated kidneys and those obtained from the non-translocated kidney.

The glomerulosclerosis of experimental diabetes in baboons closely resembles the diffuse glomerulosclerosis which occurs in diabetes in man. The development of diabetic nephropathy in baboons suggests that diabetic glomerulosclerosis in man is due to the diabetic state rather than a separate hereditary defect of the capillaries.

BIBLIOGRAPHY

1. Aagenaes, O. and Moe, H. Light and electron microscopic study of skin capillaries of diabetics. Diabetes 10: 253-259, 1961.

2. Allen, A. C. The Kidney, Medical and Surgical Diseases. pp. 554-654 Grune and Stratton, New York, 1951.

3. Banson, B. B. and Lacy, P. E. Diabetic microangiopathy in human toes with emphasis on the ultrastructural change in dermal capillaries. Amer. J. Path., 45: 41-58, 1964.

4. Beaser, S. B., Sak, M. F., Donaldson, G. W., McLaughlin, R. J., and Sommers, S. C. Alloxan diabetes in the golden hamster, Mesocricetus auratus. II Glomerulosclerosis and its relation to diabetes regulation. Diabetes, 13: 49-53, 1964.

5. Beaser, S. B., Sak, M. F., and Sommers, S. C. Influence of insulin therapy and pyelonephritis upon diabetic glomerulosclerosis in hamsters. Metabolism, 12: 704-709, 1963.

6. Becker, D., and Miller, M. Presence of diabetic glomerulosclerosis in patients with hemochromatosis. New Engl. J. Med. 263: 367-373, 1960.

7. Bell, E. T. Renal Diseases, pp. 11-24, Lea and Febiger, Philadelphia, 1947.

8. Bell, E. T. Renal vascular disease in diabetes mellitus. Diabetes 2: 376-389, 1953.

9. Bergstrand, A., and Bucht, H. Electron microscopic investigations on the glomerular lesions in diabetes mellitus. Lab. Invest. 6: 293-300, 1957.

10. Bergstrand, A. and Bucht, H. The glomerular lesions of diabetes mellitus and their electron-microscope appearances. J. Path. Bact. 77: 231-242, 1959.

11. Bloodworth, J. M. B. Diabetic microangiopathy. Diabetes 12: 99-114, 1963.

12. Bloodworth, J. M. B. Experimental diabetic glomerulosclerosis. II The dog. Arch. Path. 79: 113-125, 1965.

13. Brunschwig, A., Allen, J. G., Goldner, M. G., and Gomori, G. Alloxan J. Amer. Med. Assoc. 122: 966-966, 1943.

14. Chertack, M. M., and Sherrick, J. C. Screening for diabetes by the glucose oxidase method. J. Amer. Med. Assoc. 169: 1059-1061, 1957.

15. Dachs, S., Churg, J., Mautner, W. and Grishman, E. Diabetic nephropathy. Amer. J. Path. 44: 155-168, 1964.

16. Daysog, A., Dobson, H. L., and Brennan, J. C. Renal glomerular and vascular lesions in prediabetes and in diabetes mellitus: A study based on renal biopsies. Ann. Intern. Med. 54: 672-684, 1961.

17. de la Pena, A., and Goldzieher, J. W. Ed. in The Baboon In Medical Research: (Clinical parameters of the normal baboon), Second International Symposium. H. Vagtborg pp. 379-389. University of Texas Press, Austin, 1967.

18. Dunn, J. S., Sheehan, H. L., and McLelchic, N. G. B. Necrosis of islets of Langerhans produced experimentally. Lancet 1: 484-487, 1943.

19. Farquhar, M. D., Hopper, J.,Jr., and Moon, H. D. Diabetic glomerulosclerosis. Electron and light microscopic studies. Amer. J. Path. 35: 721-753, 1959.

20. Finck, H. Epoxy resins in electron microscopy. J. Biophysic. and Biochem. Cytol. 7: 27-30, 1960.

21. Foglia, N. G., Mancini, R. E., and Cardeza, A. F. Glomerular lesions in diabetic rat. Arch. Path. 50: 75-83, 1950.

22. Folin, O., and Wu. H. A system of blood analysis. J. Biol. Chem. 38: 81-110, 1919.

23. Gerritzen, F., Noach, E. L., van Wighe, M., and Volk, L. E. M. Insulin deficiency and diabetes. Acta Endocrinol. 25: 91-100, 1957.

24. Gibbs, G. E., Wilson, R. B., and Gifford, H. Glomerulosclerosis in the longterm alloxan diabetic monkey. Diabetes 15: 258-261, 1966.

25. Glauert, A. M., Rogers, G. E., and Glauert, R. H. A new embedding medium for electron microscopy. Nature 178: 803-803, 1956.

26. Goldenberg, S., Alex, M., Joslin, R. A., and Blumenthal, H. T. Non-atheromatous peripheral vascular disease of the lower extremity in diabetes mellitus. Diabetes 8: 261-273, 1959.

27. Goormaghtigh, N. Le Mesangium du floculus glomerulaire. Ses reactions dans la glomerulonephrite aigue et les nephrites hypertensives. Journal d'Urologie. 57: 569-585, 1951.

28. Henry, J. B. Clinical Chemistry, in Clinical Diagnosis by Laboratory Method, I. Davidson and J. B. Henry, Eds. pp. 487-600, W. B. Saunders, Philadelphia, 1969.

29. Iidaka, K., McCoy, J., and Kimmelstiel, P. The glomerular mesangium. A quantitative analysis. Lab. Invest. 19: 573-379, 1968.

30. Ireland, J. T., Patnaik, B. K., and Duncan, L. J. P. Glomerular ultrastructure in secondary diabetics and normal subjects. Diabetes, 16: 628-635, 1967.

31. Irvine, E., Rinehart, J. F., Mortimore, G. E., and Hopper, J., Jr. The ultrastructure of the renal glomerulus in intercapillary glomerulosclerosis. Amer. J. Path. 32: 647-648, 1956.

32. Jones, David B. Inflammation and repair of the glomerulus. Amer. J. Path. 27: 991-1009, 1951.

33. Kawano, K., Arakawa, M., McCoy, J., Porch, J. and Kimmelstiel, P. Quantitative study of glomeruli; focal glomerulonephritis and diabetic glomerulosclerosis. Lab. Invest. 21: 269-275, 1969.

34. Kimmelstiel, P., Kim, O. J., and Beres, J. Studies on renal biopsy specimens with the aid of the electron microscope. I. Glomeruli in diabetes. Amer. J. Clin. Path. 38:270-279, 1962.

35. Kimmelstiel, P., Osawa, G. and Beres, J. Glomerular basement membrane in diabetics. Amer. J. Clin. Path. 45: 21-31, 1966.

36. Kimmelstiel, P. and Wilson, C. Intercapillary lesions in the glomeruli of the kidney. Amer. J. Path. 12: 83-98, 1936.

37. Lannigan, R., Blainey, J. D. and Brewer, D. B. Electron microscopy of the diffuse glomerular lesion in diabetes mellitus with special reference to early changes. J. Path. Bact. 88: 255-261, 1964.

38. Leech, R. S., and Bailey, C. C. Blood alloxan and blood glutathione in rabbits injected with alloxan. J. Biol. Chem. 157: 525-542, 1945.

39. Lonergan, P., and Robbins, S. L. Absence of intercapillary glomerulosclerosis in the diabetic patient with hemochromatosis. New Engl. J. Med. 260: 367-370, 1959.

40. Luft, J. Improvements in epoxy resin embedding methods. J. Biophysic. and Biochem. Cytol. 9: 409-414, 1961.

41. Lukens, F. D. W., and Dohan, F. C. Experimental pituitary diabetes of five years duration with glomerulosclerosis. Arch. Path. 41: 19-24, 1946.

42. Lynn, J. A., Martin, J.H., and Race, G. J. Recent improvements of histologic technics for the combined light and electron microscopic examination of surgical specimens. Amer. J. Clin. Path. 45: 704-713, 1966.

43. MacDonald, M. K., and Ireland, J. T. The glomerular lesion in idiopathic and secondary diabetes, in Aetiology of Diabetes Mellitus and Its Complications, M. P. Cameron and M. O'Connor, Eds. pp. 301-314, Ciba Foundation Colloquia on Endocrinology, Vol. 15, Little, Brown and Co., Boston, 1964.

44. Miller, S. E. Examination of urine, in A Textbook of Clinical Pathology S. E. Miller, Ed., pp. 358-390, William and Wilkins, Baltimore, 1966.

45. Orskov, H., Nielson, K., Olsen, T. S., Rafaelsen, D., and Lundbaek, K. Kidney lesions in rats with severe longterm alloxan diabetes. Diabetologia, 1: 172-179, 1965.

46. Osterby, H. R., Lundbaek, K., Olsen, T. S., and Orskov, H. Kidney lesions in rats with severe longterm alloxan diabetes. III Glomerular ultrastructure. Lab. Invest. 17: 675-692, 1967.

47. Reynolds, E. S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell. Biol. 17: 208-215, 1963.

48. Sabour, M. S., MacDonald, M. K. and Robson, J. S. An electron microscopic study of the human kidney in young diabetic patients with normal renal function. Diabetes 11: 291-295, 1962.

49. Saifer, A. and Gerstenfeld, S. The photometric microdetermination of blood glucose with glucose oxidase. J. Lab. Clin. Med. 51: 448-460, 1958.

50. Salinas-Madrigal, L., Pirani, C. L., and Pollak, V. C. Glomerular "insudative" lesions of diabetic nephropathy, electron microscopic observation. Amer. J. Path. 59: 369-397, 1970.

51. Siperstein, M. D., Colwell, A. R., Sr., and Meyer, K., Eds. Proceedings of the Conference on Blood Vessel Involvement in Diabetes Mellitus. American Institute of Biological Sciences, Washington D. C., Garamond/Pridemark Press, Baltimore, 1964.

52. Siperstein, M. D., Unger, R. H., and Madison, L. L. Further electron microscopic studies of diabetic microangiopathy. Advances Metab. Dis. 1: Suppl., 1: 261-271, 1970.

53. Siperstein, M. D., Unger, R. H., and Madison, L. L. Studies of muscle capillary basement membranes in normal subjects, diabetic, and prediabetic patients. J. Clin. Invest., 47: 1973-1999, 1968.

54. Suzuki, Y. An electron microscopic study of the renal differentiation. II Glomerulus. Keio J. Med. 8:129-144, 1959.

55. Warren, S., Lecompte, P. M., and Legg, M. A. "The Pathology of Diabetes Mellitus". pp. 452-490, Lea and Febiger. Philadelphia, Pennsylvania, 1966.

56. Winer, B. J., Statistical Principles in Experimental Design. pp. 298-378, McGraw-Hill, New York, 1962.

57. Young, F. G. Permanent experimental diabetes produced by pituitary (anterior lobe) injections. Lancet II, 372-374, 1937.

58. Zacks, S. I., Pegues, J. J., and Elliott, F. A. Interstitial muscle capillaries in patients with diabetes mellitus; A light and electron microscope study. Metabolism, 11: 381-393, 1962.

APPENDIX

Figure 1

Photomicrograph of glomerulus from baboon without pancreatectomy. (Hematoxylin - eosin stain, 500X)

Figure 2

Photomicrograph of glomerulus from baboon without pancreatectomy. (PAS stain, 500X)

Figure 1

Figure 2

Figure 3

Photomicrograph of glomerulus from depancreatized baboon, nine months diabetic. The kidney was translocated.
(Hematoxylin - eosin stain, 500X)

Figure 4

Photomicrograph of glomerulus from depancreatized baboon, nine months diabetic. The kidney was translocated.
(PAS stain, 500X)

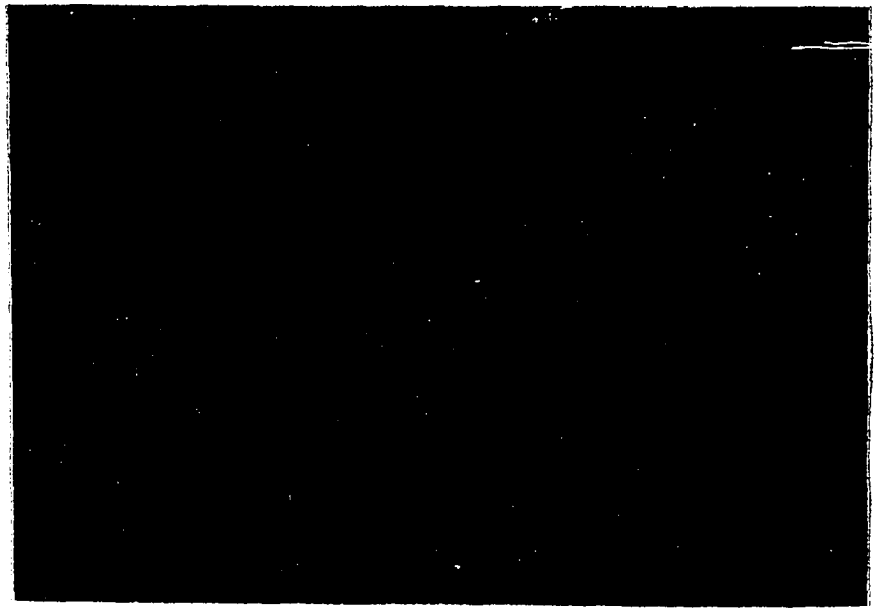

Figure 3

Figure 4

Figure 5

Photomicrograph of glomerulus from depancreatized baboon, 21 months diabetic. The kidney was translocated.
(Hematoxylin eosin stain, 500X)

Figure 6

Photomicrograph of glomerulus from depancreatized baboon, 21 months diabetic. The kidney was translocated.
(PAS stain, 500X)

Figure 5

Figure 6

Figure 7

Photomicrograph of glomerulus from depancreatized baboon, 24 months diabetic. The kidney was not translocated.
(Hematoxylin - eosin stain, 500X)

Figure 8

Photomicrograph of glomerulus from depancreatized baboon, 24 months diabetic. The kidney was not translocated.
(PAS stain, 500X)

Figure 7

Figure 8

Figure 9

Electron photomicrograph of a portion of a glomerulus from control baboon. The basement membrane (BM) forms the principal framework of the glomerulus. The inner surface of the glomerular capillary is covered with endothelium (E). The mesangial cell (M) occupies the center of the axial zone and is surrounded by loose textured basement membrane-like material. The epithelial cell has broad extensions of trabeculi with foot processes (F).

(Reynold's lead citrate and uranyl acetate, 5,780X)

Figure 9

Figure 10

Electron photomicrograph of a portion of a glomerulus from control baboon. Higher magnification showing normal capillary basement membrane (BM).

(Reynold's lead citrate and uranyl acetate, 9,700X)

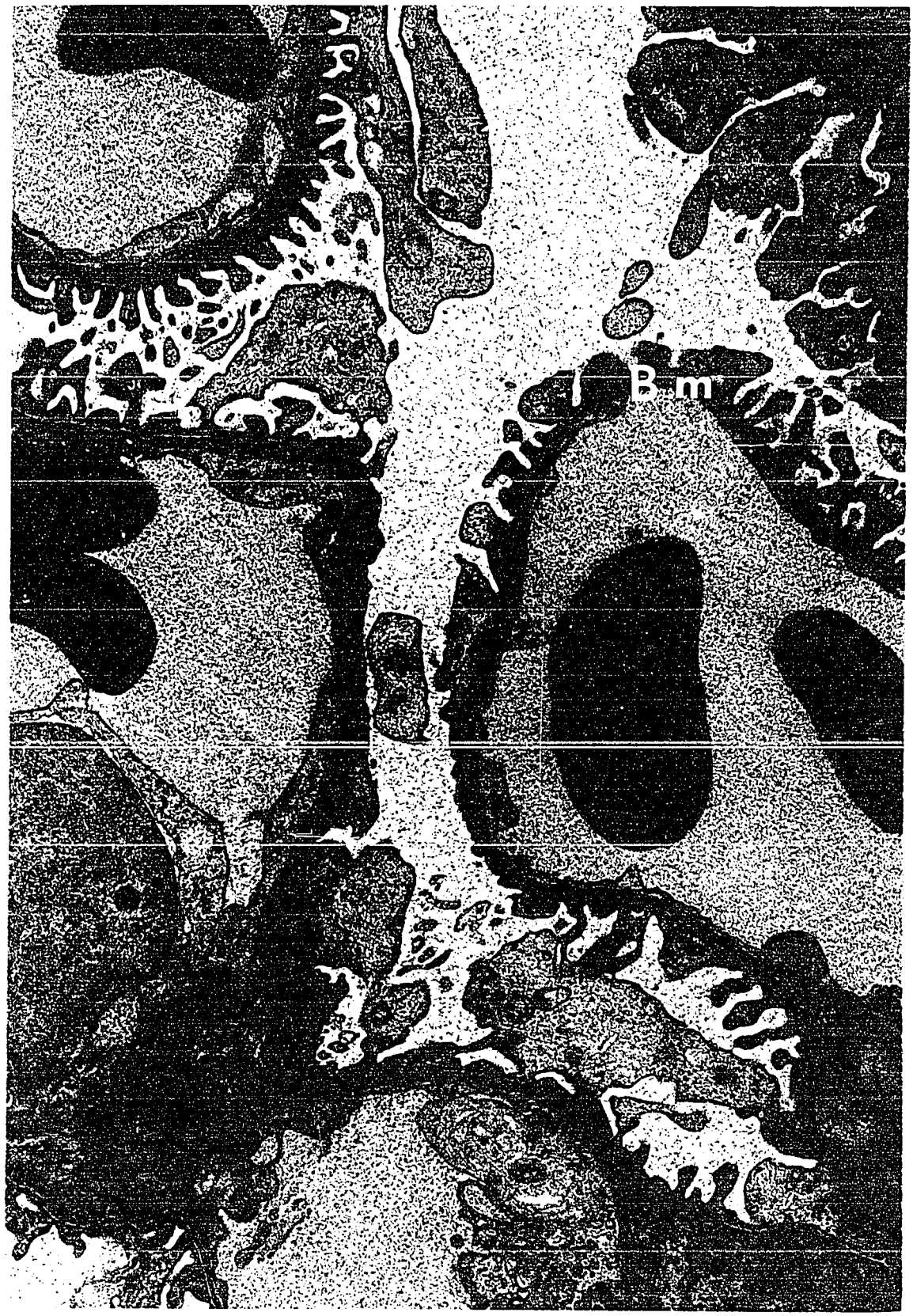

Figure 10

Figure 11

Electron photomicrograph of a portion of a glomerulus from control baboon showing uniformity of basement membrane (BM) and a normal endothelial cell (E).

(Reynold's lead citrate and uranyl acetate, 9,700X)

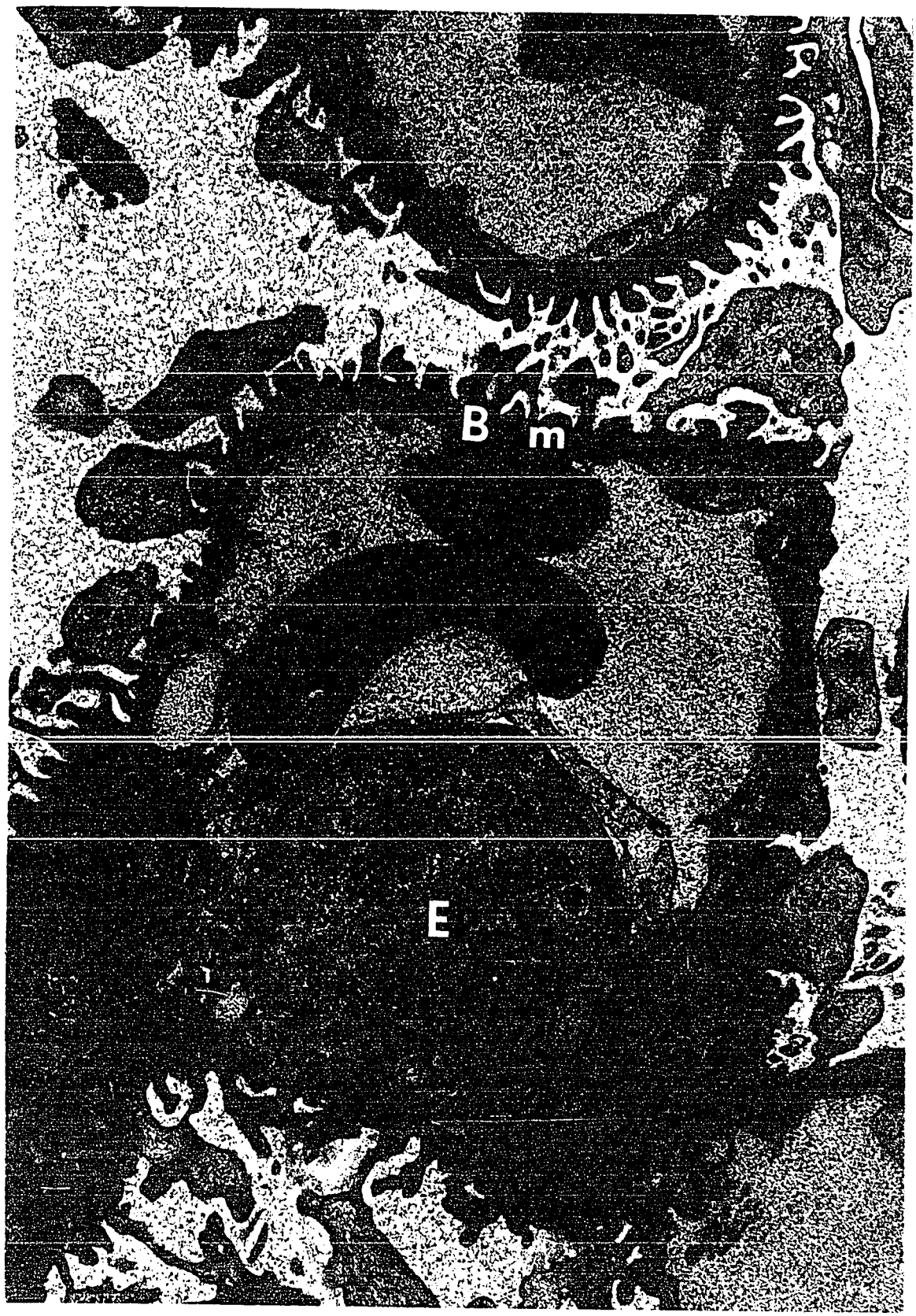

Figure 11

Figure 12

Electron photomicrograph of portion of a glomerulus from control baboon showing normal basement membrane (BM) and a normal mesangial cell (M).

(Reynold's lead citrate and uranyl acetate, 9,700X)

Figure 12

Figure 13

Electron photomicrograph of a portion of a glomerulus from depancreatized baboon, fifteen months diabetic. There is moderate glomerulosclerosis characterized by an increase in basement membrane-like trabeculae (T) and electron dense deposits (D) in the mesangium. The mesangial area contains four mesangial cells. In the peripheral capillary basement membrane there are irregular foldings (arrows). (Reynold's lead citrate and uranyl acetate, 4,670X)

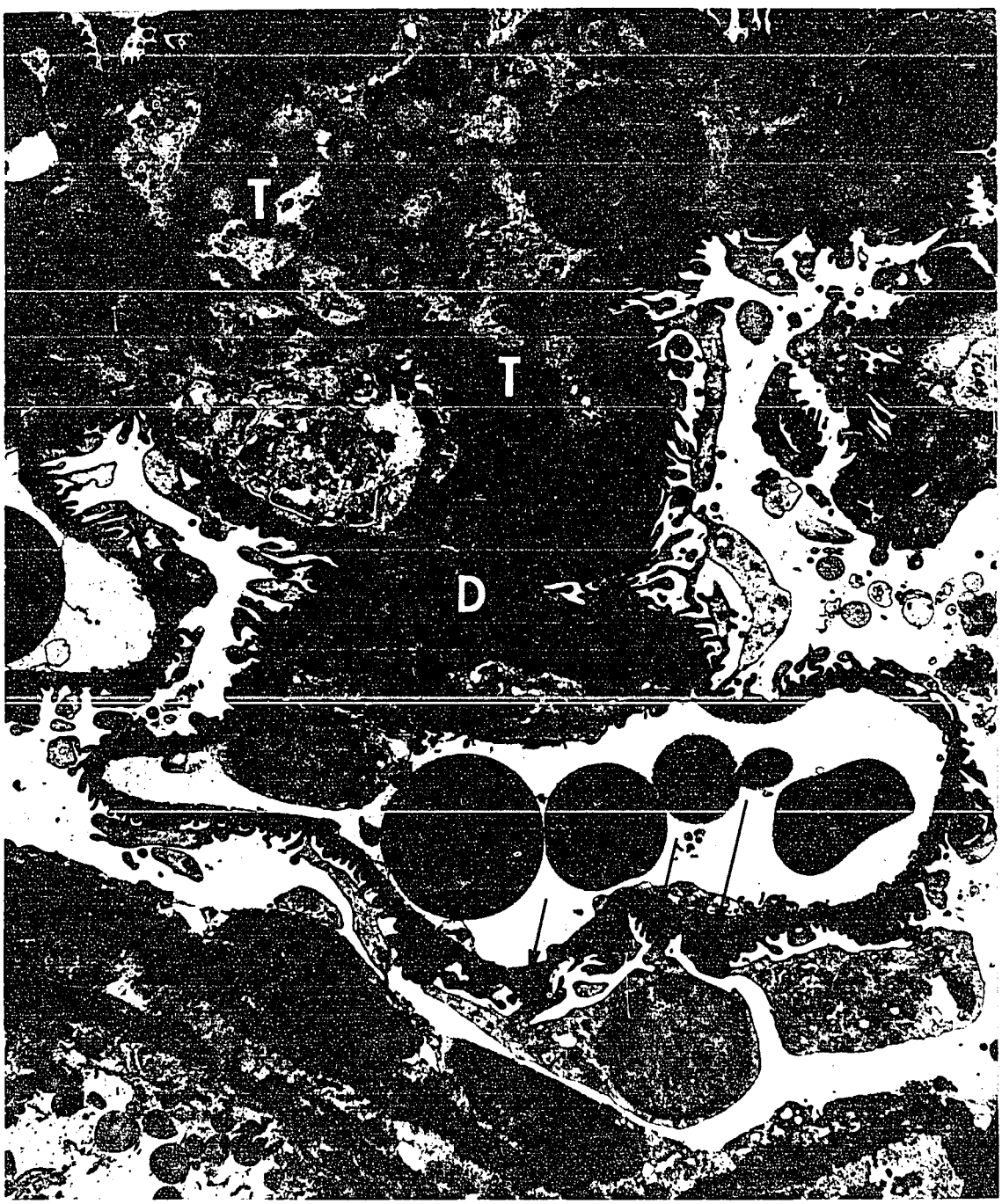

Figure 13

Figure 14

Electron photomicrograph of a portion of a glomerulus from depancreatized baboon, fifteen months diabetic. There is glomerulosclerosis characterized by increased deposits of electron dense material (D) and basement membrane-like trabeculae (T) in the mesangial area. (Reynold's lead citrate and uranyl acetate, 9,700X)

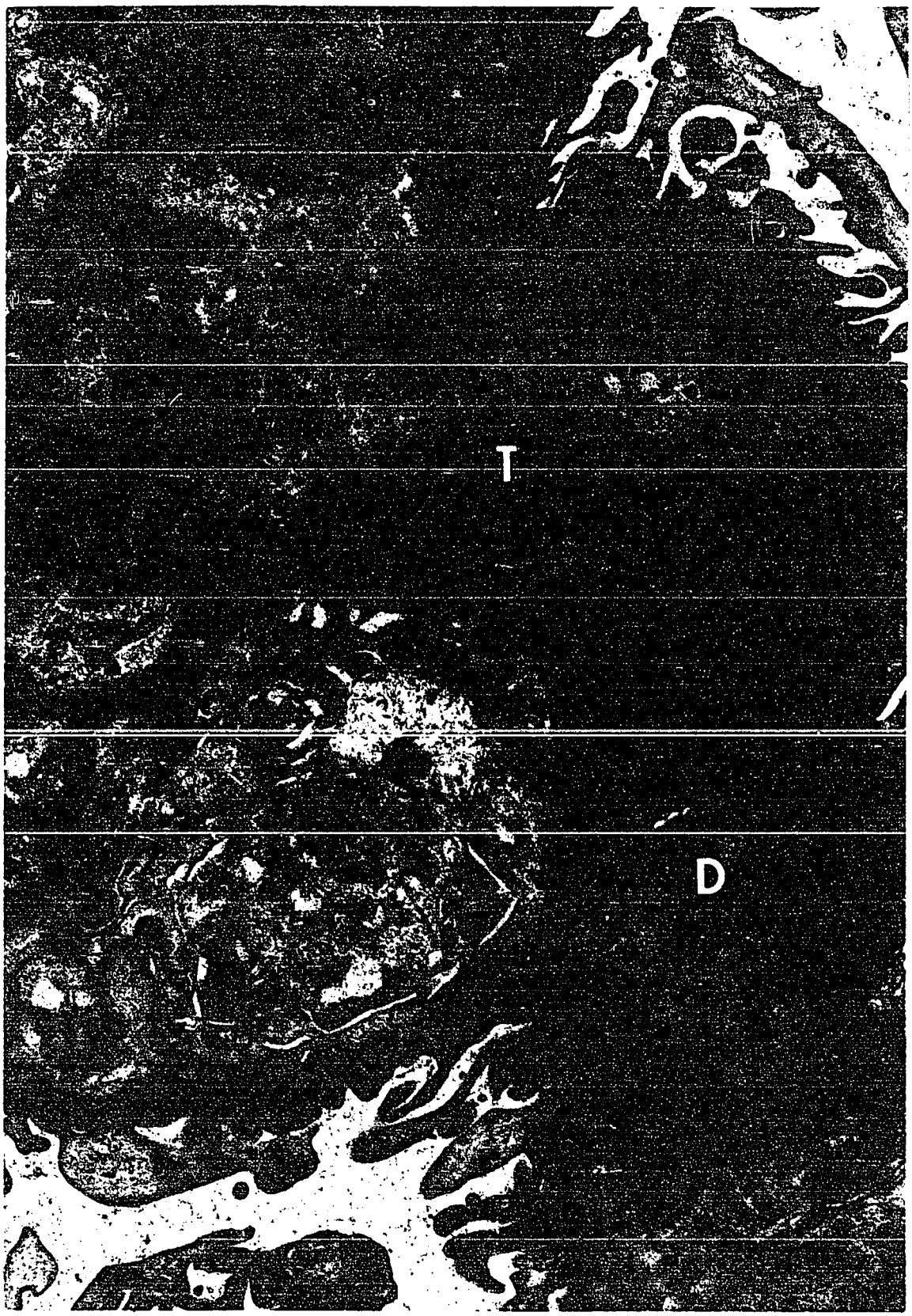

Figure 14

Figure 15

Electron photomicrograph of a portion of a glomerulus from depancreatized baboon, fifteen months diabetic. Higher magnification showing electron dense deposits (D) in the mesangial area.
(Reynold's lead citrate and uranyl acetate, 22,230X)

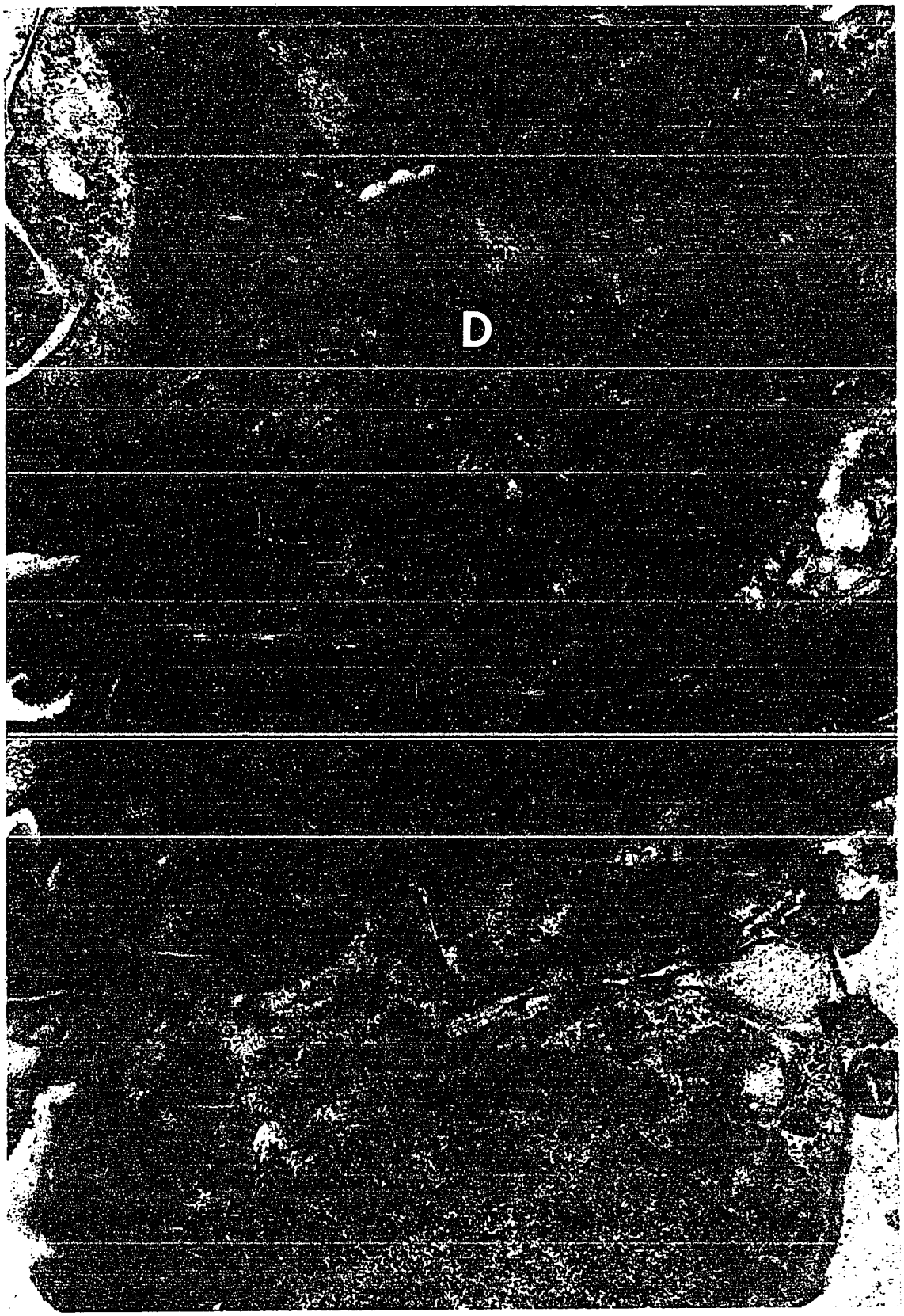

Figure 15

Figure 16

Electron photomicrograph of a portion of a glomerulus from depancreatized baboon, fifteen months diabetic. There are increased deposits of electron dense material (D) and collagenous fibrils (arrows) in the mesangium.

(Reynold's lead citrate and uranyl acetate, 9,700X)

Figure 16

Figure 17

Electron photomicrograph of a portion of a glomerulus from depancreatized baboon, twenty-one months diabetic. There is prominent glomerulosclerosis extending from the central lobular area characterized by increased basement membrane-like trabeculae (T) in the mesangium.

(Reynold's lead citrate and uranyl acetate, 4,670X)

Figure 17

Figure 18

Electron photomicrograph of a portion of a glomerulus from depancreatized baboon, twenty-one months diabetic. In addition to the glomerulosclerosis (GS), there are alterations of the peripheral capillary basement membrane with widening of the lamina rara interna (arrows). (Reynold's lead citrate and uranyl acetate, 4,670X)

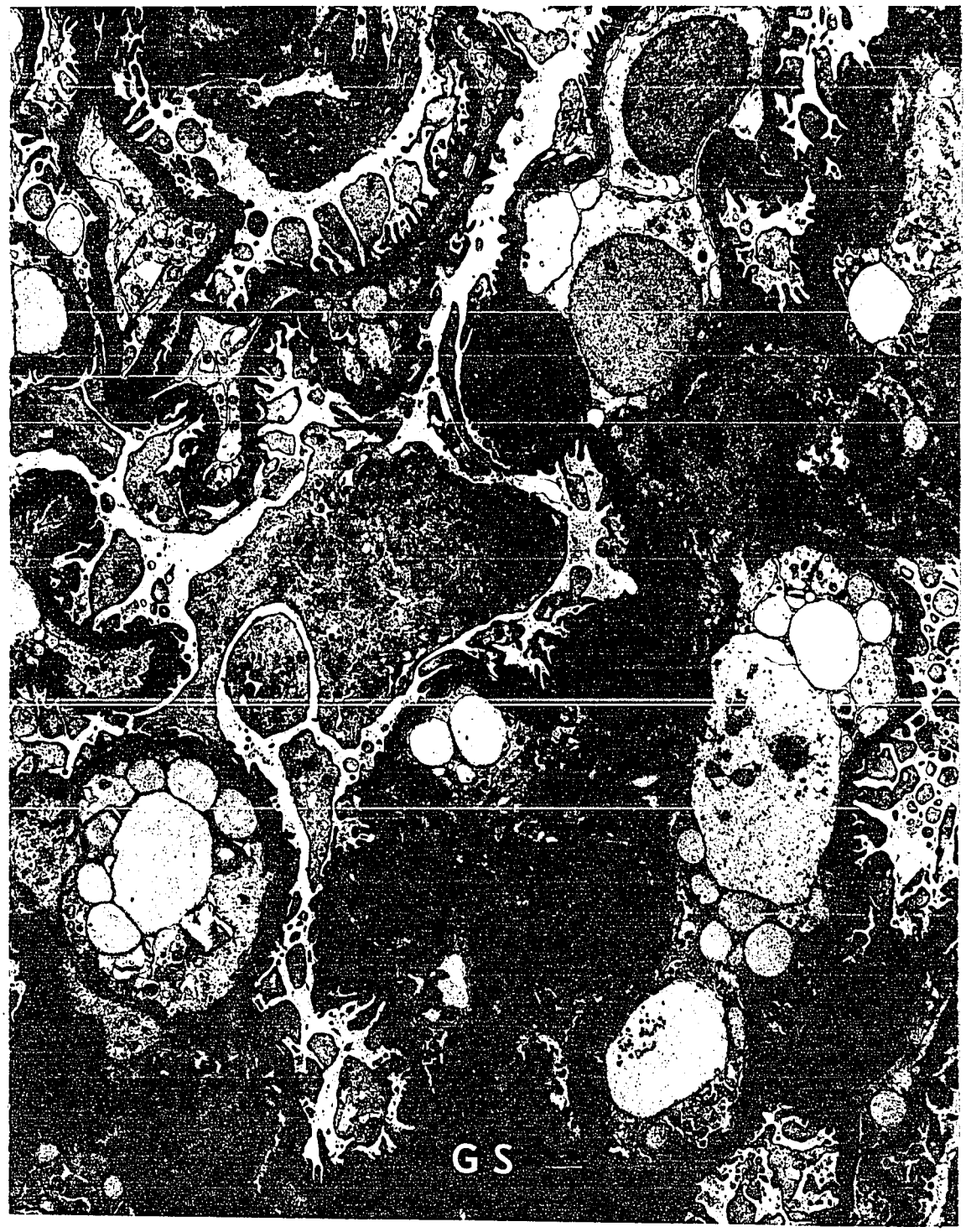

Figure 18

Figure 19

Electron photomicrograph of a portion of a glomerulus from depancreatized baboon, twenty-one months diabetic. Higher magnification shows widening of the peripheral capillary basement membrane (BM) which contains irregular densities and has a granular appearance. In general the texture of the widened basement membrane is smooth, and there are no focal area of destruction or actual holes. Occasionally there are irregular identations (arrows) of the capillary basement membrane on the endothelial edge.

(Reynold's lead citrate and uranyl acetate, 22,230X)

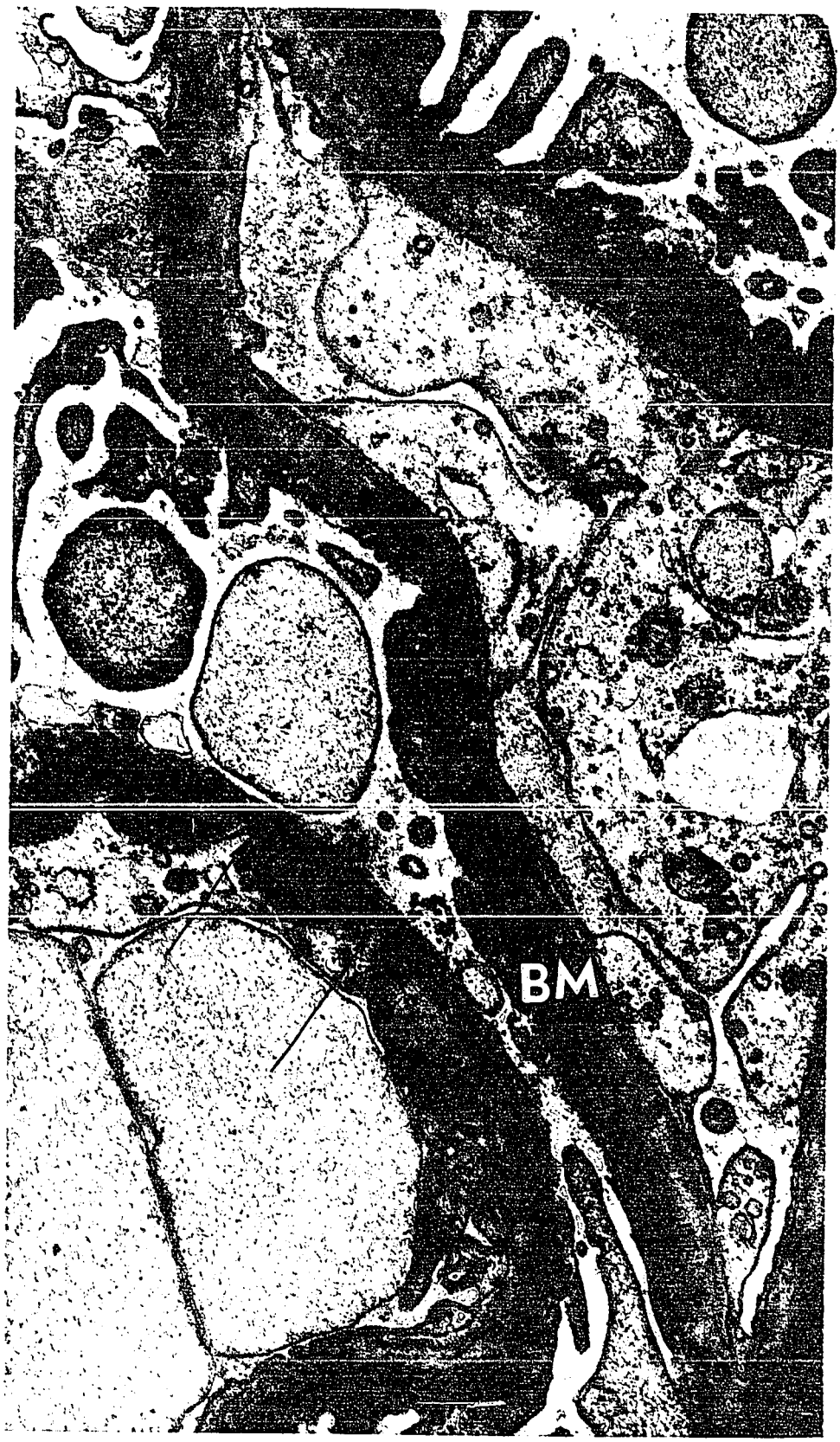

Figure 19

Figure 20

Electron photomicrograph of a portion of a glomerulus from non-translocated kidney of depancreatized baboon, twenty-four months diabetic. There is central lobular glomerulosclerosis with lipid (L) and electron dense deposits (D) in the mesangial area.
(Reynold's lead citrate and uranyl acetate, 4,670X)

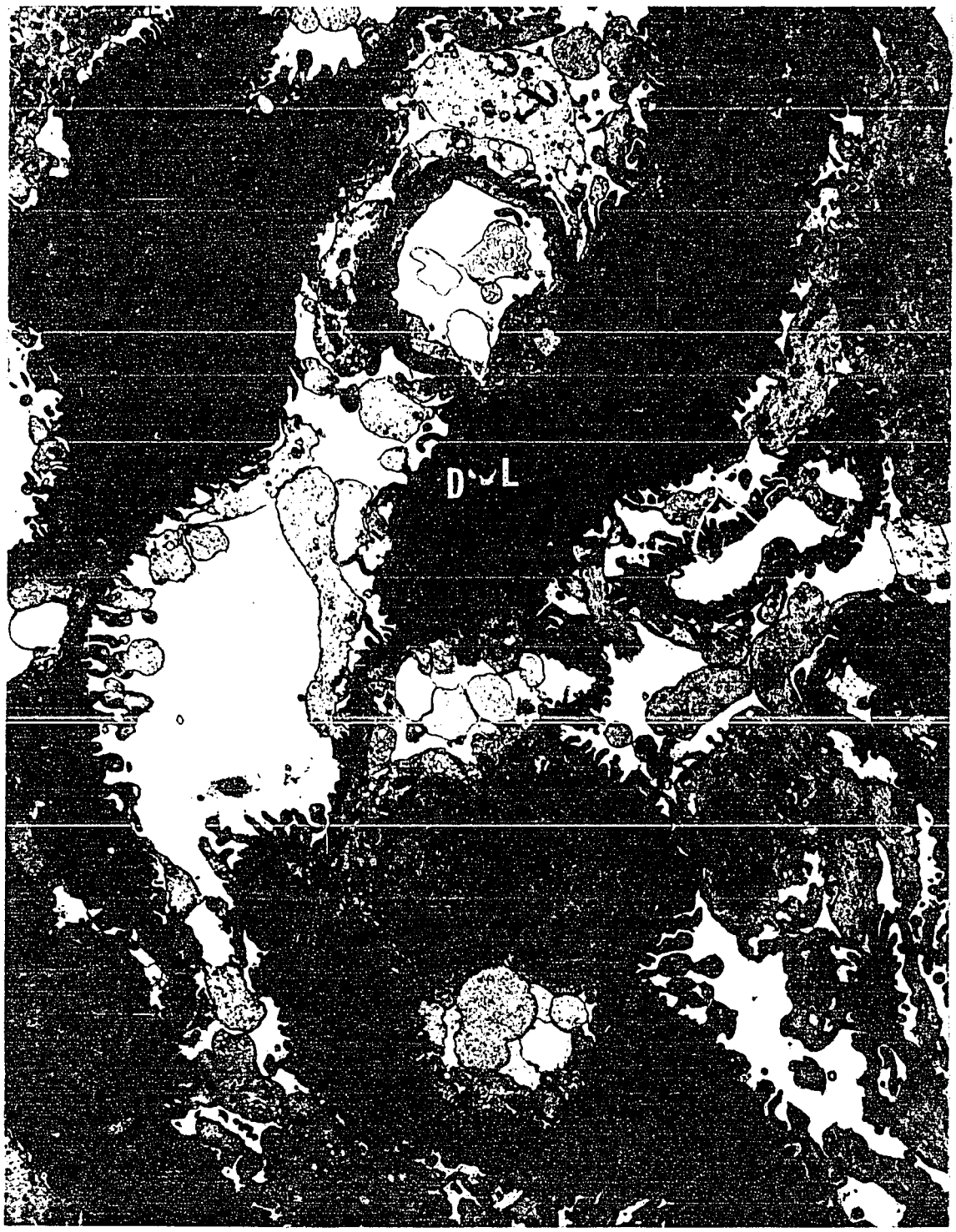

Figure 20

Figure 21

Electron photomicrograph of a portion of a glomerulus from non-translocated kidney of depancreatized baboon, twenty-four months diabetic. Higher magnification showing collagenous fibrils (arrows), and electron dense deposits (D) in the mesangium.
(Reynold's lead citrate and uranyl acetate, 22,230X)

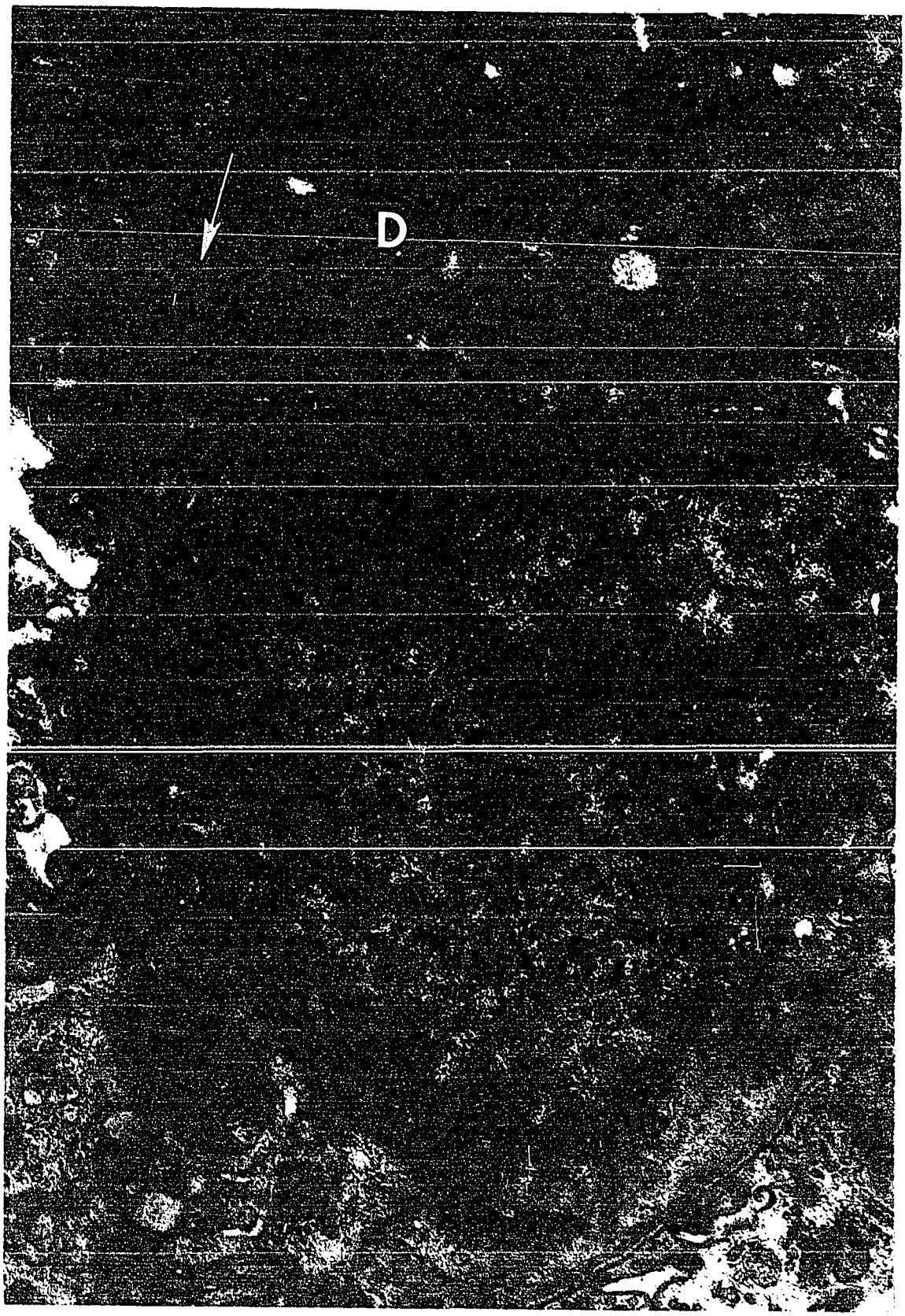

Figure 21

Figure 22

Electron photomicrograph of a portion of a glomerulus from non-translocated kidney of depancreatized baboon, twenty-four months diabetic. Higher magnification showing a crystalline structure (arrow) containing virus-like particles in the cytoplasm of an endothelial cell.

(Reynold's lead citrate and uranyl acetate, 22,230X)

Figure 22

Figure 23

Electron photomicrograph of a portion of a glomerulus from non-translocated kidney of depancreatized baboon, twenty-four months diabetic. There is prominent glomerulosclerosis. In the mesangial area, there is decreased amount of cellular material compared to the matrical(basement membrane-like trabeculae (T) and electron dense deposits (D)) material.

(Reynold's lead citrate and uranyl acetate, 9,700X)

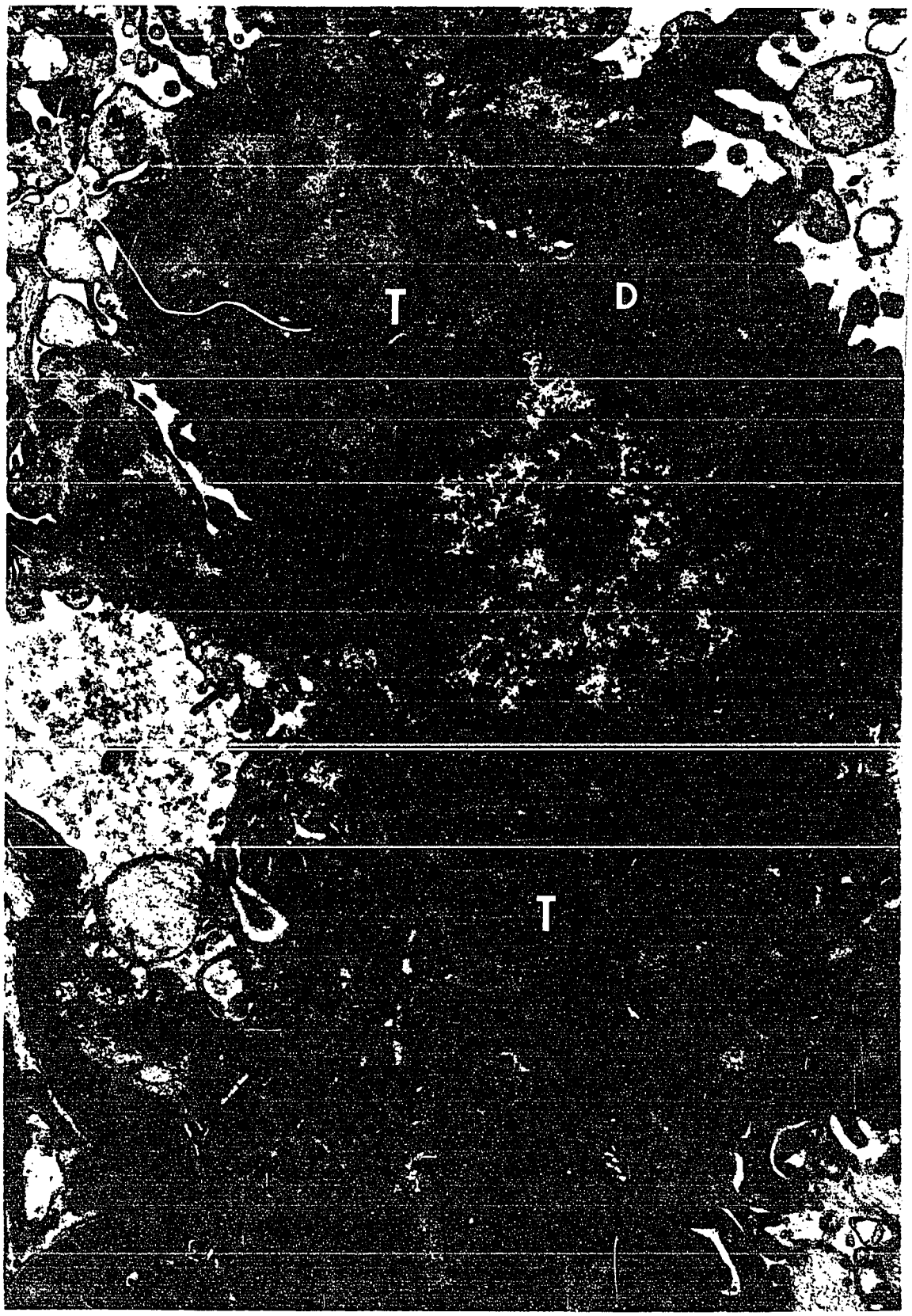

Figure 23

Figure 24

Electron photomicrograph of a portion of a glomerus from depancreatized baboon, twenty-one months diabetic. There are focal collections of electron dense granular material (arrows) in the subendothelial area. This pattern has been seen in immune complex disease in man.

(Reynold's lead citrate and uranyl acetate, 22,230X)

Figure 24